SUPPLEMENTAL MATERIAL

Supplemental Methods

Genotyping of the 12 known AMD variants was performed using primer mass extension and MALDI-TOF MS analysis (MassEXTEND methodology of Sequenom, San Diego, CA) at the Broad Institute Center for Genotyping and Analysis.

Allele-specific methylation Detection Method Development
Methods to identify allele-specific methylation were developed through the use of control mixes with known amounts of differential methylation.

Assay Development Control Mixes
To construct samples with known amounts of methylation, we first produced unmethylated replicates of the genomic DNA from clonal lymphoblast lines (LBLs) of two unrelated individuals (E44 (Coriell line GM07057) and H16 (Coriell line GM13130)) by performing whole-genome amplifications. Genomic DNA (10 ng) was amplified using the whole genome amplification phi29 kit (GE Healthcare) according to the manufacturer's manual. The DNA was split into two aliquots and one aliquot methylated with the CpG methyltransferase M.SssI (NEB). Methylated and unmethylated DNAs from the two individuals were then mixed together at various ratios to produce samples with known amounts of methylation. Further, artificial heterozygotes (AABB/BBAA) in these mixes were formed at SNPs homozygous for opposite alleles (i.e. E44-AA and H16-BB) in the two individuals.

Methylation Analyses - Affymetrix Array Data Extraction and Analysis
The median value of the representative allele probe sets was used to summarize allele probe intensities. Traditional normalizations were carried out using the Affymetrix powertools apt-probeset-summarize module [1]. Our variation of invariant probeset normalization was achieved using a combination of custom Perl and R scripts, with the quantile normalization step carried out by the normalize.quantiles.robust module of the affy package [2] in Bioconductor [3]. In order to ensure constancy of quantile adjustments, the HapMap sample NA06985_C_F3 was used as a weighted model for all normalizations.
Conventional Affymetrix 6.0 genotyping was carried out by Birdseed v1.2 [4] using default values. Arrays were run individually and dynamic modeling of clusters disabled i.e. genotypes were called from clusters derived from a reference model based on the 270 HapMap samples. Samples used were derived from GM10849 and GM12093 as previously described [5].

Methylation Analyses - Allele-specific methylation Calling
Previous experiments within the Chess lab relied on the Affymetrix 500K platform [5] and used conventional Affymetrix 500K genotyping methods for detection of allele-specific methylation [1] . Using the Affymetrix 6.0 platform allowed us access to twice as

many SNPs, but required the development and evaluation of new methods. These methods consisted of two main steps; normalization and differential methylation detection.

We attempted to remove variation between arrays of non-biological origin by the process of normalization. Initially, we examined conventional normalization methods for Affymetrix 6.0 chips i.e. median or quantile normalization of all probesets. We studied these methods using two of the most extreme sample types we could expect to encounter in an experiment i.e. a sample in which every MSRE site is unprotected from MSRE digest (unmethylated DNA + MSRE digestion) and a sample in which every site is unaffected by MSRE digestion (unmethylated DNA without MSRE digestion). A robust normalization method should be able to adjust for overall technical variation but leave true biological variation, with the MSRE digested sample distribution skewed towards lower intensities (from MSRE digested probesets) in comparison to the undigested sample, with similar high intensity ranges in the two samples (from any undigested probesets lacking MSRE sites).

When adjusting probe intensities by median normalization of all probesets (to make the overall median equal between these two samples) we found that the post-normalization MSRE digested unmethylated control mix sample ultimately had more probesets with higher intensities than the undigested post-normalization sample (Supplemental Figure 3), a result that does not reflect the underlying biology. These results are likely due to the presence of a large number of probesets with low to no signal (from MSRE digested amplicons) in the un-normalized MSRE digested sample. The presence of these probesets skews the median low; subsequent equalization of the sample medians would result in excessive amplification of undigested probeset intensities. In contrast, quantile normalization results in inappropriate amplification of digested probeset intensities so that the post-normalization MSRE digested sample no longer has probesets with lower intensities than the undigested sample, a result that is also unreflective of the underlying biology. This result is due to quantile normalization methods assuming that samples share the same underlying distribution, an unwarranted assumption in any case where samples differ in their overall methylation levels.

