中山大學附属第一医院
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Genomic Study in IgA Nephropathy

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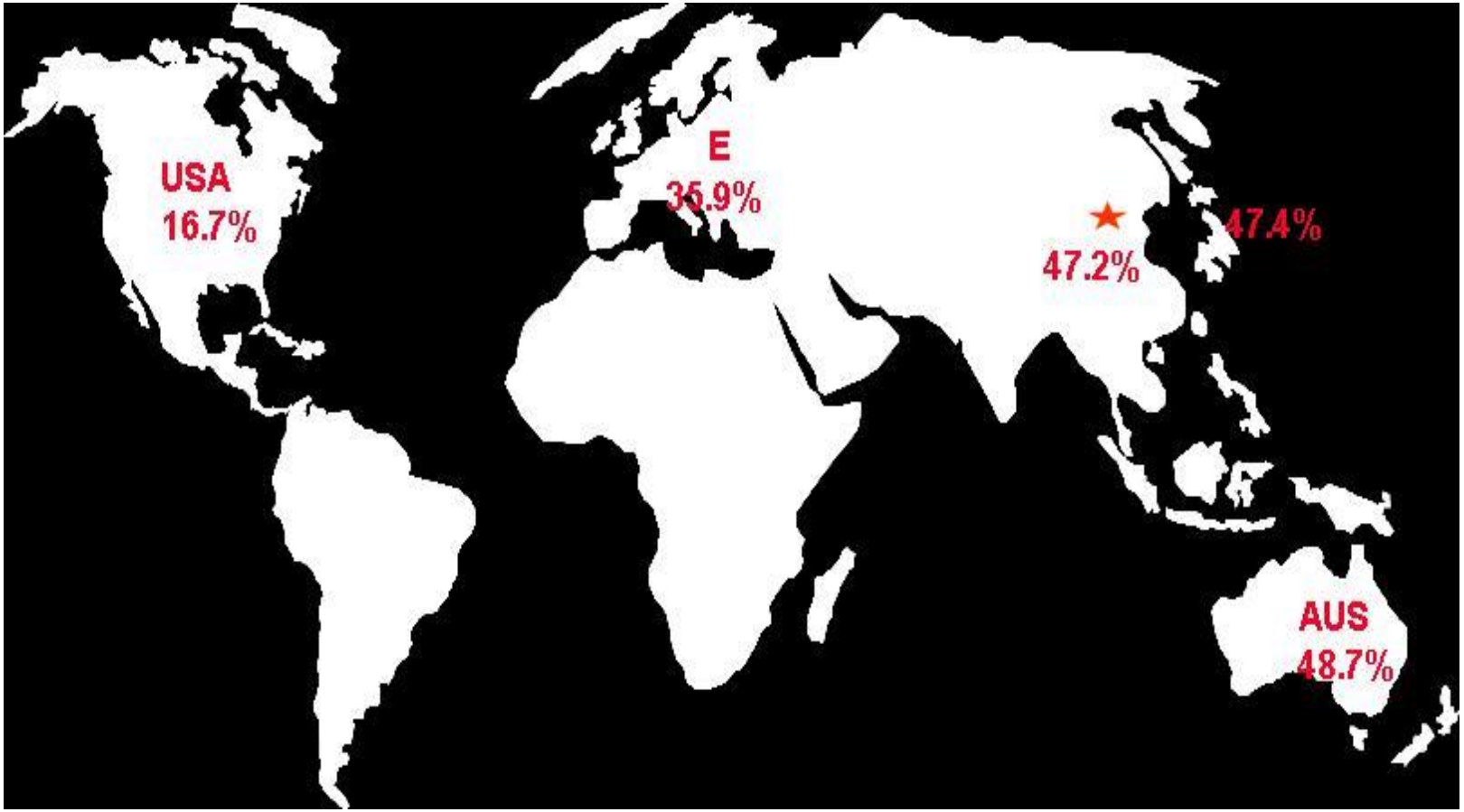
IgAN: the most common primary glomerulopathy



Challenges in IgAN: diagnosis and therapy

- **Clinical phenotype：**microscopic haematuria to massive proteinuria
- **Pathological patterns：**slightly mesangial proliferative to global sclerosis
- **Response to therapies：**Sensitive, dependent, or resistant to treatment agents
- **Clinical outcomes：**stable renal function for lifetime or progress to ESRD

Study	Patient selection	Protocols	Follow-up	Outcome
Maes BD et al. 2004 （n=34)	20<Ccr<70ml/min/, Upro>1g/d, Grade II to IV by Churg and Sobin SBP≥140 or DBP≥90	MMF (2.0/d)/Placebo(21/13)	CCr，Upro，3ys Follow up	No benefit
Frisch G et al. 2005（n=32)	20<Ccr<80ml/min/ Upro>1g/d, Glomerulosclerosis or tubulointerstitial atrophy and fibrosis ≥25% BP≥150/90 mmHg	MMF (2.0/d，n=17)/Placebo(17/15)(n=15) for 1yr	Scr,Upro, 3ys Follow up	No benefit
Chen et al 2002 （n=62）	Scr<4mg/dl, Upro≥2g/d, Grade IV-V by Lee No mention BP	OP(n=31, OP 0.8/kg.d)；MMF(n=31, 1.5~2.0/d) 6 mons	Upro，Follow up 18 mon	reducing Upro
Tang S et al	Scr<3.4mg/dl, Upro>1g/d, Haas Grade II-IV <125/85 treated with ACEI/ARB	Control (n=20), ACEI or ARB; MMF(1.5-2.0/d,n=20),ACEI or ARB，72 weeks	Upro follow up for 72 wks	reducing Upro

Pathogenesis of IgA Nephropathy

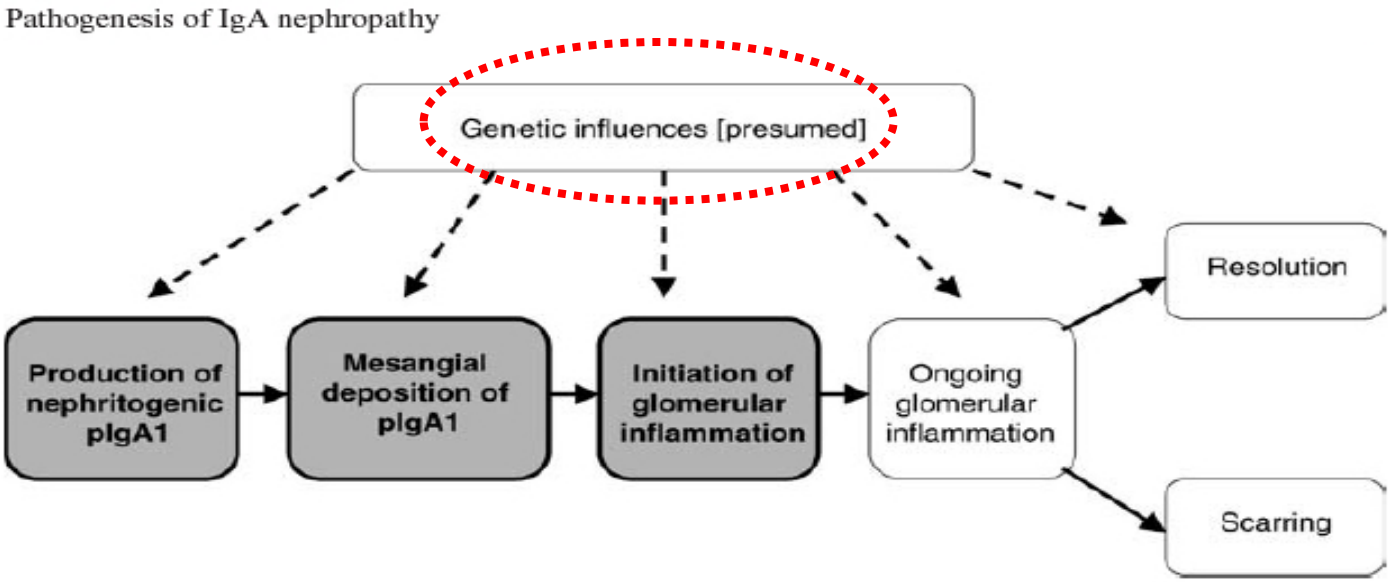
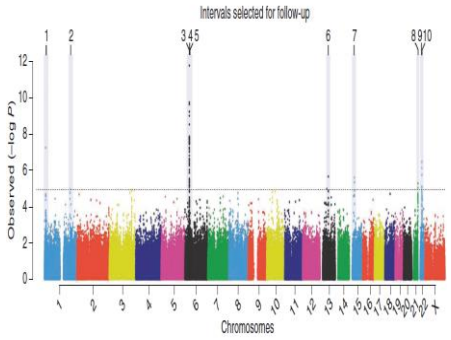


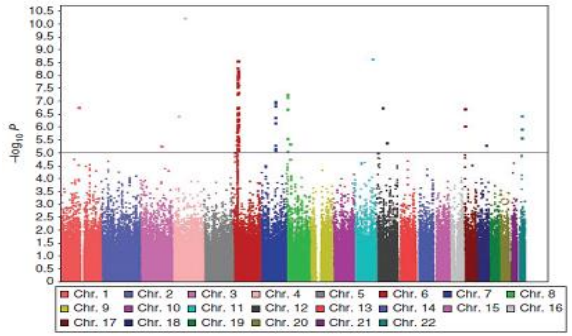
Fig. 1. Overview of pathogenesis of IgA nephropathy. Processes that appear to be IgA-specific are shown in the *grey* boxes, and are the focus of this review. 'Downstream' processes subsequent to the initiation of glomerular inflammation appear to be generic, and relevant to a wide range of mesangial proliferative glomerulonephritis. Genetic susceptibilities may be operative at all stages of the pathogenic process, but as yet none are proven (*pIgA* polymeric IgA)

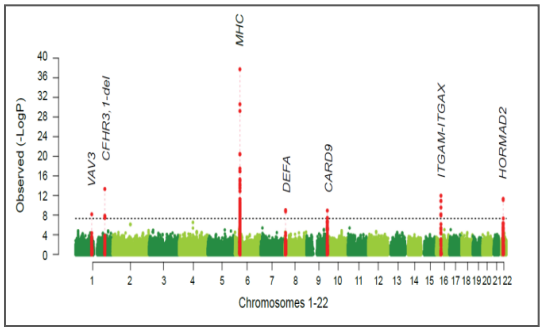
Springer Semin Immunopathol 2003;24: 477-93
Kidney Int 2004，65: 1544-1547
J Am Soc Nephrol 2005，16: 2088-2097

Tow new loci indentified in Chinese Han IgAN study

Susceptible loci	MHC	1q32	22q12	Others
IgAN study in Columbia, USA	√	√	√	16p11, 9q34, 1p13
IgAN study in SYSU, China	√	not confirmed	√	two new loci (17p13,8p23)







