Predicting acute radiation toxicity in breast cancer

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Abstract

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After surgery, radiotherapy is the second most commonly used treatment for breast cancer. Radiotherapy reduces local recurrence rates with a modest improvement in long-term overall survival. However, up to 20 % of patients may experience clinically significant side-effects (toxicity). Radiation toxicity can impact negatively on a patient's surgical outcomes and on quality of life. There are currently no clinically useful predictive tests for toxicity capable of personalising breast radiotherapy. It is also not known how patients' treatment decision-making may be influenced by prior knowledge of their personal risk of side-effects from radiotherapy. With a focus on skin toxicity, this study was designed to explore how acute radiation toxicity in the breast can be predicted more accurately, in order to give patients and clinicians better information to plan treatment.

Breast cancer patients were recruited prospectively at Leicester and seven other European and North American centres into the REQUITE cohort study. Data on acute toxicity and QoL were correlated with patient and treatment variables to identify those side-effects that could have a significant impact on QoL. Patients participating in the REQUITE study in Leicester were then interviewed to explore their attitudes towards predictive testing and whether a test for acute toxicity would influence their treatment decision-making.

The predictive power of known clinical variables associated with acute desquamation was analysed in a combination of three existing Radiogenomics cohorts. In order to investigate the addition of genetic markers to improve predictive model performance, a systematic review and meta-analysis was undertaken, which identified a number of genetic variants associated with acute breast skin toxicity. The clinical prediction model and genetic markers of acute breast toxicity failed to validate in the REQUITE breast cancer cohort, but this analysis confirmed an association of acute ulceration with SNPs near the *REV3L* gene.

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AUC	area under the curve
BCS	breast-conserving surgery
BED	biologically effective dose
BER	base excision repair
BMI	body mass index
BP	blood pressure
CI	confidence interval
CTCAE	Common Terminology Criteria for Adverse Events
CVD	cardiovascular disease
DSB	double strand break
EBRT	external beam radiotherapy
EDTA	Ethylene diamine tetra-acetic acid
EORTC	European Organisation for Research and Treatment of Cancer
ER	estrogen receptor
ESTRO	European Society for Radiotherapy and Oncology
Gy	Gray
GLM	generalised linear model
GLMM	generalised linear mixed model
GOC	Gloucestershire Oncology Centre
GWAS	genome-wide association study
HCP	healthcare professional
HuGE	Human Genome Epidemiology
IMRT	intensity-modulated radiotherapy
MICE	multiple imputation chained equations
NTCP	normal tissue complication probability
OR	odds ratio
PBI	partial breast irradiation
PROM	patient-reported outcome measure
QoL	quality of life
RGC	Radiogenomics Consortium
RMH	Royal Marsden Hospital
ROC	receiver-operating characteristic
ROS	reactive oxygen species
RS	raw score
RTOG	Radiation Therapy Oncology Group
SNP	single nucleotide polymorphism
SSB	single strand break
WBI	whole breast irradiation

Introduction

Breast cancer survival has improved markedly, with 10-year survival rates of over 80 % [1]. As a result, survivorship issues have become increasingly important, including the impact of breast cancer treatments on patients' quality of life (QoL) [2]. After surgery, radiotherapy is the second most commonly used treatment for breast cancer. About 70 % of breast cancer patients undergo radiotherapy as part of their treatment. It is indicated in the adjuvant setting after breast-conserving surgery (lumpectomy) and after mastectomy for patients with high-risk cancers [3]. Like surgery, radiotherapy is a loco-regional treatment modality. The main aim of radiotherapy is to maximise local control of the tumour while minimising damage to the normal tissue.

Breast radiotherapy

Randomised controlled trials and their meta-analysis have demonstrated that adjuvant radiotherapy after breast-conserving surgery reduces local recurrence. When such reduction exceeds 10 % in absolute terms at five years, then it is expected to lead to a modest survival benefit of a quarter of the difference [4]. In recent years, there has been a shift from the standard regimen of whole breast irradiation by external beam radiotherapy (EBRT) towards methods of delivering radiotherapy that provide shorter and more convenient schedules through hypofractionation [5], better targeting of tissues with modern planning techniques, such as intensity-modulated radiotherapy (IMRT) [6], and tailoring the extent of radiotherapy. The various clinical trials that have investigated the extent of radiotherapy also provided an insight into the natural history of early breast cancer and helped guide patient management.

Hypofractionation

Compared to the standard fractionation regimen of delivering a 50 Gy dose to the breast in 25 fractions, randomised controlled trials of hypofractionation typically involve schedules with larger doses per fraction and fewer total fractions. All of the trials in this field with mature follow-up of 10 years or more have demonstrated equivalent local and distant disease control [5, 7-10]. These trials include the RMH/GOC pilot trial, the UK START-A and START-B trials, and the Canadian Ontario trial. More recently, the UK FAST trials have investigated regimens delivering doses up to 6 Gy once per week [11], or over the course of a single week (FAST Forward) [12].

Increasing irradiation

Increasing irradiation appears beneficial in those patients with high-risk disease, where the benefit for breast cancer control is sufficiently substantial. The EORTC 22881-10882 trial is the main trial proving that addition of a tumour bed boost reduces local recurrence [13]. The absolute level of reduction depends on the background risk, but there is no risk at which there is no reduction, although there was no demonstrable improvement in survival. Regional irradiation of the internal mammary chain and medial supraclavicular nodes found an improvement in disease-free survival and breast cancer survival with a marginal improvement in overall survival [14]. In this trial, 25 % of patients had mastectomy and nearly all patients had node positive disease. The TARGIT-B (Boost) trial is currently under way to assess whether irradiation of the tumour bed during surgery improves local control [15].

Reducing irradiation

Omitting radiotherapy in selected cohorts of patients has been assessed in several published trials, such as CALGB [16], BASO-2 [17] and PRIME-2 [18]. These largely included only T1 grade 1 node-negative disease, but even in these patients omission of radiotherapy leads to a statistically significant increase in local recurrence rates from 1-2 % (1 in 50) up to 6 % (1 in 17).

Reducing the extent of radiation with partial breast irradiation (PBI) may improve outcomes in those with lower risk disease by reducing non-breast-cancer mortality [19], whilst maintaining breast cancer control. PBI is limited to the tumour bed and has been investigated in randomised controlled trials over the past two decades. The published trials found that local control remains within the respective *a priori* non-inferiority margins. In the TARGIT-A trial, local control with targeted intraoperative radiotherapy (TARGIT) was not statistically different from whole breast external beam radiotherapy (EBRT) at the pre-specified 2.5 % margin (5-year recurrence-free survival 93.9 % TARIT vs. 92.5 % EBRT) [20].

The ELIOT trial showed a significantly higher ipsilateral local recurrence rate in the intra-operative radiotherapy group, but less than the pre-specified margin of 7.5 % [21]. In the subgroups with excellent prognosis (e.g. luminal A disease), the difference was no longer statistically significant. The GEC-ESTRO trial tested the effectiveness of PBI with interstitial radiotherapy using radioactive wires and found it to be non-inferior to EBRT within a margin of 3 % [22].

A meta-analysis of the TARGIT-A and GEC-ESTRO trials demonstrated non-inferiority and a reduced breast cancer-related mortality with PBI [19]. Furthermore, the recently published results of the IMPORT-LOW trial showed that local control is non-inferior with IMRT delivered only to the tumour bed.

Radiotherapy side-effects in the breast

Radiotherapy is associated with a spectrum of side-effects (toxicity) in the surrounding normal tissues. Acute radiation toxicity occurs within 90 days of treatment and affects high turnover tissues such as the skin (Figure 1, Figure 2), whereas late radiation toxicity occurs more than 90 days after treatment and can persist for life. While acute effects in the breast mainly affect the skin due to epidermal basal cell loss and associated inflammatory response with capillary dilatation and oedema, late effects in the breast include fibrosis (scarring of the subcutaneous tissues), which can lead to atrophy (shrinkage) (Figure 3), and telangiectasia (dilated small blood vessels under the skin) (Figure 4) [23].

The incidence of different breast toxicities has been reported by several randomized controlled trials of whole-breast radiotherapy (Table 1). In the UK START B trial, the incidence of moderate or severe late toxicity ranges from 3.1 % and 4.8 % for telangiectasia to 22.0 % and 25.5 % for breast shrinkage (atrophy) for the hypo-fractionated (40 Gy in 15 fractions) and standard treatment regimen (50 Gy in 25 fractions), respectively [10]. The incidence of fibrosis reported in the Canadian hypo-fractionation trial is similar to that observed in START-B [24]. Neither trial specifically reported acute toxicity.

The UK FAST trial reported significantly lower acute skin toxicity in the hypo-fractionated 30 Gy and 28.5 Gy treatment arms compared to the standard 50 Gy arm (14.4 % and 10.4 %, respectively, versus 46.4 %) [11], while the Cambridge IMRT trial (40 Gy in 15 fractions) reported moderate to severe skin toxicity in as many as 55.4 % of patients [25]. Other observational studies indicate that between 25 and 75 % of breast cancer patients may experience a clinically significant acute skin reaction during radiotherapy [6, 26]. A recent Cochrane review of breast radiotherapy hypo-fractionation trials demonstrated a statistically significant reduction in the risk of acute skin toxicity (RR 0.32, CI 0.22-0.45) and oedema (RR 0.63, CI 0.51-0.78) compared to standard fractionation. The incidence of late toxicities was not significantly affected by hypo-fractionation, apart from telangiectasia (RR 0.68, 0.52-0.91) [5].

Trial	Acute toxicity %	Cosmesis (fair/poor) %	Shrinkage (atrophy) %	Induration (fibrosis) %	Oedema %	Telangiectasia %
START-B 50 Gy	-	21.2	25.5	15.3	8.6	4.8
START-B 40 Gy	-	26.2	22.0	12.8	4.9	3.1
Ontario 50 Gy	-	28.7	-	10.4	-	-
Ontario 42.5 Gy	-	30.2	-	11.9	-	-
Cambridge IMRT 40 Gy	36.5	55.4	10.0	11.7	4.5	4.8
FAST 50 Gy	46.4	20.9	-	-	-	-
FAST 30 Gy	14.4	35.5	-	-	-	-

Table 1. Incidence of moderate or severe breast-related toxicities reported in breast radiotherapy trials.

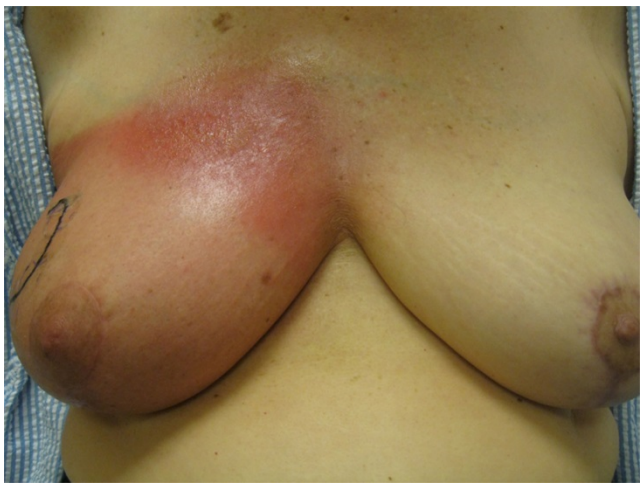

Figure 1. Mild breast radiotherapy skin reaction.

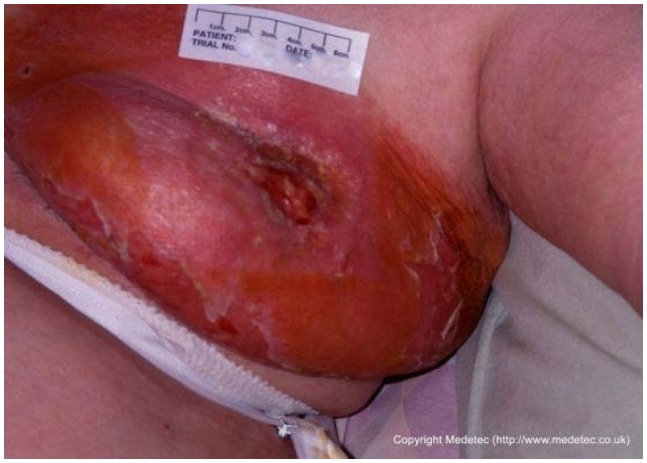

Figure 2. Clinically significant severe radiotherapy skin reaction in the breast.

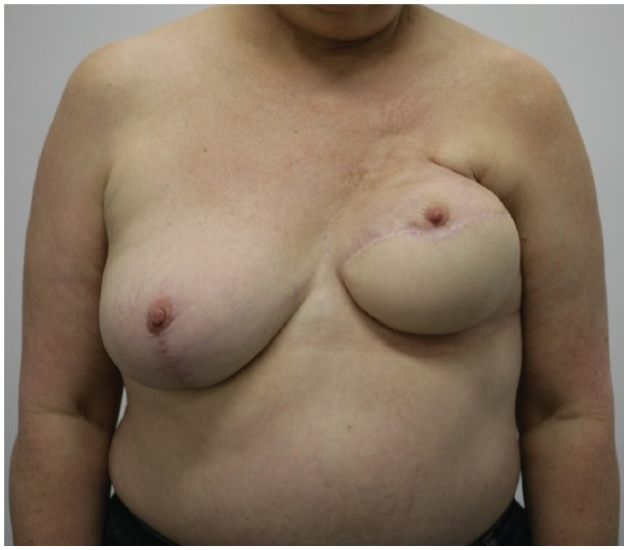

Figure 3. Patient with late fibrosis and resultant breast atrophy (shrinkage).

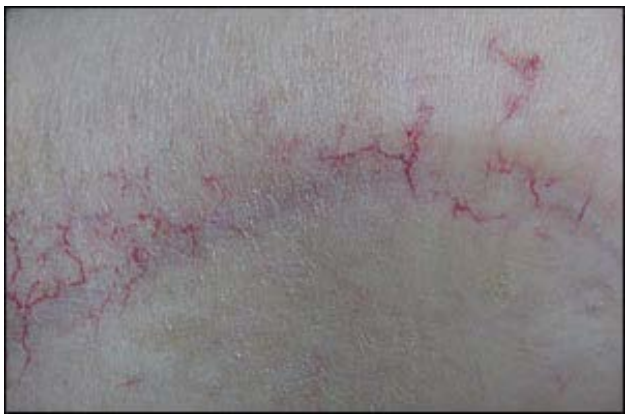

Figure 4. Close-up view of skin telangiectasiae.

Impact of radiotherapy on surgical outcomes

Against the background of growing patient expectations and technical expertise, rates of oncoplastic procedures after breast-conserving surgery (BCS, lumpectomy) [27] and immediate breast reconstruction after mastectomy continue to rise [28]. Breast reconstruction after mastectomy can involve the use of autologous tissue flaps to create a new breast mound, insertion of breast implants, either fixed-volume or expandable, under the pectoralis muscle, or both. Amongst breast surgeons there is an increasing awareness of the impact of post-operative radiotherapy on both cosmetic outcomes and quality of life (QoL) [29].

Data from recent observational studies indicates that up to 30 % of implant-based and up to 53 % of autologous immediate breast reconstructions receive post-operative radiotherapy [30, 31]. Surgeons are invariably influenced in their treatment recommendations by the potential risk of radiotherapy toxicity, which may differ according to the type of reconstruction and the timing of reconstruction. A meta-analysis found a complication rate of 33 % if radiotherapy followed implant-based procedures and 8 % following reconstruction using autologous tissue, but no difference in complication rates according to timing of breast reconstruction (immediate vs delayed i.e. after radiotherapy) [32].

In the absence of any randomized evidence, there is an on-going debate how to best manage patients opting for immediate reconstruction or other oncoplastic procedures, especially when one cannot predict with certainty whether radiotherapy is needed until the full post-operative histology is available [33]. Some centres advocate a 'delayed-immediate' approach to reconstruction, whereby an inflatable expander is placed in the standard fashion under the pectoralis muscle following mastectomy. Once the final histology result is known, if no radiotherapy is needed, this can be exchanged for a permanent implant or an autologous reconstruction can be performed. If post-

operative radiotherapy is required, the implant can be deflated to facilitate radiotherapy to the chest wall while preserving a potential space for re-expansion and subsequent definitive reconstruction with a permanent implant or autologous tissue [34].

Despite the lack of high-quality evidence, there is a clinical consensus that immediate breast reconstruction should at least be considered when post-operative radiotherapy is anticipated [35]. The evidence concerning the cosmetic impact of radiotherapy on other oncoplastic procedures remains equally scanty, but procedures such as therapeutic mammoplasty are nevertheless routinely performed with most patients indicated for post-operative radiotherapy [27].

Impact of radiotherapy on quality of life (QoL)

More than half of the women due to undergo breast radiotherapy are anxious about side-effects and changes to the appearance of their breast [36]. Late radiotherapy toxicity can permanently affect QoL. In the Scottish PRIME trial assessing omission of adjuvant radiotherapy in women over the age of 65, there was a significant increase in patient-reported breast pain, oedema and fibrosis in the patient group that underwent post-operative radiotherapy, although local recurrence rate was significantly reduced [37]. In the UK START trials, up to 40 % of women reported moderate or marked changes to the breast after radiotherapy [38].

While late side-effects of radiotherapy are concerning due to their potential irreversibility, acute radiotherapy toxicity, if sufficiently severe, may cause considerable patient morbidity and may even delay treatment. The vast majority of patients undergoing breast radiotherapy report skin changes [39]. Recent qualitative research has illustrated a predominantly negative perception of the acute treatment phase amongst patients with side-effects that affect multiple dimensions of QoL [40, 41].

In the treatment of breast cancer, patient-reported outcomes (PROs) after adjuvant radiotherapy are invariably linked to surgical outcomes [42]. Good quality evidence on patient reported outcome measures (PROMs) after breast surgery and radiotherapy is lacking. Most of the literature is concerned with PROMs after breast reconstruction, but consists largely of retrospective studies [43]. A more recently published prospective cohort study has shown how QoL can be influenced by the type of breast reconstruction performed, irrespective of whether the patient receives post-operative radiotherapy [31]. Further cohort studies collecting PROMs after breast reconstruction are currently under way [30].

Several systematic reviews have emphasized the need for robust and validated tools to assess PROs after breast surgery and radiotherapy [44-46]. Those developed and validated sufficiently in a breast cancer population include the EORTC QLQ-BR23 [47], FACT-B (Functional Assessment of Cancer Therapy – Breast cancer) [48], HBIS (Hopwood Body Image Scale) [49], and BREAST-Q questionnaires [50].

Inaccurate information about the effect of breast cancer treatments on QoL can impair doctor-patient communication and limit the patient's understanding of their clinical management. Breast cancer patients are faced with difficult treatment decisions, including the choice of breast-conserving surgery (BCS, lumpectomy) plus radiotherapy versus mastectomy, which may not entail radiotherapy in low-risk disease. These decisions should be guided by meaningful information about expected QoL and toxicity outcomes.

Radiobiological basis of radiation toxicity

Radiation is an established cancer treatment, because it permits tumour eradication and a relative preservation of normal tissues. The radiation employed in cancer treatment (radiotherapy) is predominantly made up of photons and electrons. It can be delivered by a machine outside the body (EBRT), typically a linear accelerator (linac), or from radio-active material placed inside the body near the tumour or in the tumour bed (brachytherapy). Radiation causes DNA damage and disrupts cell proliferation, thereby affecting cell survival and inducing cell death (apoptosis) [51].

DNA damage occurs through direct and indirect effects. As a direct effect, any atom or molecule in the radiation target may be excited or ionised, a process by which electrons are displaced from an atom or molecule leaving a charged ion. In the presence of oxygen and water in the target tissue, radiation generates free radicals, which are atoms or molecules with an unpaired orbital electron in the outer shell, resulting in a state of high chemical reactivity. This leads to the formation of reactive oxygen species (ROS), such as hydrogen peroxide and hydroxyl radicals (OH•). Hydroxyl radicals can cause DNA damage by abstraction of a hydrogen atom from the methyl group of thymine and each of the carbo-hydroxyl bonds of 2'-deoxyribose [23].

DNA double strand breaks (DSBs) are the principal DNA damage lesion responsible for radiation-induced cell death. The number of DSBs is proportional to radiation dose [52]. DSBs can lead to direct chromosome breakage or chromosomal translocations and deletions, which can be measured

using biological functional assays, such as counting the number of di-centric and ring chromosomal aberrations during metaphase [53].

Tissues that undergo rapid and frequent cell division are more susceptible to DNA damage. This includes cancer cells but also some normal tissues, such as skin, hair follicles, and mucosa. At the same time, rapidly dividing cells repopulate in the tissue, so the goal of radiotherapy is to administer a dose that causes sufficient DNA damage to induce cell death and limit re-population. However, because a similar effect is also seen in the normal cells within the irradiated tissue, it limits the dose that can be given before the patient will experience significant normal tissue toxicity.

The therapeutic ratio describes the balance between the probability of tumour control and the probability of normal tissue complications (NTCP) (Figure 5). To ensure that the vast majority of patients treated are not left with long-term side-effects from radiotherapy, it is usual practice to calculate dose limits according to clinical constraints published for different target tissues, below which only a relatively small proportion of patients (usually < 10 %) are likely to experience significant or severe normal tissue complications [54-56].

In the radical (curative) setting, radiation is administered in multiple small-dose fractions to maximise the differential in response between the tumour and dose-limiting normal tissues. Standard practice has evolved to radiotherapy regimens of between 3 to 8 weeks' duration to allow administration of higher doses, because the acute normal tissue toxicity observed in skin and mucosal surfaces is moderated by the regeneration of cells within those tissues over the course of treatment. However, the dose is limited by toxicity observed in late-responding tissues, such as the lung and heart, which do not regenerate during the course of radiation treatment [57].

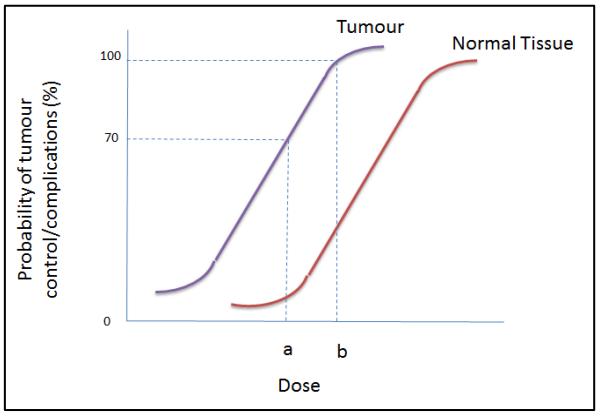

Figure 5. Graph representing therapeutic ratio. Dose 'a' has a 70 % probability of tumour control and low risk of normal tissue complications, whereas dose 'b' has an almost 100 % probability of tumour control but a much higher risk of toxicity [58].

The concept of *Four Rs of Radiobiology* summarises the biological processes that influence tumour and normal tissue responses to fractionated radiotherapy [57]:

1. Repair of tumour cell and normal cell DNA between treatment fractions:
2. Redistribution of cells into more or less radiosensitive phases of the cell cycle, with cells in mitosis most sensitive to DNA damage and cells in late S-phase most resistant:;
3. Repopulation (regeneration) of cells between fractions;
4. Re-oxygenation of tumour cells during treatment, with tumour tissue oxygenating over the course of radiotherapy, thus increasing its sensitivity to radiation.

More recently, this classification was revised into the 'Five Rs of Radiotherapy' to include 'Intrinsic Radiosensitivity' [59]. This final addition was an admission of not being able to explain the individual radiosensitivities of cells or tissues at a mechanistic level. Patients given the same radiation dose experience normal tissue toxicity to varying degrees [60]. This intrinsic radiosensitivity is at least in part determined by individual genetic variation [61].

Genetic basis of radiation toxicity

Evidence for the heritability of radiosensitivity comes from rare genetic disorders and from cell-based studies. In genetics, the heritability of a trait is the proportion of observed differences in phenotype between individuals that is due to underlying genetic differences. Initial evidence for the genetic basis of radiosensitivity came from the observation that certain individuals with specific genetic disorders, such as ataxia-telangiectasia and Nijmegen Breakage Syndrome, were hyper-sensitive to radiation and thus unable to undergo standard radiotherapy [62]. These known genetic defects affect important DNA damage response pathways, but proved to be very rare and on their own could not account for the observed inter-individual variation in radiosensitivity across the general patient population [63].

Based on data from dose fractionation studies of patients undergoing bilateral internal mammary field irradiation, the contribution of patient-specific factors to variation in radiosensitivity has been estimated to be between 49 to 90 %, with the remainder accounted for by stochastic effects [64]. Further evidence for the heritability of radiosensitivity comes from research involving functional assays of DNA damage response. Flow cytometric assays for apoptosis induction and cell cycle delay following irradiation demonstrated a significantly greater concordance in monozygotic than dizygotic twin pairs [65]. Baseline and induced micronucleus frequencies showed a heritability of 68 to 72%

and 57 to 68%, respectively, using two different models of estimating heritability [66]. Enhanced G2 chromosomal radiosensitivity, associated with early onset cancers, was found in a significant number of first-degree relatives of radiosensitive patients and cancer survivors, but not in relatives of patients with normal assay responses [67, 68].

In humans, measurements of radiosensitivity using *in vitro* assays show an approximately normal distribution, as expected for other polygenic traits such as blood pressure or height [69]. It is therefore hypothesised that radiosensitivity is a partly inherited complex polygenic trait involving a number of genes along different biological pathways. Most researchers have assumed that common genetic variants with low penetrance (i.e. modest functional effect) will account for the majority of the observed inter-individual variation.

Cellular pathways associated with radiosensitivity

Ionizing radiation causes DNA double strand breaks (DSB) as well as single strand breaks (SSB), base damage, and DNA cross-links. Cells have evolved complex repair mechanisms in response to DNA damage to maintain genomic integrity. DSBs are hardest to repair, because the DNA ends are completely separate and associated base damage impairs DNA ligation [70].

Genetic syndromes associated with radiation hypersensitivity provided the first clues as to which cellular pathways might play an important role in determining human variation in radiosensitivity. Ataxia telangiectasia (AT) was the first reported syndrome with life-threatening clinical radiotherapy toxicity and extreme cellular radiosensitivity. It is associated with mutations in *ATM* [71]. The product of *ATM* is a serine/threonine protein kinase that is activated by DNA DSBs. This requires the presence of a trimeric protein complex consisting of MRE11, RAD50 and NBS1 [72]. ATM and its downstream kinase CHK2 phosphorylate several targets that regulate DNA repair, cell cycle and apoptosis, including *H2AX*, *MDM2*, and *p53/p21* [73]. Several of these genes are mutated in other radiosensitivity syndromes, such as Nijmegen Breakage Syndrome (*NBS1*), AT-like disorder (*MRE11*), and Nijmegen breakage-like disorder (*RAD50*) [74, 75].

During S and G2 phase of the cell cycle, DNA DSB repair occurs via homologous recombination (HR). HR restores DNA DSBs using the un-damaged homologous chromosome as a template. This mechanism involves several proteins including RAD51, 52, and 54, as well as BRCA1 and 2 and XRCC2 and 3 [76]. Non-homologous end-joining (NHEJ) operates during other phases of the cell cycle or when HR is impaired. NHEJ is the main DNA DSB repair pathway in humans. It effectively links up

the ends of broken DNA in a chromosome without a template. Downstream effectors in NHEJ include XRCC4 and 5, DNA-PKcs and LIG4 [77].

DNA SSBs are repaired via the base excision repair (BER) pathway. BER allows for the quick and efficient repair of SSB as well as for the repair of damaged bases. In this pathway, specific DNA glycosylases, either with or without an endonuclease (APE) first excise the altered base and cleave the DNA at the resulting abasic site, thus generating a strand break. Then XRCC1, PARP1, PNK and LIG3 contribute to DNA re-synthesis and stabilisation [78].

Since radiation results in free radical formation in exposed tissue, genes encoding antioxidants involved in free-radical scavenging are important, for example, *SOD1* (superoxide dismutase) and *GSTA* (glutathione S-transferase). Genetic variation that predisposes to increased levels of free radicals may in turn predispose to increased radiation toxicity [79]. There is evidence from pre-clinical studies that changing levels of anti-oxidants can alter cellular radiosensitivity [80].

Formation of free radicals also induces an inflammatory response that results in the release of cytokines and growth factors, including TGFβ1 (transforming growth factor- β1), TNFα (tumour necrosis factor-α), EGF (epidermal growth factor), and interleukins [81]. There is evidence that TGFβ1 is involved in the pathogenesis of fibrosis following radiotherapy [82]. This cellular pathway has many features in common with the normal wound healing process, which becomes deregulated in radiation fibrosis [83]. Radiation fibrosis is also accompanied by pathological changes in the surrounding vasculature, in particular endothelial damage and fibrosis of the vessel wall [84].

As they involve different cellular pathways (Figure 6), it is hypothesised that variation in the above genes is expected to be associated with different clinical endpoints of radiation toxicity. For example, polymorphisms in genes involved in fibrogenesis would mainly affect fibrosis (scarring), whereas variation in genes implicated in vasculature would increase the risk of telangiectasia [85].

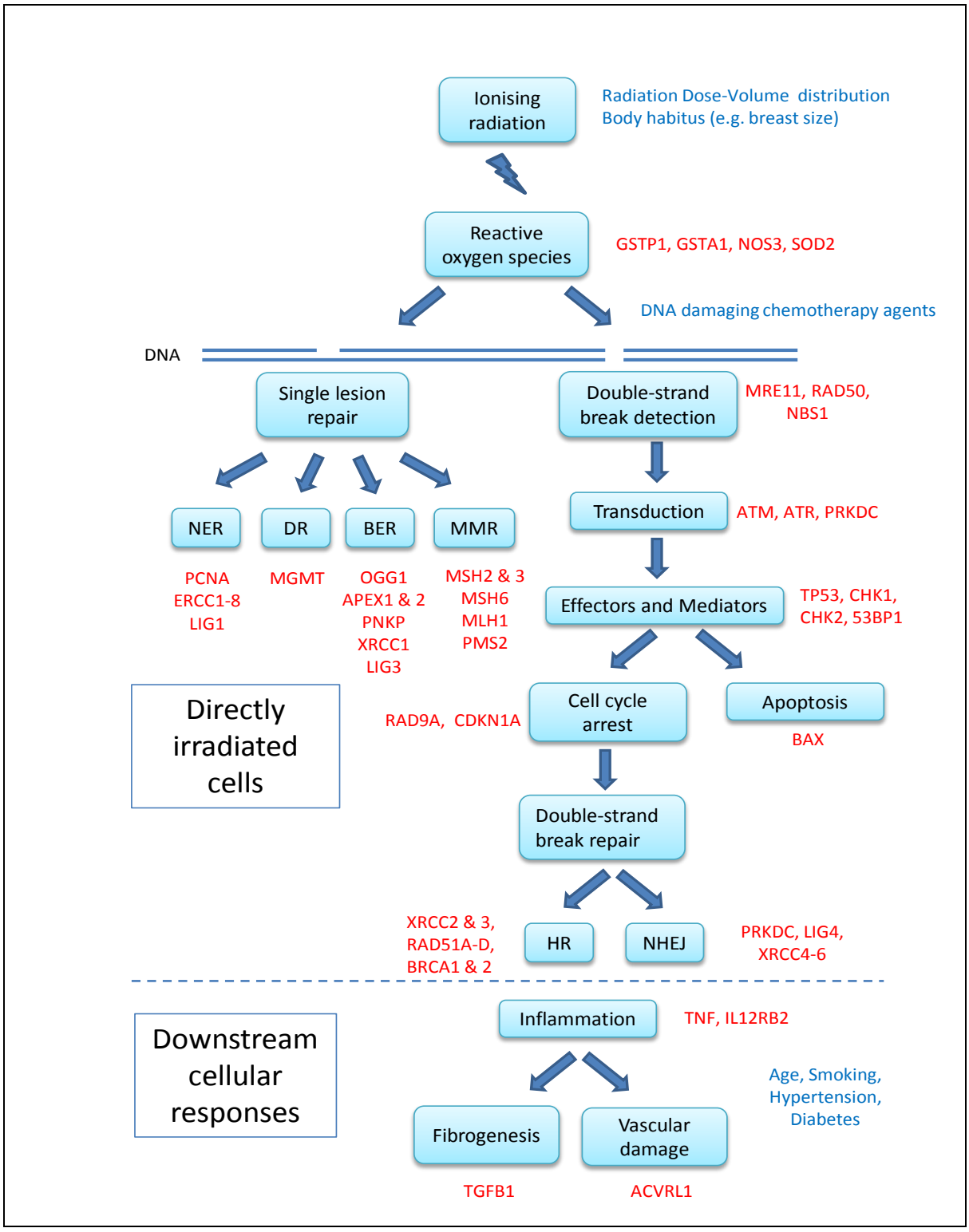

Figure 6. Cellular pathways of radiation response. Text in red are associated candidate genes, text in blue are non-genetic factors known to affect radiotherapy adverse reactions. NER Nucleotide Excision Repair, BER Base Excision Repair, DR Direct Repair, MMR Mismatch Repair, DSB Double-strand break, HR Homologous Recombination, NHEJ Non-homologous end joining (adapted from [86]).

Radiogenomics

Classic radiobiological research has focused on the identification and quantification of DNA damage induced by radiation, understanding the genes and pathways involved in DNA damage repair, and investigating signalling pathways to cell cycle arrest as well as those pathways induced by radiation that activate cell death. With the evolution of high-throughput genotyping methods and bioinformatics, radiogenomics has emerged as new research field with the aim of finding the genetic determinants of adverse reactions to radiotherapy, in parallel to the investigation of other complex genetic disease traits, such as coronary artery disease [87].

Candidate-gene approach

In order to identify genetic markers of normal tissue radiosensitivity, the main approach taken by investigators has been to type SNPs (single nucleotide polymorphisms) in the genome of patients undergoing radiotherapy. SNPs represent relatively common genetic alterations typically with low effect sizes. Through a number of case-control studies, in which SNPs at candidate loci were genotyped across patients with or without radiotherapy side-effects, several predictive genetic markers were identified. Two systematic reviews published in 2009 summarised findings of almost 60 studies conducted across a range of candidate genes involved in DNA damage response, oxidative stress response and radiation fibrogenesis [88, 89].

To foster collaboration in the field, the International Radiogenomics Consortium (RGC) was formed to facilitate the pooling of patient cohorts and datasets [90]. For example, the UK RAPPER cohort consists of patients recruited prospectively into breast and prostate radiotherapy trials in Cambridge, Manchester, and at the Royal Marsden Hospital. In 2012, the RAPPER group published an independent validation of 46 genes previously thought to be associated with radiation toxicity. After adjustment for multiple testing, none of the previously reported associations was replicated in the validation cohort of 1,613 patients [91].

These findings confirmed that associations reported in previous candidate gene studies were either false positives or true weak positives with over-inflated odds ratios. Most previous studies had been relatively under-powered to detect any genetic variant with modest functional effect on radiation toxicity. The median sample size across all SNP studies included in both 2009 systematic reviews was 101 (range 25 to 446 patients) [88, 89]. The majority of these earlier studies were also investigating multiple SNPs across different toxicity endpoints and cancer sites without adjusting for multiple testing. This would have resulted in a high probability of finding positive associations by chance and may explain the subsequent lack of replication. Within the RGC, the focus has since shifted towards larger studies with built-in replication across pooled patient cohorts. This led to the publication of

several replicated associations, in particular with late toxicity endpoints, in TNFα (n=2,036 breast cancer patients, upper quartile of overall late toxicity), ATM (n=5,456 breast and prostate cancer patients, overall acute and late toxicity), and XRCC1 (n=1,883 breast cancer patients, overall late toxicity) [92-94].

Genome-wide approach

The HapMap consortium currently estimates that there are 10 million SNPs among the three billion base pairs in the human genome [95]. Humans share large segments of DNA (haplotype blocks), which are separated by short recombination hotspots. Therefore, by genotyping a sub-set of SNPs, one can impute the genotypes of other SNPs in the same block. Microchips with up to two million tag SNPs have enabled researchers to analyse all common genetic variants. This approach has greatly advanced research into finding loci that explain the genetic component of traits (e.g. height) or the risk of developing different cancers or common diseases (e.g. coronary artery disease) [96].

Such genome-wide association studies (GWAS) have some important findings in common. The typical impact of each SNP on phenotype is small, with odds ratios of between 1.05 and 1.5. Most of the positively associated SNPs are not located in genes previously anticipated as important for the phenotype. Some are not located in genes, but in non-coding areas of the genome with regulatory or unknown function. Also, these SNPs may represent tags for further occult variants in linkage disequilibrium [97].

The experience with GWAS contrasts with the candidate gene approach to investigate the genetic determinants of radiation toxicity. Consistent with the hypothesis that radiosensitivity is an inherited complex polygenic trait, there may be there may be some genetic loci that affect the overall risk of toxicity, some that are tissue-specific (e.g. the vascular endothelium), and others that are end point-specific (e.g. erectile dysfunction in prostate cancer) [88].

The first GWAS published in the field of radiosensitivity was of a modest size (n=465) and was designed to detect SNPs associated with erectile dysfunction among prostate cancer patients treated with radiotherapy [98]. The authors identified 12 SNPs in a two-stage design based on the same patient cohort (internal replication). These 12 SNPs lie in or near genes involved in control of erectile function or cell adhesion and signalling, but not DNA damage and radiation response genes. The results are currently undergoing replication in pooled GWAS cohorts [99]. An additional moderately-sized GWAS in prostate radiotherapy (discovery cohort n=741, replication cohort n=1,001) identified a further locus associated with late toxicity in *TANC1*, which is involved in regenerating damaged muscle [100].

Several other radiogenomics GWAS are currently underway or about to be published. These are likely to provide a growing list of SNPs with evidence for predicting adverse reactions to radiotherapy in a variety of cancers. To date, the RAPPER group has conducted a phase I GWAS (discovery cohort n=1,850, replication cohort n= 1,733) to identify true associations with late radiation toxicity, with replication using several smaller cohorts [101].

Predicting acute breast radiation toxicity

Breast surgeons are invariably influenced in their treatment recommendations by the potential complications of adjuvant breast cancer treatments, particularly in view of the increasing uptake of oncoplastic procedures [29, 102]. If sufficiently severe, an acute skin reaction to radiotherapy can have detrimental effects on any form of breast reconstruction [103]. It can also predispose patients to significant chronic late toxicity [104]. This in turn leads to complications such as implant capsular contracture or scarring, which is associated with worse QoL outcomes [105].

If it was possible to stratify patients according to risk of radiation toxicity, it would enable breast surgeons to advise their patients on the most appropriate operation. More accurate information about their individual risk of radiation toxicity could also help to guide patients in the treatment decision-making process. For acute breast toxicity, the individual clinical risk factors identified in retrospective analyses have sometimes reported conflicting results, yet breast size or volume and patient body weight have been consistently associated with acute skin toxicity [25, 26, 106-111]. Nevertheless, clinical parameters alone cannot reliably predict whether a patient is particularly radiosensitive and will develop a severe radiation reaction. The time is right to develop individual risk prediction models for radiation toxicity by integrating clinical and patient factors with predictive genetic markers [112].

However, before any predictive tool can be evaluated prospectively, the predictive power of known clinical variables associated with acute radiation toxicity must be validated. Research is also needed to ensure that any such decision-making tool is acceptable to and appropriate for breast cancer patients. The goal of this study is improve the prediction of acute side-effects after breast radiotherapy by combining genetic and clinical predictors, in order to reduce the impact of radiotherapy on adverse clinical outcomes and improve patient-reported outcomes. Results of this study could be used to inform the development of future randomised-controlled interventional biomarker trials.

Patients and Methodology

In order to achieve the study goal of improving the prediction of acute breast radiation toxicity by integrating clinical and genetic predictors, the predictive power of known clinical variables associated with clinical radiation toxicity must be validated. Genetic markers reported in the literature that have previously been associated with acute breast toxicity should then be added to the model with the aim of increasing predictive power. For validation purposes, it is important to use a patient cohort recruited with reliable data capture. Research is also needed to ensure that any such predictive test is acceptable to and appropriate for breast cancer patients.

Research objectives

With the study goal in mind, this research project was designed with the following objectives:

1. To conduct a prospective cohort study of acute radiotherapy side-effects, collecting data on toxicity end-points and patient-reported outcome measures (PROMs);
2. To explore patients' attitudes to and beliefs about a predictive test for acute radiotherapy side-effects and the potential impact on patients' decision-making
3. To validate the predictive power of known clinical variables in existing cohorts of patients who underwent breast radiotherapy
4. To conduct a literature-based systematic review of published genetic markers of acute breast radiation toxicity
5. To validate published genetic markers of acute breast radiation toxicity in conjunction with validated clinical predictors in a prospectively recruited patient cohort

Study design

This research project was designed using a different methodology to address each research aim in turn. Multi-methodology is appropriate where different phases of the research project make specific demands on general methodology, and to give a more complete view of the research field [113]. It is termed mixed-methods research when both qualitative and quantitative data are collected in a planned order and then integrated at some stage during the research process [114]. A mixed methods approach serves to test consistency and increases richness and detail of research findings [115].

Phase 1

The central part of this project was a multi-centre prospective cohort study collecting clinical toxicity data and PROMs from breast cancer patients at various time-points before and after radiotherapy using a standardised data collection protocol as well as blood samples for DNA analysis and genotyping. The advantages of a prospective cohort study include reliable capture of clinical and patient variables, and baseline assessment of PROMs prior to radiotherapy to give a realistic picture of the effect of radiotherapy on QoL.

To achieve sample homogeneity and integration of data from other institutions, patients were recruited according to the methodology of the EU-funded REQUITE (Radiotherapy for Quality of life through reduced Toxicity) multi-centre prospective cohort study [116]. The clinical endpoints were acute skin toxicity (ulceration, desquamation, erythema and oedema) and change in QoL scores from PROMs questionnaires administered prior to radiotherapy, on completion of radiotherapy, and three months after radiotherapy.

Phase 2

To explore patients' attitudes and beliefs about future predictive testing for radiation toxicity, qualitative data was collected through semi-structured interviews with a sample of patients from this cohort on completion of radiotherapy in Leicester. Based on their experience of radiotherapy, participants were interviewed about their views on a predictive tool for acute radiotherapy side-effects with a focus on ensuring the acceptability and feasibility of such a predictive test. As some of the issues explored in this part of the project were personally sensitive, individual interviews were preferred to focus group. This qualitative research was conducted according to published RATS guidelines [117].

Phase 3

Known predictive clinical variables for acute radiation toxicity were analysed in three existing patient cohorts and then validated in the prospective study cohort collected in Phase 1. Previous studies reported statistical models using clinical predictors of radiation toxicity but these have not been replicated in other cohorts. Such a model must be targeted for clearly defined and specific toxicity end-points, but raises the issue of how to deal with the use of different toxicity scales in existing cohorts, such as the RTOG (Radiation Therapy Oncology Group) versus the CTCAE (Common Terminology Criteria for Adverse Events) scale [118].

Phase 4

Following published HuGE network and STROGAR guidelines [119, 120], a systematic review and meta-analysis, where appropriate, were undertaken of published genetic markers associated with acute radiotherapy side-effects. The two most recent systematic reviews of genetic markers of radiotherapy toxicity were published in 2009 [88, 89]. Since then, a number of genetic associations have been disproven for lack of replication and new candidate markers have emerged [92, 121, 122]. Moreover, there has been no previous systematic review of markers of the acute response.

Phase 5

The genetic markers identified in Phase 4 were typed in the prospectively recruited participants in project Phase 1, using commercially available Fluidigm technology. The focus of this analysis was not to replicate the genetic associations per se, but to explore the increased predictive value of a statistical model that incorporates genetic markers in addition to validated clinical predictors validated in Phase 3.

The research methods used in each phase outlined in Figure 7 will be described in further detail in the following chapters.

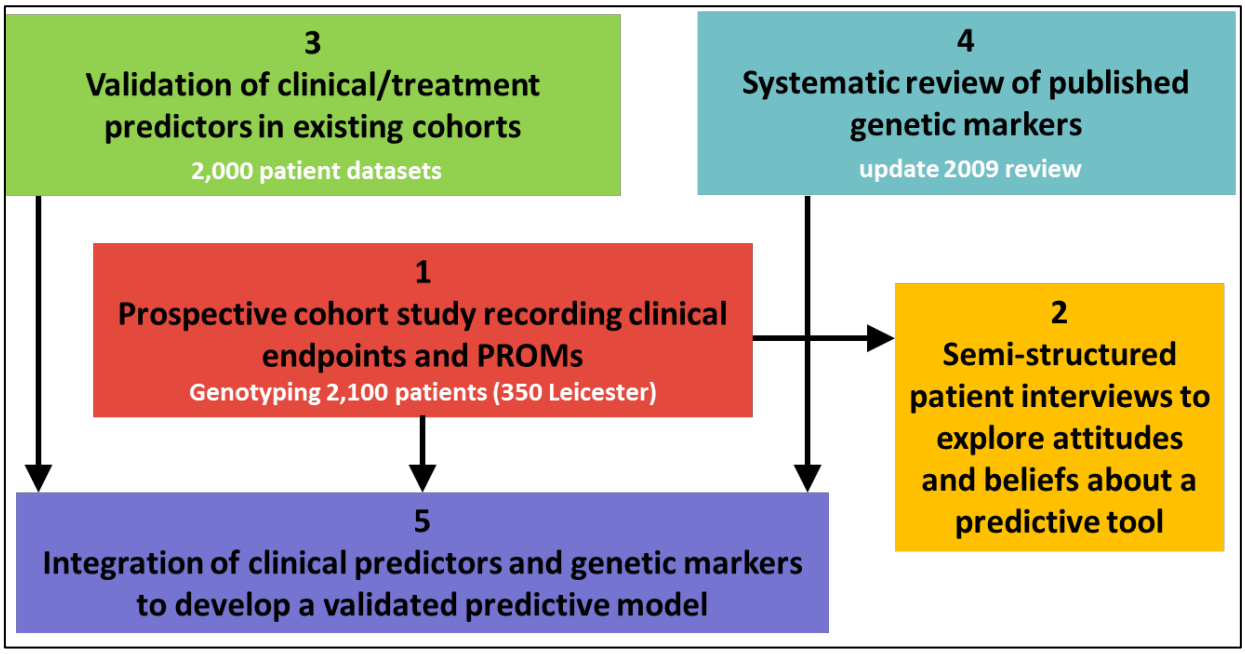

Figure 7. Overall study schema. The research project was divided into five sometimes overlapping phases.

Patients

This study involved both prospectively enrolled patients and the retrospective LeND cohort of 633 patients with documented normal tissue toxicity recruited at varying time points (2 to 15 years) after breast radiotherapy in Leicester, Nottingham and Derby [123]. For the validation of clinical predictors of acute radiation toxicity (Phase 3), two other cohorts were used: the German ISE and the Cambridge Intensity-modulated Radiotherapy (IMRT) trial cohorts. The German cohort includes a total of 478 women treated with breast radiotherapy following breast conservation recruited prospectively from centres across SW Germany [124]. The Cambridge cohort consists of 1,014 women who received adjuvant radiotherapy to the breast following breast-conserving surgery as part of the Cambridge IMRT trial, 411 of whom were randomized to intensity-modulated radiotherapy (IMRT) to improve dose homogeneity (equal distribution of the radiotherapy dose) in the irradiated breast [25].

For the multi-centre prospective validation cohort (Phase 1), patients were recruited into the EU-funded REQUITE (Radiotherapy for Quality of life through reduced Toxicity) multi-centre prospective cohort study between 2014 and 2016. The main endpoint in REQUITE is late, or long-term, radiation toxicity in a variety of cancers, but using the same methodology, data on acute toxicity were also collected on completion of radiotherapy. I recruited 350 patients following breast-conserving surgery at the Breast Unit at Glenfield Hospital and the Radiotherapy Department at the Leicester

Royal Infirmary prior to the start of their radiotherapy. In addition, access was given by the REQUITE study consortium to the data on acute toxicity and QoL for a further 1,722 breast cancer patients recruited across seven other centres in Europe and North America (total n=2,071) [116].

The qualitative study (Phase 2) involved a purposive sample of breast cancer patients recruited into the REQUITE study in Leicester who were interviewed on completion of radiotherapy. The Breast Unit at University Hospitals of Leicester is one of the largest in the UK, seeing over 800 women with a new diagnosis of breast cancer annually. These patients represent a variety of ethnic and socio-economic backgrounds as well as breast shapes and sizes.

Statistical considerations

With a conservative estimate that toxicity data would be available on 75 % of patients, based on effect sizes observed for genetic associations with radiation toxicity, a power calculation for the genetic assays showed that 1,575 patients for breast and prostate cancer each had 80% power to detect a RR of >1.56 for at least grade 2 toxicity ($\alpha = 5 \times 10^{-5}$ for 1000 SNPs, allele freq = 0.25, toxicity rate = 20%), or with more stringent criteria a 90% power to detect a RR >1.66 ($\alpha = 1 \times 10^{-5}$, allele freq = 0.25, toxicity rate = 20%).

The strength of association of predictors was assessed by calculation of the odds ratios (ORs). Toxicity scores were adjusted for clinical and patient co-variables in multivariate analysis, taking into account radiotherapy dosing schedule and other surgical factors e.g. post-operative infection. Predictor selection was based on logistic regression. The statistical model was developed on one dataset and then validated in the other cohorts.

Ethics

Human samples were collected as part of this research. Samples were anonymised and labelled with a patient identifier to allow correlation with clinical end-points. Clinical records were only accessed by the Research Fellow or by practising clinicians and entered onto designated study proformas in anonymised form. Patient data was held on a password-protected computer database physically located within the University of Leicester. Samples shipped to and stored at GCLP-compliant premises. As DNA storage is outside the remit of the UK Human Tissue Act, this legislation did not

apply. All data and experimental results were recorded and held physically within University password-protected computers. Data from semi-structured interviews was transcribed in anonymised form and transmitted using data encryption.

Patient recruitment under the REQUITE study protocol was covered by the multi-institutional UK Ethics approval (NRES Committee North West – Greater Manchester East, ref. 14/NW/0035) and equivalent approvals in other countries. Formal approval was sought from the local NHS Research Ethics Committee for additional PROMs questionnaires in project Phase 1 and patient interviews conducted in Phase 2 by way of substantial amendment. Patients were consented for interviews using an additional consent form. The study was conducted in accordance with the approved protocol, the Declaration of Helsinki, ICH Guidelines for Good Clinical Practice (ICH GCP), relevant regulations and standard operating procedures.

Study sponsor of the REQUITE study and sub-studies including the qualitative interviews was the University of Manchester, providing insurance cover for legal liabilties:

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Phase 1: The REQUITE Acute Breast study

There have been several attempts at developing predictive models that are capable of identifying patients at high risk of clinically significant side-effects before the start of their radiotherapy across different disease sites. While the majority of published models predicts late effects [125-131], there is also an increasing number of published models predicting acute toxicity [132-137]. Earlier studies have also highlighted how information from genetic marker or SNP profiling can be incorporated into clinical predictive models of radiotherapy toxicity [138, 139]. These emerging models require external validation, ideally, in a multicentre collaborative setting.

There are a number of datasets available for validation, but they are variable in terms of the data collected. With increasing international co-operation and recognition for the need to collect harmonised data, the REQUITE observational study was conceived to collect data and blood samples for biomarker assays under a unified study protocol, in order to provide a multicentre validation cohort for predictive models of radiation toxicity, to create a resource for studying the relationships between toxicity endpoints and between toxicity and QoL, and to identify and validate the most promising biomarkers of radiation toxicity [116].

While the main endpoint of REQUITE is late toxicity, it is also possible to evaluate acute toxicity endpoints using this prospective cohort design. There is a growing awareness of the impact of radiotherapy on breast cosmesis and QoL in the acute setting. If sufficiently severe, an acute radiotherapy skin reaction can affect cosmetic outcome after breast surgery and predispose the patient to late toxicity, such as scarring or implant capsular contracture [104, 105]

Individual clinical risk factors identified in retrospective analyses have sometimes reported conflicting results, yet breast size or volume and patient body weight or body mass index (BMI) have been consistently associated with acute skin toxicity [25, 26, 106-111]. At the same time, several potential genetic markers of acute skin toxicity have been reported [88, 89, 140]. Both clinical predictors and genetic markers can be validated in the REQUITE breast cohort and correlated with clinical and patient-reported QoL endpoints.

Objectives

The aim of this part of the project was to conduct a prospective cohort study of acute radiotherapy side-effects in the breast (REQUITE-AB), integrating clinical and patient-reported endpoints (research aim 1), as part of the main REQUITE study, with the following objectives:

- To recruit a prospective cohort of breast cancer patients undergoing radiotherapy according to local regimens;
- To collect standardised toxicity and non-genetic risk factor data for the study of determinants of acute radiotherapy side-effects in the breast;
- To establish a comprehensive database and sample collection as a resource for prospective validation of clinical predictor models and predictive biomarkers in later stages of the research project.

Study design

This was an international observational cohort study using the REQUITE study breast cancer sample recruited at eight participating centres in Belgium, France, Germany, Italy, Spain, UK and the USA.

Data on radiotherapy toxicity, non-genetic risk factors (e.g. radiation dosimetry, chemotherapy use, age, diabetes, smoking history, co-morbidity) and QoL was collected prospectively at specified time points (baseline, end of radiotherapy, and 3 months after radiotherapy at two centres). Pre-treatment blood samples were collected from all patients for downstream analyses.

Patient recruitment

The REQUITE study's target recruitment for breast cancer patients was 2,100 patients. Female patients over 18 years of age with primary cancer of the breast (invasive or *in situ*), due to receive adjuvant radiotherapy after breast-conserving surgery (BCS), were eligible for inclusion in the study. Patients were required to have understood information about the study and give written informed consent as part of the main REQUITE study. Patients eligible for entry into REQUITE were provided with a verbal and written explanation of the study in accordance with local research governance. After adequate time (minimum 24 hours), and provided that all queries had been addressed, patients were consented onto the study by a suitably qualified and experienced GCP (or equivalent)-trained

member of the study team. Specifically, consent was sought for use of blood samples and/or DNA in molecular genetic research and future genomic studies with potential commercialisation.

Patients were identified in the multi-disciplinary team (MDT) meetings, at outpatient clinics, and in the radiotherapy suite prior to commencement of their radiation treatment. Patient details were kept on-site in a screening and recruitment log as part of the main REQUITE study. Only a minimal dataset was recorded (hospital number and tumour site) for patients who declined to take part. Since REQUITE did not interfere with any standard or experimental diagnostic or therapeutic interventions, patients were eligible to take part in this observational study while participating in any other study or trial.

Inclusion criteria

Patients required a confirmed histological tumour diagnosis, with no evidence of distant metastatic disease, and had to be suitable for adjuvant radiotherapy, including patients who received neo-adjuvant or adjuvant chemotherapy. Patients receiving chemotherapy needed to have completed their treatment course at least two weeks prior to commencing radiotherapy. Patients were also required to have had no other malignancy for 5 years prior to enrolment, except for basal or squamous cell carcinoma of the skin. They had to give consent for breast photographs, a venous blood sample, and follow-up according to the REQUITE study schedule.

Exclusion criteria

Patients with metastatic disease were excluded, as were patients who had received prior radiotherapy to the same site (including the contralateral breast). Proton therapy, bilateral radiotherapy, and partial breast irradiation were also excluded, as were patients receiving concomitant chemo-radiation. Male breast cancer patients, patients with a breast implant *in situ*, and patients who had undergone mastectomy (prior to chest wall radiotherapy) were not eligible for inclusion in the study.

Sample collection

Pre-treatment blood samples were collected for downstream analyses: one 10 ml EDTA sample for DNA extraction to investigate genetic variation as predictor of radiation toxicity, and up to two further samples, which did not form part of this research project. Depending on the recruiting site, these included a 2.5 ml PAXgene sample for RNA collection and local storage for future studies, and/or a 10 ml Lithium Heparin sample for live cell apoptosis assay and other DNA damage assays, again processed locally by each participating study site.

Empty bar-coded 10 ml EDTA BD Vacutainer™ tubes were distributed from the Centre for Integrated Genomic Medical Research (CIGMR) at the University of Manchester to all study centres together with additional bar code labels to be used on PAXgene or Lithium Heparin sample tubes, depending on recruiting site. Frozen whole blood EDTA samples were shipped at regular intervals to CIGMR, which hosted the centralised biobank of the main REQUITE study for sample storage prior to DNA extraction. All laboratory, data and management processes within the ISO9001:2008 certified CIGMR biobank were fully ISO compliant and all processes carried out outside the biobank adhered to the ISO Quality Policy.

Bloods were collected prior to the start of chemotherapy or radiotherapy. All EDTA samples were stored at -80°C as whole blood. Study centres scanned the bar code on each sample prior to storage to provide sample tracking to the centralised REQUITE database, accessible to CIGMR, in order to warrant accurate and consistent tracking of study samples between study centres and CIGMR. Samples were shipped at regular intervals in temperature-controlled conditions by courier to CIGMR, where they were logged and any discrepancies raised with the originating centre. DNA was extracted from EDTA blood samples in batches using Nanodrop technology and diluted to a standard concentration. Undiluted and diluted DNA were stored at two different geographical sites to guard against catastrophic loss prior to genotyping.

Study endpoints

The main REQUITE study's endpoint was change in breast appearance 2 years from the start of radiotherapy, because this is considered most specific for radiotherapy [141]. Secondary endpoints were fibrosis (induration) and telangiectasia, as well as QoL and maximum grade of toxicity during the follow-up period.

The primary endpoints of the REQUITE-AB study were:

- Acute radiotherapy toxicity scored at the end-of-treatment visit
- QoL (change in PROMs) at the end of treatment and three months from the start of radiotherapy (where applicable)

Secondary endpoints in this study were:

- Surgical complications (e.g. haemato-seroma, infection)
- Maximum grade of toxicity during follow-up

Data collection

Data were collected at baseline (prior to the start of radiotherapy or within the first five days of radiotherapy) and on completion of radiation treatment (or 1-2 days prior to the last day of treatment). Selected centres also collected data three months from the start of radiotherapy. REQUITE used a centralised online database (OpenClinica™) for recording physician- and patient-reported data, as well as baseline patient data, co-morbidities, tumour characteristics, and treatment details, including surgery, radiotherapy and systemic treatments.

Toxicity was assessed using the REQUITE health professional toxicity questionnaire based on the CTCAE v4.0 [142] (see Appendix). Quality of life (QoL) was assessed by the EORTC C30 v3.0 and BR23 v1.0 questionnaires [47, 143]. The EORTC QLQ-C30 questionnaire assesses the quality-of-life of cancer patients. It has been translated and validated into 81 languages and is used in more than 3,000 studies worldwide. The QLQ-BR23 module consists of 23 items covering symptoms and side-effects related to different treatment modalities, body image and future perspective. In addition, the Hopwood Body Image Scale (HBIS) [49] was used in 293 Leicester patients only.

As side-effects are often under-reported, patient reported outcome measures (PROMs) that are both sensitive and reliable were included to improve data capture [144]. Prior to commencement of the study, the acceptability of PROMs was tested in 10 patients in each language at respective participating sites. Research physicians and patients completed questionnaires in clinic either in paper format or online using a PC or tablet. Data from paper questionnaires was entered into OpenClinica™ by local study personnel. All case record forms and patient questionnaires are available in the Appendix.

According to the main REQUITE study protocol, breast photographs (both breasts, excluding the patient's head) were taken at baseline assessment to be taken again two years following treatment to allow for assessment of change in breast appearance (skin changes, telangiectasia, shrinkage or retraction). Two anterior views were taken of the chest, one with hands on the hips and the other with hands above the head, as well as one lateral view with hands above the head.

Data handling and QC

All data entered into the REQUITE database was subject to an automatic comprehensive validation check programme to identify missing, illogical and/or inconsistent data before online submission. In addition, a dedicated REQUITE data manager reviewed any questionable data regularly, raised queries where appropriate, and corrected data entry errors with the help of the appropriate

healthcare professionals. A separate REQUITE study manager ensured compliance with the protocol by participating sites through monitoring visits and regular teleconferences.

Source verification checks of data entered by healthcare professionals were completed at each centre for approximately 5 % of patients by an independent assessor with the help of medical notes and electronic hospital records. Transcribing error checks were conducted centrally comparing database entries with scanned hard copies of toxicity and PROMs forms in a random sample of 5 % of patients per centre (minimum 5 patients). Centres with higher error rates than others were asked to repeat the QC procedure using additional patients.

Radiotherapy

Since REQUITE is not a clinical trial of radiotherapy, radiation dose and regimen were not prescribed. All radiotherapy regimens followed local standard of care decided by the treating clinician. For patients receiving chemotherapy, it was stipulated that radiotherapy should not commence until at least one month following the end of chemotherapy. The REQUITE protocol also included guidelines for heart and breast delineation on the radiotherapy planning scans [145].

Study duration

The minimum follow-up period of the main REQUITE study was 2 years. Patient recruitment commenced in April 2014 and was completed in September 2016. The follow-up period of the REQUITE-AB study was limited to the end of radiotherapy and three months after radiotherapy for PROMs at the Leicester and German centres, meaning that the final patient had reached the 3-month time point by February 2017. The study schema of the REQUITE study is outlined in Figure 8.

Patients were withdrawn from the study if they had a secondary mastectomy. Patients withdrawing from the study were not replaced. Coded blood samples and data were retained for use within REQUITE (and future medical research, as indicated on the consent form) unless the patient made a specific request otherwise.

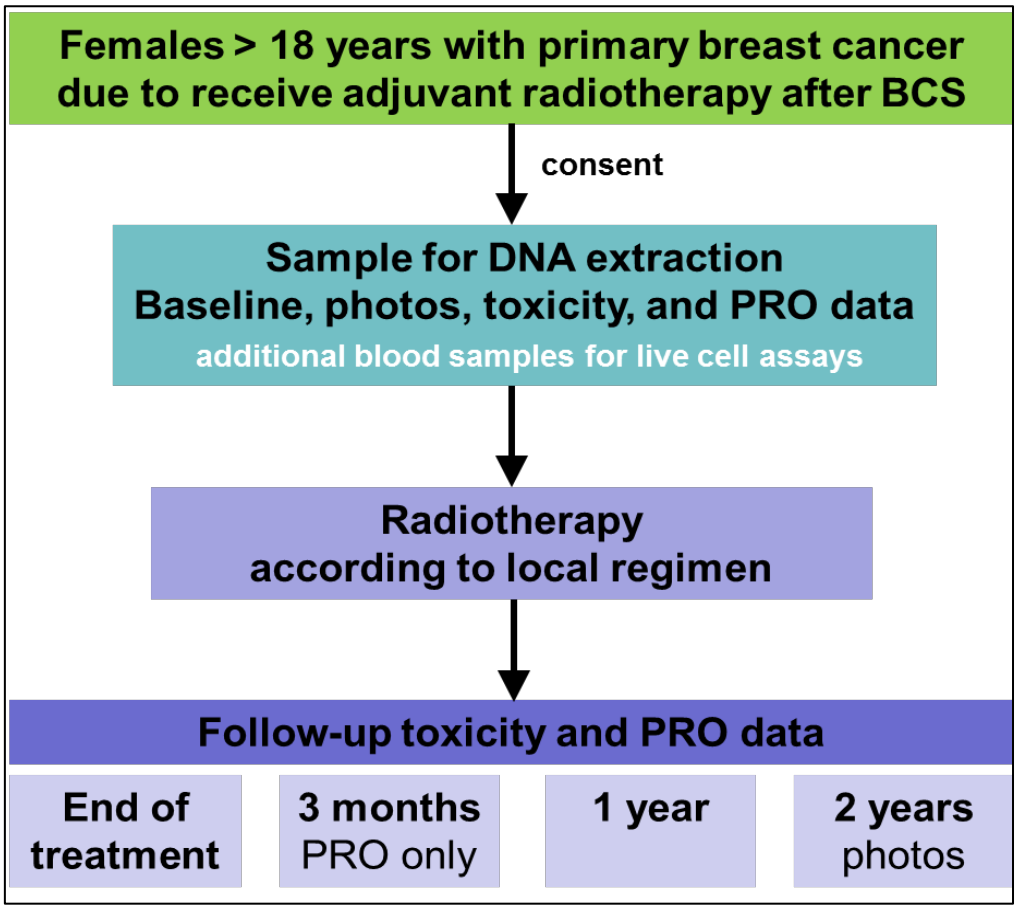


Figure 8. Study schema of the main REQUITE study. The REQUITE-AB study comprised the end-of-treatment and 3-month time points only.

Statistical methods

For descriptive statistics of patient, treatment and outcome variables, dichotomous and categorical variables were summarised using counts and percentages, and continuous variables were summarised by mean, minimum and maximum. Medians and interquartile ranges (IQR) were reported for skewed data. Breast size was calculated by adding cup and band variables coded as per REQUITE patient factor form B2a to take account of sister sizes representing the same breast volume (e.g. 34B holds an approximate breast volume equal to 32C, UK sizing).

Radiotherapy dose was calculated as biologically effective dose (BED), using the formula:

$$BED = n\,d\left(1+\frac{d}{\frac{\alpha}{\beta}}\right)$$

BED is the product of the number of fractions (n), dose per fraction (d), and a factor determined by the dose and α/β ratio for skin (10 Gy) [124]. The α/β ratio is used in radiobiology to describe the slope of the cell survival curve for different irradiated tissues [146].

As boost dose is relevant to the development of acute breast toxicity, it was incorporated into the radiotherapy dose predictor variable by adding both calculated values for BED:

$$BED_{total} = BED_{breast} + BED_{boost}$$

Multivariate analysis was carried out assessing association of primary endpoints with patient and treatment variables using logistic and ordered logistic regression, respectively. The variables included were age, BMI, breast size, smoking, alcohol (drinker yes/no), hypertension or cardiovascular disease (BP_CVD), diabetes, rheumatoid arthritis, collagen vascular disease (coll_vasc_dis), use of antidiabetic agent, ACE-inhibitor, anti-hypertensive, statin, other anti-lipid drug, amiodarone, analgesia, quadrantectomy (vs wide local excision), seroma, infection, chemotherapy, tamoxifen use, anti-HER2 therapy, BED breast, boost, BED total and use of IMRT.

The endpoints oedema, ulceration and (skin) pain scored according CTCAE v4.0 were dichotomised for the analysis, with a cut-off at grade ≥1. Erythema was analysed using ordered logistic regression according to grade. The dichotomised endpoint acute desquamation (skin loss) was defined as presence of either grade 3 erythema (moist desquamation in areas other than skin folds) or grade ≥1 ulceration.

Statistical significance was set at $p<0.05$ for all analyses of the REQUITE-AB cohort. Differences in QoL scores from baseline to end-of-treatment were compared graphically by means of box plots and assessed by paired T test. The association of QoL with clinical endpoints, patient and treatment variables was assessed using absolute PROMs scores at end-of-treatment as well as change in PROMs from baseline by multivariate logistic regression, adjusted for age, BMI, total BED, seroma, chemotherapy, tamoxifen, analgesic use, smoking and alcohol intake. For the QLQ-C30 and –BR23 tools, worsening QoL or functioning was defined as ≥ 10 point drop and worsening symptoms as ≥ 10 point increase on any scale from baseline. On the HBIS, a ≥ 10 point increase defined worsening body image.

The QLQ-C30 questionnaire is composed of both multi-item scales and single-item measures, including five functional scales, three symptom scales, a global health status / QoL scale, and six single items. Each of the multi-item scales includes a different set of items - no item occurs in more than one scale. All scales and single-item measures range in score from 0 to 100. A high score represents a higher response level. Thus a high score for a functional scale represents a *healthy level*

of functioning; a high score for the global health / QoL represents a *high QoL*, but a high score for a symptom scale / item represents a *high level of symptoms / problems*.

In the present analysis of acute breast radiation toxicity, only global health /QoL scale (QL2) and the fatigue (FA) and pain (PA) symptom scales were used. Scales are scored by first estimating the average of items (I_n) contributing to the scale (raw score, RS):

$RS = (I_1 + I_2 + \ldots + I_n)/n$

A linear transformation is then applied to standardise (S) the raw score, so that they range from 0 to 100:

$S = [(RS-1)/range] \times 100$

Range is the difference between maximum and minimum possible RS. The QLQ-C30 questionnaire is designed so that all items in any scale take the same range of possible values. All items apart from QL2 are scored 1 to 4, giving a range of 3. QoL (QL2) is scored on a 7-point scale, giving a range of 6.

The QLQ-BR23 questionnaire complements the C30 and comprises four functional and four symptom scales. While this breast cancer-specific instrument applies to patients varying in disease stage and treatment modality, only the Body Image (BI) functional and Breast (BS) and Arm (AS) symptomatic scales were used in this analysis as most relevant to radiation treatment. Scales were scored in the same way as outlined for the QLQ-C30 questionnaire by first calculating raw score and transforming to standardised scores ranging from 0 to 100.

The HBIS tool comprises 10 items scored from 0 to 3 making the maximum possible score 30. This questionnaire was administered in Leicester only. Higher scores indicate an adverse effect on body image. Scores were compared to the BI scale of the QLQ-BR23 questionnaire.

Results

A total of 2,071 breast cancer patients were recruited. The original recruitment target for Leicester was 300 breast cancer patients over a 2-year period from April 2014 to 2016. However, recruitment was extended by six months for the whole REQUITE study group as there had been an initial lag phase (Figure 9). In Leicester, I also recruited an additional 50 patients within the original recruitment period as recruitment from some participating centres remained below expectations (total n=350). Thirteen patients withdrew during the REQUITE-AB study period, leaving 2,058 patients in the analysis cohort, of which data on acute toxicity endpoints at baseline and at end-of-treatment were available for 2,058 and 2,016 patients, respectively.

QoL data were available for 1,987 patients at baseline and 1,750 patients at end-of-treatment for the QLQ-C30 questionnaire and 1,980 and 1,735 patients for the -BR23 questionnaire, respectively. Follow-up QoL data at three months was available for 301 patients for the QLQ-C30 and 298 patients for the QLQ-BR23 tool (Leicester and SW German centres only). HBIS was completed in Leicester only by 264, 261 and 240 patients at baseline, end-of-treatment, and 3 months, respectively.

In terms of QC, the mean discrepancy rate transcribing baseline forms was 0.62 % for all centres (range 0.00 % to 1.96 %), and the discrepancy rate for end-of-treatment forms was 0.30 % (0.00 % to 1.11 %).

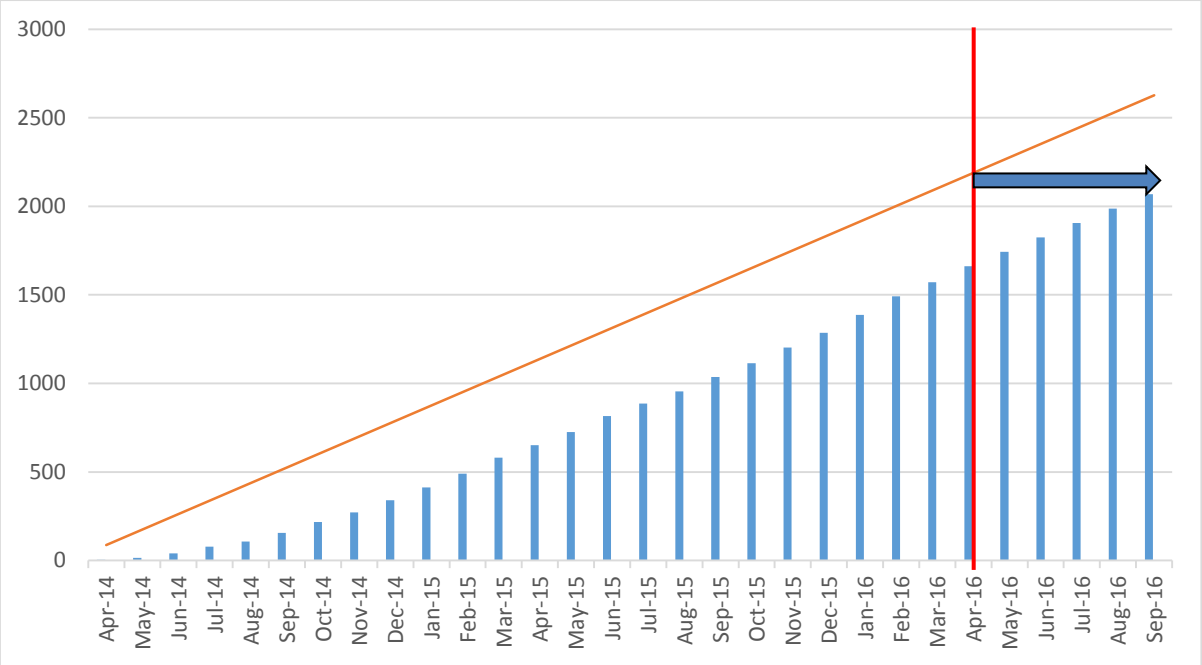


Figure 9. Graph showing cumulative recruitment figures for the breast cancer cohort of the REQUITE study in blue bars. The vertical red bar indicates original end of recruitment, which was extended by 6 months in order to achieve the original target. The diagonal brown bar represents originally projected recruitment.

Patient and treatment variables

Patient's mean age was 58.2 years (range 23 to 90 years). Mean BMI was 26.5 (13.1 to 65.4). Mean cup size was C and mean band size was 38 (UK sizing). Mean and median BED to the breast were 55.7 and 59.5 Gy, respectively (38.4 to 67.2 Gy). Mean and median total BED (including boost) were 65.9 and 67.7 Gy (44.7 to 103.2 Gy). Categorical patient variables are summarized in Table 2.

