Network analysis of GWAS data from the autoimmune disease Systemic Lupus Erythematosus and the role of vitamin D3

Kelsy Waaijenberg
3-7-2019
Supervisor: DR. S.L.M Steinbush-Coort

Maastricht University

Table of content

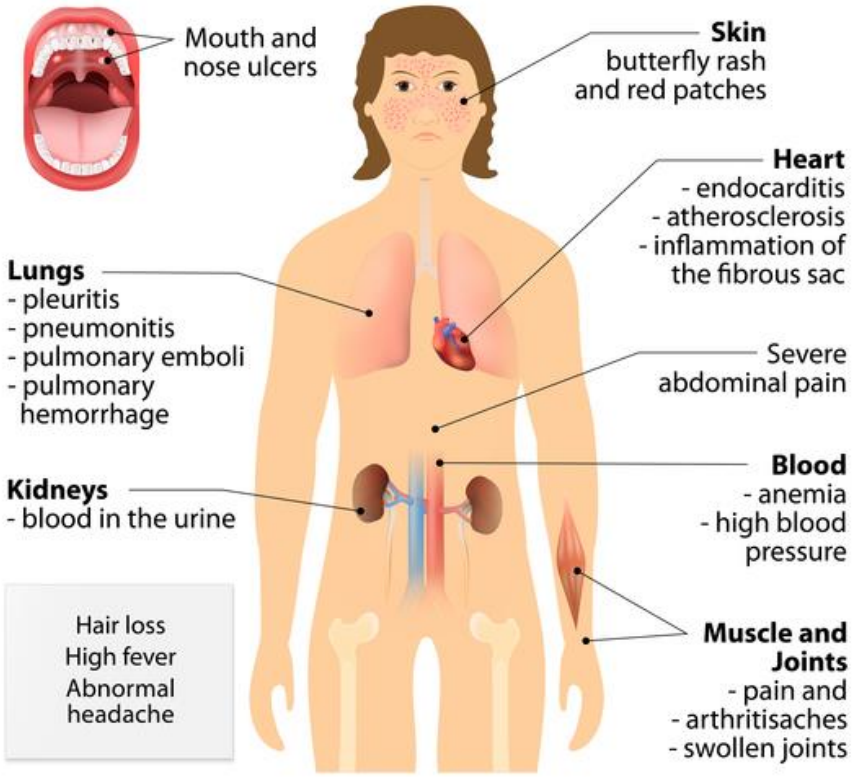
- Introduction
- Aim/Research Question
- Methods
- Results
- Discussion
- Conclusion
- Questions

Introduction

- Systemic autoimmune disease
- Dysfunction T-cells, B-cells, DC's (1)
- Production ANA's
- Loss of self-tolerance (1)
- Clinical manifestations

(1) Perl A. Pathogenic mechanisms in systemic lupus erythematosus. Vol. 43, Autoimmunity. 2010. p. 1–6.

Systemic lupus erythematosus



https://ghr.nlm.nih.gov/condition/systemic-lupus-erythematosus

https://www.medicinenet.com/image-collection/systemic_lupus_erythematosus_1_picture/picture.htm

Who are affected?

- 1 in 2.500 European population [2]
- Predominately woman (ratio 9:1)[3]
- Peak incidence: 15-40
- All age groups can be affected

(2) Johnson AE, Gordon C, Palmer RG, Bacon PA. The prevalence and incidence of systemic lupus erythematosus in Birmingham, England. Arthritis Rheum. 1995;38(4):551–8.
(3) Lam GKW, Petri M. Assessment of systemic lupus erythematosus. N Engl J Med. 2010;278(18):1022–3.

Cause?

- Not known!
- Diverse presentation
- Genetic susceptibility
- Environmental triggers

Immune response

- **Innate**
- First line of defence
- Activation complement cascade + adaptive immune system
- Antigen presentation
- DC: antigen presenters + IFN producer
- IFN-alpha → DC maturation + self-reactive T-cells (4)
- NFkB → proinflammatory transcription mediator of cytokines (5)

(4) Ronnblom L, Pascual V. The innate immune system in SLE: type I interferons and dendritic cells. Lupus. 2008 May;17(5):394–9.

(5) Sun S-C, Chang J-H, Jin J. Regulation of nuclear factor-κB in autoimmunity. Trends Immunol [Internet]. 2013/02/20. 2013 Jun;34(6):282–9. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23434408

Immune response

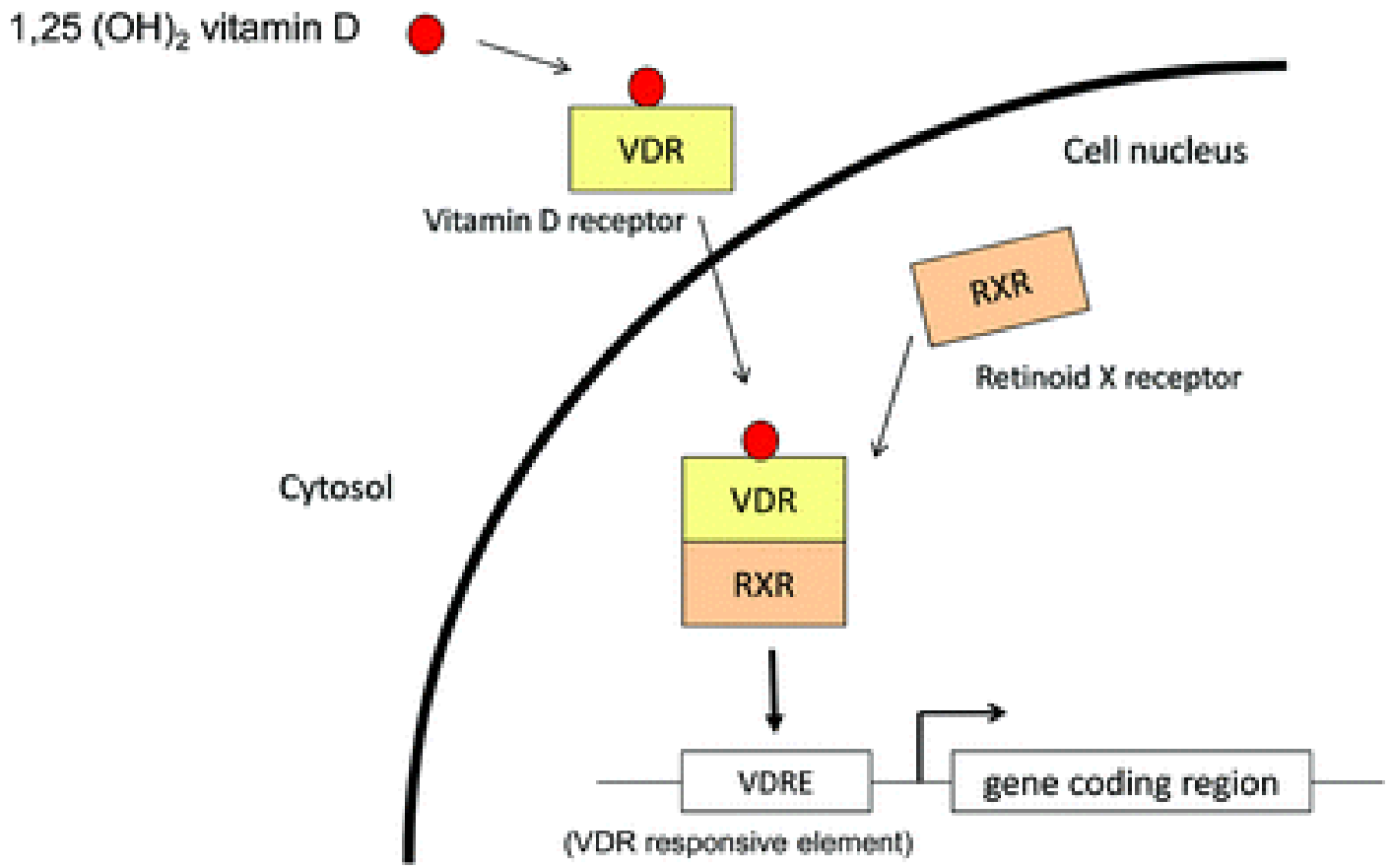
- **Adaptive**
- Triggered innate immune system
- Generates treshold level of antigens
- T and B-cell lineages

Vitamin D and SLE

- Increasing problem, global level
- Increased risk various diseases
- Common among SLE patients (6)
- Photosensitive rash
- Fever, fatigue and joint pains

(6) Liu X, Baylin A, Levy PD. Vitamin D deficiency and insufficiency among US adults: prevalence, predictors and clinical implications. Br J Nutr. 2018;119(8):928–36.