Gharavi AG, et al. Nat Genet 2011;43:321-7

Yu XQ, et al. Nat Genet 2012;44:178-82

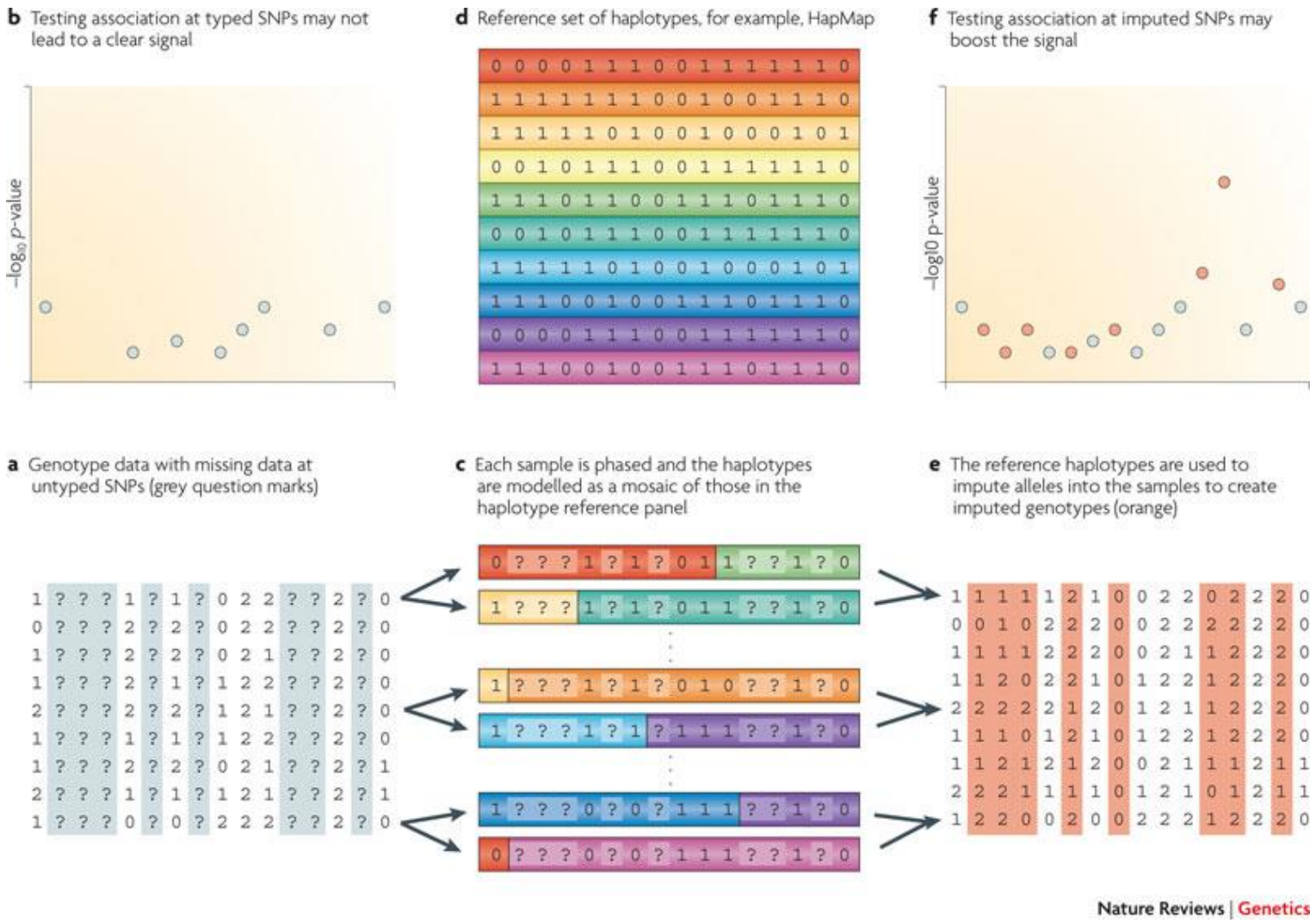
Krzysztof K, et al. Nat Genet 2014;46:1187-96

Ongoing Studies of IgAN in SYSU

- Data mining of GWAS
- GWA meta-analysis
- Identify rare and low frequency variants
- Fine mapping of susceptibility loci

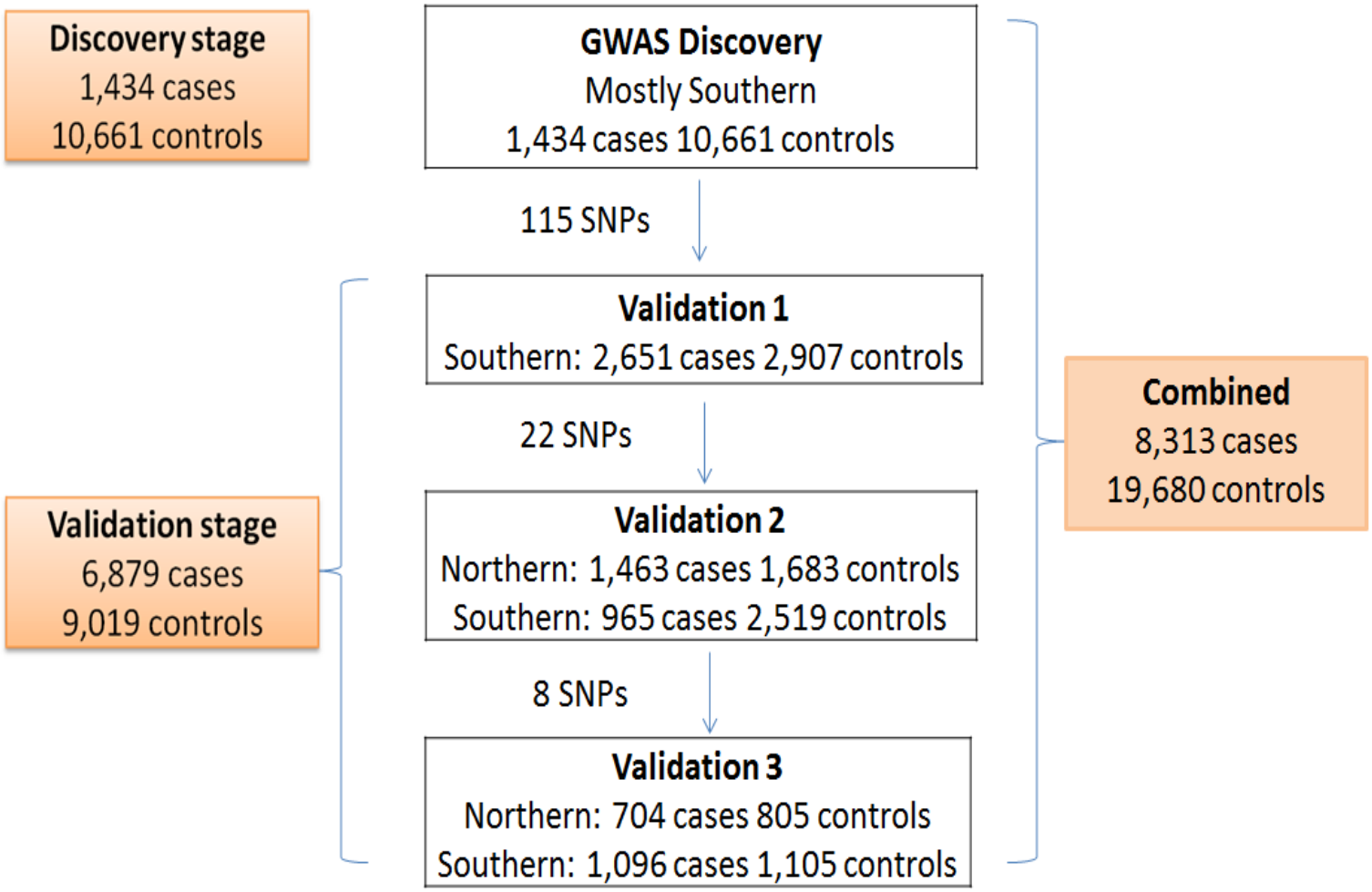
Phase II GWAS of IgAN Patients

Genotype imputation

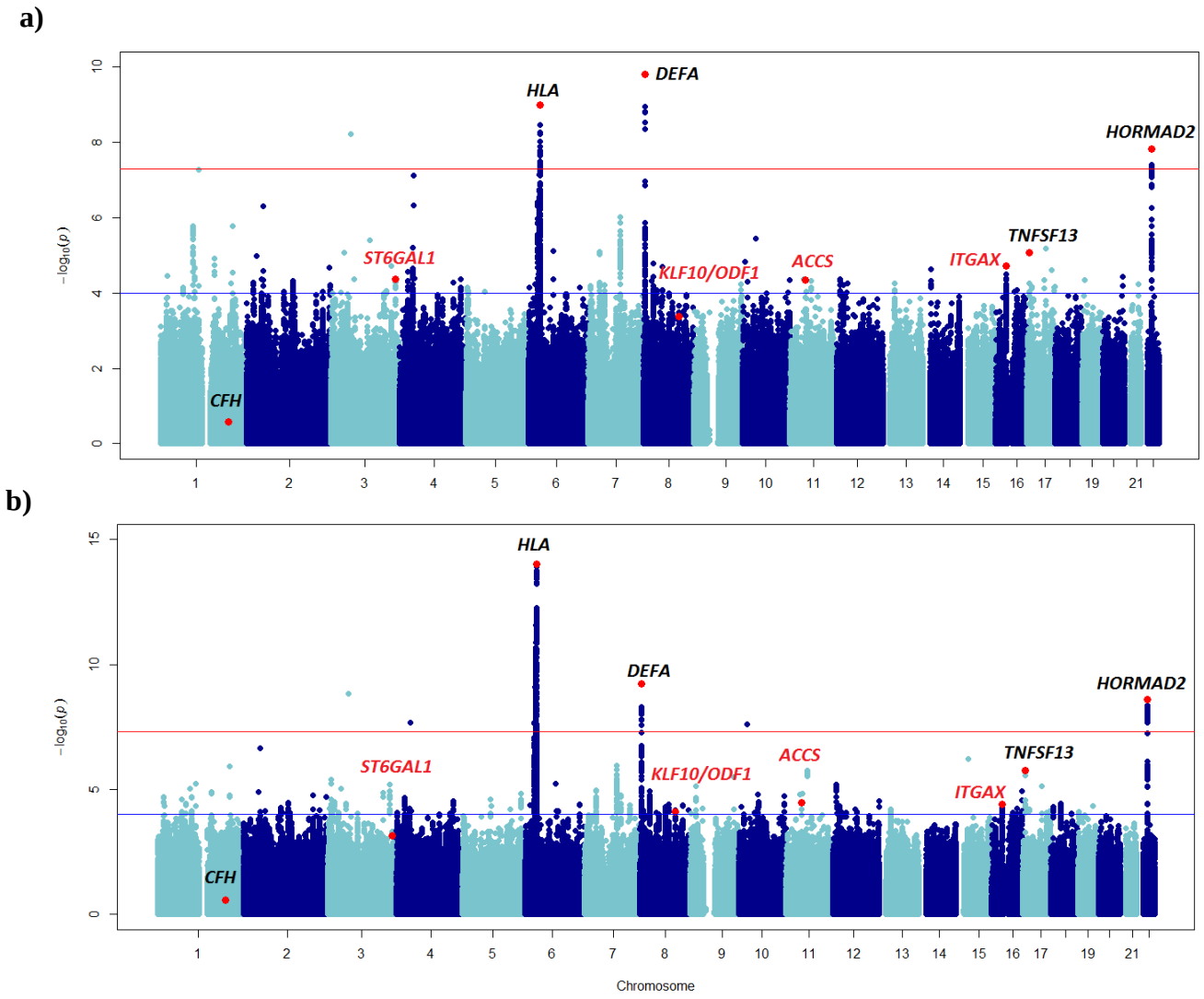


Marchini J, Howie B. Nat Rev Genet, 2010, 11(7):499-511

Study Design Flowchart



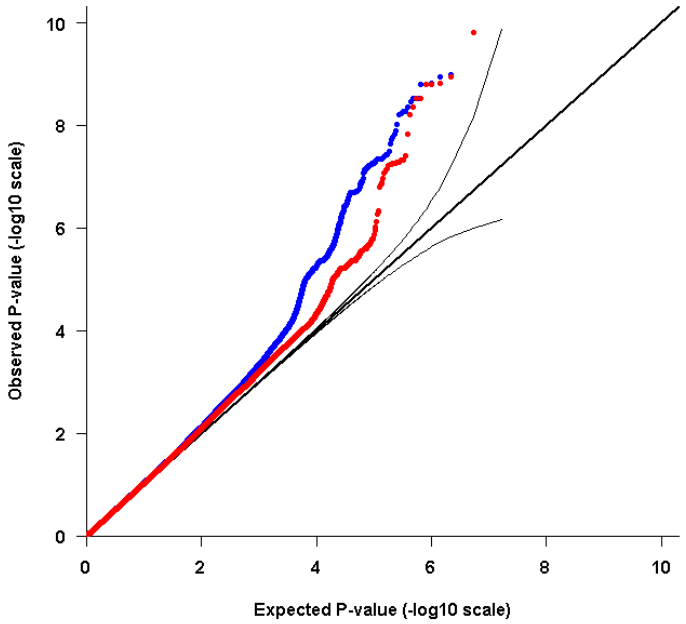
Manhattan plots



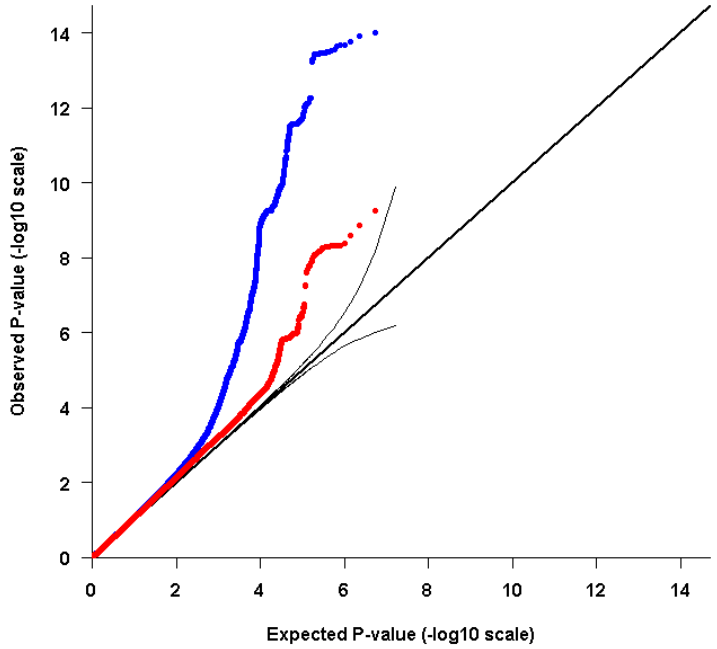
a) GEMMA[1] wald test on threshold-filtered genotypes (3.7 million SNPs) and b) Principal components (PC)-adjusted logistic regression test on genotype dosages (3.8 million SNPs). Reported SNPs within previously identified and novel loci are indicated in red.