To overcome these challenges, we developed methods tailored for the analysis of MSRE treated samples which took advantage of the fact that many amplicons lack MSRE sites and are expected to be unaffected by MSRE digestion. Specifically, probe intensities from these subsets of MSRE Negative Regions (MNRs) were quantile normalized. Normalization intensity adjustments for each MPR probe were taken from those of the MNR of closest pre-normalization intensity. The identities of MNRs were determined from the consensus human genome sequence and only amplicons with no MSRE sites or modifying SNPs from dbSNP129 [6] were permitted in the set of MNRs (Supplemental Figure 4, probeset IDs are available upon request). As the Affymetrix 6.0 platform does not separately hybridize the NspI and StyI fragment amplicons, we further filtered the MNRs to eliminate any SNPs assayed by a StyI based target amplicon with at least one MSRE site and an NspI-based target amplicon without an MSRE site (or vice versa). To validate this approach we examined the effect of MSRE digestion on MNRs and MPRs

(the latter containing at least one of each of the MSRE sites) in artificially methylated control mixes. While there was little difference in the intensity distributions of MNRs to MPRs in an unmethylated control sample before MSRE digestion, MPRs showed greatly lowered combined probe intensities relative to the MNRs after MSRE digestion (Supplemental Figure 5). Similarly, in the un-normalized data from control mixes with a 1:1 mix of unmethylated DNA from one individual and methylated DNA from the other, the A allele frequency was largely constant regardless of the A allele methylation status. In contrast, MPRs showed lower A allele frequencies when the A allele was unmethylated (Supplemental Figure 6). The effects of this normalization process are illustrated for various control mixes (Supplemental Figure 7).

Previous experiments utilizing the Affymetrix 500K platform detected allele-specific methylation as a genotype change from "AB" in undigested genomic DNA to an "AA" or "BB" genotype in the same sample after MSRE treatment. We initially explored the potential to use conventional Affymetrix 6.0 genotyping methods such as Birdseed [4], which relies on a model-based clustering algorithm to assign SNPs to one of the 3 genotypes, to perform similar analyses with our MSRE treated samples. To do so, we examined the genotyping results from identical samples before and after MSRE treatment, which were run on the Affymetrix 500K (genotyped as previously described [5]**)** and Affymetrix 6.0 platforms (genotyped with the Birdseed algorithm after normalization as detailed above). Comparison of genotypes from these two approaches showed discordant genotypes for up to 15% of the SNPs common between platforms in undigested samples. In contrast, in MSRE treated samples, the genotypes were discordant for up to 40% of the common SNPs. In particular, SNPs labeled NoCall by the 500K platform (mainly representing SNPs with biallelic lack of methylation) were always called by the Birdseed algorithm and accounted up to two-thirds of the discordance in MSRE treated samples while accounting for less than 20% of the original 500K calls. These results suggest that SNPs associated with biallelic lack of methylation were wrongly assigned to genotype clusters in our MSRE treated samples. Birdseed can use a confidence score to exclude SNPs, but we were unable to use these to identify such SNPs from true genotype calls as examination of these confidence scores revealed no difference between the distribution of confidence scores for SNPs with matching calls and those SNPs with mismatching calls between platforms (data not shown). These results indicated that the conventional clustering based methods of genotyping Affymetrix 6.0 could not be easily adapted to the detection of allele-specific methylation in MSRE treated samples.

Our method of determining ASM relies on detecting deviations from the expected allelic ratios observed in both a reference data set and MNRs (Supplemental Figures 8 and 9). As a first step, untreated samples were genotyped with Birdseed; only heterozygous SNPs were analyzed.