Variable (definition) **n=2,058**	**N**	**Proportion (%)**
BMI>25 (= overweight)	1,107	**54.0**
Smoking (ever smoked)	878	**43.1**
- ex-smoker	597	29.3
- current smoker	281	13.8
Alcohol (ever drunk any alcohol)	1,115	**55.7**
- ex-drinker	125	6.3
- current drinker	990	49.4
Hypertension (bp)	576	**27.9**
Cardiovascular disease (cvd)	143	**6.9**
Hypertension or cardiovascular disease (bp_cvd)	645	**31.3**
Diabetes	126	**6.1**
Rheumatoid arthritis	62	**3.0**
Collagen vascular disease	14	**0.7**
Anti-diabetic agent (current use)	105	**5.1**
ACE-inhibitor (current use)	144	**7.0**
Anti-hypertensives (current use)	479	**23.3**
Statins (current use)	295	**14.3**
Other lipid-lowering drug (current use)s	43	**2.1**
Amiodarone (current use)	7	**0.3**
Analgesics (current use)	201	**9.8**

Table 2. Frequency table of patient variables.

Over 80 % of all cancers were ER-positive and 13.8 % were HER2 positive. Chemotherapy was received by 636 patients (30.7 %), of whom 195 received chemotherapy in the neoadjuvant setting (before surgery). 73.0 % of patients received hormonal therapy, either in the form of tamoxifen (n=731) or an aromatase inhibitor (n=712). 56 patients were both on tamoxifen and an aromatase inhibitor. Anti-HER2 therapy was received by 7.7 % of patients (n=159) (Table 3).

Variable **n=2,058**	**N**	**Proportion**
ER status		
- Positive	1,669	**81.1 %**
- Negative	258	**12.5 %**
- Unknown	131	**6.4 %**
HER2 status		
- Positive	285	**13.8 %**
- Negative	1,503	**73.0 %**
- Unknown	270	**13.1 %**
Chemotherapy	636	**30.7 %**
Hormonal therapy		
- Tamoxifen	731	**35.5 %**
- Aromatase inhibitor	712	**34.6 %**
- Both	56	**2.7 %**
- None	555	**27.0 %**
Anti-HER2 therapy	159	**7.7 %**

Table 3. Frequency table of tumour and systemic treatment variables.

For breast-conserving tumour resection, just over 45 % of patients underwent wide local excision as opposed to quadrantectomy. However, there were clear differences between sites. While the majority of patients was treated by WLE at the two Belgian sites (Gent and Leuven) as well as in Leicester (between 87.9 % and 100 %), the remaining five centres treated the majority of patients by quadrantectomy (WLE rates 0 to 15 %). It is worth noting that the German centre comprised a total of seven sites across the country's south west region. Mammoplasty was not separately recorded in this study (Table 4). The majority of patients underwent axillary sentinel lymph node biopsy (SLNB) for staging (81.4 %). 8.1 % and 10.5 % of patients, respectively, had a planned axillary node dissection or completion axillary node dissection after positive SLNB, respectively (not shown). 11.1 % of patients received radiotherapy to the axilla. Surgical complications are shown in Table 5.

	Gent	Leuven	Montpellier	SW Germany	Milan	Spain	Leicester	Mt Sinai	Total
WLE	262	252	43	31	0	3	326	2	**919**
Quad	36	0	398	175	103	312	23	73	**1120**
% WLE	87.9 %	100.0 %	9.8 %	15.0 %	0 %	1.0 %	93.4 %	2.7 %	**45.1 %**

Table 4. Frequency table of surgical treatment modality by centre (breast conservation).

Infection	91	**4.5 %**
- Oral antbiotics	76	**3.8 %**
- Intravenous antibiotics	6	**0.3 %**
- No antibiotics	4	**0.2 %**
- Unknown	5	**0.3 %**
Haemato-seroma	260	**13.0 %**
- Delay to radiotherapy	31	**1.6 %**
- No delays	229	**11.4 %**

Table 5. Frequency table of surgical complications.

The use of radiotherapy boost (electron or photon) varied between centres from 10.3 % in Leicester, 40.8 % at Mt Sinai (US), to over 75 % in all continental European centres. Use of intensity-modulated radiotherapy (IMRT), either simple field-in-field planned or complex highly modulated, ranged from 0 % to 89.6 % of patients depending on expertise at participating centres (Table 6).

	Gent	Leuven	Montpellier	SW Germany	Milan	Spain	Leicester	Mt Sinai	Total
Boost	75.5 %	99.2 %	75.7 %	80.6 %	81.6 %	80.6 %	10.3 %	40.8 %	**67.8 %**
IMRT	89.6 %	85.1 %	12.6 %	64.6 %	1.0 %	10.5 %	88.8 %	0 %	**49.5 %**

Table 6. Frequency table of radiotherapy (RT) treatment modalities by centre (all centres whole breast RT, IMRT = intensity-modulated radiotherapy).

At least moderately positive/negative correlations (r>0.4) were observed between BMI and breast size (0.62) and chemotherapy and anti-HER2 therapy (0.41). There were expected correlations of anti-hypertensive and ACE-inhibitor treatment with hypertension (0.81 and 0.42). All other patient and treatment variables were only weakly correlated.

Clinical end-points

Acute oedema of the breast tissue was experienced post-treatment by 31.6 % of patients, excluding patients who already had post-operative oedema before radiotherapy (11.1 %). Skin ulceration affected 9.3 % patients at the end of radiotherapy. Adjusting for patients who had mild erythema pre-treatment (n=129), there were 417 patients who experienced grade 2 acute erythema (22.8 %) and 28 patients with grade 3 acute erythema (1.4 %) by the end of radiotherapy. Acute desquamation, defined as either grade 3 acute erythema or any ulceration, affected 9.5 % of patients (n=191). The distribution of patients by CTCAE grade for different acute toxicity is shown in Figure 8.

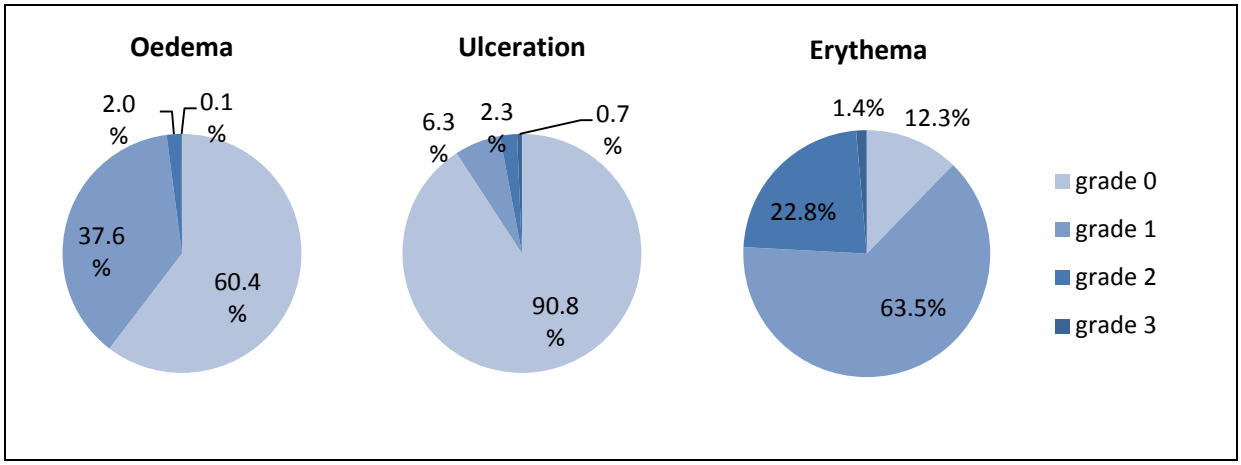


Figure 10. Distribution of patients by acute toxicity endpoints at end-of-treatment.

For the endpoint acute erythema, the proportion of patients experiencing ≥ grade 2 acute erythema (CTCAE v4.0) was broadly similar for five centres (range 11 to 18 %, including Leicester). However, 40.5 % patients in Milan, 33.3 % patients at the Spanish centres, and 74.6 % of patients at Mt Sinai (US) experienced ≥ grade 2 acute erythema (Figure 11).

Excluding two outliers (Mt Sinai and Montpellier), the frequency of acute desquamation (defined as grade 3 acute erythema or any ulceration scored according to CTCAE v4.0) ranged from 5.2 % to 12.0 % (not shown).

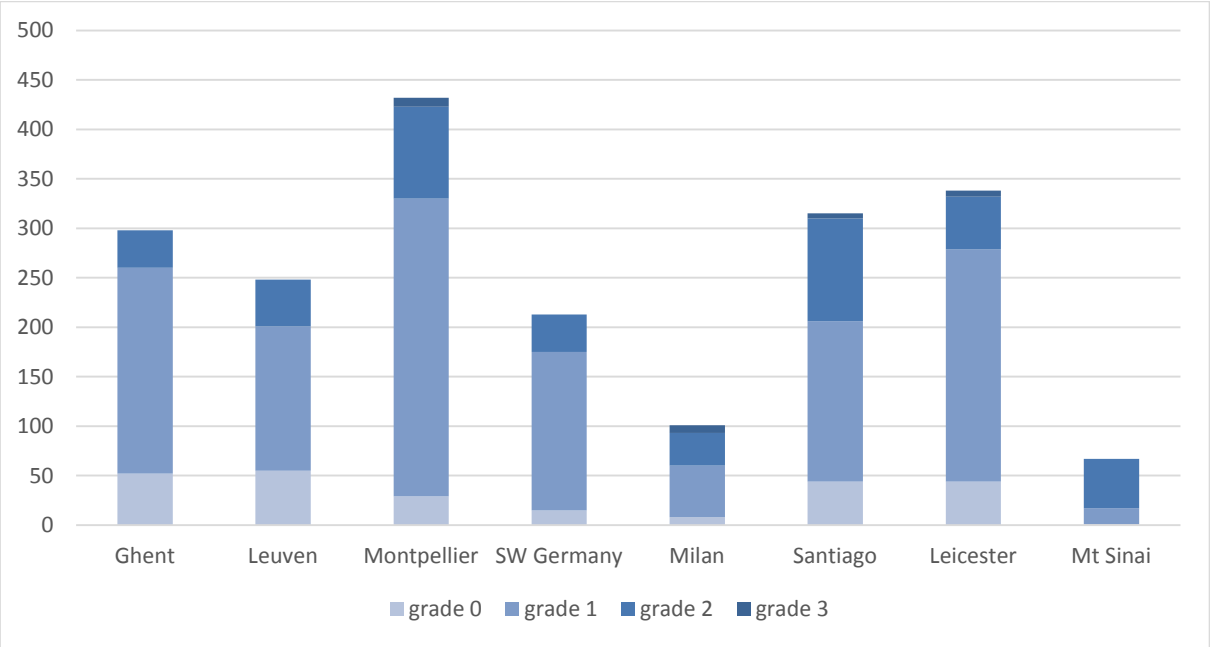


Figure 11. Distribution of patients by grade of acute erythema and by centre.

Pain (mild, moderate, or severe) was reported to the healthcare professional by 31.1 % of patients (n=639) at baseline following surgery but before radiotherapy. This proportion increased to 47.2 % (n=937) at the end of radiotherapy. Adjusting for patients who had pain both at baseline and at the end of treatment, 27.0 % (n=535) of patients developed acute pain after radiotherapy.

Acute pain was associated with acute oedema and acute erythema on univariate analysis (both p<0.001, chi square) and there were trends with acute ulceration (p=0.077) and acute desquamation (p=0.051). Both the latter endpoints were highly correlated (r=0.97). On multivariate analysis, only acute oedema and acute erythema remained significantly associated with acute pain, with odds ratios (OR) of 1.56 and 1.31, respectively (Table 7).

acute_pain	Odds Ratio	95% Conf.	Interval	p-value
acute_oedema	1.56	1.26	1.93	**0.000**
acute_ulceration	1.12	0.29	4.25	0.870
acute_desquamation	1.05	0.28	3.90	0.944
acute_erythema	1.31	1.11	1.54	**0.001**

Table 7. Association between acute pain in REQUITE breast cohort with other acute clinical toxicity endpoints (fixed effects logistic regression).

Association of clinical end-points with patient and treatment variables

On multivariate analysis, the development of acute oedema was significantly associated with patient age, breast size, drinking alcohol, quadrantectomy, and total biologically effective dose (BED) (fixed effects stepwise backward logistic regression). Age (OR=0.99 per year, CI 0.98-1.00), alcohol (0.78, 0.63-0.97) and quadrantectomy (0.49, 0.37-0.63) were protective, while breast size (OR=1.16 per sister size unit, 1.12-1.21) and total BED (OR=1.05 per Gy, 1.04-1.07) predisposed the patient to acute oedema (Table 8). Only breast size, quadrantectomy, and total BED remained significant on random effects analysis (not shown).

acute_oedema	Odds Ratio	p-value	[95% Conf.	Interval]
age	0.99	0.009	0.98	1.00
quadrantectomy	0.49	0.000	0.37	0.63
breast size	1.16	0.000	1.12	1.21
bed_total	1.05	0.000	1.04	1.07
alcohol	0.78	0.028	0.63	0.97
bed_breast	0.95	0.004	0.92	0.98

Table 8. Association of acute oedema with patient and treatment variables in the REQUITE breast cohort (stepwise backward logistic regression).

Acute ulceration was significantly associated with post-operative seroma (OR=1.68, CI 1.11-2.53), BMI (OR=1.09 per unit BMI, 1.07-1.13), radiotherapy boost (1.60, 1.06-1.13), and BED to the breast (1.11 per Gy, 1.06-1.17), while use of IMRT techniques (0.48, 0.33-0.71) and statin use (0.31, 0.16-0.65) were protective (Table 9). Only BMI and statin use remained significant on random-effects analysis (not shown).

acute_ulceration	Odds Ratio	p-value	[95% Conf.	Interval]
seroma	1.68	0.014	1.11	2.53
bmi	1.10	0.000	1.07	1.13
boost	1.60	0.026	1.06	2.41
imrt	0.48	0.000	0.33	0.71
bed_breast	1.11	0.000	1.06	1.17
statin	0.32	0.002	0.16	0.65

Table 9. Association of acute ulceration with patient and treatment variables in the REQUITE breast cohort (stepwise backward logistic regression).

Acute erythema was significantly associated with breast size (OR=1.10, CI 1.06-1.15), analgesic use (1.85, 1.34-2.54), and BED breast (1.08 per Gy, 1.05-1.10), while age (0.99 per year, 0.98-1.00), seroma formation (0.70, 0.53-0.93) and alcohol usage were protective. (Table 10) Only breast size, analgesic use and BED (breast) remained significant on random-effects analysis (not shown).

acute_erythema	Odds Ratio	p-value	[95% Conf.	Interval]
age	0.99	0.004	0.98	1.00
seroma	0.70	0.015	0.53	0.93
Breast size	1.10	0.000	1.06	1.15
analgesic	1.85	0.000	1.34	2.54
alcohol	0.82	0.045	0.67	1.00
imrt	0.73	0.003	0.59	0.90
bed_breast	1.08	0.000	1.05	1.10

Table 10. Association of acute erythema with patient and treatment variables in the REQUITE breast cohort (stepwise backward ordered logistic regression).

Acute desquamation was significantly associated with BMI (OR=1.09 per unit, 1.06-1.12), BED breast (1.13 per Gy, 1.08-1.18) and seroma formation (1.68, 1.13-2.50), whereas use of IMRT (0.48, 0.33-0.70) and statin (0.30, 0.15-0.60) were protective (Table 11). Only BMI and statin use remained significant on random effects analysis (not shown).

acute_desquamation	Odds Ratio	P>z	[95% Conf.	Interval]
imrt	0.482	0.000	0.33	0.70
bmi	1.092	0.000	1.06	1.12
bed_breast	1.129	0.000	1.08	1.18
seroma	1.682	0.010	1.13	2.50
statin	0.296	0.001	0.15	0.60

Table 11. Association of acute desquamation with patient and treatment variables in the REQUITE breast cohort (stepwise backward logistic regression).

Acute pain was significantly associated with use of IMRT (OR=1.29, CI 1.04-1.61), breast size (1.05 per sister size unit, 1.01-1.10), hypertension or cardiovascular disease (BP_CVD) (1.31, 1.02-1.68), and alcohol use (1.29, 1.03-1.61). Age (0.99, 0.98-1.00) was protective. Only age and BP or CVD remained significant on random effects analysis (not shown).

acute_pain	Odds Ratio	P>z	[95% Conf.	Interval]
age	0.99	0.023	0.98	1.00
imrt	1.29	0.022	1.04	1.61
Breast size	1.06	0.010	1.01	1.10
bp_cvd	1.31	0.037	1.02	1.68
alcohol	1.29	0.026	1.03	1.61

Table 12. Association of acute pain with patient and treatment variables in the REQUITE breast cohort (logistic regression)

Quality of life

Patient response rates for the EORTC questionnaires were 96.6 % at baseline and 85.0 % at end-of-treatment. Only Leicester and German patients completed the questionnaires at 3 months from radiotherapy. At least moderate correlations (r>0.4) were observed between QoL (global health) and fatigue (r=0.60), QoL and pain (0.52), pain and fatigue (0.55), pain and breast symptoms (0.51), and pain and arm symptoms (0.47).

Completion rates for the HBIS questionnaire by Leicester patients were 90.1 %, 89.1 %, and 81.9 % at baseline, end-of-treatment, and 3 months, respectively. HBIS scores correlated highly with body image scores on the QLQ-BR23 (r=0.90), as HBIS includes most of the -BR23 items relevant to body image, but no other correlations were observed. Higher scores indicate body image has been affected. No significant difference was observed in HBIS scores on completion of radiotherapy, compared to baseline (p=0.43, paired T test) (Figure 12).

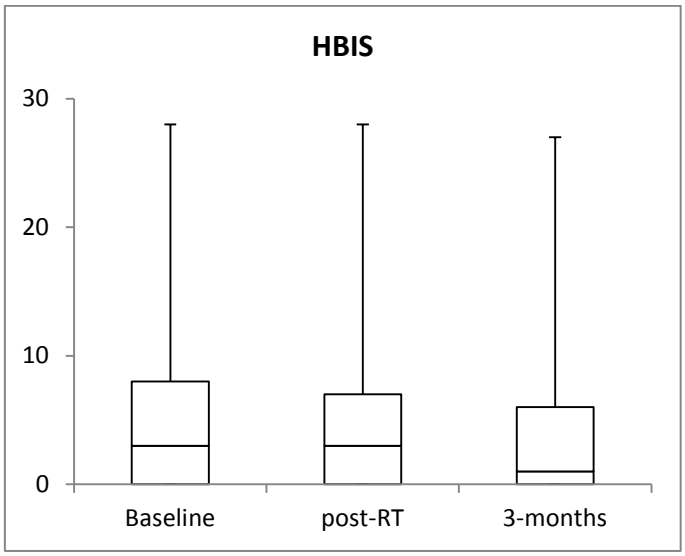


Figure 12. Box plots Hopwood body image scores (HBIS) in Leicester breast cancer patients (n=293) participating in the REQUITE study.

Distribution patterns of relevant PROMs assessed by EORTC questionnaires are shown in Figure 13. These demonstrate a small but significant decrease in QoL (global health) by the end of radiotherapy ($p<0.001$, paired T test), as well as an increase in fatigue, pain, and breast symptoms (all $p<0.001$). There was a small but non-significant decrease in body image after treatment ($p=0.26$), with no change in arm symptoms ($p=0.36$).

In the subset of patients with available data at 3 months (n=301), QoL (global health) returned to baseline levels ($p=0.88$), while increases in fatigue ($p=0.03$) and breast symptoms ($p=0.02$) and a small increase in pain ($p<0.001$) persisted (Figure 13).

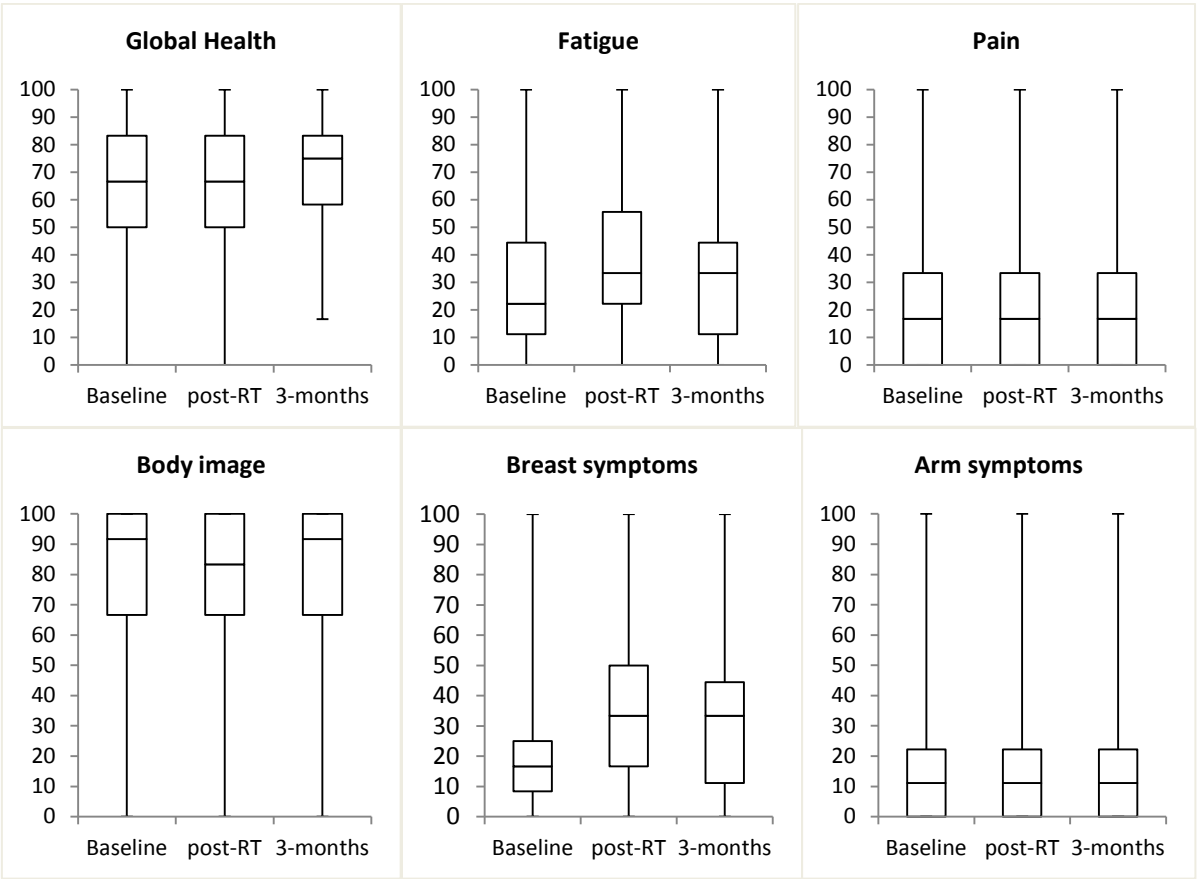


Figure 13. Boxplots showing distribution of QoL outcomes at baseline (n=1,987), end of radiotherapy (post-RT, n=1,750), and 3-months after radiotherapy (n=301).

Association of worsening quality of life with acute toxicity endpoints

For this analysis, paired data from 1,689 patients was available. Of these, 17.3 % (n=295) experienced worsening of their QoL (global health) during radiotherapy. However, there was no statistically significant association with acute clinical toxicity endpoints, adjusting for age, BMI, total radiotherapy dose, seroma, chemotherapy, tamoxifen, smoking, alcohol and analgesic use.

52 % of patients experienced worse fatigue by the end of radiotherapy. Adjusting for patient and treatment variables, this was significantly associated with acute pain toxicity (OR 1.33, CI 1.05-1.68). 39.6 % of patients reported worse pain at the end of radiotherapy. This was associated with both acute erythema (OR=1.23, CI 1.03-1.48) and healthcare professional-reported acute pain (OR=1.68, 1.32-2.12) (Table 13).

	worse_QoL				**worse_fatigue**				**worse_pain**			
Toxicity endpoint	OR	p-value	CI lower	CI upper	OR	p-value	CI upper	CI lower	OR	p-value	CI upper	CI lower
acute_oedema	1.15	0.347	0.86	1.55	0.86	0.202	0.69	1.08	0.90	0.355	0.71	1.13
acute_erythema	0.95	0.637	0.75	1.19	1.01	0.917	0.85	1.20	**1.24**	**0.021**	1.03	1.50
acute_desq	1.15	0.551	0.73	1.80	0.89	0.544	0.63	1.28	1.35	0.097	0.95	1.94
acute_pain	0.85	0.314	0.62	1.16	**1.33**	**0.018**	1.05	1.68	**1.68**	**0.000**	1.32	2.12

Table 13. Association of worse QoL, fatigue, and pain with acute toxicity endpoints in the REQUITE breast cohort adjusted for patient and treatment variables (logistic regression, acute_desq=acute desquamation).

In terms of breast-specific PROMs, 17.4 % of patient had a worse body image than before radiotherapy, but this was not associated with any toxicity in particular, after adjustment for patient and treatment variables.

56.7 % of patients reported worse breast symptoms after radiotherapy, which was significantly associated with acute erythema (OR=1.70, 1.41-2.06), acute desquamation (OR=1.77, 1.18-2.67) and acute pain (OR=1.56, 1.22-2.00). Lastly, 28.8 % of patients experienced worse arm symptoms following radiotherapy. This was not particularly associated with any acute toxicity after adjusting for patient and treatment variables (Table 14).

	worse_body image				**worse_breast**				**worse_arm**			
Toxicity endpoint	OR	p-value	CI lower	CI upper	OR	p-value	CI upper	CI lower	OR	p-value	CI upper	CI lower
acute_oedema	0.78	0.115	0.58	1.06	1.21	0.116	0.95	1.54	1.05	0.725	0.82	1.34
acute_erythema	1.15	0.213	0.92	1.45	**1.71**	**0.000**	1.41	2.06	1.14	0.187	0.94	1.38
acute_desq	1.36	0.158	0.89	2.08	**1.77**	**0.006**	1.18	2.67	0.86	0.445	0.59	1.26
acute_pain	1.26	0.135	0.93	1.70	**1.56**	**0.000**	1.22	2.00	1.25	0.091	0.97	1.61

Table 14. Association of worse body image, worse breast and worse arm symptoms with acute toxicity in the REQUITE breast cohort adjusted for patient and treatment variables (logistic regression, acute_desq=acute desquamation).

Discussion

The aim of this part of the project was to conduct a prospective cohort study of acute radiotherapy side-effects in the breast, integrating clinical and patient-reported endpoints, using the methodology of the main REQUITE study. The primary endpoints of the REQUITE-AB study were acute radiotherapy toxicity and QoL (change in PROMs) at the end of treatment. Although a six-month extension to patient recruitment became necessary, the aim was successfully achieved within the time constraints of the project, recruiting over 2,000 breast cancer patients across eight centres into the observational cohort study, including 350 patients from Leicester.

The REQUITE study sample consists only of patients undergoing breast-conserving surgery, which may explain the higher than expected rate of ER-positive tumours (81 %) and lower than expected number of patients receiving chemotherapy (30.6 %) and axillary dissection (18.6 %), although 11.1 % of patients received axillary radiotherapy. Otherwise, patients were broadly similar at baseline to what would be expected from a European and North American breast cancer population [4, 147].

As this was a non-experimental observational study, there was considerable heterogeneity in terms of treatment between different centres. In particular, there was variability in the use of radiotherapy boost as well as IMRT and surgical excision techniques (quadrantectomy vs. WLE). Nevertheless, the distribution of patients by grade of toxicity on completion of radiotherapy was broadly similar between institutions, except for the two centres with the smallest number of patients recruited (n=106 and 75, respectively).

Historically, rates of grade 3 skin toxicity (moist desquamation) reported in the literature have been in excess of 10 %, irrespective of scoring system [106, 148, 149]. Technical progress in delivering radiotherapy towards 3D-conformal or IMRT techniques [150, 151] and increasing use of hypo-fractionation [25, 152, 153] have has since facilitated a reduction in significant acute breast toxicity. In the present study, 1.4 % of patients experienced moist desquamation (CTCAE grade 3 toxicity) and 9.3 % experienced any ulceration at the end of treatment, which is comparable in proportion to more recently published data [25, 26, 108, 152, 153].

Patient and treatment factors that were significantly associated with acute toxicity differed by clinical endpoint. However, odds ratios were generally below 2 or above 0.5, indicating moderate associations at best. The endpoints acute desquamation and ulceration were highly correlated; therefore, the patient and treatment factors associated with either were the same. Only breast size or BMI were consistently associated with all endpoints, which has been commonly reported in other published cohorts [25, 26, 109, 124].

Presence of post-operative seroma was associated with acute ulceration or desquamation, whereas statin use was protective. Post-operative seroma or infection has been reported as an association with acute toxicity in the Cambridge IMRT trial cohort [25]. Statins have been previously investigated for their attenuating properties on radiation-induced normal tissue toxicity in a mouse model, where they are thought to ameliorate the effects of vasculopathy associated with radiation reaction [154], while they are generally thought to act as radio-sensitizers of cancer cells [155, 156].

Although dose has been associated with acute skin toxicity in several previous reports [106, 109], the only positive association with dose (BED) on multivariate analysis was found for acute oedema. However, these previous studies used different scoring systems, mostly the RTOG scale. For the same endpoint, acute oedema, quadrantectomy appeared protective, even on random-effects analysis. This may be accounted for by the larger excision volumes of quadrantectomy compared to WLE and hence reducing breast volume prior to radiotherapy. Mammoplastic surgical techniques, which involve a reduction of breast tissue volume to facilitate oncoplastic local resection, were not separately recorded in REQUITE.

Physician-recorded acute pain was associated with age (marginally protective), hypertension or cardiovascular disease, breast size, and alcohol use. On multivariate analysis with different toxicity endpoints , it was associated with acute erythema and oedema, whereas patient-reported pain was associated with acute erythema only, adjusting for age, BMI, total radiotherapy dose, seroma, chemotherapy, tamoxifen, smoking, alcohol and analgesic use. Nevertheless, poor association between physician-assessed data and PROMs in radiotherapy follow-up trials has been previously reported and may again account for the differences in association seen in the present cohort [42].

PROMs data for the acute phase have been under-reported in previous trials of radiotherapy [38, 157]. In the present study, QoL data collected at the end of treatment indicates worsening global health, and increase in fatigue, pain and breast symptoms, but no significant change in body image or arm symptoms. Based on the sub-group of patients for whom PROMs data was available, there was evidence that fatigue and breast symptoms may persist at 3 months from the start of radiotherapy, although long-term data after cancer treatment demonstrates that QoL after cancer treatment usually improves in the long-term [157].

In this sample, worsening fatigue, worsening pain or breast symptoms were associated with acute erythema and/or acute pain and/or desquamation on multivariate analysis adjusting for patient baseline and treatment variables. Worsening global health was not significantly associated with any acute toxicity endpoint, although multivariate analysis showed a significant association with age, chemotherapy and alcohol use. This probably reflects the fact that the QoL PROM represents a more complete measure of the patient's experience than physican-assessed symptom scales.

The findings on QoL will need to be substantiated further by comparison with other validated symptom-specific PROMs, e.g. the Multi-Fatigue Inventory [158], data of which will be available from the REQUTE cohort at a later stage. PROMs should be incorporated into any longitudinal study or trial of breast cancer treatments, as there is accumulating evidence that physician-assessed toxicity reporting is often discordant with patient-reported symptoms and only captures the patient experience of side-effects as if through a filter, an effect that is even worse if toxicity is scored based on medical record entries [159]. Breast cancer patients undergoing radiotherapy should be guided by meaningful information not just about expected toxicity but also QoL outcomes. This should serve to increase doctor-patient communication and the patient's understanding of their proposed treatment.

Conclusions

Almost 25 % of patients in the REQUITE-AB study experienced grade ≥2 skin toxicity by the end of radiotherapy, although only 9 % experienced acute desquamation. Following radiotherapy, global health decreased and fatigue, pain and breast symptoms increased and some may persist at 3 months from treatment. The recognition of acute side-effects that have significant impact on QoL scores may provide information for adjustment of treatment and management of symptoms.

Considering the heterogeneity in terms of radiation treatment and surgical excision techniques between centres, breast size and BMI were consistently associated with all toxicity endpoints, while this cohort analysis raises the possibility that statins could be used to ameliorate some of the acute radiotherapy side-effects. Patients and data from this part of the study will be used in the following chapters to explore attitudes towards a predictive test for acute radiotherapy side-effects and validate predictive models for radiation toxicity.

Phase 2: Semi-structured patient interviews

The impact of radiation toxicity on QoL is well documented in existing trials of breast radiotherapy [37, 38, 157]. More than half of all women due to undergo radiotherapy are anxious about side-effects and changes to their breast appearance [36]. Recent qualitative research has illustrated a predominantly negative perception of radiotherapy in the acute treatment phase [40]. The same authors also published a thematic analysis of semi-structured interviews with 20 women immediately after breast radiotherapy, focused on QoL in relation to skin toxicity [41]. They identified that skin toxicity affects multiple dimension of QoL, an experience which is affected by women's individual differences in terms of age, ethnicity, relationship status and attitude towards their personal appearance, and which can be modified by women's different symptom-management strategies.

According to national guidelines, patients with early breast cancer should have the option to choose between breast-conserving surgery (BCS, lumpectomy) plus radiotherapy versus mastectomy, which does not necessitate radiotherapy in low-risk disease, as equivalent treatments [3, 160]. Insufficient information about the effects of different breast cancer treatments on QoL can impair doctor-patient communication and limit the patient's understanding of their treatment. Any discussion with patients about treatment options should be guided by meaningful data about expected side-effects (toxicities) and QoL.

Women's reasons for choosing BCS vs mastectomy have been evaluated in several observational studies. Patients who choose BCS tend to have greater concern about loss of the breast, whereas patients opting for mastectomy tend to have a negative perception of radiotherapy and greater concern about tumour recurrence [161-163]. Patient's perception of her surgeon's preference for local treatment also influences that choice [164]. Because the clinical outcomes from these local treatment options are equivalent, it is important to determine patient preferences and satisfaction during the decision-making process [165]. Patient-centred decision aids have been shown to be important adjuncts for counselling women with early stage breast cancer by enhancing patient knowledge of individual treatment options and positively affecting BCS rates [166, 167], although clinicians have been concerned about their use increasing patient anxiety through information overload [168].

To guide the treatment-decision process from another angle, individual risk prediction models for treatment toxicity can be developed, by integrating clinical and patient factors with predictive biomarkers [112]. In the field of radiogenomics, several potential predictive genetic markers for radiation toxicity have been identified through a number of genetic association studies [92-94, 101].

The REQUITE study has been conceived to establish radiotherapy cohorts with patients' personal, treatment, toxicity and QoL as well as genetic data collected following a standardised protocol, and to validate predictive models of radiation toxicity [116].

However, before predictive radiogenomics testing is implemented in the clinic, it is important to gather patients' perspectives to ensure this research is relevant, appropriate, and acceptable to patients, and to explore how predictive test results should be delivered in the future.

Objectives

The objective of this part of the project was to explore patients' attitudes to, and beliefs about a predictive tool for acute radiotherapy side-effects and the impact on patients' decision-making, using acute skin toxicity as a prompt. While late radiotherapy side-effects remain a clinical concern due to their potential irreversibility, acute radiation toxicity is increasingly recognised for its impact on breast cosmesis and patients' quality of life [39, 41]. The objectives were:

- To generate a thematic description of patients' feelings and attitudes towards a radiogenomics test
- To explore how such a predictive test could impact patients' decision-making about breast cancer treatment.

Study design

This was a qualitative study conducted using semi-structured interviews with breast cancer patients enrolled in the REQUITE cohort study. It was approved by major amendment as the REQUITE-AB-QoL sub-study by the NRES Committee North West – Greater Manchester East (14/NW/0035).

Setting

Semi-structured interviews were conducted with breast cancer patients on completion of treatment in the radiotherapy department or at 6-week follow-up at University of Leicester Hospitals. These time points were chosen in anticipation that most patients had experienced toxicity by this point. One patient was interviewed in her home.

Sampling and recruitment

Eligibility criteria for the REQUITE breast cohort study were: being female, over age 18 with primary cancer of the breast, and receiving whole-breast radiotherapy after breast-conserving surgery (BCS), including patients receiving neo-adjuvant or adjuvant chemotherapy. Mastectomy patients and patients who had previous breast irradiation were excluded. To be eligible for the present qualitative study, patients were also required to give their consent to be interviewed.

Sample size was determined by data generated from participants; interviews went on until thematic saturation was reached and no new topics emerged. Patients were sampled purposively to ensure adequate representation of degree of toxicity, age, cancer type and grade, and history of chemotherapy [169].

Interview guide development

As some of the issues explored in this part of the project were personally sensitive, individual interviews were preferred to focus groups, in order to be able to probe participants' responses further for a rich picture of their views and experiences. Prior to commencement of patient interviews, an interview guide specific to this study was developed in conjunction with supervised pilot interviews at Mount Sinai in New York City (with JBS). The goal of the pilot interviews was to refine the interview technique and to ensure the questions were easy to follow and comprehend.

Pilot interviews were conducted with five female postdoctoral researchers in psychology, all of whom had no history of breast cancer or radiotherapy. During the time at Mount Sinai, the interview guide went through seven versions. The initial guide included an opening question inviting the participant to talk freely about her recent experience of breast radiotherapy, followed by an introduction of the predictive radiogenomics test using three test hypothetical results (predicting mild skin toxicity, severe skin toxicity, and an inconclusive result, respectively) as prompts to invite responses on the acceptability, practicality and implementation of a future test into the patient's treatment journey, according to the framework of Bowen *et al* [170].

In spite of the educational background of pilot interview participants, all pilot interview participants found it difficult to comprehend the concept and purpose of a predictive radiogenomics test, which was still under development and not yet used in practice. The interview guide was therefore modified to include a detailed explanation of the predictive radiogenomics test with pauses to check participants' understanding. By the end of the five initial pilot interviews, the interview guide had a more specific introduction inviting responses around how participants had experienced skin changes

during their treatment and how information received prior to treatment had prepared them for the side-effects of radiotherapy.

The interview guide was then piloted with a further five non-academic members of staff with different educational backgrounds (high school to undergraduate degree) in the Department of Cancer Studies at the University of Leicester, again all of whom had no history of breast cancer or radiotherapy. Following this set of pilot interviews, the introductory question about how participants had received information about and experienced skin changes during radiotherapy was dropped. The introduction became more focused on the question of expected vs. actual experience of their treatment as well as well as statistical vs. personal probability of experiencing side-effects from treatment:

- *Patients are told that radiotherapy can be associated with side-effects such as skin irritation, peeling, discomfort or discoloration, but aren't sure how it will affect them personally. For example, knowing that a percentage of women will have a tough time with skin changes during radiotherapy is different from knowing that I (me) will have a tough time with skin changes - does that make sense?*
- *Can you talk to me a bit about how you expected to fare during radiotherapy and whether the treatment affected you as you thought it would?*

In the final version of the interview guide (see Appendix), these introductory questions were followed by an explanation of the concept of predictive testing for radiation toxicity, using the example of acute skin toxicity. After engaging with this information and based on their own experience of radiotherapy, participants were invited to describe their views and ideas about how they personally would react to the test, and to share their thoughts on advantages and disadvantages of a predictive test for skin toxicity to themselves and their healthcare professionals.

They were then verbally presented with three standardized fictional test results: one suggesting a high likelihood of severe skin toxicity; one suggesting mild or no skin toxicity, and one inconclusive test result. Participants were also asked at what level of risk the result would influence their treatment decision, and how interested they would be in options for alternative treatment (e.g. BCS with radiotherapy vs. mastectomy +/- reconstruction without radiotherapy).

Attitudes towards testing for long-term side-effects, such as fibrosis (scarring) and breast shrinkage were also explored. Questions from the Bowen framework [170] were retained as prompts, such as those relating to the feasibility and implementation of a predictive test – in particular, acceptability, demand, and practicality, as well as integration into the treatment decision-making process. To ensure that important themes have not been missed, the interview guide concluded with the question: 'Is there anything else you would like to tell me?'

Patient interviews

Semi-structured interviews with patients were conducted by one researcher (TR). No further changes were made to the guide during interviews with patients. Interviews were audio-recorded and transcribed verbatim using professional transcription services. Patient anonymity was ensured by using only first names, initials, or the option of using a fictional name during the interview. At the start of the interview, confidentiality was stressed and participants were invited to express their views on the proposed radiogenomics test for skin toxicity.

The relationship between researcher and patient participant was considered throughout the interview [171]. Although the researcher conducting the interviews was surgically trained and worked as a research physician on the main REQUITE study, he was not involved in the patients' usual medical care, nor did he work clinically in the radiotherapy department where patients were recruited. Participants were advised that any medical or psychosocial issues raised during the interview would be referred to their usual medical and nursing team.

Data analysis

Anonymised transcripts were imported into QSR International's NVivo 10 for Windows software. Inductive thematic analysis was used to describe patients' feelings and attitudes towards a predictive test for breast radiation toxicity, and to explore how the test result could impact their treatment decision-making [172]. Thematic analysis is a particular type of qualitative analysis focused on recognising, analysing, and reporting repeating themes across a data set. It is not tied to a specific theory or epistemology, therefore one has to be explicit why this method was used.

First, thematic analysis in this study was driven by an interest in understanding whether a test for acute radiation toxicity would be acceptable to breast cancer patients. Secondly, although this approach may lose some of the depth of a more abstract analysis, the objectives were to generate a thematic description of patients' feelings and attitudes towards a radiogenomics test, and to explore how such a predictive test could impact patients' decision-making about breast cancer treatment.

Emerging themes were identified iteratively through systematic coding of the transcripts and constant comparison across transcripts until thematic saturation was achieved. Each transcript was coded independently by TR and JBS who conferred by telephone/video-conferencing after every two to three interviews.

On completion of the interviews, codes were finalised and final themes agreed in conjunction with my supervisor (JBS) at Mount Sinai, in order to prepare data for write-up according to published RATS guidelines for qualitative research [117]. Fifty-two initial codes were combined into three primary themes. Minor coding discrepancies were resolved through discussion between authors. All interviews were included in the analysis.

Participant characteristics

Table 15 summarises the participants' characteristics. Median age was 60 years (range 41 to 81). Median interview length was 30:43 minutes (23:33 to 39:11). All patients had undergone BCS plus axillary sentinel node biopsy or axillary dissection according to lymph node status and received whole breast radiotherapy. Two patients also received axillary radiotherapy. Only one patient had previous experience of personal genetic testing and was awaiting results of a BRCA1/2 mutation test.

	Number of Participants
Age group	
under 50	4
50 to 59	6
60 to 69	7
over 70	4
Ethnicity	
White European	20
Indian	1
Breast cancer stage	
Tis (DCIS)	3
T1N0	12
T1N1	4
pT0pN0	1
pT1pN1	1
Receptor status	
ER positive	12
HER2 positive	2
Triple negative	4
Not assessed (DCIS)	3
Chemotherapy	
None	12
Discussed but not received	3
Adjuvant	4
Neoadjuvant	2
Acute skin toxicity	
grade 0	3
grade 1 (mild erythema)	12
grade 2 (moderate erythema and/or patchy moist desquamation)	5
grade 3 (confluent moist desquamation)	1

Table 15. Characteristics of breast cancer patients participating in the interview study (n = 21).

Results

Twenty-one female patients with breast cancer were interviewed. Three main themes emerged from the data that described patient attitudes towards a future predictive radiogenomics test for breast radiation toxicity and its potential impact on breast cancer treatment decision-making (Table 15):

1) Willingness to undergo a radiogenomics test (subthemes – information, trusted expert)
2) Implications of a radiogenomics test (subthemes – preparation and planning, anxiety without recourse)
3) Impact on treatment decision-making (subthemes – prioritising cancer cure, preserving breast integrity, patient preferences).

Main theme	**Sub-themes (description)**
1. Willingness to undergo a radiogenomics test	• Additional information is good but may lead to information overload. • HCPs as the trusted expert should receive and explain test result and provide patient with a management plan accordingly.
2. Implications of a radiogenomics test	• Preparation and planning both for patient and HCPs • Enhances anxiety or dread, particularly in the absence of symptom modifiers, or if long-term toxicity such as scarring and chronic pain were predicted.
3. Impact on treatment decision-making	• Benefit of cancer cure is prioritised over risk of treatment side-effects, particularly acute toxicity, which is usually transient. • Preserving breast integrity is more important than avoiding acute side-effects by undergoing more surgery (e.g. mastectomy +/- reconstruction) • Individual preferences may dictate whether patients change their treatment plain to avoid radiotherapy in case of significant predicted long-term side-effects.

Table 16. Emerging themes describing patient attitudes towards a future predictive radiogenomics test for breast radiation toxicity.

Theme 1: Willingness to undergo a radiogenomics test

Participants felt a predictive radiogenomics test would be just as routine as any medical test in their journey through cancer treatment.

> *I think it's all part of the package.* (P14)

> *I think it's just one blood sample at a time when you're having blood samples done all the time and I think, you know, it's not something you get wound up about. (P1)*

> *It wouldn't have bothered me at all.* (P3)

Participants' willingness to undergo a future radiogenomics test for breast radiation toxicity stemmed from both personal interest in the test result and interest in the result being provided to their expert healthcare professionals (HCPs).

Subtheme 1.1: Information

The information which a predictive radiogenomics test could provide was appreciated as a good idea in general.

> *It's wise to be informed really, isn't it?* (P4)

> *I think information is good.* (P14)

> *I […] would have liked to have known what the end result would look like. That would have been the key thing for me really, you know, whether the radiotherapy would undo all the lovely work that the surgeon had done.* (P3)

The information was perceived as empowering patients to make informed choices about their treatment.

> *Because then they'd be more informed, better able to make a decision, better able to make choices, and I think that's quite important to have the choice rather than have somebody say 'you are having this, you are having that', and then end up looking not the way you want to look*. (P6)

> *Because information is power and if you have [it], it gives you choice.* (P16)

> *It's all about working with your doctor and his team and making informed decisions. So I think the more information you have so you can make those decisions.* (P19)

Participants felt that patient autonomy in making any treatment decision based on the information from the radiogenomics test should be respected.

> *Even if that test came back and said, yep, yours is likely to be the worst reaction ever […], you could still say 'actually, I'm still going to go with wide [local] excision and radiotherapy'. So having the test doesn't mean you're then tied to having radiotherapy or not, depending on what the result is.* (P3)

At the same time, some participants were concerned that this additional test could lead to information overload. In fact, some participants would not wish to find out this information at all, and others felt they would only want the news delivered a little bit at a time.

> *I think if you cover every avenue of treatment and side effects and everything then it's good and it's good to know, sometimes it's a lot to take in I think. (P2)*

> *It sounds an absolutely good idea, but I personally wouldn't like to know how severe it's going to be. I wouldn't like to know that this was coming to me anyway.* (P21)

> *I think if you're doing something like that you may need to ask individuals or I don't know whether you ask them or whether you can tell from actually speaking to them how much information somebody can actually take on board.* (P21)

Subtheme 1.2: Trusted expert

Participants expressed a preference for the HCPs or the doctor providing their breast cancer care to receive the radiogenomics test result.

> *I think it is important, certainly from a healthcare perspective, but not necessarily for the individual.* (P21)

In this sample, participants' interest in their HCPs receiving the test result might have been associated with a more general sense of trust in their providers and their willingness to be guided by them.

> *I would have gone along, yeah, because I trusted them to tell me what was best for me.* (P2)

> *My consultant, Dr [oncologist] and Dr [surgeon], I've just been guided by what they say. […] So I didn't sort of question it, I just went with what they said.* (P19)

Participants were particularly interested in HCPs using the test result to explain the predicted side-effects and to suggest a treatment plan accordingly.

> *It's bound to help them in the planning.* (P10)

> *I think that's going to help people make a decision along with the help from the consultant I think, yeah, I think you also need to be guided.* (P6)

Participants also expected the HCP to provide a reference frame for different levels of toxicity, for example, with the help of visual aids.

> *OK, so you've got your test result now and […] fine, you won't have any reaction, I'd still want some pictures, I'd still want to know what 'fine' looks like.* (P3)

Theme 2: Implications of a radiogenomics test

The proposed radiogenomics test generated a range of behavioural and emotional responses from patients. If they perceived the additional information as positive, some participants felt the test result would reassure and provide them with accurate expectations about the course of their treatment.

> *Well, for myself it's that the test is – well, that piece of mind – to know what to expect.* (P11)

> *I think forewarned is forearmed, isn't it, really?* (P13)

Subtheme 2.1: Preparation and planning

Some participants felt that being aware of their personal risk of radiotherapy side-effects could help them prepare and plan for side-effects.

> *Well, I think preparation. You know, preparing yourself for it.* (P13)

> *I'm OK because I know it's coming and I'll be half prepared that if it does come then don't be scared, this is all part and parcel of the treatment.* (P7)

If predicted to have severe toxicity, some participants would adjust their daily routine or use preventative measures, such as additional creams for skin side-effects.

> *It might have been helpful in sort of planning ahead and, you know, if I knew that radiotherapy was going to make me very ill then, you know, I might have been able to change things about work and all that sort of thing.* (P1)

> *Preparing yourself really, yes, making sure you have your right moisturisers, things like that Aloe Vera.* (P18)

> *I'd probably think I'd go out and buy some correct ointments that are recommended and maybe get rid of the bra and get rid of, you know, so that you're organised and prepared for it. So yes, it would be useful to know.* (P15)

If predicted to have considerable radiation toxicity, participants would be interested in, and expect, closer observation and help by the HCP.

> *Yeah, well at least they know what to look out for won't they and they'll think oh well she has got these genes so perhaps we'll keep an eye and see if this happens. I assume that'd be the best way.* (P7)

I would want to know what help was available. You know, as you're informing people of the side-effects, have you got any answers, you know, to help the patient, you know, through any sort of serious damage to their breast – you know, their skin? (P13)

Subtheme 2.2: Anxiety without recourse

Some participants were concerned that advance knowledge of the expected severity of radiotherapy side-effects could lead to anxiety, or even dread and powerlessness, particularly if they believed there were no available options for symptom management.

Well, apart from scaring the patient, because if there's no other option and they have to go through the radiotherapy then that's a scary prospect. (P16)

I think if you're told, yeah, you could get this, you could get that, it depends what sort of person you are, you know, you could go home fretting, worrying, think about and dwell on it, if you're not then I just think what will be will be, you know. (P2)

You have to take into consideration whether somebody is upbeat about it or whether they're down in the dumps, you know in some respects it could tip them over the edge I think possibly. (P21)

However, these negative emotions could be modified according to the value patients placed on having certainty from the test result.

I suppose anticipating damage and watching the damage happen might psychologically be a bit difficult, but that's weighed against being prepared for something that was going to be distressing. (P14)

If you're forewarned then you can deal with it personally because that's the way I like to -, if I know what's going to happen then it's not going to be a surprise. (P7)

But if you're pre-warned that, yes, you are going to react badly for it, you can get your mind-set into that, as well – that, yeah, it's going to be a bit difficult. (P8)

Of the side-effects mentioned during the interviews, anxiety was weighted more on long-term breast toxicity, such as fibrosis (scarring) and atrophy (shrinkage), rather than acute skin toxicity.

I don't know if that would be frightening to know that in the long term it's going to end up some sort of scarred mess or not, I mean, I believe if it's not then that's great but I don't know, I think I'd be frightened about that. (P6)

Theme 3: Impact on treatment decision-making

Whether the radiogenomics test result would influence treatment decision-making depended on participants' priorities and preferences as well as their attitude to mastectomy.

Subtheme 3.1: Prioritising cancer cure

'Cancer cure' was prioritised over the risk of treatment side-effects, particularly acute skin toxicity, which is likely to be transient.

> *You need to know that the cancer's going to go. I think my skin can get better. I'm not sure the cancer can get better.* (P20)

> *Anything to cure the cancer, I'd have gone through. No, it doesn't matter what the side-effects would have been. I'd have still done it, definitely, and I think anyone who doesn't, is risking their health.* (P11)

Participants would thus consider mastectomy if required to treat their cancer but not to avoid radiotherapy side-effects.

> *If I had to have a mastectomy because of the cancer then I'd have it, but if it was just because I was going to get side effects from the radiotherapy I wouldn't because I can cope with side effects, they will go.* (P19)

Subtheme 3.2: Preserving breast integrity

While participants would entertain the idea of mastectomy for cancer cure, preserving the integrity of their breast was important given the scenario of predicted severe acute skin toxicity.

> *So if I was told 'well if you have a mastectomy then your prognosis is the same' and I would say 'well why would I want to have that, I'd rather have the skin changes and keep my breast'.* (P14)

> *So if I was a severe, but I think having a mastectomy just for skin irritation or that then no, I wouldn't. […] No, because obviously what's months? You know you can deal with months. Once a mastectomy has gone, it's gone, isn't it?* (P18)

Subtheme 3.3: Patient preferences

Some participants appeared willing to entertain the idea of mastectomy to avoid radiotherapy under certain conditions. Chronic long-term toxicity such as fibrosis (scarring) was considered important, although it would have to be weighed against the side-effects of more surgery.

> *Maybe if somebody thought they were going to be really scarred, but then you're going to be scarred by having a mastectomy.* (P6)

Symptoms of severe and chronic pain, suffering, or sensitivity, rather than cosmetic appearance, might change some participants' treatment decision.

> *Visually, it wouldn't affect me at all, but I'm looking at it from discomfort and pain and perhaps long-term treatment.* (P13)

> *If it was me, if you said that, that your skin would come off and it'll be painful, I think I'd go for the mastectomy, I think I would say 'no I don't want radiotherapy' from this test, yeah.* (P6)

> *I would certainly consider if there was pain and oversensitivity.* (P14)

> *I did think that straightaway, so if somebody told me that I was going to -, I could avoid further suffering from radiotherapy then I would have said no just get rid of it, yeah.* (P10)

Some participants also raised concerns about significant complications affecting surrounding tissue or organs, albeit rare, which might affect their decision-making regarding treatment.

> *If I was told 'well in your case I'm sorry but the radiotherapy will severely damage your lung, then I'd have to think about whether I would then have a mastectomy.* (P14)

> *Unless I was told that the scarring was going to be really severe; it would affect my lungs/my breathing – I perhaps would go ahead with a mastectomy to avoid that.* (P20)

Treatment decision-making might also be affected if participants perceived a given side-effect to be chronic, and to require long-term maintenance or entail further suffering.

> *Depends on how bad they think it's going to be in the long term, for me, I just want things over and done with and finished, where if it's going to make it drag on and drag on then probably not, probably I'd go for the other option and get everything over and done with.* (P7)

However, some comments suggested that patient age might modify the relationship between test results, distress, and decision-making.

> *In terms of cosmetic effects I would be less worried about that but I'm 69 so if I was 35 or 55, it would probably matter more.* (P14)

> *Somebody younger might be, but as somebody who is coming up to 60, no. No, I'm not considering going on the beach or topless anymore. So you know it really doesn't matter now.* (P21)

Discussion

The clinical application of predictive radiogenomics testing raises multiple practical challenges [112]. Before such a test is implemented in practice, it is important to gather patients' perspectives to inform ongoing research this field. To date, it is not known if patients who might be offered radiogenomics testing in the future understand this form of personalised medicine and how they perceive its potential benefits and risks.

Results from the present study indicate that patients support and have confidence in the validity of a predictive radiogenomics test for breast toxicity, but they would prefer the result be provided to HCPs (rather than provided directly to patients) and for their HCP to outline a management plan for side-effects if predicted to be severe including possible alternatives to radiotherapy. Some patients would find the test result reassuring, while others might find it anxiety-provoking. Except in cases of significant chronic symptoms or end-organ damage, patients rarely felt that advance knowledge of their personal risk of acute breast radiation toxicity would influence their breast cancer treatment decision-making, which was irrespective of degree of toxicity experienced.

The themes identified in the present study are consistent with the literature from other fields on patients' reactions to receiving personalised genetic test results [173]. Participants preferred the result of this radiogenomics test to be provided to the HCP providing their usual breast cancer care, rather than provided as direct-to-consumer testing. While patients are ethically autonomous, this notion of the doctor as a trusted expert resonates with the concept that many patients may reflect back the responsibility for treatment decisions to their HCP [174]. While some patients wanted as much information on their risk as possible, others preferred not to receive too much information on personalised risk, which aligns with the concept of information 'monitors' and 'blunters' [175].

For patients in this study, predicting symptomatic side-effects such as pain was equally important as clinical signs of skin change or fibrosis. Patients also felt that the severity of long-term side-effects would more likely have an impact on their treatment decision-making than acute (short-term) toxicity. The accuracy of a future predictive radiogenomics test was not questioned by patients, although concerns about accuracy and clinical utility of genomics testing are often held by HCPs [176].

The issue of provider training in genomic testing has been raised in other fields of personalised medicine [177]. If their predicted skin toxicity were severe, patients in this study would expect their HCP to provide a management plan, which might include a spectrum of interventions from symptomatic modifiers, such as creams, advice to change their daily routine, to changing the treatment plan altogether and avoid the need for radiotherapy where clinically possible (e.g.

mastectomy +/- reconstruction). Both a patient's preferences and distress are likely to play a role in negotiating this treatment plan, and HCPs will be required to pay particular attention to a patient's expectations and beliefs [178].

The patient's own decision-making style and personal preferences have previously been shown to be important for patients with early breast cancer in choosing between mastectomy and BCS [161, 179]. Historically, the predominant model of treatment decision-making in surgery was paternalistic, driven by the surgeon's expertise, but more recently the pendulum has swung considerably towards a consumerism-dominated model, in which the patient chooses between her treatment options. This choice takes place at the time of diagnosis when breast cancer patients express high levels of anxiety and distress [180]. They may also have inaccurate perceptions of recurrence risk and expected outcomes from treatment [181].

Ideally, the process should involve shared decision-making, in which both parties share information about treatment options and take steps to arrive at a consensus treatment decision. Surgeons should also be aware of their own preconceptions, such as the perception that BCS confers greater QoL benefits compared to mastectomy [182], when studies have demonstrated a more equivocal picture in terms of QoL scores for mastectomy patients undergoing immediate breast reconstruction vs. patients undergoing BCS followed by radiotherapy [183]. This approach can improve patient satisfaction, provided that their preferred decision style is achieved [165, 179].

Limitations

There are several limitations associated with the present study. It was conducted in a single centre participating in the REQUITE study with a sample of British largely Caucasian White female breast cancer patients and therefore may not reflect the views of patients from other nationalities, ethnicities, or with different cancer types. Mastectomy patients are excluded from the main REQUITE study, so did not feature in this sample. Such patients may hold different views given their dissimilar experience of breast surgery. Some findings may also be context-specific to the UK National Health Service and may not be representative of patients in other healthcare systems.

One of the difficulties during the interviews was getting patients to understand the concept of a personalised genomics test for radiation toxicity, which as yet is not available. Only one patient in this sample had previous personal experience of genetic testing (for breast cancer predisposition), but all patients participating in this interview study also participated in the REQUITE study and were aware of the research towards predictive radiogenomics testing.

Conclusions

Before radiogenomics testing is implemented in the clinic, it is important to gather patients' perspectives on the appropriateness, delivery and implications of such a test. Using a test for acute skin toxicity as a prompt, results from the present study show that patients would be generally interested in a predictive test for breast radiation toxicity, but expect their HCP to be provided with the result. Except in cases of significant chronic symptoms and pain or significant end-organ damage, patients rarely felt that advance knowledge of their personal risk of breast radiation toxicity would influence their treatment decision-making.

As the test result may provoke emotions of anxiety and dread, it will be important how the provider presents and frames the information from the test. In discussing any treatment recommendation based on the test result, HCPs should take into account the patient's preferences, but the results indicate that many patients would prioritise cancer cure and breast integrity. Future research should explore in more detail not only how patients but also their HCPs will use the information from a predictive radiogenomics test for breast toxicity in the clinic, including predictive testing for late radiation toxicities, an endpoint in the main REQUITE study. The field should also move forward towards developing predictive models for chronic symptoms, such as pain and fatigue.

Phase 3: Clinical prediction model

Several publications – more recently observational studies – have reported acute breast radiation toxicity and correlations with clinical and treatment factors, often with conflicting results [25, 26, 107, 109-111, 124, 184]. Depending on the published study, the variables associated with acute skin toxicity were BMI, breast volume, breast or cup size, or breast diameter, boost, radiotherapy dose, dose inhomogeneity (V>107 %), smoking, time from chemotherapy, and hormonal treatment (Table 17). However, where studies reported statistical prediction models of radiation toxicity, these have never been replicated in other cohorts. The development of a validated clinical prediction model is essential before genetic markers are incorporated into the model to be validated at a later stage.

Predictive models that are capable of identifying patients at high risk of clinically significant side-effects before the start of their radiotherapy have been developed across different disease sites. While the majority of published models predict late effects [125-131], there are also an increasing number of published models predicting acute toxicity, although none of these have been for breast radiotherapy [132-137]. In order to develop a clinical prediction model for acute breast radiation toxicity, it is therefore necessary to use data from previously collected cohorts.

While there are a number of datasets available for validation, they are variable in the quality of the data collected. The REQUITE study was conceived as a multicentre validation cohort for predictive models of radiation toxicity collecting data under a unified protocol [116]. Although late toxicity remains the main endpoint in REQUITE, data collected at the end of radiation treatment can be used to validate prediction models of acute toxicity endpoints. Earlier cohorts of breast cancer patients with documented radiation toxicity have also been relatively small in size, often recruiting hundreds rather than thousands of patients (Table 17), whereas the number of breast cancer patients recruited into REQUITE exceeds 2,000.

First author	Year published	N	Significant association with acute breast toxicity on MV analysis
Twardella [124]	2003	478	BMI >25
Back [106]	2004	234	Breast size, patient weight
Barnett [25]	2011	1014	Breast volume, tamoxifen, post-op infection; V >107 % not significant
Terrazzino [110]	2012	286	Boost, breast diameter (aggregate data)
Sharp [109]	2013	391	BMI >30, dose >50 Gy, smoking
Tortorelli [111]	2013	200	Dose inhomogeneity V >107 %
Ciammella [107]	2014	212	Boost
De Langhe [26]	2014	377	BMI >25, bra cup size> D, concurrent hormonal treatment, normofractionation, smoking
Zygogianni [184]	2014	44	Time from chemo < 20 days

Table 17. Association of clinical predictor variables with acute breast toxicity in previously published studies.

Since any prediction model should be targeted for specific toxicity endpoints, this raised the issue of how to deal with the use of different toxicity scales in the previously assembled cohorts , such as the RTOG (Radiation Therapy Oncology Group) versus the CTCAE (Common Terminology Criteria for Adverse Events) scale, with variable ranges of toxicity recorded. The RTOG scale for acute radiation morbidity has separate scales mostly based on target organ or body region (e.g. larynx, upper GI, skin) [185]. Since the incorporation of the Late Effects in Normal Tissues, Sujective, Objective, Management and Analytic (LENT-SOMA) scales [186] in CTCAE, the latter are now seen as the preferred method of recording toxicity in studies of cancer treatment [142]. CTCAE v4.0 has separate scales for radiation dermatitis (erythema) and ulceration, both of which may be relevant to the acute response to radiotherapy observed in the breast (Table 18).

Toxicity	Grade 1	2a	2b	3	4
RTOG Skin	Follicular, faint or dull erythema / epilation / dry desquamation / decreased sweating	Tender or bright erythema +/-dry desquamation	Patchy moist desquamation; moderate oedema	Confluent, moist desquamation other than skin folds, pitting edema	Ulceration, hemorrhage, necrosis
CTCAE v4.0 Radiation dermatitis	Faint erythema or dry desquamation	Moderate to brisk erythema or patchy moist desquamation, mostly confined to skin folds and creases; moderate edema (tenderness is graded separately in the Pain category)		Confluent moist desquamation ≥1.5 cm diameter and not confined to skin folds; pitting edema	Skin necrosis or ulceration of full thickness dermis; may include bleeding not induced by minor trauma or abrasion
CTCAE v4.0 Skin ulceration	Combined area of ulcers <1 cm; non-blanchable erythema of intact skin with associated warmth or oedema	Combined area of ulcers 1 – 2 cm; partial thickness skin loss involving skin or subcutaneous fat		Combined area of ulcers >2 cm; full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to fascia	Any size ulcer with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures with or without full thickness skin loss

Table 18. RTOG and CTCAE v4.0 toxicity scales for acute skin reaction and ulceration.

Objectives

The aim of this part of the project was to derive a prediction model for acute breast toxicity from existing patient cohorts and then validate the model in the prospective REQUITE cohort collected in Phase 1, with the following objectives:

- To predict a unified toxicity endpoint across existing patient cohorts;
- To develop a combined model that takes account of heterogeneity between cohorts;
- To validate the model in the REQUITE patient cohort.

Data sources

Derivation cohorts

Three existing cohorts with patients who received whole breast radiotherapy after breast-conserving surgery (BCS) were used and are described in Table 19 [25, 123, 124]. Patients from the three cohorts who were treated with external beam radiotherapy (EBRT) following BCS were eligible for this analysis.

	LeND	**ISE**	**Cambridge**
Total patients in cohort (n)	663	478	1144
Eligible patients (BCS)	**390**	**all**	**all**
Location	Leicester, Nottingham, Derby	SW Germany	Cambridge
Study design	retrospective	prospective	prospective
Recruitment year (range)	2008-2010	1998-2001	2003-2007
Treatment year (range)	1995-2008	1998-2001	2003-2007
Age (median, range)	59 (33-87)	61 (27-87)	59 (26-84)
Whole breast dose (median, range in Gy)	50 (40-50)	50 (44-56)	40 (40-50)
Whole breast fractions (median, range)	25 (11-25)	25 (22-29)	15 (15-25)
Boost (proportion of patients)	10 %	90 %	65 %
Toxicity scale used	RTOG	CTCAE v2.0	RTOG
Breast measurement	Bra size	Bra size	Breast size (1-3)
BMI ≥25 (proportion)	Not available	48 %	63 %
Smoker (current or previous)	13 %	30 %	15 %
Chemotherapy	28 %	none	20 %
Diabetes	8 %	6 %	5 %
Hypertension	35 %	32 %	Not available
Cardiovascular disease	Not available	16 %	10 %
Tamoxifen use	Not available	80 %	66 %

Table 19. Summary of study characteristics of eligible patients from the three derivation cohorts.

For both LeND and Cambridge cohorts, acute skin toxicity was scored according to the RTOG scale. The German ISE study used a modified version of the Common Toxicity Criteria (CTCAE v2.0) scale for erythema (Table 20). None of the patients in ISE received chemotherapy. Information on BMI and history of cardiovascular disease was not available for the LeND cohort, while history of hypertension was not available for the Cambridge cohort.

Score	Definition
0	No change
1	Faint or dull erythema; epilation; dry desquamation
2a	Tender or bright erythema; moderate oedema
2b	Severe erythema
2c	≥ 1 moist desquamation or interruption of treatment due to side-effects
3	several or confluent areas of moist desquamation
4	ulceration, haemorrhage, necrosis

Table 20. Modified version of CTCAE v2.0 (erythema) scoring system used in the German ISE cohort.

The LeND cohort [123] consists of 633 breast cancer patients with documented normal tissue toxicity recruited at varying time points (up to several years) after breast radiotherapy +/- boost in Leicester, Nottingham and Derby (UK) between 2008 and 2010. Acute toxicity was collected from medical records. After excluding the first 154 patients without data on acute toxicity, and 119 patients who had chest wall radiotherapy following mastectomy, 390 patients treated with EBRT following BCS were included from the LeND cohort.

The Cambridge cohort [25] consists of 1,144 women who received adjuvant radiotherapy to the breast following BCS as part of the Cambridge IMRT trial (UK) following the current standard hypo-fractionated regimen with the exception of a single patient, 411 of whom were randomized to intensity-modulated radiotherapy (IMRT) to improve dose homogeneity (equal distribution of the radiotherapy dose) in the irradiated breast. Toxicity was documented weekly during treatment according to the RTOG scale. All patients from the Cambridge cohort were included.

The German ISE cohort [124] includes 478 breast cancer patients treated with conventional EBRT recruited into a prospective patient cohort documenting acute radiotherapy toxicity at baseline, at cumulative doses of 36-42 Gy and 44-50 Gy, respectively, at the end of radiotherapy and 6 weeks following radiotherapy. All patients from the ISE cohort were included.

Validation cohort

The breast cancer patient cohort of REQUITE observational study, as described in Phase 1 of the project, was used as validation cohort. Summary characteristics are described in Table 21. The REQUITE cohort comprises 2,071 patients treated by BCS and EBRT recruited prospectively across nine centres in Western and Southern Europe and North America between 2014 and 2016, with toxicity documented at baseline (pre-radiotherapy) and within two days of completion of treatment using the CTCAE v4.0 toxicity scales for radiation dermatitis and ulceration.

	REQUITE breast
Total patients in cohort (n)	2,071
Age (median, range)	58 (23-90)
Whole breast dose (median, range in Gy)	50 (28.5-56)
Whole breast fractions (median, range)	25 (5-31)
Boost (proportion)	64 %
Toxicity scale used	CTCAE v4.0
Breast measurement	Bra size
BMI ≥25 (proportion)	54 %
Smoker (current or previous)	43 %
Chemotherapy	30 %
Diabetes	6 %
Hypertension	28 %
Cardiovascular disease	7 %
Tamoxifen or aromatase inhibitor use	76 %

Table 21. Summary of study characteristics of breast cancer patients in the REQUITE cohort.

Endpoint and case definition

A case was defined as having either endpoint ≥ 1 moist desquamation **or** ≥ 1 ulceration during treatment or within 6 weeks of completion of treatment (acute phase reaction) according to the grading system used for each patient cohort:

- RTOG grade 2b: patchy moist desquamation/ moderate oedema;
- Modified CTCAE v2.0 grade 2c: ≥ 1 moist desquamation or interruption of treatment;
- CTCAE v4.0 grade 3 erythema: Confluent moist desquamation ≥1.5 cm diameter not confined to skin folds;
- CTCAE v4.0 grade 1 ulceration: combined area of ulcers < 1 cm (anywhere).

This case definition implies that skin integrity has been broken, either over the breast or in the infra-mammary fold. The distribution of patients according to toxicity grade at treatment completion is shown in Table 22. There were **171** events across the three derivation cohorts. It was noted that the lower incidence of desquamation in the Cambridge IMRT cohort reflected that virtually all patients in this study received the current standard UK hypo-fractionated regimen of 40 Gy in 15 fractions.

Toxicity	**LeND**	**ISE**	**Cambridge**
Eligible patients (n)	**390**	**478**	**1144**
grade 0	164	10	45
grade 1	120	110	654
grade 2a	48	176	418
grade 2b	15	96	26
grade 2c*	-	84	-
grade 3	41	2	1
grade 4	2	0	0
Acute desquamation (≥grade 2b RTOG or ≥2c CTCAE toxicity)	**58 (15 %)**	**86 (18 %)**	**27 (2.4 %)**

*Table 22. Distribution by grade of breast skin toxicity of eligible patients in three derivation cohorts; number and proportion of cases at the end of radiotherapy (*grade 2c only possible in modified CTCAE v2.0 scale).*

The distribution of patients according to grade of toxicity for ulceration and erythema on completion of radiotherapy in the REQUITE cohort is shown in Table 23. There were 183 patients with CTCAE v4.0 ≥ grade 1 ulceration and 28 patients with ≥ grade 3 erythema, with a total of **191** events of acute desquamation (≥ grade 1 ulceration or ≥ grade 3 erythema).

Toxicity	**Ulceration**	**Erythema**
grade 0	1,810	248
grade 1	124	1,280
grade 2	46	455
grade 3	13	28
grade 4	0	0
≥grade 1 toxicity (ulceration) OR ≥grade 3 toxicity (erythema) (%)	**183 (9.2 %)**	**28 (1.4 %)**

Table 23. Distribution by grade of breast skin toxicity in the REQUITE cohort; number and proportion of cases at the end of radiotherapy.

Exposure variables

Based on data from previously published studies [25, 26, 106, 108, 110, 111, 124, 184], the following variables were considered as candidate predictors:

- Age
- Total radiotherapy dose (BED = Biologically Effective Dose)
- BP (hypertension) or cardiovascular disease (BP_CVD)
- Breast size (pre-radiotherapy)
- Chemotherapy
- Diabetes
- Smoking

Breast volume as determined by the planning CT scan was not included as the prediction model was to be developed for use before any local breast cancer treatment including surgery.

BED was calculated using the formula:

$$BED = n\,d\left(1+\frac{d}{\frac{\alpha}{\beta}}\right)$$

BED is the product of the number of fractions (n), dose per fraction (d), and a factor determined by the dose and α/β ratio for skin (10 Gy), which is used in radiobiology to describe the slope of the cell survival curve for different irradiated tissues [146].