Quantile-quantile plots

a) •λ_{GC} = 1.039, λ_{GC} without MHC SNPs= 1.035

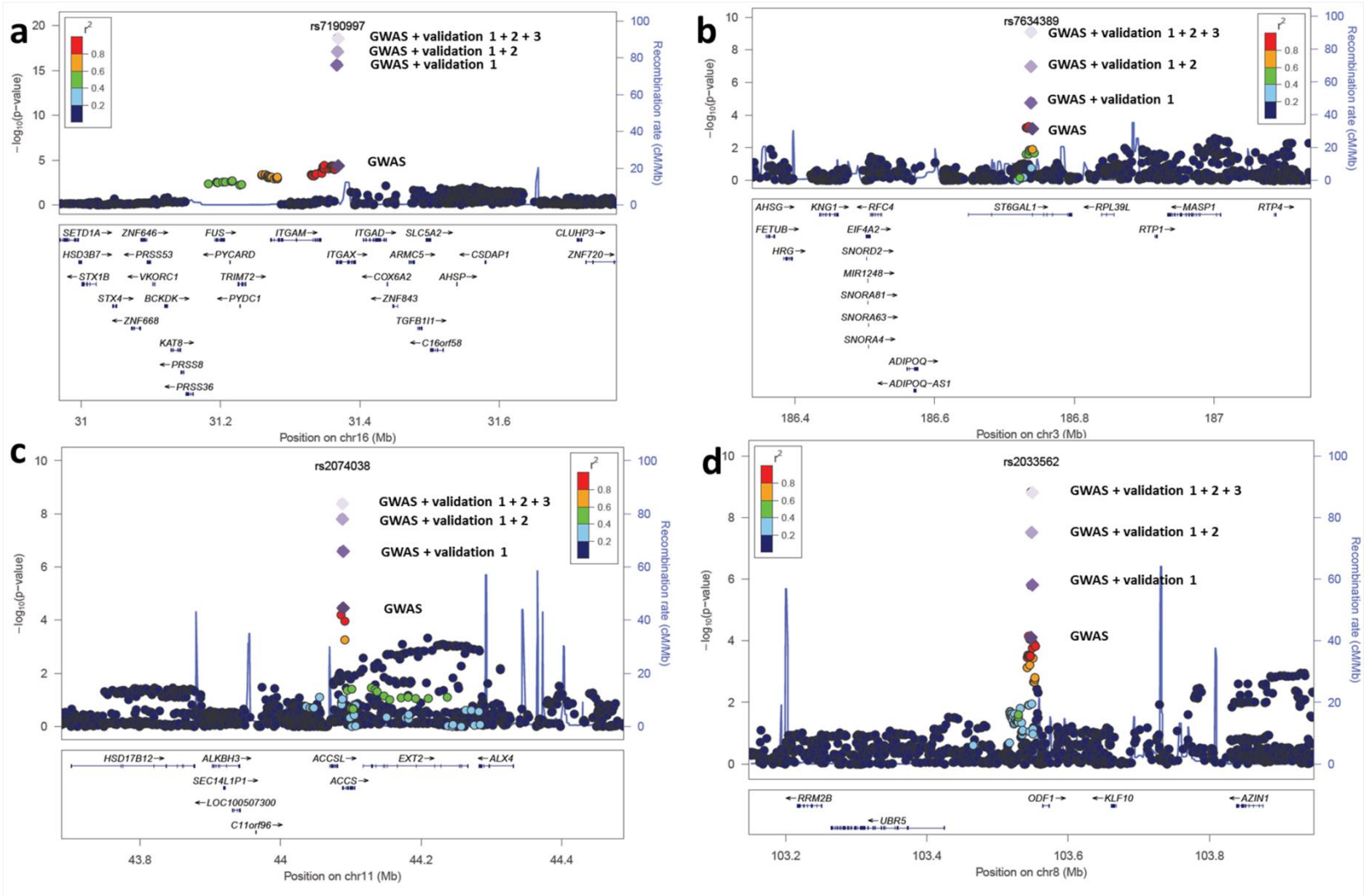


b) •λ_{GC} = 1.087, λ_{GC} without MHC SNPs= 1.077



a) GEMMA[1] wald test on threshold-filtered genotypes and b) PC-adjusted logistic regression test on genotype dosages. The 95% confidence intervals of the P-value distributions and the genomic inflation lambda GC values are indicated.

Recombination plots of the novel loci reaching genome-wide significance



showing P-values obtained in the GWAS discovery (logistic regression) and in the combined analysis of GWAS and validation 1, 2 and 3 samples (fixed effects meta-analysis).

Li M, …Yu XQ*. Nat Communications, 2015

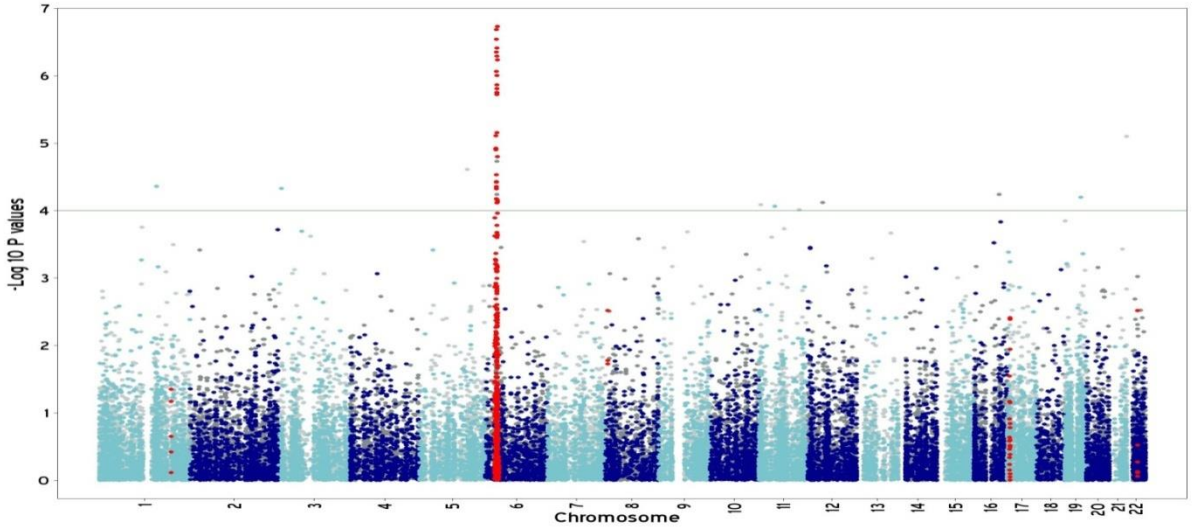
Exome-Array Study of IgAN in Chinese Population

Summary of dataset

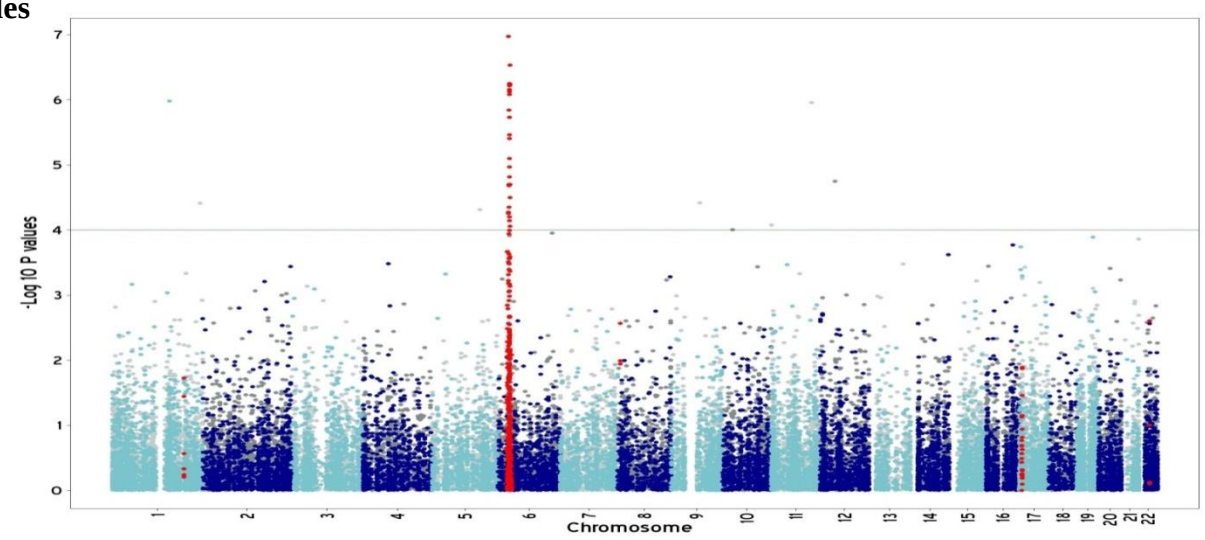
Study	N	Missing(0.01)& Heterozygosity	IBD (MZ only)	IBD in 7 Exomes (MZ only)	PCA 1KG	PCA ASN	Case	Control
IgAN	3954	-129	-33	-1	-3	0	2378	1410
AS_controls	553	-8	-4	0	-3	0		538
NPC_case+controls	2975	-15	-1	-1	0	0		2958
ES_cases	2256	-25	-46	0	-4	0		2181
SICC_controls	2576	-70	-5	-4	-21	0		2476
IMHSC_case+controls	3539	-35	-15	-3	-27	0		3459
PD_case+controls	1364	-8	-22	-1	-15	0		1318
POAG_case+control	1327	-20	-5	0	0	0		1302
Total	18544	-310	-131	-10	-73	0	**2378**	**15642**

