A cross-road of genetic predisposition of lupus nephritis and IgA nephropathy
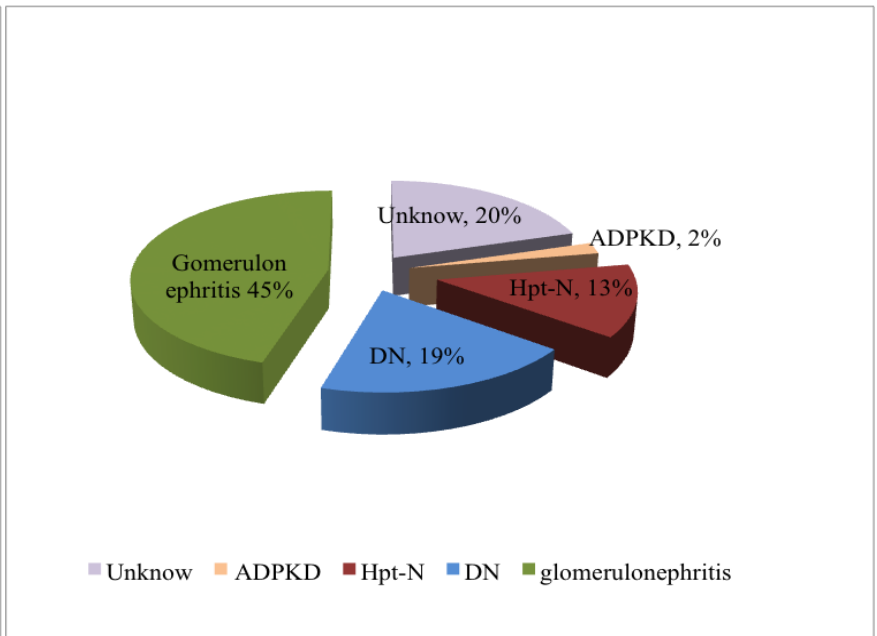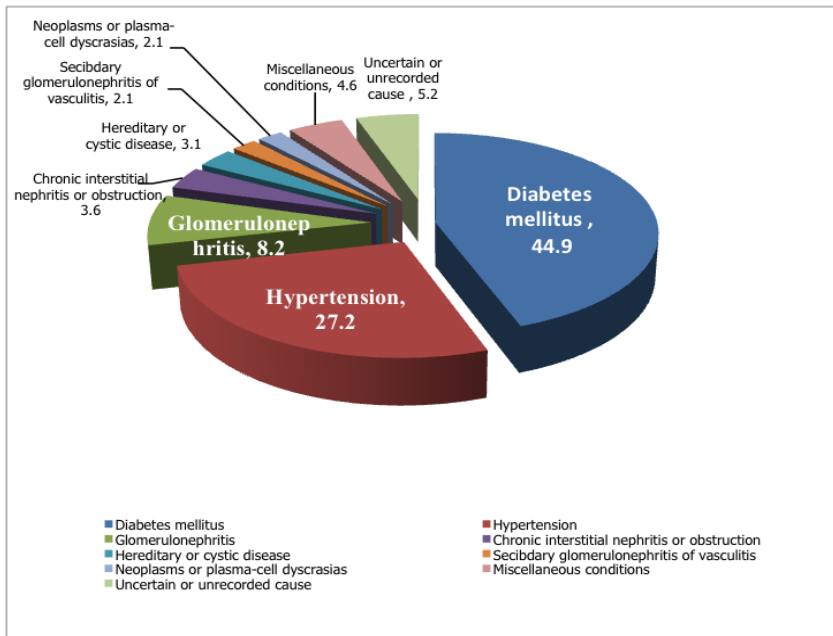
Hong Zhang

**Renal Division, Peking University First Hospital;
Peking University Institute of Nephrology
Beijing**

GN is the leading cause of ESKD in China

- **IgA nephropathy (IgAN) is the most common Primary GN in China.**
- **Lupus nephritis (LN) is the most common form of secondary GN in China.**
- **Common causative diseases for ESKD in young adults.**
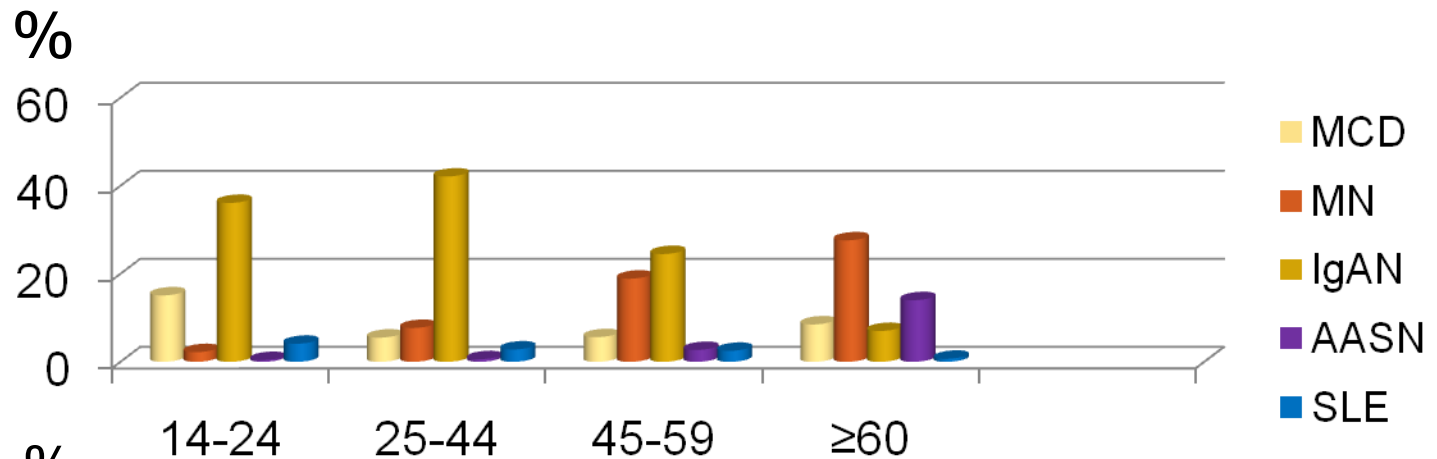