Vitamin D3 deficiency and SLE



https://selfhacked.com/blog/natural-ways-to-increase-calcitrol-and-vitamin-d-receptor-gene-expression/

VDR activation and Lupus

- VDR-activation immune cells: (7)
- Inhibition T-cell proliferation
- Inhibition immunoglobulin production, B-cells
- SNPs VDR: APAI, TAQ1, BSM1

→Increase susceptibility to SLE

(7) Bikle DD. Vitamin D Metabolism, Mechanism of Action, and Clinical Applications. Chem Biol [Internet]. 2014;21(3):319–29. Available from: http://www.sciencedirect.com/science/article/pii/S107455211400024

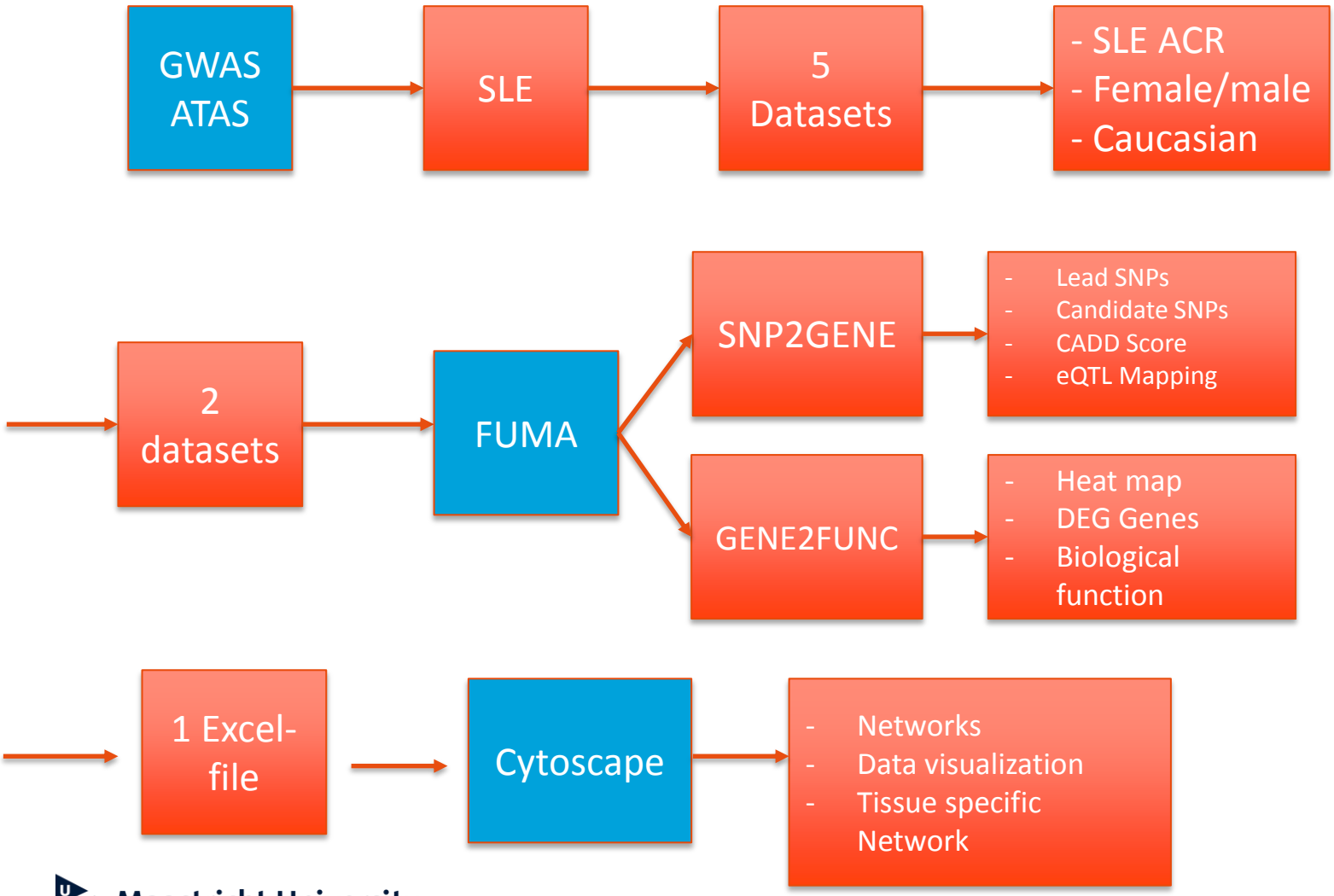
Aim/Research question

- Lot not known about the role of vitamin D3
- SNPs, Genes and Pathways involved in SLE

- Visualize and integrate SNPs, Genes and biological processes that are involved in SLE

Methods

Methods

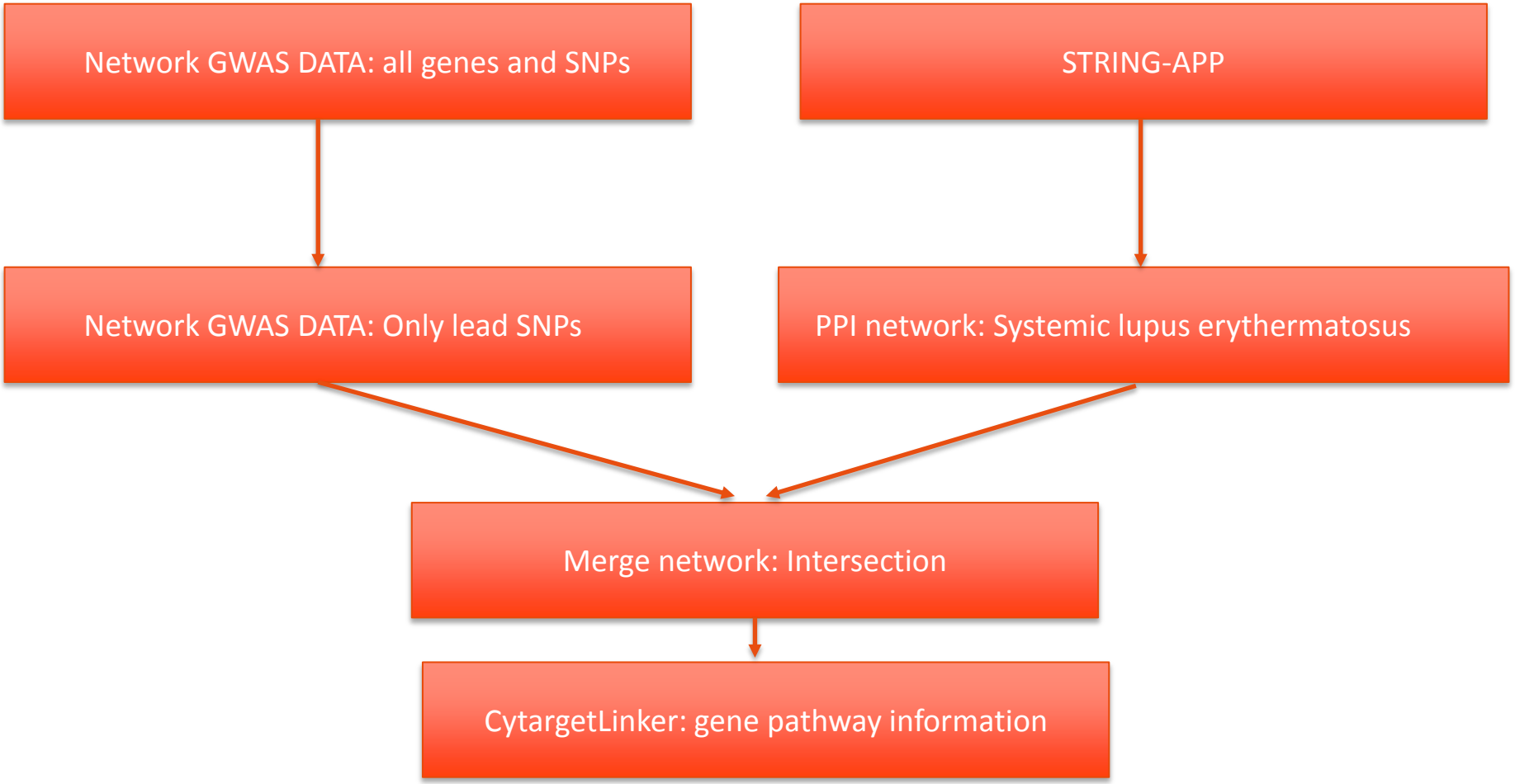

GWAS ATAS
SLE
5 Datasets
- SLE ACR
- Female/male
- Caucasian
2 datasets
FUMA
SNP2GENE
- Lead SNPs
- Candidate SNPs
- CADD Score
- eQTL Mapping
GENE2FUNC
- Heat map
- DEG Genes
- Biological function
1 Excel-file
Cytoscape
- Networks
- Data visualization
- Tissue specific Network