For each sample, heterozygous probesets with low intensity were removed from the analysis, as these SNPs should represent biallelically unmethylated SNPs for which the

allelic probe intensities represent only non-specific hybridization (Supplemental Figure 10). For probeset *i* in the sample j, we describe the probe intensity (PI) as the square root of the sum of the squared individual probeA and probeB intensities:

$$PI_{i,j} = \sqrt{probeA_{i,j}^2 + probeB_{i,j}^2}$$

Using this definition, mean baseline PI values were derived for a reference set composed of the 270 samples from the International HapMap project. Applying these HapMap statistics to the individual MSRE treated samples and probesets, within each sample we discarded any MSRE treated probesets that showed less than 20% of the average HapMap probe intensity. All samples and probesets were examined independently, i.e. the discard status of a probates in one sample had no effect on the same probeset's discard status in another sample.

Using the remaining sample probesets, our method detects allele-specific methylation based on standard normalized changes (Z-scores) in the log2 relative allele (probeA/probeB) frequency (RAF) for AB genotypes after MSRE digestion (Supplemental Figures 8 and 9). For probeset i, in sample j RAF was defined as:

$$RAF_{i,j} = \frac{probeA_{i,j}}{probeB_{i,j}}$$

To adjust for inter-probe variation in RAF values, RAF statistics were calculated for every probeset within the HapMap population. These statistics were calculated separately for all genotypes (homozygous reference allele, homozygote alternate allele and heterozygous) at that probeset within the Hapmap population. Here, for probeset i in the Hapmap population, $\mu_{i,Hapmap}$ represents the mean RAF value for Hapmap samples heterozygous at the SNP assayed by probeset i, $\sigma_{i,Hapmap}$ represents the standard deviation of this mean. To ensure that deviations from the expected heterozygous RAF values were reliably detected, probesets showing poor overall separation of genotypes were discarded from all analyses; these were defined as probesets where at least one of the baseline HapMap homozygous mean RAF values was less than 3 standard deviations ($\sigma_{i,Hapmap}$) from the mean RAF value ($\mu_{i,Hapmap}$) for Hapmap samples heterozygous at the SNP assayed by probeset i.

The heterozygous baseline statistics were applied to MSRE-treated heterozygous sample probesets to derive a standard normalized Z-score as follows, where $X_{i,j}$ is the RAF value of sample j for probeset i :

$$Z_{i,j} = \frac{X_{i,j} - \mu_{i,Hapmap}}{\sigma_{i,Hapmap}}$$

To adjust for residual inter-sample technical variability, the Z-score distribution for all MNRs within each sample was determined and lower and upper Z-score cutoffs were chosen for each sample based on their ability to exclude 95% of the MNR Z-score values. Sample SNPs with Z-scores more extreme than these cutoffs, i.e. SNPs with Z-score values lower than the 2.5th percentile or higher than the 97.5th percentile of that sample's MNR Z-scores, were called as either A (A allele methylated) or B (B allele methylated),

depending on the direction of deviation from the mean (lower Z scores=B allele methylated, high Z-scores=A allele methylated).

Supplemental Results

Detection of Differential Methylation Events

The genome-scale approach we deployed to assess ASM is designed to focus on regions outside of CpG islands, which we consider an advantage since, with the exception of X-inactivation, ASM is generally not found for CpG islands. However, to assess the performance of our approach, we began by assessing data from 186 MSRE-positive regions (MPRs) (Affymetrix target regions possessing one or more MSRE sites, for more details see Methods) overlapping CpG islands. As expected, since CpG islands are typically unmethylated, these 186 MPRs showed low median probe intensities in our samples, indicating a lack of methylation. Specifically, of the 186 CpG island MPRs, 148 have intensity values below the 15th percentile of probe intensities for all MPRs. An examination of the 10 CpG island MPRs with the highest median intensities in our samples showed this subset to be largely comprised of fairly atypical CpG islands, of small length (<300 bp in length) and internal to genes (data not shown). These data are consistent with the expected low levels of methylation on both alleles of CpG islands on autosomes.

To validate our approach to detecting ASM, we first examined whether we could observe previously described differentially methylated regions. Our approach, described in detail in the Supplementary Materials, relies on observing outlier allelic ratios after MSRE digestion as an indicator of unmethylated MSRE CpG(s) associated with one allele. Expressed as a standard Z-score, our detection method accounts for both probe set and sample based technical variation, normalizing against both the HapMap distributions of heterozygous probe set allelic ratios and the heterozygous MSRE-Negative Regions' (MNRs) allelic ratios in each individual sample.