Renal Data System. USRDS

Dialysis Registration. China.
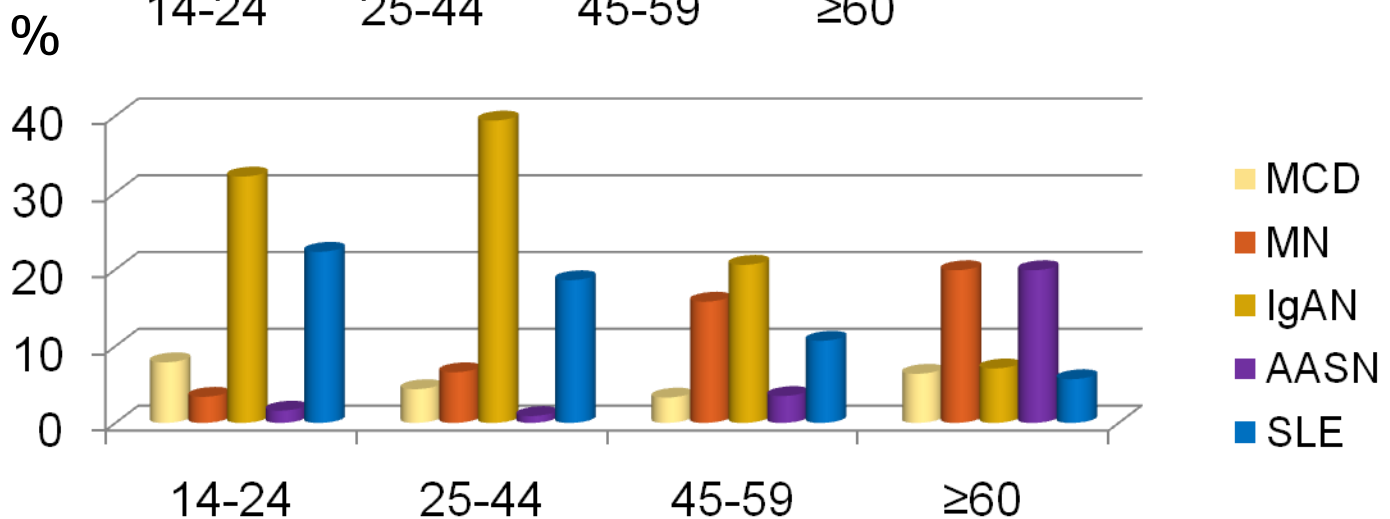
Disease composition of renal biopsy patients（5398）
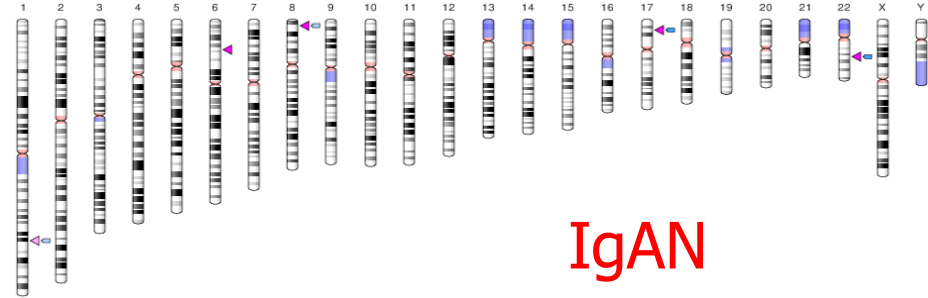
Zhou FD, et al. NDT. 2009, 2009,4(3):870-6.
Lv JC, Zhang H, et al. Nephrology 2008,9(2):221

Genome-wide association studies (GWAS) in SLE and IgA nephropathy

❖ Recent genome-wide association studies (GWAS) have identified multiple susceptibility loci for IgAN and systemic lupus erythematosus.
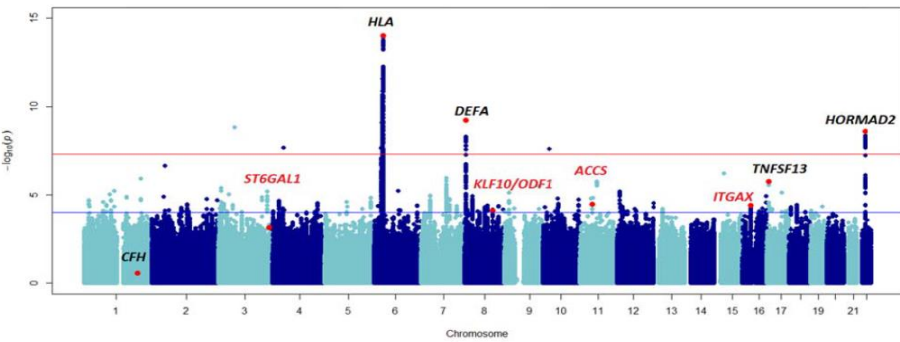
Manhattan Plot on the GWAS Results of Guangzhou, China
Li M, et al. Nat Commun. 2015;6:7270.

IgAN
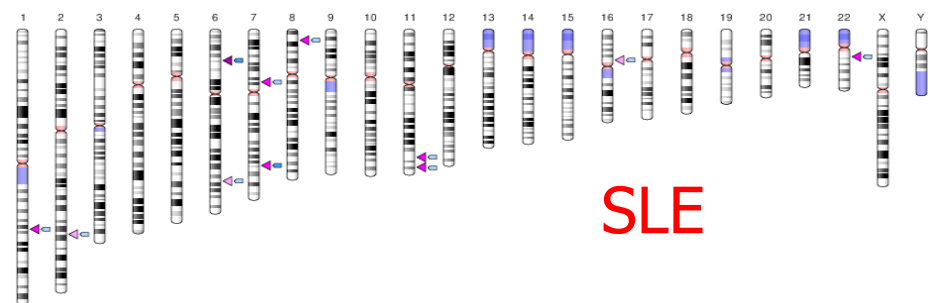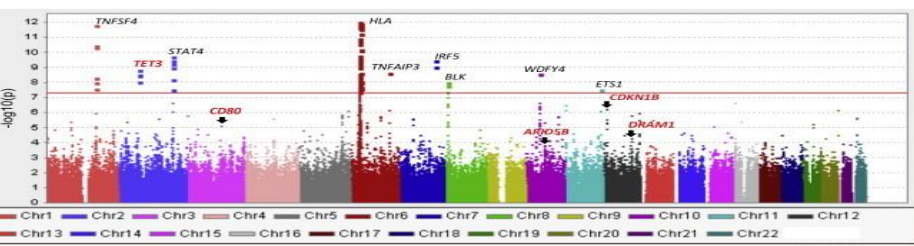
Manhattan Plot on the Meta-analysis Results of the Two SLE GWASs on Two Chinese Populations in Hong Kong and Anhui, China

SLE

From The Phenotype-Genotype Integrator (PheGenI)

4

Yang W, et al. Am J Hum Genet. 2013;92(1):41-51.