Manhattan plots from GEMMA analysis of coding SNPs in analysis 1 and 2

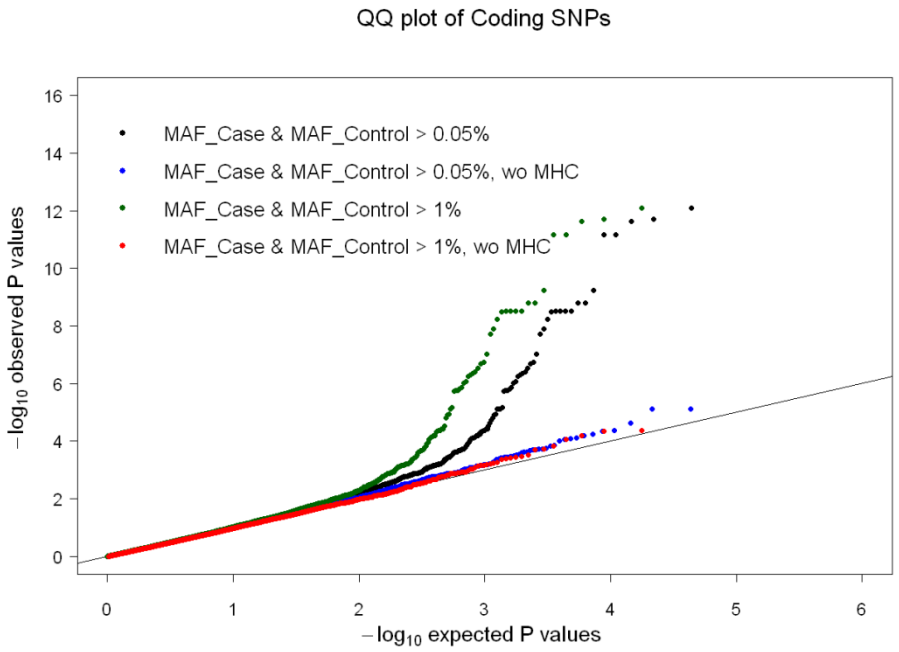
Analysis 1: 18,020 Samples

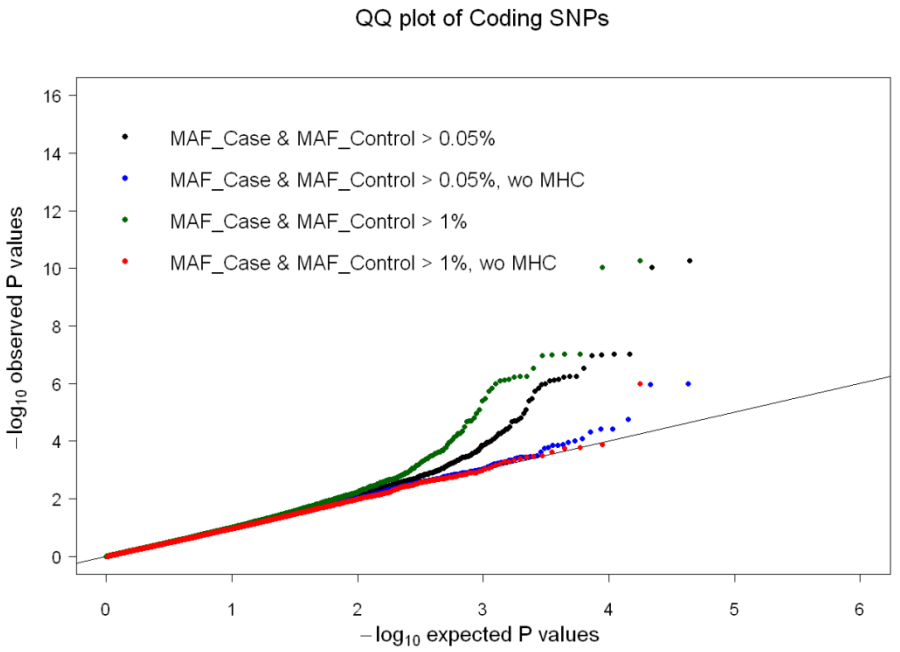


Analysis 2: 10,940 Samples



QQ plots of analysis 1 and 2 (GEMMA) in the discovery study





Analysis 1:
λgc =0.945 for 43,977 NPs
λgc =0.930 for 43,058 SNPs (wo MHC)
λgc =0.948 for 17,647 SNPs
λgc =0.924 for 17,156 SNPs (wo MHC)

Analysis 2:
λgc =0.951 for 43,868 SNPs
λgc =0.939 for 42,949 SNPs (wo MHC)
λgc =0.976 for 17,660 SNPs
λgc =0.956 for 17,161SNPs (wo MHC)

Association test results using logistic regression analyses (adjusted for PC1-5 in the discovery samples)

		Frequency cases	Frequency controls	P	OR (95% CI)
SNP 1	**Discovery**	1.436%	0.942%	5.02E-06	1.880 (1.434-2.465)
	Validation Northern	1.292%	0.646%	8.56E-03	2.028 (1.197-3.347)
chr6:43748545 A/G	**Validation Southern**	0.964%	0.415%	1.05E-04	2.313 (1.514-3.532)
		P_{het}	**I^2**	**P**	**OR**
	Validation only	0.703	0	3.02E-06	2.197
Meta analysis	**All**	0.721	0	8.53E-11	2.002
SNP 2	**Discovery**	0.929%	0.487%	2.00E-04	1.907 (1.357-2.680)
	Validation Northern	1.028%	0.704%	0.161	1.469 (0.858-2.515)
chr19:1612427 A/G	**Validation Southern**	0.964%	0.474%	5.86E-04	2.053 (1.362-3.094)
		P_{het}	**I^2**	**P**	**OR**
	Validation only	0.332	0	3.38E-04	1.815
Meta analysis	**All**	0.611	0	2.46E-07	1.859

Clinical phenotypes of IgAN cases: rare variant carriers vs non-carriers

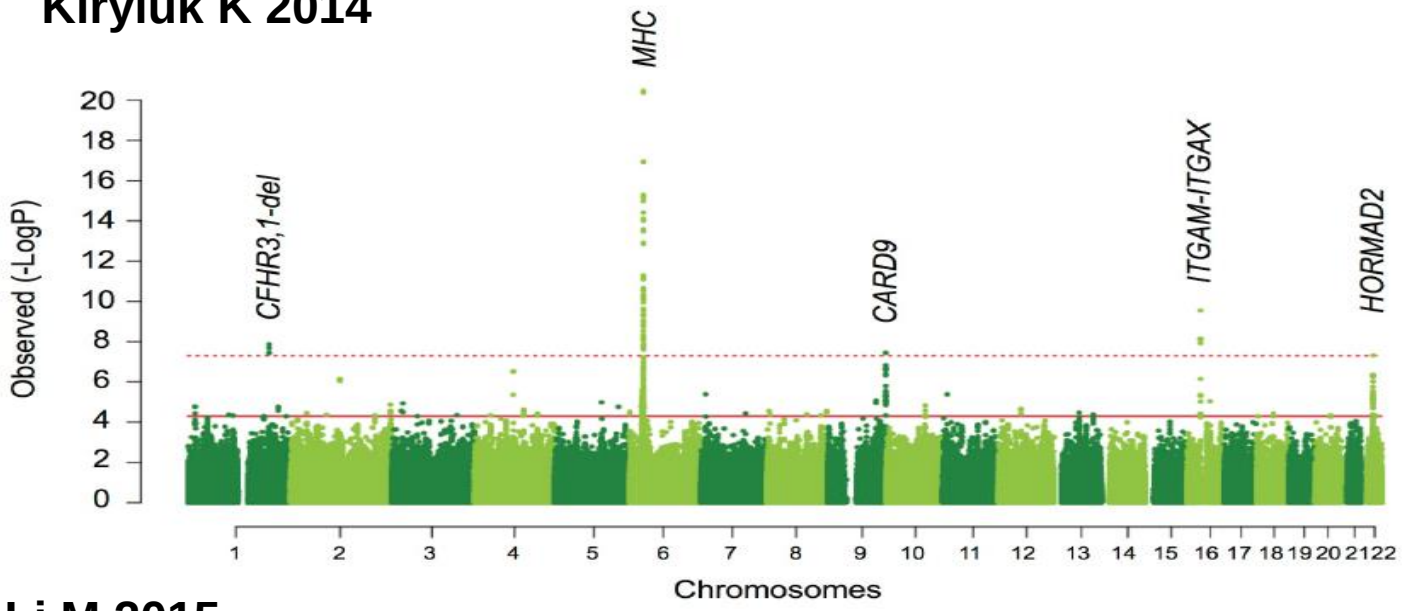
	Gene 1 V341I carriers				*Gene 2* T531M carriers				Non-carriers		
	Mean	**StDev**	**N**	**P**	**Mean**	**StDev**	**N**	**P**	**Mean**	**StDev**	**N**
Age of onset (years)	30.63	10.29	68	0.424	30.91	10.12	44	0.635	31.65	11.24	2541
Serum IgA (g/L)	2.83	1.04	80	0.252	2.82	0.88	51	0.490	3.17	7.17	2924
Glomerular filtration rate (ml/min)	98.26	47.74	70	0.036	81.81	46.69	43	0.445	121.23	792.16	2378
Urinary protein (g/24h)	1.67	1.96	82	0.883	21.02	140.15	53	0.350	14.69	199.72	2931
	Present	**Absent**	**%**	**P**	**Present**	**Absent**	**%**	**P**	**Present**	**Absent**	**%**
Hypertension	27	58	31.76%	0.811	16	41	28.07%	0.884	956	2211	30.19%
Recurrent gross hematuria	21	63	25.00%	0.466	21	36	36.84%	0.239	896	2184	29.09%
Asymptomatic hematuria	32	40	44.44%	0.717	24	21	53.33%	0.129	1067	1486	41.79%
Asymptomatic proteinuria	58	1	98.31%	0.057	31	4	88.57%	0.543	1803	173	91.24%
CKD stage	**N**	**%**		**P**	**N**	**%**		**P**	**N**	**%**	
1	42	60.00%		0.146	16	37.21%		0.681	1120	47.26%	
2	12	17.14%			12	27.91%			568	23.97%	
3	12	17.14%			10	23.26%			420	17.72%	
4	3	4.29%			2	4.65%			135	5.70%	
5	1	1.43%			3	6.98%			127	5.36%	
Clinical pattern											
1	15	35.71%		0.727	9	33.33%		0.969	491	31.06%	
2	23	54.76%			16	59.26%			963	60.91%	
3	4	9.52%			2	7.41%			127	8.03%	
Lee's Grade											
I	1	9.09%		0.608	0	0.00%		0.761	29	6.52%	
II	1	9.09%			2	28.57%			85	19.10%	
III	4	36.36%			3	42.86%			180	40.45%	
IV	5	45.45%			2	28.57%			130	29.21%	
V	0	0.00%			0	0.00%			21	4.72%	