CADD-score

- Combined Annotation Dependent Depletion (8)
- Based on diverse genomic features
- Machine Learning model
- Single CADD per SNP
- Deleteriousness + insertion/deletions variants

(8) Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res [Internet]. 2019 Jan 8 [cited 2019 Jun 20];47(D1):D886–94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30371827

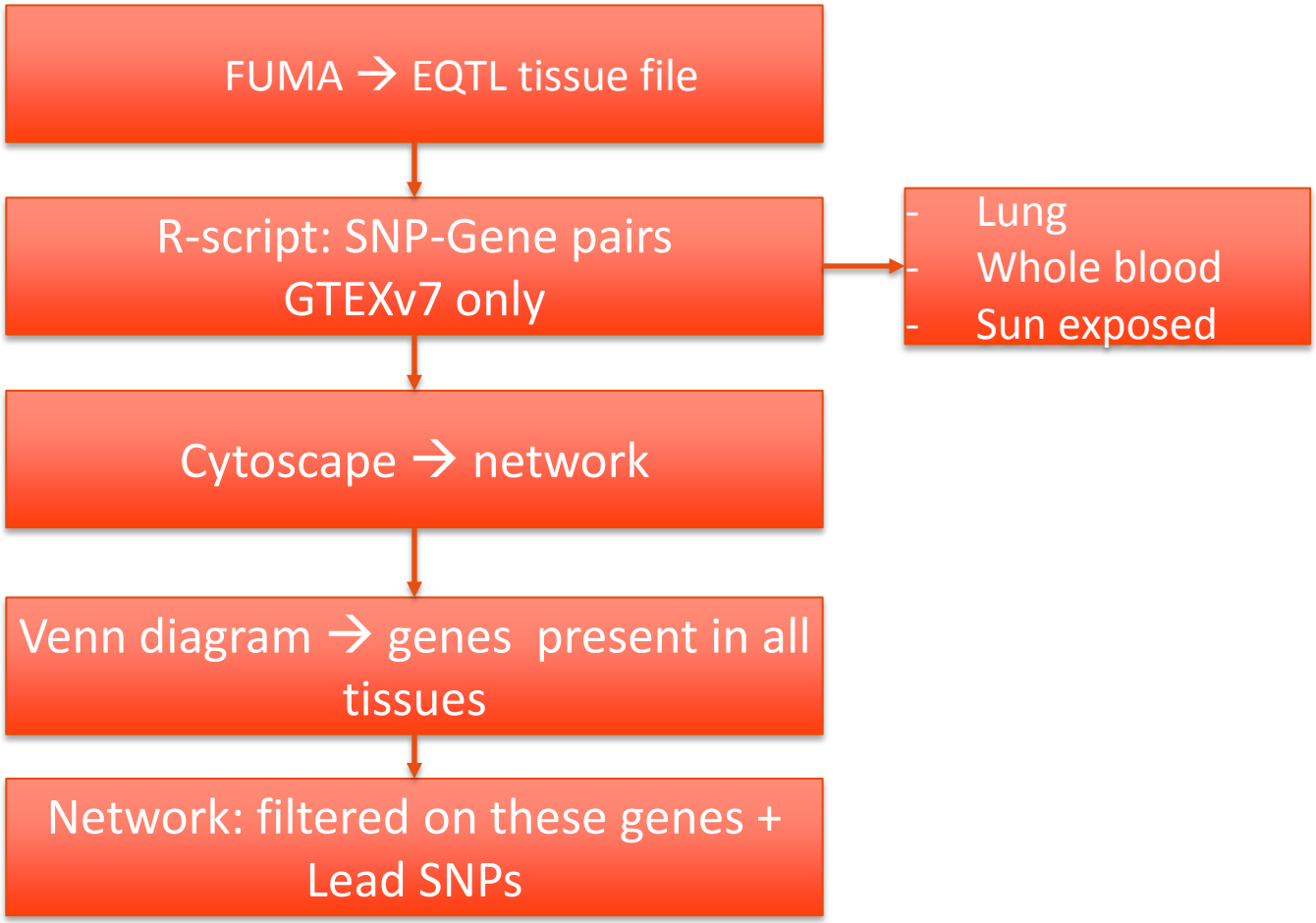
Network creation



Data visualization

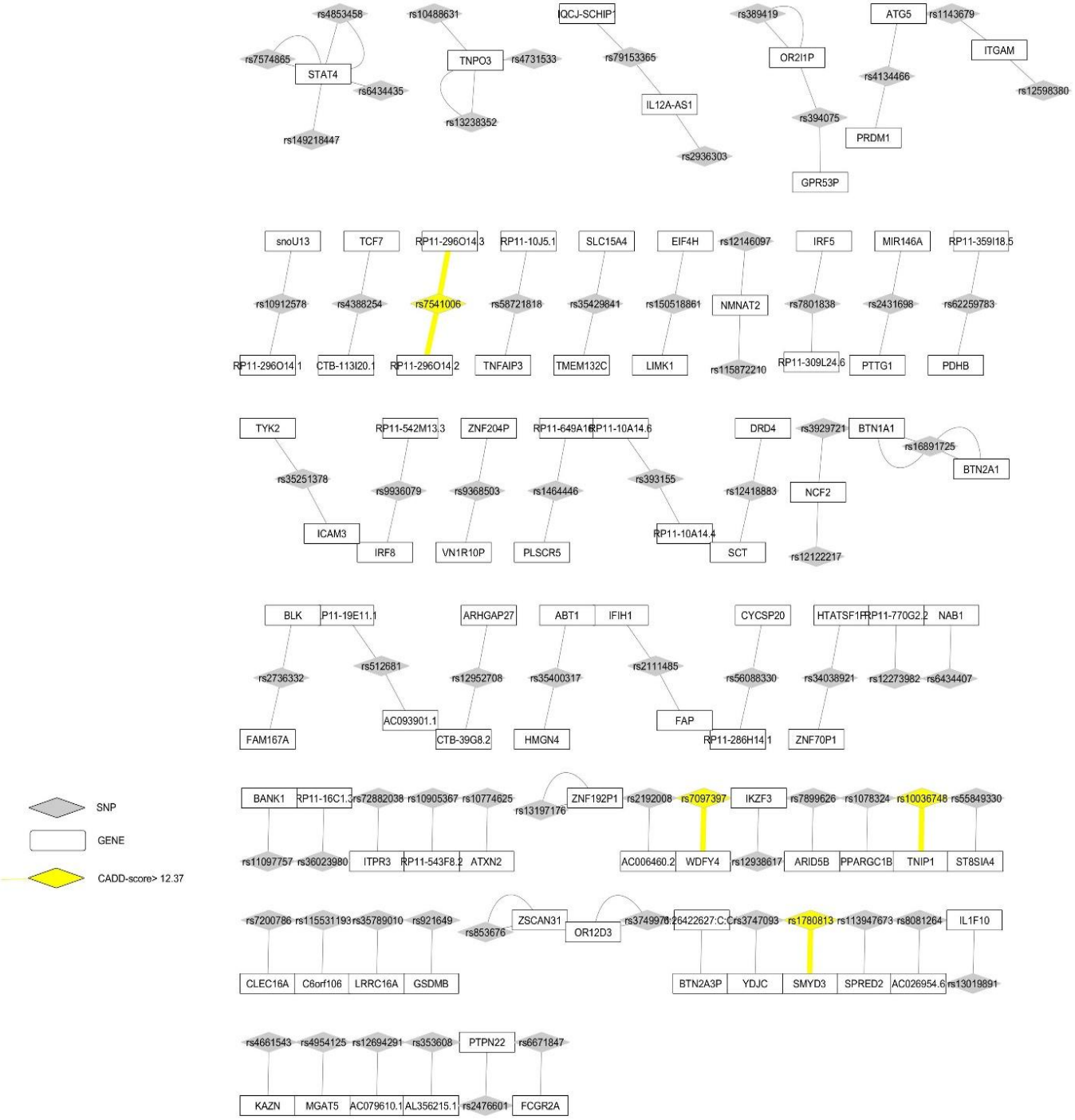
- Transcriptomics data set
- CD4 T-cells, CD19 B cells and myeloid cells
- 11 healthy subjects, 14 SLE patients
- Log2FC and p-values
- Loaded into network