As boost dose is relevant to the development of skin toxicity, it was incorporated into the radiotherapy dose predictor variable by adding both calculated values for BED:

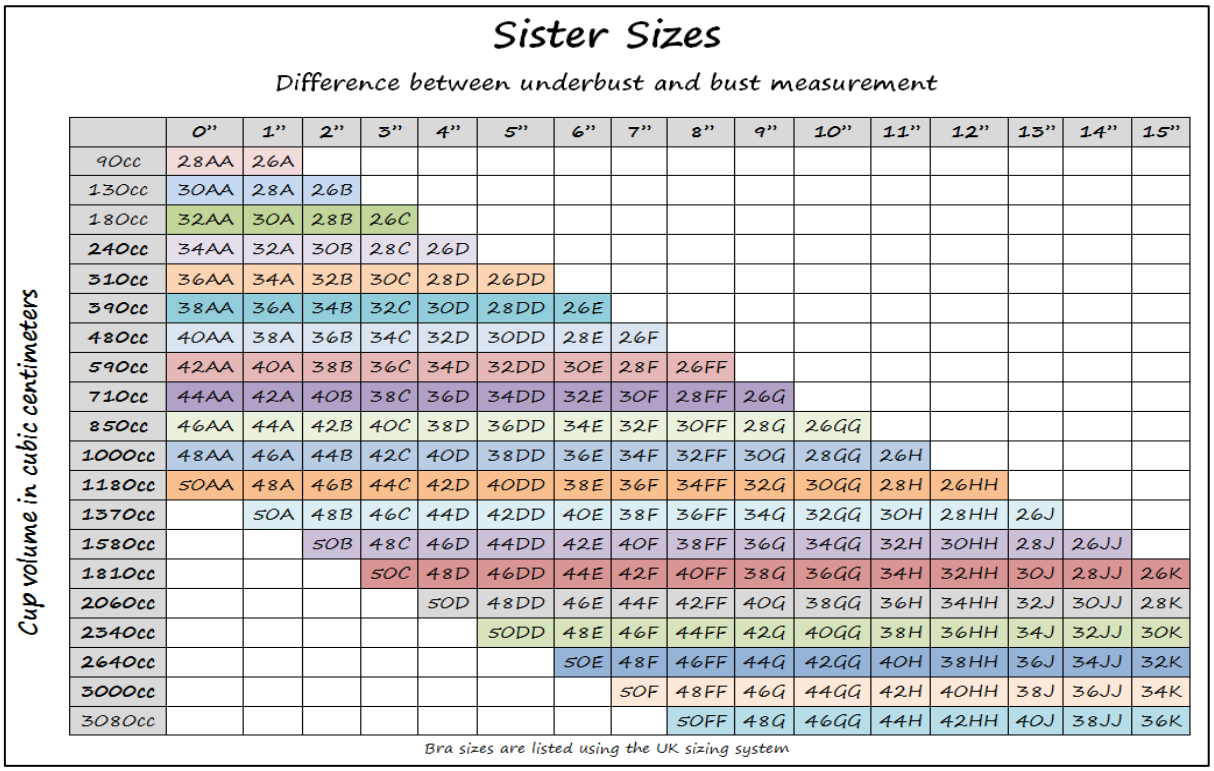
$$BED_{total} = BED_{breast} + BED_{boost}$$

Hypertension (BP) and cardiovascular disease (CVD) were combined as single factor variable defined as presence of *either* BP *or* CVD (BP_CVD). BMI or tamoxifen use were not considered as candidate predictors, because no data was available for patients from the LeND cohort (Table 19).

For patients in the LeND, ISE and REQUITE cohorts, breast size was calculated by adding cup and band sizes as captured into a single continuous variable to represent 'sister' sizes representing the same breast volume (see Table 24, e.g. 34B holds an approximate breast volume equal to 32C, UK sizing). For the Cambridge cohort, breast size was scored on an ordinal scale (1 = small, 2 = medium, 3 = large). In order to approximate these to their corresponding breast 'sister' sizes, a z-score was calculated for each patient in both the Cambridge and the LeND and ISE cohorts. A z-score indicates how many standard deviations (SD) an observation is from the mean. It can be calculated using the following formula:

$$z = (X - \mu) / \sigma$$

where z is the z-score, X is the value of the element, μ is the mean of the population, and σ is the standard deviation. Each patient in the Cambridge cohort with a valid z-score was then allocated a breast 'sister' size approximately according to the distribution of z-scores in the LeND and ISE cohorts, giving patients with 'small' breasts a breast size score of 6 (390 cc), 'medium' breasts size 9 (710 cc), and 'large breasts' size 12 (1,180 cc).



	0"	1"	2"	3"	4"	5"	6"	7"	8"	9"	10"	11"	12"	13"	14"	15"
90cc	28AA	26A														
130cc	30AA	28A	26B													
180cc	32AA	30A	28B	26C												
240cc	34AA	32A	30B	28C	26D											
310cc	36AA	34A	32B	30C	28D	26DD										
390cc	38AA	36A	34B	32C	30D	28DD	26E									
480cc	40AA	38A	36B	34C	32D	30DD	28E	26F								
590cc	42AA	40A	38B	36C	34D	32DD	30E	28F	26FF							
710cc	44AA	42A	40B	38C	36D	34DD	32E	30F	28FF	26G						
850cc	46AA	44A	42B	40C	38D	36DD	34E	32F	30FF	28G	26GG					
1000cc	48AA	46A	44B	42C	40D	38DD	36E	34F	32FF	30G	28GG	26H				
1180cc	50AA	48A	46B	44C	42D	40DD	38E	36F	34FF	32G	30GG	28H	26HH			
1370cc		50A	48B	46C	44D	42DD	40E	38F	36FF	34G	32GG	30H	28HH	26J		
1580cc			50B	48C	46D	44DD	42E	40F	38FF	36G	34GG	32H	30HH	28J	26JJ	
1810cc				50C	48D	46DD	44E	42F	40FF	38G	36GG	34H	32HH	30J	28JJ	26K
2060cc					50D	48DD	46E	44F	42FF	40G	38GG	36H	34HH	32J	30JJ	28K
2340cc						50DD	48E	46F	44FF	42G	40GG	38H	36HH	34J	32JJ	30K
2640cc							50E	48F	46FF	44G	42GG	40H	38HH	36J	34JJ	32K
3000cc								50F	48FF	46G	44GG	42H	40HH	38J	36JJ	34K
3080cc									50FF	48G	46GG	44H	42HH	40J	38JJ	36K

Table 24. Breast volume (cc) according to bra 'sister' sizes (UK sizing). For example, size 34B represents the same breast volume as size 30D (see http://www.sizechart.com/brasize/sistersize/index.html)

The following six pre-specified interactions were also considered as predictors:

- Age x BED_total
- Age x BP_CVD
- Age x chemo
- Age x diabetes
- Chemo x BED_total
- Smoking x BP_CVD

The consideration of 13 candidate predictor variables in this analysis satisfied the methodological sample size constraint of at least 10 events per variable (EPV) required to reduce issues with over-fitting in predictive model development [187].

Missing values and imputation

Data on the outcome variable as defined for this analysis were available for all patients in the derivation cohorts (n=2,012). Considering the candidate variables for this study, 20 % of cases were incomplete with observations in at least one variable missing (see Table 25). Observations on breast size were missing at random from 337 patients across the three derivation cohorts (15 %), while the remaining variables were missing in between 0.5 % and 2 % of patients. Variables which were not missing at random (BMI and tamoxifen use) were not imputed. Nevertheless, due to its correlation with breast size (r=0.53), BMI was used as auxiliary variable.

In recognition of the drawbacks of mean or regression imputation, multiple imputation (MI) was used to replace missing values in order to minimise bias from analysing only complete cases. Multiple imputation is an iterative form of stochastic imputation that uses the distribution of observed data to estimate multiple missing observations that reflect the uncertainty around its true value. The objective of MI is not to predict missing values as close as possible to the true ones but to handle missing data in a way resulting in valid statistical inference. The values for these observations can then be used in the analysis of interest and the results combined across imputed datasets [188].

Using Multivariate Imputation by Chained Equations (MICE) [189] and 'just another variable' approach based on all candidate predictors and interactions, *m* imputed datasets were created for missing variables using the *augment* option, which were then combined across all datasets using Rubin's rules to obtain final model estimates [190]. Rubin's rules assume that across all complete datasets, the model is fit on the same effects and in the same order, that categorical variables have the same set of categories, and that the reference category remains the same. The number of imputations (*m*=10) was determined by the percentage of incomplete observations per variable to reduce the error associated with estimating the regression coefficients, standard errors and the resulting p-values [191].

Variable	Number of observations	Missing observations
acute_desquamation	1,999	13
age	2,011	1
BMI	1,576	436
breast size	1,675	337
bp_cvd	1,968	44
chemo	2,002	10
diabetes	1,971	41

Table 25. Distribution of missing data cross three combined derivation cohorts (n=2,012).

Statistical modelling

The model was developed in Stata™ version 14.1 using stepwise multiple logistic regression to model the probability of acute desquamation (≥ 1 moist desquamation or ulceration). Fractional polynomials were used to model potential non-linear relationships between continuous predictors and the outcome variable. Fractional polynomials increase the flexibility afforded by the family of conventional polynomial models.

Initially, both a fixed generalised linear model (GLM, *logit*) and generalised linear mixed model (GLMM, *xtmelogit*) model were developed to check for random effects between the three study cohorts making up the derivation cohort. GLMMs are an extension of mixed models and GLMs to allow for inclusion of both fixed and random effects and response variables from different distributions, such as binary responses. Similar to GLMs, a link function is applied, such as the logit link. However, interpretation of model coefficients in GLMMs can be more complex because of the random effects. Because calculated odds ratios for individual predictor variables holding all other predictors constant will be conditional upon the individual patient cohort, it is more useful in GLMMs to calculate average fixed effects marginalizing the random effects. Similarly, probability calculations based upon GLMM are usually displayed as average marginal probability.

Stepwise backwards selection was used to determine the candidate variables to be included in the final prediction model (with $p<0.2$ taken conservatively to warrant inclusion). Continuous variables were kept as continuous, rather than dichotomising, to avoid loss of predictive power. After elimination, each excluded predictor was re-inserted into the final model to further check whether they became statistically significant at this stage.

The equation for the log odds of adverse acute toxicity was formed using the estimated β coefficients multiplied by the predictors included in the model together with the intercept across cohorts. The predicted risk of adverse acute toxicity can thus be calculated:

$$predicted\ risk = \frac{e^{\log\ odds}}{1 + e^{log\ odds}}$$

Model performance

Discrimination of the fitted models was assessed using the c-statistic (AUC from the logistic model, plotting sensitivity over 1-specificity). The area under the curve (AUC) indicates the probability that a patient with the adverse outcome had a higher predicted probability than a patient without the adverse outcome, for random pairs of patients with and without the outcome. A c-statistic of 1 indicates perfect discrimination, whereas 0.5 indicates no discrimination.

Calibration of the fitted models was examined by the Hosmer-Lemeshow "goodness-of-fit" test and by checking the calibration (regression) slope of the fit of observed versus predicted probabilities across all patients A slope of 1 indicates perfect calibration and would be expected across the imputed datasets as the model is being developed in the same data (apparent performance).

Overall performance was assessed by calculating a Brier score, which are the average square distances from the predicted and observed outcomes.

Internal validation

Bootstrapping was used to internally validate developed models by repeating the multiple imputation (*m*=10) and variable selection procedure in each of 100 bootstrap samples with replacement. Each model was then applied to the original dataset to test model performance (c-statistic and calibration slope). To control for optimism (over-fitting), the average difference in AUC for the model developed in each of the bootstrap samples and the original dataset was applied to the original AUC.

A shrinkage factor was estimated by averaging the calibration slopes from each of the bootstrap samples. Each calibration slope was estimated using the outcomes of patients in the original dataset and the linear predictor calculated from the bootstrap model coefficients in the original dataset as a single co-variable [192]. The β coefficients in the original dataset were then multiplied by the shrinkage factor in the final model and the intercept was re-estimated to ensure that overall calibration was maintained.

External validation

The final optimism-corrected model developed in the derivation cohorts was applied to each patient in the REQUITE validation cohort to predict the log odds of adverse acute desquamation on the basis of the presence or absence of one or more of the predictor variables. Performance of the final model was assessed by calculating the c-statistic (AUC) and examining the calibration plot across tenths of predicted risk.

Results

Examining fractional polynomials for the continuous candidate predictors age and BED, demonstrated no improvement in model deviance. Hence both age and BED were kept as linear predictors. The trace plots of imputed values for the derivation cohort (example shown in Figure 14) showed convergence on the mean. Comparing p-values in the full model for the imputed versus non-imputed data, significant associations did not change apart from for the factor diabetes and the interaction age x diabetes (p=0.076 vs p=0.033, and p=0.11 vs p=0.046, respectively).

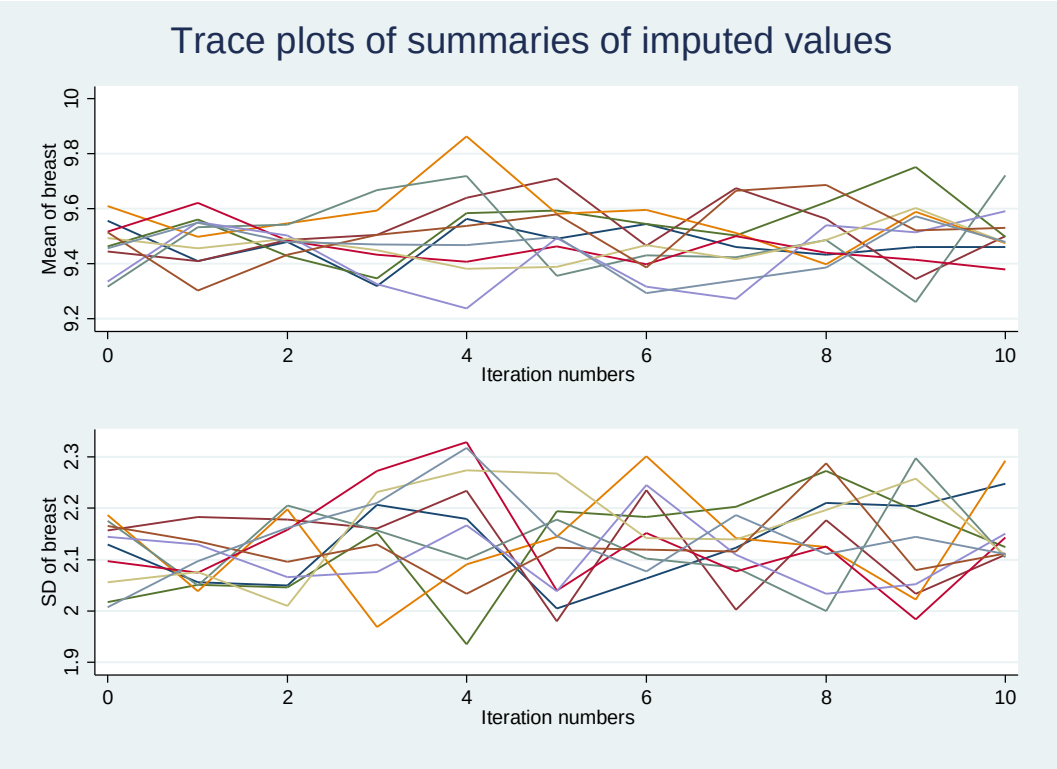


Figure 14. Trace plots of summaries of imputed values for the variable breast size.

Model development, performance and internal validation

The initial logistic regression model with variable odds ratios is shown in Table 26. At this stage, variables that satisfied the p<0.2 stepwise inclusion threshold were: age, BED_total, BP_CVD, diabetes, smoking, and the interactions age x BED_total, age x diabetes, and chemo x BED_total. Using a mixed modelling approach (*xtmelogit*) with study cohort as the higher level variable, the variable smoking and interaction of chemo x BED_total fell outside the inclusion threshold, but other

variable did not change significantly (not shown). It was therefore decided to proceed with a fixed-effects logistic regression procedure (*logit*), which also made the interpretation of model coefficients and applying the final model to the validation cohort easier.

Acute_desquamation	Odds Ratio	Std. Err.	p-value	[95% Conf.	Interval]
Age	1.16	0.099	0.082	0.98	1.37
BED_total	1.23	0.098	0.009	1.05	1.44
BP_CVD	7.36	9.969	0.141	0.52	104.75
Breast size	1.47	0.064	0	1.35	1.61
Chemo	56.45	254.707	0.372	0.01	394612.1
Diabetes	69.3	165.464	0.076	0.64	7469.76
Smoking	1.52	0.376	0.091	0.94	2.47
Age x BED_total	1	0.001	0.08	1	1
Age x BP_CVD	0.97	0.021	0.242	0.93	1.02
Age x chemo	1.03	0.031	0.343	0.97	1.09
Age x diabetes	0.94	0.036	0.11	0.87	1.01
BP_CVD x smoking	1.17	0.472	0.688	0.53	2.58
Chemo x BED_total	0.91	0.057	0.135	0.81	1.03

Table 26. Initial logistic regression model for acute desquamation using all predictor variables in the development cohort.

The initial model contained high standard errors for BP_CVD, chemo, and diabetes due to collinearity. Following stepwise exclusion from the model of all interaction terms apart from age x BED_total and of chemotherapy, the final logistic prediction model for acute desquamation contained seven of the 13 candidate predictor variables, which satisfied the inclusion criteria $p<0.2$ (Table 27).

Acute_desquamation	Odds Ratio	Std. Err.	p-value	[95% Conf.	Interval]
Age	1.14	0.085	0.081	0.98	1.32
Age x BED_total	1.00	0.001	0.073	1.00	1.00
BED_total	1.21	0.083	0.006	1.06	1.38
BP_CVD	1.70	0.323	0.005	1.17	2.47
Breast size	1.46	0.063	0	1.34	1.58
Diabetes	1.52	0.446	0.157	0.85	2.70
Smoking	1.67	0.320	0.007	1.15	2.43

Table 27. Final logistic regression model for acute desquamation in the development cohort.

Table 28 shows apparent and internal validation performance statistics of the risk prediction model in the derivation cohort. Apparent performance was estimated directly from the dataset used to develop the prediction model. Test performance was determined by developing the model in each bootstrap sample, calculating performance in the bootstrap sample, and then applying the bootstrap model to the original dataset (not shown). Average optimism was the average difference between model performance in the bootstrap dataset versus test performance in the original dataset.

After correcting for optimism, the final risk prediction model was able to discriminate patients with and without acute desquamation following breast radiotherapy with a c-statistic (AUC) of 0.74 (CI 0.71-0.77). Agreement between observed and predicted proportions showed good calibration (Hosmer-Lemeshow p=0.4279) with a Brier score of 0.0689 (Figure 15), but a shrinkage factor of 0.89 (optimism-corrected calibration slope) was applied to adjust predictor coefficients in the final model.

The final risk prediction model equation to calculate the log odds of a patient's developing acute desquamation was:

Log odds (acute_desq) = -16.5158 + 0.115334*age -0.00181*age*BED_total + 0.167934*BED_total + 0.474004*BP_CVD + 0.334303*breast_size * 0.370598*diabetes + 0.457858*smoking.

Measure	**Apparent performance**	**Average optimism**	**Optimism-corrected**
c-statistic (AUC)	0.77 (0.73-0.81)	+0.03	0.74 (0.71-0.77)
calibration slope	0.97 (0.75-1.19)	+0.08	0.89 (0.68-1.10)

Table 28. Model diagnostics (with 95 % CI) in the derivation cohort.

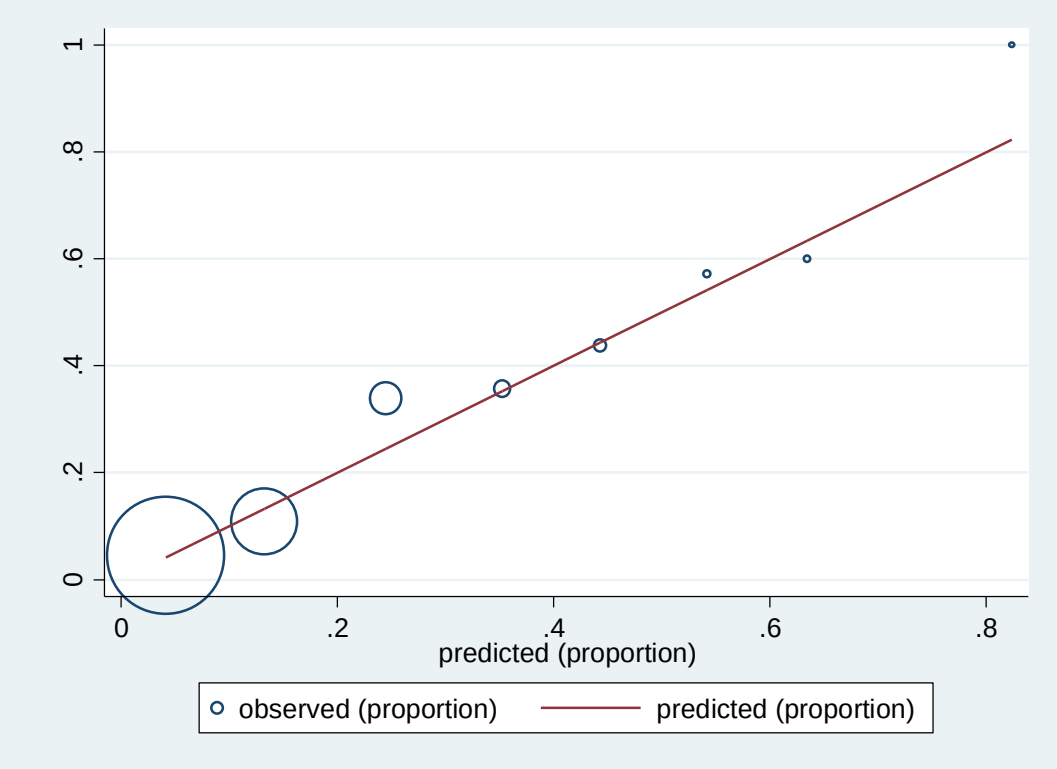


Figure 15. Calibration plot in the derivation cohort.

To illustrate, using two examples of predicted probabilities in the derivation cohort:

1. A woman aged 50, non-smoker, not diabetic, and no history of either hypertension or cardiovascular disease, wearing UK bra size 30A, receiving standard breast radiotherapy at 40 Gy in 15 fractions, would have a **0.4 % predicted probability** of acute desquamation following radiotherapy;

2. A woman aged 50, who is a smoker with diabetes and a history of either hypertension or cardiovascular disease, wearing UK bra size 42F, receiving standard breast radiotherapy at 40 Gy in 15 fractions plus a boost of 12.5 Gy in 5 fractions, would have a **67 % predicted probability** of acute desquamation following radiotherapy.

Model validation in the REQUITE-AB cohort

Applying the final risk prediction model to the validation cohort gave a c-statistic (AUC) of 0.62 (CI 0.58-0.66) with relatively poor calibration (slope = 0.42, 0.27-0.58, Hosmer-Lemeshow p<0.001) and a Brier score of 0.1008 (Figure 16). The mean predicted probability of acute desquamation following radiotherapy in the REQUITE breast cohort was 16.7 % versus an actual probability of 9.5 %.

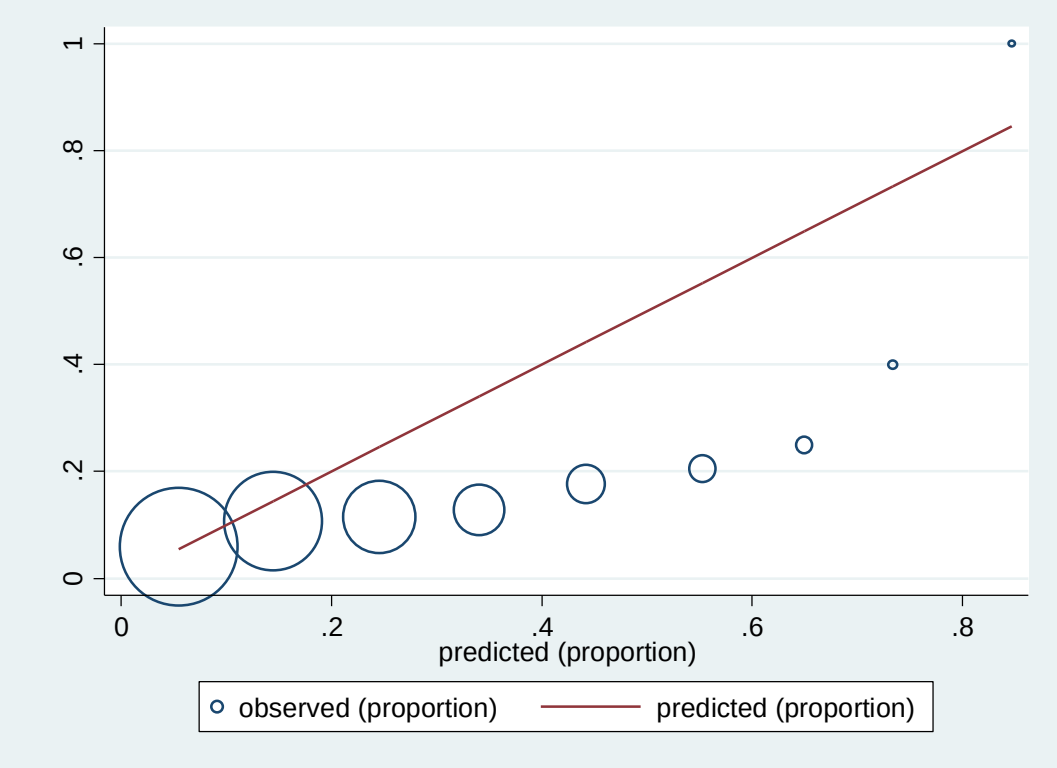


Figure 16. Calibration plot in the REQUITE-AB validation cohort.

Development of new model in REQUITE-AB cohort

Applying the same statistical method as used to develop a prediction model in the derivation cohorts, only the four variables age, BED total, breast size, and diabetes satisfied the including criteria p<0.2 in the new final model (Table 29). Compared to the derivation model, the odds ratios in the new final model were smaller ranging from 1.14 for each unit increase in sister breast size to 0.47 for diabetes, which turned out to be protective in the REQUITE cohort.

This new model was able to discriminate patients with and without acute desquamation following breast radiotherapy with a c-statistic (AUC) of 0.67 (CI 0.63-0.71). Agreement between observed and predicted proportions in the new model showed good calibration (Hosmer-Lemeshow p=0.5128) with a Brier score of 0.0848 (Figure 17).

Acute desquamation	Odds Ratio	Std. Err.	p-value	[95% Conf.	Interval]
Age	1.015032	.0078399	0.053	.9997821	1.030515
BED_total	1.061981	.0092083	0.000	1.044086	1.080184
Breast size	1.143899	.0360281	0.000	1.075421	1.216738
Diabetes	0.474087	.1946445	0.069	.212023	1.060067

Table 29. New logistic regression model to predict acute desquamation developed in the REQUITE cohort.

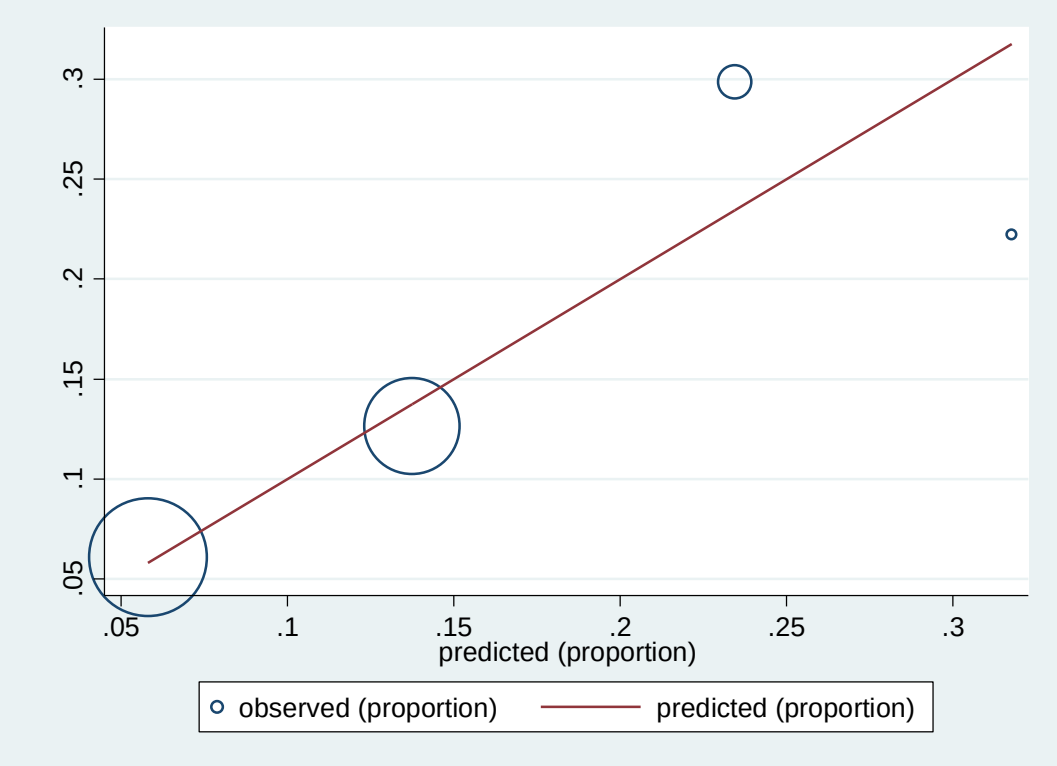


Figure 17. Calibration plot for new model developed in the REQUITE-AB cohort.

Discussion

Accurate prediction models can inform patients and clinicians about the future course of a condition or an illness, thereby helping guide decisions about treatment. For a prediction model to be valuable, it should not only have predictive ability in the derivation cohort but must also perform well in a validation cohort. In the present study, the model to predict the risk of acute desquamation following breast radiotherapy demonstrated useful discrimination after internal validation with an AUC of 0.74 and good calibration. However, the drop in performance (discrimination and calibration) in the REQUITE validation cohort was a lot more than expected from internal validation. Reasons why a predictive model may perform substantially differently between derivation and validation cohorts include over-fitting, missing important predictor variables, inter-observer variability leading to measurement errors of predictors, or differences in the patient cohort case mix.

The model developed across the three derivation cohorts in this study included clinically relevant predictors which satisfied the relatively loose criteria for inclusion in the model ($p<0.2$). The purpose of multivariate prediction modeling is to predict outcome with consideration of the joint effects of predictors. The purpose is estimation rather than testing for association with risk factors, and it is therefore reasonable to include clinical predictors despite non-significant association or collinearity, to ensure that important predictors are not missed [193].

In order to address over-fitting, bootstrapping was used as an internal validation technique in this part of the project, but other studies with reasonably large datasets have also used split sample training-validation or cross validation. Nevertheless, the literature suggests that internal validation nearly always yields optimistic results given that the derivation and validation sets come from the same cohort [194]. Therefore, it is possible that a different internal validation method may have produced similar results to the bootstrapping method used in the present study.

However, using an alternative statistical method to traditional logistic regression modelling, such as Lasso techniques or data mining techniques such as machine learning algorithms, may have identified other predictor variables or potential interactions in patients with several marginal risk factors [195]. Machine learning algorithms are used with increasing frequency, in particular in the context of 'big data' such as electronic health records [196].

The distribution of predictor and outcome variables in the present study was broadly similar between derivation and the REQUITE validation cohorts, but there was heterogeneity between the centres in either cohort with regards to treatment variables, such as dose fractionation, use of boost treatment or IMRT and inclusion of patients who received prior adjuvant chemotherapy. Poor generalisability of the prediction model to the REQUITE cohort could also be due to differences in radiotherapy

techniques over time. The patients in the derivation cohorts were on average treated more than 10 years prior to those of the REQUITE cohort. Certainly, there has been widespread update of intensity-modulated radiotherapy over that time, with almost 50 % of patients in the REQUITE cohort treated in this way. And although the event rate of acute desquamation was roughly the same between both the derivation (171/2,012) and validation cohorts (191/2,072), measurement error due to inter-observer variability and use of different scales in assessing toxicity endpoints cannot be excluded.

The majority of published prediction research continues to focus solely on model development and validation studies are more scarcely reported. When a validation study shows disappointing results, as in this case, one can often be tempted to reject the initial model and develop a new model from the validation cohort data, shown above for the REQUITE cohort. Interestingly, the new model included fewer predictors than the model originally developed in the derivation cohort, with an opposite effect for the presence or absence for diabetes.

However, several methods have been described in the literature to update prior predictive models with data from the patients of the validation cohort [197]. Despite the application of a shrinkage factor calculated from 100 bootstrap models in the derivation cohort in the final predictive model, the calibration curve in the validation cohort suggests that the predicted probabilities in the REQUITE patients were too low apart from the in the highest decile of the calibration curve.

Although calibration could be further improved using shrinkage and re-calibration, this would not change the model's discriminatory power. To improve discrimination in a new set of patients, the model would need to be revised, for example, by additional adjustment to regression coefficients of predictors with different strength in the derivation compared to the validation cohort (e.g. diabetes), stepwise selection of additional predictors, and re-estimation of all regression coefficients in the validation population. These approaches to update the model need to be balanced against the fact that the information in the original model will be neglected.

As the commonly published AUC of the ROC curve can be relatively insensitive for assessing differences in discriminatory power between predictive models and is not directly clinically relevant, novel performance measures, such as net re-classification improvement (NRI) and net benefit (NB) would also be important to consider [198]. Clinical prediction models should also ideally recommend decisions instead of simply providing personal risk estimates. Risk models without recommending clinical decisions are less likely to change treatment decision-making behaviour than those that translate risk into a decision recommendation [199]. Nevertheless, whether a validated prediction model is of actual benefit in clinical practice is ideally determined by a clinical trial randomising

patients or clinicians to use of the prediction rule versus usual care or clinical judgement, which is best achieved using a cluster randomized design [200].

Conclusions

The aim of this part of the project was to derive a prediction model for acute breast toxicity from existing patient cohorts and validate the model in the REQUITE cohort. Using a unified endpoint of acute desquamation across three derivation cohorts, the final model failed to validate in REQUITE patients due to potential cohort heterogeneity, particularly in terms of radiotherapy techniques. However, statistical techniques to update predictive models may serve to improve the performance of the prediction rule in the future.

In the subsequent parts of this research project, the application of genetic markers associated with acute breast toxicity in REQUITE patients will be investigated. Ultimately, the addition of validated genetic markers to a clinical prediction model should further improve model performance.

Phase 4: Systematic review and meta-analysis of genetic markers

There is considerable variation between individual patients' tissue reaction in response to radiotherapy [201]. It is now understood that individual sensitivity to radiation is at least in part determined by genetic variation [202]. The main approach taken by investigators to identify genetic markers of normal tissue toxicity has been to type SNPs (single nucleotide polymorphisms) in the genome of patients undergoing radiotherapy. SNPs represent relatively common genetic alterations typically with low effect sizes [95]. Through a number of case-control studies, in which SNPs at candidate loci were genotyped across patients with or without radiotherapy side-effects, several predictive genetic markers have been identified [86].

Two systematic reviews of genetic markers of radiotherapy toxicity were published in 2009. In the first review by Andreassen and Alsner [88], 58 publications were identified that studied candidate genetic variants, of which most were related to DNA damage response, followed by genetic variants involved in oxidative stress or fibrogenesis. The majority of studies were undertaken in breast and prostate radiotherapy in relation to a variety of acute and late toxicity endpoints. The other review by Popanda *et al* [89] covered data from 32 published studies summarized according to genes involved in oxidative stress response, fibrogenesis, DNA damage signalling and DNA repair, all of which were also included in the Andreassen and Alsner review.

Of the DNA damage response genes, polymorphisms in *XRCC1* have been the most frequently studied variants, yet with some conflicting results [203-211]. Equally, earlier studies of *ATM* variants produced inconsistent results [208, 212], but a later individual patient meta-analysis (n=2,759 breast cancer, n=2,697 prostate cancer patients) undertaken under the auspices of the Radiogenomics Consortium has found a replicated association of the *ATM* rs1801516 variant with both early and late toxicity in breast and prostate cancer [94].

Amongst genes involved in oxidative stress response, *GSTP1* variants have been studied in relation to acute breast toxicity [213], while *GSTA1* and *NOS3* variants have been studied in relation to the development of telangiectasia in the breast [79, 214]. TGFB1 was the most researched gene involved in fibrogenesis. Initial results indicated an association with late breast fibrosis [215, 216], however, this was not replicated in a subsequent study by the same authors [208]. Subsequent studies showing an association with breast fibrosis [85, 217] have since been disproven through lack of replication in the RAPPER cohort [218]. Moreover, a patient-level meta-analysis (n=2,782) of the *TGFB* rs1800469 SNP failed to demonstrate any association with late breast radiation toxicity [122].

Since the publication of the two systematic radiogenomics reviews, many genetic associations have been disproven for lack of replication [140] and new candidate markers have emerged [219, 220]. Several genome wide association studies (GWAS) are also underway with published data on late toxicity outcomes [98, 221-223]. There have been two literature-based systematic reviews of single SNP variants in relation to multiple toxicity endpoints, covering *XRCC1* Arg399Gln (rs25487), Arg194Trp (rs1799712), -77T>C (rs3213235)and Arg280His (rs25489) [224], and the *ATM* Asp1853Asn (rs1801516) and TP53 Arg72Pro (rs1042522) polymorphisms [225], respectively. However, neither of the two earlier general systematic reviews [88, 89] have been updated, nor has there been a systematic review specifically of genetic markers of acute clinical endpoints in the breast.

Objectives

The aim was to conduct a literature-based systematic review of published genetic markers of acute breast radiation toxicity, with the following objectives:

- To identify genetic markers that have been tested for an association with acute radiation toxicity in breast cancer patients
- To assess strength, consistency, and risk of bias among published associations between each genetic marker and the phenotype
- To determine the pooled effect size of reported genetic markers for each replicated association

Search strategy

Articles included in the two existing 2009 systematic reviews [88, 89] covering genetic association with radiation toxicity were screened for relevant studies covering acute toxicity endpoints in breast cancer patients. All articles indexed in the following databases were searched with the following terms, with the last search performed on 1st May 2017:

Web of Science Core Collection™	breast cancer AND radiotherapy AND toxicity AND genetic
Ovid SP/Medline	breast cancer AND (radiotherapy NOT chemotherapy) AND toxicity AND (polymorphism OR genetic marker)
Pubmed	breast cancer AND (radiotherapy NOT chemotherapy) AND toxicity AND (polymorphism OR genetic marker)
JSTOR	breast cancer AND radiotherapy AND toxicity AND genetic
Cochrane Library	breast cancer AND radiotherapy AND toxicity AND genetic

Reference list in published studies were searched to identify any additional records. Data covering genetic markers of acute breast radiation toxicity presented at conferences attended by members of the international Radiogenomics Consortium [226] were also identified.

Inclusion and exclusion criteria

Population and condition of interest

Acute radiation toxicity experienced by breast cancer patients undergoing radiation treatment, which is predominantly erythema (redness) of the skin over the breast. Other acute toxicity endpoints, such as pain or fatigue, were also considered.

Interventions or exposures

Any defined and identifiable genetic variant, including single nucleotide polymorphism (SNP) or copy number variant (CNV).

Comparison or control group

Patients without toxicity.

Outcomes of interest

Prevalence or incidence and severity of acute skin reaction (erythema) and any additional identified acute toxicity endpoint following whole breast external beam radiotherapy.

Study designs

Any study design, but cohort, case-control, and cross-sectional studies were expected. Any studies reporting acute breast radiation toxicity endpoints and genetic associations were included.

Exclusion criteria

Studies not reporting acute breast radiation toxicity endpoints.

Data extraction

Using the above search strategy and inclusion criteria, the review was conducted according to published HuGE network guidelines for the systematic review and meta-analysis of genetic association studies [120]. The HuGE guidelines provide guidance on the format of the review, potential conflict of interest, submission of a protocol, planning the review, search strategy, handing bias and confounding, and on presentation of individual studies. The review will be reported according to the PRISMA guidelines [227].

The review protocol was prospectively registered (PROSPERO 2015: CRD42015024248) [228]. Abstracts of studies were screened by two independent investigators (Tim Rattay and Kerstie Johnson) to see if they fulfil the inclusion criteria. Where there were disagreements, a third reviewer (Dr Christopher Talbot) was consulted. Duplicate records or duplicate publication of datasets were excluded.

Eligible studies proceeded to full-text qualitative assessment by two methodologically trained independent reviewers (TR and KJ) according to published STROGAR criteria [229], a radiogenomics extension of the STREGA statement for the reporting of genetic association studies [230]. Data was extracted in duplicate and entered into a spreadsheet (Table 30). Where possible, full genotype frequencies were extracted or requested from authors for both cases and controls. Literature references were tracked in EndNote™.

Reference	First author
	Year of publication
Study design	
Population	Country of study
	N of total patients
	N of breast patients with acute toxicity endpoint
	Ethnicity
	Age range
Phenotype(s)	Acute toxicity endpoint
	Grading scale used to score toxicity
	Time points acute toxicity scored
Exposures	Genotype(s)/polymorphism(s)
	HWE assessment
	Genotyping methods and accuracy
	Radiotherapy regime and dose
	Other exposures reported (e.g. BMI, smoking)
Results	Phenotype
	Genotype(s) or haplotype or combined genotypes
	Measures of association (e.g. OR, 95 % CI)
	Interactions
Data source	Publication, online database, correspondence with authors

Table 30. Data fields extracted from published studies following full-text qualitative assessment.

Statistical methods

Odds ratios and 95 % confidence intervals (CI) were extracted from individual studies. Where >1 report was identified for same association in the same study population, the publication with the largest sample size was included. For polymorphisms assessed in two or more studies for the same phenotype, a meta-analysis was performed using the *metan* package (Stata 14.1 for Windows, StataCorp, College Station, TX, USA). The Z test was used to determine statistical significance of association. The fixed effect model based on the Mantel-Haenszel method and the random-effects model based on the Dersimonian-Laird method were used to pool data from different studies. The fixed effect analysis weights each study by the inverse of its variance and it assumes that all studies in the analysis share a common true effect size. The random effects analysis includes the variance within each study, but also the variance between studies, and it assumes that the studies were drawn from populations that differ from each other in ways that could impact on the common true effect.

In the meta-analysis, genotype or allelic frequencies were used rather than the individual studies' pre-calculated effect sizes. Because the underlying model of inheritance remains unknown, the allelic association test (additive model of inheritance) was used for all variants included in the meta-analysis [231]. Any SNPs in linkage disequilibrium were identified using the Broad Institute's SNAP pairwise LD (r^2>0.9) and pooled together in the meta-analysis. For each variant, Hardy-Weinberg equilibrium was assessed in the control groups in each study included in the meta-analysis as the sum of squared differences (chi square) between observed and expected genotypes, with a significance level set at $p<0.05$. However, studies were not excluded for violation of HWE alone [232].

Statistical heterogeneity amongst pooled candidate SNP studies was assessed by Cochran's Q test (the weighted sum of squared differences between individual study effects and the pooled effect) with a significance level of $p<0.05$. As sensitivity analysis, the influence of individual studies on the summary effect estimate was assessed for all variants using the *metainf* command. Risk of bias was assessed qualitatively in phenotype and genotype definitions. Funnel plot asymmetry was tested using the *metabias* command according to the method of Begg and Mazumdar [233].

Results

Study selection

Figure 18 shows the study selection process. A total of 134 articles were identified through database searching and from the two earlier systematic reviews [88, 89]. After exclusion of duplicates (n = 58), a further 42 records were excluded after screening the title and abstract as they did not fulfil the inclusion criteria for this review. Thirty-four articles proceeded to full-text assessment for eligibility, of which 3 were excluded; one because patients in the study underwent intra-operative partial breast irradiation [210], another because the study involved gene expression analysis but no germline genotyping [234], and another earlier study with no detailed information available by different tumour site [235].

Thus, 31 full-text articles were included in the systematic review investigating a total of 175 genetic variants, including four publications covering various BRCA1 and BRCA2 mutations [236-239] and two publications covering various ATM mutations [240, 241]. Of those variants, 46 were investigated in at least 2 or more studies across 22 publications which were included in the final meta-analysis stage.

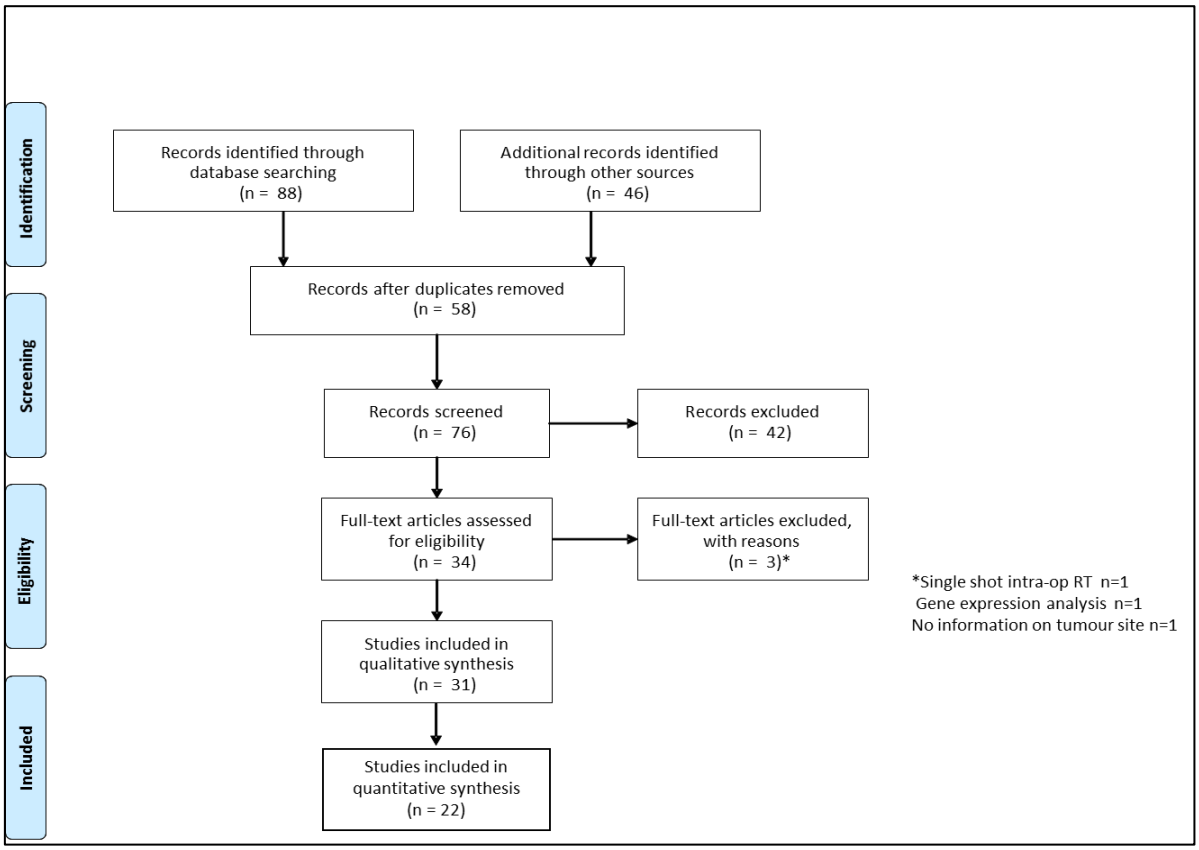


Figure 18. PRISMA flow diagram for study selection process

Study characteristics

Study characteristics are summarized in Table 31. There were 31 publications between 1998 and 2017 investigating genetic associations with acute radiation toxicity enrolling between 11 and 1,357 breast cancer patients. The majority of studies were in European (n = 19) or North American (n = 7) populations. Two studies were performed in Japanese populations and one each in Chinese, Korean, and Indian patients, respectively. A further single study came from Argentina. None of the studies explicitly reported patients' ethnicity.

While earlier studies up to about 2003 were mostly case series of either BRCA [236, 237, 239] or ATM mutation carriers [240-243], later designs included case control [209, 244-246] and retrospective [121, 238, 247, 248] as well as prospective cohort studies [26, 203-206, 249-255] conducted in a general breast cancer patient population, including both BCS and mastectomy patients. Several studies also included at least some patients who had chemotherapy.

In a couple of cases, there were multiple publications covering different genetic variants from the same patient cohort; the prospective multi-centre German cohort of 446 patients treated by BCS and radiotherapy but no chemotherapy [203, 204, 213, 249, 255], and the French case-control study, which matched 70 'radiosensitive' patients with 184 other breast cancer patients treated at the same hospital [209, 244, 245]. The two largest patient cohorts overlapped, as the sample of 1,357 patients in the individual patient-level meta-analysis for *ATM* rs1801516 [94] included 925 breast cancer patients from the earlier publication by the RAPPER consortium attempting to replicate previously published genetic associations with radiation toxicity [140].

Between studies, the total whole-breast radiation dose given ranged from 40 to 57.7 Gy with between 12 % and 100 % of patients receiving a boost dose. Although not specified in all publications, radiotherapy technique varied between cobalt therapy for patients treated before 1996 in one retrospective cohort study [242] and either multi-beam or field-in-field IMRT in a more recent prospective cohort study [26].

The case or endpoint definition varied between studies according to the toxicity scale and relative cut-off used. The majority of publications used either RTOG or CTCAE scales with toxicity defined as either grade 2 or 3 skin reaction, while the modified CTCAE cut-off grade 2c (inframammary desquamation or at least one interruption of treatment) was used in publications based on the largest German cohort [203, 204, 213, 249, 255] on one Italian study [253]. The endpoint of 'radiosensitivity' was not further defined in the three French publications [209, 244, 245], whereas the two publications used a cut-off based on having a STAT (standardised total average toxicity) score in the upper quartile to facilitate comparison across different patient cohorts included in each of

those consortia [94, 140]. There were no publications on patient-reported endpoints such as pain or fatigue.

In terms of genotyping strategy, there was no published GWAS for acute breast radiation toxicity. Apart from earlier studies covering ATM and BRCA mutation carriers, all studies used a candidate gene approach covering variants in genes involved in oxidative stress response, fibro- and angio-genesis, DNA damage signalling and DNA repair (Table 31), as highlighted by a previous review [258]. The two Japanese studies employed high-throughput SNP techniques to type 999 and 3,144 variants, respectively. In the Barnett *et al* publication, the SNPs were selected after a comprehensive literature search of previous genetic association studies so that they could be validated. Genotyping QC was infrequently reported in earlier studies before 2003 and was not clear in a few later publications [94, 238, 252].

First author	Year	Country	Study population	N breast	Cases	Controls	Study design	Case definition	Variants genotyped
Gaffney [236]	1998	USA	21 breast cancer cases out of 50 known BRCA carriers treated by BCS or Mx + RT	21	6	15	Case series	RTOG grade 3 skin toxicity	BRCA 1 and 2 mutations (various)
Weissberg [241]	1998	USA	13 AT-heterozygote cancer patients from 270 AT families	11	11		Case series	RTOG grade 2 skin toxicity	ATM mutations (various)
Oppitz [243]	1999	Germany	20 cancer patients with severe toxicity	11	11		Case series	RTOG grade 3 skin toxicity	ATM mutations (various)
Leong [237]	2000	USA	22 radiosensitive patients or with secondary malignancies	12	3	9	Case series	RTOG grade 3 toxicity	BRCA1 and 2 mutations (various)
Pierce [239]	2000	USA	71 breast cancer cases in known BRCA carriers and 213 age-matched controls	284	80	204	Case-control study	RTOG grade 2 toxicity	BRCA1 and 2 mutations (various)
Iannuzi [240]	2002	USA	46 patients BCS + RT, with skin reaction scored retrospectively	46	23	23	Case series	RTOG grade 2 skin toxicity	ATM mutations (various)
Angele [244]	2003	France	70 radiosensitive and 184 other breast cancer patients (single hospital)	254	70	184	Case-control study	Grade not defined	ATM variants
Bremer [242]	2003	Germany	10 ATM mutation carriers out of a cohort of 1,000 breast cancer patients	10	1	9	Case series	RTOG grade 2 skin toxicity	ATM mutations (various)
Moullan [209]	2003	France	70 radiosensitive and 184 other breast cancer patients (single hospital)	254	70	184	Case-control study	Grade not defined	XRCC1
Chang-Claude [204]	2005	Germany	446 patients BCS + RT from four hospitals, no chemotherapy	446	77	369	Prospective cohort study	CTCAE v2.0 grade 2c skin toxicity	XRCC1, XPD
Ahn [249]	2006	Germany	446 patients BCS + RT from four hospitals, no chemotherapy	446	77	369	Prospective cohort study	CTCAE v2.0 grade 2c skin toxicity	MnSOD, CAT, MPO, eNOS
Ambrosone [213]	2006	Germany	446 patients BCS + RT from four hospitals, no chemotherapy	446	77	369	Prospective cohort study	CTCAE v2.0 grade 2c skin toxicity	GSTM1, GSTT1, GSTA1, GSTP1
Brem [245]	2006	France	66 radiosensitive and 181 other breast cancer patients (single hospital)	247	66	181	Case-control study	Grade not defined	XRCC1
Popanda [203]	2006	Germany	446 patients BCS + RT from four hospitals, no chemotherapy	446	77	369	Prospective cohort study	CTCAE v2.0 grade 2c skin toxicity	XRCC3, XRCC2, NBS
Tan [255]	2006	Germany	446 patients BCS + RT from four hospitals, no chemotherapy	446	77	369	Prospective cohort study	CTCAE v2.0 grade 2c skin toxicity	TP53, p53PIN4, p21
Ho [247]	2007	USA	131 patients from three centres	131	51	80	Retrospective cohort study	RTOG grade 2 toxicity	ATM exome
Suga [206]	2007	Japan	399 patients BCS + RT from 10 institutions	399	109	290	Prospective cohort study	RTOG grade 2 toxicity	999 SNPs in 137 genes
Isomura [246]	2008	Japan	77 patients with grade 2 and 79 matched patients with grade 0-1 reaction	156	77	79	Case-control study	RTOG grade 2 skin toxicity	3,144 SNPs in 494 genes

First author	Year	Country	Study population	N breast	Cases	Controls	Study design	Case definition	Variants genotyped
Zhou [256]	2010	China	119 patients treated at a single hospital	119	69	50	Case series	RTOG grade 2 skin toxicity	XRCC1 -77T>C, Arg194Trp, Arg280His, Arg399Gln
Mangoni [253]	2011	Italy	87 patients BCS + RT, of whom 26 also had chemotherapy	87	8	79	Prospective cohort study	CTCAE v2.0 grade 2c skin toxicity	XRCC1, XRCC3, XPD, MSH2, MLH1, MSH3, GSTM1, GSTT1
Murray [121]	2011	UK	480 patients BCS or Mx recruited >3 years after RT from three centres	480			Retrospective cohort study	Mean acute score on CTCAE v3.0	LIG3, RAD9A, PTTG1
Raabe [205]	2012	Germany	83 patients BCS and RT recruited at single centre	83	46	47	Prospective cohort study	RTOG grade 2 skin toxicity	GSTP1, TGFB1, SOD2, XRCC1, XPD, ATM
Barnett [140]	2012	UK	1,613 patients enrolled in UK RT trials recruited into the RAPPER cohort	942			Clinical trials	STAT score	92 variants in 46 genes
Terrazzino [110]	2012	Italy	286 patients BCS + RT of whom 110 received chemo	286	89	196	Retrospective cohort study	RTOG grade 2 skin toxicity	MSH2, MSH3, XRCC1, XPD, eNOS, GSTP1, GSTA1, SOD2, TGFb1,TP53
Borghini [251]	2014	Italy	59 patients BCS + RT	59	24	35	Prospective cohort study	RTOG grade 1 skin toxicity	GSTM1, GSTT1, XRCC1, XRCC3, H2Ax
De Langhe [26]	2014	Belgium	377 patients BCS + RT from two centres, of whom 134 had chemotherapy	377	220	157	Prospective cohort study	CTCAE v3.0 grade 2 skin toxicity	XRCC3, LIG3 , MLH1; 5 SNPs based on [257]
Park [238]	2014	S. Korea	213 patients BCS + RT +/- chemo, who underwent BRCA mutation testing	213	57	156	Retrospective cohort study	RTOG grade 2 skin toxicity	BRCA 1 and 2 mutations (various)
Cordoba [252]	2016	Argentina	80 patients BCS + RT, 25 received neoadjuvant chemotherapy	80	32	48	Prospective cohort study	RTOG grade 2 skin toxicity	GSTP1, SOD2, NOS3, GSTA1
Baijer [250]	2016	France	113 patients from the French CO-HO-RT study	113	44	69	Prospective cohort study	Grade not defined	15 SNPs in TRAIL/TNFSF
Andreassen [94]	2016	UK Denmark Spain	1,357 patients including 925 from Cambridge IMRT trial; others from Danish registry & Spanish cohorts	1,357			Individual patient-level meta-analysis	STAT score	ATM rs1801516 SNP
Mumbrekar [254]	2017	India	135 patients BCS or Mx + RT, with toxicity data available for 126	126	44	38	Prospective cohort study	RTOG grade 2 skin toxicity	22 SNPs in 18 genes

Table 31. Study characteristics of 31 studies eligible for this systematic review (BCS = breast-conserving surgery, Mx = mastectomy, RT = radiotherapy). Case and control frequencies are shown for studies with a binary endpoint.

Results from individual studies

The published results from individual studies are detailed in Table 32, with any significant individual odds ratios indicated. This does not reflect the total number of genetic variants investigated but it shows the 175 variants across 31 studies, for which data was published in the literature. Where indicated, individual studies' authors were contacted successfully to obtain raw genotyping frequencies for use in the subsequent meta-analysis.

Many studies did not use specific genetic models for calculating odds ratios. Instead, odds ratios were typically calculated individually for various genotypes or combinations of genotypes, as indicated in Table 32. Interactions were not always assessed or published. The majority of associations in these individual publications remained non-significant, with the exception of *ATM* rs1801516 [94, 244], *GSTP1* rs1695 [213], *PTTG1* rs2961952 in a Japanese cohort [206], *XRCC1* rs3213235 [110, 256], *MSH2* rs2303428 [253], *LIG3* rs3744355 [121, 206], *RAD9A* rs2286620 [121, 206], *eNOS* rs1799983 [110], and *MLH1* rs1800734 [26].

First author	Year	Genotype	rs ID	Controls WT	Controls het	Controls homo	Phenotype	Cases WT	Cases het	Cases homo	OR (CI)	Genetic model	Interactions
Gaffney	1998	BRCA1 mutation (various)		n/a	11		moist desquamation	n/a	3		n/a		
Gaffney	1998	BRCA2 mutation (various)		n/a	10		moist desquamation	n/a	3		n/a		
Weissberg	1998	ATM mutation (various)		n/a	11		RTOG grade 2+	n/a	8		n/a		
Oppitz	1999	ATM 3161 C>G	rs1800057	10	1		RTOG grade 3+	8	1		n/a		
Oppitz	1999	ATM 3285 9delT		10	1		RTOG grade 3+	8	1		n/a		
Oppitz	1999	ATM 3403 15delA		10	1		RTOG grade 3+	8	1		n/a		
Oppitz	1999	ATM 7927 +23insAlu		10	1		RTOG grade 3+	8	1		n/a		
Leong	2000	BRCA1 mutation (various)		3	0	0	moist desquamation	3	0	0	n/a		
Leong	2000	BRCA2 mutation (various)		3	0	0	moist desquamation	3	0	0	n/a		
Pierce	2000	BRCA1 mutation (various)		213	54		RTOG grade 2+	pooled frequencies for carriers			n/a		
Pierce	2000	BRCA2 mutation (various)		213	17		RTOG grade 2+	pooled frequencies for carriers			n/a		
Iannuzi	2002	ATM mutation (various)		40	6		RTOG grade 2+	19	4		2.21 (0.36-13.47)	carriers vs WT	
Angele	2003	ATM 2119 T>C		248	6	0	Radiosensitive	70	0	0	n/a		adjusted for chemotherapy
Angele	2003	ATM 2572 T>C	rs1800056	243	11	0	Radiosensitive	68	2	0	0.62 (0.13-2.99)	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM 3161 C>G	rs1800057	234	20	0	Radiosensitive	66	4	0	0.63 (0.20-1.97)	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM 4148 C>T		253	1	0	Radiosensitive	69	1	0	n/a		adjusted for chemotherapy
Angele	2003	ATM 4258 C>T	rs1800058	247	7	0	Radiosensitive	67	3	0	2.43 (0.52-11.28)	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM 4473 T>C		252	2	0	Radiosensitive	69	1	0	2.13 (0.12-36.61)	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM 4578 C>T	rs1800889	232	21	1	Radiosensitive	63	7	0	0.99 (0.35-2.75)	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM 5089 A>G		253	1	0	Radiosensitive	69	1	0	n/a		adjusted for chemotherapy
Angele	2003	ATM 5557 G>A	rs1801516	192	56	6	Radiosensitive	51	15	4	**6.76 (1.19-38.43)**	**homozygotes**	adjusted for chemotherapy
Angele	2003	ATM 5558 A>T	rs1801673	247	7	0	Radiosensitive	68	2	0	0.97 (0.18-5.27)		adjusted for chemotherapy
Angele	2003	ATM IVS22-77 T>C		90	**123**	41	Radiosensitive	32	**27**	11	**0.45 (0.24-0.85)**	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM IVS38-15 G>C		252	2	0	Radiosensitive	69	1	0	2.13 (0.12-36.61)	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM IVS38-8 T>C		236	18	0	Radiosensitive	65	5	0	1.16 (0.39-3.42)	heterozygote carriers vs WT	adjusted for chemotherapy
Angele	2003	ATM IVS48+238 C>G		89	122	43	Radiosensitive	31	28	11	**0.50 (0.27-0.94)**	heterozygote carriers vs WT	adjusted for chemotherapy
Bremer	2003	ATM 1066-6 T->G		n/a	7		RTOG grade 2+	n/a	0		n/a		
Moullan	2003	XRCC1 Arg194Trp	rs1799782	219	34	1	Radiosensitive	56	13	1	1.98 (0.92-4.17)	allelic model	adjusted for age
Moullan	2003	XRCC1 Arg280His	rs25489	214	39	1	Radiosensitive	60	9	1	0.96 (0.42-2.04)	allelic model	adjusted for age
Moullan	2003	XRCC1 Arg399Gln	rs25487	109	113	32	Radiosensitive	24	37	9	1.30 (0.85-1.99)	allelic model	adjusted for age
Bremer	2004	ATM 3801delG		n/a	2		RTOG grade 2+	n/a	1		n/a		
Bremer	2005	ATM 7775 C>G		n/a	1		RTOG grade 2+	n/a	0		n/a		
Chang-Claude	2005	APE Asp148Gln	rs3136820	121	220	104	CTCAE 2c+	23	38	16	0.88 (0.52-1.49)	heterozygote carriers vs WT	**0.19 (0.06-0.56) if BMI<25 and XRCC1 399Gln carrier**
Chang-Claude	2005	XPD Asp312Asn	rs1799793	173	213	56	CTCAE 2c+	33	38	5	0.87 (0.54-1.41)	heterozygote carriers vs WT	nil significant
Chang-Claude	2005	XPD Lys751Gln	rs1052559	165	219	57	CTCAE 2c+	33	33	10	0.83 (0.40-1.70)	heterozygote carriers vs WT	nil significant
Chang-Claude	2005	XRCC1 Arg194Trp	rs1799782	396	45	2	CTCAE 2c+	70	7	0	0.77 (0.35-1.70)	heterozygote carriers vs WT	nil significant
Chang-Claude	2005	XRCC1 Arg280His	rs25489	395	48	2	CTCAE 2c+	71	5	1	0.51 (0.20-1.31)	heterozygote carriers vs WT	nil significant
Chang-Claude	2005	XRCC1 Arg399Gln	rs25487	181	204	61	CTCAE 2c+	31	36	10	0.96 (0.58-1.57)	heterozygote carriers vs WT	**0.19 (0.06-0.56) if BMI<25 and XRCC1 399Gln carrier**
Ahn	2006	CAT -329 T>C	rs1001179	233	162	22	CTCAE 2c+	43	31	1	0.95 (0.59-1.54)	heterozygote carriers vs WT	nil significant
Ahn	2006	eNOS Glu298Asp	rs1799983	187	174	65	CTCAE 2c+	33	32	9	0.64 (0.30-1.35)	heterozygote carriers vs WT	**6.39 (2.53-16.15) if WT and BMI>25**
Ahn	2006	MnSOD Ex2+24 T>C	rs1799725	113	204	111	CTCAE 2c+	24	34	17	0.71 (0.41-1.20)	heterozygote carriers vs WT	nil significant
Ahn	2006	MPO -642 G>A	rs2333227	251	133	13	CTCAE 2c+	44	22	3	1.45 (0.43-4.86)	heterozygote carriers vs WT	**3.61 (1.78-7.35) if WT and BMI>25**
Ambrosone	2006	GSTA1 -52 G>A	rs3957357	149	194	87	CTCAE 2c+	29	35	12	1.09 (0.54-1.62)	heterozygote carriers vs WT	adjusted for BMI, boost, smoking, alcohol
Ambrosone	2006	GSTM1 deletion	allelic deletion	215	213		CTCAE 2c+	36	39		1.23 (0.74-2.03)		adjusted for BMI, boost, smoking, alcohol
Ambrosone	2006	GSTP1 Ile105Val	rs1695	176	213	38	CTCAE 2c+	27	39	**10**	**2.28 (1.04-4.99)**	**homozygotes**	adjusted for BMI, boost, smoking, alcohol
Ambrosone	2006	GSTT1 deletion	allelic deletion	384	55		CTCAE 2c+	69	6		0.73 (0.29-1.66)		adjusted for BMI, boost, smoking, alcohol
Brem	2006	XRCC1 -77 T>C	rs3213235	90	107	50	Radiosensitive	24	27	15	1.10 (0.51-2.40)	homozygotes	**WT significant in combination with other XRCC1 alleles**

First author	Year	Genotype	rs ID	Controls WT	Controls het	Controls homo	Phenotype	Cases WT	Cases het	Cases homo	OR (CI)	Genetic model	Interactions
Popanda	2006	NBS1 Glu185Gln	rs1805794	196	210	39	CTCAE 2c+	36	35	6	0.93 (0.59-1.49)	heterozygote carriers vs WT	nil significant
Popanda	2006	XRCC2 Arg188His	rs3218536	387	55	3	CTCAE 2c+	71	5	1	0.60 (0.26-1.39)	heterozygote carriers vs WT	nil significant
Popanda	2006	XRCC3 Thr241Met	rs861539	156	212	76	CTCAE 2c+	21	41	14	1.33 (0.80-2.21)	heterozygote carriers vs WT	nil significant
Tan	2006	p21 Ser31Arg	rs1801270	387	55	3	CTCAE 2c+	68	8	1	1.02 (0.50-2.05)	heterozygote carriers vs WT	nil significant
Tan	2006	p53 PIN3 A2 duplication	rs17878362	326	111	8	CTCAE 2c+	60	16	1	0.79 (0.45-1.36)	heterozygote carriers vs WT	nil significant
Tan	2006	TP53 Arg72Pro	rs1042522	256	164	35	CTCAE 2c+	46	26	5	0.76 (0.47-1.22)	heterozygote carriers vs WT	nil significant
Ho	2007	ATM 378 T>A		117	14		RTOG grade 2+	42	9		3.2 (1.0-10.2)		
Ho	2007	ATM 5557 G>A	rs1801516	116	15		RTOG grade 2+	44	7		1.4 (0.5-4.2)		
Ho	2007	ATM variant (various)		80	51		RTOG grade 2+	27	24		1.7 (0.9-3.6)		
Suga	2007	ALAD 3'UTR	rs818707	342	52	5	CTCAE 2+	100	8	1	2.20 (1.14-5.74)	recessive model	
Suga	2007	BAX	rs918456	147	194	57	CTCAE 2+	30	66	13	**0.56 (0.33-0.89)**	recessive model	
Suga	2007	CD44 3'UTR C>T	rs8193	145	197	57	CTCAE 2+	27	64	18	**0.48 (0.28-0.76)**	recessive model	
Suga	2007	COMT IVS1 +2329 C>T	rs3087869	194	156	49	CTCAE 2+	52	50	7	0.95 (0.61-1.49)	recessive model	
Suga	2007	LIG3 -19314 G>C	rs3744355	207	165	27	CTCAE 2+	49	46	14	0.68 (0.43-1.06)	recessive model	
Suga	2007	MAD2L2	rs2294638	134	191	74	CTCAE 2+	48	45	16	1.87 (1.18-2.97)	recessive model	
Suga	2007	MAP3K7	rs3757244	351	48	0	CTCAE 2+	102	7	0	2.40 (1.15-7.54)	recessive model	
Suga	2007	MAT1A	rs2282367	334	62	3	CTCAE 2+	98	9	2	2.04 (1.09-4.67)	recessive model	
Suga	2007	NEIL3	rs3805169	258	127	14	CTCAE 2+	70	30	9	0.97 (0.62-1.56)	recessive model	
Suga	2007	NFE2L2	rs1806649	352	47	0	CTCAE 2+	90	19	0	**0.51 (0.27-0.99)**	recessive model	
Suga	2007	OGG1	rs2075747	188	168	43	CTCAE 2+	51	53	5	0.98 (0.62-1.52)	recessive model	
Suga	2007	PTTG1	rs2961950	181	177	41	CTCAE 2+	59	41	9	1.62 (1.04-2.54)	recessive model	
Suga	2007	PTTG1	rs2961952	162	185	52	CTCAE 2+	35	56	18	**0.61 (0.37-0.95)**	recessive model	
Suga	2007	PTTG1	rs3811999	297	97	15	CTCAE 2+	88	19	2	1.92 (1.15-3.46)	recessive model	
Suga	2007	RAD17	rs3756402	304	91	4	CTCAE 2+	91	17	1	1.83 (1.07-3.51)	recessive model	
Suga	2007	RAD9A	rs2286620	237	141	21	CTCAE 2+	76	31	2	1.85 (1.17-3.04)	recessive model	
Suga	2007	RAD9A	rs917570	290	104	5	CTCAE 2+	87	22	0	1.69 (1.03-3.03)	recessive model	
Suga	2007	REV3L	rs190246	135	196	68	CTCAE 2+	31	51	27	0.71 (0.43-1.14)	recessive model	
Suga	2007	REV3L	rs240962	121	197	81	CTCAE 2+	24	56	29	**0.56 (0.32-0.92)**	recessive model	
Suga	2007	SH3GL1	rs243336	164	175	60	CTCAE 2+	34	58	17	**0.56 (0.34-0.89)**	recessive model	
Suga	2007	SH3GL1	rs73234	180	163	48	CTCAE 2+	39	53	13	**0.60 (0.37-0.95)**	recessive model	
Suga	2007	TGFb3	rs2268622	137	186	76	CTCAE 2+	29	55	25	**0.61 (0.36-0.98)**	recessive model	
Suga	2007	TGFbR3	rs1926261	155	167	77	CTCAE 2+	35	46	28	0.67 (0.41-1.06)	recessive model	
Suga	2007	TGFbR3	rs913060	268	110	21	CTCAE 2+	65	36	8	0.63 (0.40-1.01)	recessive model	
Suga	2007	XRCC1 Arg399Gln	rs25487	327	142	19	CTCAE 2+	59	36	14	0.86 (0.54-1.34)	recessive model	
Isomura	2008	ABCA1 intron variant	rs2230806	42	111		CTCAE 2+	13	63		2.91 (1.30-6.78)		
Isomura	2008	ABCA1 intron variant	rs2253304	42	111		CTCAE 2+	13	64		3.02 (1.35-7.04)		
Isomura	2008	ABCA1 intron variant	rs2487058	41	107		CTCAE 2+	13	61		2.83 (1.26-6.67)		
Isomura	2008	IL12RB2 intron variant	rs379056	81	74		CTCAE 2+	49	28		2.50 (1.25-5.06)		
Isomura	2008	IL12RB2 intron variant	rs3790566	80	74		CTCAE 2+	48	28		2.45 (1.23-4.97)		
Isomura	2008	IL12RB2 intron variant	rs3790568	85	70		CTCAE 2+	51	26		2.52 (1.26-5.13)		
Zhou	2010	XRCC1 -77 T>C	rs3213235	74	21	7	CTCAE 2+	45	18	6	**3.66 (1.04-17.95)**	heterozygote carriers vs WT	adjusted for RT dose, age, smoking, ER+
Zhou	2010	XRCC1 Arg194Trp	rs1799782	50	42	10	CTCAE 2+	32	25	6	0.89 (0.35-2.04)	heterozygote carriers vs WT	adjusted for RT dose, age, smoking, ER+
Zhou	2010	XRCC1 Arg280His	rs25489	80	18	4	CTCAE 2+	55	12	2	0.90 (0.26-3.01)	heterozygote carriers vs WT	adjusted for RT dose, age, smoking, ER+
Zhou	2010	XRCC1 Arg399Gln	rs25487	58	34	10	CTCAE 2+	39	24	6	1.16 (0.47-3.59)	heterozygote carriers vs WT	adjusted for RT dose, age, smoking, ER+
Mangoni	2011	GSTM1 deletion	allelic deletion	42		45	CTCAE 2c+	3		5	1.24 (0.27-5.65)		
Mangoni	2011	GSTT1 deletion	allelic deletion	63		24	CTCAE 2c+	5		3	0.80 (0.15-4.34)		
Mangoni	2011	MGMT Leu84Phe	rs12917	58		29	CTCAE 2c+	6		2	0.58 (0.10-3.51)	Carriers & homozygotes vs WT	
Mangoni	2011	MLH1 Ile219Val	rs1799977	10		77	CTCAE 2c+	1		7	1.35 (0.09-19.28)	Carriers & homozygotes vs WT	