Submitted to Nature Genetics 2015

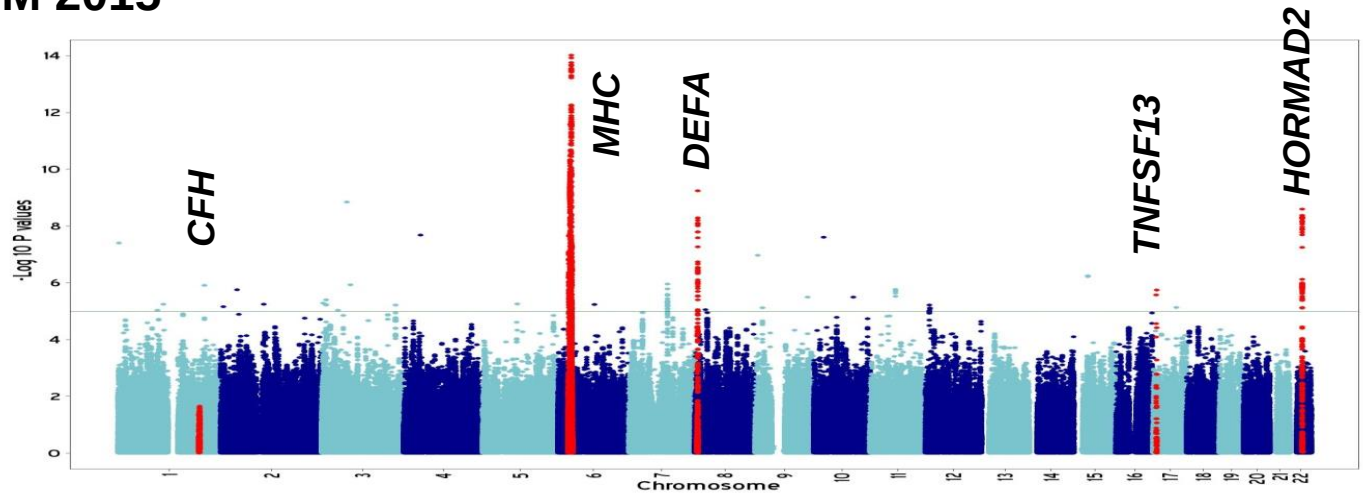
DEFA Copy Number Polymorphism in IgAN

Multiple susceptible loci from GWAS

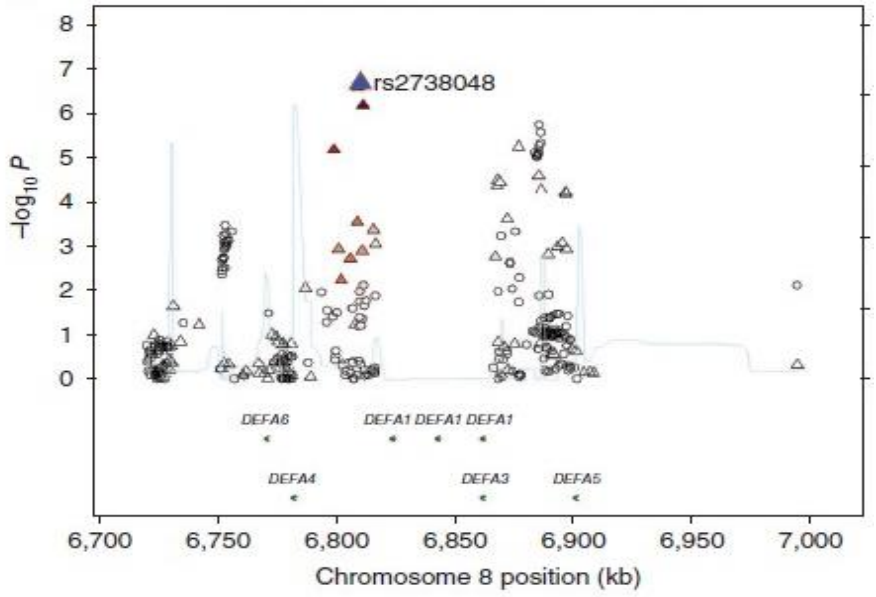
Kiryluk K 2014



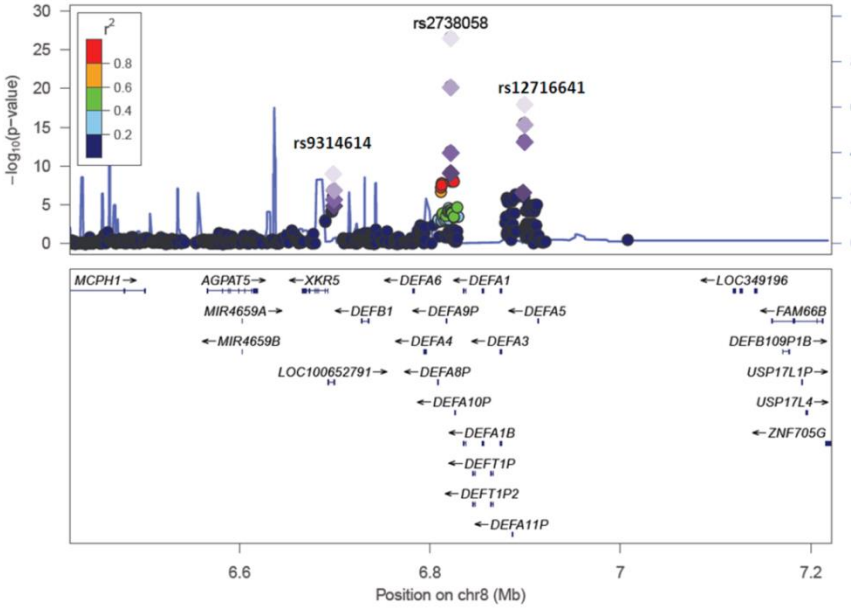
Li M 2015



Regional association plots of susceptible signals in *DEFA* region



Yu XQ*, et al. *Nat Genet*, 2011,12.

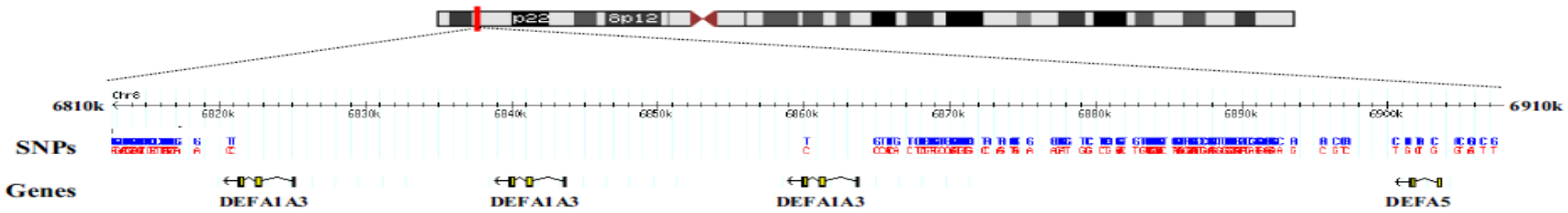


Li M, et al. *Nat Commun*, 2015,6.

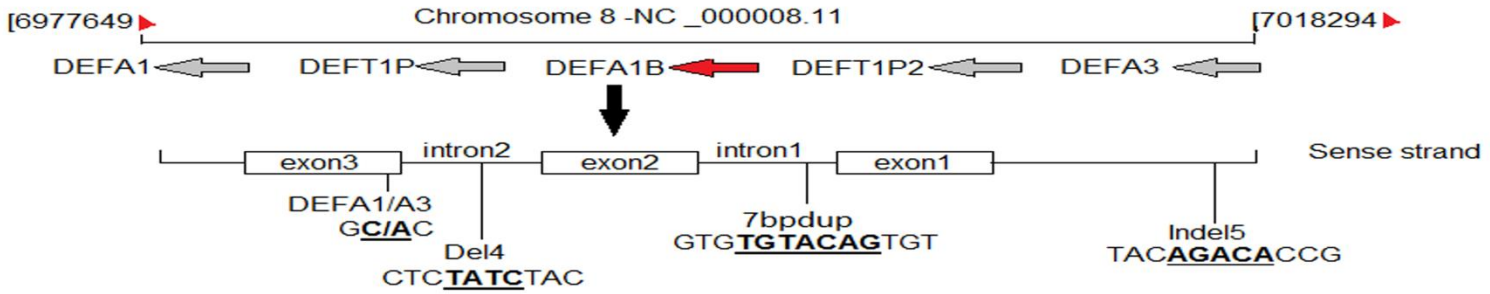
The association of *rs2738048* was first reported by our group, and has already been replicated by other following study. In ancestry-specific analyses, the association of *rs2738048* was evident only in Asian cohorts (P=1.3*10^{-7} in Asians, P=0.58 in Europeans).

DEFA copy number polymorphisms(CNPs)

- The detected association at 8p23.1 is within a single coherent LD block (~100kb) where several members of the *DEFA* family reside, including *DEFA1A3* and *DEFA5*.



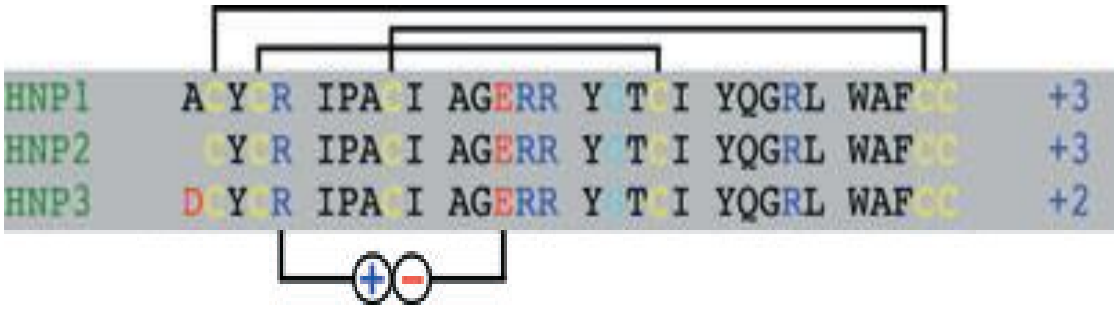
- *DEFA1A3* which near telomere exhibits accelerated rates of duplication and rearrangement, contains all types of structural variations including deletion, duplication, inversion and translocation.



Alpha-defensin (HNP1-3)

- *DEFA1A3* gene directly encoding inactive pre-prodefensins (about 96 amino acides) in promyelocytes (the precursor cells of neutrophils) in bone marrow, where they are packaged into azurophilic granules. They are finally cleaved to give the mature defensins (HNP1-3, 29-30 amino acids long) in mature neutrophils.

- HNP1-3 are very important components of innate immunity. They can act as endogenous antibiotis and involved in the inflammation response to infection , and also act as immune modulators that chemoattract T cells, immature dendritic cells and monocytes, and induce the release of chemokines and cytokines.