Tissue specific network creation



Results

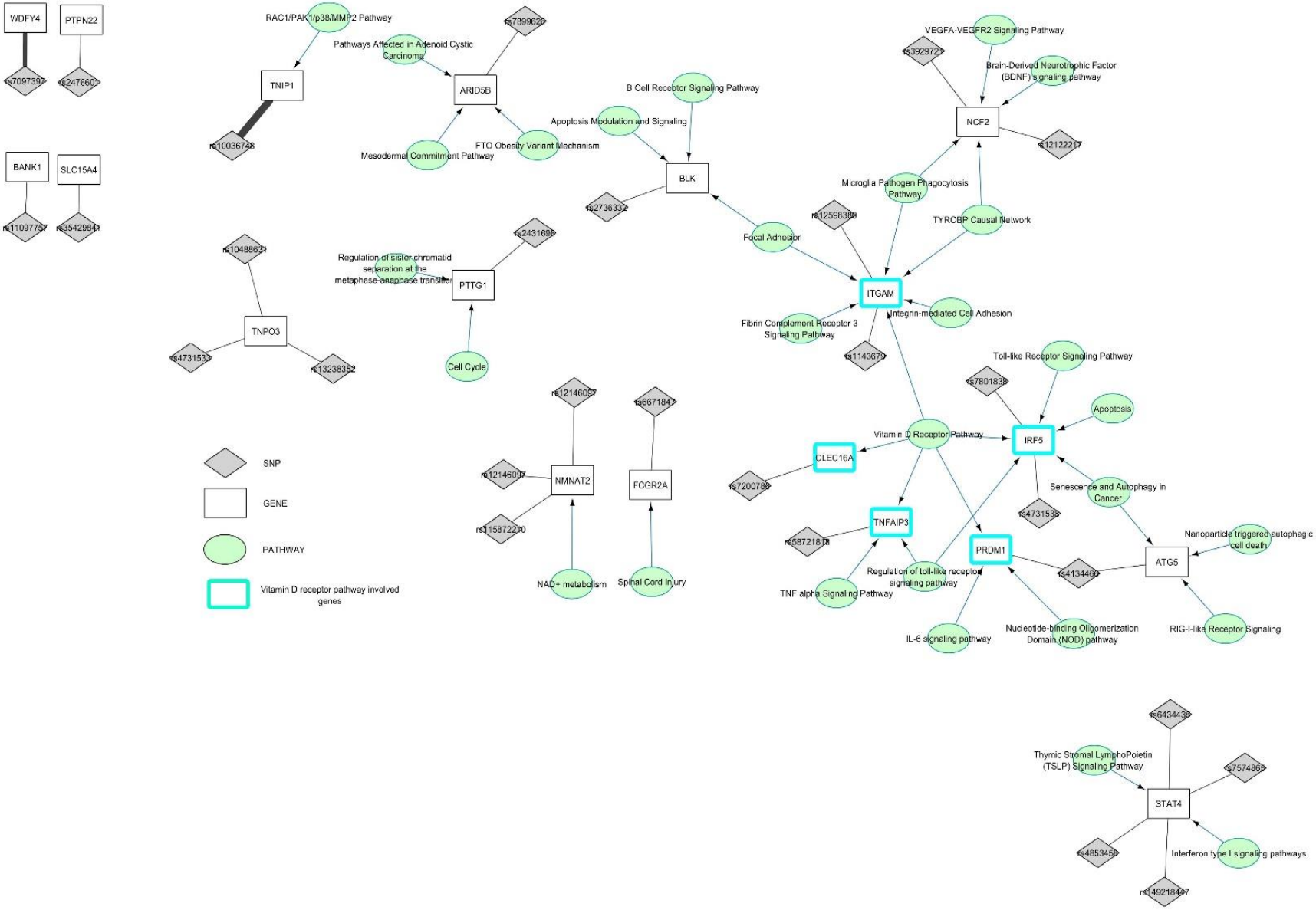
Gwa


rs4853458
rs10488631
IQCJ-SCHIP1
rs389419
ATG5
rs1143679
rs7574865
TNPO3
rs4731533
rs79153365
OR2I1P
ITGAM
STAT4
rs4134466
rs6434435
IL12A-AS1
rs12598380
rs13238352
rs394075
rs149218447
rs2936303
PRDM1
GPR53P
snoU13
TCF7
RP11-296O14.3
RP11-10J5.1
SLC15A4
EIF4H
rs12146097
IRF5
MIR146A
RP11-359I18.5
rs10912578
rs4388254
rs7541006
rs58721818
rs35429841
rs150518861
NMNAT2
rs7801838
rs2431698
rs62259783
RP11-296O14.1
CTB-113I20.1
RP11-296O14.2
TNFAIP3
TMEM132C
LIMK1
rs115872210
RP11-309L24.6
PTTG1
PDHB
TYK2
RP11-542M13.3
ZNF204P
RP11-649A16
RP11-10A14.6
DRD4
rs3929721
BTN1A1
rs16891725
BTN2A1
rs35251378
rs9936079
rs9368503
rs1464446
rs393155
rs12418883
NCF2
ICAM3
IRF8
VN1R10P
PLSCR5
RP11-10A14.4
SCT
rs12122217
BLK
P11-19E11.1
ARHGAP27
ABT1
IFIH1
CYCSP20
HTATSF1P
RP11-770G2.2
NAB1
rs2736332
rs512681
rs12952708
rs35400317
rs2111485
rs56088330
rs34038921
rs12273982
rs6434407
AC093901.1
FAP
FAM167A
CTB-39G8.2
HMGN4
RP11-286H14.1
ZNF70P1
SNP
GENE
CADD-score> 12.37
BANK1
RP11-16C1.3
rs72882038
rs10905367
rs10774625
rs13197176
ZNF192P1
rs2192008
rs7097397
IKZF3
rs7899626
rs1078324
rs10036748
rs55849330
rs11097757
rs36023980
ITPR3
RP11-543F8.2
ATXN2
AC006460.2
WDFY4
rs12938617
ARID5B
PPARGC1B
TNIP1
ST8SIA4
rs7200786
rs115531193
rs35789010
rs921649
rs853676
ZSCAN31
OR12D3
rs3749976
26422627:C:G
rs3747093
rs1780813
rs113947673
rs8081264
IL1F10
CLEC16A
C6orf106
LRRC16A
GSDMB
BTN2A3P
YDJC
SMYD3
SPRED2
AC026954.6
rs13019891
rs4661543
rs4954125
rs12694291
rs353608
PTPN22
rs6671847
KAZN
MGAT5
AC079610.1
AL356215.1
rs2476601
FCGR2A