First author	Year	Genotype	rs ID	Controls WT	Controls het	Controls homo	Phenotype	Cases WT	Cases het	Cases homo	OR (CI)	Genetic model	Interactions
Mangoni	2011	MSH2 gIVS12-6 T>C	rs2303428	78		9	CTCAE 2c+	6		2	**10.92 (1.61-73.89)**	Carriers & homozygotes vs WT	
Mangoni	2011	MSH3 Ala1045Thr	rs26279	11		76	CTCAE 2c+	0		8	n/a	Carriers & homozygotes vs WT	
Mangoni	2011	XPD Asp312Asn	rs1799793	29		58	CTCAE 2c+	3		5	0.88 (0.19-4.14)	Carriers & homozygotes vs WT	
Mangoni	2011	XPD Lys751Gln	rs13181	28		59	CTCAE 2c+	4		4	0.95 (0.21-4.29)	Carriers & homozygotes vs WT	
Mangoni	2011	XRCC1 Arg194Trp	rs1799782	77		10	CTCAE 2c+	7		1	1.81 (0.19-17.08)	Carriers & homozygotes vs WT	
Mangoni	2011	XRCC1 Arg399Gln	rs25487	41		46	CTCAE 2c+	2		6	3.04 (0.58-15.9)	Carriers & homozygotes vs WT	
Mangoni	2011	XRCC3 Thr241Met	rs861539	29		58	CTCAE 2c+	0		8	n/a	Carriers & homozygotes vs WT	
Murray	2011	LIG3 +1508 3'end C>T	rs1052536	not shown			mean acute score						
Murray	2011	LIG3 +2033 exon 21	rs3744357	not shown			mean acute score						
Murray	2011	LIG3 -19314 G>C	rs3744355	329	73	5	mean acute score				**not shown p 0.046**	individual patient data obtained	
Murray	2011	PTTG1 -1993 C>T	rs3811999	not shown			mean acute score						
Murray	2011	PTTG1 -6399 A>G	rs2910190	not shown			mean acute score						
Murray	2011	PTTG1 Intron4	rs2961951	not shown			mean acute score						
Murray	2011	RAD9A +1103 of exon11	rs2286620	not shown			mean acute score				**not shown p 0.046**	individual patient data obtained	
Murray	2011	RAD9A Intron9	rs2255990	not shown			mean acute score						
Barnett	2012	ABCA1	rs2230806	309	232	35	RTOG grade 2+	180	125	32	individual patient data obtained from author		
Barnett	2012	ALAD	rs818707	445	126	9	RTOG grade 2+	274	55	7	individual patient data obtained from author		
Barnett	2012	APEX	rs1130409	166	286	128	RTOG grade 2+	78	177	81	individual patient data obtained from author		
Barnett	2012	ARTEMIS	rs35441642	480	97	4	RTOG grade 2+	295	39	3	individual patient data obtained from author		
Barnett	2012	ATM	rs11212570	461	107	9	RTOG grade 2+	279	55	2	individual patient data obtained from author		
Barnett	2012	ATM	rs11212592	379	184	18	RTOG grade 2+	224	100	12	individual patient data obtained from author		
Barnett	2012	ATM	rs17503908	462	114	5	RTOG grade 2+	266	67	4	individual patient data obtained from author		
Barnett	2012	ATM	rs1800054	566	12	0	RTOG grade 2+	330	5	0	individual patient data obtained from author		
Barnett	2012	ATM	rs1800056	566	13	0	RTOG grade 2+	326	11	0	individual patient data obtained from author		
Barnett	2012	ATM	rs1800057	548	31	0	RTOG grade 2+	311	21	0	individual patient data obtained from author		
Barnett	2012	ATM	rs1800058	559	19	1	RTOG grade 2+	319	15	0	individual patient data obtained from author		
Barnett	2012	ATM	rs1800889	525	54	1	RTOG grade 2+	308	29	0	individual patient data obtained from author		
Barnett	2012	ATM	rs227060	265	255	59	RTOG grade 2+	164	140	33	individual patient data obtained from author		
Barnett	2012	ATM	rs4986761	572	8	0	RTOG grade 2+	326	9	1	individual patient data obtained from author		
Barnett	2012	ATM	rs4987889	559	22	0	RTOG grade 2+	324	12	1	individual patient data obtained from author		
Barnett	2012	ATM	rs4988023	442	127	11	RTOG grade 2+	237	92	8	individual patient data obtained from author		
Barnett	2012	ATM	rs639923	526	46	2	RTOG grade 2+	304	29	2	individual patient data obtained from author		
Barnett	2012	ATM	rs664677	192	278	111	RTOG grade 2+	118	158	60	individual patient data obtained from author		
Barnett	2012	BAX	rs918546	130	311	139	RTOG grade 2+	92	175	69	individual patient data obtained from author		
Barnett	2012	CD44	rs8193	251	260	70	RTOG grade 2+	153	139	45	individual patient data obtained from author		
Barnett	2012	CDKN1A	rs1801270	501	80	0	RTOG grade 2+	293	39	3	individual patient data obtained from author		
Barnett	2012	eNOS	rs1799983	252	250	72	RTOG grade 2+	149	147	40	individual patient data obtained from author		
Barnett	2012	EPDR1	rs1376264	276	244	61	RTOG grade 2+	157	146	34	individual patient data obtained from author		
Barnett	2012	ERCC2	rs1052555	263	251	67	RTOG grade 2+	149	145	43	individual patient data obtained from author		
Barnett	2012	ERCC2	rs1799787	284	238	58	RTOG grade 2+	158	141	37	individual patient data obtained from author		
Barnett	2012	ERCC2	rs1799793	254	255	64	RTOG grade 2+	140	147	45	individual patient data obtained from author		
Barnett	2012	ERCC4	rs1799801	294	238	48	RTOG grade 2+	167	143	27	individual patient data obtained from author		
Barnett	2012	ERCC4	rs1800067	494	80	4	RTOG grade 2+	288	45	2	individual patient data obtained from author		
Barnett	2012	GSTP1	rs1695	241	273	66	RTOG grade 2+	136	162	38	individual patient data obtained from author		
Barnett	2012	HIF1A	rs12435848	376	184	20	RTOG grade 2+	238	86	12	individual patient data obtained from author		
Barnett	2012	HIF1A	rs2301106	435	134	10	RTOG grade 2+	265	67	4	individual patient data obtained from author		
Barnett	2012	HIF1A	rs2301111	353	198	26	RTOG grade 2+	232	94	10	individual patient data obtained from author		
Barnett	2012	HIF1A	rs2301113	352	204	25	RTOG grade 2+	222	99	16	individual patient data obtained from author		

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Barnett	2012	IL12RB2	rs3790568	524	56	0	RTOG grade 2+	313	23	0	individual patient data obtained from author		
Barnett	2012	Ku70/XRCC6	rs132788	260	248	70	RTOG grade 2+	122	170	43	individual patient data obtained from author		
Barnett	2012	KU70/XRCC6	rs2267437	189	269	122	RTOG grade 2+	119	155	63	individual patient data obtained from author		
Barnett	2012	LIG3	rs3744355	473	103	5	RTOG grade 2+	284	52	1	individual patient data obtained from author		
Barnett	2012	LIG4	rs12856974	514	59	1	RTOG grade 2+	298	35	1	individual patient data obtained from author		
Barnett	2012	LIG4	rs1805386	394	163	24	RTOG grade 2+	241	84	12	individual patient data obtained from author		
Barnett	2012	LIG4	rs1805388	408	158	15	RTOG grade 2+	231	96	10	individual patient data obtained from author		
Barnett	2012	MAD2L2	rs2294638	151	293	137	RTOG grade 2+	93	172	71	individual patient data obtained from author		
Barnett	2012	MAP3K7	rs3757244	579	1	0	RTOG grade 2+	337	0	0	individual patient data obtained from author		
Barnett	2012	MAT1A	rs2282367	283	246	52	RTOG grade 2+	149	153	33	individual patient data obtained from author		
Barnett	2012	MLH1	rs1799977	272	248	60	RTOG grade 2+	148	149	40	individual patient data obtained from author		
Barnett	2012	MLH1	rs1800734	358	185	36	RTOG grade 2+	206	116	15	individual patient data obtained from author		
Barnett	2012	MRE11	rs2155209	260	251	67	RTOG grade 2+	146	151	38	individual patient data obtained from author		
Barnett	2012	MRE11	rs569143	204	259	113	RTOG grade 2+	118	168	51	individual patient data obtained from author		
Barnett	2012	MSH2	rs2303428	460	109	2	RTOG grade 2+	270	58	7	individual patient data obtained from author		
Barnett	2012	NEIL3	rs3805169	508	70	1	RTOG grade 2+	278	53	3	individual patient data obtained from author		
Barnett	2012	NFE2L2	rs1806649	310	220	48	RTOG grade 2+	174	136	27	individual patient data obtained from author		
Barnett	2012	PAH	rs1126758	215	260	106	RTOG grade 2+	120	168	48	individual patient data obtained from author		
Barnett	2012	PRKDC	rs2213178	338	209	32	RTOG grade 2+	215	103	18	individual patient data obtained from author		
Barnett	2012	PTTG1	rs2961950	267	249	61	RTOG grade 2+	132	169	33	individual patient data obtained from author		
Barnett	2012	PTTG1	rs2961952	327	211	39	RTOG grade 2+	165	154	18	individual patient data obtained from author		
Barnett	2012	PTTG1	rs3811999	203	270	108	RTOG grade 2+	125	172	40	individual patient data obtained from author		
Barnett	2012	Rad21	rs1050838	413	145	21	RTOG grade 2+	218	109	10	individual patient data obtained from author		
Barnett	2012	RAD9A	rs2286620	500	79	2	RTOG grade 2+	298	38	1	individual patient data obtained from author		
Barnett	2012	RAD9A	rs917570	177	299	102	RTOG grade 2+	95	179	61	individual patient data obtained from author		
Barnett	2012	REV3L	rs190246	455	122	4	RTOG grade 2+	264	71	2	individual patient data obtained from author		
Barnett	2012	REV3L	rs240962	453	122	5	RTOG grade 2+	263	71	3	individual patient data obtained from author		
Barnett	2012	REV3L	rs240962	455	121	4	RTOG grade 2+	262	72	2	individual patient data obtained from author		
Barnett	2012	SART1	rs2276015	579	1	0	RTOG grade 2+	336	0	0	individual patient data obtained from author		
Barnett	2012	SH3GL1	rs73234	191	268	122	RTOG grade 2+	118	153	64	individual patient data obtained from author		
Barnett	2012	SOD2	rs4880	162	272	139	RTOG grade 2+	102	161	73	individual patient data obtained from author		
Barnett	2012	TGFB1	rs1466338	238	272	69	RTOG grade 2+	152	146	38	individual patient data obtained from author		
Barnett	2012	TGFB1	rs1466345	281	248	52	RTOG grade 2+	170	144	22	individual patient data obtained from author		
Barnett	2012	TGFB1	rs1800469	277	252	48	RTOG grade 2+	166	143	25	individual patient data obtained from author		
Barnett	2012	TGFB1	rs1982073	224	273	84	RTOG grade 2+	123	174	37	individual patient data obtained from author		
Barnett	2012	TGFB1	rs4803455	139	299	141	RTOG grade 2+	74	189	74	individual patient data obtained from author		
Barnett	2012	TGFB1	rs8110090	534	42	5	RTOG grade 2+	310	25	1	individual patient data obtained from author		
Barnett	2012	TGFB3	rs2268622	417	147	15	RTOG grade 2+	242	89	5	individual patient data obtained from author		
Barnett	2012	TGFBR3	rs1926261	337	212	32	RTOG grade 2+	197	124	15	individual patient data obtained from author		
Barnett	2012	TP53	rs1042522	336	206	39	RTOG grade 2+	193	119	24	individual patient data obtained from author		
Barnett	2012	XPC	rs2228000	311	238	27	RTOG grade 2+	180	133	23	individual patient data obtained from author		
Barnett	2012	XPC	rs2228001	203	299	79	RTOG grade 2+	140	150	47	individual patient data obtained from author		
Barnett	2012	XRCC1	rs12611088	232	275	73	RTOG grade 2+	142	150	45	individual patient data obtained from author		
Barnett	2012	XRCC1	rs1799778	238	264	74	RTOG grade 2+	142	151	44	individual patient data obtained from author		
Barnett	2012	XRCC1	rs1799782	495	81	5	RTOG grade 2+	301	35	1	individual patient data obtained from author		
Barnett	2012	XRCC1	rs2023614	494	78	8	RTOG grade 2+	300	35	2	individual patient data obtained from author		
Barnett	2012	XRCC1	rs25487	238	266	74	RTOG grade 2+	142	151	44	individual patient data obtained from author		
Barnett	2012	XRCC1	rs2854496	398	161	22	RTOG grade 2+	220	102	15	individual patient data obtained from author		

First author	Year	Genotype	rs ID	Controls WT	Controls het	Controls homo	Phenotype	Cases WT	Cases het	Cases homo	OR (CI)	Genetic model	Interactions
Barnett	2012	XRCC1	rs3213235	572	6	0	RTOG grade 2+	333	3	0	individual patient data obtained from author		
Barnett	2012	XRCC1	rs3213266	491	74	9	RTOG grade 2+	294	39	2	individual patient data obtained from author		
Barnett	2012	XRCC1	rs3213282	188	275	112	RTOG grade 2+	94	165	77	individual patient data obtained from author		
Barnett	2012	XRCC1	rs3213334	320	227	33	RTOG grade 2+	203	115	19	individual patient data obtained from author		
Barnett	2012	XRCC1 Arg280His	rs25489	540	41	0	RTOG grade 2+	295	36	5	individual patient data obtained from author		
Barnett	2012	XRCC3	rs1799794	359	195	26	RTOG grade 2+	215	104	18	individual patient data obtained from author		
Barnett	2012	XRCC3	rs3212079	507	71	3	RTOG grade 2+	280	55	1	individual patient data obtained from author		
Barnett	2012	XRCC3	rs3212090	262	256	60	RTOG grade 2+	143	150	44	individual patient data obtained from author		
Barnett	2012	XRCC3	rs3212102	545	35	0	RTOG grade 2+	324	11	0	individual patient data obtained from author		
Barnett	2012	XRCC3	rs861534	238	258	85	RTOG grade 2+	153	148	36	individual patient data obtained from author		
Barnett	2012	XRCC5	rs3835	446	127	8	RTOG grade 2+	277	58	1	individual patient data obtained from author		
Raabe	2012	ATM 5557 G>A	rs1801516	63	18	2	RTOG grade 2+	35	9	2	1.38 (0.20-10.59)		breast volume no effect
Raabe	2012	GSTP1 Ile105Val	rs1695	37	38	8	RTOG grade 2+	18	26	2	1.20 (0.24-4.35)		breast volume no effect
Raabe	2012	SOD2 Val16Ala	rs4880	excluded deviated from HWE			RTOG grade 2+				n/a		
Raabe	2012	TGFB1 C-509T	rs1800469	29	40	14	RTOG grade 2+	15	22	9	1.59 (0.42-6.24)		9.58 (1.23-104.30) if <750 cc volume
Raabe	2012	XPD Gln751Lys	rs13181	34	38	10	RTOG grade 2+	15	24	7	3.44 (0.81-16.01)		3.95 (0.91-22.75) if >750 cc volume
Raabe	2012	XRCC1 Arg399Gln	rs25487	36	33	14	RTOG grade 2+	19	20	7	1.04 (0.29-3.73)		breast volume no effect
Terrazzino	2012	eNOS 894 G>T	rs1799983	118	125	42	RTOG grade 2+	32	38	19	**2.04 (1.05-3.98)**	recessive model	
Terrazzino	2012	GSTA1 -69 C>T	rs3957356	85	136	64	RTOG grade 2+	24	41	24	1.44 (0.80-2.58)	recessive model	
Terrazzino	2012	GSTP1 Ile105Val	rs1695	132	130	23	RTOG grade 2+	47	35	7	0.96 (0.38-2.42)	recessive model	
Terrazzino	2012	MSH2 gIVS12-6 T>C	rs2303428	229	52	4	RTOG grade 2+	70	17	2	2.23 (0.30-16.09)	recessive model	
Terrazzino	2012	MSH3 Ala1045Thr	rs26279	134	122	29	RTOG grade 2+	41	36	12	1.64 (0.75-3.60)	recessive model	
Terrazzino	2012	SOD2 Val16Ala	rs4880	72	136	77	RTOG grade 2+	24	44	21	0.77 (0.43-1.38)	recessive model	
Terrazzino	2012	TGFB1 C-509T	rs1800469	123	130	32	RTOG grade 2+	43	37	9	0.85 (0.37-1.91)	recessive model	
Terrazzino	2012	TGFB1 T869C	rs1982073	112	127	46	RTOG grade 2+	35	40	14	0.96 (0.48-1.90)	recessive model	
Terrazzino	2012	TP53 Arg72Pro	rs1042522	167	106	12	RTOG grade 2+	56	31	2	0.43 (0.09-1.99)	recessive model	
Terrazzino	2012	XPD Gln751Lys	rs1052559	116	130	39	RTOG grade 2+	37	42	10	0.73 (0.34-1.57)	recessive model	
Terrazzino	2012	XRCC1 Arg194Trp	rs1799782	253	31	1	RTOG grade 2+	79	10	0	n/a	recessive model	
Terrazzino	2012	XRCC1 Arg399Gln	rs25487	113	137	35	RTOG grade 2+	35	42	12	1.17 (0.56-2.48)	recessive model	
Terrazzino	2012	XRCC1 T-77C	rs3213235	98	136	51	RTOG grade 2+	32	47	10	**0.48 (0.29-1.01)**	recessive model	
Borghini	2014	GSTM1 deletion	allelic deletion	35	24		RTOG grade 1+	10	14		**2.4 (1.1-5.3)**		**HR 2.4 (1.1-5.5) controlling for BMI**
Borghini	2014	GSTT1 deletion	allelic deletion	41	18		RTOG grade 1+	16	8		p=0.7		
Borghini	2014	H2AX 1057 T>C	rs7350	24	21	14	RTOG grade 1+	9	11	4	p=0.34		
Borghini	2014	H2AX -1420 G>A	rs8551	20	30	10	RTOG grade 1+	12	8	4	p=0.07		
Borghini	2014	XRCC1 Arg399Gln	rs25487	30	20	9	RTOG grade 1+	9	9	6	p=0.1		
Borghini	2014	XRCC3 Thr241Met	rs861539	33	21	5	RTOG grade 1+	11	12	1	p=0.14		
De Langhe	2014	LCP2 unknown location	rs4867592	not shown			CTCAE 2+	not shown					
De Langhe	2014	LIG3 -19314 G>C	rs3744355				CTCAE 2+	not shown					
De Langhe	2014	LTHA4 5' flanking T>C	rs7970524				CTCAE 2+	not shown					
De Langhe	2014	MLH1 Ile219Val	rs1800734	227	124	51	CTCAE 2+	146	60	12	**0.52 heterozygote**		
De Langhe	2014	NDUFB6	rs12003093				CTCAE 2+	not shown					
De Langhe	2014	PHLDA3	rs3888929				CTCAE 2+	not shown					
De Langhe	2014	VDR	rs4760658				CTCAE 2+	not shown					
De Langhe	2014	XRCC3 Thr241Met	rs861539				CTCAE 2+	not shown					
Park	2014	BRCA1 mutation (various)		193	20		RTOG grade 2+	53	4		0.58 (0.19-1.79)		
Park	2015	BRCA2 mutation (various)		189	24		RTOG grade 2+	48	9		1.15 (0.64-3.67)		
Andreassen	2016	ATM 5557 G>A	rs1801516	recessive model			z acute skin in upper quartile	pooled frequencies for WT & carriers vs homozygotes			**1.71 (1.11-2.66)**	homozygotes	co-variates mininmal effect

First author	Year	Genotype	rs ID	Controls WT	Controls het	Controls homo	Phenotype	Cases WT	Cases het	Cases homo	OR (CI)	Genetic model	Interactions
Baijer	2016	TRAiL	rs1131532	not shown			acute dermatitis	not shown			3.90 (0.88-17.3)		
Baijer	2016	TRAIL	rs1131535	not shown			acute dermatitis	not shown			1.58 (0.63-3.96)		
Baijer	2016	TRAIL	rs3815496	not shown			acute dermatitis	not shown			3.88 (0.87-17.22)		
Cordoba	2016	eNOS3 G894T	rs1799983	30	34	16	RTOG grade 2+	14	12	7	0.70 (0.28-1.75)		homozyotes if treated with chemo p=0.04
Cordoba	2016	GSTA1 C69T	rs3957356	15	50	14	RTOG grade 2+	5	21	7	1.71 (0.53-5.49)		
Cordoba	2016	GSTP1 A313G	rs1695	25	38	17	RTOG grade 2+	10	14	9	1.08 (0.41-2.82)		
Cordoba	2016	SOD2 T47C	rs4880	18	45	17	RTOG grade 2+	9	18	6	0.63 (0.22-1.82)		
Mumbrekar	2017	BAX	rs918546	24	40	17	RTOG grade 2+	13	18	11	0.94 (0.42-2.11)	dominant model	
Mumbrekar	2017	CD44 3'UTR C>T	rs8193	38	26	11	RTOG grade 2+	12	22	5	2.31 (1.02-5.23)	dominant model	
Mumbrekar	2017	LIG3 -19314 G>C	rs3744355	65	10	2	RTOG grade 2+	34	8	1	1.43 (0.55-3.74)	dominant model	
Mumbrekar	2017	MAD2L2	rs2294638	24	41	14	RTOG grade 2+	13	18	12	1.58 (0.57-4.41)	heterozygote carriers vs WT	
Mumbrekar	2017	MAT1A	rs2282367	54	21	2	RTOG grade 2+	35	8	0	0.59 (0.23-1.47)	heterozygote carriers vs WT	
Mumbrekar	2017	NEIL3	rs3805169	63	15	0	RTOG grade 2+	37	7	0	0.79 (0.30-2.13)	dominant model	
Mumbrekar	2017	OGG1	rs2075747	31	32	7	RTOG grade 2+	22	14	3	0.62 (0.14-2.60)	heterozygote carriers vs WT	
Mumbrekar	2017	PTTG1	rs2961950	30	36	12	RTOG grade 2+	13	21	8	1.39 (0.63-3.10)	dominant model	
Mumbrekar	2017	PTTG1	rs2961952	39	33	4	RTOG grade 2+	16	22	5	1.78 (0.83-3.82)	dominant model	
Mumbrekar	2017	PTTG1 -1993 C>T	rs3811999	35	39	3	RTOG grade 2+	23	14	4	0.65 (0.30-1.40)	dominant model	
Mumbrekar	2017	RAD9A	rs917570	58	20	1	RTOG grade 2+	29	14	1	1.43 (0.64-3.16)	dominant model	
Mumbrekar	2017	REV3L	rs190246	59	21	0	RTOG grade 2+	31	13	0	1.18 (0.52-2.67)	heterozygote carriers vs WT	
Mumbrekar	2017	REV3L	rs240962	56	20	2	RTOG grade 2+	24	14	2	1.63 (0.71-3.76)	heterozygote carriers vs WT	
Mumbrekar	2017	SH3GL1	rs243336	42	30	7	RTOG grade 2+	20	19	5	1.26 (0.47-3.36)	homozygotes	
Mumbrekar	2017	SH3GL1	rs73234	65	10	2	RTOG grade 2+	34	8	1	0.88 (0.39-1.99)	dominant model	
Mumbrekar	2017	TGFBR3	rs1926261	58	20	0	RTOG grade 2+	27	13	0	1.40 (0.61-2.81)	dominant model	
Mumbrekar	2017	XRCC1 Arg399Gln	rs25487	32	35	9	RTOG grade 2+	20	18	5	0.89 (0.26-3.03)	homozygotes	

*Table 32. Genotype frequencies and odds ratios for defined phenotype (case) for individual studies in order of publication. Significant odds ratios (OR) indicated in **bold** (WT = wild type, het = heterozygote (carrier), homo = homozygote).*

Meta-analysis

Publications grouping various BRCA mutations or ATM mutations were excluded from this part of the analysis [236-241]. In order that analysis using the allelic association test was possible, aggregate genotype frequencies by allele (e.g. AA, Aa,, aa) were obtained from the authors of two publications [121, 140], but were not available for three further publications [26, 94, 253], apart from details on the *MLH1* rs1800734 variant [26] and the *GSTM1* and *GSTT1* deletions [253], respectively. Data on the majority of patients included in the patient-based meta-analysis of *ATM* rs1801516 was available through the earlier publication by the RAPPER consortium [140].

Thus, a meta-analysis was feasible for 44 genetic variants for which data was available in ≥2 publications. Despite a relative lack of statistical heterogeneity, due to clinical heterogeneity between the different studies, a random effects model was chosen for each analysis using the Der Simonian & Laird method.

Forest plots for each of the 44 variants in the analysis and sensitivity plots, excluding one study at a time, where applicable, are shown in Figure 19, extending over several pages. The most investigated variant was *XRCC1* Arg399Gln rs25487 (9 studies, total n = 3,235 patients), followed by *XRCC1* Arg194Trp rs1799782 (5 studies, n = 2,301) and *GSTP* Ile105Val rs1695 (5 studies, n = 2,035). All three showed no association with acute toxicity.

Eight genetic markers showed a significant association ($p<0.05$) with the endpoint in meta-analysis with between two and four studies per variant (Table 33). The 'A' allele of *ATM* 5557 G>A rs1801516 (pooled OR 1.27, CI 1.02 to 1.58) and 'A' allele of *PTTG1* A>G rs2961952 (1.24, 1.05 to 1.47) were positively associated with acute skin toxicity, while the 'A' allele of *IL12RB2* intron G>A variant rs3790568 (0.70, 0.49 to 0.99), the 'T' allele of *PTTG1* -1993 C>Trs3811999(0.81, 0.68 to 0.96), and the 'T' allele of *RAD9A* 1103 C>T rs2286620 (0.73, 0.55 to 0.96) appeared to be protective.

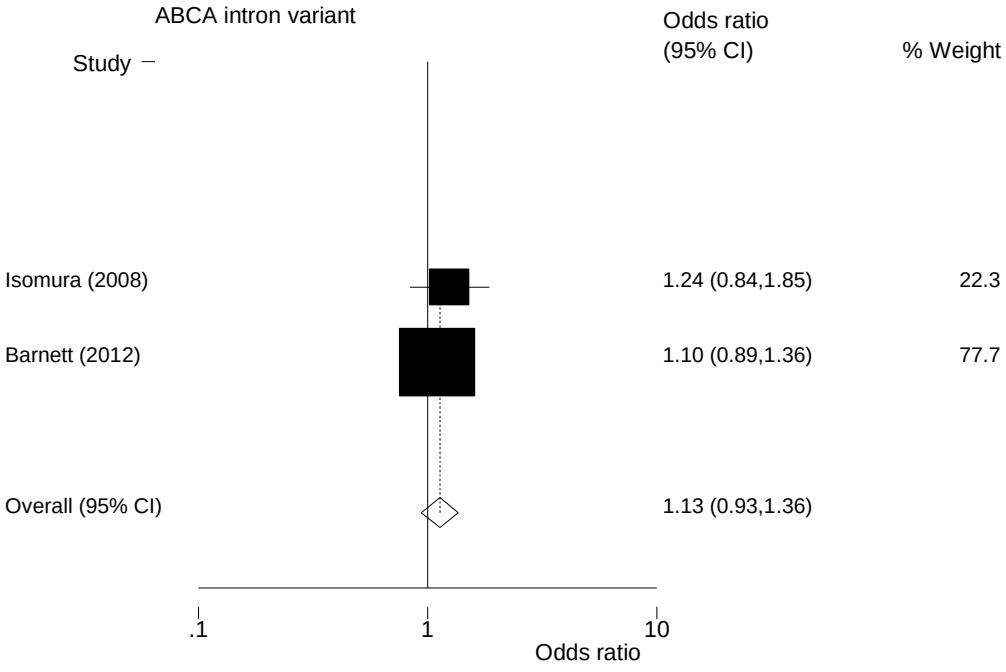
Sensitivity analyses were undertaken for markers with more than 2 publications to assess the influence of individual studies on the summary effect estimate. Only those sensitivity analyses that were significant are presented in Figure 19. By excluding data from Barnett *et al* [140], both the 'C' allele of *LIG3* -19314 G>C rs3744355 (1.38, 1.07 to 1.80) and the 'T' allele of *REV3L* intron C>T rs240962 (1.38, 1.05 to 1.82) show a significant association, while the exclusion of Mumbrekar *et al* [254] renders the association with the 'C' allele of *NEIL3* intron C>T variant rs3805169 significant (1.31, 1.02 to 1.69). There were seven studies which deviated from HWE in the control group for at least one genetic variant (not shown) [26, 206, 213, 246, 249, 252, 256]. On sensitivity analysis, this did not affect the meta-analysis results for any of those markers.

Variant	Studies (n)	Patients (n)	Allele frequencies				pooled allelic OR	CI upper	CI lower	p-value
			Controls		Cases					
			A (n)	a (n)	A (n)	a (n)				
ATM 5557 G>A rs1801516	4	1,552	1,842	254	857	151	1.27	1.02	1.58	0.036
IL12RB intron G>A rs3790568	2	1,148	1,344	126	777	49	0.70	0.49	0.99	0.044
LIG3 -19314 G>C rs3744355*	3	1,112	1,450	316	352	106	1.38	1.07	1.80	0.014
NEIL3 intron T>C rs3805169**	3	1,53	1,870	242	860	114	1.31	1.02	1.69	0.038
PTTG1 intron G>A rs2961952	3	1,541	1,485	619	664	314	1.24	1.05	1.47	0.012
PTTG1 -1993 C>T rs3811999	3	1,544	1,476	658	677	297	0.81	0.68	0.96	0.015
RAD9A 1103 C>T rs2286620	2	1,426	1,694	266	817	75	0.73	0.55	0.96	0.025
REV3L intron C>T rs240962*	2	626	571	383	166	132	1.38	1.05	1.82	0.022

Table 33. Genetic variants with significant association on meta-analysis (exluding Barnett et al [140], **excluding Mumbrekar et al [254]). A/a denote the reference and variant allele, respectively. Significance level set at 0.05. OR = odds ratio, CI = confidence interval.*

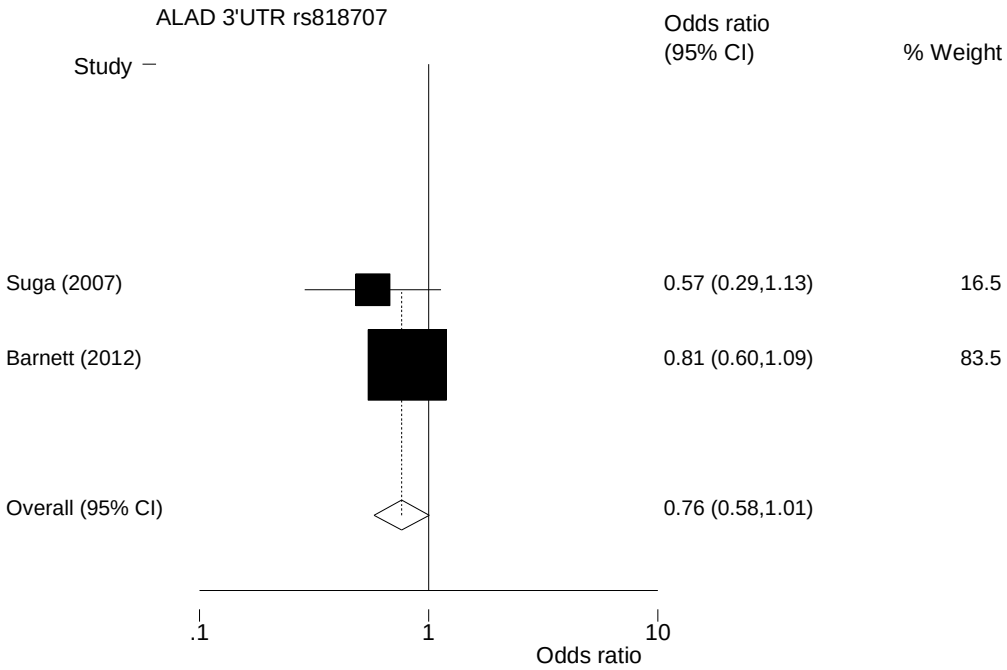
Figure 19. Forest plots for 44 genetic variants included in the meta-analysis. Significant plots are indicated in bold. Sensitivity plots are also shown for LIG3 -19314 G>C rs3744355, NEIL3 rs3805169, and REV3L rs240962.

ABCA Arg219Lys G>A rs2230806

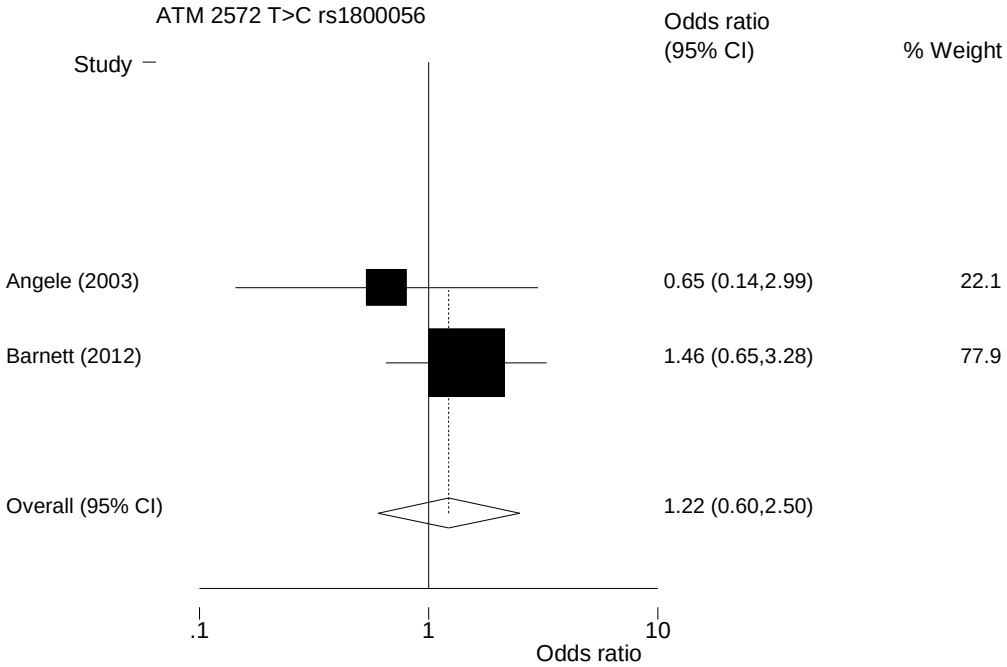


Heterogeneity chi-squared = 0.30 (d.f. = 1) p = 0.586
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 1.26 p = 0.209

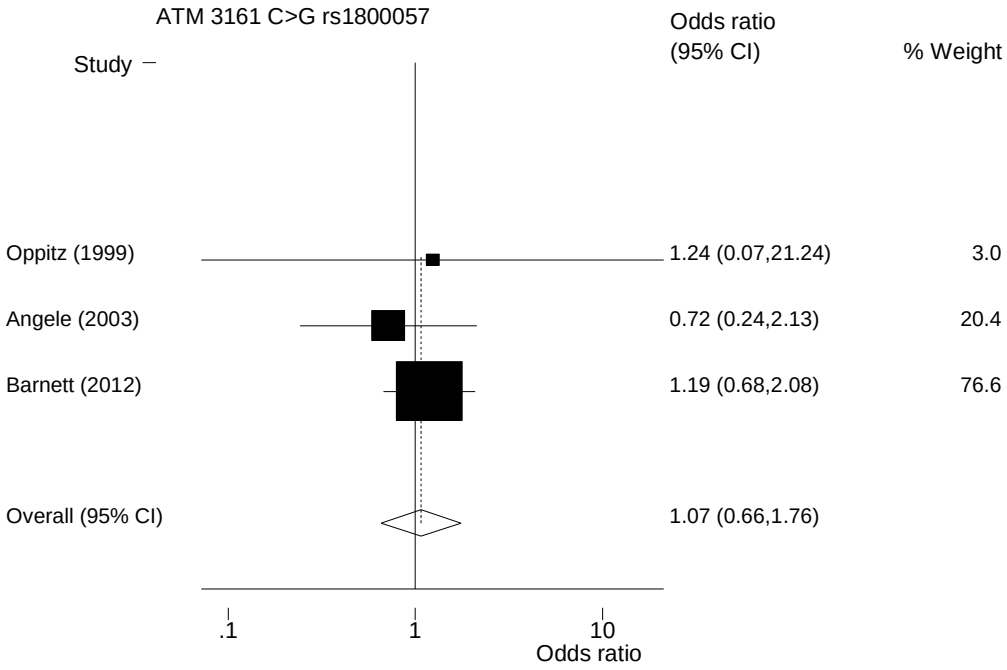
ALAD 3’UTR G>A rs818707



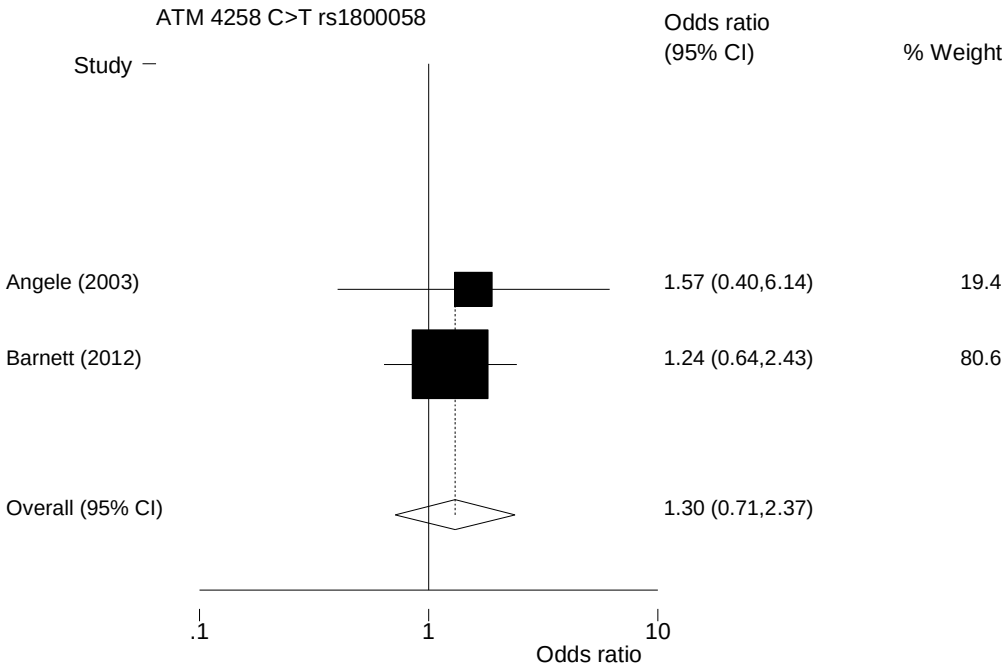
Heterogeneity chi-squared = 0.82 (d.f. = 1) p = 0.365Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 1.91 p = 0.056



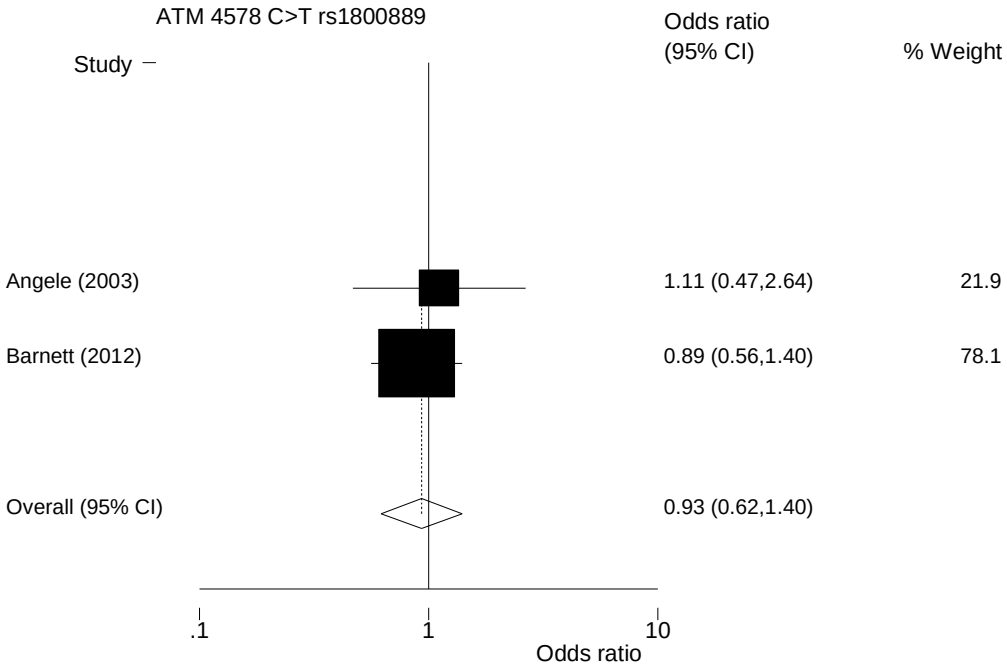
Heterogeneity chi-squared = 0.84 (d.f. = 1) p = 0.360
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.55 p = 0.579



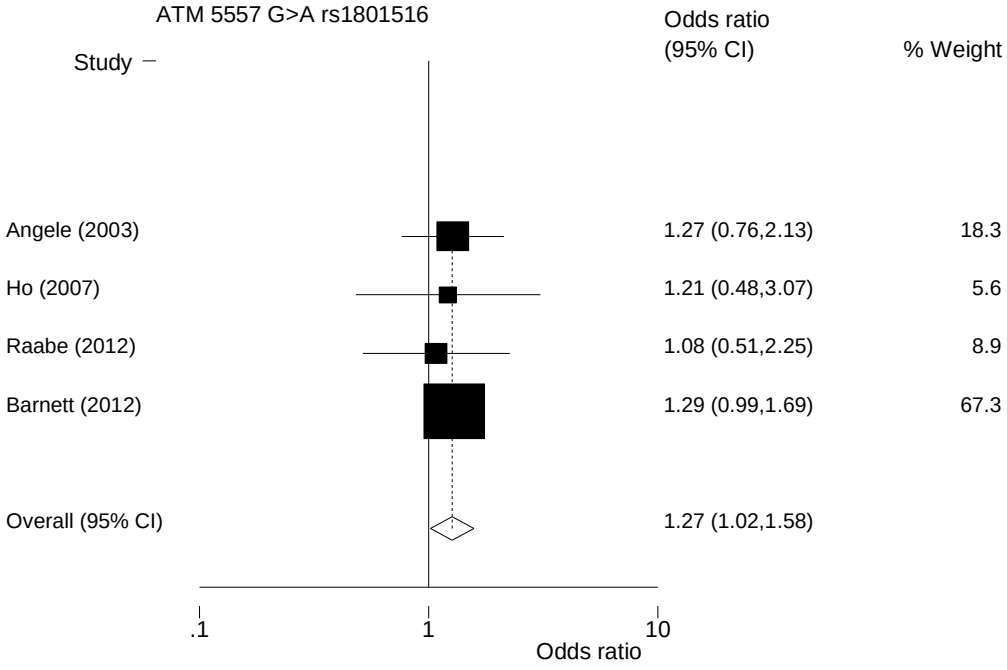
Heterogeneity chi-squared = 0.66 (d.f. = 2) p = 0.720
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.28 p = 0.780



Heterogeneity chi-squared = 0.09 (d.f. = 1) p = 0.766
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.86 p = 0.391

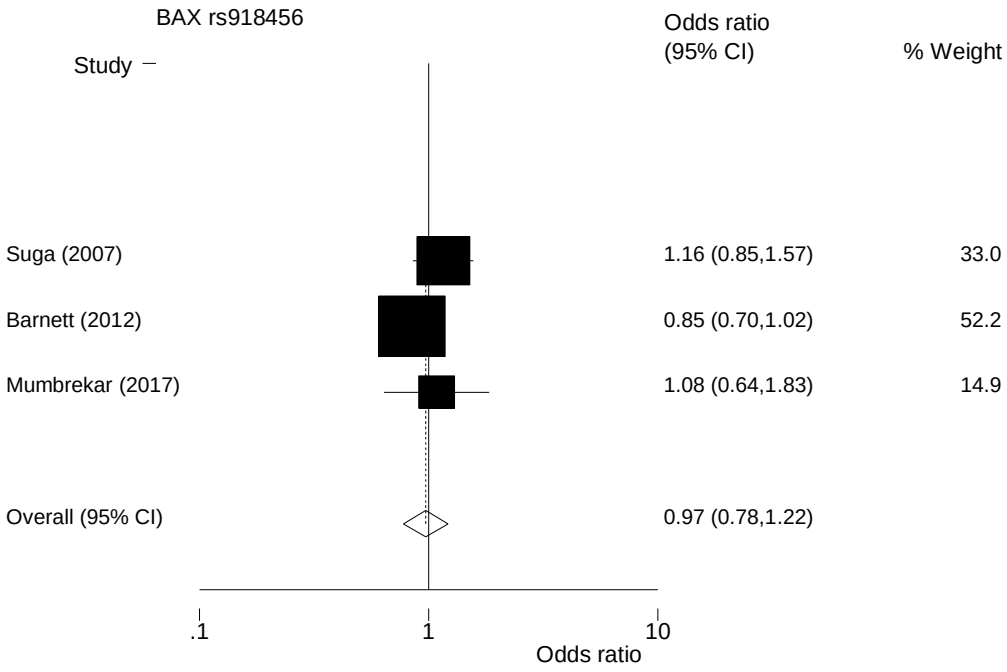


Heterogeneity chi-squared = 0.20 (d.f. = 1) p = 0.653
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.35 p = 0.730

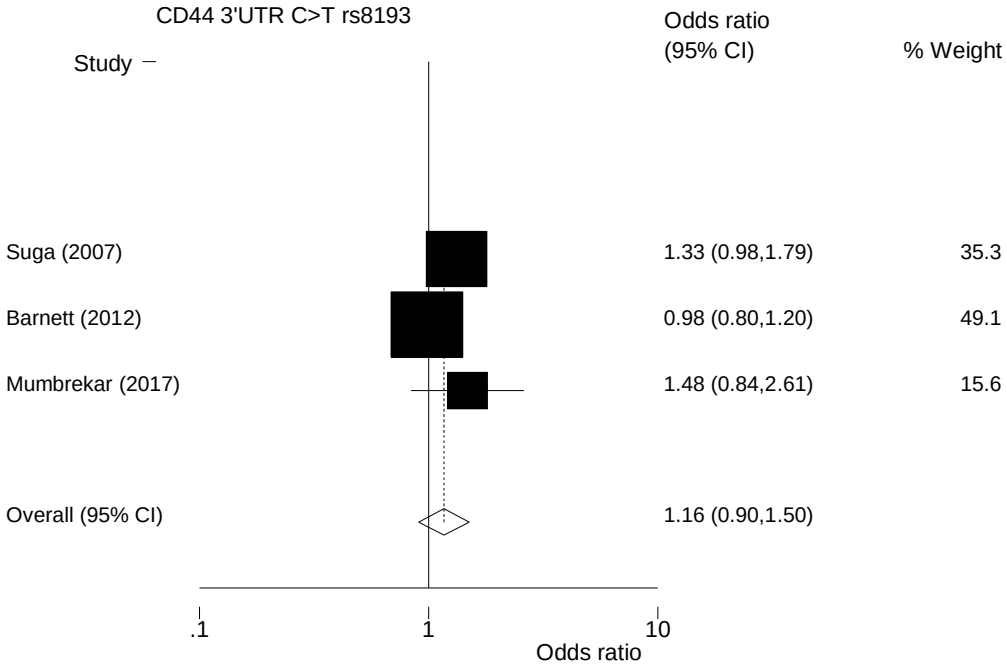


Heterogeneity chi-squared = 0.22 (d.f. = 3) p = 0.974
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 2.09 p = 0.036

BAX g12844 T>G rs918456

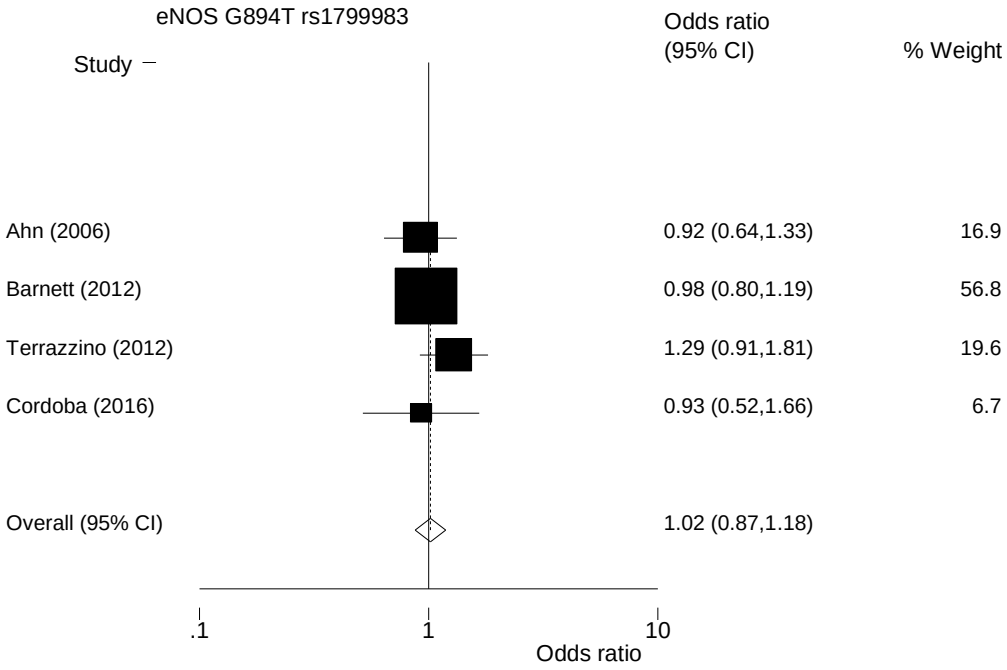


Heterogeneity chi-squared = 3.25 (d.f. = 2) p = 0.197
Estimate of between-study variance Tau-squared = 0.0157
Test of OR=1 : z= 0.25 p = 0.806



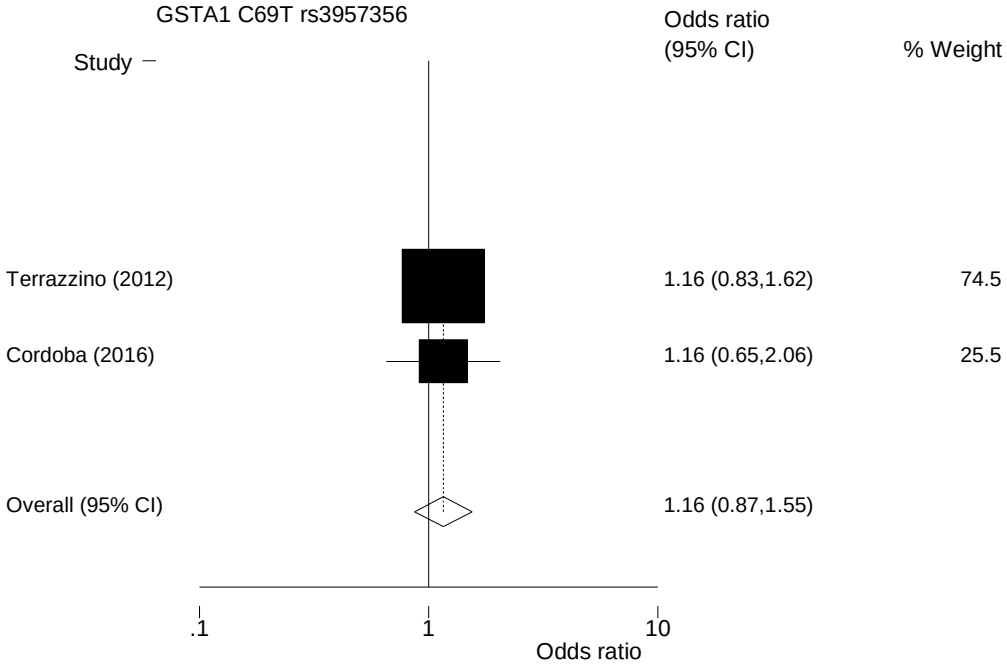
Heterogeneity chi-squared = 3.80 (d.f. = 2) p = 0.150
Estimate of between-study variance Tau-squared = 0.0237
Test of OR=1 : z= 1.17 p = 0.243

eNOS c.894 G>T rs1799983



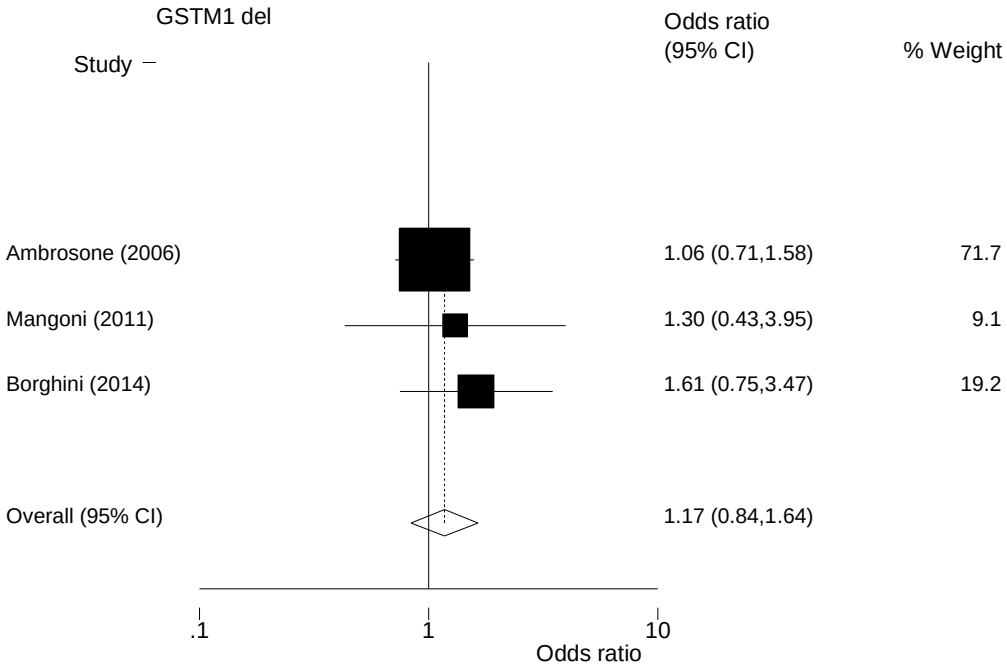
Heterogeneity chi-squared = 2.36 (d.f. = 3) p = 0.501
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.21 p = 0.832

GSTA -69 C>T rs1799983



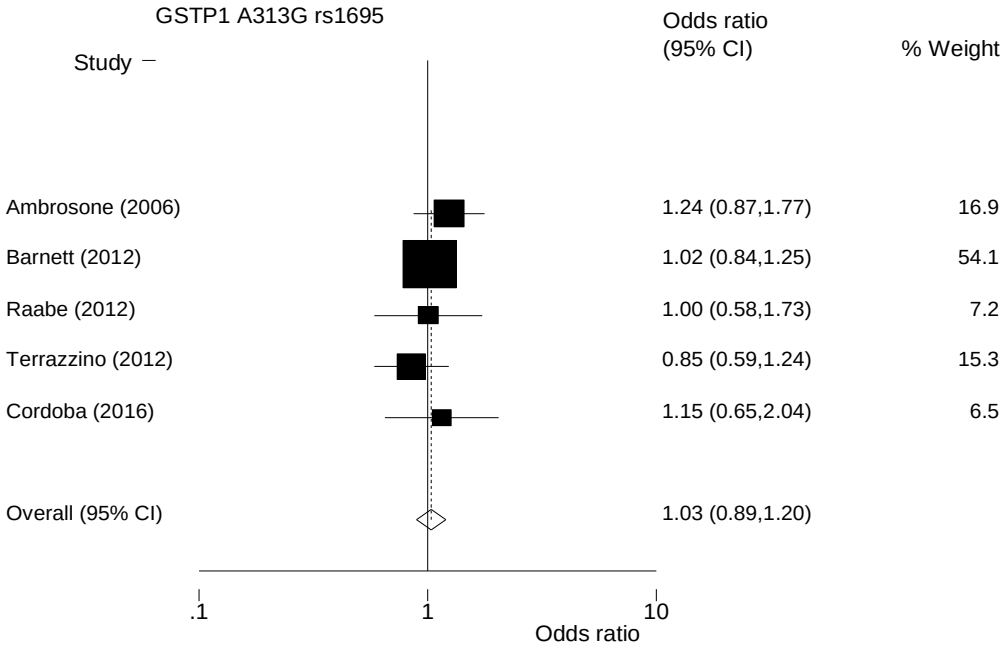
Heterogeneity chi-squared = 0.00 (d.f. = 1) p = 0.998
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.99 p = 0.320

GSTM1 wt/null



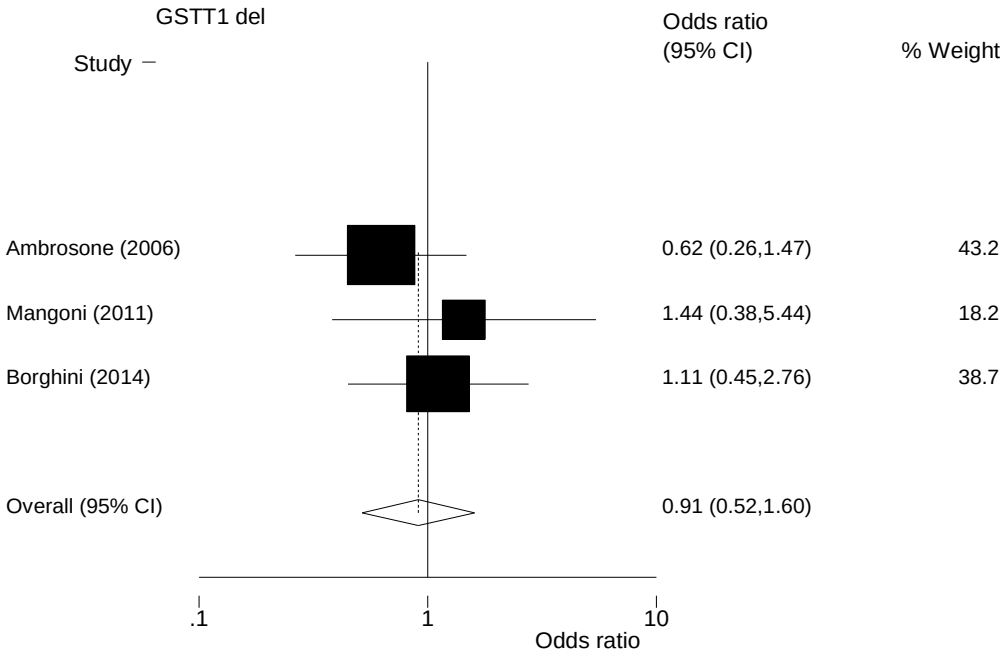
Heterogeneity chi-squared = 0.94 (d.f. = 2) p = 0.624
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.92 p = 0.356

GSTP c.313 A>G rs1695



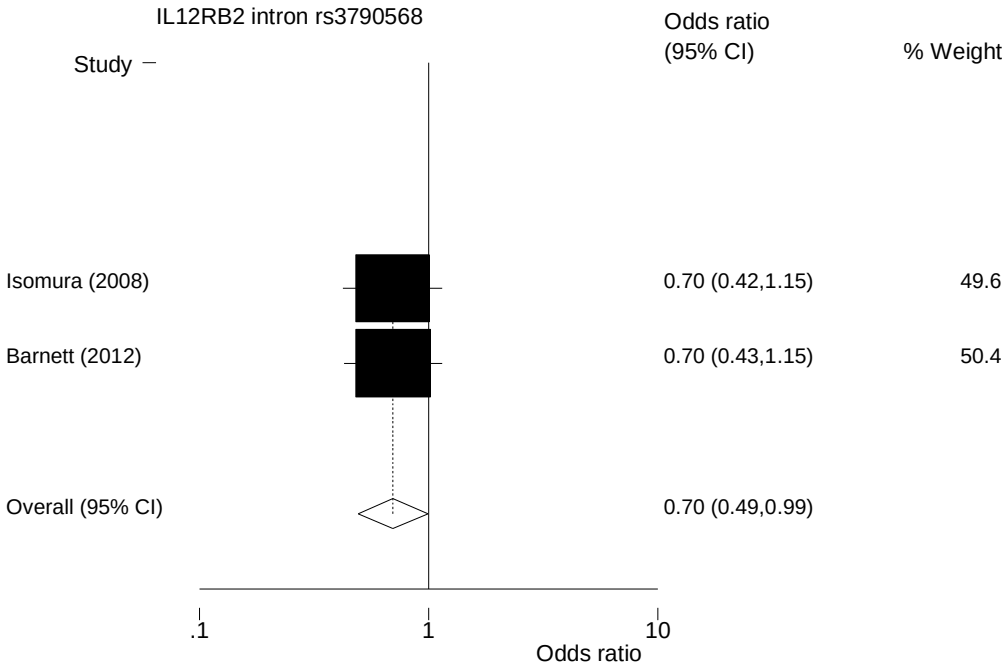
Heterogeneity chi-squared = 2.21 (d.f. = 4) p = 0.697
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.44 p = 0.660

GSTT1 wt/null

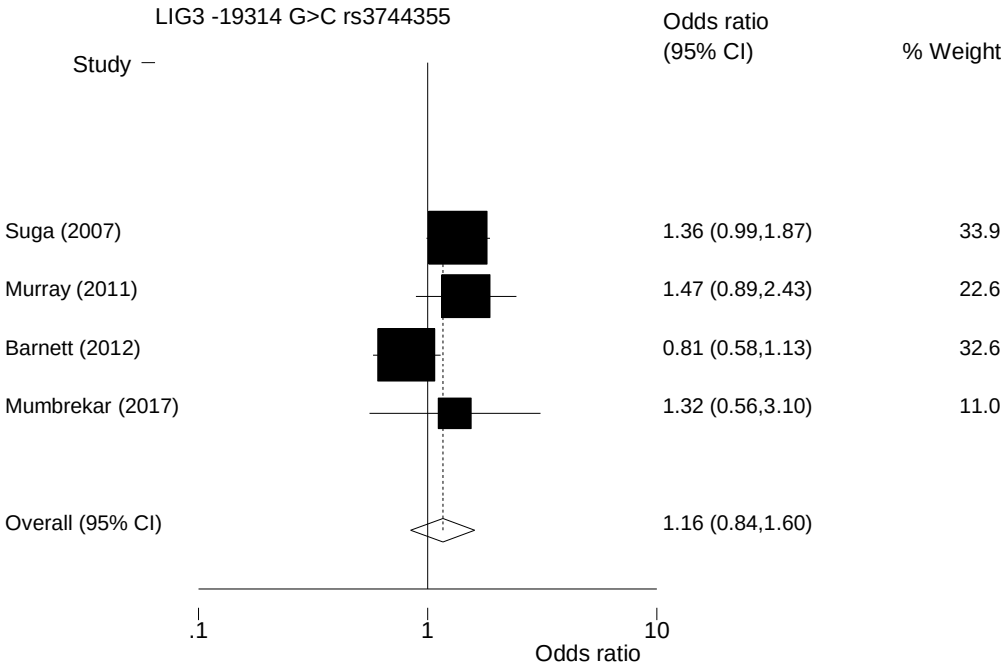


Heterogeneity chi-squared = 1.39 (d.f. = 2) p = 0.500
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.33 p = 0.738

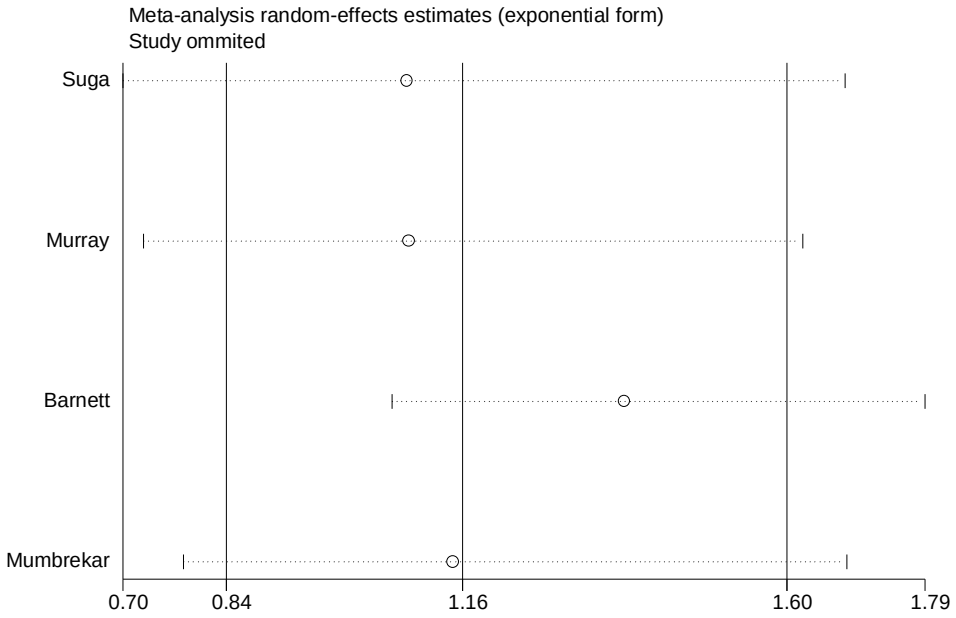
IL12RB2 intron G>A rs3790568



Heterogeneity chi-squared = 0.00 (d.f. = 1) p = 0.993
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 2.01 p = 0.044

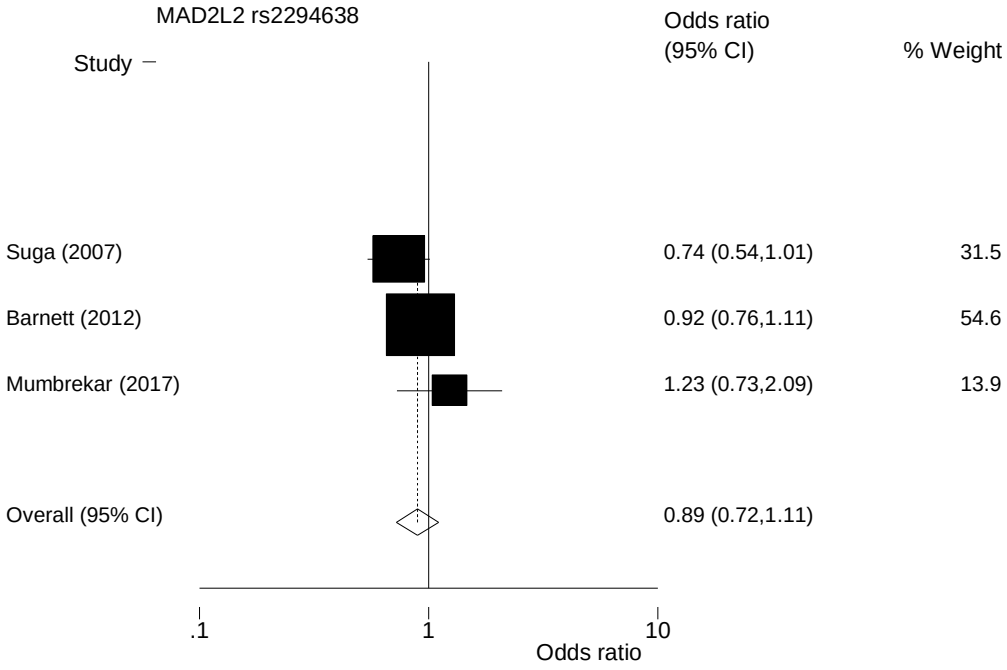


Heterogeneity chi-squared = 6.16 (d.f. = 3) p = 0.104
Estimate of between-study variance Tau-squared = 0.0521
Test of OR=1 : z= 0.93 p = 0.355



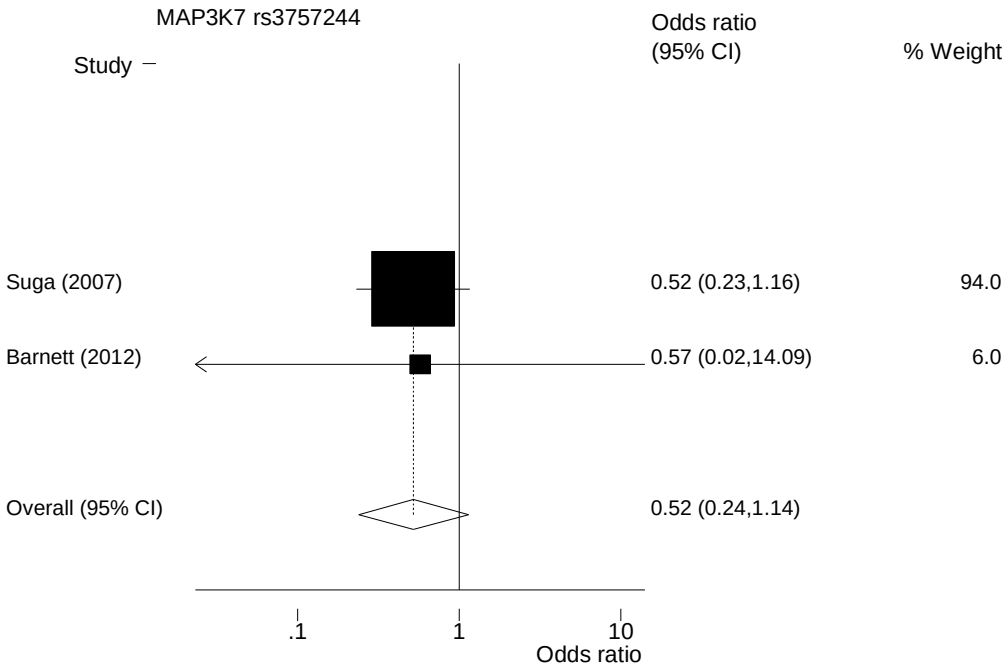
Sensitivity analysis for LIG3 -19314 G>C rs3744355 (excluding Barnett 2012), see 3rd line.
Heterogeneity chi-squared = 0.08 (d.f. = 2) p = 0.962
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 2.46 p = 0.014

MAD2L2 intron G>A rs2282367



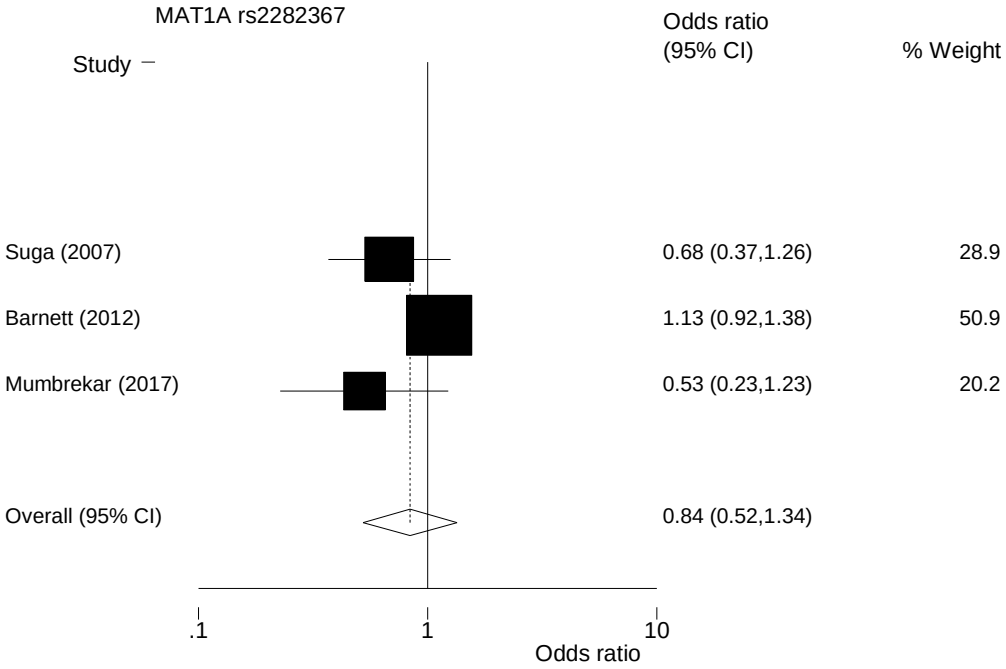
Heterogeneity chi-squared = 2.94 (d.f. = 2) p = 0.230
Estimate of between-study variance Tau-squared = 0.0120
Test of OR=1 : z= 1.03 p = 0.303

MAP3K7 G>A rs3757244

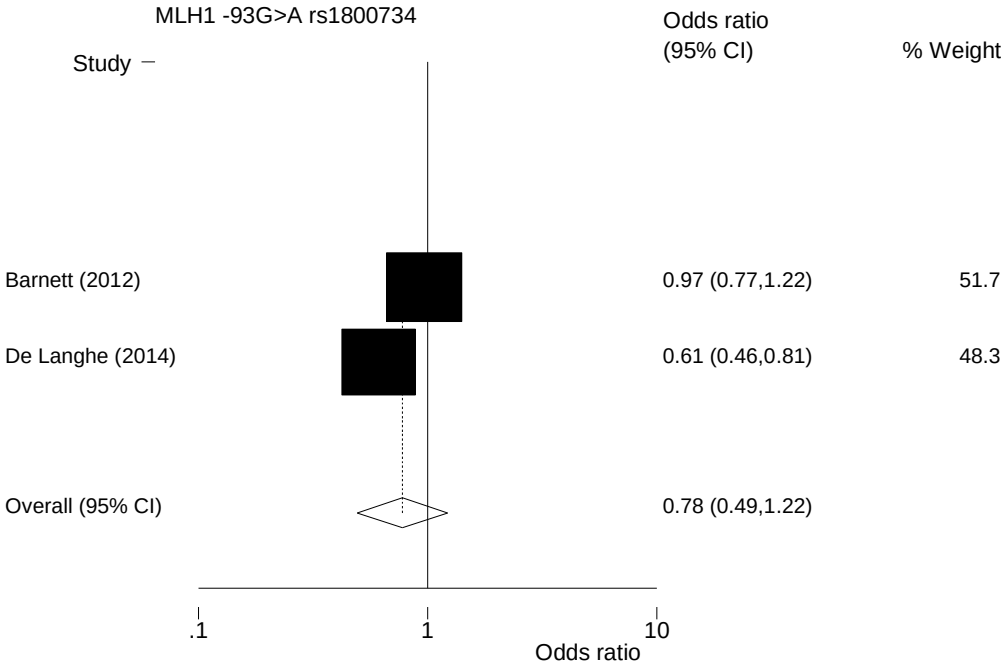


Heterogeneity chi-squared = 0.00 (d.f. = 1) p = 0.953
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 1.63 p = 0.103

MAT1A intron G>A rs2282367

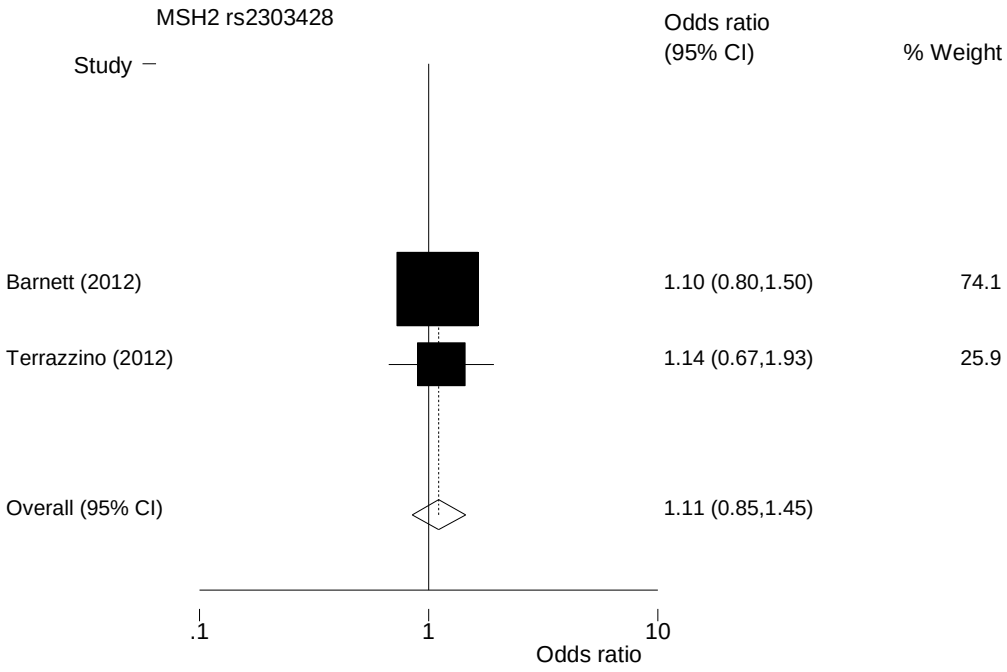


Heterogeneity chi-squared = 2.33 (d.f. = 1) p = 0.087
Estimate of between-study variance Tau-squared = 0.1039
Test of OR=1 : z= 0.20 p = 0.459



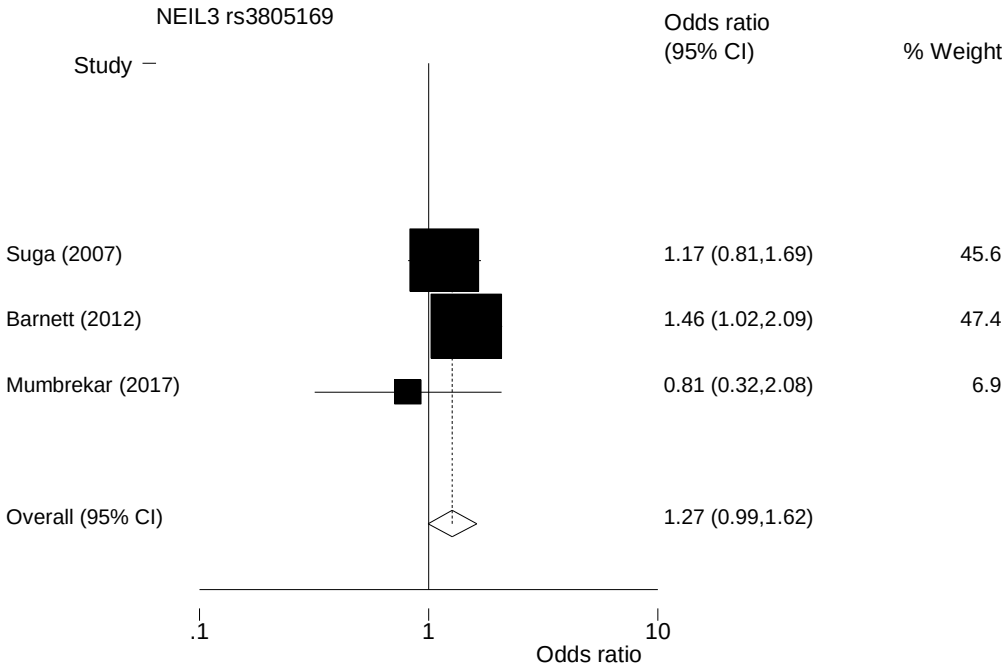
Heterogeneity chi-squared = 6.18 (d.f. = 1) p = 0.013
Estimate of between-study variance Tau-squared = 0.0897
Test of OR=1 : z= 1.10 p = 0.271

MSH2 intron T>C rs2302428

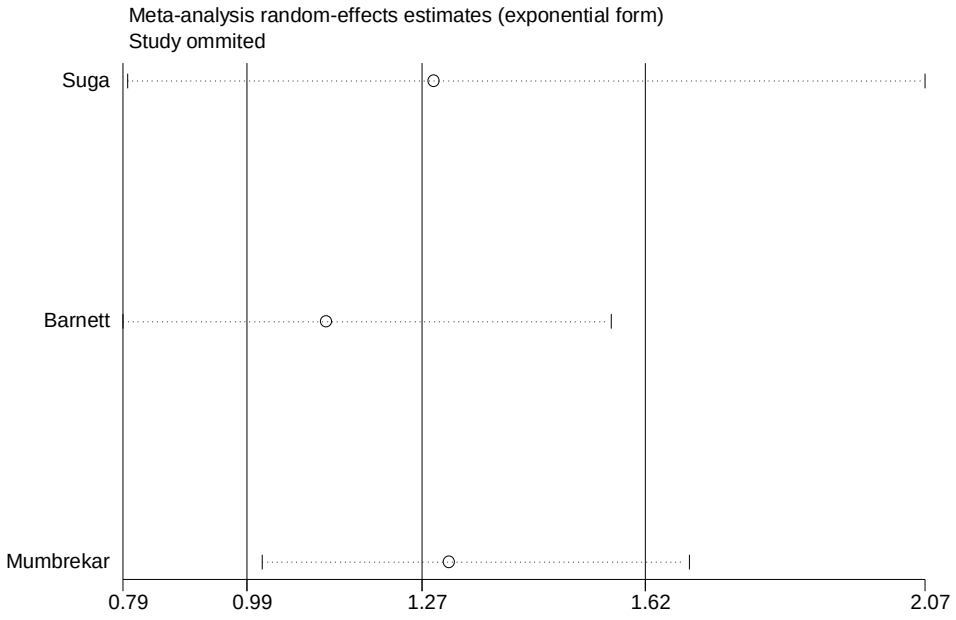


Heterogeneity chi-squared = 0.01 (d.f. = 1) p = 0.908
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.74 p = 0.460

NEIL3 intron T>C rs3805169



Heterogeneity chi-squared = 1.65 (d.f. = 2) p = 0.438
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 1.89 p = 0.059



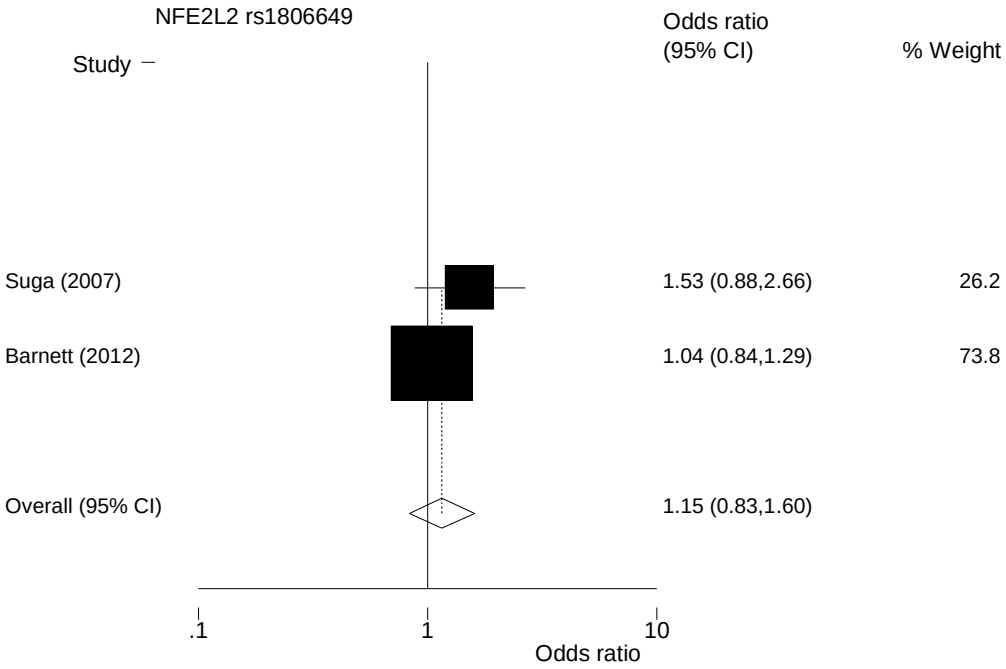
Sensitivity analysis for NEIL3 rs3805169 (excluding Mumbrekar 2017), see 3rd line.