Analysis of MPRs proximal to the imprinted genes *SNRPN* and *H19* [30,31] revealed clear patterns of ASM consistent with imprinting (Supplemental Figure 11A and 11B). These MPRs showed consistently outlying Z-scores (i.e. strong differential methylation) with no consistent allelic preference of allele, a pattern consistent with random paternal or maternal inheritance of alleles in our study population. Our approach also replicated findings of sequence influenced ASM [12,13]. Of the 5 ASM loci and the 8 imprinted DMRs from Schalkwyk et al. [13] that we could assay, we observe monoallelic methylation in 5/5 and 7/8 respectively, with the methylation associated allele consistent with that observed by Schalkwyk et al. For example, MPRs assaying the same genomic regions as the rs6494120 and rs943049 variants, showed strong allelic preference of methylation with the C and G alleles respectively, (Supplemental Figure 11C and 11D).

We further validated a subset of these monoallelic methylation events by targeted next-generation bisulfite sequencing in an independent collection of 70 healthy control subjects. We examined 10 regions by bisulfite PCR (Supplemental Table 2); two positive control MPRs previously shown to demonstrate ASM, one MPR that showed no evidence of ASM and seven MPRs with evidence of ASM. All PCRs were designed so that both SNP and all MSRE CpGs would fit within typical 454 pyrosequencing read lengths. These experiments confirmed the presence of ASM at three of the seven MPRs with microarray based evidence of ASM, as defined by rejection of the chi-square null-

hypothesis of no association between methylation state and allele ($p<0.05$) for at least one MSRE CpG within the amplicon (Supplemental Table 4). We also validated a lack of ASM at the MPR without microarray-based evidence of ASM and further confirmed the presence of ASM at the two positive control MPRs. For all ASM MPRs, the allele associated with methylation was consistent between the microarray and sequencing analyses.

Levels of Allele-Specific Methylation

We further examined the levels of ASM in our samples (Supplemental Figure 12). Consistent with previous results [11], whole blood samples showed a range of ASM levels, with an unadjusted median of 7.4% of MPRs exhibiting methylation associated with only one allele. Our method relies on finding outlier allelic ratios at MPRs within the bottom and top 2.5 percentiles of the MNR allelic ration distribution (for more details, see Methods), and as such is expected to have 5% false positive rate. Accounting for this, we observed ASM in an adjusted median of 2.4% of MPRs (Supplemental Table 3).

Supplemental References

1. **Affymetrix Power Tools** [http://www.affymetrix.com/partners_programs/programs/developer/tools/powertools.affx]
2. Gautier L, Cope L, Bolstad BM, Irizarry RA: **affy--analysis of Affymetrix GeneChip data at the probe level**. *Bioinformatics* 2004, **20**(3):307-315.
3. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J *et al*: **Bioconductor: open software development for computational biology and bioinformatics**. *Genome Biol* 2004, **5**(10):R80.
4. Korn JM, Kuruvilla FG, Mccarroll SA, Wysoker A, Nemesh J, Cawley S, Hubbell E, Veitch J, Collins PJ, Darvishi K *et al*: **Integrated genotype calling and association analysis of SNPs, common copy number polymorphisms and rare CNVs**. *Nat Genet* 2008, **40**(10):1253-1260.
5. Hellman A, Chess A: **Extensive sequence-influenced DNA methylation polymorphism in the human genome**. *Epigenetics & Chromatin* 2010, **3**(1):11.
6. **Database of Single Nucleotide Polymorphisms (dbSNP). (dbSNP Build ID: 129).** [http://www.ncbi.nlm.nih.gov/SNP/]
7. Yu Y, Bhangale TR, Fagerness J, Ripke S, Thorleifsson G, Tan PL, Souied EH, Richardson AJ, Merriam JE, Buitendijk GHS *et al*: **Common variants near FRK/COL10A1 and VEGFA are associated with advanced age-related macular degeneration**. *Human Molecular Genetics* 2011, **20**(18):3699-3709.