Advanced approaches of genetics in complex diseases

GWAS

Imputation

Association Testing

Independent Replication

Sequencing

Exome
Whole Genome
Gene-wise Analysis

Independent Replication

Informatics

eQTL

DNase1 sensitivity

TF binding sites

Histone Marks

Enhancer Analysis

miRNA target

Damaging AA

Conservation

Pathway Analysis

Directed Studies

Specific Cell Types

RNAseq

Functional Assays

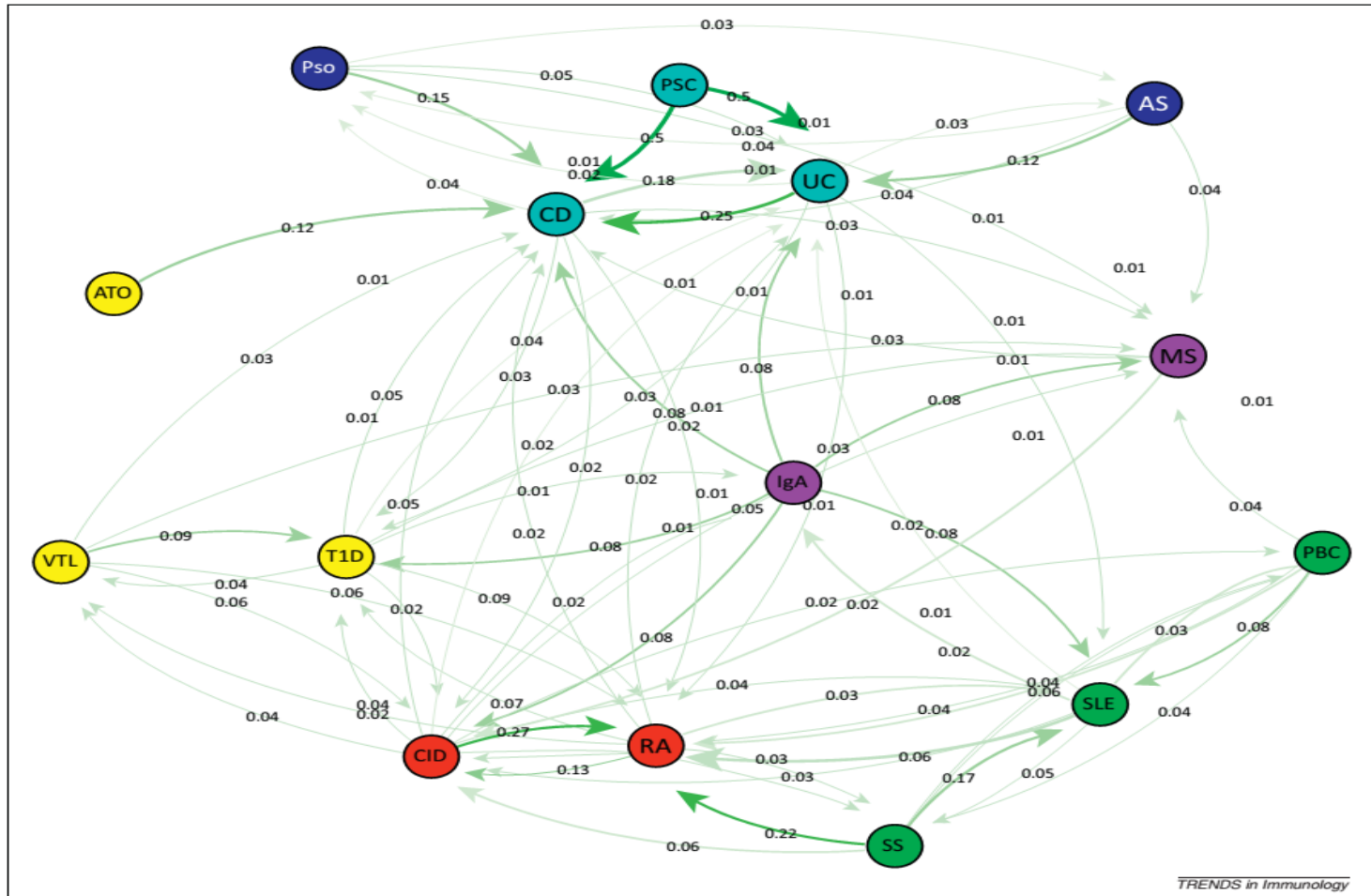**(SNP array vs. sequencing)**

Seldin MF. J Autoimmun. 2015;64:1-12.

Shared genetics in immune-related diseases

❖ Different complex diseases share common risk bases, i.e., pleiotropy

# Concomitance of chronic inflammatory and autoimmune diseases

Table 2 | Concomitance of chronic inflammatory and autoimmune diseases

Disease	Other diseases seen in patients	Familiar clustering	Refs
Ankylosing spondylitis	Ulcerative colitis (5%), Crohn's disease (3%), psoriasis (16%)	IBD* (7%), psoriasis (10%)	94
Asthma	Crohn's disease, ulcerative colitis	ND	95
AIT disease (including Graves' and Hashimoto's disease)	Rheumatoid arthritis (1.4–17.6%), T1D (3–15%), coeliac disease (5.4%)	ND	4,96
Coeliac disease	Asthma (24.6%), SLE (2.4%), AIT (5%), T1D (5%), psoriasis	ND	97–99
Crohn's disease	Rheumatoid arthritis (1.7–1.6%), asthma (7.1–13.0%), psoriasis (1.7–1.9%), coeliac disease (19%), multiple sclerosis (0.4%)	Psoriasis (10%)	95,100–102
Multiple sclerosis	AIT (0.5–3.9%), psoriasis (6%), IBD (3%)*, rheumatoid arthritis (0.35–2.4%), T1D (0–2.6%)	AIT (10%), psoriasis (6%), IBD (3%)*, rheumatoid arthritis (2%)	4
Psoriasis	Crohn's disease, ulcerative colitis, coeliac disease	ND	95
Rheumatoid arthritis	Asthma (10.0%), T1D (0.3–6%), AIT (0.5–9.8%)	AIT (2.1%), T1D (0.44), SLE (0.36%), multiple sclerosis (0.29%)	4,85,99
SLE	Diabetes mellitus (11.6%)	ND	103
T1D	Coeliac disease (4–9%), AIT (0–24%), asthma (5%), rheumatoid arthritis (2%)	Coeliac disease (6%), AIT (8%) (2.7%), rheumatoid arthritis (0.97%)	4,99,104,105
Ulcerative colitis	Rheumatoid arthritis (1.1–1.6%), asthma (7.9–12.0%), psoriasis (1.7–1.8%), multiple sclerosis (0.4–0.54%)	ND	95,102

*No discrimination made between Crohn's disease and ulcerative colitis. AIT, autoimmune thyroid disease; IBD, inflammatory bowel disease; ND, not determined; SLE, systemic lupus erythematosus; T1D, type 1 diabetes.

Zhernakova A, et al. Nat Rev Genet. 2009;10(1):43–55.