Heterogeneity chi-squared = 0.72 (d.f. = 1) p = 0.397

Estimate of between-study variance Tau-squared = 0.0000

Test of OR=1 : z= 2.08 p = 0.038

NFE2L2 intron C>T rs1806649

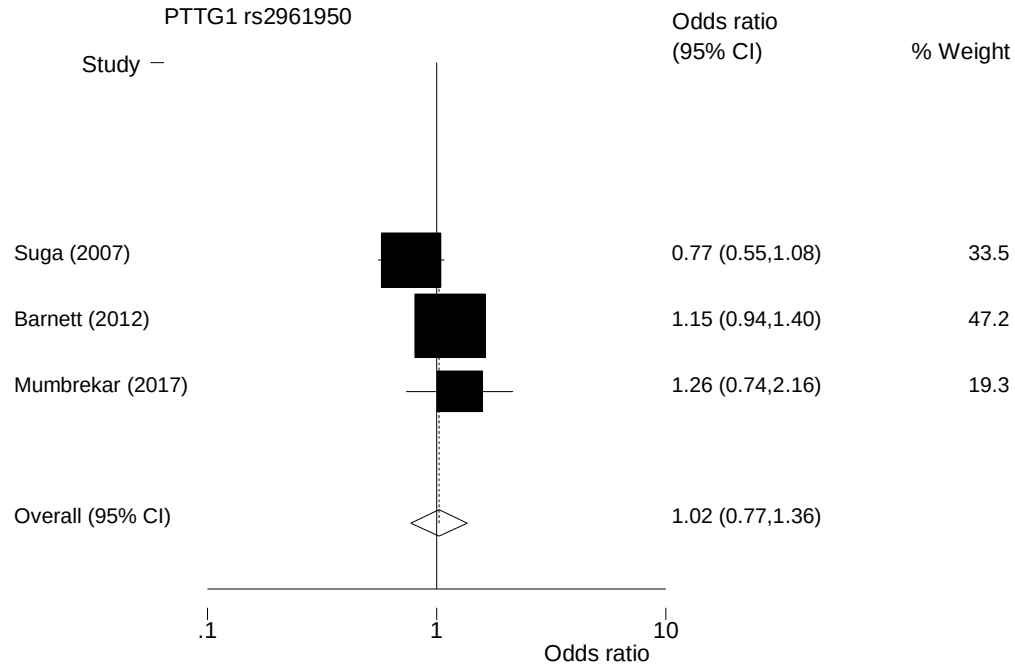


Heterogeneity chi-squared = 1.57 (d.f. = 1) p = 0.210

Estimate of between-study variance Tau-squared = 0.0261

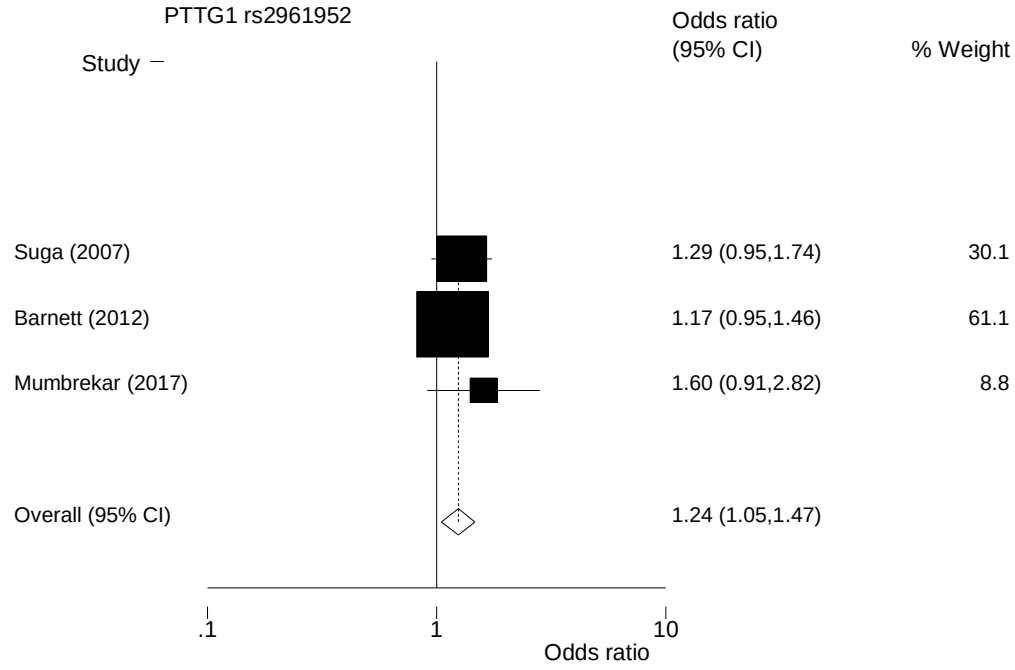
Test of OR=1 : z= 0.85 p = 0.395

PTTG intron G>A rs2961950



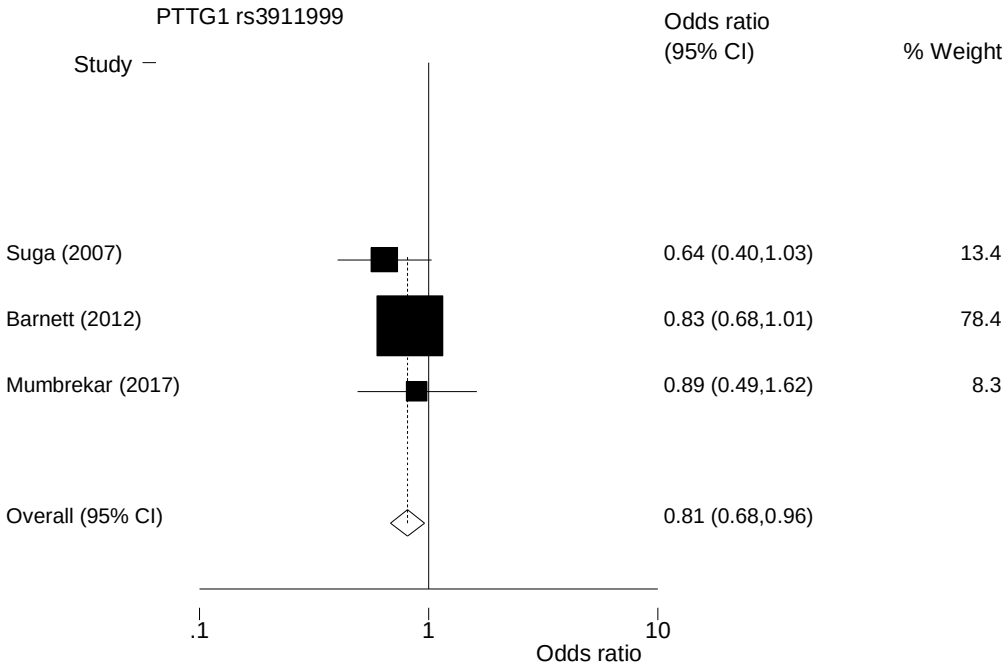
Heterogeneity chi-squared = 4.42 (d.f. = 2) p = 0.110
Estimate of between-study variance Tau-squared = 0.0345
Test of OR=1 : z= 0.15 p = 0.879

PTTG intron G>A rs2961952



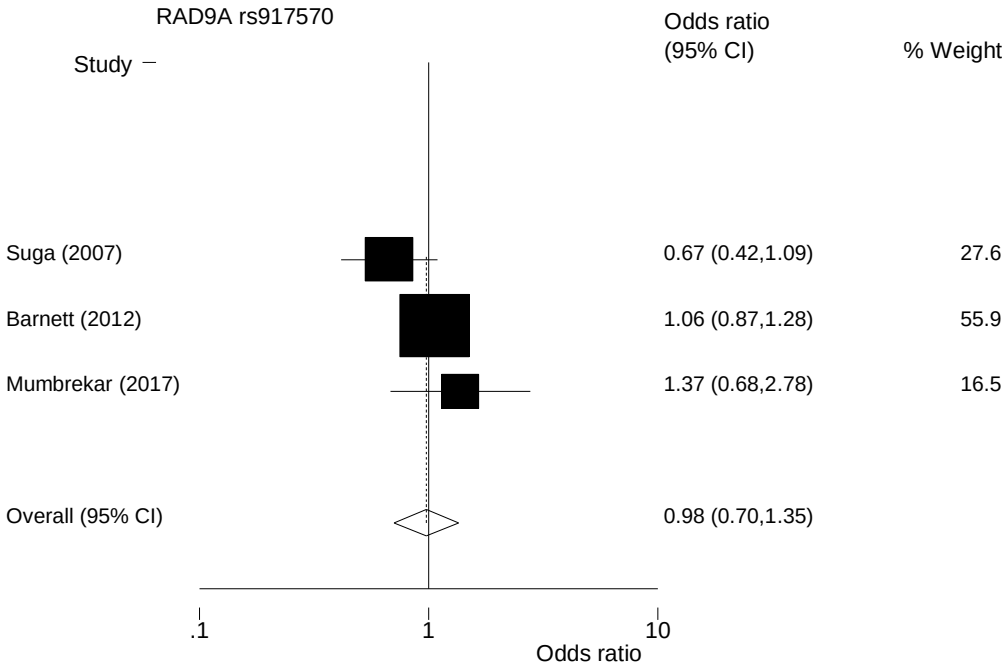
Heterogeneity chi-squared = 1.10 (d.f. = 2) p = 0.578
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 2.53 p = 0.012

PTTG -1993 C>T rs3811999



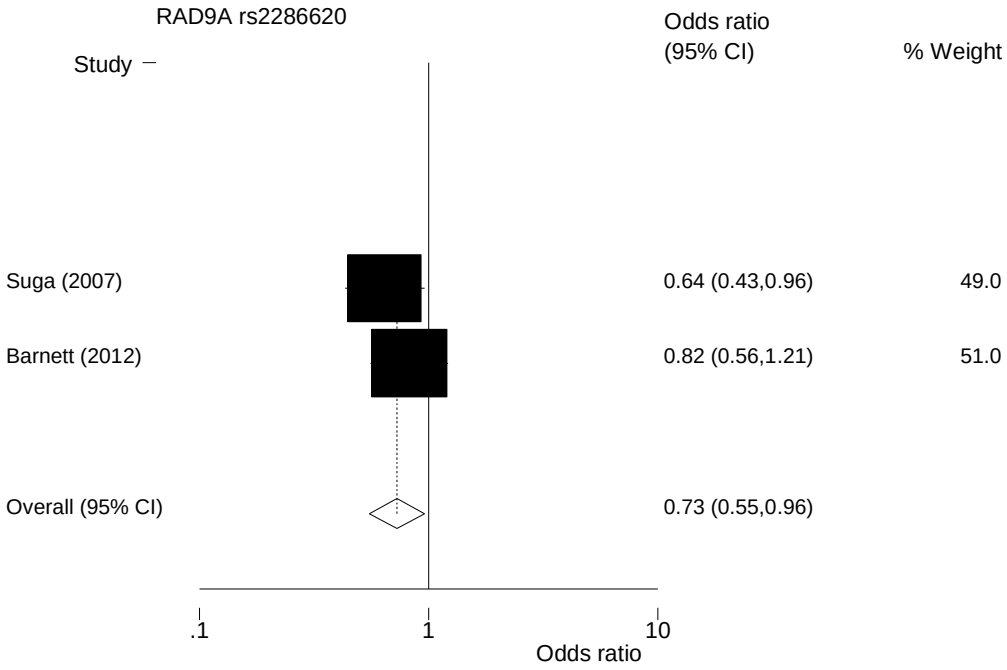
Heterogeneity chi-squared = 1.09 (d.f. = 2) p = 0.580
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 2.44 p = 0.015

RAD9A 1103 C>T



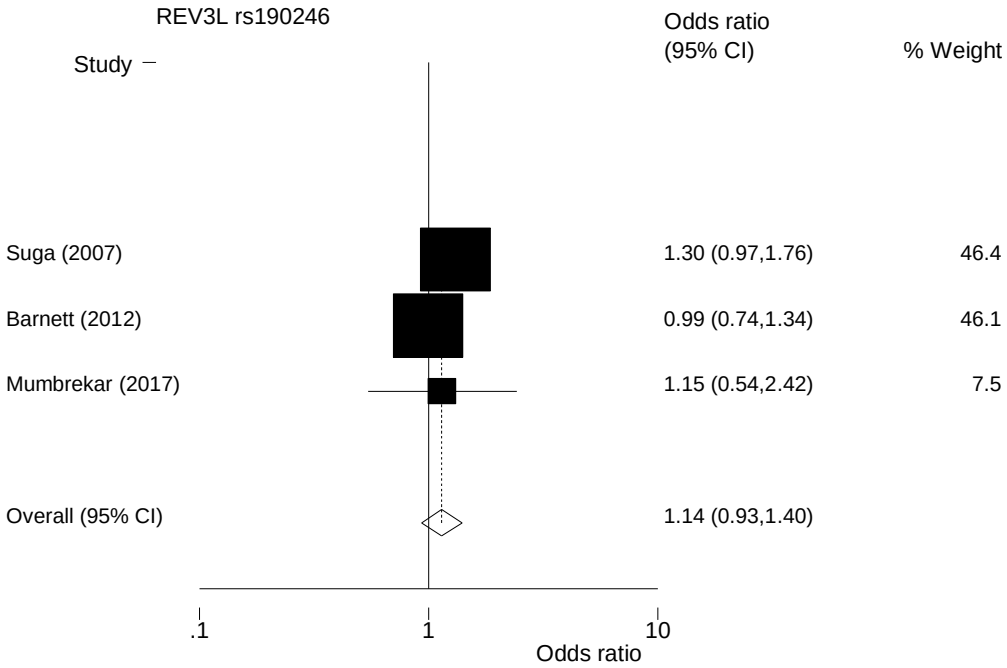
Heterogeneity chi-squared = 3.67 (d.f. = 2) p = 0.160
Estimate of between-study variance Tau-squared = 0.0403
Test of OR=1 : z= 0.15 p = 0.882

RAD9A intron C>T rs2286620



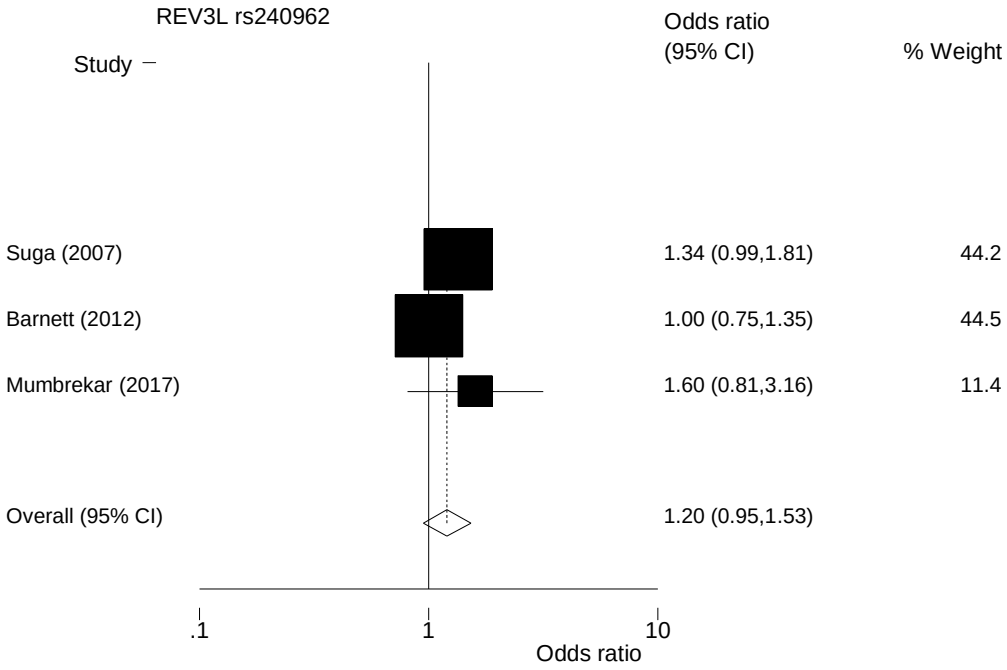
Heterogeneity chi-squared = 0.74 (d.f. = 1) p = 0.391
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 2.24 p = 0.025

REV3L intron G>T rs190246

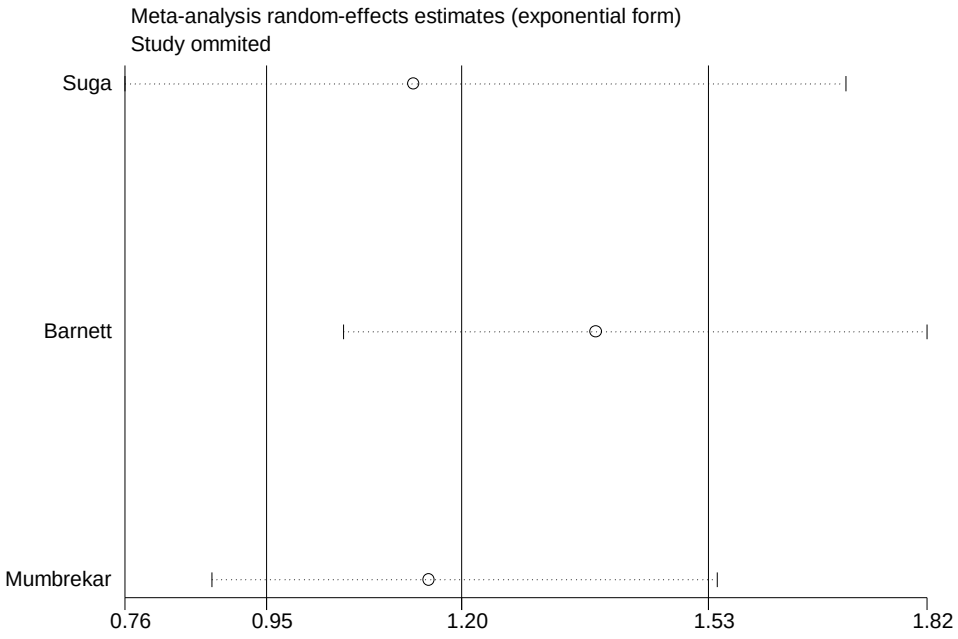


Heterogeneity chi-squared = 1.56 (d.f. = 2) p = 0.457
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 1.25 p = 0.211

REV3L intron C>T rs240962

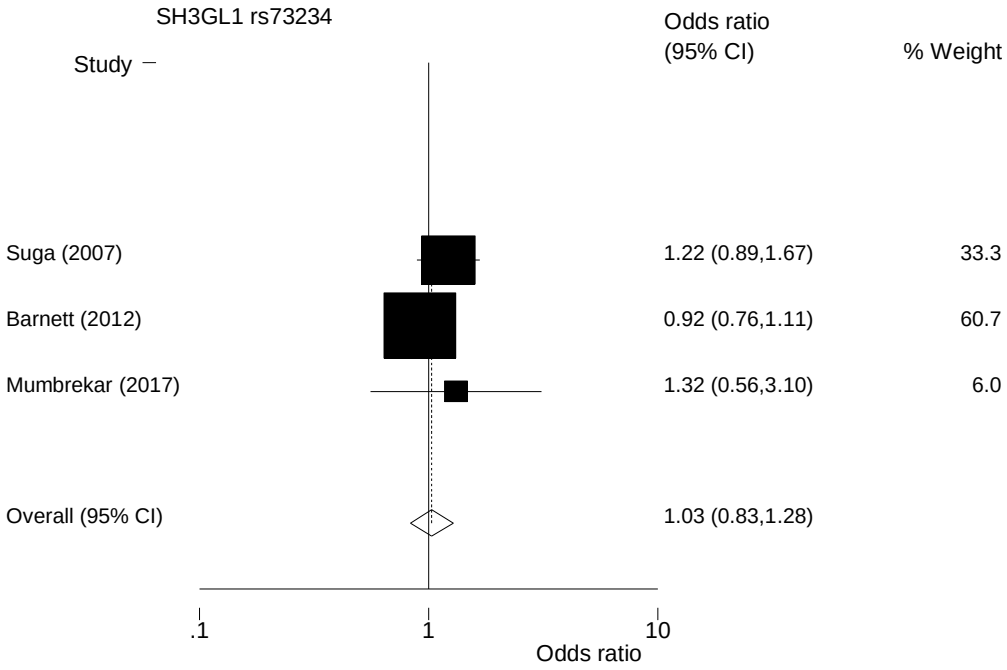


Heterogeneity chi-squared = 2.56 (d.f. = 2) p = 0.279
Estimate of between-study variance Tau-squared = 0.0103
Test of OR=1 : z= 1.51 p = 0.131



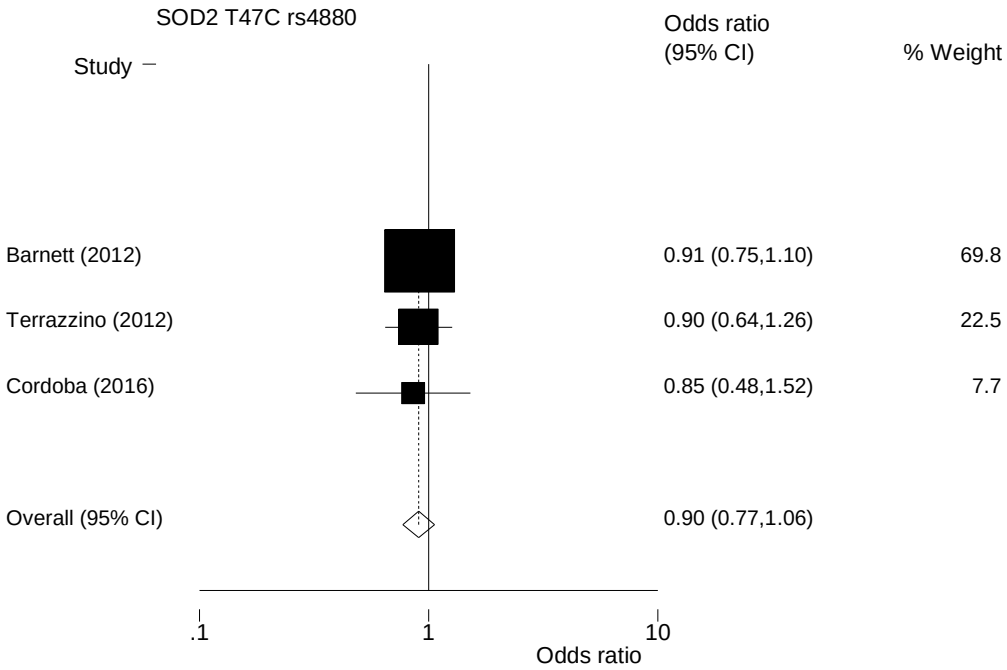
Sensitivity analysis for REV3L rs240962 (excluding Barnett 2012), see 2nd line.
Heterogeneity chi-squared = 0.21 (d.f. = 1) p = 0.645
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 2.29 p = 0.022

SH3GL1 intron G>C rs73234



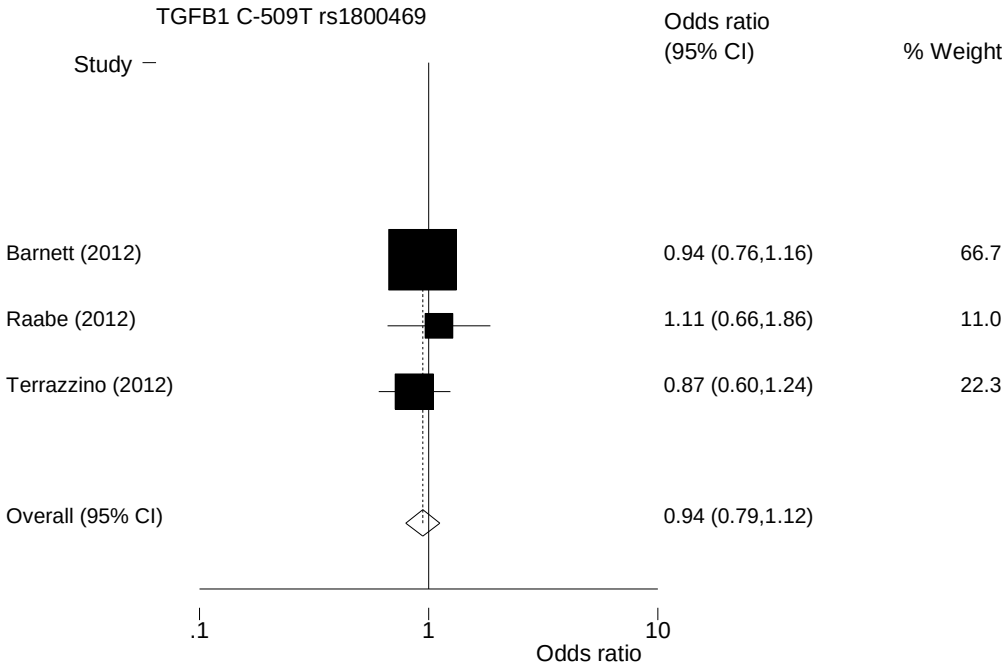
Heterogeneity chi-squared = 2.66 (d.f. = 2) p = 0.264
Estimate of between-study variance Tau-squared = 0.0103
Test of OR=1 : z= 0.27 p = 0.789

SOD2 47 T>C rs4880



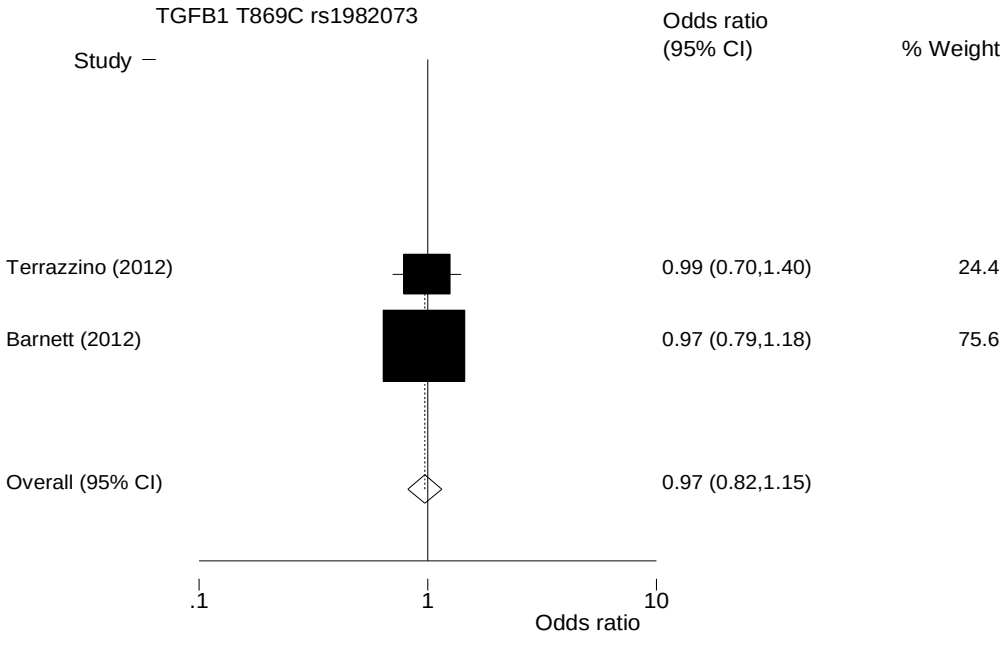
Heterogeneity chi-squared = 0.04 (d.f. = 2) p = 0.978
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 1.23 p = 0.220

TGFb1 -509 C>T rs1800469



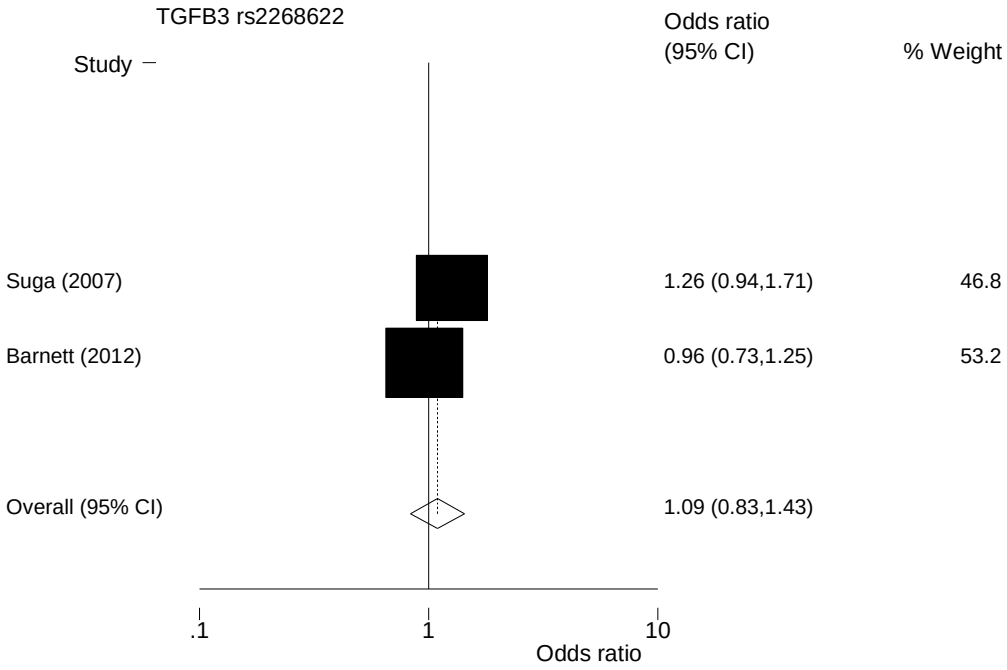
Heterogeneity chi-squared = 0.59 (d.f. = 2) p = 0.746
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.70 p = 0.484

TGFb1 869 T>C rs1982073



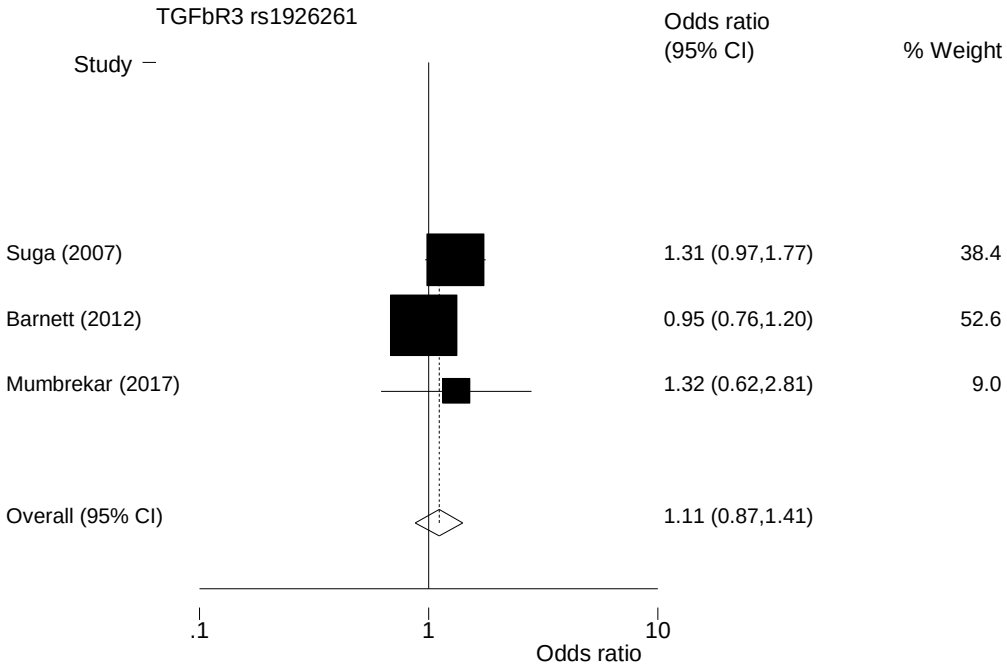
Heterogeneity chi-squared = 0.02 (d.f. = 1) p = 0.898
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.33 p = 0.740

TGFb3 intron T>C rs2268622



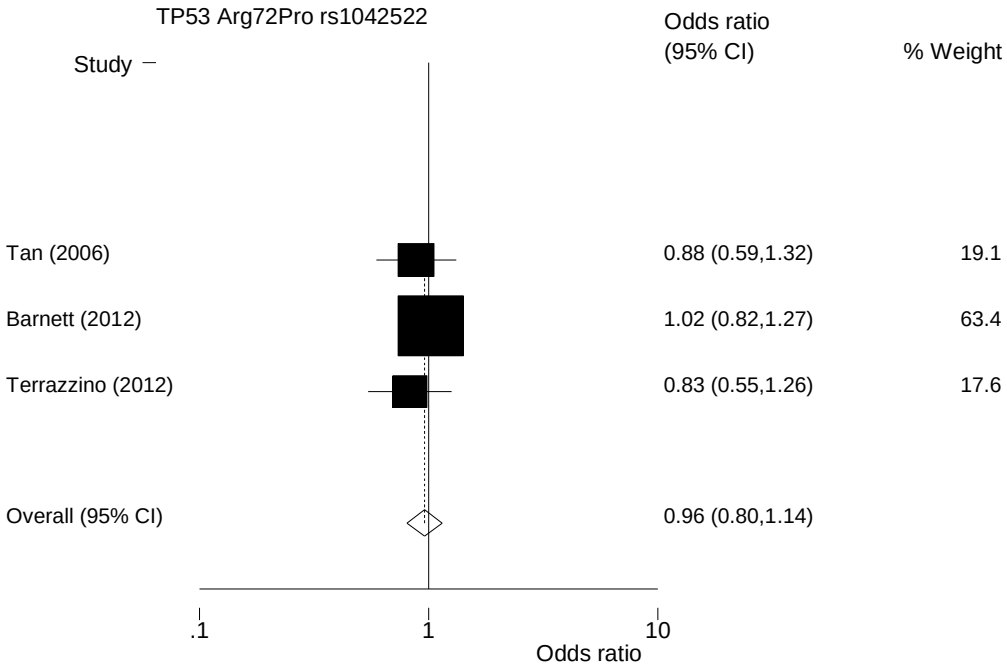
Heterogeneity chi-squared = 1.84 (d.f. = 1) p = 0.175
Estimate of between-study variance Tau-squared = 0.0177
Test of OR=1 : z= 0.63 p = 0.532

TGFbR3 intron G>A rs1926261



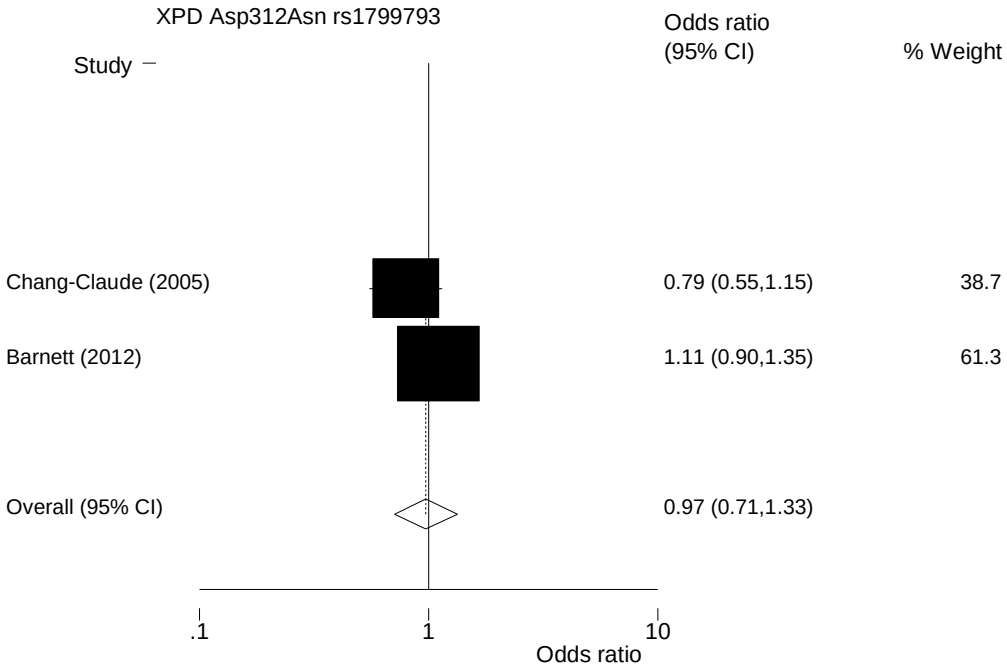
Heterogeneity chi-squared = 2.96 (d.f. = 2) p = 0.227
Estimate of between-study variance Tau-squared = 0.0151
Test of OR=1 : z= 0.85 p = 0.397

TP53 Arg72Pro G>C rs1042522



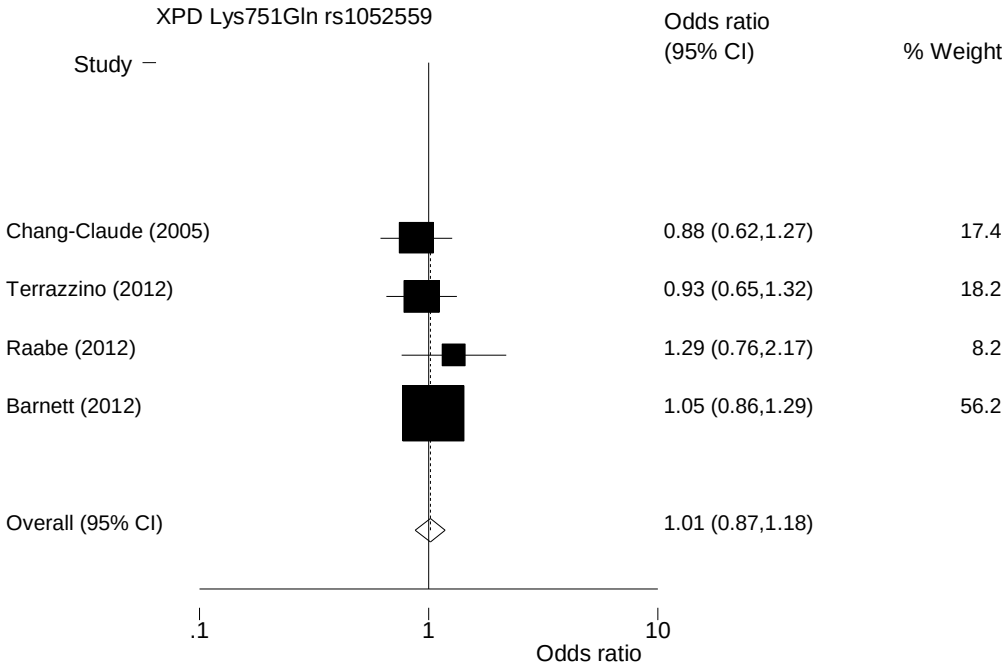
Heterogeneity chi-squared = 0.96 (d.f. = 2) p = 0.617
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.48 p = 0.630

XPD Asp312Asn C>T



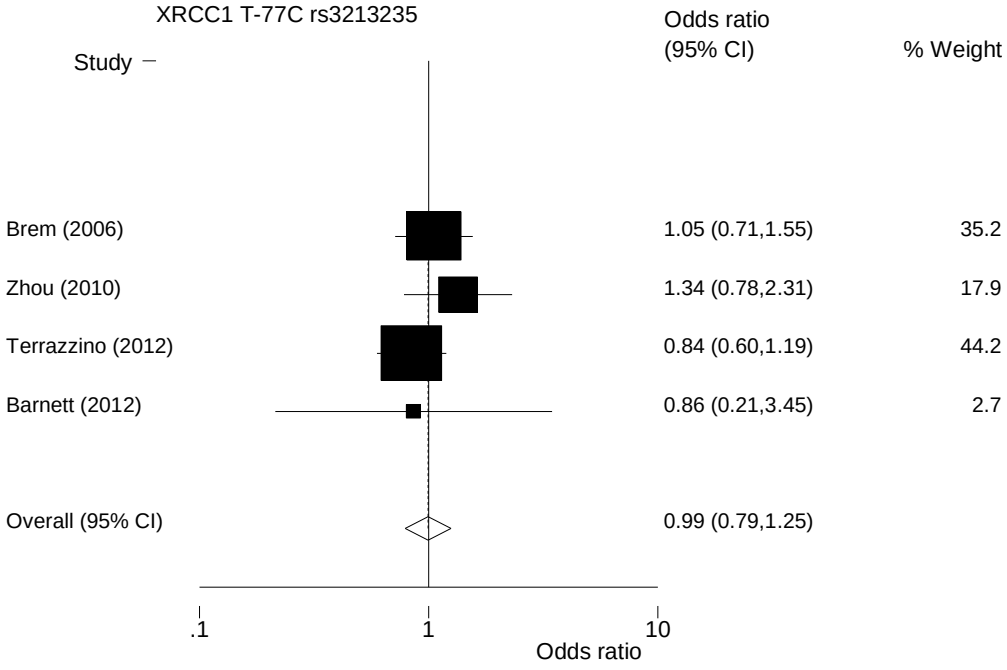
Heterogeneity chi-squared = 2.40 (d.f. = 1) p = 0.122
Estimate of between-study variance Tau-squared = 0.0320
Test of OR=1 : z= 0.17 p = 0.864

XPD Gln751Lys T>G rs1052559 (merged into rs13181)



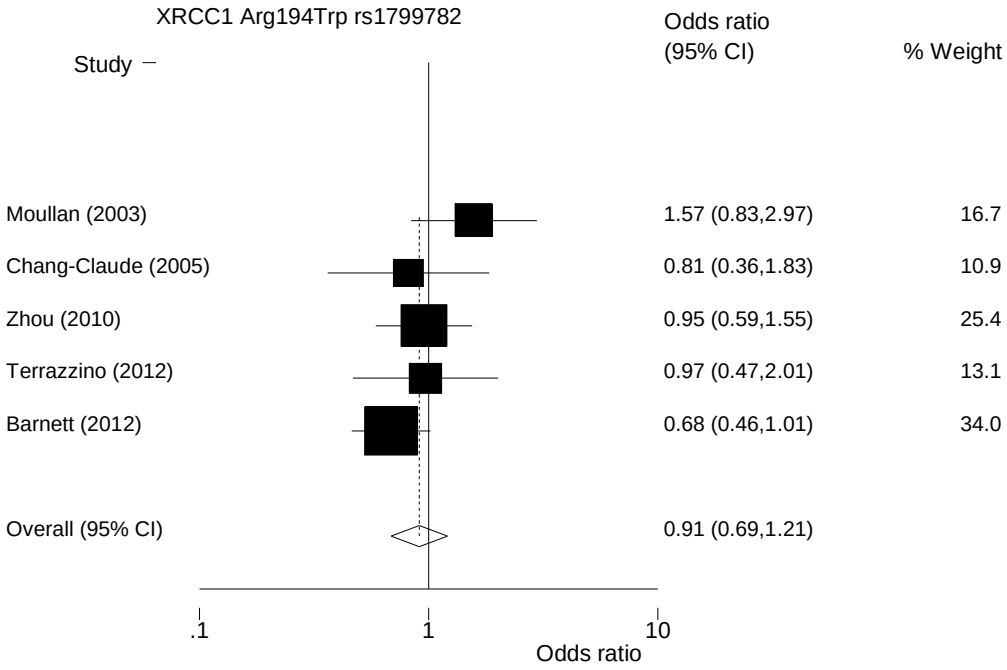
Heterogeneity chi-squared = 1.72 (d.f. = 3) p = 0.632
612 Estimate of between-study variance Tau-squared = 0.0000
613 Test of OR=1 : z= 0.19 p = 0.850

XRCC1 -77 T>C rs3213235



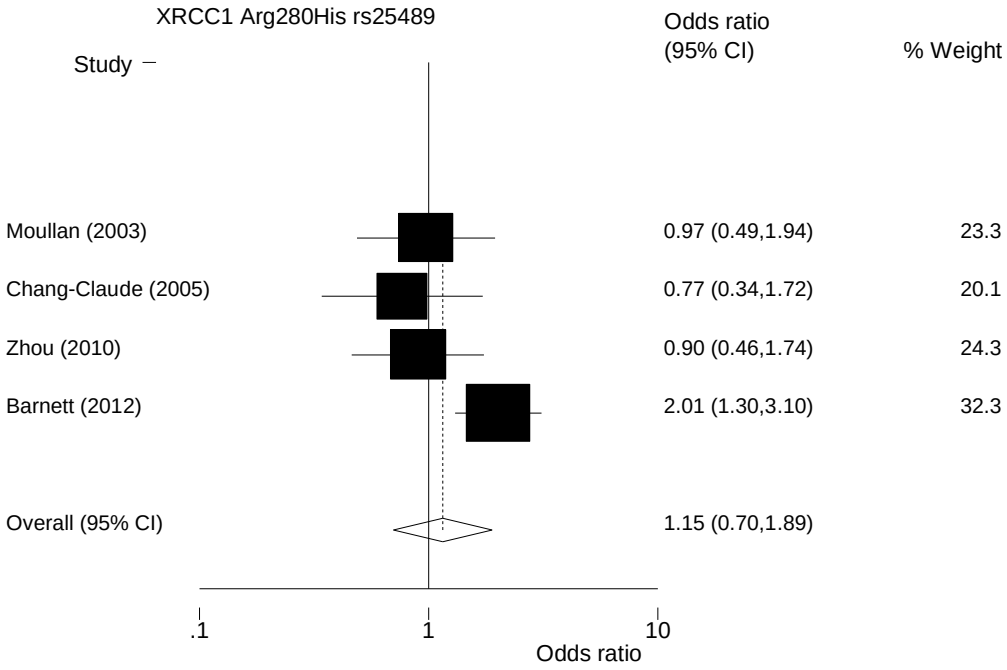
Heterogeneity chi-squared = 2.18 (d.f. = 3) p = 0.536
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 0.08 p = 0.937

XRCC1 Arg194Trp C>T rs1799782



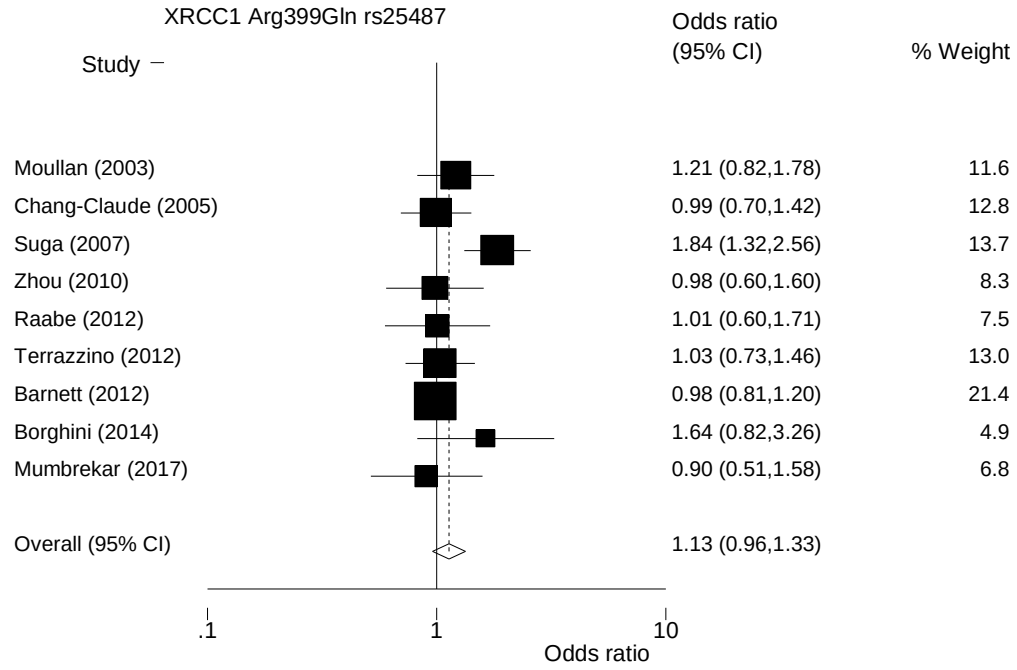
Heterogeneity chi-squared = 5.00 (d.f. = 4) p = 0.288
Estimate of between-study variance Tau-squared = 0.0211
Test of OR=1 : z= 0.64 p = 0.521

XRCC1 Arg280His G>A rs25489



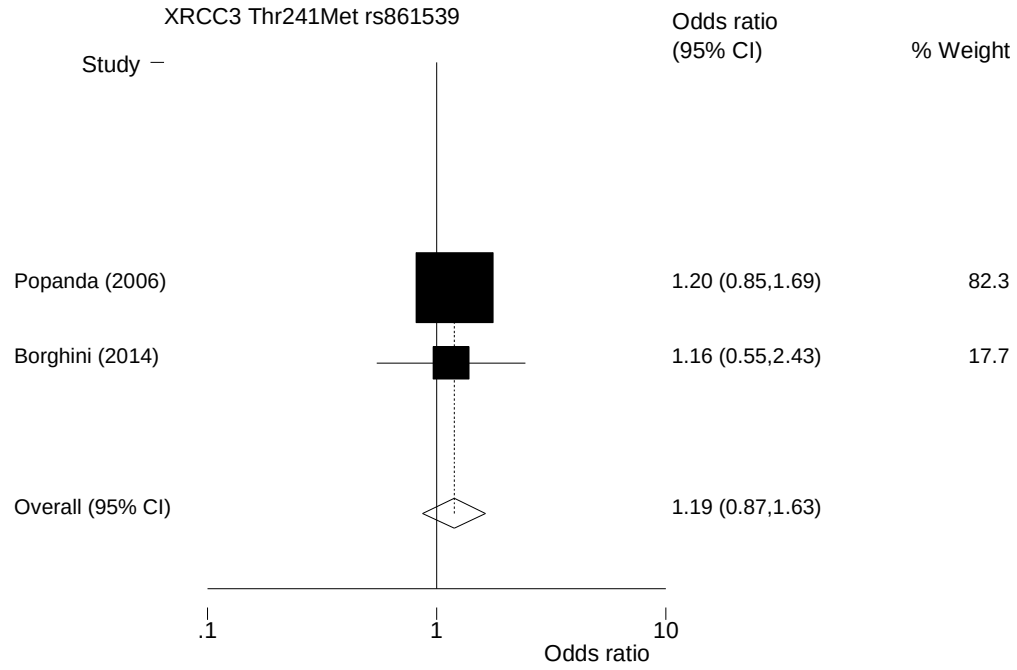
Heterogeneity chi-squared = 7.40 (d.f. = 3) p = 0.060
Estimate of between-study variance Tau-squared = 0.1502
Test of OR=1 : z= 0.55 p = 0.582

XRCC1 Arg399Gln G>A rs25487



Heterogeneity chi-squared = 13.05 (d.f. = 8) p = 0.110
Estimate of between-study variance Tau-squared = 0.0231
Test of OR=1 : z= 1.44 p = 0.150

XRCC3 Thr241Met G>A rs861539



Heterogeneity chi-squared = 0.01 (d.f. = 1) p = 0.933
Estimate of between-study variance Tau-squared = 0.0000
Test of OR=1 : z= 1.08 p = 0.279

Publication bias

Funnel plots showing scatterplots of the log odds ratios individual studies over each study standard error of the log odds ratio as a measure of study size are shown in Figure 20 to Figure 27 for the five genetic markers significant on meta-analysis and three genetic markers significant after sensitivity analysis. The funnel plots demonstrate no evidence of asymmetry in any of the comparisons, indicating absence of publication bias.

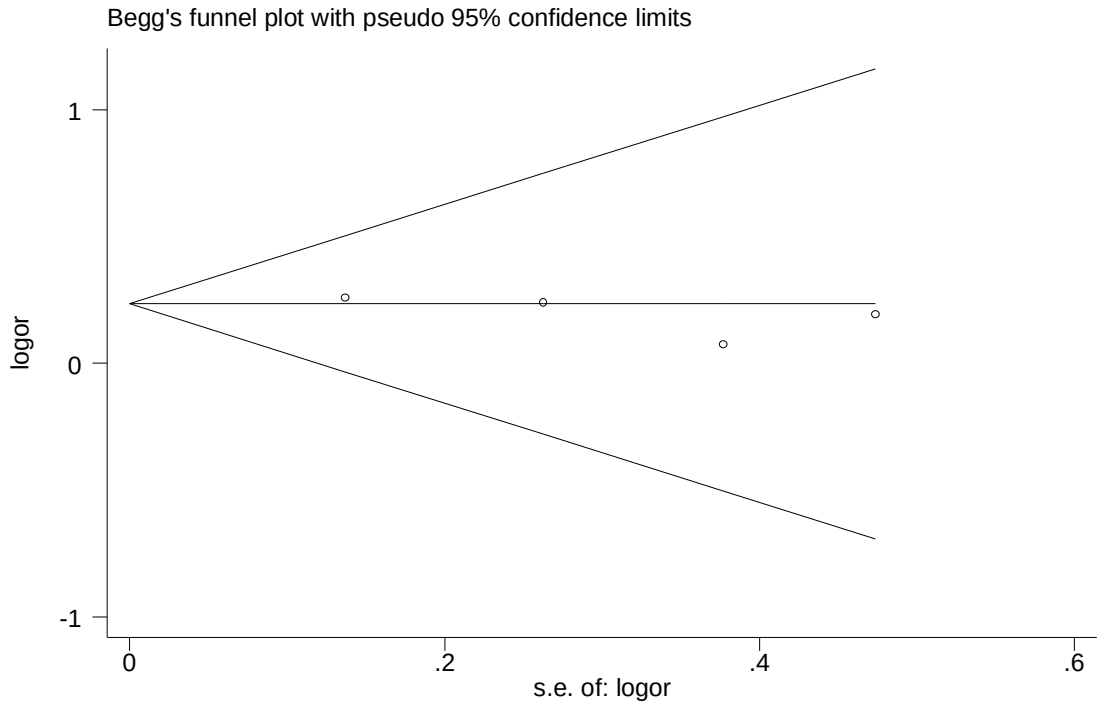


Figure 20. Funnel plot for ATM 5557 G>A rs1801516.

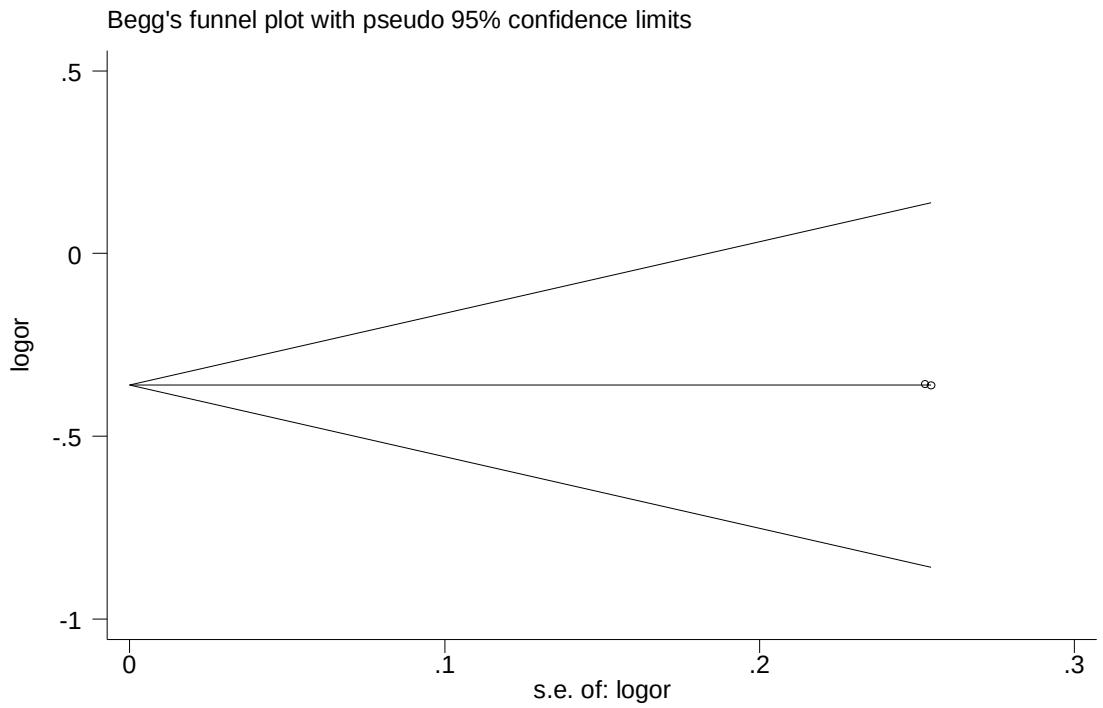


Figure 21. Funnel plot for IL12RB intron rs3790568.

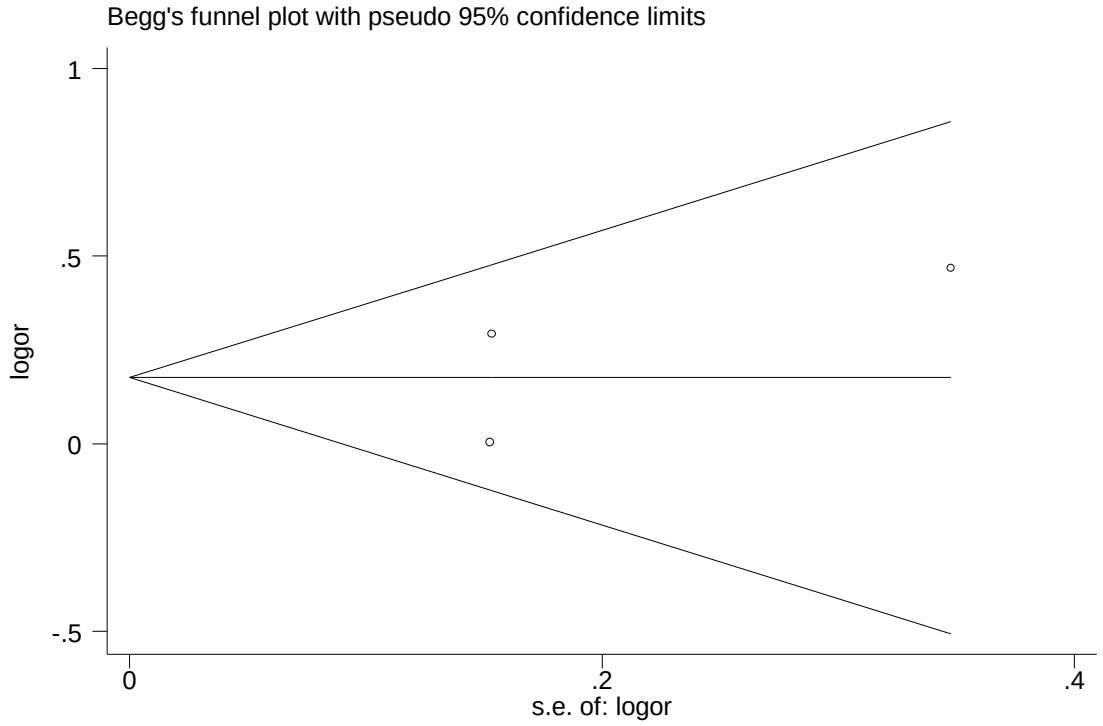


Figure 22. Funnel plot for LIG3 -19314 G>C rs3744355 excluding Barnett et al [140].

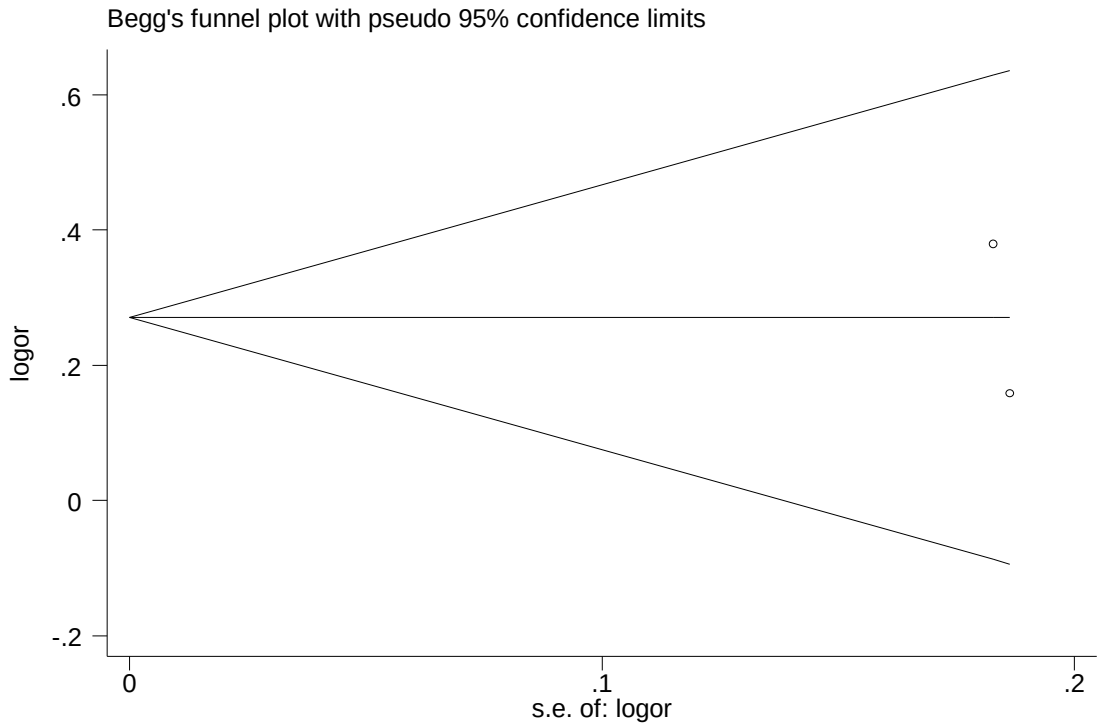


Figure 23. Funnel plot for NEIL3 rs3805169 excluding Mumbrekar et al [254].

.