Supplemental Figure Legends

Supplemental Figure 1 - Manhattan plot of case-control analyses.
Chi-square tests for significant enrichment of ASM in cases or controls did not yield any genome-wide significant results after Bonferroni correction for multiple testing (-log10 (p-values)=5.4 on the y-axis). Noted in green are log10 (p-values) for individual MPR amplicons within a 1MB windows surrounding for 14 variants implicated in AMD [7]: rs1061170 (chr1:194925860); rs1410996 (chr1:194963556); rs1713985 (chr4:57481207); rs10033900 (chr4:110878516); rs641153 (chr6:32022159); rs1999930 (chr6:116493827); rs4711751 (chr6:43936560); rs9332739 (chr6:32011783); rs13278062 (chr8:23138916); rs10490924 (chr10:124204438); rs10468017 (chr15:56465804); rs3764261 (chr16:55550825); rs2230199 (chr19:6669387); and rs9621532 (chr22:31414511)).

Supplemental Figure 2 - Individual assessment of methylation-sensitive restriction enzyme digest efficacy.
Density plots are shown for assayed probe intensities for amplicons with single MSRE sites for each of the five MSRE enzymes used as well as for MNRs (UNCUT). Intensities are expressed as the assayed total intensities for these amplicons normalized against the total intensities for these amplicons in the HapMap samples. All MSREs with the exception of HhaI exhibited reduced overall intensities as compared to amplicons without MSRE sites (MNRs/UNCUT).

Supplemental Figure 3 - Assessment of traditional Affymetrix SNP6.0 array normalization methods.
Scatter plots (left panels) and histograms (right panels) of un-normalized (top panels), median normalized (middle panels) and quantile normalized (bottom panels) probe intensities for both MSRE undigested and MSRE digested samples of an unmethylated control are shown.

Supplemental Figure 4 - Method of MNR selection.
Amplicons were chosen as MNRs based on two criteria: 1) assay based size selection of amplicons results in a final amplicon size range of 200-1200 bp and 2) bioinformatic prediction of MSRE site locations. These criteria allow selection of 3 classes of MNR amplicons expected to show no effect of MSRE digestion: a) those with both NspI and StyI amplicons of 200-1200bp with no MSRE sites (1st alternative) and those with either b) NspI amplicons of 200-1200bp with no MSRE sites and StyI amplicons outside this size range (2nd alternative, top) or c) StyI amplicons of 200-1200bp with no MSRE sites and NspI amplicons outside this size range (2nd alternative, bottom).

Supplemental Figure 5 - MPRs show lower combined probe intensities relative to MNRs after MSRE digest.
Scatter plot (top panel) and density plots (bottom panels) of total probe intensities before (top panel and bottom left panel) and after MSRE digestion (top panel and bottom right panel) for amplicons with (MPRs - in red) and without (MNRs - in blue) MSRE sites in an unmethylated control sample. MPRs show a pronounced shift to lower intensities after MSRE digestion.

Supplemental Figure 6 - Allele frequencies vary with MSRE digest for MPRs but not MNRs in control methylation mixes.
Scatter plots of A allele frequencies (probe A intensity/ (probe A intensity + probe B intensity) for amplicons with (MPRs - in red) and without (MNRs - in blue) MSRE sites from two 50:50 E44-H16 control mix samples, one where one sample has H16 methylated and E44 unmethylated (y-axis) and the other H16 unmethylated and E44 methylated (x-axis). In the top panel, MPRs where E44 contributes the A allele are shown and in the bottom panel, E44 contributes the B allele. MSRE digest does not change the distribution for MNRs but shifts those of MPRs towards the axis of the mix with the methylated A allele.