Pleiotropic effects: SLE and IgAN
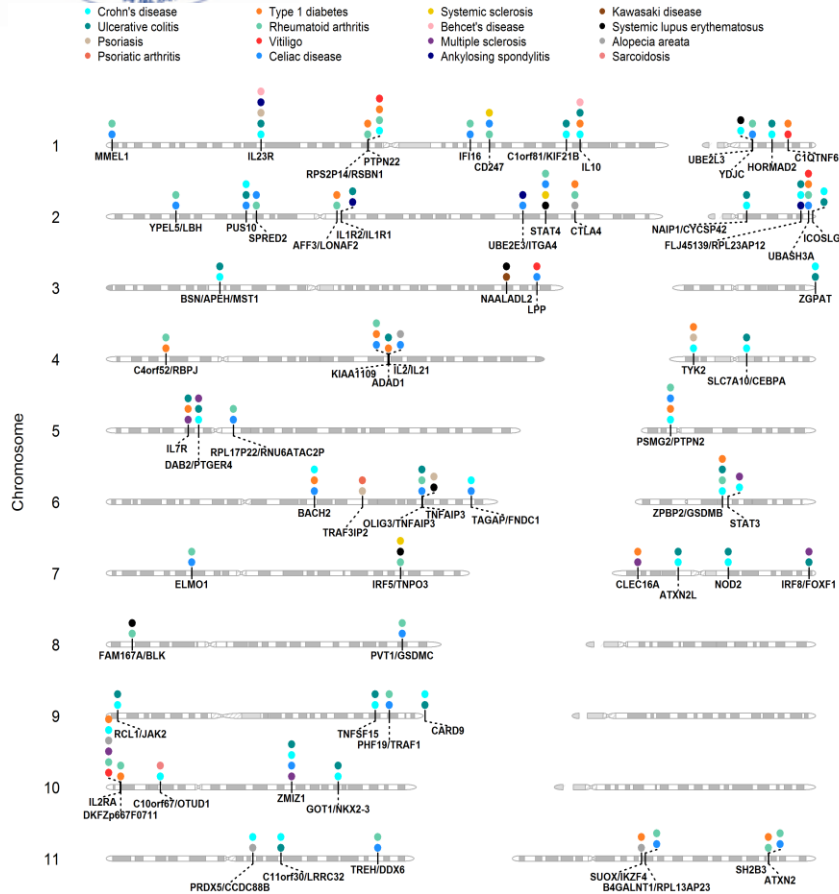
A comprehensive analysis of shared loci between systemic lupus erythematosus (SLE) and sixteen autoimmune diseases

Ramos PS, et al. PLoS Genet. 2011;7(12):e1002406.
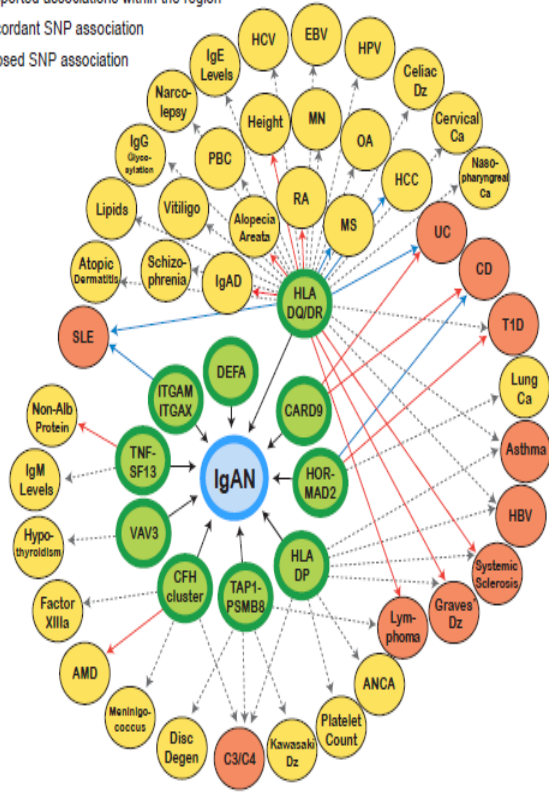
Pleiotropic effects of IgAN GWAS loci

Kiryluk K, et al. Nat Genet. 2014;46(11):1187-96.
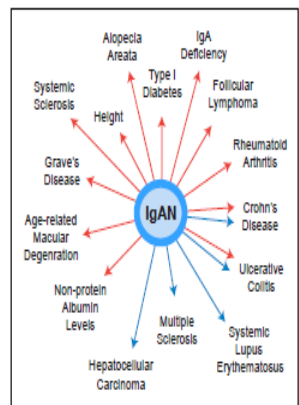
Questions?

* **LN: no specific GWAS conducted for LN in the strict sense.**
* **Pleiotropy: no available study conducted in a given population.**
* **Complex diseases: rigid significance p value in GWAS may obscure identification of genes/pathways shared between diseases**

* **What loci/genes were shared or specific for IgAN and LN in Chinese?**

Similarity between IgAN and LN

- ❖ **Geography: higher prevalence among Asians;**

- ❖ **Immune-mediated disease:**

  circulating immune complexes;

- ❖ **Clinical manifestation: chronic course; renal involvement;**

- ❖ **Pathological findings: morphological similarities;**

- ❖ **ESKD common pathways:**

  inflammation, complement activation; RAAS

- ❖ **Co-occurrences: SLE with IgAN**

Significance

- **Shared gene/pathway:**
  - **Similar pathogenesis**
  - **Same target—refined therapy for different forms of glomerulonephritis**

    Eculizumab: aHUS, DDD, C3 glomerulonephritis

- **Specific gene/pathway:**
  - **Different pathogenesis**
  - **Targeted therapy-- less adverse effect**
  - **Glucocorticosteroid, immunosuppressant**

Parkes M, et al. Nat Rev Genet. 2013;14(9):661-73.

Flow Chart of Study Design

Diseases: IgAN/LN

GWAS or nsSNP data available

Samples:
1100 IgAN vs 1000 Controls; 500 LN vs 500 Controls

Genetic variants （p<1*10^{-5})
Catalogue of published GWAS and nsSNP Scan

Genetic association and genetic replication

In silico analysis
Gene annotation, eQTL, Differential gene expression analysis, Gene ontology analysis

**Shared associated genes/pathways between diseases**

✧ SLE-SNPs: IgAN

Most of risk alleles of SLE were protective alleles for IgAN

Chr	Bp	Locus	SNP	Major /minor allele	MAF case/control (%)	Trend test p-values	Allele OR (95% CI) by SLE risk allele*	SLE risk allele OR
1	194953541	CFH	rs6677604	G/<u>A</u>	4.10/7.26	8.41×10^{-6}	0.55 (0.42-0.72)	1.19
1	234106500	LYST	rs9782955	<u>C</u>/T	12.87/10.71	3.31×10^{-2}	0.81 (0.67-0.98)	1.18
3	58345217	PXK	rs6445975	<u>T</u>/G	23.79/19.79	2.01×10^{-3}	0.79 (0.68-0.92)	1.20
6	32508322	HLA-DRA	rs9501626	C/<u>A</u>	11.39/16.26	4.91×10^{-6}	0.66 (0.55-0.79)	1.86
6	32694832	HLA-DRB1	rs9271366	A/<u>G</u>	12.60/18.65	6.96×10^{-8}	0.63 (0.53-0.75)	1.26
8	11377591	BLK	rs7812879	<u>C</u>/T	26.59/23.23	1.23×10^{-2}	0.83 (0.72-0.96)	1.45
8	11381089	BLK	rs2254546	<u>G</u>/A	26.63/23.12	9.32×10^{-3}	0.83 (0.72-0.95)	1.42
8	11381382	BLK	rs2736340	<u>T</u>/C	29.94/26.94	3.33×10^{-2}	0.86 (0.75-0.99)	1.35
22	20247190	UBE2L3	rs131654	<u>T</u>/G	46.48/49.94	2.63×10^{-2}	1.15 (1.02-1.30)	1.28
22	20269675	UBE2L3	rs5754217	G/<u>T</u>	47.32/43.74	2.11×10^{-2}	1.16 (1.02-1.31)	1.20