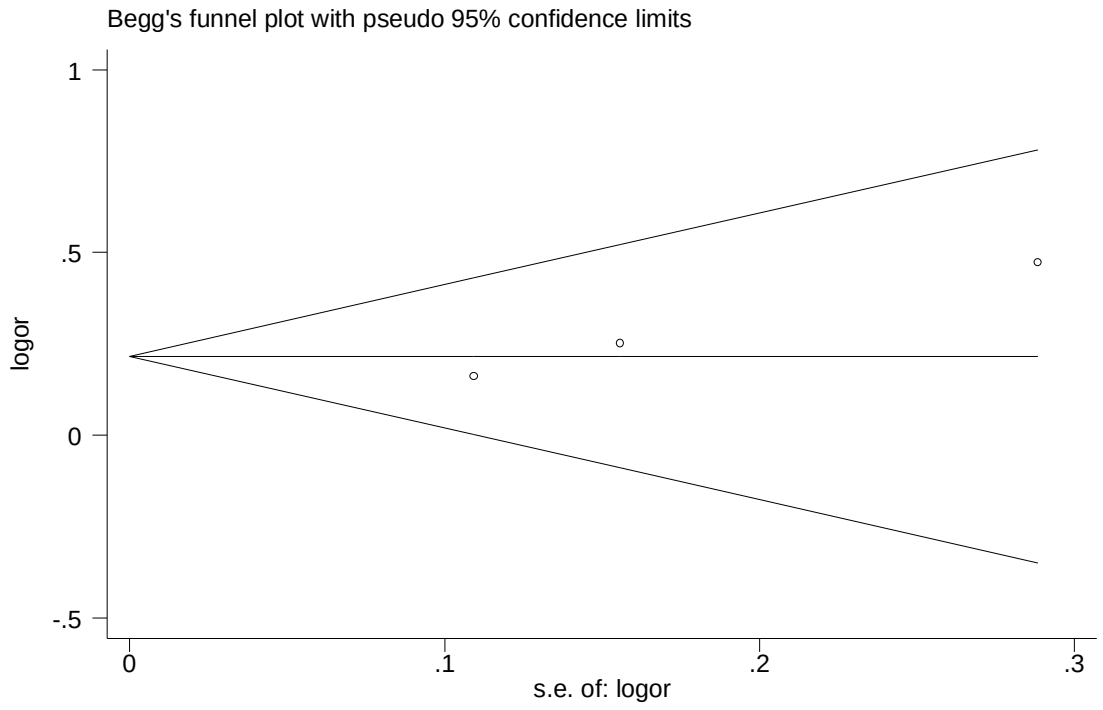


Figure 24. Funnel plot for PTTG rs2961952.

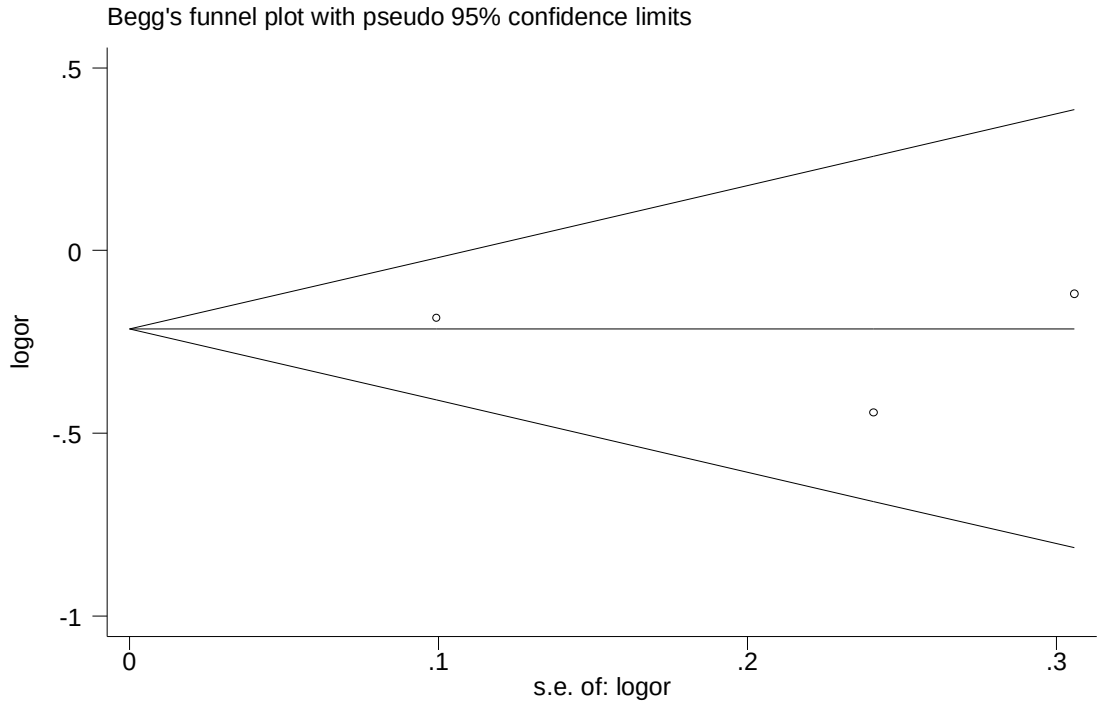


Figure 25. Funnel plot for PTTG1 rs3811999.

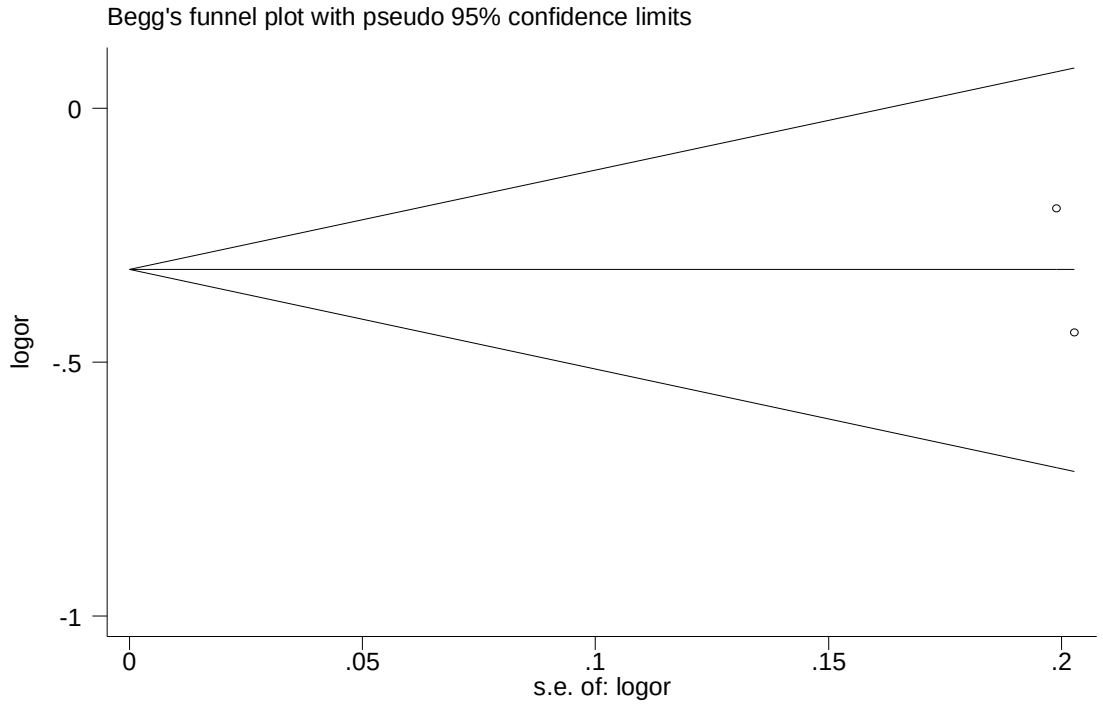


Figure 26. Funnel plot for RAD9A rs2286620.

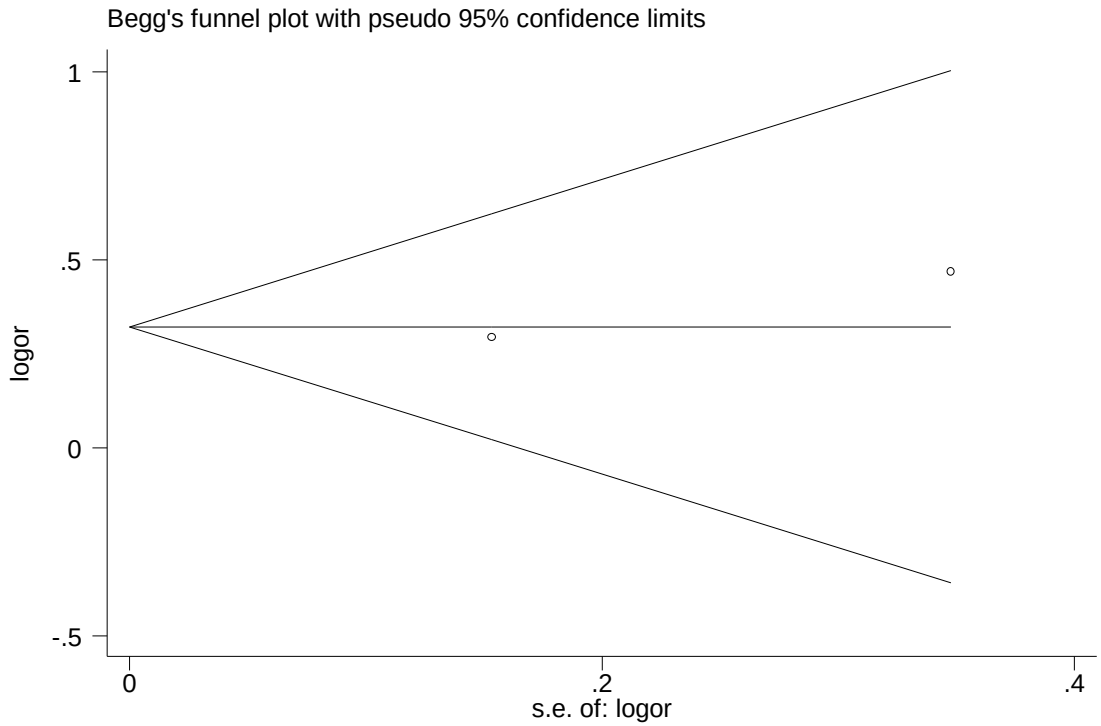


Figure 27. Funnel plot for REV3L rs240962 excluding Barnett et al [140].

Discussion

Systematic reviews of gene-disease association studies are accepted as a key method of establishing the genetic components of complex diseases [259]. The aim of this part of the project was to conduct a literature-based systematic review of published genetic markers of acute breast radiation toxicity, in order to identify genetic markers associated with acute radiation toxicity in breast cancer patients, and in part, to update the two existing systematic reviews published in 2009, which covered a range of acute and late radiation toxicity endpoints across different cancer types [88, 89]. A total of 13 further relevant studies have been published in the literature since 2009, including publications with ≥ 1,000 patients [94, 140], whereas the largest number of patients in publications prior to 2010 was 446.

To date, there has been no published GWAS of acute breast radiation toxicity, although late toxicity endpoints have been examined [222]. The largest single study to date failed to replicate any of the previous genetic associations with toxicity, including acute breast toxicity [140]. In a meta-analysis for four *XRCC1* variants published in 2012, a significant association of both the 399Gln rs25487 and 280His rs25489 alleles with any toxicity (early or late) was demonstrated on subgroup analysis only with studies grouped either by genotyping method or adjuvant treatment (radiotherapy alone vs radiotherapy and chemotherapy) [224]. A more recent meta-analysis of the *ATM* rs1801516 and *TP53* rs1042522 using a fixed-effects model despite clinical heterogeneity failed to demonstrated any overall association with toxicity from breast or prostate radiotherapy [225].

The results of the present study include a number of genetic variants that may be associated with breast skin toxicity and confirm a lack of association with other variants on the basis of candidate gene studies combining up to 3,235 patients. Five variants in genes implicated in either DNA repair or cell cycle arrest were significant at $p<0.05$, albeit with relatively low ORs below 1.5, or above 0.66 for the protective variants. Of these, the *ATM* 5557 G>A rs1801516 variant had previously been replicated in a patient-based meta-analysis of 1,357 patients with an OR of 1.71 (CI 1.11-2.66) including the data in the present review [94].

PTTG (Pituitary Tumour-Transforming Gene) represents a homologue of yeast securin proteins, which prevent sister chromatid separation before anaphase [260]. Previous single cohort studies failed to demonstrate any significant association with acute skin toxicity [140, 206, 254], but the present meta-analysis shows a significant association for the 'A' allele of *PTTG1* intron G>A rs2961952 and the 'T' allele of *PTTG1* rs3811999. Although the PTTG1 intron rs2961950 G>A variant is in moderate LD (r^2=0.81, distance 4,784 bp) with rs2961952, the former variant 'A' allele did not demonstrate any association with acute skin toxicity. *RAD9A* encodes a protein required for cell cycle arrest and DNA damage repair. The *RAD9A* 1103 rs2286620 T variant allele showed a protective effect combining

data from two studies in a Japanese and UK population, whereas individually, only the Japanese study showed a significant effect [140, 206]. The 'A' allele of the rs3790568 intron variant of *IL12RB*, implicated in T cell signalling and fibrogenesis, also proved to be protective against breast skin toxicity combining the data from a Japanese study by Isomura *et al* [246] and the UK study by Barnett *et al* [140].

Sensitivity analysis excluding one study at a time also rendered the meta-analysis significant at $p<0.05$ for three further genetic markers. Excluding Barnett *et al* [140], the *LIG3* rs3744355 variant 'C' allele was significantly associated with acute skin toxicity based on three studies in Japanese, UK, and Indian cohorts. *LIG3* is involved in base excision repair of single strand DNA lesions [261]. Similarly, the *REV3L* rs240962 variant 'T' allele became significant based on the same Japanese and Indian study [206, 254]. The latter may be accounted for by ethnic differences between Asian and European populations, due to the difference in frequency of the 'T' allele (0.11 in the UK vs. 0.18 in the Indian and 0.47 in the Japanese study). *REV3L* encodes a subunit of DNA polymerase zeta implicated in DNA repair [262]. Excluding the study by Mumbrekar *et al* [254], the *NEIL3* rs3805169 'C' variant also becomes significant based on the Japanese study by Suga e*t al* [206] and the UK RAPPER consortium replication study [140]. *NEIL3* encodes a further DNA glycosylase implicated in single-lesion base excision repair [263].

Limitations

This systematic review and meta-analysis was based on published data of candidate SNP studies, except where indicated that data was obtained directly from the authors. As such, the results are limited by the constraints of working with published data rather than individual patient-level data. There is also risk of bias from incomplete retrieval of data or incomplete reporting of results.

Studies used a range of endpoint definitions for acute toxicity (e.g. grade 2, grade 1) and a number of different toxicity rating scales (e.g. RTOG, CTCAE). In the absence of individual patient-level data, it was not possible to calculate a statistically unified endpoint, for example, by working with residuals or calculating standardized average toxicity scores [264]. There was also considerable heterogeneity in terms of patients' treatment characteristics between the studies. Some excluded patients with adjuvant chemotherapy and the radiotherapy fractionation schedule differed depending on study year and location. In particular, the more recent studies by Barnett *et al* [140] and De Langhe *et al* [26] used a hypo-fractionated regime with image-modulated radiotherapy techniques, which is associated with reduced acute skin toxicity overall. Due to the relatively low number of studies per single variant, further sensitivity analysis based on treatment parameter was not possible.

In absence of a clear mode of inheritance, the allelic (additive) model was used for calculating odds ratios for individual variants. If the exact model of inheritance could be established for individual markers, then another model (e.g. dominant, recessive) may have given different results. The p-values indicated for associations in this analysis were significant at the 0.05 level before applying any correction for testing multiple genetic markers or applying genome-wide significance thresholds. This probably implies that the true genetic variants associated with acute breast radiation toxicity are yet to be identified. The results for variants significant after exclusion of individual studies from the meta-analysis would also benefit from further investigation of influence of ethnicity and other patient parameters known to play a role in the development of radiation toxicity.

Conclusions

The present systematic review and meta-analysis has shown no association with acute breast radiation toxicity for several genetic markers previously significant in single studies, indicating that those results were probably false positives. It has also confirmed a number of variants that may be associated with acute toxicity, some of which may not have been previously significant in individual studies. These variants and other SNPs in the genes involved will be genotyped in the REQUITE-AB cohort in the next study phase.

Phase 5: Genotying in the REQUITE-AB cohort

In order to identify genetic predictors of radiation toxicity, the main approach taken by investigators has been to type SNPs in the genome of patients undergoing radiotherapy. Numerous studies have reported on genetic variations modifying the clinical radiosensitivity risk, predominantly in pathways based on mechanistic understanding of radiation pathogenesis [86, 265]. Acute skin toxicity is initiated by depletion of acutely responding epithelial tissues and damage to micro vessels [266].

Earlier studies in radiogenomics involved candidate SNP association studies in relatively small patient cohorts [88, 89]. Previously reported associations with acute breast radiation toxicity included genes involved in major DNA repair pathways (ATM, LIG3, MLH1, RAD9A, XRCC1) and genes regulating the cellular response to oxidative stress (GSTP1) [26, 94, 121, 206, 209, 213]. Although none of these reported associations independently replicated in a study conducted by the RAPPER group [140], the meta-analysis in this research project demonstrated some SNPs associated significantly with acute breast (skin) toxicity at the 0.05 level across several publications including the RAPPER validation cohort.

More recently, promising genetic associations have emerged from multi-institutional cohorts with built-in replication, in particular for late breast toxicity [92]. At the same time, several small to medium-sized genome-wide association studies (GWAS) have been published in the field of radiogenomics [98, 100, 101, 223]. This has been facilitated by the availability of micro-chips with up to two million tag SNPs, which enable researchers to analyse all common genetic variants, including copy number variation (CNV), to identify those loci that explain the genetic components of traits like susceptibility to radiation toxicity. Such studies need to be powered adequately to detect SNPs with small to moderate odds ratios of between 1.1 and 1.5.

Objectives

The main objective of this study phase was to validate published genetic markers of acute breast radiation toxicity in conjunction with validated clinical predictors in a prospectively recruited patient cohort, in particular:

- To evaluate the association between replicated genetic markers identified in the systematic review (phase 4) with toxicity endpoints in the REQUITE-AB breast cancer cohort;
- To assess the effect of adding genetic markers to the clinical and treatment predictors associated with toxicity endpoints in the REQUITE-AB cohort (phase 1).

Study population

This study was conducted in the REQUITE breast cancer patient cohort described in study phase 1, using a single stage design with a panel of SNPs determined by the results of the meta-analysis undertaken in study phase 4. The patients were recruited prospectively following breast-conserving surgery across eight participating centres between 2014 and 2016, with documented toxicity at baseline and end-of-treatment. As this constituted an interim genotyping stage of the REQUITE cohort study, samples from only 1,876 patients out of 2,072 were available for analysis. In particular, for logistical reasons, all patient samples from Mount Sinai were excluded.

Phenotypes

The toxicity phenotypes acute erythema, acute ulceration, acute desquamation, and acute oedema scored according to CTCAE v4.0, as well as physician-recorded acute pain, were investigated. Overall acute toxicity was measured as a Standardized Total Average Toxicity (STAT) acute score, combining all skin toxicities. STAT scores were calculated as described elsewhere [264]: First, standardized z-scores were calculated per study for each toxicity endpoint (i) and each individual patient (k) of the respective study:

$$Z_{k,i} = \frac{(s_{k,i} - mean_i)}{standard\ deviation_i}$$

Then, STAT scores were calculated as the average of all non-missing Z scores for an individual patient:

$$STAT_k = mean\ Z_{k,i}$$

Sample collection

DNA was extracted from pre-treatment anonymised bar-coded EDTA blood samples collected as described in study phase 1 from the whole REQUITE breast cancer cohort. Briefly, frozen whole blood EDTA samples from participating centres were shipped regularly to the Centre for Integrated Genomic Medical Research (CIGMR) at the University of Manchester, which hosted the centralised biobank of the main REQUITE study for sample storage prior to DNA extraction. All laboratory, data and management processes within the ISO9001:2008 certified CIGMR biobank were fully ISO compliant and all processes carried out outside the biobank adhered to the ISO Quality Policy.

All EDTA samples were stored at -80°C as whole blood. Study centres scanned the bar code on each sample prior to storage to provide sample tracking to the centralised REQUITE database, accessible

to CIGMR. On arrival by courier, CIGMR logged all samples and raised any discrepancies raised with the originating centre. DNA was extracted from EDTA blood samples in batches using Nanodrop technology and diluted to a standard concentration. Undiluted and diluted DNA were stored at two different geographical sites to guard to guard against catastrophic loss prior to genotyping.

Genotyping strategy

Candidate SNPs significantly associated with acute skin toxicity identified in the systematic review were: *ATM* 5557 G>A (rs1801516), *IL12RB2* intron G>A (rs3790568), *LIG3* -19314 G>C (rs3744355), *NEIL3* intron T>C (rs3805169), *PTTG1* G>A (rs2961952), *PTTG1* -1993 C>T (rs3811999), *RAD9A* 1103 C>T (rs2286620), and *REV3L* intron C>T (rs240962).

As SNPs identified in the literature tend to be surrogate markers rather than functional variants, each potential susceptibility locus should be studied in depth in order to identify the most plausible causative variants. Accordingly, using the Broad Institute SNAP Proxy Search programme, 1,000 Genomes and Hapmap3 were searched for SNPs in relatively close linkage disequilibrium (LD) (r^2 between 0.95 and 0.7) with these seven SNPs. This yielded 31 SNPs for *ATM* rs1801516, 2 SNPs for *IL12RB2* rs3790568, 16 SNPs for *NEIL3* rs3805169, 11 *SNPs* for PTTG1 rs2961952, 44 SNPs for *RAD9A* rs2286620, and 54 SNPs for *REV3L* rs240962 (detail not shown). There were no SNPs in LD with *LIG3* rs3744355. All SNPs in LD for *IL12RB2* rs3790568, *NEIL3* rs3805169, and *PTTG1* rs2961952 were selected for further genotyping. The SNPs in LD for the remaining three SNPs were selected on minor allele frequency, but those with exactly the same r^2 or D' values were excluded (seeTable 34).

In addition to those 73 SNPs, 6 SNPs which showed a trend towards significance ($p \leq 0.2$) in the earlier meta-analysis in Phase 4 (*ALAD* 3'UTR rs818707, *XRCC1* Arg399Gln rs25487, *MAP3K7* rs3757244, *REV3L* rs190246, rs2230806, *SOD2* T47C rs4880) and 16 additional SNPs included in the validation study by Barnett *et al* [140] as previously associated with breast toxicity were selected for genotyping in the cohort, as well two SNPs significant in an analysis of acute breast toxicity from a collaborating study (rs13116075 and rs1801260).

A total of 96 SNPs were genotyped using a custom-made 96.96 Fluidigm SNP array (Illumina, San Diego, CA, USA) by the REQUITE commercial partner Source Bioscience in their facilities in Nottingham, UK. This was preceded by a specific target amplification step to provide sufficient template molecules to use on the Fluidigm integrated fluidic circuits. 5% of the samples from each

plate were duplicated on another plate as a reproducibility check. Duplicated samples were required to give greater than 99% concordance before the assay is accepted.

Statistical methods

STATA version 14.1 for Research and PLINK version1.9 [267] were used for statistical analysis. First, logistic regression without adjustment for co-variates was used for dichotomised toxicity endpoints applying an additive genetic model of inheritance with Bonferroni correction for 96 tests ($p<0.0005$). The association of both individual SNPs and groups of SNPs within a single locus (gene sets) was assessed, the latter using the PLINK set-test function

In the PLINK set-based test (--set-test), SNPs in LD (--set $r^2>0.7$) are grouped as sets and standard single SNP analysis is carried out. For each set of SNPs, a mean SNP statistic is calculated from the single SNP statistics of a maximum amount (--set-max 99999) of independent SNPs below a p-value threshold (--set-p 0.1). If SNPs are not independent (i.e. $r^2>0.7$), the SNP with the lowest p-value in the single SNP analysis is selected. The same analysis is then repeated a cross a number (--mperm 10,000) of permutated datasets, keeping LD between SNPs constant. An empirical p-value (emp1) is then derived by calculating the number of times the test statistic of the simulated SNP sets exceeds that of the original SNP set.

Overall toxicity was assessed in terms of STAT (acute) score using multivariate linear regression [264]. For each toxicity endpoint, residuals were then calculated for each patient using clinical covariates that were significantly associated with the toxicity endpoint in a first regression model to assess toxicity not explained by patient- and treatment-related factors. In a second regression model, the residuals from the first regression model were used to assess associations with genotype. Study centre was included as predictor variable to take into account cohort heterogeneity. Hardy-Weinberg equilibrium (HWE) was assessed by chi square test.

Results

The characteristics of the patient cohort were described earlier in the results from phase 1 of the project. Briefly, the incidences of dichotomised endpoints in the cohort were 31.6 % for acute oedema, 9.3 % for acute ulceration, 24.2 % for ≥grade 2 erythema, 9.5 % for acute desquamation, and 27.0 % for acute pain.

During the genotyping analysis, three patient samples (RQ-30116-0, RQ-34185-7 and RQ45223-2) did not produce any data and were repeated on the final array run. There were 9 SNP assays which failed to produce consistent results across the runs with call rates <96 % (rs11227756, rs11786715, rs1616208, rs3811999, rs4255546, rs4930192, rs557953, rs79890820 and rs111501), leaving 87 SNPs for the final analysis (Table 34). Of these, a number deviated from HWE: rs596917, rs354523, rs2961935, rs4880, rs7791642, rs818707 and rs10890838 (all p-values <0.01).

rs number	Chr	Coordinate	Gene	Major allele	Minor allele	Genotype frequencies	MAF % (dbSNP)	HWE (p-value)	Call rate %
rs3790568	1	67370377	IL12RB2	G	A	7/205/1641	0.17	0.833	99.00
rs6685568	1	67389614	IL12RB2	A	G	11/260/1574	0.18	0.8693	98.05
rs12409092	1	67390948	IL12RB2	T	C	9/211/1634	0.17	0.42	99.53
rs4849101	2	112687260	non-coding	T	C	343/941/570	0.49	0.2029	99.17
rs2881208	2	199272885	SATB2	C	T	271/930/655	0.44	0.04675	99.47
rs1801260	4	55435202	CLOCK	T	C	133/722/994	0.23	0.9055	99.23
rs13116075	4	139008878	intron variant	A	G	41/479/1334	0.07	0.8567	99.35
rs17064666	4	177346497	NEIL3	C	T	29/376/1451	0.21	0.4304	96.81
rs2877985	4	177347743	NEIL3	A	G	24/421/1403	0.21	0.2492	98.76
rs3805169	4	177351762	NEIL3	T	C	10/261/1588	0.15	1	99.29
rs17064704	4	177354496	NEIL3	C	T	9/235/1614	0.13	0.8544	99.23
rs17064705	4	177354515	NEIL3	G	T	4/234/1617	0.13	0.1802	99.11
rs575018	5	101043689	non-coding	T	C	229/829/791	0.28	0.6084	99.59
rs505994	5	101048059	non-coding	A	C	231/826/799	0.28	0.4424	99.82
rs2961935	5	160401392	PTTG1	T	C	96/774/960	0.38	0.0001453	99.00
rs2961944	5	160408651	PTTG1	G	A	127/727/1005		0.8113	99.00
rs2910190	5	160415458	PTTG1	G	A	125/711/1005		1	99.17
rs2910201	5	160423365	PTTG1	T	C	194/843/821	0.47	0.3199	99.35
rs2961950	5	160424738	PTTG1	G	A	192/843/815	0.48	0.2485	99.35
rs2961951	5	160427271	PTTG1	G	T	116/701/1030	0.27	0.8535	99.65
rs2961952	5	160429522	PTTG1	G	A	132/708/1015	0.48	0.5889	98.82
rs2961911	5	160434568	PTTG1	T	C	123/703/1030	0.29	0.8548	99.29
rs17142289	6	6550516	LY81-AS6	A	G	3/90/1767	0.07	0.1217	99.47
rs596917	6	41420606	non-coding	G	T	134/827/829	0.47	0.0001892	98.88
rs3757244	6	90587350	MAP3K7	C	0	0/0/1816	0.01	1	99.41
rs6934341	6	111098387	SLC16A10	G	A	37/474/1347	0.23	0.5804	99.41
rs4947106	6	111100163	SLC16A10	C	T	34/475/1350	0.22	0.3518	99.17

rs number	Chr	Coordinate	Gene	Major allele	Minor allele	Genotype frequencies	MAF % (dbSNP)	HWE (p-value)	Call rate %
rs9384787	6	111113707	SLC16A10	A	T	35/482/1343	0.34	0.3128	99.53
rs193281	6	111166487	SLC16A10	C	T	33/472/1353	0.20	0.3027	99.47
rs395564	6	111176176	SLC16A10	T	C	35/477/1342	0.24	0.3564	99.59
rs377716	6	111181705	SLC16A10	A	G	34/476/1350	0.21	0.308	99.53
rs354525	6	111186193	SLC16A10	A	G	30/483/1338	0.29	0.07719	98.82
rs354523	6	111188122	SLC16A10	G	A	30/556/1267	0.28	0.000288	99.05
rs354542	6	111193483	SLC16A10	C	T	34/478/1348	0.28	0.308	99.35
rs354538	6	111196863	SLC16A10	A	G	35/476/1349	0.22	0.4044	99.35
rs434034	6	111204180	SLC16A10	G	A	34/472/1353	0.22	0.3999	99.41
rs354546	6	111211927	SLC16A10	C	T	23/436/1400	0.26	0.09985	97.75
rs354547	6	111213919	SLC16A10	T	G	33/477/1349	0.28	0.2637	99.53
rs354526	6	111216278	SLC16A10	G	T	24/439/1396	0.25	0.1258	98.94
rs354527	6	111217390	SLC16A10	G	A	20/434/1401	0.19	0.03646	99.00
rs354551	6	111224337	SLC16A10	G	A	34/469/1356	0.20	0.3995	99.23
rs191631	6	111226862	SLC16A10	T	G	24/433/1400	0.21	0.1506	99.11
rs3912092	6	111251609	intron variant	C	A	14/332/1514	0.18	0.4269	99.23
rs240986	6	111265852	MFSD4B	T	A	19/404/1437	0.20	0.1209	99.11
rs190246	6	111277043	MFSD4B	C	A	17/404/1424	0.20	0.04525	99.41
rs190245	6	111294270	POLZ	G	A	16/388/1456	0.26	0.08206	99.41
rs240998	6	111307676	REV3L	C	T	17/414/1429	0.26	0.02837	99.47
rs240962	6	111321243	REV3L	C	T	17/412/1431	0.26	0.03604	99.65
rs4880	6	159692840	SOD2	C	T	476/811/501	0.41	0.0000865	99.47
rs882460	7	31494170	intron variant	T	C	117/691/1043	0.22	0.8525	99.47
rs7791642	7	67241411	near TYW1	A	G	2/308/1543	0.09	0.0001287	99.41
rs10280848	7	68639201	intron variant	C	T	337/896/617	0.44	0.7037	98.64
rs12531679	7	136162189	LOC105375523	G	A	21/346/1492	0.04	0.8045	97.52
rs8178046	8	47929148	PRKDC	C	T	1/73/1786	0.01	0.5329	99.70
rs6475752	9	23658013	LOC101929563	G	T	142/671/1037	0.29	0.0246	99.17
rs2230806	9	104858586	ABCA1	G	A	130/722/1003	0.44	1	99.59
rs818704	9	113386036	ALAD	G	A	44/561/1216	0.09	0.0304	99.59
rs818707	9	113387687	ALAD	G	A	16/454/1369	0.09	0.0007167	99.00
rs7037705	9	122145255	NDUFA8	A	C	7/202/1647	0.15	0.6728	99.17
rs2001635	11	67094924	RAD9A, POLD4 etc	G	A	22/302/1527	0.35	0.1291	99.35
rs608273	11	67158297	RAD9A, POLD4 etc	C	T	19/269/1567	0.35	0.06441	99.11
rs674499	11	67195954	RAD9A, POLD4 etc	G	A	19/267/1571	0.35	0.06211	99.53
rs4542419	11	67203126	RAD9A, POLD4 etc	C	T	20/290/1543	0.35	0.1496	99.35
rs3927807	11	67231384	RAD9A, POLD4 etc	G	A	14/269/1572	0.35	0.5262	99.41
rs2071007	11	67282821	RAD9A, POLD4 etc	A	G	21/296/1542	0.35	0.1212	99.35
rs872110	11	67397320	RAD9A, POLD4 etc	A	G	21/286/1545	0.35	0.08168	95.56
rs2286620	11	67399513	RAD9A, POLD4 etc	T	C	10/258/1589	0.17	1	99.41
rs1638586	11	67407126	RAD9A, POLD4 etc	G	A	20/294/1536	0.38	0.1573	99.76
rs1558256	11	67412217	RAD9A, POLD4 etc	A	G	22/299/1533	0.35	0.0958	99.29
rs1558257	11	67412554	RAD9A, POLD4 etc	G	A	2/213/1589	0.10	0.06007	99.17
rs1790733	11	67418529	RAD9A, POLD4 etc	T	C	21/295/1534	0.36	0.1208	95.57
rs4754296	11	108135455	ATM	T	G	37/485/1336	0.07	0.4138	99.17

rs number	Chr	Coordinate	Gene	Major allele	Minor allele	Genotype frequencies	MAF % (dbSNP)	HWE (p-value)	Call rate %
rs73006226	11	108202001	ATM	C	A	27/406/1361	0.06	0.6729	99.17
rs4988023	11	108298268	ATM	A	C	34/461/1364	0.07	0.5683	99.05
rs1801516	11	108304735	ATM	G	A	34/455/1368	0.07	0.6326	99.53
rs3092993	11	108364388	ATM	C	A	33/459/1363	0.07	0.5031	99.35
rs10890838	11	108432578	ATM	C	T	0/492/1340	0.14	<0.0001	99.29
rs7115351	11	108454062	ATM	C	T	44/491/1324	0.19	0.9297	99.59
rs11212650	11	108456491	ATM	A	G	40/478/1342	0.15	0.7855	98.94
rs17108024	11	108462809	ATM	C	T	46/487/1327	0.21	0.86	96.75
rs4753840	11	108467613	ATM	A	G	40/475/1345	0.08	0.8553	99.35
rs4754317	11	108488434	ATM	G	A	32/486/1340	0.13	0.1181	99.65
rs72993806	11	108488962	ATM	C	G	34/482/1336	0.09	0.2325	99.76
rs7947933	11	108489579	ATM	A	C	33/485/1341	0.12	0.1682	98.16
rs3744355	17	34962027	LIG3	G	C	20/339/1489	0.13	0.8992	98.64
rs17798101	18	24540972	intron variant	T	C	44/460/1351	0.11	0.5184	99.00
rs25487	19	43551574	XRCC1	G	A	236/855/769	0.26	0.9597	99.00
rs1799778	19	43554989	XRCC1	C	A	233/856/765	0.28	0.8004	99.41

Table 34. List of successfully typed candidate SNPs in the REQUITE-AB cohort ordered by chromosome and gene (MAF = minor allele frequency) Genotype frequencies are presented in order of minor allele homozygote, heterozygote, and major allele homozygote. Call rates are averaged across Fluidigm plates..

Association of genetic markers with toxicity endpoints

None of the individual candidate SNPs were significantly associated with any of the acute toxicity endpoints (see Appendix). The only nominally significant associations were seen for acute pain with SNPs on chromosome 1 in the *IL12RB2* gene, namely, rs3790568 (p=0.009), rs6685568 (p=0.006), and rs12409092 (p=0.004) (Table 35).

rs number	Chr	Coordinate	Gene	patients (n)	Major allele	Proportion affected	Minor allele	Proportion affected	p-value association	OR
rs3790568	1	67370377	IL12RB2	1720	G	0.052	A	0.075	0.008826	1.479
rs6685568	1	67389614	IL12RB2	1711	A	0.069	G	0.097	0.005521	1.447
rs12409092	1	67390948	IL12RB2	1720	T	0.054	C	0.08	0.003665	1.526

Table 35. Results for genetic association (of minor allele) with toxicity endpoint pain in the REQUITE-AB cohort (OR=odds ratio, CI= confidence interval).

Association with acute toxicity model residuals

Multivariate regression modelling for all five toxicity endpoints and STAT acute revealed cup (breast) size to be consistently associated with all endpoints and different variables reaching statistical significant, depending on endpoint (Table 36). None of the individual candidate SNPs were significantly associated with any of the acute toxicity model residuals (see Appendix). The most promising trend for association seen in this analysis was for acute desquamation and *LIG3* rs3744355 (p=0.05) (Table 37). Using gene set-based testing, there was a significant association of the *SLC16A10* rs191631 variant with acute ulceration model residual (p= 1 x 10^{-8}) (Table 38).

Toxicity endpoint	**Significant clinical predictors on MVA**
Acute oedema	centre, cup (breast) size, BED breast
Acute ulceration	centre, cup (breast) size, statin use, alcohol use
Acute erythema (biv)	BP, cup (breast size), BED breast
Acute desquamation	centre, cup (breast) size, statin use, alcohol use
Acute pain	centre, cup (breast) size, analgesic use, BED breast

Table 36. Clinical predictors significantly associated (p<0.05) with acute toxicity endpoints used to calculate model residuals (MVA = multivariate analysis, BED = biologically effective dose).

rs number	Chr	Coordinate	Gene	Major allele	Minor allele	p-value association
rs3790568	1	67370377	IL12RB2	G	A	0.9173
rs6685568	1	67389614	IL12RB2	A	G	0.7259
rs12409092	1	67390948	IL12RB2	T	C	0.8815
rs4849101	2	112687260	non-coding	T	C	0.6012
rs2881208	2	199272885	SATB2	C	T	0.08532
rs1801260	4	55435202	CLOCK	T	C	0.07242
rs13116075	4	139008878	intron variant	A	G	0.8997
rs17064666	4	177346497	NEIL3	C	T	0.1559
rs2877985	4	177347743	NEIL3	A	G	0.6513
rs3805169	4	177351762	NEIL3	T	C	0.6909
rs17064704	4	177354496	NEIL3	C	T	0.6212
rs17064705	4	177354515	NEIL3	G	T	0.924
rs575018	5	101043689	non-coding	T	C	0.8395
rs505994	5	101048059	non-coding	A	C	0.8486
rs2961935	5	160401392	PTTG1	T	C	0.991
rs2961944	5	160408651	PTTG1	G	A	0.7151
rs2910190	5	160415458	PTTG1	G	A	0.7282

rs number	Chr	Coordinate	Gene	Major allele	Minor allele	p-value association
rs2910201	5	160423365	PTTG1	T	C	0.3136
rs2961950	5	160424738	PTTG1	G	A	0.2835
rs2961951	5	160427271	PTTG1	G	T	0.7839
rs2961952	5	160429522	PTTG1	G	A	0.5862
rs2961911	5	160434568	PTTG1	T	C	0.8552
rs17142289	6	6550516	LY81-AS6	A	G	0.7688
rs596917	6	41420606	non-coding	G	T	0.298
rs3757244	6	90587350	MAP3K7	C	0	NA
rs6934341	6	111098387	SLC16A10	G	A	0.3125
rs4947106	6	111100163	SLC16A10	C	T	0.4142
rs9384787	6	111113707	SLC16A10	A	T	0.2876
rs193281	6	111166487	SLC16A10	C	T	0.2715
rs395564	6	111176176	SLC16A10	T	C	0.2795
rs377716	6	111181705	SLC16A10	A	G	0.3225
rs354525	6	111186193	SLC16A10	A	G	0.3261
rs354523	6	111188122	SLC16A10	G	A	0.6191
rs354542	6	111193483	SLC16A10	C	T	0.3478
rs354538	6	111196863	SLC16A10	A	G	0.3349
rs434034	6	111204180	SLC16A10	G	A	0.3098
rs354546	6	111211927	SLC16A10	C	T	0.2573
rs354547	6	111213919	SLC16A10	T	G	0.3142
rs354526	6	111216278	SLC16A10	G	T	0.2743
rs354527	6	111217390	SLC16A10	G	A	0.3008
rs354551	6	111224337	SLC16A10	G	A	0.288
rs191631	6	111226862	SLC16A10	T	G	0.1796
rs3912092	6	111251609	intron variant	C	A	0.7477
rs240986	6	111265852	MFSD4B	T	A	0.3686
rs190246	6	111277043	MFSD4B	C	A	0.3581
rs190245	6	111294270	POLZ	G	A	0.1425
rs240998	6	111307676	REV3L	C	T	0.1851
rs240962	6	111321243	REV3L	C	T	0.1666
rs4880	6	159692840	SOD2	C	T	0.8723
rs882460	7	31494170	intron variant	T	C	0.2718
rs7791642	7	67241411	near TYW1	A	G	0.6827
rs10280848	7	68639201	intron variant	C	T	0.195
rs12531679	7	136162189	LOC105375523	G	A	0.8607
rs8178046	8	47929148	PRKDC	C	T	0.7804
rs6475752	9	23658013	LOC101929563	G	T	0.2229
rs2230806	9	104858586	ABCA1	G	A	0.1906
rs818704	9	113386036	ALAD	G	A	0.5466
rs818707	9	113387687	ALAD	G	A	0.2926
rs7037705	9	122145255	NDUFA8	A	C	0.514
rs2001635	11	67094924	RAD9A, POLD4 etc	G	A	0.6119
rs608273	11	67158297	RAD9A, POLD4 etc	C	T	0.5466
rs674499	11	67195954	RAD9A, POLD4 etc	G	A	0.5636

rs number	Chr	Coordinate	Gene	Major allele	Minor allele	p-value association
rs4542419	11	67203126	RAD9A, POLD4 etc	C	T	0.9296
rs3927807	11	67231384	RAD9A, POLD4 etc	G	A	0.6498
rs2071007	11	67282821	RAD9A, POLD4 etc	A	G	0.7005
rs872110	11	67397320	RAD9A, POLD4 etc	A	G	0.8598
rs2286620	11	67399513	RAD9A, POLD4 etc	T	C	0.8874
rs1638586	11	67407126	RAD9A, POLD4 etc	G	A	0.6753
rs1558256	11	67412217	RAD9A, POLD4 etc	A	G	0.5004
rs1558257	11	67412554	RAD9A, POLD4 etc	G	A	0.3556
rs1790733	11	67418529	RAD9A, POLD4 etc	T	C	0.5244
rs4754296	11	108135455	ATM	T	G	0.4451
rs73006226	11	108202001	ATM	C	A	0.6261
rs4988023	11	108298268	ATM	A	C	0.4342
rs1801516	11	108304735	ATM	G	A	0.4621
rs3092993	11	108364388	ATM	C	A	0.4431
rs10890838	11	108432578	ATM	C	T	0.4459
rs7115351	11	108454062	ATM	C	T	0.5712
rs11212650	11	108456491	ATM	A	G	0.4634
rs17108024	11	108462809	ATM	C	T	0.6618
rs4753840	11	108467613	ATM	A	G	0.386
rs4754317	11	108488434	ATM	G	A	0.2975
rs72993806	11	108488962	ATM	C	G	0.3381
rs7947933	11	108489579	ATM	A	C	0.3335
rs3744355	17	34962027	LIG3	G	C	0.0516
rs17798101	18	24540972	intron variant	T	C	0.8404
rs25487	19	43551574	XRCC1	G	A	0.272
rs1799778	19	43554989	XRCC1	C	A	0.2201

Table 37. Results for genetic association (of minor allele) with acute desquamation model residual in the REQUITE-AB cohort.

Gene set	NSNP	NSIG	ISIG	Emp1	SNPs
IL12RB2	3	0	0	1	NA
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	4	1	1×10^{-6}	rs191631
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

Table 38. Results for the PLINK gene-set association test with acute ulceration model residual in the REQUITE-AB cohort (NSNP= number of SNPs, NSIG= number of significant SNPs at p<0.1, ISIG= number of independently significant SNPs, Emp1 = empirical set-based p-value, derived by calculating the number of times the permuted set-test statistic exceeds that for the original SNP set).

Discussion

No individual associations or trends in the meta-analysis described earlier (phase 4) were replicated in this analysis, although nominally significant associations were seen for SNPs in the *IL12RB* gene and acute pain as well as the previously replicated *LIG3* rs3744355 variant and acute desquamation [121]. Applying a Bonferroni correction for 96 independent tests, none of these associations remained significant ($p<0.0005$). However, this correction may have been too conservative, as the several SNPs were selected on the basis of being in moderate LD with each other. Using gene set-based testing, there was a significant association of the *SLC16A10* rs191631 variant with acute ulceration. This variant was originally selected as candidate SNP as in linkage disequilibrium with the *REV3L* rs240962 variant ($r^2=0.915$, $D'=1$) identified in the meta-analysis based on two Asian association studies [206, 254].

There have been no previous reports on the association of the rs191631 SNP with radiation toxicity. The *SLC16A10* gene encodes a member of the solute carrier family-16 proteins responsible for transporting aromatic amino acids across the plasma membrane. SLC16A10 mediates Na+-independent transport of tryptophan, tyrosine, phenylalanine, and L-DOPA. The *SLC16A10* gene is found near the *REV3L* (*REV3* like) gene on chromosome 6, which encodes the DNA-directed polymerase zeta (*POLZ*) catalytic subunit, which in turn functions in trans-lesion DNA synthesis in a complex with *REV3L* and *MAD2L2* and has been implicated in platinum drug resistance [268]. It has been postulated that two specialised DNA polymerases are required for successful trans-lesion synthesis, with *POLZ* extending the DNA primer after *POLN* has inserted a nucleotide opposite the lesion [268]. This plays an important role in so-called DNA damage bypass, which are cellular mechanisms for tolerating unrepaired damage during the DNA replication [269].

Despite the harmonised and unified data collection method of the REQUITE cohort study, this analysis failed to replicate any of the previously reported significant associations with acute breast toxicity. While adjustment for centre to account for genetic variation was undertaken in this analysis, heterogeneity in toxicity reporting highlighted to some degree in phase 1 of the project could have reduced statistical power in this genotyping study. Further replication studies are required to investigate associations of individual SNPs with acute breast toxicity endpoints.

Conclusions

The accuracy of acute breast radiation toxicity prediction may be improved by the addition of genetic markers significantly associated with respective endpoints. The SNPs investigated in this analysis require further replication in future genetic association studies, but the *SLC16A10* rs191631 variant may be associated with acute ulceration following breast radiotherapy through gene-gene interactions with other *SLC16A10* and *REV3L* variants. These genetic markers should be validated in future prediction models in conjunction with clinical and treatment predictors.

General Discussion

Breast cancer is the most common malignancy in the UK and the second most common cancer worldwide [270]. More than 53,000 new cases are diagnosed in the UK per year, with one in eight women affected by the condition at some point in their lives [271]. Breast cancer constitutes a major public health issue with significant resource implications [272]. Given that per-patient healthcare costs are estimated to be in excess of £12,000 in the first 15 months following diagnosis (approximately £600m per annum), identifying the right treatment for the right patient is therefore both a clinical and research priority.

After surgery, radiotherapy is the second most commonly used treatment for breast cancer. It is personalised at the mechanistic level through the planning stage, through targeting of tissue, and through specific mode of delivery. However, there are currently no clinically useful tests capable of personalising radiotherapy by predicting whether or not a patient will develop serious normal tissue complications [273]. Radiation toxicity can impact negatively on a patient's surgical outcomes and on quality of life [105]. Little is known how patients' treatment decision-making may be influenced by prior knowledge of their personal risk of side-effects from radiotherapy.

With a focus on skin toxicity, this study was designed to explore how acute radiation toxicity can be predicted more accurately, in order to give breast cancer patients and clinicians better information to plan treatment. At first, breast cancer patients were recruited prospectively at Leicester and seven other European and North American centres into the REQUITE cohort study. Data on toxicity and QoL in the acute treatment phase were correlated with patient and treatment variables to identify those acute side-effects that could have a significant impact on QoL. In the REQUITE cohort, apart from the patient's breast size and BMI, the treatment variables post-operative seroma and radiation dose were significantly associated with acute toxicity. On the other hand, statin use appeared protective for acute ulceration or desquamation, and quadrantectomy (larger excision volume) was protective against oedema. Those QoL endpoints significantly associated with toxicity were fatigue, pain and breast symptoms, but not global health (overall QoL). However, global health remained associated with age, chemotherapy and alcohol use in the multivariate analysis.

Patients with breast cancer participating in the REQUITE study in Leicester were then interviewed to explore their attitudes towards predictive testing for radiotherapy side-effects and whether a test for acute toxicity would influence their treatment decision-making. The emerging themes from the patient interviews were: (1) Patients viewed a predictive radiogenomics test mainly as a medical test to be used by HCPs; (2) Undergoing the test may provoke anxiety and dread, therefore it will be

important how the HCP presents and frames the test results; and (3) In discussing treatment options after the test, the HCP should take into account the patient's preferences and priorities, which might include cancer cure and breast integrity.

In the third phase of the study, the predictive power of known clinical variables associated with acute skin toxicity (acute desquamation) was analysed in a combination of three existing Radiogenomics cohorts. However, the model failed to validate in the REQUITE breast cancer patients cohort, which may be attributed to cohort heterogeneity in terms of radiation treatment and will therefore require novel and different approaches to predictive modelling in the future.

In order to investigate the addition of genetic markers to improve future model performance, a systematic review and meta-analysis undertaken as part of this project identified a number of genetic variants associated with acute breast skin toxicity, some of which had not been significant in previous individual studies. Subsequent validation of these and other genetic markers in the REQUITE breast cancer cohort failed to confirm any significant individual associations, but identified an association between markers near the *REV3L* gene with acute ulceration.

Surgical context

The results of the present study demonstrate that a predictive test for acute breast toxicity has the potential to personalise breast cancer care. In the future, any such test could be tailored towards different endpoints, e.g. pain or fibrosis (scarring), depending on clinical and patient need. Nevertheless, there is accumulating evidence that early reactions to radiotherapy can be associated with the development of late toxicity [104]. Late toxicity, such as fibrosis or atrophy (shrinkage) not only affects patient QoL but also affects cosmetic outcomes from breast surgery.

This study also shows that patients have the confidence in a predictive test, but would prefer the result to be provided to healthcare professionals. As the vast majority of patients with a new diagnosis of breast cancer first see a surgeon, breast surgeons are uniquely placed in using this predictive information to guide the patient through treatment planning as part of the multi-disciplinary breast cancer team. Surgery remains the primary treatment for the majority of patients with early or locally advanced breast cancer, and treatment planning must take into account the impact of radiotherapy on cosmesis and QoL, which is particularly important in the setting of breast reconstruction and oncoplastic surgery.

While patients should be encouraged to be actively involved in decision-making regarding their breast cancer treatment, this study has confirmed that some patients may find this as a burden. It is the responsibility of the multidisciplinary team including the surgeon to ensure that patients are given all relevant evidence and personalised information in an understandable manner to be able to choose the treatment of the patient's true preference. Breast conservation on the one hand and breast reconstruction on the other hand, are often routinely offered with the assumption that both will improve QoL over mastectomy [274].

However, to date there is no level 1 evidence regarding optimal practice in breast reconstruction [43]. Moreover, a range of oncoplastic and reconstructive techniques are available, which makes decision-making for breast reconstruction complex with reports of dissatisfaction and regret not uncommon [275]. Informed clinical decision-making is also hampered by a lack of high-quality short and long-term PROMs and QoL data for the different reconstructive techniques [276]. Specific controversies in breast reconstruction include the type and timing of surgery in the context of adjuvant radiotherapy. A well designed randomised trial addressing this issue failed to recruit [31], so reconstructive techniques involving implants would still not currently be recommend when the expectation of adjuvant radiotherapy is high [29]. Having a personalised test to predict the severity of radiation toxicity in the breast may therefore help to guide surgeons and patients in choosing the most appropriate reconstructive technique.

While long-term PROMs data for breast conservation are available from radiotherapy trials, these fail to show a convincing or significant benefit of different fractionation regimens and techniques over another in terms of quality of life [38, 157]. An earlier Cochrane review found no difference in patient satisfaction whether they have immediate or delayed or no reconstruction [277]. Similar evidence is emerging from recent observational studies that breast conservation and breast reconstruction may lead to similar QoL outcomes, provided that the reconstructed breast does not require irradiation [278]. This opens up the possibility of using a personalised predictive test of radiotherapy side-effects to counsel patients towards treatment options that avoid radiation if toxicity is predicted to be severe to ensure optimal QoL outcomes.

It also highlights that it may be more important to predict QoL for each patient according to different treatment options, rather than recommending one treatment based on empirical evidence for its QoL benefit or lack thereof. Carefully conducted research is required to help the decision-making process and specific aids may reduce decisional conflict and increase knowledge about options [279]. In the context of breast reconstruction, there are several examples of interventions that support shared decision-making by clarifying pre-surgical expectations but they required further evaluation to confirm efficacy [280, 281].

Future directions

Further research should focus on validation of predictive models for both toxicity and QoL endpoints using available data from existing trials or patient cohorts. For example, the German ISE study collected PROMs data which was never published. While clinical data from the Cambridge IMRT trial cohort was made available for the present study, QoL data for the Cambridge IMRT trial cohort had been published elsewhere [157].

Despite the unified protocol, data collection methods in the REQUITE breast cohort could have been improved, in particular as the CTCAE v4.0 scale used for toxicity scoring did not specifically differentiate patients with mild and more severe desquamation over the breast, although skin loss was separately captured by the endpoint ulceration. This may have contributed to the heterogeneity observed in terms of acute endpoint distribution, in addition to the heterogeneity in terms of treatment already highlighted in this report.

The results from the qualitative interview study with Leicester patients merit similar evaluation in other countries, and interviews are under way or have been completed in at least two further REQUITE centres (Ghent and Milan). Moreover, since patients regard the test as for use by HCPs, clinicians' views on a predictive test for radiation toxicity should also be explored.

The p-values indicated for associations in the systematic review and meta-analysis were significant at the 0.05 level but none were significant at 0.01 or smaller thresholds commonly used in genome-wide association studies. This probably implies that the true genetic variants associated with acute breast radiation toxicity are yet to be identified. The results for variants significant after exclusion of individual studies from the meta-analysis would also benefit from further investigation of influence of ethnicity and other patient parameters known to play a role in the phenotype.

The interim candidate SNP validation undertaken in the REQUITE breast cohort highlights the challenges in linking genotype with phenotype, but a focus on validation rather than discovery will enable definitive findings regarding the importance of individual genetic variants that are starting to emerge from meta-analyses of existing GWAS. The GWAS data of the REQUITE cohort and analysis of the acute breast toxicity phenotypes are therefore eagerly anticipated. Where it is feasible to conduct high-throughput genotyping retrospectively in radiogenomics cohort used in the present study, this would enable emerging variants from one GWAS to be validated in the REQUITE GWAS together with clinical predictors to assess any improvement in model performance through the addition of SNPs or other genetic variants. Limited SNP data has been made available for the LeND and ISE cohorts, but integration with patient and treatment data for predictive modelling was beyond the scope of this project.

To date it is not known whether genetic loci that affect risk for normal tissue toxicity in radiotherapy also affect tumour radiosensitivity. Breast tumours are genetically diverse from normal tissue. They are characterised by copy number variations (CNV) and single nucleotide polymorphisms (SNP) in regions that regulate genes involved in DNA damage control and hence in the response to radiation [282]. Furthermore, several gene expression studies have been published with the aim of developing a genetic radiosensitivity signature for both tumours and normal tissue, but none have been validated on a large scale for clinical use [283-285].

In the clinical context, the finding that statin use and certain surgical techniques (quadrantectomy) were protected for acute radiation toxicity merits further investigation in cross-sectional analyses and possible future interventional trials. The clinical rationale behind using predictive information about radiation toxicity to direct treatment is summarised in Figure 28 and Figure 29. The ability to identify those patients likely to develop toxicity could enable radiation oncologists to individualise radiotherapy dose, which should improve survival and decrease morbidity. Similarly, surgeons could offer patients alternative local treatment that avoids radiotherapy altogether while maintaining tumour control and QoL outcomes.

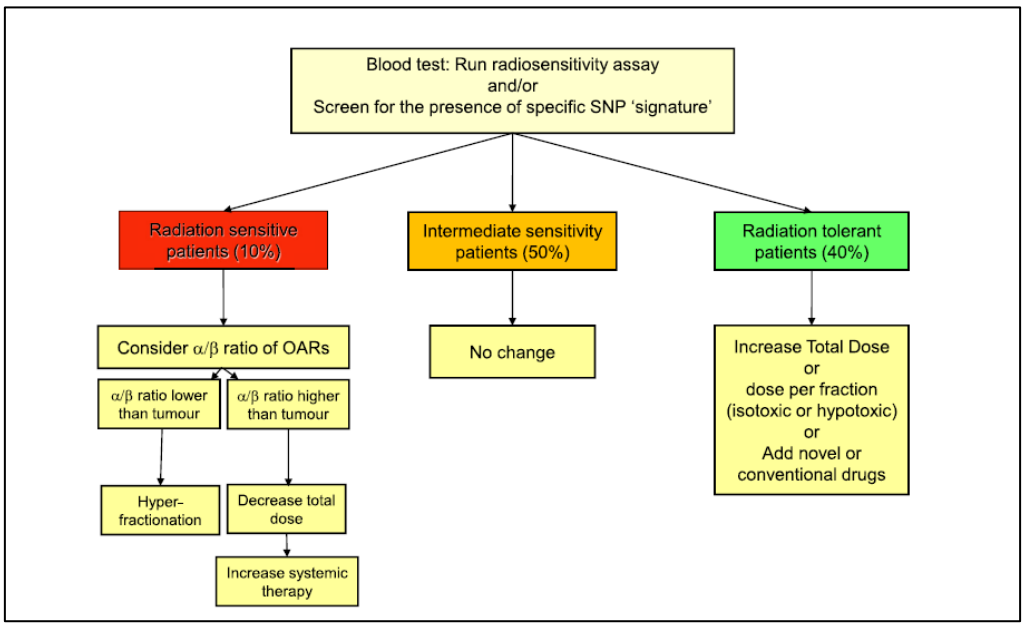


Figure 28. Schema for radiation treatment modification based on a predictive test of radiosensitivity showing the population of radiotherapy patients divided into three groups (from [112]).

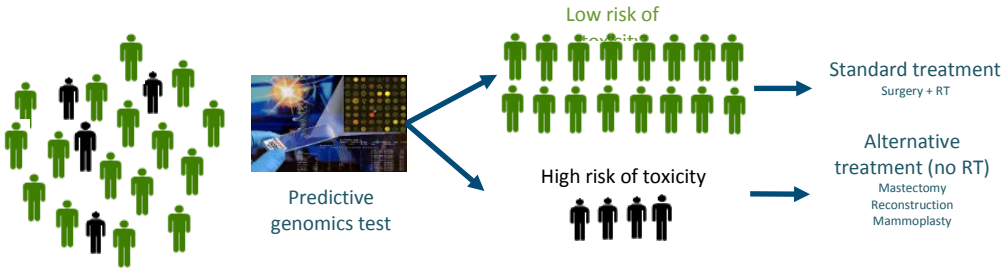


Figure 29. Schema for surgical treatment modification for breast cancer patients based on predicted risk of radiation toxicity.

Ultimately, this will lead to impact studies designed to evaluate the effect of predictive tests on clinician behavior and patient outcomes. This is often done comparing outcomes between clinicians provided with output from the predictive model to a control group without the predictive model. Although this is best done using a site-randomized controlled trial approach, it may also be assessed using a pre-post study design [286]. A potential intermediate step using decision modeling techniques or Markov modeling could be used to estimate the potential consequences and benefits of using a predictive model. If such an interim analysis did not reveal improved patient outcomes, this would obviate the need for formal impact studies.

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Appendix

1. REQUITE study pro-formas
2. REQUITE-AB-QoL interview guide
3. PLINK outputs from interim candidate genotyping in REQUITE-AB cohort
4. Accepted manuscripts

REQUITE
Validating Predictive Models and Biomarkers of Radiotherapy Toxicity to Reduce Side-Effects and Improve Quality-of-Life in Cancer Survivors

B2a

BREAST PATIENT FACTORS – BASELINE

(to be completed pre-radiotherapy)

Study Number RQ☐☐☐☐☐-☐

Patient Initials ☐☐☐☐

Date of Birth (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Date Completed (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Name + Signature of Person completing the CRF _____

Height (cm) ☐☐☐

Weight at cancer diagnosis (kg) ☐☐☐

Age at start of radiotherapy (yrs) ☐☐

Bra cup size ☐
1=AA
2=A
3=B
4=C
5=D
6=E/DD
7=F (E Italy)
8=G (F Italy
9=H (FF Italy)
10=J (G Italy)
11> J (G Italy)

Band size ☐
1=28 (UK)
2=30 (UK)
3=32 (UK) 70 (EU) 85 (Fr) 1 (It)
4=34 (UK) 75 (EU) 90 (Fr) 2 (It)
5=36 (UK) 80 (EU) 95 (Fr) 3 (It)
6=38 (UK) 85 (EU) 100 (Fr) 4 (It)
7=40 (UK) 90 (EU) 105 (Fr) 5 (It)
8=42 (UK) 95 (EU) 110 (Fr) 6 (It)
9=44 (UK) 100 (EU) 115 (Fr) 7 (It)
10>above

Smoker ☐
0=Never
1=Ex before cancer diagnosis
2=Ex since cancer diagnosis
3=Current
7=Do not wish to answer

If ever smoker
Duration of smoking (yrs) ☐☐
No. of tobacco products a day ☐☐☐

If ex smoker before cancer diagnosis: Time since quitting smoking (yrs) ☐☐

Tobacco product _____

Alcohol intake ☐
0=Never
1=Previously consumed alcohol, but stopped BEFORE cancer diagnosis
2=Previously consumed alcohol, but stopped AT cancer diagnosis
3=Current
7=Do not wish to answer

Previous alcohol consumption: Approximate number of alcoholic drinks a week ☐☐☐
777=Do not wish to answer
888=Not applicable

Current alcohol consumption: Approximate number of alcoholic drinks a week ☐☐☐
777=Do not wish to answer
888=Not applicable

Menopausal status at time of cancer diagnosis	□	1=Pre 2=Post 3=Peri	If postmenopausal, age of menopause (yrs)	□□	
			If postmenopausal, use of menopausal hormone replacement therapy?	□	0=No 1=Yes
Diabetes	□	0=No 1=Yes	If yes, duration (yrs)	□□	
History of heart disease	□	0=No 1=Yes	If yes, duration (yrs)	□□	
Rheumatoid Arthritis	□	0=No 1=Yes	If yes, duration (yrs)	□□	
Systemic Lupus Erythematosus	□	0=No 1=Yes	If yes, duration (yrs)	□□	
Other collagen vascular disease	□	0=No 1=Yes	If yes, duration (yrs)	□□	
Hypertension	□	0=No 1=Yes	If yes, duration (yrs)	□□	
Depression	□	0=No 1=Yes	If yes, duration (yrs)	□□	
Medication at cancer diagnosis					
On anti-diabetic drug?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
On ACE inhibitor?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
On other anti-hypertensive drug?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
On statin?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
On other lipid-lowering drugs?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
On amiodarone?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
On analgesics?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
On anti-depressant?	□	0=No 1=Yes	If yes, duration (yrs)	□□	
Family history of breast cancer in first degree relative	□	0=No 1=Yes	Family history of radiotherapy toxicity	□	0=No 1=Yes 9=Not known

Other co-morbidity _____

Previous Malignancies? ☐ 0=No 1=Yes

Which type?

ICD-10 / ICD-O-3 coding: □□□.□ / □□□□/□

Date of diagnosis (dd/mm/yyyy) □□/□□/□□□□

Therapy received for previous malignancy

Surgery ☐ 0=No 1=Yes
Hormonal therapy ☐ 0=No 1=Yes
Chemo therapy ☐ 0=No 1=Yes
Radio therapy ☐ 0=No 1=Yes
Other therapy ☐ 0=No 1=Yes
No therapy ☐ 0=No 1=Yes

Date of last therapy for previous malignancy (dd/mm/yyyy) □□/□□/□□□□

Ethnicity ☐☐

1=White (European or American European)
2=White and Black Caribbean Mixed
3=White and Black African Mixed
4=White and Asian Mixed
5=Hispanic American
6=Turkish
7=Indian
8=Pakistani
9=Bangladeshi
10=Chinese
11=Japanese
12=Other Asian
13=Black Caribbean
14=Black African
15=Northern African
16=African American
17=Jewish Ashkenazi
18=Jewish Sephardi
19=Any Other Ethnic Background; please specify _____
77=Patient refused to give answer

Highest educational/professional qualification received

☐

1=Primary school
2=Secondary school (Please select an option _____)
3=Professional school (e.g. technical. Please specify type _____)
4=University (or equivalent)
5=Others, please specify _____
7=Do not wish to answer

Options for "Secondary school":
a. UK: GSCE / O level
b. UK: A level
c. US: High school
d. B: Algemeen Secundair Onderwijs
e. GER: Hauptschule
f. GER: Realschule/Mittlere Reife
g. GER: Gymnasium/Abitur
h. CH: Realschule
i. CH: Sekundarschule
j. CH: Gymnasium / Matura
k. F: college
l. F: lycée/baccalaureate
m. I: scuola secondaria di primo grado
n. I: scuola secondaria di secondo grado
o. NL: voortgezet onderwijs
p. SP: Educación Secundaria Obligatoria/Bachillerato
q. Other, please specify
_____

Net household income (average) per month

☐☐

1=<1.000 €
2=1.000-<2.000€
3=2.000-<3.000€
4=3.000-<4.000€
5=4.000-<5.000€
6=5.000-<6.000€
7=6.000-<7.000€
8=7.000-<8.000€
9=8.000€ and higher
77= Do not wish to answer

Number of household members ☐☐

REQUITE
Validating Predictive Models and Biomarkers of Radiotherapy Toxicity to Reduce Side-Effects and Improve Quality-of-Life in Cancer Survivors

BREAST CLINICAL AND TREATMENT DATA COLLECTION FORM
(to be completed at end of radiotherapy)

Study Number RQ☐☐☐☐☐-☐

Patient Initials ☐☐☐☐

Date of Birth (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Date Completed (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Name + Signature of Person completing the CRF _____

Primary Surgery & Tumour Pathology

Surgery	☐ 0=No 1=Yes	Surgery Date (dd/mm/yyyy)	☐☐/☐☐/☐☐☐☐	
Type of surgery	☐ 1=Segmentectomy/Quadrantectomy 2=Wide local excision 9=Not known			
Axillary Surgery	☐ 0=No 1=Yes 9=Not known	If Yes… please state type	☐	1=Sentinel node biopsy 2=Planned axillary dissection 3=Sentinel node biopsy plus axillary dissection
Number of Nodes Involved	☐☐	Number of Nodes examined	☐☐	
Persistent post operative haematoma/ haematoseroma	☐ 0=No 1=Yes, with delayed RT 2=Yes, without delayed RT 9=Not known	Post operative oedema	☐	0=No 1=Yes 9=Not known
Post operative infection	☐ 0=No 1=Yes 9=Not known	If yes, infection requiring antibiotics	☐	0=No 1=Yes, oral 2=Yes, intravenous 9=Not known
Delayed healing >3 wks following surgery?	☐ 0=No 1=Yes 9=Not known			
Side of Primary	☐ 1=Left 2=Right	Quadrant	☐	1=Upper 12h 2=Upper Outer 3=Upper Inner 4=Central 5=Lower 6h 6=Lower Outer 7=Lower Inner 8=Other: specify_____ 9=Not specified
Locality	☐ 1=Unifocal 2=Multifocal 3=Multicentric >1 quadrant			

Histological Grade □ 1=Well 2=Moderate 3=Poor 4=Undifferentiated 9=Not known

Histological Type □ 1=Infiltrating ductal 2=Infiltrating lobular 3=DCIS 4=Tubular 5=Other : specify_____ 9=Not known

Pathological tumour size (mm) □□□

Pathologic UICC stage □ T (Tis, 1a-c, 2, 3, 4a-d, X) □ N (0-3, X)

□ M (0,1, X) □ R (0, 1, 2, X)

If neo-adjuvant chemotherapy, clinical UICC stage □ T (Tis, 1a-c, 2, 3, 4a-d, X) □ N (0-3, X) □ M (0,1, X)

Ki-67 status (%) □□□

ER Status □ 1=Positive (>10%) 2=Negative (≤10%) 9=Not known

HER-2 status □ 1=Positive 2=Negative 9=Not known

PR Status □ 1=Positive (>10%) 2=Negative (≤10%) 9=Not known

Neo-adjuvant chemotherapy □ 0=No 1=Yes

If yes,

Anthracycline Chemotherapy □ 0=No 1=Yes 9=Not known

Date Started (dd/mm/yyyy) □□/□□/□□□□

Date of last dose (dd/mm/yyyy) □□/□□/□□□□

If yes, Number of cycles □□

If yes, Drugs used _____

Non-anthracycline Chemotherapy □ 0=No 1=Yes 9=Not known

Date Started (dd/mm/yyyy) □□/□□/□□□□

Date of last dose (dd/mm/yyyy) □□/□□/□□□□

If yes, Number of cycles □□

If yes, Drugs used _____

Adjuvant chemotherapy □ 0=No 1=Yes

If yes,

Anthracycline Chemotherapy □ 0=No 1=Yes 9=Not known

Date Started (dd/mm/yyyy) □□/□□/□□□□

Date of last dose (dd/mm/yyyy) □□/□□/□□□□

If yes, Number of cycles □□

If yes, Drugs used _____

Non-anthracycline Chemotherapy □ 0=No 1=Yes 9=Not known

Date Started (dd/mm/yyyy) □□/□□/□□□□

Date of last dose (dd/mm/yyyy) □□/□□/□□□□

If yes, Number of cycles □□

If yes, Drugs used _____

Other systemic treatment □ 0=No 1=Yes

If yes,

Tamoxifen □ 0=No 1=Yes 9=Not known

If yes, (planned) Date Started (dd/mm/yyyy) □□/□□/□□□□

Aromatase Inhibitor □ 0=No 1=Yes 9=Not known

If yes, (planned) Date Started (dd/mm/yyyy) □□/□□/□□□□

Anti-HER2 / targeted therapy □ 0=No 1=TKI 2=MAb 9=Not known

If yes, (planned) Date Started (dd/mm/yyyy) □□/□□/□□□□

If yes, Number of cycles □

If yes, Drug 1 used _____

More than 1 drug?

□ 1=Yes 0=No

If yes, Number of cycles □

If yes, Drug 2 used _____

Date Started (dd/mm/yyyy) □□/□□/□□□□

Radiotherapy

*If information is **Not Known** fill boxes with **9's,** if **Not Applicable** with **8's***

Whole breast radiotherapy (<u>without</u> boost dose)

Date whole breast radiotherapy started (dd/mm/yyyy) □□/□□/□□□□

Date whole breast radiotherapy finished (dd/mm/yyyy) □□/□□/□□□□

Radiotherapy interrupted >3 days due to complications □
0=No
1=Yes
9=Not known

If yes, number of days interrupted □□

If yes, give <u>detailed</u> reason _____

IMRT □
0=No
1=Yes
9=Not known

Type of IMRT □
1=Simple field in field
2=Complex highly modulated

Treatment position □
1=Prone
2=Supine

3D □
0=No
1=Yes
9=Not known

Treated breast □
1=Left
2=Right

Axillary levels treated □
0=None
1=I
2=II
3=III
4=I-III
5=Other: specify_____
9=Not known

Supraclavicular fossa □
0=No
1=Yes
9=Not known

Delivered whole breast dose (Gy) □□•□□

Photon (MV) □□□

Number of fractions □□

Dose per fraction (Gy) □•□□

Fractions per week □□

Second Photon: □
0=No
1=Yes

If mixed: second Photon (MV) □□□

Number of fractions □□

Dose per fraction (Gy) □•□□

Fractions per week □□

Additional Parameters (cumulative if with boost, otherwise whole breast RT only)

CT breast volume (cm^3) (see definition below) □□□□

Max skin dose (Gy) □□□•□□

*<u>Definition for **breast**</u>:*
A wire can be used on the CT scan around the palpable breast tissue to define the peripheral edges of the breast. The deep edge is the superficial side of the pectoral muscle/thoracic wall. The superficial edge is the skin. Any visible glandular breast tissue outside these margins should also be included.

Breast delineation □ 1=Definition above
2=Others _____

*<u>Definition for **skin**</u>:*
Skin is defined as the difference between the body contour and an inner isotropic contour from the body (5mm).