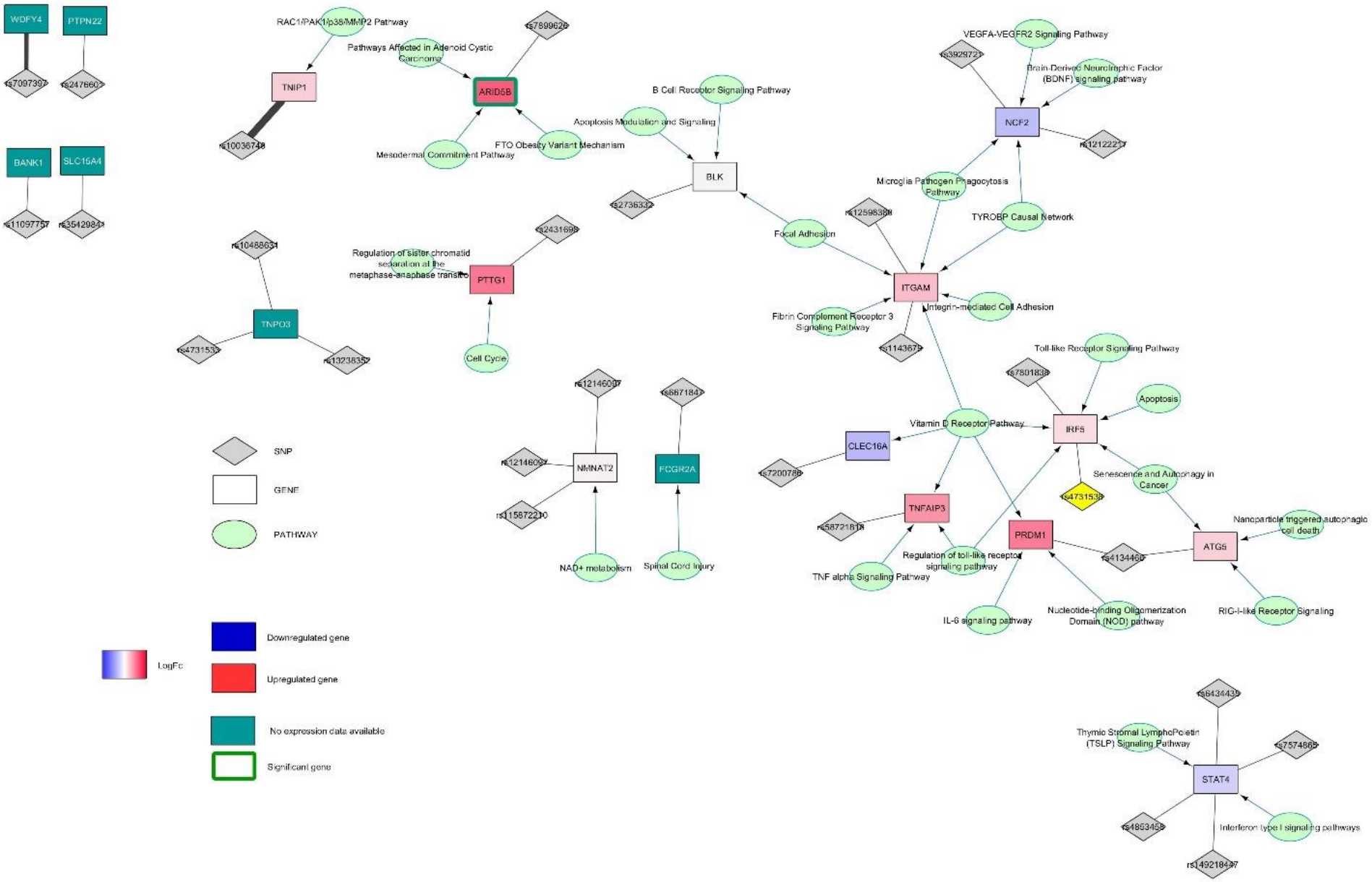
Maas

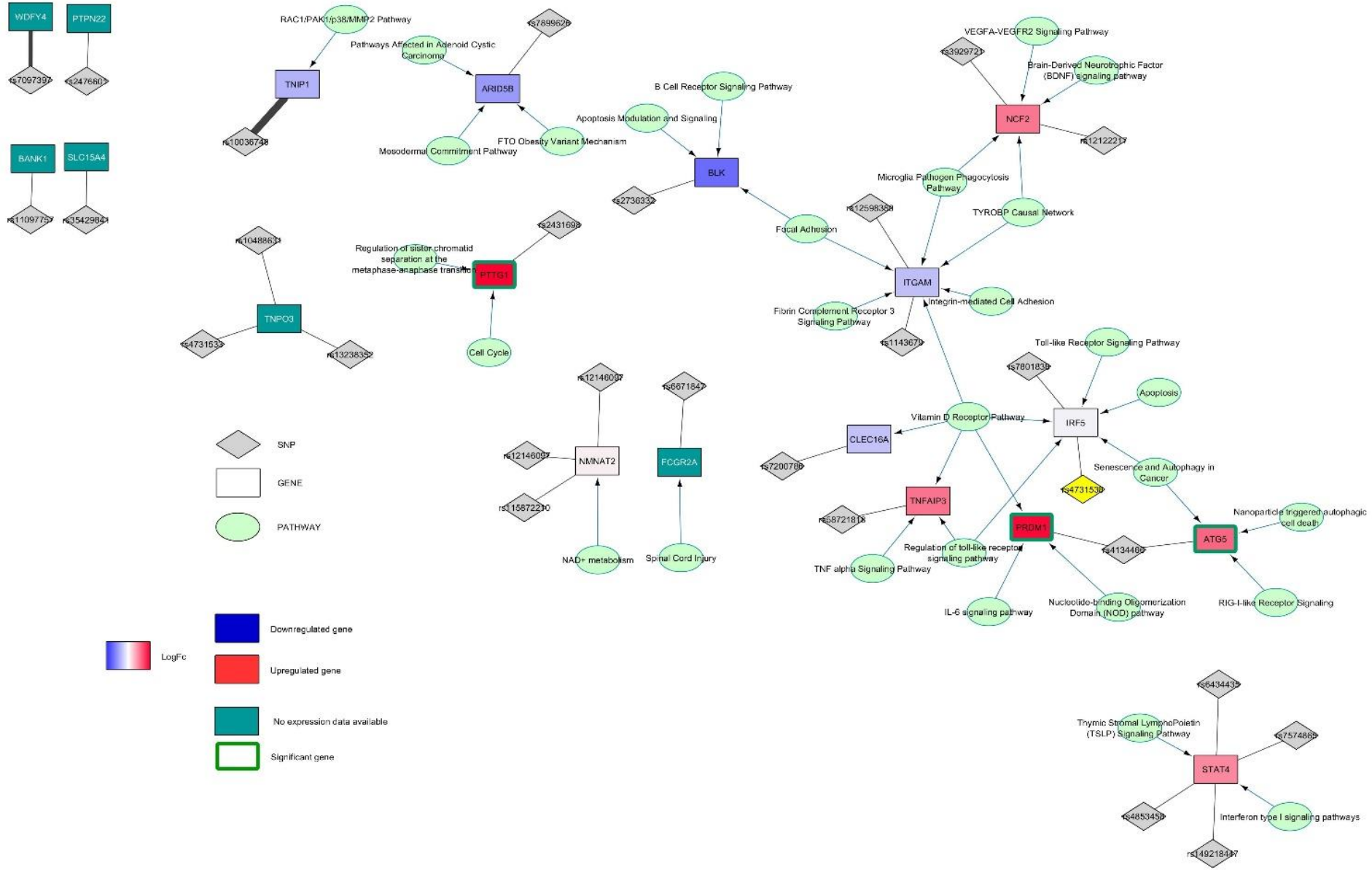
String-disease network

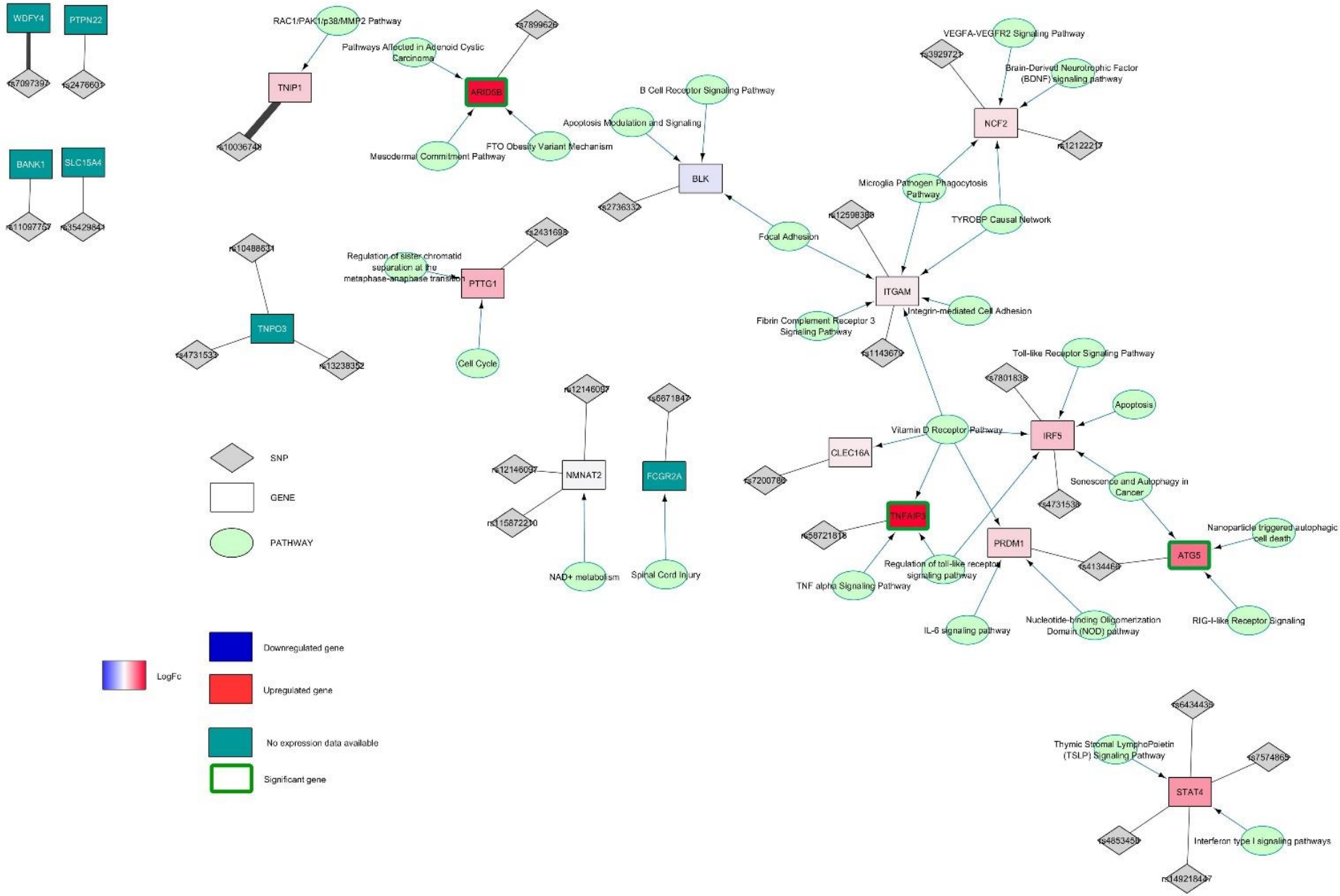
Gene name	Function	Degree	Betweenness Centrality
IL6	Cytokine, involved in pro-inflammatory and anti-inflammatory reactions	50	0.11509439
TNF	Cytokine, involved in systemic inflammation	49	0.07317615
IL10	Anti-inflammatory cytokine	44	0.03216241
CD40	Co-stimulatory protein on antigen presenting cells required for their activation	41	0.07909398
CTLA4	Protein receptor that function's as immune checkpoint and downregulates immune response	40	0.03508523


WDFY4
PTPN22
rs7097397
rs2476601
BANK1
SLC15A4
rs11097757
rs35429841
RAC1/PAK1/p38/MMP2 Pathway
Pathways Affected in Adenoid Cystic Carcinoma
rs7899620
TNIP1
ARID5B
rs10036748
Mesodermal Commitment Pathway
FTO Obesity Variant Mechanism
B Cell Receptor Signaling Pathway
Apoptosis Modulation and Signaling
BLK
rs2736332
VEGFA-VEGFR2 Signaling Pathway
rs3929727
Brain-Derived Neurotrophic Factor (BDNF) signaling pathway
NCF2
rs12122217
Microglia Pathogen Phagocytosis Pathway
rs12598389
TYROBP Causal Network
Focal Adhesion
rs10488631
rs243169