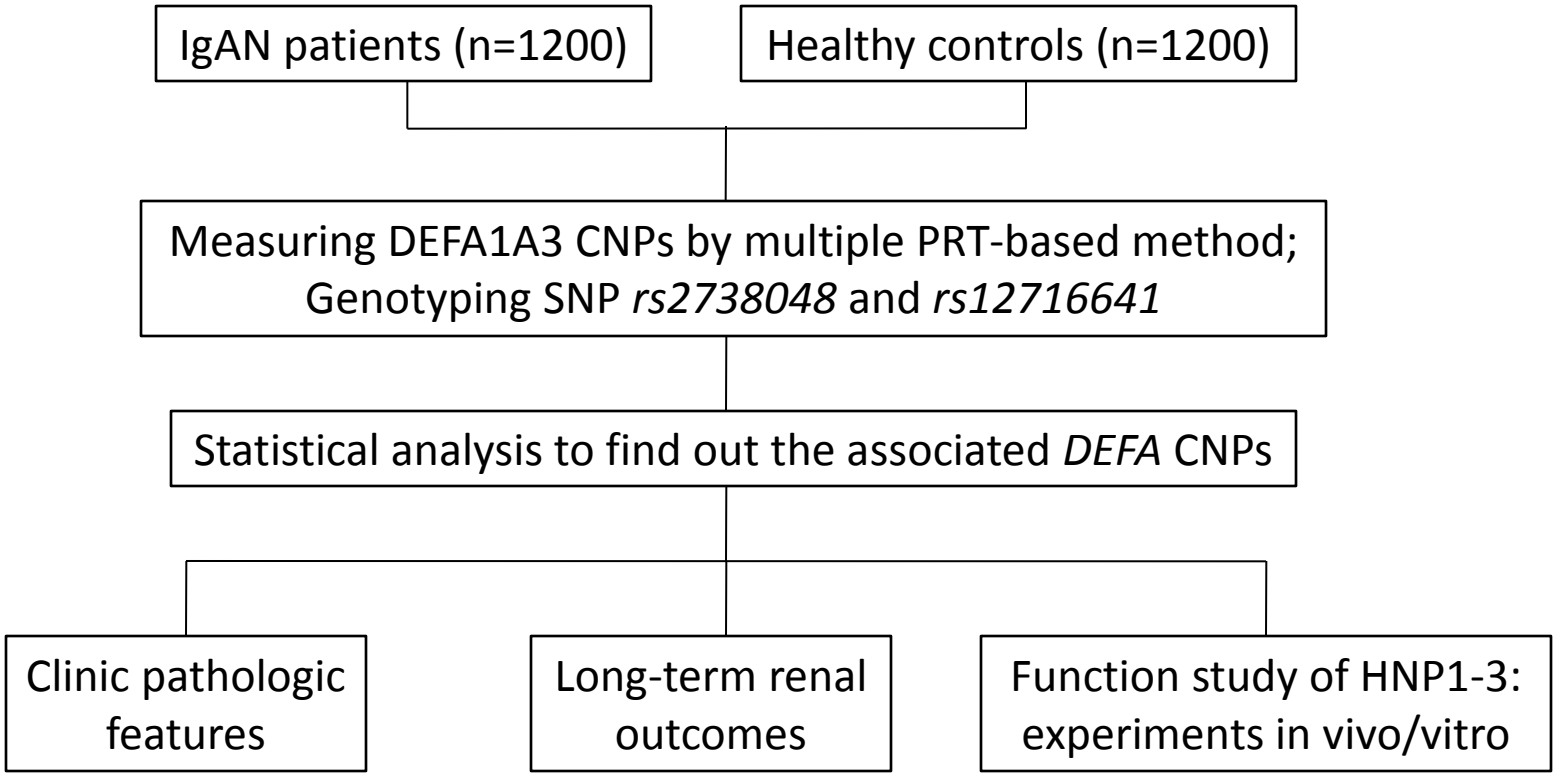


Immunological Reviews 2012 Vol. 245: 84–112

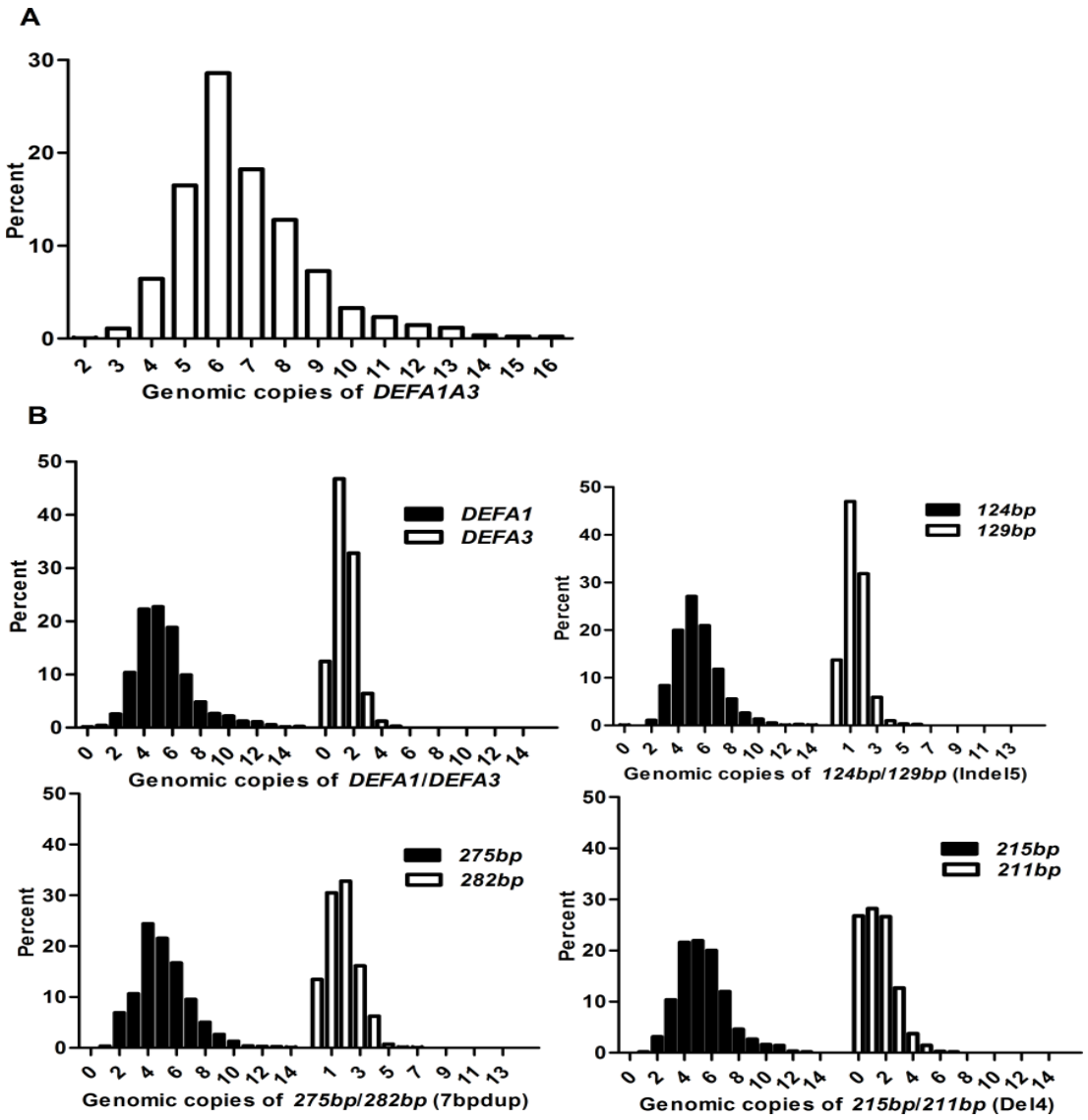
Questions

- Can *DEFA1A3* CNPs explain the previous detected GWAS SNP association?
- Is there any association effect between *DEFA1A3* CNPs and IgAN?
- What's the potential effect of *DEFA1A3* in the pathogenesis of IgA nephropathy?

Study Design



Distributions of *DEFA* CNVs in Southern Chinese (n=2376)



Multiple Associated *DEFA* CNPs

	Cohort I (197cases/199controls)		Cohort II (992cases/988controls)		Meta-analysis (fixed effects)			
Variant	*P*	OR[a](95%CI)	*P*	OR[a](95%CI)	*P*	OR[a](95%CI)	*Q* test	I^2
DEFA1A3	4.06×10^{-2}	0.90(0.82,0.99)	3.06×10^{-8}	0.88(0.84,0.92)	3.99×10^{-9}	0.88(0.84,0.92)	0.62	0
DEFA1	3.22×10^{-1}	0.96(0.87,1.04)	6.72×10^{-5}	0.91(0.87,0.95)	6.71×10^{-5}	0.92(0.88,0.96)	0.36	0
DEFA3	1.89×10^{-2}	0.75(0.59,0.95)	1.11×10^{-3}	0.83(0.75,0.93)	6.55×10^{-5}	0.82(0.76,0.88)	0.42	0
129bp	1.89×10^{-1}	0.85(0.68,1.08)	3.42×10^{-2}	0.89(0.80,0.99)	1.37×10^{-2}	0.88(0.80,0.99)	0.73	0
124bp	1.51×10^{-1}	0.91(0.80,1.03)	5.39×10^{-8}	0.86(0.81,0.91)	2.89×10^{-8}	0.87(0.82,0.91)	0.39	0
282bp	4.00×10^{-1}	0.93(0.79,1.10)	6.28×10^{-1}	0.98(0.90,1.07)	4.11×10^{-1}	0.97(0.74,1.27)	0.60	0
275bp	3.92×10^{-1}	0.96(0.86,1.06)	9.50×10^{-8}	0.87(0.83,0.92)	2.51×10^{-7}	0.89(0.83,0.91)	0.10	62[b]
215bp	9.54×10^{-1}	1.00(0.90,1.10)	2.98×10^{-1}	0.97(0.93,1.02)	3.28×10^{-1}	0.98(0.89,1.08)	0.68	0
211bp	1.09×10^{-3}	0.75(0.63,0.89)	8.50×10^{-14}	0.75(0.69,0.81)	3.50×10^{-16}	0.75(0.70,0.80)	0.99	0
rs2738048	3.15×10^{-2}	0.73(0.54,0.97)	8.88×10^{-3}	0.82(0.71,0.95)	9.58×10^{-4}	0.80(0.70,0.92)	0.45	0
rs12716641	7.37×10^{-2}	0.73(0.53,1.03)	3.85×10^{-5}	0.72(0.61,0.84)	6.99×10^{-6}	0.72(0.62,0.83)	0.89	0

[a]: OR per copy of CNV

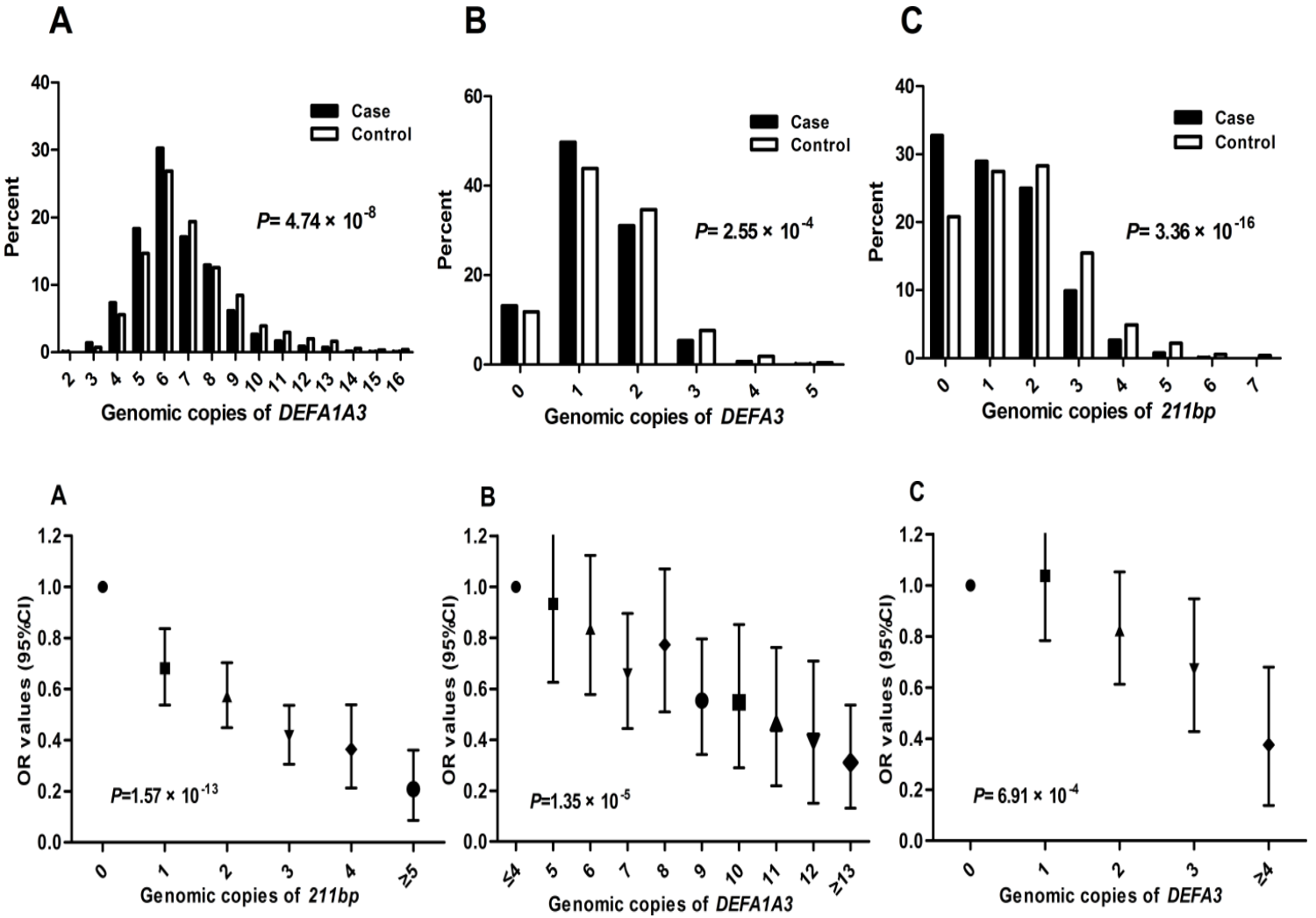
[b]**:**The random-effects meta-analysis p-value for *275bp* is 0.026, OR estimate is 0.902

Three Independently Associated *DEFA* CNPs

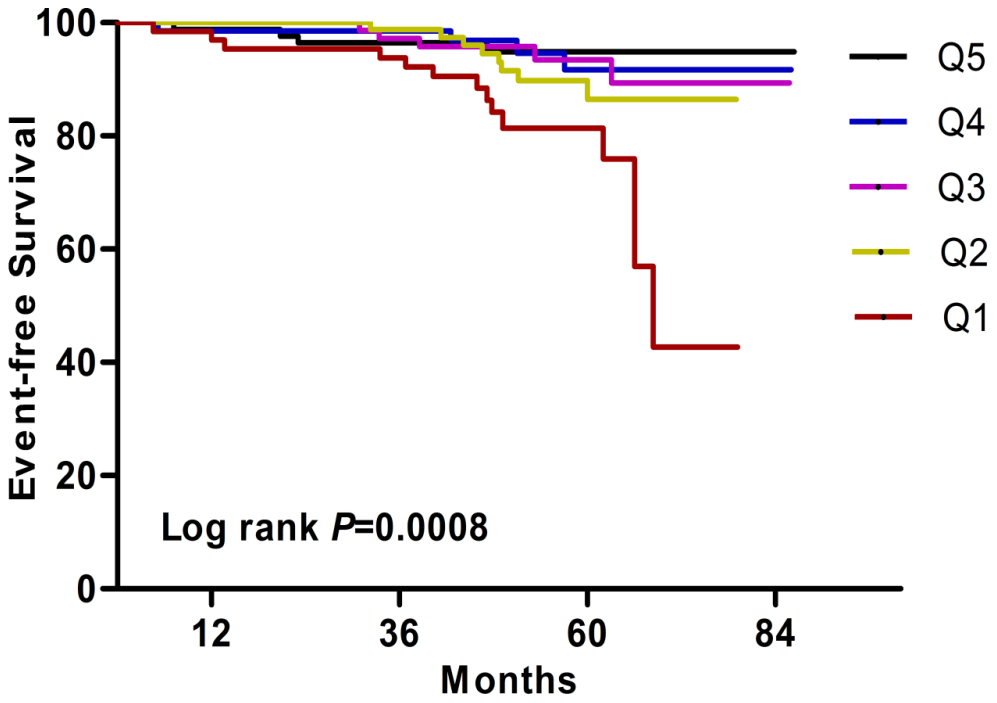
	Unconditional [b]		Condition on *211bp*		Condition on *DEFA1A3 &211bp*	
Variable	*P*	OR (95%CI) [a]	*P*	OR (95%CI) [a]	*P*	OR (95%CI) [a]
DEFA1A3	3.99×10^{-9}	0.88(0.84,0.92)	3.07×10^{-3}	0.93(0.89,0.98)		
DEFA1	6.71×10^{-5}	0.92(0.88,0.96)	NS		NS	
DEFA3	6.55×10^{-5}	0.82(0.76,0.88)	1.35×10^{-3}	0.85(0.77,0.94)	3.14×10^{-3}	0.86(0.78,0.95)
129bp	1.37×10^{-2}	0.88(0.80,0.99)	NS		NS	
124bp	2.89×10^{-8}	0.87(0.82,0.91)	3.07×10^{-2}	0.94(0.89,0.99)	NS	
275bp	2.51×10^{-7}	0.89(0.83,0.91)	NS		NS	
211bp	3.50×10^{-16}	0.75(0.70,0.80)				
rs2738048	9.58×10^{-4}	0.80(0.70,0.92)	NS		NS	
rs12716641	6.99×10^{-6}	0.72(0.62,0.83)	6.18×10-3	0.86(0.78,0.96)		

[a]: OR per copy of CNV
[b]: from the meta-analysis of the two independent cohorts
NS: not significant, $P\geq0.05$.