Skin delineation □ 1=Definition above
2=Others
_____

Internal mammary volume (cm^3) □□□□

Whole breast Hot spots (incl. skin) with >107% of Prescribed Dose (cm^3) □□□

If yes, quadrant: □

1=Upper 12h
2=Upper Outer
3=Upper Inner
4=Central
5=Lower 6h
6=Lower Outer
7=Lower Inner
8=Other: specify_____

Mean Heart Dose (Gy) (see definition) □□•□□

*Definition for **heart**:*
Superiorly the heart starts just inferior to the left pulmonary artery. It includes the atria, ventricles, auricles, vessels and fat tissue within the pericardium. Since the cardiac vessels run in the fatty tissue within the pericardium, they should be included in the contours, even if there is no heart muscle visible in that area. Inferiorly, the heart blends with the diaphragm. Feng et al 2011 PMID: 20421148

Mean Ipsilateral Lung Dose (Gy) □□•□□

Heart delineation □

1=Definition above
2=Left ventricle only
3=Others
_____

Boost (additional to the whole breast radiation) □

0=No
1=Yes

If yes,
Boost sequence □

1=Sequential including IORT boost
2=Simultaneous integrated
3=Simultaneous integrated and sequential

Date boost started (dd/mm/yyyy) □□/□□/□□□□

Date boost finished (dd/mm/yyyy) □□/□□/□□□□

Breast boost type □

1=Electrons
2=Photons
3=Brachytherapy
4=Intra-operative boost
5=Electrons+Photons

Bolus □

0=No
1=Yes
9=Not known

If photon boost or brachytherapy:

Photon energy (MV or kV) □□

Photon/brachytherapy Boost Volume (cm^3) □□□

Boost dose (Gy) □□•□□

Number of boost fractions □□

Dose per fraction (Gy) □□•□□

Fractions per week □□

Second Photon: □ 0=No 1=Yes

If yes, second Photon (MV or kV) □□

Photon/brachytherapy Boost Volume (cm^3) □□□

boost dose (Gy) □□•□□

Number of boost fractions □□

Dose per fraction (Gy) □□•□□

Fractions per week □□

If electron boost:

Electron energy (MeV) □□

Electron boost field size (cm) □□•□x□□•□

Circular electron boost diameter (cm) □□

Electron boost dose (Gy) □□•□□

Number of boost fractions □□

Dose per fraction (Gy) □□•□□

Fractions per week □□

REQUITE
Validating Predictive Models and Biomarkers of Radiotherapy Toxicity to Reduce Side-Effects and Improve Quality-of-Life in Cancer Survivors

B5

HEALTH PROFESSIONAL BREAST TOXICITY DATA (CTCAEv4.0)

To be completed by the Doctor or Research Nurse ONLY

Study Number RQ☐☐☐☐☐-☐

Patient Initials ☐☐☐☐

Date of Birth (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Date Completed (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Name + Signature of Person completing the CRF _____

Time Point

- ☐ Pre-radiotherapy (RT)
- ☐ End of RT
- ☐ 6 weeks after end of RT*
- ☐ 1 year after RT start
- ☐ 2 years after RT start
- ☐ 3 years after RT start*
- ☐ 4 years after RT start*

* if available

If information is **Not Known** fill boxes with 9's, if **Not Applicable** with 8's

Is there atrophy within the treated breast? ☐
- 0 = None
- 1 = Minimal asymmetry; minimal atrophy
- 2 = Moderate asymmetry; moderate atrophy
- 3 = Asymmetry >1/3 of breast volume; severe atrophy

Is there any nipple retraction of the treated breast? ☐
- 0 = None
- 1 = Asymptomatic; asymmetry with slight retraction and/or thickening of the nipple areolar complex
- 2 = Symptomatic; asymmetry of nipple areolar complex with moderate retraction and/or thickening of the nipple areolar complex

Is there oedema of the treated breast? ☐
- 0 = None
- 1 = Swelling or obscuration of anatomic architecture on close inspection
- 2 =Readily apparent obscuration of anatomic architecture; obliteration of skin folds; readily apparent deviation from normal anatomic contour; limiting instrumental activities of daily living (ADL)
- 3= Gross deviation from normal anatomic contour; limiting self care ADL

Is there any skin ulceration? ☐
- 0 = None
- 1 = Combined area of ulcers <1cm nonblanchable erythema of intact skin with associated warmth or edema
- 2 = Combined area of ulcers 1-2cm; partial thickness skin loss involving skin or subcutaneous fat
- 3 = Combined area of ulcers >2cm; full thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to fascia
- 4 = Any ulcer size with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures with or without full thickness skin loss

Is there any telangiectasia of the tumour bed? ☐
- 0 = None
- 1 = Telangiectasia covering <10% of the treated breast
- 2 = Telangiectasia covering >10% of the treated breast; associated with psychosocial impact

Is there any telangiectasia outside the tumour bed? ☐
- 0 = None
- 1 = Telangiectasia covering <10% of the treated breast
- 2 = Telangiectasia covering >10% of the treated breast; associated with psychosocial impact

Is there any skin induration (fibrosis) of the tumour bed? ☐
- 0 = None
- 1 = Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)
- 2 = Moderate induration, able to slide skin, unable to pinch skin, limiting instrumental activities for daily living (ADL)
- 3 = Severe induration; unable to slide or pinch skin; affecting activities for daily living; limiting self care ADL

Is there any skin induration (fibrosis) outside the tumour bed? ☐
- 0 = None
- 1 = Mild induration, able to move skin parallel to plane (sliding) and perpendicular to skin (pinching up)
- 2 = Moderate induration, able to slide skin, unable to pinch skin, limiting instrumental activities for daily living (ADL)
- 3 = Severe induration; unable to slide or pinch skin; affecting activities for daily living; limiting self care ADL

Erythema ☐
- 0 = None
- 1 = Faint erythema or dry desquamation
- 2 = Moderate to brisk erythema; patchy moist desquamation, mostly confined to skin folds and creases; moderate oedema
- 3 = Moist desquamation in areas other than skin folds and creases; bleeding induced by minor trauma or abrasion
- 4 = Life-threatening consequences; skin necrosis or ulceration of full thickness dermis; spontaneous bleeding from involved site; skin graft indicated

Arm lymphoedema ☐
- 0 = None
- 1 = 5-10% inter limb discrepancy in volume or circumference at point of greatest visible difference; swelling or obscuration of anatomic architecture on close inspection
- 2 = >10-30% inter limb discrepancy in volume or circumference at point of greatest visible difference; Readily apparent obscuration of anatomic architecture; obliteration of skin folds; readily apparent deviation from normal anatomic contour; limiting instrumental activities of daily living (ADL)
- 3 = > 30% inter-limb discrepancy in volume; gross deviation from normal anatomic contour; limiting self care ADL

Skin hyperpigmentation ☐
- 0 = None
- 1 = Hyperpigmentation or depigmentation covering <10% body surface area (BSA); no psychosocial impact
- 2 = Hyperpigmentation or depigmentation covering >10% BSA; associated with psychosocial impact

Pneumonitis ☐
- 0 = None
- 1 = Asymptomatic; clinical or diagnostic observations only; intervention not indicated
- 2 = Symptomatic; medical intervention indicated; limiting instrumental activities of daily living (ADL)
- 3 = Severe symptoms; limiting self care ADL; oxygen indicated
- 4 = Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)

Questions for the patient

Pain

Have you had any pain in your treated breast in the last two weeks? ☐ 0=No 1=Yes

If yes, how severe is the pain? ☐ 1=Mild 2=Moderate, limiting usual activities 3=Severe, stopping activities

Are you taking any medication for this pain? ☐ 0=No 1=Yes

If yes, please give name of medication and how often you take this.

drug name _____

dose and frequency _____

Swollen arm

Do you have a swollen arm? ☐ 0=No 1=Yes

If yes, does your swollen arm interfere with normal activity? ☐ 1=No 2=Limiting activity 3=Limiting self care

---

Mid-upper arm circumference

Left arm (mm) ☐☐☐

Right arm (mm) ☐☐☐

☐☐/☐☐/☐☐☐☐ Measuring Date (dd/mm/yyyy)

If information is **Not Known** fill boxes with 9's, if **Not Applicable** with 8's

PATIENT QUESTIONNAIRE – BASELINE

To be completed pre-radiotherapy

Study Number RQ☐☐☐☐☐-☐

Patient Initials ☐☐☐☐

Date of Birth (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Date Completed (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

B6a_B EORTC C30 (version 3)

Instructions:

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the <u>past</u> week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4

-- Please go on to the next page --

During the past week:	Not at All	A Little	Quite a Bit	Very Much
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7
Very poor ---------- Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7
Very poor ---------- Excellent

-- Please go on to the next page --

B6b_B EORTC BR23 (Version 1.0)

Instructions:

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:		Not at All	A Little	Quite a Bit	Very Much
31.	Did you have a dry mouth?	1	2	3	4
32.	Did food and drink taste different than usual?	1	2	3	4
33.	Were your eyes painful, irritated or watery?	1	2	3	4
34.	Have you lost any hair?	1	2	3	4
35.	Answer this question only if you had any hair loss: Were you upset by the loss of your hair?	1	2	3	4
36.	Did you feel ill or unwell?	1	2	3	4
37.	Did you have hot flushes?	1	2	3	4
38.	Did you have headaches?	1	2	3	4
39.	Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
40.	Have you been feeling less feminine as a result of your disease or treatment?	1	2	3	4
41.	Did you find it difficult to look at yourself naked?	1	2	3	4
42.	Have you been dissatisfied with your body?	1	2	3	4
43.	Were you worried about your health in the future?	1	2	3	4

During the past four weeks:		Not at All	A Little	Quite a Bit	Very Much
44.	To what extent were you interested in sex?	1	2	3	4
45.	To what extent were you sexually active? (with or without intercourse)	1	2	3	4
46.	Answer this question only if you have been sexually active: To what extent was sex enjoyable for you?	1	2	3	4

-- Please go on to the next page --

During the <u>past</u> week:		Not at All	A Little	Quite a Bit	Very Much
47.	Did you have any pain in your arm or shoulder?	1	2	3	4
48.	Did you have a swollen arm or hand?	1	2	3	4
49.	Was it difficult to raise your arm or to move it sideways?	1	2	3	4
50.	Have you had any pain in the area of your affected breast?	1	2	3	4
51.	Was the area of your affected breast swollen?	1	2	3	4
52.	Was the area of your affected breast oversensitive?	1	2	3	4
53.	Have you had skin problems on or in the area of your affected breast (e.g., itchy, dry, flaky)?	1	2	3	4

-- Please go on to the next page --

B6c_B Hopwood Body Image Scale (HBIS)

Instructions:

We are interested in how you feel about your appearance, and about any changes that may have resulted from your disease or treatment. Please answer all of the questions yourself by circling the number under the reply that comes closest to the way you have been feeling about yourself, during the past week. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

During the <u>past</u> week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
1. Have you been feeling self-conscious about your appearance?	1	2	3	4
2. Have you felt less physically attractive as a result of your disease or treatment?	1	2	3	4
3. Have you been dissatisfied with your appearance when dressed?	1	2	3	4
4. Have you been feeling less feminine as a result of your disease or treatment?	1	2	3	4
5. Did you find it difficult to look at yourself naked?	1	2	3	4
6. Have you been feeling less sexually attractive as a result of your disease or treatment?	1	2	3	4
7. Did you avoid people because of the way you felt about your appearance?	1	2	3	4
8. Have you been feeling the treatment has left your body less whole?	1	2	3	4
9. Have you felt dissatisfied with your body?	1	2	3	4
10. Have you been dissatisfied with the appearance of your scar?	1	2	3	4

-- Please go on to the next page –

B6d_B Multidimensional Fatigue Inventory (MFI)

Instructions:

By means of the following statements we would like to get an idea how you have been feeling **in the past week**. There is, for example, the statement "I feel relaxed". If you think that this is **entirely true**, that indeed you have been feeling relaxed lately, please, place an X in the extreme left box, like this:

yes, that is true ☒1 ☐2 ☐3 ☐4 ☐5 no, that is not true

The more you **disagree** with the statement, the more you place an X in the direction of "no, that is not true". Please do not miss out a statement and place only one X in a box for each statement.

1.	I feel fit.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
2.	Physically I feel only able to do a little.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
3.	I feel very active.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
4.	I feel like doing all sorts of nice things.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
5.	I feel tired.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
6.	I think I do a lot in a day.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
7.	When I am doing something, I can keep my thoughts on it.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
8.	Physically I can take on a lot.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
9.	I dread having to do things.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
10.	I think I do very little in a day.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
11.	I can concentrate well.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
12.	I am rested.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
13.	It takes a lot of effort to concentrate on things.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
14.	Physically I feel I am in a bad condition.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
15.	I have a lot of plans.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
16.	I tire easily.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
17.	I get little done.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
18.	I don't feel like doing anything.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
19.	My thoughts easily wander.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
20.	Physically I feel I am in an excellent condition.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true

For the following questions please circle the number between 0 and 10 that best applies to you

21.	How did you feel in the past week?	not tired at all	0	1	2	3	4	5	6	7	8	9	10	entirely tired
22.	How did you feel in the year before cancer diagnosis?	not tired at all	0	1	2	3	4	5	6	7	8	9	10	entirely tired

-- Please go on to the next page --

B6e_B Global Physical Activity Questionnaire (GPAQ)

Instructions:

Next we are going to ask you about the time you spend doing different types of physical activity **in a typical week in the year before cancer diagnosis**. Please answer these questions even if you do not consider yourself to be a physically active person.

Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, seeking employment. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

	Questions	Response	Code
Activity at work			
1	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *carrying or lifting heavy loads, digging or construction work* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 4*	P1
2	In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days ⌊__⌋	P2
3	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P3 (a-b)
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking *or carrying light loads* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 7*	P4
5	In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days ⌊__⌋	P5
6	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P6 (a-b)

-- Please go on to the next page --

Travel to and from places

The next questions exclude the physical activities at work that you have already mentioned.
Now we would like to ask you about the usual way you travel to and from places.
For example to work, for shopping, to market, to place of worship.

7	Do you walk or use a bicycle (*pedal cycle*) for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days ⌊__⌋	P8
9	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P9 (a-b)

Recreational activities

The next questions exclude the work and transport activities that you have already mentioned.
Now we would like to ask you about sports, fitness and recreational activities (leisure).

10	Do you do any vigorous-intensity sports, fitness or recreational (*leisure*) activities that cause large increases in breathing or heart rate like *running or football,* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 13*	P10
11	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P11
12	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P12 (a-b)
13	Do you do any moderate-intensity sports, fitness or recreational *(leisure*) activities that causes a small increase in breathing or heart rate such as brisk walking, (*cycling, swimming, volleyball*) for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P16*	P13
14	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P14
15	How much time do you spend doing moderate-intensity sports, fitness or recreational (*leisure*) activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P15 (a-b)

Sedentary behaviour

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.

16	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P16 (a-b)

Thank you very much.

REQUITE
Validating Predictive Models and Biomarkers of Radiotherapy Toxicity to Reduce Side-Effects and Improve Quality-of-Life in Cancer Survivors

B6_F

PATIENT QUESTIONNAIRE – FOLLOW-UP

Study Number RQ☐☐☐☐☐-☐

Patient Initials ☐☐☐☐

Date of Birth (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Date Completed (dd/mm/yyyy) ☐☐/☐☐/☐☐☐☐

Time Point

- ☐ End of radiotherapy (RT)
- ☐ 3 months after RT start
- ☐ 1 year after RT start
- ☐ 2 years after RT start
- ☐ 3 years after RT start*
- ☐ 4 years after RT start*

B6a_F EORTC C30 (version 3)

Instructions:

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4

Please go on to the next page

During the past week:	Not at All	A Little	Quite a Bit	Very Much
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4
17. Have you had diarrhoea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7
Very poor ---------- Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7
Very poor ---------- Excellent

Please go on to the next page

B6b_F EORTC BR23 (Version 1.0)

Instructions:

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:		Not at All	A Little	Quite a Bit	Very Much
31.	Did you have a dry mouth?	1	2	3	4
32.	Did food and drink taste different than usual?	1	2	3	4
33.	Were your eyes painful, irritated or watery?	1	2	3	4
34.	Have you lost any hair?	1	2	3	4
35.	Answer this question only if you had any hair loss: Were you upset by the loss of your hair?	1	2	3	4
36.	Did you feel ill or unwell?	1	2	3	4
37.	Did you have hot flushes?	1	2	3	4
38.	Did you have headaches?	1	2	3	4
39.	Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
40.	Have you been feeling less feminine as a result of your disease or treatment?	1	2	3	4
41.	Did you find it difficult to look at yourself naked?	1	2	3	4
42.	Have you been dissatisfied with your body?	1	2	3	4
43.	Were you worried about your health in the future?	1	2	3	4

During the past four weeks:		Not at All	A Little	Quite a Bit	Very Much
44.	To what extent were you interested in sex?	1	2	3	4
45.	To what extent were you sexually active? (with or without intercourse)	1	2	3	4
46.	Answer this question only if you have been sexually active: To what extent was sex enjoyable for you?	1	2	3	4

Please go on to the next page

During the <u>past</u> week:		Not at All	A Little	Quite a Bit	Very Much
47.	Did you have any pain in your arm or shoulder?	1	2	3	4
48.	Did you have a swollen arm or hand?	1	2	3	4
49.	Was it difficult to raise your arm or to move it sideways?	1	2	3	4
50.	Have you had any pain in the area of your affected breast?	1	2	3	4
51.	Was the area of your affected breast swollen?	1	2	3	4
52.	Was the area of your affected breast oversensitive?	1	2	3	4
53.	Have you had skin problems on or in the area of your affected breast (e.g., itchy, dry, flaky)?	1	2	3	4

Please go on to the next page

B6c_F Hopwood Body Image Scale (HBIS)

Instructions:

We are interested in how you feel about your appearance, and about any changes that may have resulted from your disease or treatment. Please answer all of the questions yourself by circling the number under the reply that comes closest to the way you have been feeling about yourself, during the past week. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

During the <u>past</u> week:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
1. Have you been feeling self-conscious about your appearance?	1	2	3	4
2. Have you felt less physically attractive as a result of your disease or treatment?	1	2	3	4
3. Have you been dissatisfied with your appearance when dressed?	1	2	3	4
4. Have you been feeling less feminine as a result of your disease or treatment?	1	2	3	4
5. Did you find it difficult to look at yourself naked?	1	2	3	4
6. Have you been feeling less sexually attractive as a result of your disease or treatment?	1	2	3	4
7. Did you avoid people because of the way you felt about your appearance?	1	2	3	4
8. Have you been feeling the treatment has left your body less whole?	1	2	3	4
9. Have you felt dissatisfied with your body?	1	2	3	4
10. Have you been dissatisfied with the appearance of your scar?	1	2	3	4

-- Please go on to the next page –

B6d_F Multidimensional Fatigue Inventory (MFI)

Instructions:

By means of the following statements we would like to get an idea how you have been feeling **in the past week**. There is, for example, the statement "I feel relaxed". If you think that this is **entirely true**, that indeed you have been feeling relaxed lately, please, place an X in the extreme left box, like this:

yes, that is true ☒1 ☐2 ☐3 ☐4 ☐5 no, that is not true

The more you **disagree** with the statement, the more you place an X in the direction of "no, that is not true". Please do not miss out a statement and place only one X in a box for each statement.

1. I feel fit.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
2. Physically I feel only able to do a little.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
3. I feel very active.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
4. I feel like doing all sorts of nice things.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
5. I feel tired.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
6. I think I do a lot in a day.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
7. When I am doing something, I can keep my thoughts on it.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
8. Physically I can take on a lot.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
9. I dread having to do things.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
10. I think I do very little in a day.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
11. I can concentrate well.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
12. I am rested.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
13. It takes a lot of effort to concentrate on things.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
14. Physically I feel I am in a bad condition.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
15. I have a lot of plans.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
16. I tire easily.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
17. I get little done.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
18. I don't feel like doing anything.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
19. My thoughts easily wander.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true
20. Physically I feel I am in an excellent condition.	yes, that is true	☐1	☐2	☐3	☐4	☐5	no, that is not true

For the following question please circle the number between 0 and 10 that best applies to you

21. How did you feel in the past week?	not tired at all	0	1	2	3	4	5	6	7	8	9	10	entirely tired

-- Please go on to the next page --

B6e_F Global Physical Activity Questionnaire (GPAQ)

Instructions:

Next we are going to ask you about the time you spend doing different types of physical activity **in a typical week in the past month**. Please answer these questions even if you do not consider yourself to be a physically active person.

Think first about the time you spend doing work. Think of work as the things that you have to do such as paid or unpaid work, study/training, household chores, harvesting food/crops, seeking employment. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate, 'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

	Questions	Response	Code
Activity at work			
1	Does your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like *carrying or lifting heavy loads, digging or construction work* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 4*	P1
2	In a typical week, on how many days do you do vigorous-intensity activities as part of your work?	Number of days ⌊__⌋	P2
3	How much time do you spend doing vigorous-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P3 (a-b)
4	Does your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as brisk walking *or carrying light loads* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 7*	P4
5	In a typical week, on how many days do you do moderate-intensity activities as part of your work?	Number of days ⌊__⌋	P5
6	How much time do you spend doing moderate-intensity activities at work on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P6 (a-b)

Please go on to the next page

Travel to and from places

The next questions exclude the physical activities at work that you have already mentioned.
Now we would like to ask you about the usual way you travel to and from places.
For example to work, for shopping, to market, to place of worship.

7	Do you walk or use a bicycle (*pedal cycle*) for at least 10 minutes continuously to get to and from places?	Yes 1 No 2 *If No, go to P 10*	P7
8	In a typical week, on how many days do you walk or bicycle for at least 10 minutes continuously to get to and from places?	Number of days ⌊__⌋	P8
9	How much time do you spend walking or bicycling for travel on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P9 (a-b)

Recreational activities

The next questions exclude the work and transport activities that you have already mentioned.
Now we would like to ask you about sports, fitness and recreational activities (leisure).

10	Do you do any vigorous-intensity sports, fitness or recreational (*leisure*) activities that cause large increases in breathing or heart rate like *running or football,* for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P 13*	P10
11	In a typical week, on how many days do you do vigorous-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P11
12	How much time do you spend doing vigorous-intensity sports, fitness or recreational activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P12 (a-b)
13	Do you do any moderate-intensity sports, fitness or recreational *(leisure*) activities that causes a small increase in breathing or heart rate such as brisk walking, (*cycling, swimming, volleyball*) for at least 10 minutes continuously?	Yes 1 No 2 *If No, go to P16*	P13
14	In a typical week, on how many days do you do moderate-intensity sports, fitness or recreational (*leisure*) activities?	Number of days ⌊__⌋	P14
15	How much time do you spend doing moderate-intensity sports, fitness or recreational (*leisure*) activities on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P15 (a-b)

Sedentary behaviour

The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.

16	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes ⌊__⌋⌊__⌋ : ⌊__⌋⌊__⌋ hrs mins	P16 (a-b)

Thank you very much.

Aim & Objectives

- To explore patients' attitudes to and beliefs about a predictive tool for acute radiotherapy side-effects and the impact on patients' decision-making
- To generate a thematic description of the patients' feelings, attitudes and concerns about any such predictive test

Patient Details

Initials_____ Study ID_____ Date_____

Start time_____ End time_____ Interview length_____

Interview location_____ Interviewer_____

Introduction

I am Dr Tim Rattay and I want to thank you for agreeing to talk with me today. I am a breast surgeon but I have also been working with the University on the REQUITE study which you are taking part in. The aim of the interview today is to get your views, thoughts and feelings on what we are developing as part of the REQUITE study; that is, a test which is going to tell us how breast cancer patients will react to radiotherapy, in order to reduce the side-effects from treatment for all patients.

As you know from the consent form, I will be recording this interview. Your answers will be typed up later so that I can compare the opinions of different patients. I will put everyone's answers together at the end and I may use specific quotes, but there will be no way to link those quotes to you.

Do you have any questions before we start the interview? _____

Before we start, I need to remind you about a couple more things:

- As part of this interview I can<u>not</u> give you any advice on your own treatment but I can let you know who to contact if you have any concerns.
- This interview is completely anonymous. I want you to feel free to discuss what you like and what you don't like. And to further protect your privacy, if you like, I can call you by your first name only, by your initials, or even by a fictional name.

What would you like me to call you? _____

Interview

OK, let's begin. For recording purposes, this is recording XXX (ID#) conducted by [interviewer] on DD/MMM/YEAR.

As a surgeon, I have seen that some women with breast cancer struggle with the decision between having a lumpectomy plus radiotherapy or mastectomy alone, given that the cure rates are the same. An important part of this decision is the patients' expectations of radiation treatment. Patients are told that radiotherapy can be associated with side-effects such as skin irritation, peeling, discomfort or discoloration, but aren't sure how it will affect them personally.

- For example, knowing that a percentage of women will have a tough time with skin changes during radiotherapy is different from knowing that I (me) will have a tough time with skin changes - does that make sense?

_____

_____

_____

- Can you talk to me a bit about how you expected to fare during radiotherapy and whether the treatment affected you as you thought it would?

_____

_____

_____

At the moment, we cannot tell in advance whether a particular woman will have only very mild skin changes or very severe ones. That's why I am working with a team of researchers on developing a new kind of test to give us just that information. In the future, this test will be able to tell a given patient her own individual risk of having skin changes, and the severity of those skin changes.

- I've found that this idea can be a little difficult to explain clearly. So far, does it make sense to you? - Or would you like me to clarify?

_____

_____

_____

Great, now let me describe this test to you:

1. It will have two parts. First, it will involve asking some questions about your general health and breast shape and size.

2. Second, it will also require taking some blood to send for genetic testing – no more than one tube-full. We want to send the blood for genetic testing because we know that the extent to which someone develops skin changes during radiotherapy is related to their genes.

The test will be done shortly after diagnosis, but before any treatment starts, because the goal of the test is to help make treatment decisions. After the test results are back, which should take about a week, patients will have the option to receive personalised information about their individual risk of getting skin changes from radiotherapy. The test will also tell how severe those skin changes might be. The information can then be used to help plan treatment.

- Again, I realise this may sound a bit complex. Just to be sure that we are on the same page; can I ask you to tell me in your own words what the test is about?

_____

_____

- Looking back now at your own treatment, how interested would you have been in having this test?

_____

_____

- What would you have seen as the advantages of having this test? - How can you imagine it being helpful to patients or their care providers?

_____

_____

- What would you imagine being the disadvantages of this test? - What negative effects could you imagine for patients or care providers?

_____

_____

Now I'd like you to imagine **three** scenarios.

1. First, imagine if your own test results had indicated that you would only have mild skin changes from radiotherapy. How would you have felt?

_____

_____

2. Second, imagine if the test came back with an inconclusive result. What I mean is that based on the test results, we can't tell how your skin will react during radiotherapy. As much as we'd like all our tests to be perfect, some people will have an inconclusive score. How would you have felt?

_____

_____

3. Third, imagine if the test came back with a result indicating that you were likely to have a very severe skin reaction. What I mean by that is the breast skin sloughing off, which is often painful. How would you have felt?

_____

_____

[See prompts to probe.]

- So, overall how important do you think such a test is on a scale from 0-10 and why?

_____

_____

- Before we wrap up, is is there anything else you would like me to know? [Address any concerns, provide referrals if necessary.]

_____

_____

Thank you again.

Prompts

Adapted from Bowen (2009) framework for the design and evaluation of feasibility studies.

Topic	Prompts	☑
Acceptability	• What implications would the test result have for you? • How would this test have helped you to prepare for treatment? • How would this test have helped you make a decision about treatment? • If you decided to go ahead with RT anyway, how would your treatment experience be affected by the result? • How do you feel about the fact that this test will include some genetic information?	
Demand	• How interested would you have been in this test? • If you imagine that you are talking about this test to a friend who has just been diagnosed with breast cancer. What would you say to them?	
Implementation	• What level of result would you need to influence your decision? • How certain would you want to be before acting on the result? • How do you think the extra information from the test would help your surgeon?	
Practicality	• How would you want the test results be delivered? • What could we do to make the test report clear and helpful? • Who should give you the test result? • How long would you like to spend with […] discussing the results? • How do you feel about getting a printed copy of the test result?	
Adaptation	• Is there any other way the test could have been of value to you? • What if the test told you about the risk of long-term side-effects?	
Integration	• What other information would have helped you to make a decision about radiation treatment? • When faced with a whole lot of information at the beginning of your treatment, how do you think this test would fit in?	
Expansion	• How can we make sure that this test fits in well with your cancer care pathway?	

Gene sets

IL12RB2
rs3790568
rs6685568
rs12409092
END

NEIL3
rs17064666
rs2877985
rs3805169
rs17064704
rs17064705
END

PTTG1
rs2961935
rs2961944
rs2910190
rs2910201
rs2961950
rs2961951
rs2961952
rs2961911
END

POLZ
rs6934341
rs4947106
rs9384787
rs193281
rs395564
rs377716
rs354525
rs354523
rs354542
rs354538
rs434034
rs354546
rs354547
rs354526
rs354527
rs354551
rs191631
rs3912092
rs240986
rs190246
rs190245
rs240998
rs240962
END

ALAD
rs818704
rs818707
END

RAD9A
rs2001635
rs608273
rs674499
rs4542419
rs3927807
rs2071007
rs872110
rs2286620
rs1638586

rs1558256
rs1558257
rs1790733
END

ATM
rs4754296
rs73006226
rs4988023
rs1801516
rs3092993
rs10890838
rs7115351
rs11212650
rs17108024
rs4753840
rs4754317
rs72993806
rs7947933
END

XRCC1
rs25487
rs1799778
END

ST8SIA4
rs575018
rs505994
END

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CHR	SNP	TEST	A1	A2	GENO	O(HET)	E(HET)	P
1	rs3790568	ALL(NP)	A	G	7/205/1641	0.1106	0.1112	0.833
1	rs6685568	ALL(NP)	G	A	11/260/1574	0.1409	0.1412	0.8693
1	rs12409092	ALL(NP)	C	T	9/211/1634	0.1138	0.1159	0.42
2	rs4849101	ALL(NP)	C	T	343/941/570	0.5076	0.4925	0.2029
2	rs2881208	ALL(NP)	T	C	271/930/655	0.5011	0.4786	0.04675
4	rs1801260	ALL(NP)	C	T	133/722/994	0.3905	0.3916	0.9055
4	rs13116075	ALL(NP)	G	A	41/479/1334	0.2584	0.2568	0.8567
4	rs17064666	ALL(NP)	T	C	29/376/1451	0.2026	0.2065	0.4304
4	rs2877985	ALL(NP)	G	A	24/421/1403	0.2278	0.2216	0.2492
4	rs3805169	ALL(NP)	C	T	10/261/1588	0.1404	0.1397	1
4	rs17064704	ALL(NP)	T	C	9/235/1614	0.1265	0.1269	0.8544
4	rs17064705	ALL(NP)	T	G	4/234/1617	0.1261	0.1219	0.1802
5	rs575018	ALL(NP)	C	T	229/829/791	0.4484	0.4538	0.6084
5	rs505994	ALL(NP)	C	A	231/826/799	0.445	0.4532	0.4424
5	rs2961935	ALL(NP)	C	T	96/774/960	0.423	0.3885	0.0001453
5	rs2961944	ALL(NP)	A	G	127/727/1005	0.3911	0.3885	0.8113
5	rs2910190	ALL(NP)	A	G	125/711/1005	0.3862	0.3858	1
5	rs2910201	ALL(NP)	C	T	194/843/821	0.4537	0.4431	0.3199
5	rs2961950	ALL(NP)	A	G	192/843/815	0.4557	0.4433	0.2485
5	rs2961951	ALL(NP)	T	G	116/701/1030	0.3795	0.3776	0.8535
5	rs2961952	ALL(NP)	A	G	132/708/1015	0.3817	0.3867	0.5889
5	rs2961911	ALL(NP)	C	T	123/703/1030	0.3788	0.3806	0.8548
6	rs17142289	ALL(NP)	G	A	3/90/1767	0.04839	0.05028	0.1217
6	rs596917	ALL(NP)	T	G	134/827/829	0.462	0.4246	0.0001892
6	rs3757244	ALL(NP)	0	C	0/0/1816	0	0	1
6	rs6934341	ALL(NP)	A	G	37/474/1347	0.2551	0.2514	0.5804
6	rs4947106	ALL(NP)	T	C	34/475/1350	0.2555	0.2494	0.3518
6	rs9384787	ALL(NP)	T	A	35/482/1343	0.2591	0.2527	0.3128
6	rs193281	ALL(NP)	T	C	33/472/1353	0.254	0.2476	0.3027
6	rs395564	ALL(NP)	C	T	35/477/1342	0.2573	0.2515	0.3564
6	rs377716	ALL(NP)	G	A	34/476/1350	0.2559	0.2497	0.308
6	rs354525	ALL(NP)	G	A	30/483/1338	0.2609	0.2503	0.07719
6	rs354523	ALL(NP)	A	G	30/556/1267	0.3001	0.2772	0.000288
6	rs354542	ALL(NP)	T	C	34/478/1348	0.257	0.2505	0.308
6	rs354538	ALL(NP)	G	A	35/476/1349	0.2559	0.2505	0.4044
6	rs434034	ALL(NP)	A	G	34/472/1353	0.2539	0.2483	0.3999
6	rs354546	ALL(NP)	T	C	23/436/1400	0.2345	0.2257	0.09985
6	rs354547	ALL(NP)	G	T	33/477/1349	0.2566	0.2494	0.2637
6	rs354526	ALL(NP)	T	G	24/439/1396	0.2361	0.2277	0.1258
6	rs354527	ALL(NP)	A	G	20/434/1401	0.234	0.2229	0.03646
6	rs354551	ALL(NP)	A	G	34/469/1356	0.2523	0.2471	0.3995
6	rs191631	ALL(NP)	G	T	24/433/1400	0.2332	0.2255	0.1506
6	rs3912092	ALL(NP)	A	C	14/332/1514	0.1785	0.1748	0.4269
6	rs240986	ALL(NP)	A	T	19/404/1437	0.2172	0.2094	0.1209
6	rs190246	ALL(NP)	A	C	17/404/1424	0.219	0.2092	0.04525
6	rs190245	ALL(NP)	A	G	16/388/1456	0.2086	0.2003	0.08206

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6	rs240998	ALL(NP)	T	C	17/414/1429	0.2226	0.2119	0.02837
6	rs240962	ALL(NP)	T	C	17/412/1431	0.2215	0.211	0.03604
6	rs4880	ALL(NP)	T	C	476/811/501	0.4536	0.4999	8.638e-05
7	rs882460	ALL(NP)	C	T	117/691/1043	0.3733	0.3749	0.8525
7	rs7791642	ALL(NP)	G	A	2/308/1543	0.1662	0.1542	0.0001287
7	rs10280848	ALL(NP)	T	C	337/896/617	0.4843	0.4885	0.7037
7	rs12531679	ALL(NP)	A	G	21/346/1492	0.1861	0.1869	0.8045
8	rs8178046	ALL(NP)	T	C	1/73/1786	0.03925	0.03951	0.5329
9	rs6475752	ALL(NP)	T	G	142/671/1037	0.3627	0.383	0.0246
9	rs2230806	ALL(NP)	A	G	130/722/1003	0.3892	0.3893	1
9	rs818704	ALL(NP)	A	G	44/561/1216	0.3081	0.2929	0.0304
9	rs818707	ALL(NP)	A	G	16/454/1369	0.2469	0.2294	0.0007167
9	rs7037705	ALL(NP)	C	A	7/202/1647	0.1088	0.1096	0.6728
11	rs2001635	ALL(NP)	A	G	22/302/1527	0.1632	0.1695	0.1291
11	rs608273	ALL(NP)	T	C	19/269/1567	0.145	0.1518	0.06441
11	rs674499	ALL(NP)	A	G	19/267/1571	0.1438	0.1508	0.06211
11	rs4542419	ALL(NP)	T	C	20/290/1543	0.1565	0.1622	0.1496
11	rs3927807	ALL(NP)	A	G	14/269/1572	0.145	0.1473	0.5262
11	rs2071007	ALL(NP)	G	A	21/296/1542	0.1592	0.1653	0.1212
11	rs872110	ALL(NP)	G	A	21/286/1545	0.1544	0.1614	0.08168
11	rs2286620	ALL(NP)	C	T	10/258/1589	0.1389	0.1385	1
11	rs1638586	ALL(NP)	A	G	20/294/1536	0.1589	0.1642	0.1573
11	rs1558256	ALL(NP)	G	A	22/299/1533	0.1613	0.1679	0.0958
11	rs1558257	ALL(NP)	A	G	2/213/1589	0.1181	0.1131	0.06007
11	rs1790733	ALL(NP)	C	T	21/295/1534	0.1595	0.1656	0.1208
11	rs4754296	ALL(NP)	G	T	37/485/1336	0.261	0.2556	0.4138
11	rs73006226	ALL(NP)	A	C	27/406/1361	0.2263	0.2235	0.6729
11	rs4988023	ALL(NP)	C	A	34/461/1364	0.248	0.2441	0.5683
11	rs1801516	ALL(NP)	A	G	34/455/1368	0.245	0.242	0.6326
11	rs3092993	ALL(NP)	A	C	33/459/1363	0.2474	0.243	0.5031
11	rs10890838	ALL(NP)	T	C	0/492/1340	0.2686	0.2325	4.618e-17
11	rs7115351	ALL(NP)	T	C	44/491/1324	0.2641	0.263	0.9297
11	rs11212650	ALL(NP)	G	A	40/478/1342	0.257	0.255	0.7855
11	rs17108024	ALL(NP)	T	C	46/487/1327	0.2618	0.2628	0.86
11	rs4753840	ALL(NP)	G	A	40/475/1345	0.2554	0.2539	0.8553
11	rs4754317	ALL(NP)	A	G	32/486/1340	0.2616	0.2522	0.1181
11	rs72993806	ALL(NP)	G	C	34/482/1336	0.2603	0.2529	0.2325
11	rs7947933	ALL(NP)	C	A	33/485/1341	0.2609	0.2525	0.1682
17	rs3744355	ALL(NP)	C	G	20/339/1489	0.1834	0.1841	0.8992
18	rs17798101	ALL(NP)	C	T	44/460/1351	0.248	0.2518	0.5184
19	rs25487	ALL(NP)	A	G	236/855/769	0.4597	0.4589	0.9597
19	rs1799778	ALL(NP)	A	C	233/856/765	0.4617	0.4588	0.8004

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Acute oedema

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	0	0	1	NA
NEIL3	5	1	1	0.1239	rs2877985
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

Acute oedema

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR
1	rs3790568	67370377	A	0.06166	0.05697	G	0.3181	0.5727	1.088
1	rs6685568	67389614	G	0.07962	0.07459	A	0.2842	0.594	1.073
1	rs12409092	67390948	C	0.06672	0.05856	T	0.9248	0.3362	1.149
4	rs17064666	177346497	T	0.1098	0.1209	C	0.9527	0.329	0.8967
4	rs2877985	177347743	G	0.1123	0.1349	A	3.636	0.05654	0.8117
4	rs3805169	177351762	C	0.06734	0.08	T	1.829	0.1763	0.8303
4	rs17064704	177354496	T	0.05976	0.07271	C	2.102	0.1471	0.8106
4	rs17064705	177354515	T	0.05892	0.0688	G	1.272	0.2595	0.8475
5	rs575018	101043689	C	0.3617	0.3431	T	1.222	0.2689	1.085
5	rs505994	101048059	C	0.36	0.3426	A	1.069	0.3011	1.08
5	rs2961935	160401392	C	0.2581	0.2646	T	0.1704	0.6797	0.9671
5	rs2961944	160408651	A	0.2593	0.2647	G	0.1224	0.7264	0.9722
5	rs2910190	160415458	A	0.2547	0.2624	G	0.2448	0.6207	0.9606
5	rs2910201	160423365	C	0.3356	0.3268	T	0.279	0.5974	1.04
5	rs2961950	160424738	A	0.335	0.328	G	0.1777	0.6733	1.032
5	rs2961951	160427271	T	0.2559	0.2471	G	0.3284	0.5666	1.048
5	rs2961952	160429522	A	0.2686	0.2553	G	0.7298	0.393	1.071
5	rs2961911	160434568	C	0.2589	0.2506	T	0.2867	0.5923	1.044
6	rs6934341	111098387	A	0.1433	0.1503	G	0.3093	0.5781	0.9457
6	rs4947106	111100163	T	0.1414	0.1491	C	0.3778	0.5388	0.94
6	rs9384787	111113707	T	0.1439	0.1514	A	0.3539	0.5519	0.9422
6	rs193281	111166487	T	0.1391	0.1483	C	0.5399	0.4625	0.9282
6	rs395564	111176176	C	0.1436	0.1503	T	0.2842	0.594	0.9479
6	rs377716	111181705	G	0.1423	0.149	A	0.2888	0.591	0.9474
6	rs354525	111186193	G	0.1441	0.1488	A	0.1401	0.7082	0.9631
6	rs354523	111188122	A	0.1562	0.1684	G	0.8624	0.3531	0.9142
6	rs354542	111193483	T	0.1423	0.1498	C	0.3621	0.5474	0.9413
6	rs354538	111196863	G	0.1439	0.149	A	0.1618	0.6875	0.9605
6	rs434034	111204180	A	0.14	0.1486	G	0.4752	0.4906	0.9327
6	rs354546	111211927	T	0.1254	0.1319	C	0.3013	0.583	0.9435
6	rs354547	111213919	G	0.1406	0.1495	T	0.5107	0.4748	0.9304
6	rs354526	111216278	T	0.1263	0.1336	G	0.3756	0.54	0.9373
6	rs354527	111217390	A	0.1237	0.1299	G	0.2736	0.6009	0.9457
6	rs354551	111224337	A	0.1364	0.1491	G	1.047	0.3062	0.9011
6	rs191631	111226862	G	0.1248	0.1321	T	0.3733	0.5412	0.9372
6	rs3912092	111251609	A	0.09175	0.09918	C	0.5058	0.477	0.9175
6	rs240986	111265852	A	0.1136	0.1216	T	0.4879	0.4849	0.9258
6	rs190246	111277043	A	0.1139	0.122	C	0.4919	0.4831	0.9253
6	rs190245	111294270	A	0.1103	0.1139	G	0.1042	0.7469	0.9644
6	rs240998	111307676	T	0.1153	0.1224	C	0.3847	0.5351	0.9342
6	rs240962	111321243	T	0.1145	0.122	C	0.4347	0.5097	0.93
9	rs818704	113386036	A	0.1794	0.1761	G	0.0612	0.8046	1.023
9	rs818707	113387687	A	0.1369	0.1309	G	0.2491	0.6177	1.053
11	rs2001635	67094924	A	0.09677	0.09214	G	0.2013	0.6537	1.056

							acute_oedema		
11	rs608273	67158297	T	0.08347	0.08231	C	0.01427	0.9049	1.015
11	rs674499	67195954	A	0.08347	0.08129	G	0.05058	0.8221	1.029
11	rs4542419	67203126	T	0.09122	0.08689	C	0.1857	0.6665	1.055
11	rs3927807	67231384	A	0.07953	0.08013	G	0.003961	0.9498	0.9918
11	rs2071007	67282821	G	0.09428	0.08824	A	0.3556	0.5509	1.076
11	rs872110	67397320	G	0.09122	0.08737	A	0.1462	0.7022	1.048
11	rs2286620	67399513	C	0.07251	0.07645	T	0.1782	0.6729	0.9445
11	rs1638586	67407126	A	0.09407	0.0886	G	0.2895	0.5906	1.068
11	rs1558256	67412217	G	0.09713	0.0905	A	0.4167	0.5186	1.081
11	rs1558257	67412554	A	0.05332	0.06297	G	1.279	0.258	0.8381
11	rs1790733	67418529	C	0.09576	0.08901	T	0.4378	0.5082	1.084
11	rs4754296	108135455	G	0.1484	0.1495	T	0.007782	0.9297	0.9913
11	rs73006226	108202001	A	0.1274	0.1266	C	0.004593	0.946	1.007
11	rs4988023	108298268	C	0.138	0.1422	A	0.1117	0.7383	0.9665
11	rs1801516	108304735	A	0.1368	0.1405	G	0.09104	0.7629	0.9695
11	rs3092993	108364388	A	0.1366	0.1417	C	0.172	0.6783	0.9584
11	rs10890838	108432578	T	0.1337	0.1332	C	0.001961	0.9647	1.005
11	rs7115351	108454062	T	0.1515	0.156	C	0.1256	0.723	0.9658
11	rs11212650	108456491	G	0.1481	0.149	A	0.004369	0.9473	0.9934
11	rs17108024	108462809	T	0.1524	0.1555	C	0.06091	0.8051	0.9761
11	rs4753840	108467613	G	0.1456	0.149	A	0.07154	0.7891	0.9736
11	rs4754317	108488434	A	0.1515	0.1439	G	0.3703	0.5429	1.062
11	rs72993806	108488962	G	0.1501	0.1453	C	0.1445	0.7039	1.039
11	rs7947933	108489579	C	0.1515	0.1442	A	0.3422	0.5586	1.06
19	rs25487	43551574	A	0.3662	0.3547	G	0.4575	0.4988	1.051
19	rs1799778	43554989	A	0.3682	0.3534	C	0.7643	0.382	1.066

Acute pain

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR
1	rs3790568	67370377	A	0.07541	0.05227	G	6.858	0.008826	1.479
1	rs6685568	67389614	G	0.09688	0.06901	A	7.7	0.005521	1.447
1	rs12409092	67390948	C	0.08041	0.05419	T	8.443	0.003665	1.526
4	rs17064666	177346497	T	0.1152	0.119	C	0.09467	0.7583	0.9646
4	rs2877985	177347743	G	0.1198	0.1307	A	0.745	0.3881	0.9057
4	rs3805169	177351762	C	0.06996	0.0781	T	0.6696	0.4132	0.8879
4	rs17064704	177354496	T	0.06379	0.0705	C	0.4989	0.48	0.8983
4	rs17064705	177354515	T	0.06276	0.06682	G	0.1903	0.6627	0.9351
5	rs575018	101043689	C	0.3536	0.3487	T	0.07388	0.7858	1.022
5	rs505994	101048059	C	0.3529	0.3478	A	0.08119	0.7757	1.023
5	rs2961935	160401392	C	0.2735	0.2584	T	0.8224	0.3645	1.08
5	rs2961944	160408651	A	0.2778	0.2575	G	1.507	0.2196	1.109
5	rs2910190	160415458	A	0.2764	0.2541	G	1.823	0.1769	1.122
5	rs2910201	160423365	C	0.3454	0.3241	T	1.441	0.23	1.1
5	rs2961950	160424738	A	0.3458	0.3249	G	1.389	0.2386	1.098
5	rs2961951	160427271	T	0.2703	0.2423	G	2.931	0.08691	1.158
5	rs2961952	160429522	A	0.2819	0.2513	G	3.435	0.06382	1.169
5	rs2961911	160434568	C	0.2737	0.2456	T	2.951	0.08582	1.157
6	rs6934341	111098387	A	0.1492	0.1488	G	0.0009118	0.9759	1.003
6	rs4947106	111100163	T	0.1471	0.1475	C	0.0008582	0.9766	0.9969
6	rs9384787	111113707	T	0.1471	0.1505	A	0.06214	0.8032	0.974
6	rs193281	111166487	T	0.144	0.1469	C	0.04523	0.8316	0.9775
6	rs395564	111176176	C	0.1464	0.1499	T	0.06801	0.7943	0.9727
6	rs377716	111181705	G	0.1461	0.1482	A	0.02417	0.8765	0.9836
6	rs354525	111186193	G	0.1477	0.1482	A	0.001202	0.9723	0.9963
6	rs354523	111188122	A	0.1625	0.1659	G	0.05818	0.8094	0.9757
6	rs354542	111193483	T	0.1471	0.1485	C	0.01141	0.9149	0.9887
6	rs354538	111196863	G	0.1471	0.1485	A	0.01141	0.9149	0.9887
6	rs434034	111204180	A	0.1451	0.1471	G	0.02414	0.8765	0.9836
6	rs354546	111211927	T	0.132	0.1298	C	0.02946	0.8637	1.019
6	rs354547	111213919	G	0.1471	0.1475	T	0.0008582	0.9766	0.9969
6	rs354526	111216278	T	0.1337	0.131	G	0.04548	0.8311	1.024
6	rs354527	111217390	A	0.1327	0.1268	G	0.2194	0.6395	1.054
6	rs354551	111224337	A	0.143	0.1467	G	0.07954	0.7779	0.9703
6	rs191631	111226862	G	0.132	0.1296	T	0.03469	0.8522	1.021
6	rs3912092	111251609	A	0.09568	0.09763	C	0.03064	0.861	0.9779
6	rs240986	111265852	A	0.1163	0.1206	T	0.127	0.7216	0.9593
6	rs190246	111277043	A	0.1167	0.121	C	0.1222	0.7266	0.9599
6	rs190245	111294270	A	0.107	0.1149	G	0.4374	0.5084	0.9234
6	rs240998	111307676	T	0.1152	0.1221	C	0.319	0.5722	0.9361
6	rs240962	111321243	T	0.1163	0.121	C	0.1501	0.6985	0.9558
9	rs818704	113386036	A	0.1806	0.1765	G	0.0807	0.7764	1.028
9	rs818707	113387687	A	0.1393	0.1312	G	0.398	0.5281	1.072
11	rs2001635	67094924	A	0.1012	0.09238	G	0.6454	0.4218	1.107

							acute_pain		
11	rs608273	67158297	T	0.08884	0.08173	C	0.4652	0.4952	1.095
11	rs674499	67195954	A	0.08866	0.08084	G	0.568	0.4511	1.106
11	rs4542419	67203126	T	0.09129	0.08864	C	0.06049	0.8057	1.033
11	rs3927807	67231384	A	0.08848	0.07802	G	1.042	0.3074	1.147
11	rs2071007	67282821	G	0.09259	0.0908	A	0.02729	0.8688	1.022
11	rs872110	67397320	G	0.09298	0.08846	A	0.176	0.6748	1.056
11	rs2286620	67399513	C	0.08642	0.07214	T	2.055	0.1517	1.217
11	rs1638586	67407126	A	0.09627	0.08968	G	0.3679	0.5442	1.081
11	rs1558256	67412217	G	0.09607	0.09255	A	0.1034	0.7478	1.042
11	rs1558257	67412554	A	0.07006	0.05652	G	2.225	0.1358	1.258
11	rs1790733	67418529	C	0.09588	0.09059	T	0.2358	0.6273	1.064
11	rs4754296	108135455	G	0.143	0.1503	T	0.2989	0.5846	0.9433
11	rs73006226	108202001	A	0.1274	0.1262	C	0.009477	0.9224	1.011
11	rs4988023	108298268	C	0.1348	0.1418	A	0.2875	0.5918	0.943
11	rs1801516	108304735	A	0.1327	0.1404	G	0.3548	0.5514	0.9365
11	rs3092993	108364388	A	0.133	0.1413	C	0.4097	0.5221	0.932
11	rs10890838	108432578	T	0.1323	0.133	C	0.002797	0.9578	0.9941
11	rs7115351	108454062	T	0.1461	0.1563	C	0.5704	0.4501	0.9234
11	rs11212650	108456491	G	0.142	0.1501	A	0.369	0.5436	0.9371
11	rs17108024	108462809	T	0.144	0.157	C	0.9123	0.3395	0.9037
11	rs4753840	108467613	G	0.143	0.1485	A	0.1734	0.6771	0.9565
11	rs4754317	108488434	A	0.1379	0.1488	G	0.6771	0.4106	0.9149
11	rs72993806	108488962	G	0.1381	0.1493	C	0.7095	0.3996	0.913
11	rs7947933	108489579	C	0.1379	0.149	A	0.7102	0.3994	0.913
19	rs25487	43551574	A	0.3601	0.358	G	0.01383	0.9064	1.009
19	rs1799778	43554989	A	0.3588	0.3582	C	0.001023	0.9745	1.003

Acute ulceration

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	0	0	1	NA
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	2	1	0.1857	rs191631
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

Acute ulceration

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR
1	rs3790568	67370377	A	0.05814	0.05854	G	0.0008912	0.9762	0.9928
1	rs6685568	67389614	G	0.07558	0.07629	A	0.002199	0.9626	0.99
1	rs12409092	67390948	C	0.0614	0.06121	T	0.0002106	0.9884	1.003
4	rs17064666	177346497	T	0.1012	0.119	C	0.9616	0.3268	0.8333
4	rs2877985	177347743	G	0.1287	0.1274	A	0.004617	0.9458	1.012
4	rs3805169	177351762	C	0.07225	0.07625	T	0.07113	0.7897	0.9436
4	rs17064704	177354496	T	0.06358	0.069	C	0.1438	0.7045	0.9162
4	rs17064705	177354515	T	0.06358	0.06577	G	0.0245	0.8756	0.9644
5	rs575018	101043689	C	0.3538	0.3487	T	0.03563	0.8503	1.023
5	rs505994	101048059	C	0.3517	0.3479	A	0.01994	0.8877	1.017
5	rs2961935	160401392	C	0.2573	0.263	T	0.05136	0.8207	0.971
5	rs2961944	160408651	A	0.2688	0.2623	G	0.06775	0.7946	1.034
5	rs2910190	160415458	A	0.2645	0.2594	G	0.0433	0.8352	1.027
5	rs2910201	160423365	C	0.3064	0.3321	T	0.9399	0.3323	0.8882
5	rs2961950	160424738	A	0.3035	0.3331	G	1.245	0.2646	0.8722
5	rs2961951	160427271	T	0.2514	0.2498	G	0.004262	0.9479	1.009
5	rs2961952	160429522	A	0.263	0.2593	G	0.02244	0.8809	1.019
5	rs2961911	160434568	C	0.2514	0.2535	T	0.006987	0.9334	0.9892
6	rs6934341	111098387	A	0.1705	0.1457	G	1.531	0.2159	1.206
6	rs4947106	111100163	T	0.1676	0.1444	C	1.353	0.2447	1.193
6	rs9384787	111113707	T	0.1705	0.1467	A	1.399	0.2369	1.196
6	rs193281	111166487	T	0.1705	0.1426	C	1.96	0.1616	1.236
6	rs395564	111176176	C	0.1734	0.1454	T	1.943	0.1634	1.233
6	rs377716	111181705	G	0.1705	0.1443	A	1.72	0.1897	1.219
6	rs354525	111186193	G	0.1657	0.1453	A	1.032	0.3098	1.168
6	rs354523	111188122	A	0.1792	0.1629	G	0.6041	0.437	1.122
6	rs354542	111193483	T	0.1705	0.1449	C	1.636	0.2008	1.213
6	rs354538	111196863	G	0.1705	0.1449	A	1.636	0.2008	1.213
6	rs434034	111204180	A	0.1705	0.1432	G	1.882	0.1701	1.23
6	rs354546	111211927	T	0.159	0.1267	C	2.876	0.08992	1.302
6	rs354547	111213919	G	0.1705	0.1441	T	1.75	0.1858	1.221
6	rs354526	111216278	T	0.159	0.1283	G	2.587	0.1077	1.285
6	rs354527	111217390	A	0.1541	0.1252	G	2.335	0.1265	1.273
6	rs354551	111224337	A	0.1705	0.1422	G	2.019	0.1553	1.24
6	rs191631	111226862	G	0.159	0.1266	T	2.905	0.08832	1.304
6	rs3912092	111251609	A	0.1069	0.09569	C	0.4534	0.5007	1.132
6	rs240986	111265852	A	0.1387	0.117	T	1.416	0.234	1.216
6	rs190246	111277043	A	0.1404	0.1172	C	1.579	0.209	1.23
6	rs190245	111294270	A	0.1358	0.1103	G	2.047	0.1525	1.268
6	rs240998	111307676	T	0.1416	0.1179	C	1.672	0.196	1.235
6	rs240962	111321243	T	0.1416	0.1173	C	1.766	0.1839	1.242
9	rs818704	113386036	A	0.1726	0.1776	G	0.0522	0.8193	0.966
9	rs818707	113387687	A	0.1529	0.1308	G	1.315	0.2514	1.2
11	rs2001635	67094924	A	0.07514	0.0956	G	1.543	0.2142	0.7686

							acute_ulcer		
11	rs608273	67158297	T	0.06647	0.0844	C	1.326	0.2495	0.7725
11	rs674499	67195954	A	0.06647	0.08364	G	1.225	0.2684	0.7802
11	rs4542419	67203126	T	0.0843	0.08872	C	0.07546	0.7835	0.9456
11	rs3927807	67231384	A	0.06647	0.08135	G	0.9422	0.3317	0.8041
11	rs2071007	67282821	G	0.0896	0.09027	A	0.001754	0.9666	0.9917
11	rs872110	67397320	G	0.07803	0.08974	A	0.5312	0.4661	0.8585
11	rs2286620	67399513	C	0.07225	0.07547	T	0.04661	0.8291	0.954
11	rs1638586	67407126	A	0.07849	0.09163	G	0.654	0.4187	0.8444
11	rs1558256	67412217	G	0.07558	0.09445	A	1.319	0.2508	0.7839
11	rs1558257	67412554	A	0.04491	0.0614	G	1.462	0.2266	0.7188
11	rs1790733	67418529	C	0.07267	0.09316	T	1.576	0.2094	0.7629
11	rs4754296	108135455	G	0.159	0.1481	T	0.2902	0.5901	1.087
11	rs73006226	108202001	A	0.1407	0.1254	C	0.6408	0.4234	1.142
11	rs4988023	108298268	C	0.1532	0.1395	A	0.4832	0.487	1.116
11	rs1801516	108304735	A	0.1503	0.1382	G	0.3839	0.5355	1.103
11	rs3092993	108364388	A	0.1503	0.1389	C	0.3348	0.5628	1.096
11	rs10890838	108432578	T	0.1412	0.1326	C	0.198	0.6563	1.076
11	rs7115351	108454062	T	0.1618	0.1538	C	0.1553	0.6936	1.062
11	rs11212650	108456491	G	0.159	0.1476	A	0.3174	0.5732	1.091
11	rs17108024	108462809	T	0.159	0.154	C	0.05873	0.8085	1.038
11	rs4753840	108467613	G	0.1647	0.1461	A	0.8622	0.3531	1.153
11	rs4754317	108488434	A	0.1618	0.1448	G	0.7309	0.3926	1.141
11	rs72993806	108488962	G	0.1618	0.1453	C	0.6841	0.4082	1.136
11	rs7947933	108489579	C	0.1618	0.145	A	0.7118	0.3988	1.139
19	rs25487	43551574	A	0.3237	0.3621	G	2.007	0.1566	0.8432
19	rs1799778	43554989	A	0.3208	0.3622	C	2.332	0.1268	0.8318

Acute desquamation

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	0	0	1	NA
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	1	1	0.1806	rs1799778
ST8SIA4	2	0	0	1	NA

Acute desquamation

CHR	SNP	BP	A1	F_A	F_U	A2	CHISQ	P	OR
1	rs3790568	67370377	A	0.05707	0.05866	G	0.01529	0.9016	0.9712
1	rs6685568	67389614	G	0.07609	0.07623	A	0.0001023	0.9919	0.9979
1	rs12409092	67390948	C	0.06011	0.06135	T	0.008809	0.9252	0.9785
4	rs17064666	177346497	T	0.1054	0.1186	C	0.5621	0.4534	0.8753
4	rs2877985	177347743	G	0.1284	0.1274	A	0.003142	0.9553	1.009
4	rs3805169	177351762	C	0.07568	0.07589	T	0.0002125	0.9884	0.997
4	rs17064704	177354496	T	0.06757	0.06859	C	0.005398	0.9414	0.9841
4	rs17064705	177354515	T	0.06757	0.06534	G	0.02697	0.8695	1.037
5	rs575018	101043689	C	0.3544	0.3486	T	0.04861	0.8255	1.026
5	rs505994	101048059	C	0.3525	0.3478	A	0.03112	0.86	1.021
5	rs2961935	160401392	C	0.265	0.2621	T	0.01413	0.9054	1.015
5	rs2961944	160408651	A	0.2757	0.2615	G	0.3455	0.5567	1.075
5	rs2910190	160415458	A	0.2705	0.2587	G	0.2396	0.6245	1.063
5	rs2910201	160423365	C	0.3135	0.3315	T	0.4862	0.4856	0.921
5	rs2961950	160424738	A	0.3108	0.3325	G	0.7072	0.4004	0.9053
5	rs2961951	160427271	T	0.2568	0.2492	G	0.1004	0.7514	1.041
5	rs2961952	160429522	A	0.2703	0.2584	G	0.2419	0.6229	1.063
5	rs2961911	160434568	C	0.2568	0.2529	T	0.02597	0.872	1.02
6	rs6934341	111098387	A	0.1676	0.1458	G	1.245	0.2646	1.179
6	rs4947106	111100163	T	0.1622	0.1448	C	0.7985	0.3715	1.143
6	rs9384787	111113707	T	0.1703	0.1466	A	1.472	0.225	1.195
6	rs193281	111166487	T	0.1649	0.1431	C	1.27	0.2597	1.182
6	rs395564	111176176	C	0.1676	0.1459	T	1.238	0.2658	1.179
6	rs377716	111181705	G	0.1649	0.1447	A	1.075	0.2998	1.167
6	rs354525	111186193	G	0.163	0.1454	A	0.8151	0.3666	1.145
6	rs354523	111188122	A	0.1757	0.1632	G	0.3773	0.5391	1.093
6	rs354542	111193483	T	0.1649	0.1453	C	1.008	0.3155	1.161
6	rs354538	111196863	G	0.1649	0.1453	A	1.008	0.3155	1.161
6	rs434034	111204180	A	0.1649	0.1436	G	1.207	0.272	1.177
6	rs354546	111211927	T	0.1486	0.1277	C	1.294	0.2554	1.193
6	rs354547	111213919	G	0.1649	0.1445	T	1.1	0.2943	1.169
6	rs354526	111216278	T	0.1486	0.1292	G	1.102	0.2939	1.177
6	rs354527	111217390	A	0.144	0.1261	G	0.955	0.3284	1.166
6	rs354551	111224337	A	0.1649	0.1427	G	1.32	0.2507	1.186
6	rs191631	111226862	G	0.1514	0.1272	T	1.714	0.1904	1.223
6	rs3912092	111251609	A	0.1027	0.09608	C	0.1666	0.6831	1.077
6	rs240986	111265852	A	0.1324	0.1175	T	0.7065	0.4006	1.146
6	rs190246	111277043	A	0.1339	0.1178	C	0.8133	0.3672	1.158
6	rs190245	111294270	A	0.1351	0.1102	G	2.073	0.1499	1.262
6	rs240998	111307676	T	0.1405	0.1178	C	1.625	0.2024	1.225
6	rs240962	111321243	T	0.1405	0.1172	C	1.72	0.1896	1.232
9	rs818704	113386036	A	0.1694	0.178	G	0.163	0.6864	0.942
9	rs818707	113387687	A	0.1456	0.1314	G	0.5717	0.4496	1.126
11	rs2001635	67094924	A	0.07838	0.09538	G	1.132	0.2874	0.8065

							acute_desq		
11	rs608273	67158297	T	0.06757	0.08441	C	1.242	0.265	0.786
11	rs674499	67195954	A	0.06757	0.08364	G	1.14	0.2856	0.7939
11	rs4542419	67203126	T	0.08424	0.08876	C	0.08391	0.7721	0.9444
11	rs3927807	67231384	A	0.06757	0.08134	G	0.8567	0.3547	0.8184
11	rs2071007	67282821	G	0.08919	0.09032	A	0.00522	0.9424	0.9862
11	rs872110	67397320	G	0.07838	0.08979	A	0.5357	0.4642	0.8621
11	rs2286620	67399513	C	0.06757	0.07603	T	0.3421	0.5586	0.8807
11	rs1638586	67407126	A	0.0788	0.09169	G	0.6679	0.4138	0.8474
11	rs1558256	67412217	G	0.07609	0.09454	A	1.339	0.2473	0.7888
11	rs1558257	67412554	A	0.04469	0.06155	G	1.625	0.2024	0.7133
11	rs1790733	67418529	C	0.07337	0.09323	T	1.573	0.2098	0.7701
11	rs4754296	108135455	G	0.1622	0.1477	T	0.5499	0.4584	1.117
11	rs73006226	108202001	A	0.1397	0.1254	C	0.5908	0.4421	1.132
11	rs4988023	108298268	C	0.1568	0.139	A	0.8654	0.3522	1.151
11	rs1801516	108304735	A	0.1541	0.1376	G	0.7462	0.3877	1.141
11	rs3092993	108364388	A	0.1541	0.1384	C	0.6737	0.4118	1.133
11	rs10890838	108432578	T	0.1484	0.1317	C	0.7867	0.3751	1.149
11	rs7115351	108454062	T	0.1703	0.1528	C	0.7775	0.3779	1.138
11	rs11212650	108456491	G	0.1649	0.1469	A	0.8493	0.3567	1.147
11	rs17108024	108462809	T	0.1676	0.153	C	0.5401	0.4624	1.114
11	rs4753840	108467613	G	0.1676	0.1457	A	1.266	0.2604	1.181
11	rs4754317	108488434	A	0.1676	0.144	G	1.478	0.2241	1.197
11	rs72993806	108488962	G	0.1676	0.1445	C	1.407	0.2355	1.192
11	rs7947933	108489579	C	0.1676	0.1442	A	1.449	0.2287	1.195
19	rs25487	43551574	A	0.3216	0.3626	G	2.428	0.1192	0.8334
19	rs1799778	43554989	A	0.3189	0.3627	C	2.772	0.09593	0.8227

Acute erythema

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	0	0	1	NA
NEIL3	5	1	1	0.2826	rs17064705
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	2	1	0.2027	rs10890838
XRCC1	2	0	0	1	NA
ST8SIA4	2	1	1	0.07143	rs575018

Acute erythema

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	rs3790568	67370377	1812	0.01894	0.04505	9.76e-05	0.4203	0.6743
1	rs6685568	67389614	1804	0.04441	0.04014	0.0006788	1.106	0.2687
1	rs12409092	67390948	1813	0.04315	0.04378	0.0005362	0.9857	0.3244
4	rs17064666	177346497	1816	0.01912	0.03264	0.0001892	0.586	0.558
4	rs2877985	177347743	1808	-0.005974	0.03234	1.889e-05	-0.1847	0.8535
4	rs3805169	177351762	1819	0.04024	0.04014	0.000553	1.003	0.3161
4	rs17064704	177354496	1818	0.04412	0.04194	0.0006092	1.052	0.2929
4	rs17064705	177354515	1815	0.07183	0.04362	0.001493	1.647	0.09981
5	rs575018	101043689	1810	0.03866	0.02209	0.001691	1.75	0.08033
5	rs505994	101048059	1816	0.03326	0.02203	0.001255	1.51	0.1313
5	rs2961935	160401392	1789	0.009696	0.0254	8.15e-05	0.3817	0.7028
5	rs2961944	160408651	1818	-0.01127	0.02422	0.0001193	-0.4654	0.6417
5	rs2910190	160415458	1801	-0.0136	0.02434	0.0001735	-0.5588	0.5764
5	rs2910201	160423365	1817	0.006566	0.02278	4.577e-05	0.2882	0.7732
5	rs2961950	160424738	1809	0.003111	0.02285	1.026e-05	0.1362	0.8917
5	rs2961951	160427271	1806	0.01476	0.02459	0.0001997	0.6003	0.5484
5	rs2961952	160429522	1814	0.01566	0.02403	0.0002342	0.6515	0.5148
5	rs2961911	160434568	1816	0.01013	0.02431	9.572e-05	0.4167	0.6769
6	rs6934341	111098387	1817	0.007296	0.03021	3.213e-05	0.2415	0.8092
6	rs4947106	111100163	1818	0.009494	0.03049	5.338e-05	0.3113	0.7556
6	rs9384787	111113707	1819	0.02205	0.03028	0.0002916	0.7281	0.4667
6	rs193281	111166487	1817	0.01001	0.03065	5.873e-05	0.3265	0.7441
6	rs395564	111176176	1813	0.005105	0.03041	1.557e-05	0.1679	0.8667
6	rs377716	111181705	1819	0.007154	0.03047	3.034e-05	0.2348	0.8144
6	rs354525	111186193	1810	0.009202	0.03077	4.948e-05	0.2991	0.7649
6	rs354523	111188122	1812	0.00947	0.02997	5.517e-05	0.316	0.752
6	rs354542	111193483	1819	0.006964	0.03044	2.881e-05	0.2288	0.8191
6	rs354538	111196863	1819	0.009185	0.03037	5.034e-05	0.3024	0.7624
6	rs434034	111204180	1818	0.009124	0.03051	4.923e-05	0.299	0.765
6	rs354546	111211927	1818	0.001447	0.03236	1.102e-06	0.04473	0.9643
6	rs354547	111213919	1818	0.007258	0.03056	3.105e-05	0.2375	0.8123
6	rs354526	111216278	1818	0.000938	0.03218	4.679e-07	0.02915	0.9767
6	rs354527	111217390	1814	0.01202	0.03279	7.413e-05	0.3665	0.714
6	rs354551	111224337	1818	0.01465	0.03058	0.0001263	0.479	0.632
6	rs191631	111226862	1816	0.01742	0.03229	0.0001604	0.5395	0.5896
6	rs3912092	111251609	1819	-0.01199	0.03624	6.024e-05	-0.3309	0.7408
6	rs240986	111265852	1819	0.008469	0.03346	3.526e-05	0.2531	0.8002
6	rs190246	111277043	1805	0.007754	0.0336	2.954e-05	0.2308	0.8175
6	rs190245	111294270	1819	0.03451	0.03432	0.0005564	1.006	0.3147
6	rs240998	111307676	1819	0.02731	0.03354	0.0003649	0.8144	0.4155
6	rs240962	111321243	1819	0.02761	0.03359	0.0003717	0.822	0.4112
9	rs818704	113386036	1781	-0.02307	0.02863	0.000365	-0.8059	0.4204
9	rs818707	113387687	1799	-0.04195	0.03276	0.0009116	-1.281	0.2005
11	rs2001635	67094924	1810	0.002198	0.0358	2.085e-06	0.0614	0.951

				acute_erythema				
11	rs608273	67158297	1814	-0.01991	0.03768	0.000154	-0.5284	0.5973
11	rs674499	67195954	1817	-0.02371	0.03776	0.0002172	-0.628	0.5301
11	rs4542419	67203126	1812	-0.01495	0.03676	9.145e-05	-0.4069	0.6842
11	rs3927807	67231384	1814	-0.02706	0.03888	0.0002673	-0.696	0.4865
11	rs2071007	67282821	1818	-0.02513	0.03631	0.0002637	-0.6921	0.489
11	rs872110	67397320	1811	-0.01614	0.03661	0.0001074	-0.4408	0.6594
11	rs2286620	67399513	1816	-0.04693	0.04031	0.0007466	-1.164	0.2445
11	rs1638586	67407126	1809	-0.01836	0.0365	0.00014	-0.5031	0.615
11	rs1558256	67412217	1813	-0.01623	0.03596	0.0001125	-0.4515	0.6517
11	rs1558257	67412554	1763	-0.04879	0.04667	0.0006202	-1.045	0.296
11	rs1790733	67418529	1809	-0.01909	0.03624	0.0001535	-0.5267	0.5985
11	rs4754296	108135455	1817	0.03069	0.03017	0.00057	1.017	0.3091
11	rs73006226	108202001	1754	0.04375	0.03275	0.001018	1.336	0.1817
11	rs4988023	108298268	1818	0.03706	0.03079	0.000797	1.204	0.2289
11	rs1801516	108304735	1816	0.03948	0.03089	0.0008999	1.278	0.2013
11	rs3092993	108364388	1814	0.03702	0.03095	0.000789	1.196	0.2318
11	rs10890838	108432578	1792	0.06161	0.03427	0.001802	1.798	0.07241
11	rs7115351	108454062	1818	0.04881	0.02944	0.001512	1.658	0.09745
11	rs11212650	108456491	1819	0.0414	0.02995	0.001051	1.382	0.167
11	rs17108024	108462809	1819	0.04632	0.0293	0.001373	1.581	0.1141
11	rs4753840	108467613	1819	0.04397	0.02999	0.001182	1.466	0.1428
11	rs4754317	108488434	1817	0.04404	0.03064	0.001137	1.437	0.1508
11	rs72993806	108488962	1811	0.04126	0.03053	0.001009	1.351	0.1767
11	rs7947933	108489579	1818	0.04373	0.03055	0.001127	1.432	0.1525
19	rs25487	43551574	1819	-0.007062	0.02214	5.597e-05	-0.3189	0.7498
19	rs1799778	43554989	1813	-0.008295	0.02227	7.659e-05	-0.3724	0.7096