Regulation of sister chromatid separation at the metaphase-anaphase transition
PTTG1
TNPO3
rs4731533
rs13238352
Cell Cycle
ITGAM
Integrin-mediated Cell Adhesion
Fibrin Complement Receptor 3 Signaling Pathway
rs1143679
Toll-like Receptor Signaling Pathway
rs7801838
Apoptosis
Vitamin D Receptor Pathway
IRF5
CLEC16A
rs7200786
Senescence and Autophagy in Cancer
rs4731532
rs12146097
rs6671847
rs12146097
NMNAT2
FCGR2A
rs115872230
NAD+ metabolism
Spinal Cord Injury
TNFAIP3
rs58721818
PRDM1
Nanoparticle triggered autophagic cell death
ATG5
rs4134466
Regulation of toll-like receptor signaling pathway
TNF alpha Signaling Pathway
IL-6 signaling pathway
Nucleotide-binding Oligomerization Domain (NOD) pathway
RIG-I-like Receptor Signaling
SNP
GENE
PATHWAY
Vitamin D receptor pathway involved genes
rs6434431
Thymic Stromal LymphoPoietin (TSLP) Signaling Pathway
rs7574865
STAT4
rs4853458
Interferon type I signaling pathways
rs149218437

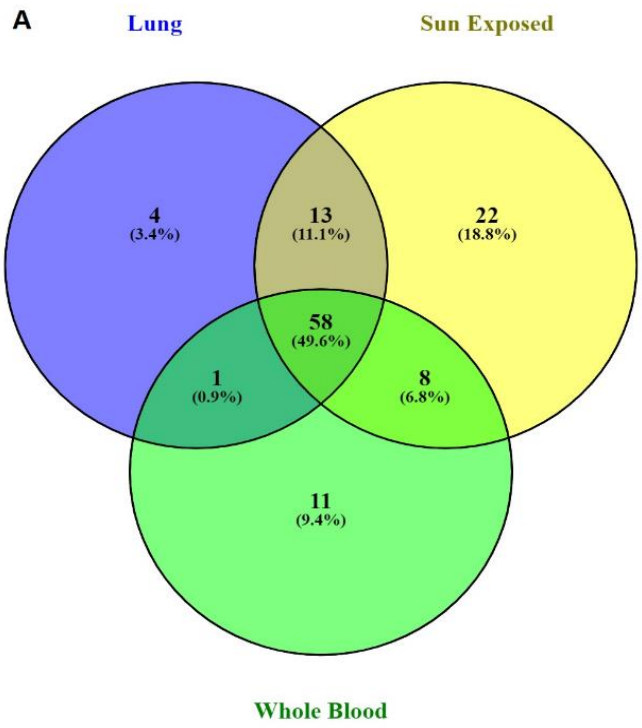
Pathway Name	Degree	Betweenness Centrality
Vitamin D Receptor Pathway	5	0.63642473
Focal Adhesion	2	0.22580645
Microglia Pathogen Phagocytosis Pathway	2	0.13104839
TYROBP Causal Network	2	0.13104839
Senescence and Autophagy in Cancer	2	0.09341398