SLE-SNPs: IgAN

Regional analysis showed similar association
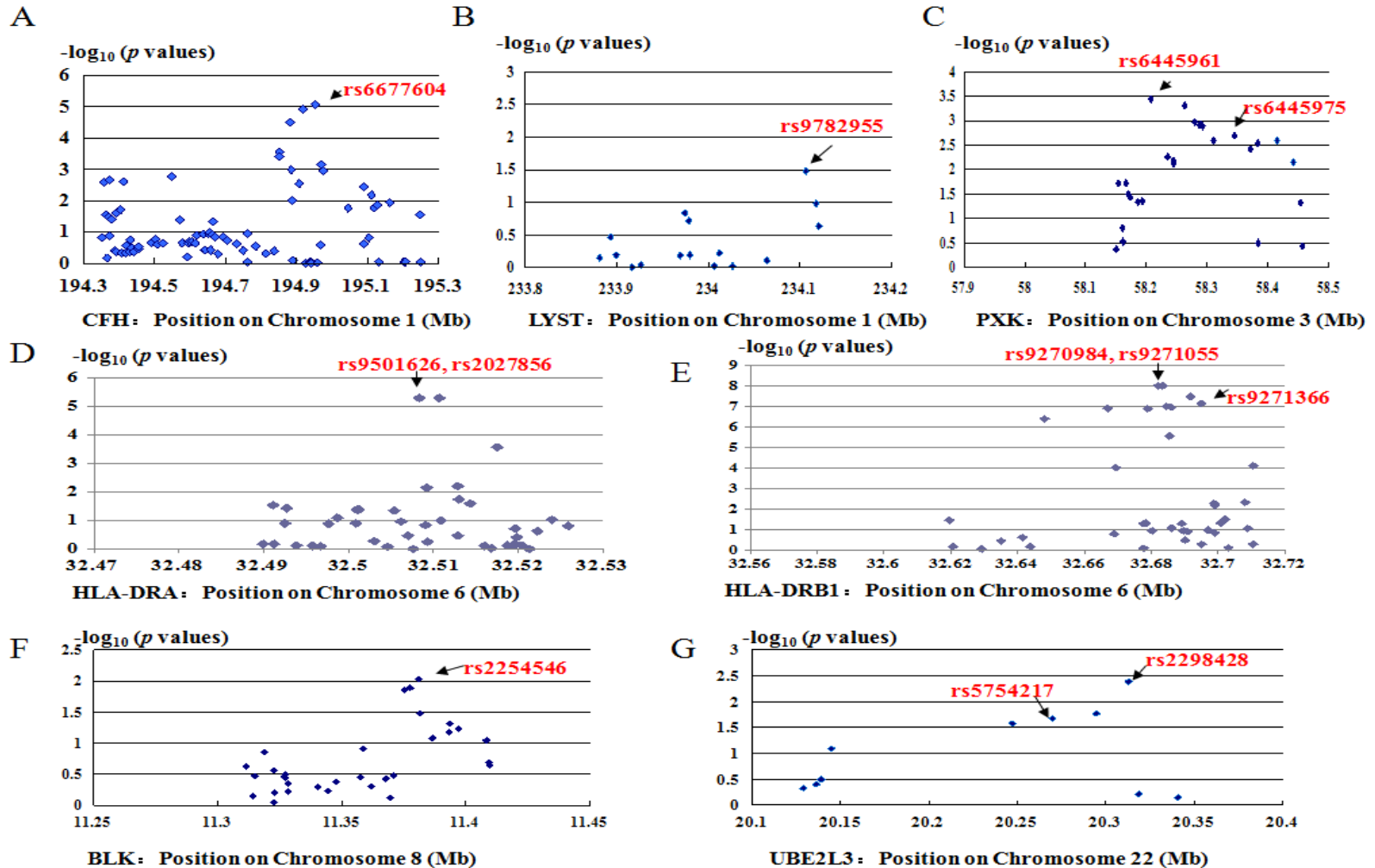
✧ SLE-SNPs: IgAN

eQTL analysis indicates potential function significance

SNP (ref.)	Gene	HapMap 3 unrelated individuals				Children siblings
		CEU (n=165)	**CHB** (n=137)	**JPT** (n=113)	**YRI** (n=203)	of British descent (n=405)
rs6445961-A	PXK	0.27 (4.10×10^{-3})	-0.20 0.07	-0.18 0.10	0.02 0.83	/
rs2298428-C	UBE2L3	-0.28 (3.30×10^{-3})	-0.28 (0.01)	-0.43 (5.00×10^{-5})	—	-0.390 (8.50×10^{-5})
rs6677604-A	CFH	0.12 (0.22)	0.02 (0.84)	0.26 (0.03)	0.11 (0.26)	—
rs9501626-A	HLA-DRA	—	—	—	—	—
rs9270984-G	HLA-DRB1	0.59 (1.00×10^{-11})	0.72 (1.30×10^{-13})	0.68 (1.40×10^{-12})	0.68 (4.90×10^{-16})	—
rs9271366-G	HLA-DRB1	0.63 (4.70×10^{-13})	0.74 (3.80×10^{-15})	0.75 (3.10×10^{-16})	0.73 (3.90×10^{-19})	0.878 (4.00×10^{-17})
rs2254546-G	BLK	0.02 (0.82)	-0.43 (8.20×10^{-5})	-0.51 (1.10×10^{-6})	-0.06 (0.57)	/

SLE-SNPs: IgAN

Differential gene expression analysis suggested involvement of the associated genes in IgAN

Candidate genes	Samples								
	Renal biopsies						**Whole blood**		
	Experiment E-GEOD-37460			**Experiment E-GEOD-35489**			**Experiment E-GEOD-14795**		
	IgAN (n=27)	Controls (n=27)	p	IgAN (n=25)	Controls (n=6)	p	IgAN (n=12)	Controls (n=8)	p
CFH	9.41 ± 0.94	8.95 ± 0.64	$\mathbf{4.09\times10^{-2}}$	5.72 ± 0.32	5.51 ± 0.14	0.14	96.90 ± 56.10	88.11 ± 61.04	0.74
HLA-DRA	11.59 ± 0.33	10.89 ± 0.54	$\underline{\mathbf{6.56\times10^{-7}}}$	9.42 ± 0.76	8.62 ± 0.27	$\underline{\mathbf{2.56\times10^{-4}}}$	8576.43 ± 2251.01	8638.24 ± 2355.87	0.95
HLA-DRB1	13.10 ± 0.26	12.52 ± 0.51	$\underline{\mathbf{4.22\times10^{-6}}}$	11.31 ± 0.65	10.43 ± 0.28	$\underline{\mathbf{5.58\times10^{-5}}}$	16661.58 ± 5086.23	15779.10 ± 3730.21	0.68
PXK	—	—	—	—	—	—	—	—	—
BLK	4.91 ± 0.25	4.82 ± 0.17	0.14	4.48 ± 0.13	4.44 ± 0.13	0.53	372.31 ± 148.09	245.60 ± 104.07	$\mathbf{3.75\times10^{-2}}$
UBE2L3	9.58 ± 0.18	9.66 ± 0.29	0.21	7.94 ± 0.13	7.75 ± 0.16	$\mathbf{3.24\times10^{-3}}$	492.78 ± 94.12	362.57 ± 132.65	$\mathbf{1.90\times10^{-2}}$