Distribution of different DEFA subunits CNPs in IgAN



Genetic scores of *DEFA* CNPs associated with renal outcomes



Q5: average GS=9.57;

Q4: average GS=7.22;

Q3: average GS=5.95;

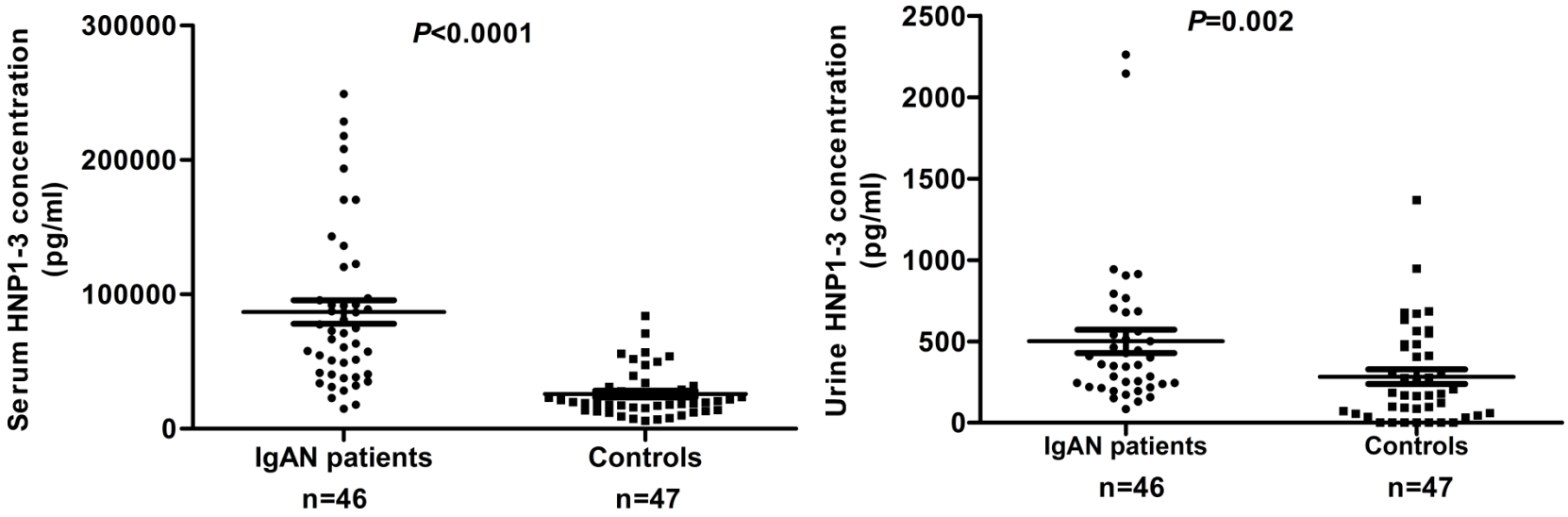
Q2: average GS=4.52;

Q1: average GS=3.06.

Questions

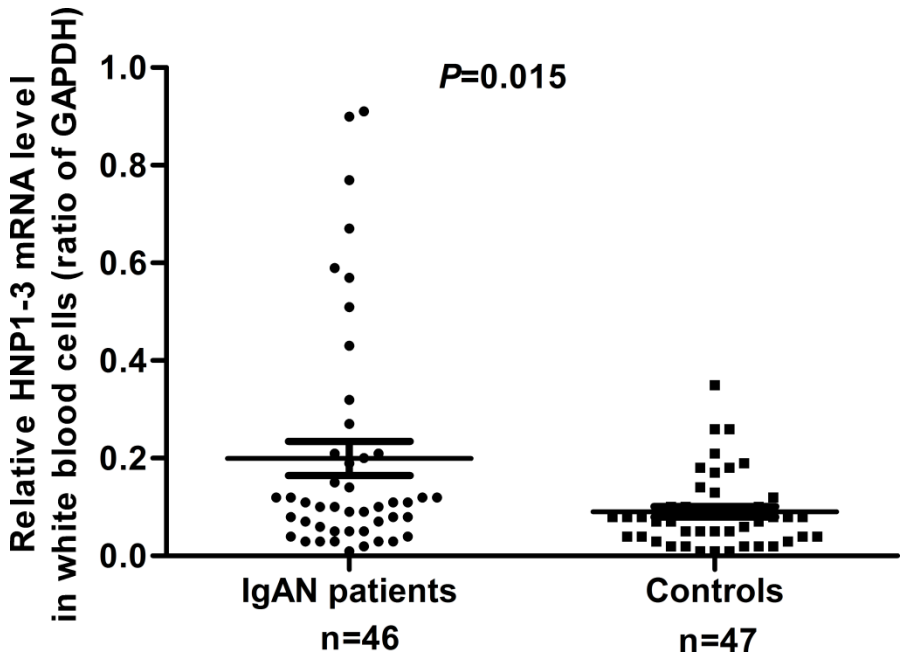
- Can *DEFA1A3* CNPs explain the previous detected GWAS SNP association?
- Is there any association effect between *DEFA1A3* CNPs and IgAN?
- What's the potential effect of *DEFA1A3* in the pathogenesis of IgA nephropathy?

Serum/Urine level of HNP1-3 in IgA Patients



Serum and urine level of HNP1-3 are both significantly increased in IgAN patients

HNP1-3 mRNA level of WBC in IgAN Patients



HNP1-3 mRNA is up-regulated in IgAN patients

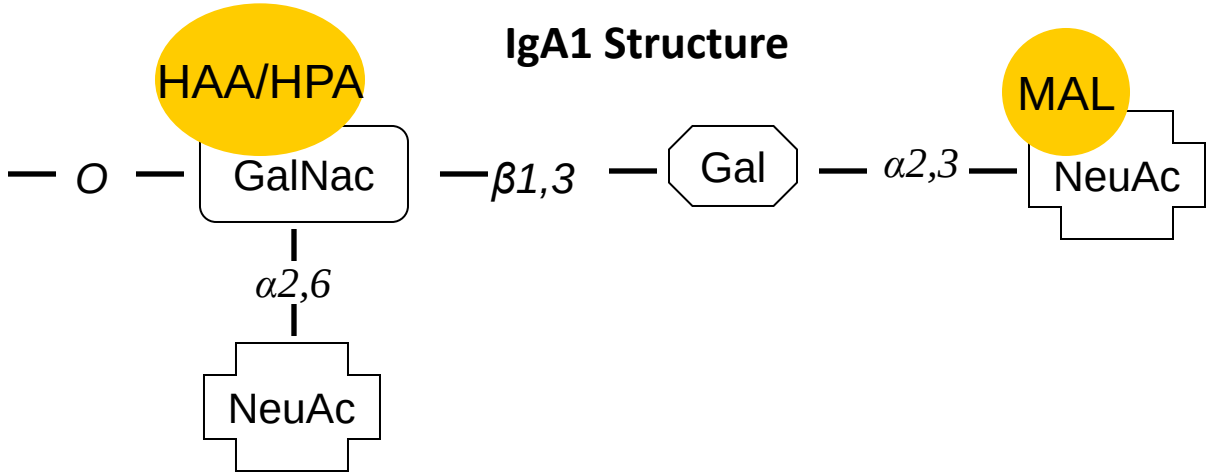
Correlations between CNs and expression of HNP1-3

	Total			Cases			Controls		
	Serum HNP1-3	Urine HNP1-3	HNP1-3 mRNA	Serum HNP1-3	Urine HNP1-3	HNP1-3 mRNA	Serum HNP1-3	Urine HNP1-3	HNP1-3 mRNA
DEFA1A3	-0.10	-0.19*	0.03	-0.11	-0.18	0.09	0.06	-0.20	-0.21
DEFA1	-0.05	-0.22**	0.08	-0.09	-0.27**	0.11	0.09	-0.20	-0.11
DEFA3	-0.10	0.07	-0.16	0.02	0.24*	-0.12	-0.11	0.01	-0.22
215bp	-0.10	-0.16	0.06	-0.07	-0.23*	0.11	-0.05	-0.06	-0.21
211bp	-0.00	--0.07	-0.05	-0.06	0.06	-0.03	0.26*	-0.25	-0.02

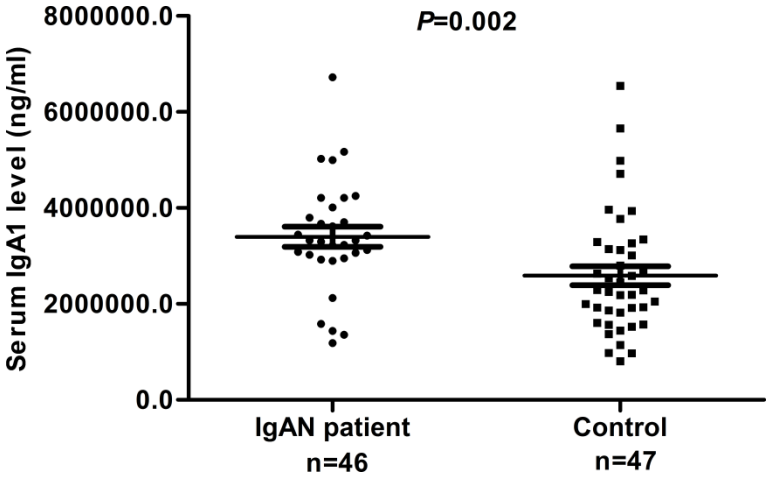
*: $p<0.05$; **: $p<0.01$

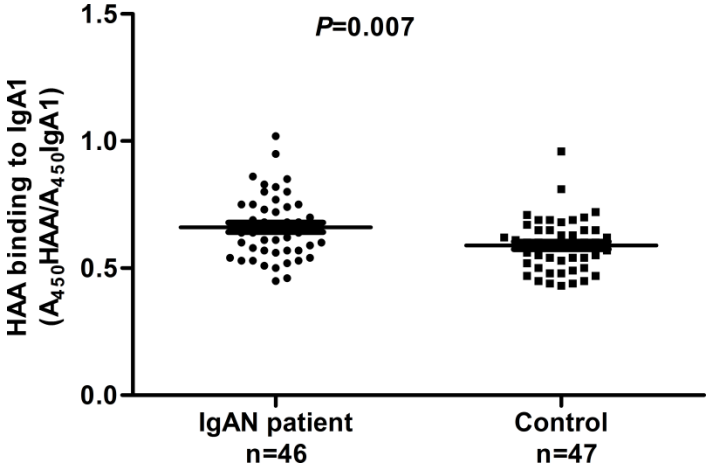
Correlation between *DEFA1A3* CNVs and pathogenic IgA1

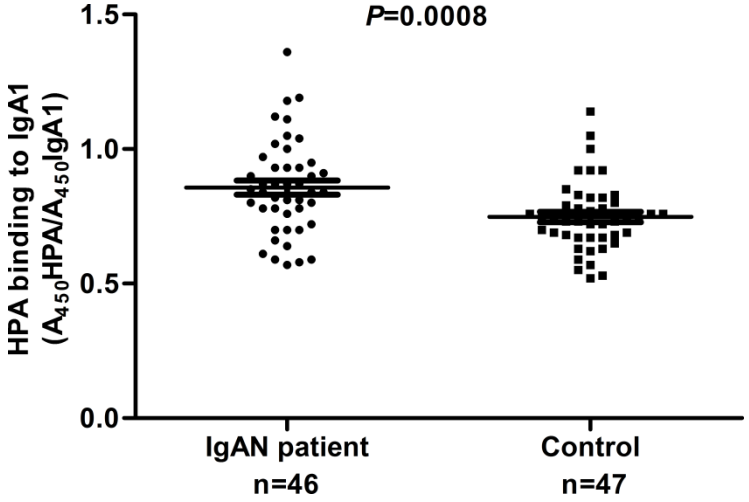
- Pathogenic IgA1 :
- galactose-deficient IgA1,**HAA/HPA** is binding to GalNac. Serum IgA1 of patients have **more** galactose deficient IgA1 (HAA/HPA-binding IgA1);
- **a2,6**- sialylated O-glycans (NeuAc attached to GalNAc, prevent subsequent Gal attachment). **MAL** is binding to **a2,3**-sialylated O-glycans (NeuAc attached to Gal). Serum IgA1 of patients have **less** a2,3-sialylated O-glycans (MAL-binding IgA1).