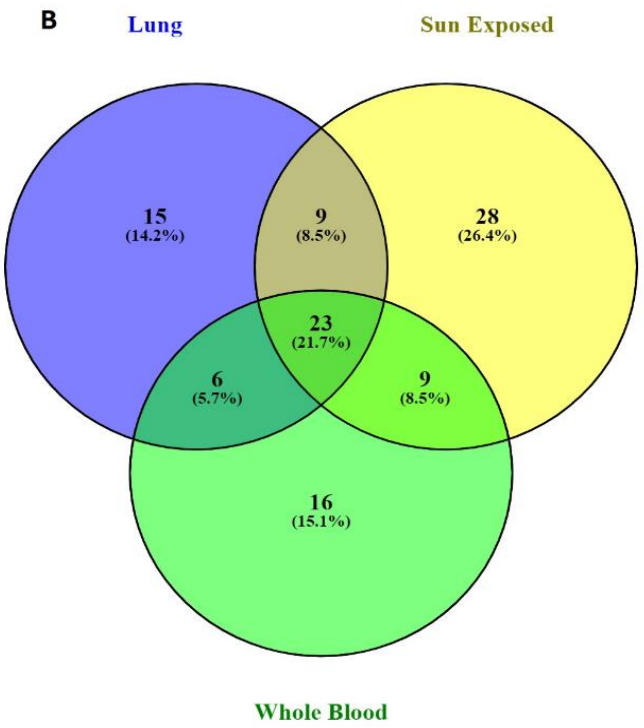

WDFY4
PTPN22
rs7097397
rs247660
BANK1
SLC19A4
rs11097757
rs3529841
RAC1/PAK1/p38/MMP2 Pathway
Pathways Affected in Adenoid Cystic Carcinoma
rs7899626
TNIP1
ARID5B
rs10036748
Mesodermal Commitment Pathway
FTO Obesity Variant Mechanism
Apoptosis Modulation and Signaling
B Cell Receptor Signaling Pathway
BLK
rs2736337
VEGFA-VEGFR2 Signaling Pathway
rs3929721
Brain-Derived Neurotrophic Factor (BDNF) signaling pathway
NCF2
rs12122217
Microglia Pathogen Phagocytosis Pathway
TYROBP Causal Network
rs12598388
Focal Adhesion
ITGAM
Integrin-mediated Cell Adhesion
Fibrin Complement Receptor 3 Signaling Pathway
rs1143679
rs10488631
TNPO3
rs4731533
rs13238357
rs2431698
Regulation of sister chromatid separation at the metaphase-anaphase transition
PTTG1
Cell Cycle
rs12146007
rs6671847
rs12146007
NMNAT2
FCGR2A
rs11587210
NAD+ metabolism
Spinal Cord Injury
Toll-like Receptor Signaling Pathway
rs7801838
Apoptosis
Vitamin D Receptor Pathway
IRF5
CLEC16A
rs7200786
Senescence and Autophagy in Cancer
rs4731538
TNFAIP3
rs58721818
Nanoparticle triggered autophagic cell death
PRDM1
ATG5
rs4134460
Regulation of toll-like receptor signaling pathway
TNF alpha Signaling Pathway
IL-6 signaling pathway
Nucleotide-binding Oligomerization Domain (NOD) pathway
RIG-I-like Receptor Signaling
SNP
GENE
PATHWAY
LogFc
Downregulated gene
Upregulated gene
No expression data available
Significant gene
rs6434435
Thymic Stromal LymphoPoietin (TSLP) Signaling Pathway
rs7574865
STAT4
rs4803455
Interferon type I signaling pathways
rs149218437


WDFY4
PTPN22
rs7097397
rs2476601
BANK1
SLC15A4
rs11097757
rs35429841
RAC1/PAK1/p38/MMP2 Pathway
Pathways Affected in Adenoid Cystic Carcinoma
rs7899626
TNIP1
ARID5B
rs10036748
Mesodermal Commitment Pathway
FTO Obesity Variant Mechanism
B Cell Receptor Signaling Pathway
Apoptosis Modulation and Signaling
BLK
rs2736332
VEGFA-VEGFR2 Signaling Pathway
rs3929721
Brain-Derived Neurotrophic Factor (BDNF) signaling pathway
NCF2
rs12122217
Microglia Pathogen Phagocytosis Pathway
TYROBP Causal Network
rs12598358
Focal Adhesion
rs10488631
rs2431698
Regulation of sister chromatid separation at the metaphase-anaphase transition
PTTG1
TNPO3
rs4731533
rs13238352
Cell Cycle
ITGAM
Integrin-mediated Cell Adhesion
Fibrin Complement Receptor 3 Signaling Pathway
rs1143679
Toll-like Receptor Signaling Pathway
rs7801839
Apoptosis
Vitamin D Receptor Pathway
IRF5
CLEC16A
rs7200786
Senescence and Autophagy in Cancer
rs4731530
rs12146007
rs6671847
NMNAT2
FCGR2A
rs115872210
NAD+ metabolism
Spinal Cord Injury
TNFAIP3
rs8721818
PRDM1
ATG5
Nanoparticle triggered autophagic cell death
Regulation of toll-like receptor signaling pathway
rs4134466
TNF alpha Signaling Pathway
IL-6 signaling pathway
Nucleotide-binding Oligomerization Domain (NOD) pathway
RIG-I-like Receptor Signaling
SNP
GENE
PATHWAY
LogFc
Downregulated gene
Upregulated gene
No expression data available
Significant gene
rs6434435
Thymic Stromal LymphoPoietin (TSLP) Signaling Pathway
rs7574865
STAT4
rs4853458
Interferon type I signaling pathways
rs149218437


WDFY4
PTPN22
rs7097397
rs2476601
BANK1
SLC15A4
rs11097757
rs35429841
RAC1/PAK1/p38/MMP2 Pathway
TNIP1
rs10036748
Pathways Affected in Adenoid Cystic Carcinoma
rs7899626
ARID5B
Mesodermal Commitment Pathway
FTO Obesity Variant Mechanism
B Cell Receptor Signaling Pathway
Apoptosis Modulation and Signaling
BLK
rs2736337
VEGFA-VEGFR2 Signaling Pathway
rs3929721
Brain-Derived Neurotrophic Factor (BDNF) signaling pathway
NCF2
rs12122217
Microglia Pathogen Phagocytosis Pathway
rs12598388
TYROBP Causal Network
Focal Adhesion
ITGAM
Integrin-mediated Cell Adhesion
Fibrin Complement Receptor 3 Signaling Pathway
rs1143679
rs10488631
TNPO3
rs4731533
rs13238352
Regulation of sister chromatid separation at the metaphase-anaphase transition
PTTG1
rs2431693
Cell Cycle
Toll-like Receptor Signaling Pathway
rs7801838
Apoptosis
IRF5
Vitamin D Receptor Pathway
CLEC16A
rs7200786
Senescence and Autophagy in Cancer
rs4731538
TNFAIP3
rs58721818
Nanoparticle triggered autophagic cell death
ATG5
PRDM1
rs4134466
Regulation of toll-like receptor signaling pathway
TNF alpha Signaling Pathway
IL-6 signaling pathway
Nucleotide-binding Oligomerization Domain (NOD) pathway
RIG-I-like Receptor Signaling
rs12146037
NMNAT2
rs115872210
NAD+ metabolism
rs6671847
FCGR2A
Spinal Cord Injury
SNP
GENE
PATHWAY
LogFc
Downregulated gene
Upregulated gene
No expression data available
Significant gene
rs6434435
Thymic Stromal LymphoPoietin (TSLP) Signaling Pathway
rs7574865
STAT4
rs4853458
Interferon type I signaling pathways
rs149218447