Supplemental Figure 7 - Assessment of MNRs based quantile interpolation normalization method in control methylation mixes.
Scatterplots are shown of the probe A intensity values of un-normalized (left panels) and normalized (right panels) of two replicates from two separate reciprocal 50:50 E44:H16 control mixes. In these 50:50 mixes, only one of the samples is methylated; in the top panels, the E44 sample is methylated and the H16 unmethylated, in the bottom panels the H16 sample is methylated and the E44 sample unmethylated. The MNR quantile interpolated normalization based method used greatly reduced variation between replicates.

Supplemental Figure 8 - Method of MPR selection.
MPRs were filtered for quality and potential technical artifacts by multiple criteria. Of the ~910,000 amplicons on the Affymetrix SNP6.0 array, ~~150,000 had no predicted MSRE sites, and were used to normalize between arrays but discarded from downstream analyses. We also removed amplicons with MSRE sites that did not perform well on our HapMap reference set; ~70,000 amplicons had no calls across the entire HapMap samples, and ~180,000 had poor separation (or low "discernability") between the 3 log2 (A/B) distributions for the 3 genotype classes (AA, AB and BB). We also removed any amplicon predicted with the potential to have a SNP which could add or remove an MSRE site, resulting in a final number of ~230,000 potential MPRs available to assay in each sample.

Supplemental Figure 9 - Method of allele-specific methylation determination.
Multiple criteria were required to be met before a MPR was determined to exhibit ASM. Of the ~230,000 MPRs passing quality checks (Supplemental Figure 8), given a typical heterozygosity level of 25% at any given SNP, approximately 60,000 could be expected to be heterozygous in any given sample. In the scatter plot in A), the 4 different states after MSRE digest expected are compared to the typical distribution of probe intensities observed within the HapMap samples for the same MPR (here portrayed by red squares in all scatter plots): a) biallelic methylation (yellow circle) would look like unchanged, AB genotype calls; b) monoallelic A methylation (green circle) would resemble AA genotype calls; c) monoallelic B methylation (blue circle) would resemble BB genotype calls and finally, d) biallelic lack of methylation would resemble a "NoCall". The primary calling method relies on feature extraction by way of conversion of 2-dimensional A and B probe intensity data (B, first plot) from heterozygotes to log2 (A/B) values and is compared against the typical log2 (A>B) distribution observed for this MPR within the HapMap samples (B, 2nd plot). Put simply, MPRs diverging from this distribution after MSRE treatment are called ASM. Using this method, biallelic unmethylated states have the potential to result in false positive ASM calls as any log2(A/B) value would be based on background noise, so are filtered out by removing MPRS with low total intensities (highlighted here with a red quarter-circle, for further information on how this filter was devised, see Supplemental Figure 10). To account for inter-probeset variation we standard normalized all post-MSRE treatment MPR log2 (A/B) relative allele frequency (RAF) values against the standard deviation of the HapMap RAFs for the same probeset derive a Z-score (i.e. (C). Two example plots are show here, one for a MPR with a wide HapMap log2 (A/B) distribution (C, left), and one for a MPR with a narrow HapMap log2 (A/B) distribution (C, right); standard normalization results in these different example MPRs having comparable Z-score distributions. To account any further inter-sample variability we used the distribution of Z-scores for a sample's MNR population (which is expected to have identical distributions within the sample population) to determine the final ASM call thresholds. MPRs with values outside of boundaries containing 95% of the MNRs for a sample were called ASM (D).

Supplemental Figure 10 - Derivation of intensity ratio cutoff.
To filter out potential false positives derived from biallelic unmethylated MPRs a filter based on the intensity ratio of the MSRE treated MPRs as compared to that of the HapMap reference samples. The threshold was chosen to screen out biallelic unmethylated MPRS while still passing any ASM MPRs (which would be expected to show reduced overall intensities as compared to biallelic methylated MPRs). The final value of this filter was based on observation of this intensity ratio in 1:1 unmethylated control mixes, which are expected to model the properties of monoallelically methylated MPRs at all assayed amplicons. At an intensity ratio value of 0.2, only 0.2% of these mock ASM MPRs were filtered out.