Residual of acute desquamation

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	rs3790568	67370377	1698	-0.00215	0.02071	6.353e-06	-0.1038	0.9173
1	rs6685568	67389614	1689	0.006448	0.01839	7.287e-05	0.3506	0.7259
1	rs12409092	67390948	1698	-0.002987	0.02003	1.31e-05	-0.1491	0.8815
2	rs4849101	112687260	1698	0.005219	0.009982	0.0001611	0.5228	0.6012
2	rs2881208	199272885	1700	-0.01747	0.01015	0.001742	-1.722	0.08532
4	rs1801260	55435202	1694	0.01967	0.01094	0.001906	1.798	0.07242
4	rs13116075	139008878	1700	0.001716	0.01361	9.358e-06	0.1261	0.8997
4	rs17064666	177346497	1701	-0.02116	0.0149	0.001185	-1.42	0.1559
4	rs2877985	177347743	1694	-0.006688	0.0148	0.0001207	-0.452	0.6513
4	rs3805169	177351762	1704	-0.007352	0.01848	9.295e-05	-0.3978	0.6909
4	rs17064704	177354496	1703	-0.00955	0.01932	0.0001436	-0.4943	0.6212
4	rs17064705	177354515	1700	-0.001922	0.02015	5.358e-06	-0.09539	0.924
5	rs575018	101043689	1696	0.002048	0.01011	2.422e-05	0.2026	0.8395
5	rs505994	101048059	1702	0.001923	0.01007	2.145e-05	0.191	0.8486
5	rs2961935	160401392	1674	0.0001315	0.01167	7.598e-08	0.01127	0.991
5	rs2961944	160408651	1703	0.00403	0.01104	7.832e-05	0.365	0.7151
5	rs2910190	160415458	1688	0.003876	0.01115	7.165e-05	0.3476	0.7282
5	rs2910201	160423365	1702	-0.01049	0.01041	0.0005974	-1.008	0.3136
5	rs2961950	160424738	1695	-0.01124	0.01048	0.0006793	-1.073	0.2835
5	rs2961951	160427271	1691	0.003095	0.01128	4.454e-05	0.2743	0.7839
5	rs2961952	160429522	1699	0.00599	0.011	0.0001746	0.5444	0.5862
5	rs2961911	160434568	1701	0.00203	0.01112	1.96e-05	0.1825	0.8552
6	rs17142289	6550516	1704	-0.008829	0.03003	5.079e-05	-0.294	0.7688
6	rs596917	41420606	1636	0.01168	0.01122	0.0006629	1.041	0.298
6	rs3757244	90587350	1662	NA	NA	NA	NA	NA
6	rs6934341	111098387	1702	0.01393	0.01379	0.0006	1.01	0.3125
6	rs4947106	111100163	1703	0.01137	0.01393	0.000392	0.8167	0.4142
6	rs9384787	111113707	1704	0.01472	0.01384	0.0006643	1.064	0.2876
6	rs193281	111166487	1702	0.01539	0.014	0.0007112	1.1	0.2715
6	rs395564	111176176	1699	0.01502	0.01388	0.0006892	1.082	0.2795
6	rs377716	111181705	1704	0.01377	0.01391	0.0005752	0.9897	0.3225
6	rs354525	111186193	1695	0.0138	0.01405	0.0005695	0.9822	0.3261
6	rs354523	111188122	1697	0.006821	0.01372	0.0001458	0.4972	0.6191
6	rs354542	111193483	1704	0.01305	0.0139	0.000518	0.9392	0.3478
6	rs354538	111196863	1704	0.01337	0.01387	0.0005464	0.9646	0.3349
6	rs434034	111204180	1703	0.01417	0.01394	0.0006064	1.016	0.3098
6	rs354546	111211927	1703	0.01671	0.01474	0.0007544	1.133	0.2573
6	rs354547	111213919	1703	0.01405	0.01396	0.0005955	1.007	0.3142
6	rs354526	111216278	1703	0.01603	0.01466	0.0007026	1.094	0.2743
6	rs354527	111217390	1699	0.01543	0.01491	0.0006309	1.035	0.3008
6	rs354551	111224337	1703	0.01483	0.01396	0.0006637	1.063	0.288
6	rs191631	111226862	1701	0.01976	0.01472	0.00106	1.342	0.1796
6	rs3912092	111251609	1704	0.005301	0.01648	6.08e-05	0.3217	0.7477
6	rs240986	111265852	1704	0.0137	0.01523	0.0004749	0.8993	0.3686

				res_desq				
6	rs190246	111277043	1690	0.01407	0.0153	0.0005003	0.9192	0.3581
6	rs190245	111294270	1704	0.02299	0.01567	0.001263	1.467	0.1425
6	rs240998	111307676	1704	0.02028	0.0153	0.001032	1.326	0.1851
6	rs240962	111321243	1704	0.0212	0.01532	0.001124	1.384	0.1666
6	rs4880	159692840	1636	0.001507	0.009375	1.581e-05	0.1607	0.8723
7	rs882460	31494170	1696	0.01239	0.01127	0.000713	1.099	0.2718
7	rs7791642	67241411	1697	-0.007518	0.01839	9.862e-05	-0.4089	0.6827
7	rs10280848	68639201	1696	-0.01273	0.009816	0.0009911	-1.296	0.195
7	rs12531679	136162189	1703	-0.002799	0.01594	1.812e-05	-0.1756	0.8607
8	rs8178046	47929148	1704	0.009588	0.03439	4.567e-05	0.2788	0.7804
9	rs6475752	23658013	1696	0.01323	0.01085	0.0008768	1.219	0.2229
9	rs2230806	104858586	1701	0.01448	0.01106	0.001008	1.309	0.1906
9	rs818704	113386036	1668	-0.007949	0.01318	0.0002182	-0.603	0.5466
9	rs818707	113387687	1684	0.0159	0.0151	0.0006586	1.053	0.2926
9	rs7037705	122145255	1700	0.01339	0.02052	0.0002509	0.6527	0.514
11	rs2001635	67094924	1696	-0.008435	0.01662	0.000152	-0.5075	0.6119
11	rs608273	67158297	1699	-0.01059	0.01756	0.0002142	-0.603	0.5466
11	rs674499	67195954	1702	-0.01016	0.01758	0.0001962	-0.5776	0.5636
11	rs4542419	67203126	1697	0.001505	0.01703	4.608e-06	0.08838	0.9296
11	rs3927807	67231384	1700	-0.008194	0.01804	0.0001214	-0.4541	0.6498
11	rs2071007	67282821	1703	0.006486	0.01686	8.698e-05	0.3847	0.7005
11	rs872110	67397320	1696	-0.003014	0.01706	1.842e-05	-0.1767	0.8598
11	rs2286620	67399513	1701	-0.002655	0.01876	1.18e-05	-0.1416	0.8874
11	rs1638586	67407126	1696	-0.007056	0.01684	0.0001036	-0.419	0.6753
11	rs1558256	67412217	1698	-0.01124	0.01667	0.0002678	-0.674	0.5004
11	rs1558257	67412554	1649	-0.02006	0.0217	0.0005182	-0.9241	0.3556
11	rs1790733	67418529	1695	-0.01074	0.01687	0.0002394	-0.6367	0.5244
11	rs4754296	108135455	1702	0.01058	0.01385	0.0003431	0.7638	0.4451
11	rs73006226	108202001	1650	0.007323	0.01503	0.000144	0.4873	0.6261
11	rs4988023	108298268	1703	0.01106	0.01414	0.0003596	0.7823	0.4342
11	rs1801516	108304735	1701	0.01044	0.01419	0.0003184	0.7356	0.4621
11	rs3092993	108364388	1699	0.01091	0.01422	0.0003467	0.7672	0.4431
11	rs10890838	108432578	1679	0.01198	0.01572	0.0003465	0.7624	0.4459
11	rs7115351	108454062	1703	0.007661	0.01353	0.0001886	0.5664	0.5712
11	rs11212650	108456491	1704	0.01007	0.01374	0.0003159	0.7334	0.4634
11	rs17108024	108462809	1704	0.005889	0.01346	0.0001125	0.4375	0.6618
11	rs4753840	108467613	1704	0.01193	0.01376	0.0004415	0.8671	0.386
11	rs4754317	108488434	1702	0.0147	0.01411	0.0006384	1.042	0.2975
11	rs72993806	108488962	1697	0.01347	0.01406	0.0005413	0.9581	0.3381
11	rs7947933	108489579	1703	0.0136	0.01406	0.0005498	0.9673	0.3335
17	rs3744355	34962027	1693	-0.03154	0.01619	0.00224	-1.948	0.05155
18	rs17798101	24540972	1699	0.00275	0.01365	2.391e-05	0.2014	0.8404
19	rs25487	43551574	1704	-0.0112	0.01019	0.000709	-1.099	0.272
19	rs1799778	43554989	1698	-0.01257	0.01025	0.0008865	-1.227	0.2201

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Residual of acute desquamation

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	0	0	1	NA
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

Residual of acute erythema

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	rs3790568	67370377	1744	0.03364	0.02835	0.0008077	1.187	0.2355
1	rs6685568	67389614	1735	0.03359	0.02521	0.001023	1.332	0.183
1	rs12409092	67390948	1744	0.04776	0.02751	0.001727	1.736	0.08275
2	rs4849101	112687260	1744	0.002944	0.01361	2.685e-05	0.2163	0.8288
2	rs2881208	199272885	1746	0.03062	0.01387	0.002787	2.208	0.02738
4	rs1801260	55435202	1740	0.01943	0.01493	0.0009736	1.301	0.1933
4	rs13116075	139008878	1746	0.03704	0.01857	0.002275	1.994	0.04627
4	rs17064666	177346497	1747	-0.004553	0.02034	2.87e-05	-0.2238	0.8229
4	rs2877985	177347743	1740	-0.01495	0.02017	0.0003159	-0.7411	0.4587
4	rs3805169	177351762	1750	-0.001751	0.02515	2.773e-06	-0.06962	0.9445
4	rs17064704	177354496	1749	-0.01039	0.02629	8.935e-05	-0.3951	0.6928
4	rs17064705	177354515	1746	-0.0001625	0.02745	2.009e-08	-0.00592	0.9953
5	rs575018	101043689	1742	0.02192	0.01377	0.001455	1.592	0.1116
5	rs505994	101048059	1748	0.01994	0.01374	0.001205	1.452	0.1468
5	rs2961935	160401392	1720	-0.00453	0.01588	4.737e-05	-0.2853	0.7755
5	rs2961944	160408651	1749	-0.009259	0.0151	0.0002152	-0.6132	0.5398
5	rs2910190	160415458	1734	-0.009375	0.01522	0.0002189	-0.6158	0.5381
5	rs2910201	160423365	1748	-0.001836	0.01423	9.529e-06	-0.129	0.8974
5	rs2961950	160424738	1740	-0.003082	0.01428	2.679e-05	-0.2158	0.8292
5	rs2961951	160427271	1737	-0.001183	0.01538	3.408e-06	-0.0769	0.9387
5	rs2961952	160429522	1745	-0.003164	0.015	2.551e-05	-0.2109	0.833
5	rs2961911	160434568	1747	-0.001957	0.01519	9.52e-06	-0.1289	0.8975
6	rs17142289	6550516	1750	-0.01833	0.04085	0.0001152	-0.4487	0.6537
6	rs596917	41420606	1682	0.003961	0.01537	3.952e-05	0.2577	0.7967
6	rs3757244	90587350	1706	NA	NA	NA	NA	NA
6	rs6934341	111098387	1748	-0.0001623	0.01884	4.248e-08	-0.008613	0.9931
6	rs4947106	111100163	1749	0.0002169	0.01903	7.436e-08	0.0114	0.9909
6	rs9384787	111113707	1750	0.001309	0.01892	2.737e-06	0.06917	0.9449
6	rs193281	111166487	1748	0.002092	0.01913	6.851e-06	0.1094	0.9129
6	rs395564	111176176	1745	0.002323	0.01897	8.601e-06	0.1224	0.9026
6	rs377716	111181705	1750	4.216e-05	0.01902	2.812e-09	0.002217	0.9982
6	rs354525	111186193	1741	0.005509	0.01916	4.752e-05	0.2875	0.7738
6	rs354523	111188122	1743	0.002208	0.01872	7.988e-06	0.1179	0.9061
6	rs354542	111193483	1750	-0.00103	0.019	1.683e-06	-0.05424	0.9568
6	rs354538	111196863	1750	0.001473	0.01895	3.455e-06	0.07771	0.9381
6	rs434034	111204180	1749	0.001602	0.01906	4.042e-06	0.08403	0.933
6	rs354546	111211927	1749	-0.008672	0.02012	0.0001063	-0.431	0.6665
6	rs354547	111213919	1749	0.0001429	0.01908	3.213e-08	0.007492	0.994
6	rs354526	111216278	1749	-0.007875	0.02001	8.865e-05	-0.3936	0.694
6	rs354527	111217390	1745	-0.002708	0.02042	1.01e-05	-0.1327	0.8945
6	rs354551	111224337	1749	0.005491	0.01908	4.743e-05	0.2879	0.7735
6	rs191631	111226862	1747	0.0009516	0.02007	1.288e-06	0.04742	0.9622
6	rs3912092	111251609	1750	-0.00308	0.02252	1.07e-05	-0.1368	0.8912
6	rs240986	111265852	1750	-0.004884	0.02085	3.139e-05	-0.2343	0.8148

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6	rs190246	111277043	1736	-0.006078	0.02098	4.838e-05	-0.2896	0.7721
6	rs190245	111294270	1750	0.008083	0.02144	8.129e-05	0.377	0.7062
6	rs240998	111307676	1750	0.0066	0.02094	5.684e-05	0.3152	0.7526
6	rs240962	111321243	1750	0.007662	0.02097	7.637e-05	0.3654	0.7149
6	rs4880	159692840	1682	-0.01784	0.01289	0.001139	-1.384	0.1665
7	rs882460	31494170	1743	0.01556	0.0153	0.0005938	1.017	0.3093
7	rs7791642	67241411	1743	-0.04367	0.02507	0.00174	-1.742	0.08164
7	rs10280848	68639201	1742	-0.005169	0.01336	8.606e-05	-0.387	0.6988
7	rs12531679	136162189	1749	-0.03265	0.02166	0.001299	-1.507	0.1319
8	rs8178046	47929148	1750	0.03563	0.04655	0.0003349	0.7653	0.4442
9	rs6475752	23658013	1741	-0.001046	0.01483	2.858e-06	-0.0705	0.9438
9	rs2230806	104858586	1746	0.03352	0.01506	0.002833	2.226	0.02613
9	rs818704	113386036	1713	-0.01387	0.01791	0.0003505	-0.7746	0.4387
9	rs818707	113387687	1730	-0.006554	0.02051	5.91e-05	-0.3196	0.7493
9	rs7037705	122145255	1746	0.006891	0.02778	3.528e-05	0.2481	0.8041
11	rs2001635	67094924	1742	0.002596	0.02244	7.691e-06	0.1157	0.9079
11	rs608273	67158297	1745	-0.01008	0.02366	0.0001041	-0.426	0.6701
11	rs674499	67195954	1748	-0.01191	0.02373	0.0001444	-0.5021	0.6157
11	rs4542419	67203126	1743	-0.01041	0.02307	0.0001168	-0.4511	0.652
11	rs3927807	67231384	1745	-0.006645	0.02444	4.24e-05	-0.2719	0.7858
11	rs2071007	67282821	1749	-0.01502	0.02277	0.0002491	-0.6597	0.5095
11	rs872110	67397320	1742	0.002352	0.023	6.013e-06	0.1023	0.9185
11	rs2286620	67399513	1747	-0.008991	0.02531	7.231e-05	-0.3552	0.7225
11	rs1638586	67407126	1740	0.003537	0.0229	1.373e-05	0.1545	0.8772
11	rs1558256	67412217	1744	0.003752	0.02254	1.59e-05	0.1665	0.8678
11	rs1558257	67412554	1695	0.003121	0.02912	6.788e-06	0.1072	0.9146
11	rs1790733	67418529	1740	0.004404	0.02274	2.158e-05	0.1936	0.8465
11	rs4754296	108135455	1748	0.007969	0.01891	0.0001018	0.4215	0.6734
11	rs73006226	108202001	1692	0.01773	0.02047	0.0004439	0.8664	0.3864
11	rs4988023	108298268	1749	0.01654	0.01929	0.0004207	0.8575	0.3913
11	rs1801516	108304735	1747	0.01737	0.01936	0.0004607	0.8968	0.3699
11	rs3092993	108364388	1745	0.01709	0.0194	0.0004452	0.8811	0.3784
11	rs10890838	108432578	1725	0.02684	0.02136	0.0009157	1.257	0.209
11	rs7115351	108454062	1749	0.01768	0.01847	0.0005246	0.9575	0.3384
11	rs11212650	108456491	1750	0.01453	0.01875	0.0003435	0.7751	0.4384
11	rs17108024	108462809	1750	0.01608	0.01838	0.0004378	0.875	0.3817
11	rs4753840	108467613	1750	0.01539	0.01878	0.0003844	0.8199	0.4124
11	rs4754317	108488434	1748	0.01785	0.01925	0.0004919	0.9269	0.3541
11	rs72993806	108488962	1743	0.01503	0.01917	0.0003529	0.7839	0.4332
11	rs7947933	108489579	1749	0.01737	0.01919	0.0004685	0.9049	0.3656
17	rs3744355	34962027	1738	0.0116	0.02192	0.0001614	0.5294	0.5966
18	rs17798101	24540972	1745	0.02656	0.0186	0.001168	1.428	0.1536
19	rs25487	43551574	1750	-0.009424	0.01387	0.0002641	-0.6796	0.4969
19	rs1799778	43554989	1744	-0.01038	0.01394	0.0003181	-0.7445	0.4567

Residual of acute erythema

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	1	1	0.2	rs12409092
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

Residual of acute oedema

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	rs3790568	67370377	1744	0.0007354	0.03247	2.944e-07	0.02265	0.9819
1	rs6685568	67389614	1735	0.00513	0.02878	1.834e-05	0.1783	0.8585
1	rs12409092	67390948	1744	0.01361	0.03153	0.000107	0.4318	0.666
2	rs4849101	112687260	1744	-0.004305	0.01558	4.382e-05	-0.2763	0.7823
2	rs2881208	199272885	1746	0.01382	0.01587	0.0004347	0.8709	0.3839
4	rs1801260	55435202	1740	-0.01053	0.01709	0.0002183	-0.6161	0.5379
4	rs13116075	139008878	1746	0.008525	0.0213	9.18e-05	0.4001	0.6891
4	rs17064666	177346497	1747	-0.01471	0.02328	0.0002289	-0.632	0.5274
4	rs2877985	177347743	1740	-0.03959	0.02308	0.00169	-1.715	0.0865
4	rs3805169	177351762	1750	-0.03415	0.02876	0.0008062	-1.188	0.2352
4	rs17064704	177354496	1749	-0.03589	0.03005	0.000816	-1.194	0.2325
4	rs17064705	177354515	1746	-0.0321	0.03139	0.0005993	-1.023	0.3066
5	rs575018	101043689	1742	0.01525	0.01579	0.0005358	0.9658	0.3343
5	rs505994	101048059	1748	0.01303	0.01572	0.0003936	0.8291	0.4071
5	rs2961935	160401392	1720	-0.001486	0.01817	3.895e-06	-0.0818	0.9348
5	rs2961944	160408651	1749	-0.001385	0.01727	3.68e-06	-0.08018	0.9361
5	rs2910190	160415458	1734	-0.002525	0.01741	1.214e-05	-0.145	0.8847
5	rs2910201	160423365	1748	0.0115	0.01627	0.0002859	0.7066	0.4799
5	rs2961950	160424738	1740	0.00862	0.01633	0.0001603	0.5278	0.5977
5	rs2961951	160427271	1737	0.01118	0.01759	0.000233	0.6358	0.525
5	rs2961952	160429522	1745	0.01729	0.01714	0.0005832	1.009	0.3133
5	rs2961911	160434568	1747	0.01033	0.01735	0.0002032	0.5955	0.5516
6	rs17142289	6550516	1750	-0.03762	0.04672	0.0003707	-0.8051	0.4208
6	rs596917	41420606	1682	0.006231	0.01751	7.536e-05	0.3558	0.722
6	rs3757244	90587350	1706	NA	NA	NA	NA	NA
6	rs6934341	111098387	1748	-0.009772	0.02156	0.0001177	-0.4533	0.6504
6	rs4947106	111100163	1749	-0.01133	0.02177	0.0001552	-0.5207	0.6026
6	rs9384787	111113707	1750	-0.01282	0.02164	0.0002007	-0.5923	0.5537
6	rs193281	111166487	1748	-0.01532	0.02186	0.0002811	-0.7006	0.4836
6	rs395564	111176176	1745	-0.009995	0.02168	0.0001219	-0.461	0.6448
6	rs377716	111181705	1750	-0.009683	0.02175	0.0001134	-0.4452	0.6562
6	rs354525	111186193	1741	-0.007947	0.02199	7.512e-05	-0.3615	0.7178
6	rs354523	111188122	1743	-0.01298	0.02142	0.000211	-0.6062	0.5445
6	rs354542	111193483	1750	-0.01176	0.02173	0.0001675	-0.5412	0.5885
6	rs354538	111196863	1750	-0.006257	0.02168	4.765e-05	-0.2886	0.7729
6	rs434034	111204180	1749	-0.0133	0.02179	0.0002132	-0.6103	0.5417
6	rs354546	111211927	1749	-0.01234	0.02302	0.0001644	-0.536	0.592
6	rs354547	111213919	1749	-0.01447	0.02182	0.0002518	-0.6633	0.5072
6	rs354526	111216278	1749	-0.01314	0.02288	0.0001888	-0.5744	0.5658
6	rs354527	111217390	1745	-0.0127	0.02335	0.0001698	-0.5441	0.5865
6	rs354551	111224337	1749	-0.01863	0.02182	0.0004171	-0.8538	0.3933
6	rs191631	111226862	1747	-0.01297	0.02297	0.0001827	-0.5646	0.5724
6	rs3912092	111251609	1750	-0.02237	0.02575	0.0004318	-0.869	0.385
6	rs240986	111265852	1750	-0.02003	0.02384	0.0004035	-0.84	0.401

				res_oedema				
6	rs190246	111277043	1736	-0.01859	0.02395	0.0003471	-0.776	0.4379
6	rs190245	111294270	1750	-0.00849	0.02453	6.855e-05	-0.3462	0.7293
6	rs240998	111307676	1750	-0.01278	0.02395	0.0001629	-0.5337	0.5936
6	rs240962	111321243	1750	-0.01323	0.02398	0.0001741	-0.5517	0.5812
6	rs4880	159692840	1682	0.01116	0.01476	0.0003402	0.7561	0.4497
7	rs882460	31494170	1743	0.0136	0.01751	0.0003463	0.7766	0.4375
7	rs7791642	67241411	1743	-0.004205	0.02869	1.234e-05	-0.1466	0.8835
7	rs10280848	68639201	1742	0.01336	0.01528	0.0004394	0.8746	0.3819
7	rs12531679	136162189	1749	0.0204	0.02479	0.0003876	0.823	0.4106
8	rs8178046	47929148	1750	0.04685	0.05325	0.0004428	0.8799	0.379
9	rs6475752	23658013	1741	0.02957	0.01698	0.001741	1.742	0.08177
9	rs2230806	104858586	1746	-0.01355	0.01725	0.0003537	-0.7855	0.4323
9	rs818704	113386036	1713	0.01332	0.02053	0.000246	0.6488	0.5165
9	rs818707	113387687	1730	0.007521	0.02348	5.935e-05	0.3203	0.7488
9	rs7037705	122145255	1746	0.01523	0.03179	0.0001317	0.4792	0.6318
11	rs2001635	67094924	1742	0.001576	0.02566	2.167e-06	0.06141	0.951
11	rs608273	67158297	1745	-0.01046	0.02707	8.558e-05	-0.3862	0.6994
11	rs674499	67195954	1748	-0.008125	0.02713	5.137e-05	-0.2995	0.7646
11	rs4542419	67203126	1743	0.00612	0.02639	3.089e-05	0.2319	0.8166
11	rs3927807	67231384	1745	-0.01489	0.02791	0.0001633	-0.5336	0.5937
11	rs2071007	67282821	1749	0.008368	0.02605	5.906e-05	0.3212	0.7481
11	rs872110	67397320	1742	-0.006132	0.02627	3.132e-05	-0.2334	0.8154
11	rs2286620	67399513	1747	-0.02488	0.02894	0.0004233	-0.8596	0.3901
11	rs1638586	67407126	1740	0.001422	0.02616	1.701e-06	0.05437	0.9566
11	rs1558256	67412217	1744	0.003192	0.02574	8.824e-06	0.124	0.9013
11	rs1558257	67412554	1695	-0.04895	0.03314	0.001286	-1.477	0.1399
11	rs1790733	67418529	1740	0.001541	0.02602	2.02e-06	0.05925	0.9528
11	rs4754296	108135455	1748	0.00484	0.02161	2.871e-05	0.2239	0.8229
11	rs73006226	108202001	1692	0.00939	0.02349	9.456e-05	0.3998	0.6894
11	rs4988023	108298268	1749	0.002328	0.02207	6.372e-06	0.1055	0.916
11	rs1801516	108304735	1747	0.002692	0.02211	8.494e-06	0.1217	0.9031
11	rs3092993	108364388	1745	4.274e-06	0.02216	2.134e-11	0.0001928	0.9998
11	rs10890838	108432578	1725	0.01474	0.02441	0.0002116	0.6039	0.546
11	rs7115351	108454062	1749	0.0008062	0.02112	8.342e-07	0.03818	0.9696
11	rs11212650	108456491	1750	0.007895	0.02145	7.75e-05	0.3681	0.7129
11	rs17108024	108462809	1750	0.002797	0.02103	1.012e-05	0.133	0.8942
11	rs4753840	108467613	1750	0.002812	0.02148	9.804e-06	0.1309	0.8959
11	rs4754317	108488434	1748	0.02012	0.02202	0.0004778	0.9136	0.361
11	rs72993806	108488962	1743	0.0147	0.02192	0.0002582	0.6706	0.5026
11	rs7947933	108489579	1749	0.01961	0.02195	0.0004568	0.8936	0.3717
17	rs3744355	34962027	1738	0.02222	0.02513	0.0004502	0.8842	0.3767
18	rs17798101	24540972	1745	0.00455	0.02131	2.615e-05	0.2135	0.831
19	rs25487	43551574	1750	0.007021	0.01586	0.000112	0.4426	0.6581
19	rs1799778	43554989	1744	0.0104	0.01593	0.0002445	0.6527	0.514

res_oedema.qassoc.set

Residual of acute oedema

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	0	0	1	NA
NEIL3	5	1	1	0.1646	rs2877985
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

Residual of acute pain

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	rs3790568	67370377	1720	0.07837	0.03171	0.003543	2.472	0.01354
1	rs6685568	67389614	1711	0.08127	0.02806	0.004883	2.896	0.003828
1	rs12409092	67390948	1720	0.08415	0.03079	0.004328	2.733	0.006344
2	rs4849101	112687260	1720	-0.02027	0.01528	0.001023	-1.326	0.1849
2	rs2881208	199272885	1722	-0.01985	0.01552	0.0009504	-1.279	0.201
4	rs1801260	55435202	1717	0.005666	0.01674	6.682e-05	0.3385	0.735
4	rs13116075	139008878	1722	0.02391	0.02078	0.0007688	1.15	0.2502
4	rs17064666	177346497	1723	-0.01055	0.0227	0.0001254	-0.4646	0.6423
4	rs2877985	177347743	1716	-0.02258	0.02254	0.0005851	-1.002	0.3166
4	rs3805169	177351762	1726	-0.02651	0.02806	0.0005173	-0.9446	0.345
4	rs17064704	177354496	1725	-0.01908	0.0293	0.0002462	-0.6513	0.5149
4	rs17064705	177354515	1722	-0.01339	0.0306	0.0001113	-0.4377	0.6617
5	rs575018	101043689	1718	0.001166	0.01544	3.322e-06	0.07551	0.9398
5	rs505994	101048059	1724	0.001269	0.01537	3.957e-06	0.08255	0.9342
5	rs2961935	160401392	1696	0.01405	0.01777	0.000369	0.7908	0.4292
5	rs2961944	160408651	1725	0.01637	0.01688	0.0005453	0.9696	0.3324
5	rs2910190	160415458	1711	0.01977	0.017	0.0007905	1.163	0.2451
5	rs2910201	160423365	1724	0.01641	0.01591	0.0006175	1.031	0.3025
5	rs2961950	160424738	1716	0.01582	0.01598	0.000572	0.9905	0.3221
5	rs2961951	160427271	1713	0.02395	0.01716	0.001137	1.396	0.163
5	rs2961952	160429522	1721	0.02606	0.01676	0.001404	1.554	0.1203
5	rs2961911	160434568	1723	0.02282	0.01697	0.001049	1.344	0.1791
6	rs17142289	6550516	1726	-0.05939	0.04563	0.0009813	-1.301	0.1933
6	rs596917	41420606	1661	0.0007332	0.01718	1.098e-06	0.04269	0.966
6	rs3757244	90587350	1682	NA	NA	NA	NA	NA
6	rs6934341	111098387	1724	0.005872	0.02107	4.512e-05	0.2787	0.7805
6	rs4947106	111100163	1725	0.005672	0.02126	4.132e-05	0.2668	0.7896
6	rs9384787	111113707	1726	0.002175	0.02113	6.141e-06	0.1029	0.9181
6	rs193281	111166487	1724	0.001236	0.02137	1.944e-06	0.05786	0.9539
6	rs395564	111176176	1721	-0.0001829	0.02117	4.343e-08	-0.00864	0.9931
6	rs377716	111181705	1726	0.003045	0.02124	1.192e-05	0.1433	0.886
6	rs354525	111186193	1717	0.004492	0.0215	2.546e-05	0.209	0.8345
6	rs354523	111188122	1719	0.0007506	0.02091	7.508e-07	0.0359	0.9714
6	rs354542	111193483	1726	0.004462	0.02122	2.563e-05	0.2102	0.8335
6	rs354538	111196863	1726	0.004428	0.02117	2.537e-05	0.2091	0.8344
6	rs434034	111204180	1725	0.002922	0.02129	1.093e-05	0.1372	0.8909
6	rs354546	111211927	1725	0.009305	0.02247	9.948e-05	0.414	0.6789
6	rs354547	111213919	1725	0.006571	0.02131	5.518e-05	0.3083	0.7579
6	rs354526	111216278	1725	0.01019	0.02236	0.0001205	0.4557	0.6487
6	rs354527	111217390	1721	0.01763	0.02281	0.0003472	0.7727	0.4398
6	rs354551	111224337	1725	0.0008245	0.02131	8.687e-07	0.03869	0.9691
6	rs191631	111226862	1723	0.01127	0.02244	0.0001465	0.5021	0.6157
6	rs3912092	111251609	1726	-0.0006717	0.02517	4.131e-07	-0.02669	0.9787
6	rs240986	111265852	1726	-0.005986	0.02331	3.825e-05	-0.2568	0.7974

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6	rs190246	111277043	1712	-0.005599	0.02342	3.343e-05	-0.2391	0.8111
6	rs190245	111294270	1726	-0.01353	0.02398	0.0001847	-0.5644	0.5726
6	rs240998	111307676	1726	-0.01028	0.02341	0.0001118	-0.4391	0.6606
6	rs240962	111321243	1726	-0.005396	0.02345	3.072e-05	-0.2301	0.818
6	rs4880	159692840	1658	-0.01183	0.01449	0.000402	-0.8161	0.4146
7	rs882460	31494170	1719	-0.005743	0.01715	6.531e-05	-0.3349	0.7378
7	rs7791642	67241411	1719	0.004149	0.02819	1.261e-05	0.1472	0.883
7	rs10280848	68639201	1718	0.004606	0.01498	5.511e-05	0.3075	0.7585
7	rs12531679	136162189	1725	0.01499	0.02436	0.0002198	0.6155	0.5383
8	rs8178046	47929148	1726	-0.04591	0.05245	0.0004442	-0.8753	0.3815
9	rs6475752	23658013	1717	-0.002372	0.01661	1.19e-05	-0.1428	0.8864
9	rs2230806	104858586	1722	-0.007984	0.01691	0.0001296	-0.4721	0.6369
9	rs818704	113386036	1689	0.01094	0.02016	0.0001745	0.5425	0.5875
9	rs818707	113387687	1707	0.0227	0.0229	0.0005761	0.9914	0.3216
9	rs7037705	122145255	1722	0.04736	0.03104	0.001352	1.526	0.1272
11	rs2001635	67094924	1718	0.01579	0.02501	0.0002323	0.6315	0.5278
11	rs608273	67158297	1721	0.01565	0.02635	0.0002051	0.5938	0.5527
11	rs674499	67195954	1724	0.01677	0.02641	0.0002341	0.6349	0.5256
11	rs4542419	67203126	1719	0.00743	0.02566	4.883e-05	0.2896	0.7722
11	rs3927807	67231384	1721	0.02698	0.02722	0.0005713	0.9912	0.3217
11	rs2071007	67282821	1725	0.005316	0.02538	2.546e-05	0.2095	0.8341
11	rs872110	67397320	1718	0.01119	0.02558	0.0001115	0.4374	0.6618
11	rs2286620	67399513	1723	0.04163	0.0282	0.001265	1.476	0.1401
11	rs1638586	67407126	1716	0.01633	0.0255	0.0002392	0.6404	0.522
11	rs1558256	67412217	1720	0.009085	0.0251	7.626e-05	0.362	0.7174
11	rs1558257	67412554	1671	0.03798	0.03245	0.0008203	1.171	0.2419
11	rs1790733	67418529	1716	0.01352	0.02539	0.0001654	0.5324	0.5945
11	rs4754296	108135455	1724	-0.009115	0.02119	0.0001074	-0.4301	0.6672
11	rs73006226	108202001	1668	-0.005112	0.0229	2.991e-05	-0.2232	0.8234
11	rs4988023	108298268	1725	-0.01354	0.02164	0.0002273	-0.6259	0.5314
11	rs1801516	108304735	1723	-0.01538	0.02172	0.0002913	-0.7081	0.479
11	rs3092993	108364388	1721	-0.01623	0.02175	0.0003239	-0.7463	0.4556
11	rs10890838	108432578	1702	-0.003071	0.02388	9.723e-06	-0.1286	0.8977
11	rs7115351	108454062	1725	-0.01794	0.02069	0.0004358	-0.8668	0.3862
11	rs11212650	108456491	1726	-0.01364	0.02102	0.0002442	-0.6489	0.5165
11	rs17108024	108462809	1726	-0.02247	0.0206	0.0006902	-1.091	0.2753
11	rs4753840	108467613	1726	-0.01074	0.02105	0.000151	-0.5103	0.6099
11	rs4754317	108488434	1724	-0.01981	0.02158	0.0004888	-0.9177	0.3589
11	rs72993806	108488962	1719	-0.02016	0.0215	0.0005118	-0.9376	0.3486
11	rs7947933	108489579	1725	-0.0197	0.02151	0.0004866	-0.9158	0.3599
17	rs3744355	34962027	1714	0.00809	0.02455	6.339e-05	0.3295	0.7419
18	rs17798101	24540972	1721	0.01393	0.02078	0.0002612	0.6702	0.5028
19	rs25487	43551574	1726	-0.004602	0.01558	5.06e-05	-0.2954	0.7678
19	rs1799778	43554989	1720	-0.005552	0.01566	7.318e-05	-0.3546	0.723

Residual of acute pain

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	3	1	0.01317	rs6685568
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

Residual of acute ulceration

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	rs3790568	67370377	1698	0.0004901	0.02007	3.516e-07	0.02442	0.9805
1	rs6685568	67389614	1689	0.005842	0.01782	6.369e-05	0.3278	0.7431
1	rs12409092	67390948	1698	-0.0002204	0.01941	7.602e-08	-0.01135	0.9909
2	rs4849101	112687260	1698	0.006171	0.009671	0.00024	0.638	0.5235
2	rs2881208	199272885	1700	-0.01517	0.009837	0.001398	-1.542	0.1233
4	rs1801260	55435202	1694	0.0198	0.0106	0.002057	1.867	0.06201
4	rs13116075	139008878	1700	-0.007126	0.01319	0.0001719	-0.5402	0.5891
4	rs17064666	177346497	1701	-0.02168	0.01444	0.001324	-1.501	0.1335
4	rs2877985	177347743	1694	-0.004122	0.01434	4.884e-05	-0.2875	0.7738
4	rs3805169	177351762	1704	-0.008715	0.01791	0.0001391	-0.4865	0.6266
4	rs17064704	177354496	1703	-0.01205	0.01872	0.0002435	-0.6437	0.5199
4	rs17064705	177354515	1700	-0.004946	0.01953	3.777e-05	-0.2533	0.8001
5	rs575018	101043689	1696	0.001788	0.009795	1.968e-05	0.1826	0.8551
5	rs505994	101048059	1702	0.001619	0.009759	1.619e-05	0.1659	0.8682
5	rs2961935	160401392	1674	-0.002655	0.01131	3.298e-05	-0.2348	0.8144
5	rs2961944	160408651	1703	0.001361	0.0107	9.512e-06	0.1272	0.8988
5	rs2910190	160415458	1688	0.001024	0.01081	5.323e-06	0.09473	0.9245
5	rs2910201	160423365	1702	-0.01214	0.01009	0.0008518	-1.204	0.2288
5	rs2961950	160424738	1695	-0.01291	0.01016	0.0009541	-1.272	0.2037
5	rs2961951	160427271	1691	0.001303	0.01094	8.398e-06	0.1191	0.9052
5	rs2961952	160429522	1699	0.003159	0.01067	5.171e-05	0.2962	0.7671
5	rs2961911	160434568	1701	0.0004753	0.01078	1.144e-06	0.04408	0.9648
6	rs17142289	6550516	1704	-0.01379	0.0291	0.0001318	-0.4737	0.6358
6	rs596917	41420606	1636	0.01028	0.0109	0.000544	0.943	0.3458
6	rs3757244	90587350	1662	NA	NA	NA	NA	NA
6	rs6934341	111098387	1702	0.01523	0.01337	0.0007629	1.139	0.2548
6	rs4947106	111100163	1703	0.01483	0.01349	0.0007099	1.099	0.2718
6	rs9384787	111113707	1704	0.01589	0.01341	0.000824	1.185	0.2363
6	rs193281	111166487	1702	0.0188	0.01356	0.001128	1.386	0.166
6	rs395564	111176176	1699	0.01858	0.01345	0.001122	1.381	0.1675
6	rs377716	111181705	1704	0.01722	0.01348	0.0009581	1.278	0.2016
6	rs354525	111186193	1695	0.01494	0.01361	0.0007109	1.097	0.2726
6	rs354523	111188122	1697	0.01128	0.01329	0.0004243	0.8482	0.3964
6	rs354542	111193483	1704	0.01651	0.01347	0.0008824	1.226	0.2204
6	rs354538	111196863	1704	0.01683	0.01344	0.0009214	1.253	0.2104
6	rs434034	111204180	1703	0.01763	0.01351	0.0009998	1.305	0.1921
6	rs354546	111211927	1703	0.0249	0.01428	0.001784	1.744	0.08142
6	rs354547	111213919	1703	0.01754	0.01352	0.0009874	1.297	0.1949
6	rs354526	111216278	1703	0.02416	0.0142	0.001698	1.701	0.08909
6	rs354527	111217390	1699	0.02361	0.01444	0.001574	1.635	0.1021
6	rs354551	111224337	1703	0.01823	0.01352	0.001067	1.348	0.1778
6	rs191631	111226862	1701	0.02528	0.01426	0.001846	1.773	0.07645
6	rs3912092	111251609	1704	0.00959	0.01597	0.0002119	0.6006	0.5482
6	rs240986	111265852	1704	0.01908	0.01476	0.0009804	1.292	0.1964

				res_ulcer				
6	rs190246	111277043	1690	0.01955	0.01482	0.00103	1.319	0.1874
6	rs190245	111294270	1704	0.0252	0.01518	0.001616	1.66	0.09713
6	rs240998	111307676	1704	0.02294	0.01482	0.001405	1.547	0.1219
6	rs240962	111321243	1704	0.0238	0.01484	0.001508	1.603	0.1091
6	rs4880	159692840	1636	0.003671	0.009065	0.0001003	0.4049	0.6856
7	rs882460	31494170	1696	0.01043	0.01092	0.0005382	0.9551	0.3397
7	rs7791642	67241411	1697	-0.003796	0.01782	2.678e-05	-0.2131	0.8313
7	rs10280848	68639201	1696	-0.0103	0.009515	0.000691	-1.082	0.2793
7	rs12531679	136162189	1703	0.0006531	0.01545	1.05e-06	0.04226	0.9663
8	rs8178046	47929148	1704	0.01587	0.03333	0.0001331	0.4761	0.6341
9	rs6475752	23658013	1696	0.01256	0.01052	0.0008409	1.194	0.2326
9	rs2230806	104858586	1701	0.01418	0.01071	0.001029	1.323	0.186
9	rs818704	113386036	1668	-0.003791	0.01276	5.294e-05	-0.297	0.7665
9	rs818707	113387687	1684	0.02127	0.01462	0.001256	1.454	0.146
9	rs7037705	122145255	1700	0.004934	0.01989	3.624e-05	0.2481	0.8041
11	rs2001635	67094924	1696	-0.01198	0.01611	0.0003266	-0.7439	0.457
11	rs608273	67158297	1699	-0.01154	0.01701	0.0002711	-0.6783	0.4977
11	rs674499	67195954	1702	-0.01112	0.01704	0.0002504	-0.6525	0.5142
11	rs4542419	67203126	1697	0.0009274	0.0165	1.863e-06	0.0562	0.9552
11	rs3927807	67231384	1700	-0.009321	0.01749	0.0001673	-0.533	0.5941
11	rs2071007	67282821	1703	0.005974	0.01634	7.854e-05	0.3655	0.7148
11	rs872110	67397320	1696	-0.003525	0.01654	2.683e-05	-0.2132	0.8312
11	rs2286620	67399513	1701	0.004006	0.01818	2.859e-05	0.2204	0.8256
11	rs1638586	67407126	1696	-0.007353	0.01632	0.0001199	-0.4506	0.6523
11	rs1558256	67412217	1698	-0.01146	0.01615	0.0002969	-0.7097	0.478
11	rs1558257	67412554	1649	-0.01833	0.02101	0.0004621	-0.8726	0.383
11	rs1790733	67418529	1695	-0.01106	0.01635	0.00027	-0.6762	0.499
11	rs4754296	108135455	1702	0.00905	0.01342	0.0002674	0.6744	0.5002
11	rs73006226	108202001	1650	0.007124	0.01456	0.0001453	0.4894	0.6247
11	rs4988023	108298268	1703	0.009383	0.0137	0.0002756	0.6848	0.4935
11	rs1801516	108304735	1701	0.008729	0.01376	0.0002369	0.6346	0.5258
11	rs3092993	108364388	1699	0.009245	0.01378	0.000265	0.6707	0.5025
11	rs10890838	108432578	1679	0.006003	0.01523	9.259e-05	0.3941	0.6936
11	rs7115351	108454062	1703	0.004556	0.01311	7.099e-05	0.3475	0.7282
11	rs11212650	108456491	1704	0.006534	0.01332	0.0001415	0.4907	0.6237
11	rs17108024	108462809	1704	0.002825	0.01305	2.754e-05	0.2165	0.8286
11	rs4753840	108467613	1704	0.01073	0.01333	0.0003805	0.8049	0.421
11	rs4754317	108488434	1702	0.01074	0.01367	0.0003626	0.7852	0.4324
11	rs72993806	108488962	1697	0.009579	0.01363	0.0002914	0.7029	0.4822
11	rs7947933	108489579	1703	0.009658	0.01363	0.000295	0.7085	0.4787
17	rs3744355	34962027	1693	-0.02407	0.0157	0.001389	-1.533	0.1254
18	rs17798101	24540972	1699	0.0006315	0.01323	1.343e-06	0.04773	0.9619
19	rs25487	43551574	1704	-0.01032	0.00988	0.0006404	-1.044	0.2965
19	rs1799778	43554989	1698	-0.01174	0.00993	0.0008232	-1.182	0.2374

res_ulcer.qassoc.set

Residual of acute ulceration

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	0	0	1	NA
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	4	1	1e-06	rs191631
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

rSTAT (acute)

CHR	SNP	BP	NMISS	BETA	SE	R2	T	P
1	rs3790568	67370377	1747	0.04964	0.04395	0.0007305	1.129	0.2589
1	rs6685568	67389614	1738	0.0658	0.039	0.001637	1.687	0.09177
1	rs12409092	67390948	1747	0.06431	0.04258	0.001305	1.51	0.1312
2	rs4849101	112687260	1747	-0.002534	0.02103	8.319e-06	-0.1205	0.9041
2	rs2881208	199272885	1749	-0.01191	0.02149	0.0001758	-0.5542	0.5795
4	rs1801260	55435202	1743	0.03682	0.02311	0.001455	1.593	0.1114
4	rs13116075	139008878	1749	0.02877	0.02878	0.0005715	0.9995	0.3177
4	rs17064666	177346497	1750	-0.04226	0.03154	0.001026	-1.34	0.1805
4	rs2877985	177347743	1743	-0.04065	0.03128	0.0009689	-1.299	0.194
4	rs3805169	177351762	1753	-0.03727	0.03895	0.0005226	-0.9569	0.3388
4	rs17064704	177354496	1752	-0.04185	0.04071	0.0006035	-1.028	0.3041
4	rs17064705	177354515	1749	-0.02036	0.04248	0.0001315	-0.4794	0.6317
5	rs575018	101043689	1745	0.02257	0.02138	0.0006388	1.056	0.2913
5	rs505994	101048059	1751	0.0204	0.02129	0.0005247	0.9582	0.3381
5	rs2961935	160401392	1723	0.0002784	0.02465	7.411e-08	0.01129	0.991
5	rs2961944	160408651	1752	0.005024	0.02338	2.638e-05	0.2149	0.8299
5	rs2910190	160415458	1737	0.005533	0.0236	3.167e-05	0.2344	0.8147
5	rs2910201	160423365	1751	-0.004211	0.02203	2.09e-05	-0.1912	0.8484
5	rs2961950	160424738	1743	-0.007887	0.02213	7.293e-05	-0.3563	0.7216
5	rs2961951	160427271	1740	0.01678	0.02385	0.0002847	0.7036	0.4818
5	rs2961952	160429522	1748	0.02271	0.02323	0.0005473	0.9778	0.3283
5	rs2961911	160434568	1750	0.01424	0.02351	0.0002099	0.6058	0.5447
6	rs17142289	6550516	1753	-0.06627	0.06328	0.0006258	-1.047	0.2952
6	rs596917	41420606	1685	0.01969	0.02369	0.0004102	0.831	0.4061
6	rs3757244	90587350	1709	NA	NA	NA	NA	NA
6	rs6934341	111098387	1751	0.0161	0.02921	0.0001736	0.5511	0.5816
6	rs4947106	111100163	1752	0.01326	0.02947	0.0001157	0.4501	0.6527
6	rs9384787	111113707	1753	0.01478	0.0293	0.0001453	0.5044	0.614
6	rs193281	111166487	1751	0.01608	0.02963	0.0001685	0.5429	0.5873
6	rs395564	111176176	1748	0.01763	0.02937	0.0002063	0.6002	0.5484
6	rs377716	111181705	1753	0.0162	0.02945	0.0001727	0.5499	0.5825
6	rs354525	111186193	1744	0.01864	0.02973	0.0002256	0.627	0.5308
6	rs354523	111188122	1746	0.005982	0.02901	2.439e-05	0.2062	0.8366
6	rs354542	111193483	1753	0.01436	0.02943	0.0001361	0.4881	0.6255
6	rs354538	111196863	1753	0.01854	0.02936	0.0002277	0.6315	0.5278
6	rs434034	111204180	1752	0.01581	0.02952	0.0001638	0.5354	0.5924
6	rs354546	111211927	1752	0.0204	0.03117	0.0002447	0.6545	0.5129
6	rs354547	111213919	1752	0.01606	0.02954	0.0001689	0.5437	0.5867
6	rs354526	111216278	1752	0.01991	0.03099	0.0002358	0.6425	0.5207
6	rs354527	111217390	1748	0.02496	0.03156	0.0003581	0.7908	0.4292
6	rs354551	111224337	1752	0.01524	0.02954	0.0001519	0.5157	0.6061
6	rs191631	111226862	1750	0.02823	0.03109	0.0004712	0.9078	0.3641
6	rs3912092	111251609	1753	-0.005321	0.03488	1.329e-05	-0.1525	0.8788
6	rs240986	111265852	1753	0.006161	0.0323	2.078e-05	0.1908	0.8487

					rSTAT			
6	rs190246	111277043	1739	0.006974	0.03246	2.658e-05	0.2149	0.8299
6	rs190245	111294270	1753	0.02615	0.03321	0.000354	0.7875	0.4311
6	rs240998	111307676	1753	0.02167	0.03243	0.0002548	0.6681	0.5042
6	rs240962	111321243	1753	0.02547	0.03248	0.000351	0.7841	0.4331
6	rs4880	159692840	1685	-0.004863	0.01975	3.601e-05	-0.2462	0.8056
7	rs882460	31494170	1745	0.02875	0.02371	0.0008428	1.213	0.2255
7	rs7791642	67241411	1746	-0.02925	0.03884	0.0003251	-0.7531	0.4515
7	rs10280848	68639201	1745	-0.01376	0.02072	0.0002527	-0.6638	0.5069
7	rs12531679	136162189	1752	-0.002247	0.03355	2.564e-06	-0.06699	0.9466
8	rs8178046	47929148	1753	0.02418	0.07214	6.416e-05	0.3352	0.7375
9	rs6475752	23658013	1744	0.03053	0.02299	0.001012	1.328	0.1843
9	rs2230806	104858586	1749	0.02847	0.02336	0.0008496	1.219	0.2231
9	rs818704	113386036	1716	-0.005241	0.02782	2.071e-05	-0.1884	0.8506
9	rs818707	113387687	1733	0.03514	0.03174	0.0007075	1.107	0.2684
9	rs7037705	122145255	1749	0.04105	0.04303	0.0005207	0.954	0.3402
11	rs2001635	67094924	1745	-0.003504	0.03479	5.82e-06	-0.1007	0.9198
11	rs608273	67158297	1748	-0.01645	0.03664	0.0001154	-0.4489	0.6536
11	rs674499	67195954	1751	-0.01521	0.03671	9.812e-05	-0.4143	0.6787
11	rs4542419	67203126	1746	0.004483	0.03569	9.045e-06	0.1256	0.9001
11	rs3927807	67231384	1748	-0.007408	0.03781	2.199e-05	-0.1959	0.8447
11	rs2071007	67282821	1752	0.008417	0.03524	3.259e-05	0.2388	0.8113
11	rs872110	67397320	1745	-0.001947	0.03558	1.718e-06	-0.05473	0.9564
11	rs2286620	67399513	1750	0.004071	0.03916	6.18e-06	0.1039	0.9172
11	rs1638586	67407126	1743	0.0005824	0.03541	1.554e-07	0.01645	0.9869
11	rs1558256	67412217	1747	-0.008936	0.03485	3.767e-05	-0.2564	0.7977
11	rs1558257	67412554	1698	-0.02828	0.04506	0.0002322	-0.6276	0.5303
11	rs1790733	67418529	1743	-0.007636	0.03524	2.698e-05	-0.2167	0.8285
11	rs4754296	108135455	1751	0.01253	0.02927	0.0001048	0.4282	0.6686
11	rs73006226	108202001	1694	0.01764	0.03179	0.000182	0.555	0.5789
11	rs4988023	108298268	1752	0.01384	0.02986	0.0001228	0.4636	0.643
11	rs1801516	108304735	1750	0.01262	0.02998	0.0001013	0.4209	0.6739
11	rs3092993	108364388	1748	0.01159	0.03003	8.534e-05	0.386	0.6995
11	rs10890838	108432578	1728	0.02634	0.03309	0.0003669	0.7959	0.4262
11	rs7115351	108454062	1752	0.006105	0.02859	2.606e-05	0.2135	0.8309
11	rs11212650	108456491	1753	0.01259	0.02903	0.0001074	0.4337	0.6646
11	rs17108024	108462809	1753	0.001688	0.02846	2.008e-06	0.05929	0.9527
11	rs4753840	108467613	1753	0.01635	0.02907	0.0001806	0.5624	0.5739
11	rs4754317	108488434	1751	0.0238	0.0298	0.0003646	0.7987	0.4246
11	rs72993806	108488962	1746	0.01811	0.02969	0.0002132	0.6098	0.542
11	rs7947933	108489579	1752	0.02182	0.02971	0.0003081	0.7344	0.4628
17	rs3744355	34962027	1741	-0.02109	0.03408	0.0002202	-0.6189	0.5361
18	rs17798101	24540972	1748	0.02609	0.02878	0.0004704	0.9065	0.3648
19	rs25487	43551574	1753	-0.01888	0.02147	0.0004412	-0.8791	0.3795
19	rs1799778	43554989	1747	-0.02024	0.02158	0.000504	-0.9381	0.3483

rSTAT (acute) gene set analysis

SET	NSNP	NSIG	ISIG	EMP1	SNPS
IL12RB2	3	1	1	0.1757	rs6685568
NEIL3	5	0	0	1	NA
PTTG1	8	0	0	1	NA
POLZ	23	0	0	1	NA
ALAD	2	0	0	1	NA
RAD9A	12	0	0	1	NA
ATM	13	0	0	1	NA
XRCC1	2	0	0	1	NA
ST8SIA4	2	0	0	1	NA

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Abstract: Background and purpose
In the field of radiogenomics, several potential predictive genetic markers have been identified that are associated with individual susceptibility to radiotoxicity. Predictive models of radiotoxicity incorporating radiogenomics and other biomarkers are being developed as part of the ongoing multi-centre REQUITE trial. The purpose of this study was to explore patient attitudes towards future predictive radiogenomics testing for breast radiotoxicity.

Patients and methods
Twenty-one semi-structured interviews were conducted with breast cancer patients taking part in the REQUITE study at one centre. Inductive thematic analysis was used to generate common themes.

Results
Three emerging themes were identified describing attitudes and feelings towards a predictive radiogenomics test for breast radiotoxicity: Theme 1 - willingness to undergo a test (subthemes - information, trusted expert), Theme 2 - implications of a test (subthemes - preparation and planning, anxiety without recourse), and Theme 3 - impact on treatment decision-making (subthemes - prioritising cancer cure, preserving breast integrity, patient preferences).

Conclusions
Results from the present study indicate that patients support and have confidence in the validity of a radiogenomics test for breast radiotoxicity, but they would prefer the result be provided to healthcare professionals. Except in cases of significant chronic symptoms and pain or significant end-organ damage, participants in this study rarely felt that advance knowledge of their personal risk of breast radiotoxicity would influence their treatment decision-making. These findings provide

a number of insights that will allow us to anticipate how patients are likely to engage with predictive radiogenomics testing in the future.

*Covering Letter

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Dr Charlotte E Coles
Editor-in-Chief
Clinical Oncology
Elsevier

Dear Charlotte

As discussed via email, please find attached our paper entitled 'The patient perspective on radiogenomics testing for breast radiotoxicity' for submission to *Clinical Oncology*.

The aim of this study was to explore patient attitudes towards future predictive radiogenomics testing for breast radiotoxicity, using semi-structured interviews. We recruited patients from the prospective REQUITE cohort study, which has been previously described in one for your editorials.

We believe that this is the first study exploring patient views on this aspect of personalized medicine and hope that you will find it suitable for publication in *Clinical Oncology*.

Kind regards

Yours sincerely

Tim Rattay
NIHR Clinical Research Fellow
Honorary SpR in Breast Surgery

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Research Highlights

- In the field of radiogenomics, several potential predictive genetic markers have been identified that are associated with individual susceptibility to radiotoxicity.
- Before these are implemented in clinical practice, it is important to gather patients' perspectives on predictive radiogenomics testing.
- Using a test for acute skin toxicity as a prompt, breast cancer patients enrolled in the prospective REQUITE cohort study underwent semi-structured interviews with subsequent inductive thematic analysis.
- Participants expressed confidence in the validity of a predictive test for breast radiotoxicity, but would prefer the result to be provided to healthcare professionals rather than directly to patients.
- Except in cases of significant chronic side-effects or end-organ damage, participants rarely felt that advance knowledge of their personal risk of breast radiotoxicity would influence their treatment decision-making.

*Title Page

The patient perspective on radiogenomics testing for breast radiotoxicity

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Abstract

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Conclusions

Results from the present study indicate that patients support and have confidence in the validity of a radiogenomics test for breast radiotoxicity, but they would prefer the result be provided to healthcare professionals. Except in cases of significant chronic symptoms and pain or significant end-organ damage, participants in this study rarely felt that advance knowledge of their personal risk of breast radiotoxicity would influence their treatment decision-making. These findings provide a number of insights that will allow us to anticipate how patients are likely to engage with predictive radiogenomics testing in the future.

Introduction

Breast cancer survival has improved markedly, with current predicted 10-year survival rates in excess of 80 % [1]. Survivorship issues and quality of life (QoL) are an increasingly important research focus in cancer care [2]. Over 70 % of breast cancer patients undergo radiotherapy. Radiotherapy reduces the risk of local recurrence and contributes to a reduction in overall mortality [3-5], but can be associated with side-effects (toxicity) in the breast. Acute toxicity occurs within 90 days of treatment and includes erythema (reddening) and fatigue. Late (long-term) toxicity, such as fibrosis, shrinkage, and telangiectasia can occur months and years after treatment [6]. Patients are affected by radiation toxicity to varying degrees [7]. Individual sensitivity to radiotherapy depends on various clinical factors, including dosimetry, body habitus, and smoking, but genetic variation is also an important contributor [8-10].

The impact of radiotoxicity on QoL is well documented in existing breast radiotherapy trials [11-13]. The majority of women due to undergo radiotherapy are anxious about side-effects and changes to their breast appearance [14]. To guide the treatment decision-making process, individual risk prediction models for radiotoxicity are currently being developed by integrating clinical and patient factors with predictive biomarkers [15]. Several potential predictive genetic markers for radiotoxicity have been identified through genetic association studies [16-19].

However, before predictive genetic (radiogenomics) testing is implemented in the clinic, it is important to gather patients' perspectives to ensure this research is relevant and appropriate, and to explore how such predictive test results should be delivered in the future. The aim of the present study was to explore the views of breast cancer patients enrolled in the ongoing REQUITE cohort study [20] on future predictive radiogenomics testing for breast radiotoxicity, using acute skin toxicity as a prompt. While late radiotherapy side-effects remain a clinical concern, acute radiotoxicity is increasingly recognised for its impact on breast cosmesis and patients' quality of life [21, 22]. The objectives of the study were to generate a thematic description of patients' feelings and attitudes towards a radiogenomics test, and to explore how such a predictive test could impact patients' breast cancer treatment decision-making.

Patients and methods

Study design

This qualitative study was conducted using semi-structured interviews with breast cancer patients enrolled in the REQUITE cohort study. It was approved by major amendment as the REQUITE-AB-QoL sub-study by the NRES Committee North West – Greater Manchester East (14/NW/0035).

Setting

Semi-structured interviews were conducted with breast cancer patients on completion of treatment in the radiotherapy department or at 6-week follow-up at University of Leicester Hospitals. These time points were chosen in anticipation that most patients had experienced toxicity by this point. One patient was interviewed in her home. Interviews were preferred over focus groups, as the issues explored were potentially personally sensitive.

Sampling and recruitment

Eligibility criteria for the REQUITE breast cohort study were: being female, over age 18 with primary cancer of the breast, and receiving whole-breast radiotherapy after breast-conserving surgery (BCS), including patients receiving neo-adjuvant or adjuvant chemotherapy. Mastectomy patients and patients with previous breast irradiation were excluded. For the present qualitative study, patients were required to give additional consent to be interviewed.

Sample size was determined by data generated from participants; interviews continued until thematic saturation was reached and no new topics emerged. Participants were sampled purposively to ensure adequate representation of degree of toxicity, age, cancer stage, and history of chemotherapy [23].

Patient interviews

Interviews were semi-structured and conducted by one researcher (TR) following an interview guide developed specifically for this study (see Supplementary Material). Pilot interviews were conducted with five female postgraduate researchers in psychology and five female non-academic university staff, all of whom had no history of breast cancer or radiotherapy. Two authors (TR and JBS) reviewed the pilot interviews and changes were made to the interview guide, particularly because pilot participants found it difficult to comprehend the concept and purpose of predictive radiogenomics testing. No further changes were made to the guide during interviews with patient participants.

Interviews were audio-recorded and transcribed verbatim using professional transcription services. Anonymity was ensured by using only first names, initials, or the option of using a fictional name during the interview. At the start of the interview, the concept of radiogenomics testing for radiotoxicity was explained, using the example of a test for acute skin toxicity, and the participant's understanding was confirmed. Participants were invited to express their perceived pros and cons of this proposed test. Participants were verbally presented with three standardized fictional case vignettes: one with test results suggesting a high likelihood of severe skin toxicity; one suggesting

mild or no skin toxicity, and one inconclusive test result. Based on their own experience of radiotherapy, participants were invited to describe their reaction to the different test results.

Following the initial discussion, the interview guide further inquired about the feasibility and implementation of a predictive test for breast skin toxicity as well as integration of the test result into treatment decision-making [24]. Participants were asked about perceived advantages and disadvantages for themselves and their healthcare professionals, and the level of predicted toxicity risk that would influence their treatment decision-making (e.g. BCS + radiotherapy vs. mastectomy +/- reconstruction without radiotherapy). Attitudes towards testing for long-term toxicity were also explored.

The relationship between researcher and participant was carefully considered [25]. Although the researcher conducting the interviews was surgically trained and worked as a research physician on the main REQUITE study, he was not involved in the participants' usual medical care, nor did he work clinically in the radiotherapy department where participants were recruited. Participants were advised that any medical issues raised during the interview would be referred to their usual medical team.

Data analysis

Anonymised transcripts were imported into NVivo 10 for Windows software. Inductive thematic analysis was used to describe participants' feelings and attitudes towards a predictive test for breast radiotoxicity, and to explore how the test result could impact their treatment decision-making [26]. Emerging themes were identified through systematic coding and constantly compared across transcripts. Each transcript was coded independently by TR and JBS who conferred after every two to three interviews. Fifty-two initial codes were combined into three primary themes. Minor coding discrepancies were resolved through discussion between authors. All interviews were included in the analysis.

Results

Twenty-one female patients were interviewed. Three main themes emerged from the data regarding patient attitudes towards a future predictive radiogenomics test for breast radiotoxicity: 1) Willingness to undergo a test (subthemes – information, trusted expert), 2) Implications of a test (subthemes – preparation and planning, anxiety without recourse), and 3) Impact on treatment decision-making (subthemes – prioritising cancer cure, preserving breast integrity, patient preferences) (Table 1).

Participants' characteristics

Table 2 summarises participants' characteristics. Median age was 60 years (range 41 to 81). Median interview length was 30:43 minutes (23:33 to 39:11). All participants had undergone BCS plus axillary sentinel node biopsy or axillary dissection and received whole breast radiotherapy. Two participants also received axillary radiotherapy. Only one participant had previous experience of personal genetic testing and was awaiting results of a BRCA1/2 mutation test.

Theme 1: Willingness to undergo a radiogenomics test

Participants felt a predictive radiogenomics test would be just as routine as any medical test in their journey through cancer treatment. *I think it's all part of the package.* (P14) *I think it's just one blood sample at a time when you're having blood samples done all the time.* (P1) *It would have just been one lesser thing in a long line of worse things that you've had to have done.* (P3)

Information

Participants had a personal interest in the information a future predictive radiogenomics test could provide. *It's wise to be informed really, isn't it?* (P4) The information was perceived as empowering patients to make informed choices about their treatment. *Because then they'd be more informed, better able to make a decision, better able to make choices, and I think that's quite important to have the choice rather than have somebody say 'you are having this, you are having that', and then end up looking not the way you want to look*. (P6)

Participants felt that patient autonomy in making any treatment decision based on the radiogenomics test should be respected. *Even if that test came back and said, yep, yours is likely to be the worst reaction ever […], you could still say 'actually, I'm still going to go with wide [local] excision and radiotherapy'. So having the test doesn't mean you're then tied to having radiotherapy or not.* (P3)

Some participants were concerned that this additional test could lead to information overload. While some would not wish to find out this information at all, others felt they would want the news but delivered a little bit at a time. *It sounds an absolutely good idea, but I personally wouldn't like to know how severe it's going to be. I wouldn't like to know that this was coming my way.* (P21)

Trusted expert

Participants preferred the healthcare professional (HCP) or doctor providing their breast cancer care to receive the radiogenomics test result. *I think it is important, certainly from a healthcare perspective, but not necessarily for the individual.* (P21) In this sample, this attitude might have been associated with a more general sense of participants' trust in their healthcare providers and willingness to be guided by them. *I would have gone along, yeah, like I said, because I trusted them to tell me what was best for me.* (P2) *Dr [oncologist] and Dr [surgeon], I've just been guided by what they say. […] So I didn't sort of question it, I just went with what they said.* (P19)

Participants were particularly interested in HCPs using the test result to provide an individual risk estimate for side-effects as well as a reference frame for different predicted levels of toxicity, for example, with the help of visual aids. *It's bound to help them in the planning.* (P10) *That's going to help people make a decision along with the help from the consultant […], I think you also need to be guided.* (P6) *OK, so you've got your test result now and fine […] you won't have any reaction. I'd still want some pictures, I'd still want to know what 'fine' looks like.* (P3)

Theme 2: Implications of a radiogenomics test

The proposed radiogenomics test generated a range of behavioural and emotional responses from particpants. If they perceived the additional information as positive, participants felt the test result would reassure and provide them with accurate expectations about the course of their treatment. *Well, for myself it's that the test is – well, that piece of mind – to know what to expect.* (P11)

Preparation and planning

Some participants felt that being aware of their personal risk of radiotherapy side-effects could help them prepare and plan for side-effects. *Well, I think preparing yourself for it. I think forewarned is forearmed, isn't it, really?* (P13) *I'm OK because I know it's coming and I'll be half prepared that if it does come then don't be scared, this is all part and parcel of the treatment.* (P7) If predicted to have severe toxicity, some participants were prepared to adjust their daily routine or use preventative measures, such as additional creams, to counteract side-effects. *It might have been helpful in sort of planning ahead. If I knew that radiotherapy was going to make me very ill then, you know, I might have been able to change things about work.* (P1) *Preparing yourself really, yes, making sure you have your right moisturisers, things like Aloe Vera.* (P18)

If predicted to have severe radiotoxicity, participants also expected closer observation and intervention by the HCP. *Yeah, well at least they know what to look out for, and they'll think oh well she has got these genes so perhaps we'll keep an eye and see if this happens. I assume that'd be the best way.* (P7) *I would want to know what help was available. You know, as you're informing people of the side-effects, have you got any answers, you know, to help the patient through any sort of serious damage to their breast – you know, their skin?* (P13)

Anxiety without recourse

Some participants were concerned that advance knowledge of severe radiotherapy side-effects could lead to feelings of anxiety, dread and powerlessness, particularly if there were no available options for symptom management. *Because if there's no other option and they have to go through the radiotherapy then that's a scary prospect.* (P16) *I think if you're told, yeah, you could get this, you could get that, it depends what sort of person you are, you could go home fretting, worrying, think about and dwell on it. If you're not then I just think what will be will be.* (P2) This anxiety was weighted more on long-term breast toxicity, such as fibrosis (scarring) and atrophy (shrinkage), rather than acute skin toxicity. *I don't know if that would be frightening to know that in the long term it's going to end up some sort of scarred mess or not, I mean, I believe if it's not then that's great but I don't know, I think I'd be frightened about that.* (P6)

However, these emotions of anxiety and dread were modified according to the value participants placed on having certainty from the test result. *I suppose anticipating damage and watching the damage happen might psychologically be a bit difficult, but that's weighed against being prepared for something that was going to be distressing.* (P14)

Theme 3: Impact on treatment decision-making

Whether the radiogenomics test result would influence treatment decision-making depended on participants' priorities and treatment preferences as well as their attitude to mastectomy.

Prioritising cancer cure

'Cancer cure' was prioritised over the risk of treatment side-effects, particularly acute skin toxicity, which is likely to be transient. *You need to know that the cancer's going to go. I think my skin can get better. I'm not sure the cancer can get better.* (P20) *Anything to cure the cancer, I'd have gone through. No, it doesn't matter what the side-effects would have been. I'd have still done it, definitely, and I think anyone who doesn't, is risking their health.* (P11) Accordingly, participants might consider mastectomy if required by their cancer but not to avoid radiotherapy side-effects. *If I had to have a mastectomy because of the cancer then I'd have it, but if it was just because I was going to get side effects from the radiotherapy I wouldn't because I can cope with side effects.* (P19)

Preserving breast integrity

Preserving the integrity of their breast was important even in the scenario of predicted severe acute skin toxicity. *So if I was told 'well if you have a mastectomy then your prognosis is the same', I would say 'well why would I want to have that, I'd rather have the skin changes and keep my breast'.* (P14) *I think having a mastectomy just for skin irritation or that, then no, I wouldn't. […] No, because obviously what's months? You know you can deal with months. Once a mastectomy has gone, it's gone, isn't it?* (P18)

Patient preferences

Some participants appeared willing to entertain the idea of mastectomy to avoid radiotherapy under certain conditions. Chronic long-term toxicity such as fibrosis (scarring) was considered important, although it would have to be weighed against the side-effects of a more extensive mastectomy. *Maybe if somebody thought they were going to be really scarred, but then you're going to be scarred by having a mastectomy.* (P6)

Symptoms of severe or chronic pain and sensitivity might change participants' treatment decision. *If it was me, if you said that, that your skin would come off and it'll be painful, I think I'd go for the mastectomy, I think I would say 'no I don't want radiotherapy' from this test, yeah.* (P6) *I would certainly consider if there was pain and oversensitivity.* (P14) If participants perceived a given side-effect to be chronic, to require long-term maintenance or entail further suffering, this might reach the threshold for changing their treatment decision. *Depends on how bad they think it's going to be in the long term, for me, I just want things over and done with and finished, where if it's going to make it drag on and drag on then probably not, probably I'd go for the other option and get everything over and done with.* (P7)

Other participants raised concerns about significant complications affecting surrounding vital organs, albeit rare, which might affect their decision-making regarding treatment. *If I was told 'well in your case I'm sorry but the radiotherapy will severely damage your lung, then I'd have to think about whether I would then have a mastectomy.* (P14)

Some comments suggested that patient preferences and hence the impact on treatment decision making might differ according to age. *In terms of cosmetic effects I would be less worried about that but I'm 69 so if I was 35 or 55, it would probably matter more.* (P14) *Somebody younger might be, but as somebody who is coming up to 60, no.* (P21)

Discussion

The clinical application of predictive radiogenomics testing raises several practical challenges [15]. The proposed study was designed to assess how patients who might be offered radiogenomics testing in the future, understand this form of personalised medicine and how they perceive its potential benefits and risks.

The themes identified in the present study are consistent with the literature from other fields on patients' reactions to receiving personalised genetic test results [27]. Participants preferred the result of this radiogenomics test to be provided to their HCP or doctor, rather than provided as direct-to-consumer testing. While patients are ethically autonomous, this notion of the doctor as a trusted expert resonates with the concept that many patients may reflect back the responsibility for treatment decisions to their HCP [28]. While some patients wanted as much information on their risk as possible, others preferred not to receive too much information on personalised risk, which aligns with the concept of information 'monitors' and 'blunters' [29].

The issue of provider training in genomic testing has been raised in other fields of personalised medicine [30]. If their predicted skin toxicity were severe, participants in this study would expect their HCP to provide a management plan, which might include a spectrum of interventions from symptomatic modifiers, such as creams, advice to change their daily routine, to changing the treatment plan altogether and avoid the need for radiotherapy where clinically possible. Both anxiety and patient preferences are likely to play a role in negotiating this treatment plan, and HCPs will be required to pay particular attention to a patient's expectations and decision-making style [31, 32].

For participants in this study, predicting symptomatic side-effects such as pain was equally important as clinical signs of skin change or fibrosis. Participants also felt that the severity of long-term side-effects would more likely have an impact on their treatment decision-making than acute (short-term) toxicity. The accuracy of a future predictive radiogenomics test was not questioned by participants, although concerns about accuracy and clinical utility of genomics testing are often held by providers [33].

Limitations

There are several limitations associated with the present study. It was conducted in a single centre participating in the REQUITE study with a sample of British, largely Caucasian White female breast cancer patients and therefore may not reflect the views of patients from other nationalities, ethnicities, with different cancer types, or with different healthcare systems. Mastectomy patients are excluded from the main REQUITE study, so did not feature in this sample.

Conclusions

Before radiogenomics testing is implemented in the clinic, it is important to gather patients' perspectives on the appropriateness, delivery and implications of such a test. Using a test for acute skin toxicity as a prompt, results from the present study indicate that patients would support and have confidence in the validity of a predictive radiogenomics test for breast toxicity, but they would prefer the result be provided to HCPs (rather than provided directly to patients). Except in cases of significant chronic symptoms or end-organ damage, participants rarely felt that advance knowledge of their personal risk of breast radiotoxicity would influence their treatment decision-making.

As the test result may provoke emotions of anxiety and dread, it will be important how the provider presents and frames the information from the test. In discussing any treatment recommendation based on the test result, HCPs should take into account the patient's preferences, but the results indicate that many patients would prioritise cancer cure and breast integrity. Future research should explore in more detail not only how patients but also their HCPs will use the information from a predictive radiogenomics test in the clinic.

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Conflict of interest statement

The authors have no conflict of interests to disclose.

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Tables

Table 1. Emerging themes describing patient attitudes towards a future predictive radiogenomics test for breast radiotoxicity.

Main theme	**Sub-themes (description)**
1. Willingness to undergo a radiogenomics test	• Additional information is good but may lead to information overload. • HCPs as the trusted expert should receive and explain test result and provide patient with a management plan accordingly.
2. Implications of a radiogenomics test	• Preparation and planning both for patient and HCPs • Enhances anxiety or dread, particularly in the absence of symptom modifiers, or if long-term toxicity such as scarring and chronic pain were predicted.
3. Impact on treatment decision-making	• Benefit of cancer cure is prioritised over risk of treatment side-effects, particularly acute toxicity, which is usually transient. • Preserving breast integrity is more important than avoiding acute side-effects by undergoing more surgery (e.g. mastectomy +/- reconstruction) • Individual preferences may dictate whether patients change their treatment plain to avoid radiotherapy in case of significant predicted long-term side-effects.

Table 2. Participant characteristics (n = 21).

	Number of Participants
Age group	
under 50	4
50 to 59	6
60 to 69	7
over 70	4
Ethnicity	
White European	20
Indian	1
Breast cancer stage	
Tis (DCIS)	3
T1N0	12
T1N1	4
pT0pN0	1
pT1pN1	1
Receptor status	
ER positive	12
HER2 positive	2
Triple negative	4
Not assessed (DCIS)	3
Chemotherapy	
None	12
Discussed but not received	3
Adjuvant	4
Neoadjuvant	2
Acute skin toxicity	
grade 0	3
grade 1 (mild erythema)	12
grade 2 (moderate erythema and/or patchy moist desquamation)	5
grade 3 (confluent moist desquamation)	1

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The patient perspective on radiogenomics testing for breast radiotoxicity

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