✧ SLE-SNPs: IgAN

Joint effects of the alleles

Number of protective alleles	Frequency (cases/controls %)	OR (95% CI)	p
≤2	5.4/1.9	1.00(Reference)	
3	13.5/10.3	0.46(0.25–0.83)	9.11×10^{-3}
4	25.7/19.5	0.46(0.26–0.82)	6.68×10^{-3}
5	26.3/25.4	0.36(0.21–0.64)	2.73×10^{-4}
6	19.0/21.4	0.31(0.18–0.55)	3.06×10^{-5}
7	6.4/13.3	0.17(0.09–0.31)	1.44×10^{-9}
≥8	3.7/8.0	0.16(0.08–0.31)	8.77×10^{-9}

✧ **SLE-SNPs: IgAN**

Pathway analysis highlighted four shared pathways
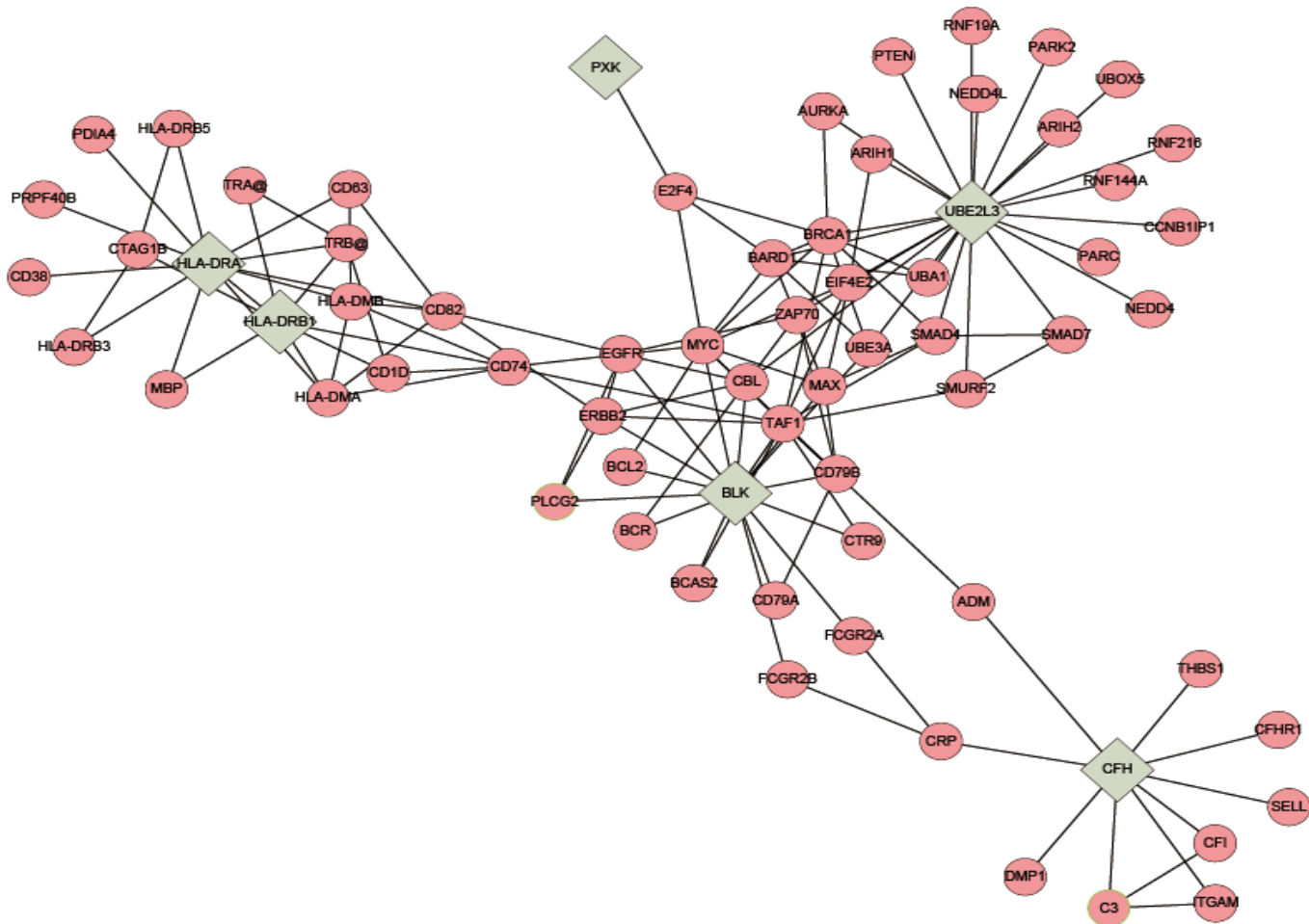
✧ SLE-SNPs: IgAN

Connected genes in the four pathways were also differentially expressed in renal tissues from IgAN

Candidate gene	Renal biopsies					
	Experiment E-GEOD-37460			Experiment E-GEOD-35489		
	IgAN (n=27)	Controls (n=27)	p	IgAN (n=25)	Controls (n=6)	p
C3	9.18±1.64	7.90±0.66	6.11×10^{-4}	8.69±1.42	7.39±0.22	1.54×10^{-4}
CD74	11.38±0.21	11.14±0.77	0.13	10.09±0.57	9.31±0.25	6.69×10^{-5}
EGFR	7.16±0.16	7.41±0.28	2.14×10^{-4}	6.88±0.13	6.95±0.21	0.27
HLA-DMA	10.83±0.25	10.30±0.44	2.37×10^{-6}	8.45±0.64	7.84±0.14	1.56×10^{-4}
HLA-DMB	10.78±0.40	10.15±0.44	1.62×10^{-6}	8.05±0.52	7.65±0.13	1.79×10^{-3}
ITGAM	7.21±0.80	6.42±0.66	2.15×10^{-4}	4.73±0.24	4.67±0.15	0.59
SMAD7	8.83±0.36	8.45±0.38	3.65×10^{-4}	6.65±0.30	7.22±0.16	1.17×10^{-4}
PTEN	7.20±0.35	6.87±0.28	3.90×10^{-4}	6.62±0.18	6.45±0.05	2.57×10^{-4}
EIF4E2	9.06±0.32	8.55±0.29	1.01×10^{-7}	7.58±0.26	7.50±0.22	0.51
PDIA4	9.08±0.19	8.73±0.20	2.16×10^{-8}	7.15±0.19	6.64±0.19	3.59×10^{-4}
RNF144A	8.80±0.33	8.44±0.31	1.30×10^{-4}	6.20±0.19	6.18±0.09	0.77
NEDD4L	8.25±0.30	8.58±0.27	7.56×10^{-5}	8.43±0.17	8.37±0.24	0.44