Supplemental Figure 11 - Detection of previously identified allele-specific methylation events.
Shown are the standard scores (or Z-scores) of heterozygote samples after probeset normalization against the log2 (A/B) distribution of heterozygote undigested HapMap samples for four MPRs found in genomic regions known to be associated with allele-specific methylation; rs220030 is a SNP within the imprinted *SNRPN* locus (A); rs2107425 is a SNP located ~2kb upstream of the imprinted *H19* locus (B); rs6494120 is an intergenic SNP located ~11kb upstream of *GCNT3* (C) and rs943049 is an intergenic SNP located ~75kb upstream of *ATP12A* (D). Red and blue circles denote MSRE treated and untreated samples respectively. Open and closed circles denote samples for which an allele-specific methylation event was and was not observed, respectively. Standard scores with a negative value denote allele-specific methylation of the B allele (i.e. log2 (A/B)<0) and those with a positive value denote allele-specific methylation of the A allele (i.e. log2 (A/B)>0) (base identities of the A and B alleles are indicated for each variant). For MPRs within known imprinted regions (panels A and B), an approximately equal number of allele-specific methylation events at the A and B alleles is observed, consistent with a pattern of allele-specific methylation based on allelic parent-of-origin within our sample population. The differential methylation patterns of MPRs found in genomic regions previously associated with cis-regulated allele-specific methylation (panels C and D) are consistent with previous results, i.e. only one allele is associated with methylation.

Supplemental Figure 12 - Levels of allele-specific methylation in whole blood samples.
Boxplots are shown for the percentage of MPRs with allele-specific methylation in the sample set. Circles denote values of individual samples, whiskers have maximum 1.5 inter-quartile range. Untreated (blue circles and first boxplot) and MSRE treated (red circles and last boxplots). MPRs show a median level of 0% allele-specific methylation in all samples after adjusting for the false positive rate of 5%; by definition, a Z-score cutoff that excludes 95% of MNRs dictates a minimum 5% positive rate. The adjusted median level of ASM in MPRs in MSRE treated samples is 2.4%.

Supplemental Table Legends

Supplemental Table 1 - Sample metadata.
Details about AMD stage, twin-pair concordance, use in CNV/methylation analyses of samples are shown.

Supplemental Table 2 – Bisulfite PCR assays of ASM loci.
For each amplicon, this table reports the intended purpose of the amplicon; known ASM regions, or as confirmation of the microarray results, like regions without ASM (Putative Non-ASM), or regions with with ASM ("Putative ASM"). The table also reports the sequences of the bisulfite PCR primers, the melting temperature used for the bisulfite PCR, the predicted product length and the genomic region amplified.

Supplemental Table 3 – Fishers Tests.
Categorical classifications and results forFisher's exact testing (p-values) of the association between methylation status (ASM or biallelic) and case/ control status are shown for all MPRs.

Supplemental Table 4 – Bisulfite Sequencing Independently Confirms Microarray ASM Assay.
Results from microarray and next-generation bisulfite sequencing ASM assays of ten variant-containing regions. For each amplicon region the table shows the variant and its categorization. "Putative ASM" variants have microarray evidence of ASM, "Known ASM" variants have been previously shown to exhibit ASM and "Putative Non-ASM" variants have no evidence of ASM in the microarray study. For the bisulfite next generation sequencing analyses, the table shows the MSRE CpG genomic position; the number reads with of methylated or unmethylated CpGs associated with the reference or alternate alleles and the unadjusted a Chi-square p-values of those associations. For MSRE CpGs with pvalues of less than 0.05, the allele with the highest number percentage of methylated reads was designated the ASM allele (REF=reference, ALT=alternate). For all MSRE CpGs that were called ASM by bisulfite sequencing in the sequencing assay, the ASM allele matched that observed in the microarray assay.

Supplemental Table 5 - MPR ASM levels are significantly higher than expected from MNR allele-specific methylation levels.
Shown are numbers of heterozygous MPRs assessed for ASM within each sample, the expected number of ASM MPRs based on the MNR Z-score distribution (i.e. 95% non-ASM, or 5% ASM), the actual observed number, fold increase over expected and the associated chi-square based p-value.