Venn diagram



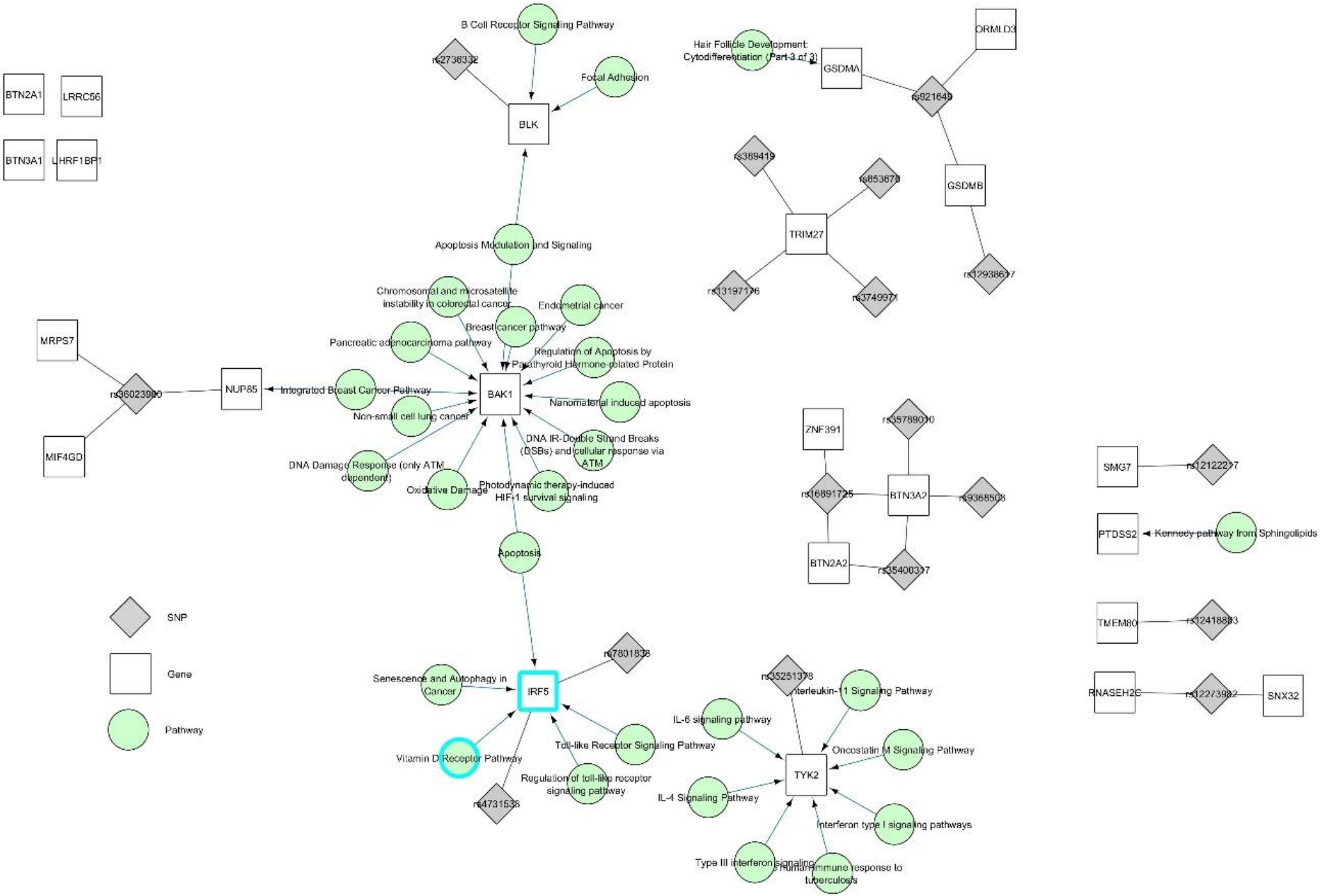


Gene clustering

Class	Genes	Number of genes
Regulation of immune response	BTN3A1, BTN2A2, TRIM27, TYK2, BTN2A1, BTN3A2, IRF5, BLK, NUP85	9
Regulation of apoptosis	BAK1, GSDMB, GSDMA, IRF5, PTDSS2	5
Catabolic process	MRPS7, SMG7, ORMDL3, RNASEH2C	4
RNA/DNA- binding	ZNF391, LRRC56, UHRF1BP1,	4
Regulation of translation	MIF4GD	1
Fertilization	TMEM80	1
Endocytosis	SNX32	1

Pathway Clustering

Class	Number of pathways
Immune system	15
Cancer	12
Differentiation, migration, adhesion and cell survival	11
Apoptosis	6
Angiogenesis	2
Cell cycle regulation	2
Lipid signalling	2
Insulin	2
Synaptic activity and plasticity	2


BTN2A1
LRRC56
BTN3A1
LHRF1BP1
B Cell Receptor Signaling Pathway
rs2736332
Focal Adhesion
BLK
Apoptosis Modulation and Signaling
Chromosomal and microsatellite instability in colorectal cancer
Endometrial cancer
Breast cancer pathway
Pancreatic adenocarcinoma pathway
Regulation of Apoptosis by Parathyroid Hormone-related Protein
MRPS7
rs36023990
NUP85
Integrated Breast Cancer Pathway
BAK1
Nanomaterial induced apoptosis
Non-small cell lung cancer
DNA IR-Double Strand Breaks (DSBs) and cellular response via ATM
MIF4GD
DNA Damage Response (only ATM dependent)
Oxidative Damage
Photodynamic therapy-induced HIF-1 survival signaling
Apoptosis
Hair Follicle Development: Cytodifferentiation (Part 3 of 3)
GSDMA
ORMDL3
rs921643
GSDMB
rs389419
rs853678
TRIM27
rs13197176
rs3749971
rs12938617
ZNF391
rs35789030
rs16891725
BTN3A2
rs9368508
BTN2A2
rs35400337
SMG7
rs12122297
PTDSS2
Kennedy pathway from Sphingolipids
TMEM80
rs12418883
RNASEH2C
rs12273982
SNX32
SNP
Gene
Pathway
Senescence and Autophagy in Cancer
IRF5
rs7801838
Vitamin D Receptor Pathway
Toll-like Receptor Signaling Pathway
Regulation of toll-like receptor signaling pathway
rs4731538
rs35251378
Interleukin-11 Signaling Pathway
IL-6 signaling pathway
Oncostatin M Signaling Pathway
TYK2
IL-4 Signaling Pathway
Interferon type I signaling pathways
Type III interferon signaling
Human immune response to tuberculosis

Discussion

Lead SNPs + genes

- **TNIP1 + rs10036748**
- Associated with SLE pathogenesis
- Encodes ABIN1 → clinical features
- Photosensitivity + vasculitis in Chinese population
- **SMYD3 + rs1780813**
- H3-Hk histone methyltransferase
- Autoreactive B-cells + target auto-antibodies in SLE
- **WDFY4 + rs709739**
- Overproduction + increased survival B-cells
- Knockout mice → autophagy activity + cell death increased
- No Literature SNP
- **RP11-296O14.2 and RP11-296O14.3 + rs7541006**
- No information available in literature

Genes and variants involved in VDR-pathway

- VDR pathway is present → role pathogenesis
- All of the genes identified as target genes

- **ITGAM + rs12598380**
- Complement receptor 3, phagocytosis
- Previously confirmed
- Rs1143679 → skin, joint, kidney + immunological disorders
- Strong LD
- **STAT4**
- Key role INF-alpha signalling
- Raised IFN-alpha correlates with disease severity
- Type I interferon singallling pathway connected

Genes and variants involved in VDR-pathway

- **IRF5 + rs7574865**
- Cell adhesion, apoptosis, cell cycle regulation and early immune response, expression IFN type I genes
- rs10181656 and rs7582694, which were in perfect LD
- Rs7574865, high signifance SLE
- **CLEC16A + rs7200786**
- Highly expressed B-cells, NK-cells and DC-cells
- Regulates autophagy
- No literature
- **TNFAIP3 + rs58721818**
- Key regulator NFkB production
- No literature SNP

Transcriptomics data of genes involved in the VDR-pathway

	CD4 T	CD19 B	Myeolid
ARID5B	SIG UP		SIG UP
ATG5		SIG UP	SIG UP
PTTG1		SIG UP	SIG UP
VDR pathway genes			
ITGAM	UP	DOWN	UP
CLEC16A	DOWN	DOWN	UP
PRDM1	UP	SIG UP	UP
TNFAIP3	UP	UP	UP
IRF5	UP	DOWN	UP

- **ATG5 and PRDM1 + Rs4134466**
- ATG5 regulator autophagy
- PRDM1 – ATG5 region → elevated expression B-cells

Venn diagrams

- Regulation of immune response
- Pathways related to immune system
- Auto-immune disorder

Tissue specific network

- Vitamin D3 pathway present → connected to IRF5
- **BAK1**
- Apoptotic regulator
- Variants associated with increased risk SLE
- **TYK2**
- Regulates cytokine signals
- Component of Type I and III interferon signaling pathways
- Binding to IFN-alpha → IFNAR phosphorylates → biding of IRF5 and IRF3
- Variants associated with SLE

Limitations

- Unbalanced female/male ratio
- (1) 93% patients, 62% control
- (2) 92,7% patients, 40% control
- Pathway information missing
- Transcriptomic dataset not complete

Future research

- Gender specific differences
- Effect of variants (SNPs)
- Differences between tissues

Conclusion

- Evidence Vitamin D receptor pathway is involved in SLE
- Genetic variants increase risk of SLE
- Intergrative systems biology → insights into disease mechanism
- More research is needed

Thank you for your attention!