✧ IgAN-SNPs: SLE-LN

MTMR3 was identified as a novel susceptibility gene to lupus nephritis in Northern Han Chinese by shared gene analysis with IgA nephropathy, with similar risk
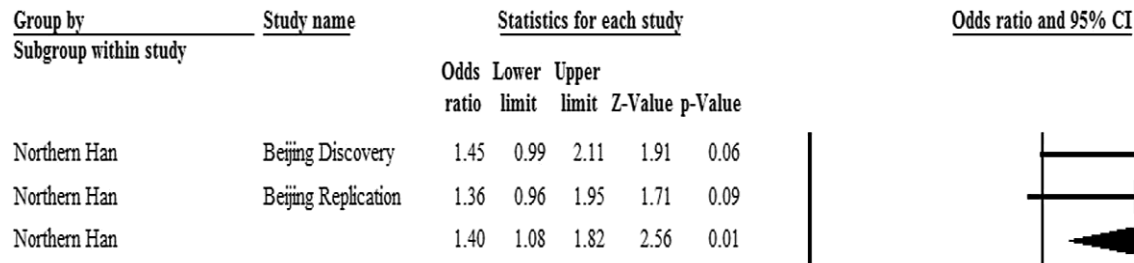
SNP	Chr.	Bp	Candidate Gene	Minor Allele	Frequency (Case/Control %)	P	OR (95% CI) in LN	OR in IgAN
rs6677604	1	194953541	CFH	A	7.36/7.45	0.94		
rs2523946	6	30049922	HLA-A	C	50.30/48.59	0.45		
rs660895	6	32685358	HLA-DRB1	G	12.60/19.01	8.97×10^{-5}	0.61 (0.48-0.79)	1.34
rs2856717	6	32778286	HLA-DQB1	T	33.06/24.14	1.09×10^{-5}	1.55 (1.28-1.89)	0.73
rs1794275	6	32779226	HLA-DQB1	T	14.68/12.90	0.25		
rs9275596	6	32789609	HLA-DQB1	C	29.33/20.82	1.22×10^{-5}	1.58 (1.29-1.94)	0.63
rs9357155	6	32917826	PSMB8	A	23.08/19.62	0.06	1.23	0.71
rs2071543	6	32919607	PSMB8	A	24.60/20.93	0.05	1.23	0.73
rs3129269	6	33205592	HLA-DPB2	T	25.81/26.16	0.86		
rs2738058	8	6810195	DEFA	G	33.87/31.79	0.32		
rs3803800	17	7403693	TNFSF13	A	30.54/32.60	0.33		
rs12537	22	28753460	MTMR3	T	25.10/29.28	3.66×10^{-2}	0.81 (0.66-0.98)	0.78
rs9983	22	28753744	MTMR3	A	11.49/7.45	2.07×10^{-3}	1.61 (1.19-2.19)	1.18
rs2412971	22	28824371	HORMAD2	A	36.39/40.24	0.08	0.85	0.75
rs2412973	22	28859631	HORMAD2	A	36.79/40.14	0.13		

MTMR3 associations could be replicated in Northern Han SLE

Cohort	Northern Han Chinese (878 LN vs. 556 non-LN)						
	Beijing Discovery (500/240)				Beijing Replication (378/316)		
SNP	MAF	P	OR (95% CI)		MAF	P	OR (95% CI)
rs12537	25.10/25.21	0.96	0.99 (0.77-1.28)		24.14/23.95	0.94	1.01 (0.79-1.29)
rs9983	11.49/8.40	**0.06**	1.42 (0.97-2.07)		11.45/8.74	**0.09**	1.36 (0.96-1.95)

Group by Subgroup within study	Study name	Statistics for each study					Odds ratio and 95% CI
		Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	
Northern Han	Beijing Discovery	1.45	0.99	2.11	1.91	0.06	
Northern Han	Beijing Replication	1.36	0.96	1.95	1.71	0.09	
Northern Han		1.40	1.08	1.82	2.56	0.01	

✧ **IgAN-SNPs: SLE-LN**

Rs9983 was annotated as functional by **HaploReg**

variant	Ref	Alt	GERP cons	SiPhy cons	Enhancer histone marks	Motifs changed	GENCODE genes	dbSNP funcannot
rs9983	G	A			HMEC	4 altered motifs	MTMR3	3'-UTR

IgAN-SNPs: SLE-LN

Prediction of rs9983 as miRNA target

Location	dbSNP ID	Variant type	Wobble base pair	Ancestral Allele	Allele	miR ID	Conservation	miRSite	Function Class	Exp Support	context+ score change
30423744	rs9983	SNP	Y	G	G	hsa-miR-3136-5p	6	gagTCAG TCAgtg	D	N	0.02
						hsa-miR-345-5p	8	GAGTCA Gtcagtg	D	N	0.004
						hsa-miR-4439	6	gagTCAG TCAgtg	D	N	0.029
						hsa-miR-4513	6	gaGTCAG TCAgtg	D	N	-0.058
						hsa-miR-6855-3p	6	gaGTCAG TCAgtg	D	N	-0.026
						hsa-miR-6857-3p	6	gagTCAG TCAgtg	D	N	0.02

✧ **IgAN-SNPs:** **SLE-LN**

e-SNPs for MTMR3 showed genotype-expression correlations, esp. rs9983
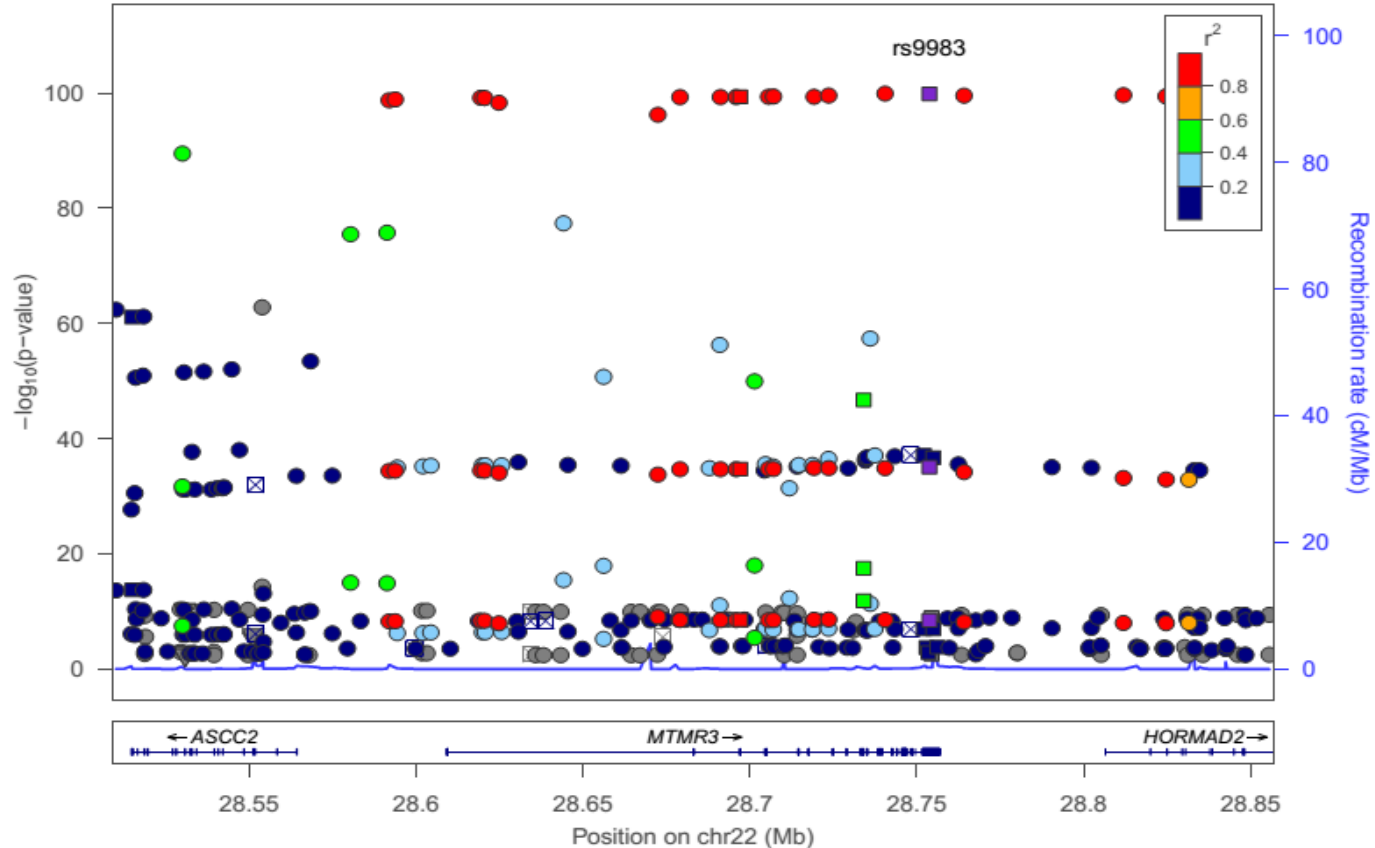
✧ IgAN-SNPs: SLE-LN

Rs9983 eQTL effect could be replicated by different databases

Study	Population	Tissue	Number	Effect	P value
MRCA	UK 405 siblings	LCL	405	**-0.32**	**4.36×10^{-9}**
MRCE	UK 550 siblings	LCL	550	**-0.41**	**1.14×10^{-9}**
Blood eQTL	Multiple	Non-transformed peripheral blood	5311	NA	**1.28×10^{-100}**
MuTHER	Twin-A	Fat	74/82	**0.23/0.24**	**0.04/0.02**
	Twin-L	LCL	76/84	-0.06/**0.27**	0.64/**0.02**
	Twin-S	Skin	79/87	0.10/**0.29**	0.37/**7.70×10^{-3}**
HapMap	CEU	LCL	109	0.04	0.70
	CHB	LCL	80	-0.05	0.64
	GIH	LCL	82	**-0.27**	**0.016**
	JPT	LCL	82	-0.10	0.39
	LWK	LCL	82	-0.08	0.50
	MEX	LCL	45	-0.04	0.81
	MKK	LCL	138	0.02	0.84
	YRI	LCL	108	0.00	0.97

✧ IgAN-SNPs: SLE-LN

❖ **MTMR3 mRNA expressions were down-regulated in glomeruli**

  ❖ LN (5.92 ± 0.15 versus 6.18 ± 0.17; p = 5.94×10^{-6};

     32 LN patients versus 14 controls)

  ❖ IgAN(6.42±0.14 versus 6.74±0.34; p = 9.64×10^{-5};

     27 LN patients versus 27 controls)

Summary

We identified shared alleles/pathway but with different effect between IgAN and LN

Chr	Locus	SNP	Allele OR by SLE risk allele in IgAN	SLE risk allele OR
1	*CFH*	rs6677604	0.55	1.19
1	*LYST*	rs9782955	0.81	1.18
3	*PXK*	rs6445975	0.79	1.20
6	*HLA-DRA*	rs9501626	0.66	1.86
6	*HLA-DRB1*	rs9271366	0.63	1.26
8	*BLK*	rs2254546	0.83	1.42
22	*UBE2L3*	rs5754217	1.16	1.20
22	*MTMR3*	rs9983	1.18	1.40

Cumulative Effects of Variants Identified by Genome-wide Association Studies in IgA Nephropathy

Xu-Jie Zhou, Yuan-Yuan Qi, Ping Hou, Ji-Cheng Lv, Su-Fang Shi, Li-Jun Liu, Na Zhao & Hong Zhang

Genetic association of *PRDM1-ATG5* intergenic region and autophagy with systemic lupus erythematosus in a Chinese population

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Article

Association of Systemic Lupus Erythematosus Susceptibility Genes with IgA Nephropathy in a Chinese Cohort

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Gene–Gene Interaction of *BLK*, *TNFSF4*, *TRAF1*, *TNFAIP3*, and *REL* in Systemic Lupus Erythematosus

Xu-jie Zhou,[1] Xiao-lan Lu,[2] Swapan K. Nath,[3] Ji-cheng Lv,[1] Sai-nan Zhu,[4] Hai-zhen Yang,[4] Lian-xiang Qin,[1] Ming-hui Zhao,[1] Yin Su,[2] International Consortium on the Genetics of Systemic Lupus Erythematosus, Nan Shen,[5] Zhan-guo Li,[2] and Hong Zhang[1]

Research Article

Detecting Genetic Associations between *ATG5* and Lupus Nephritis by *trans*-eQTL

Yue-miao Zhang,[1] Fa-juan Cheng,[1,2] Xu-jie Zhou,[1] Yuan-yuan Qi,[1] Ping Hou,[1] Ming-hui Zhao,[1] and Hong Zhang[1]

Identification of *MTMR3* as a Novel Susceptibility Gene for Lupus Nephritis in Northern Han Chinese by Shared-Gene Analysis With IgA Nephropathy

Xu-jie Zhou,[1] Swapan K. Nath,[2] Yuan-yuan Qi,[1] Fa-juan Cheng,[1] Hai-zhen Yang,[3] Yan Zhang,[4] Wanling Yang,[4] Jian-yang Ma,[5] Ming-hui Zhao,[1] Nan Shen,[6] and Hong Zhang[1]

❖ **We have some progression,**

But we still have to do a lot ... ...

致 谢

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