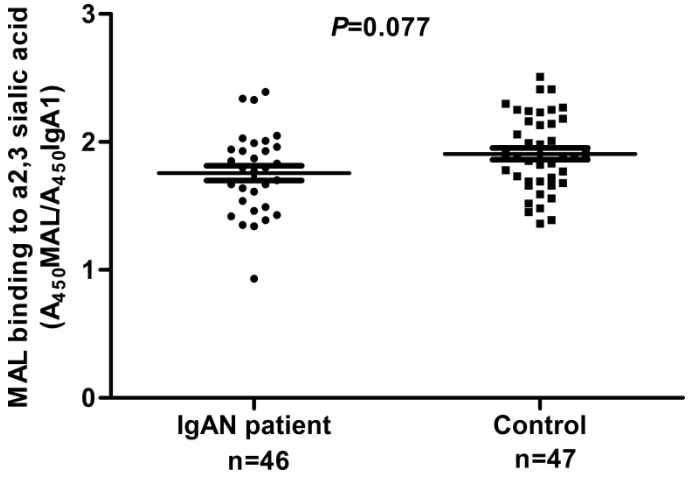


Serum IgA1, galactose-deficient IgA1 and a2,3-sialylated IgA1

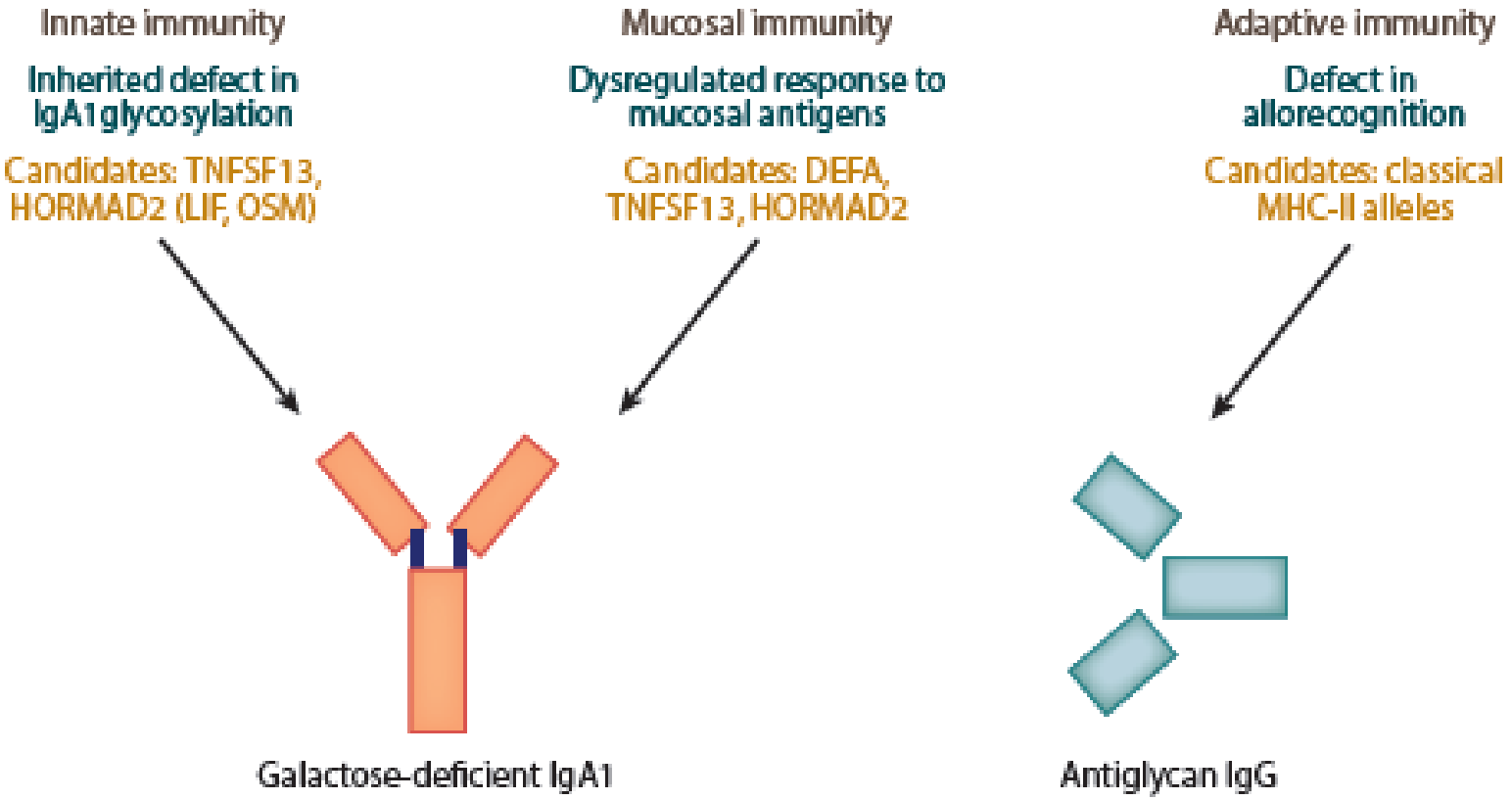








The protective effect of *211bp* and *DEFA3* in IgAN may through down-regulating the pathogenic IgA1



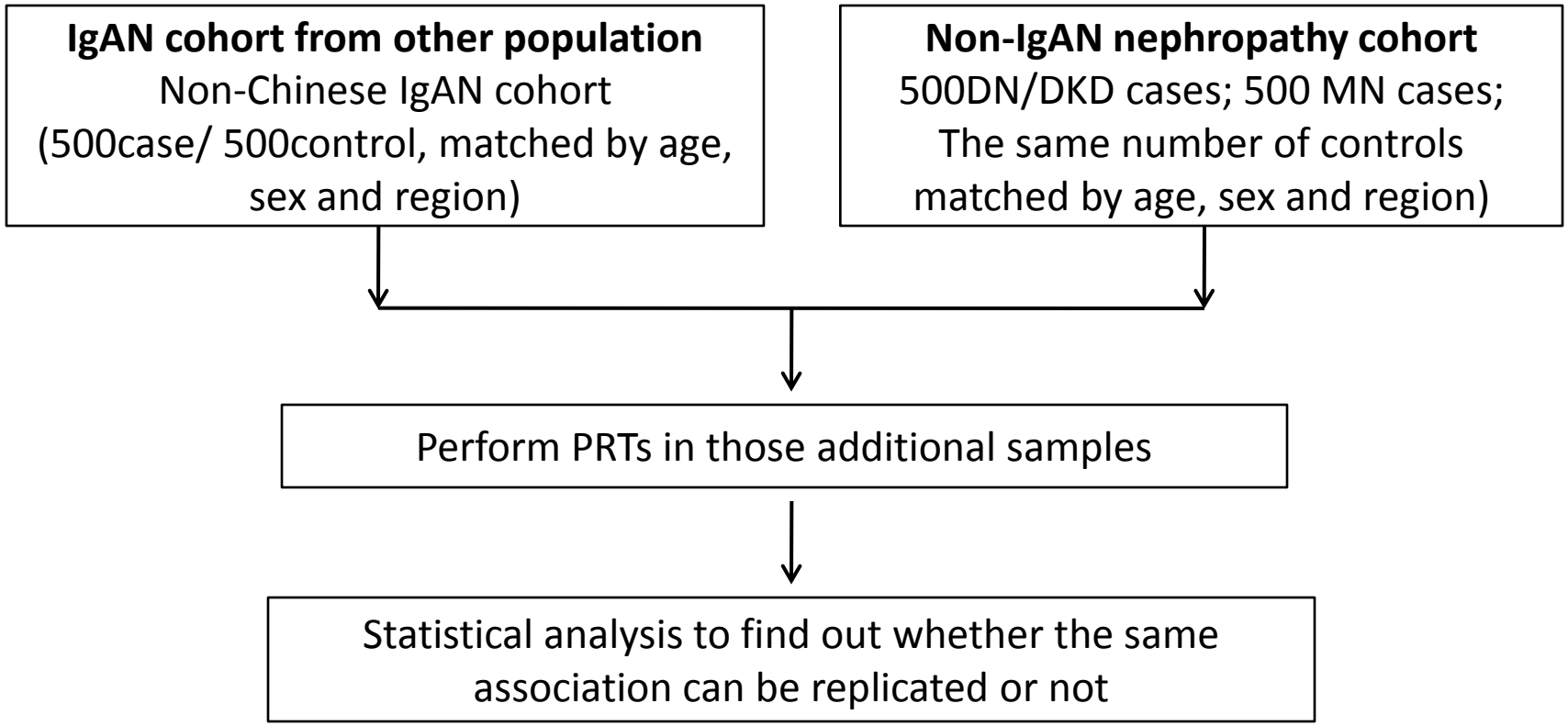
Annu. Rev. Med. 2013. 64:23.1–23.18

Questions

- Can *DEFA1A3* CNPs explain the previous detected GWAS SNP association?
- Is there any association effect between *DEFA1A3* CNPs and IgAN?
- What's the potential effect of *DEFA1A3* in the pathogenesis of IgA nephropathy?

DEFA CNV is IgAN Specific or Not?

Validation in other kidney disease population non-IgAN nephropathy cohort



DN/DKD cohort

Origin	Cases			Controls		
	Sample size	Mean age	M/F (%)	Sample size	Mean age	M/F (%)
Southern Chinese	331	57.83	67.2/32.8	329	57.51	68.7/31.3
Singaporean Chinese	475	60.53	58.7/41.3	457	58.06	58.4/41.6
Total	806	59.43	62.2/37.8	786	57.83	62.7/37.3

CN Distributions in DKD cases and controls

Gene/ alleles	**Cases** (n=806)		**Control** (n=786)		*P* value
	Median	Interquartile range	Mean	Interquartile range	
DEFA1A3	7.00	(6.00,8.00)	7.00	(6.00,8.00)	0.952
DEFA1	6.00	(4.00,7.00)	6.00	(4.00,7.00)	0.690
DEFA3	1.00	(1.00,2.00)	2.00	(1.00,2.00)	0.991
215bp	6.00	(5.00,7.00)	6.00	(5.00,7.00)	0.612
211bp	1.00	(0.00,2.00)	1.00	(0.00,2.00)	0.222

Association analysis in DKD adjusted by age and sex

Variants	DKD cohort (806 cases/786 controls)	
	P	OR[a](95%CI)
DEFA1A3	0.448	0.98(0.94,1.03)
DEFA1	0.487	0.99(0.94,1.03)
DEFA3	0.778	0.99(0.89,1.09)
215bp	0.884	1.00(0.96,1.05)
211bp	0.158	0.94(0.87,1.02)

MN cohort

Origin	Cases			Controls		
	Sample size	Mean age	M/F(%)	Sample size	Mean age	M/F(%)
Southern Chinese	493	45.97	53.3/46.7	500	45.67	53.2/46.8

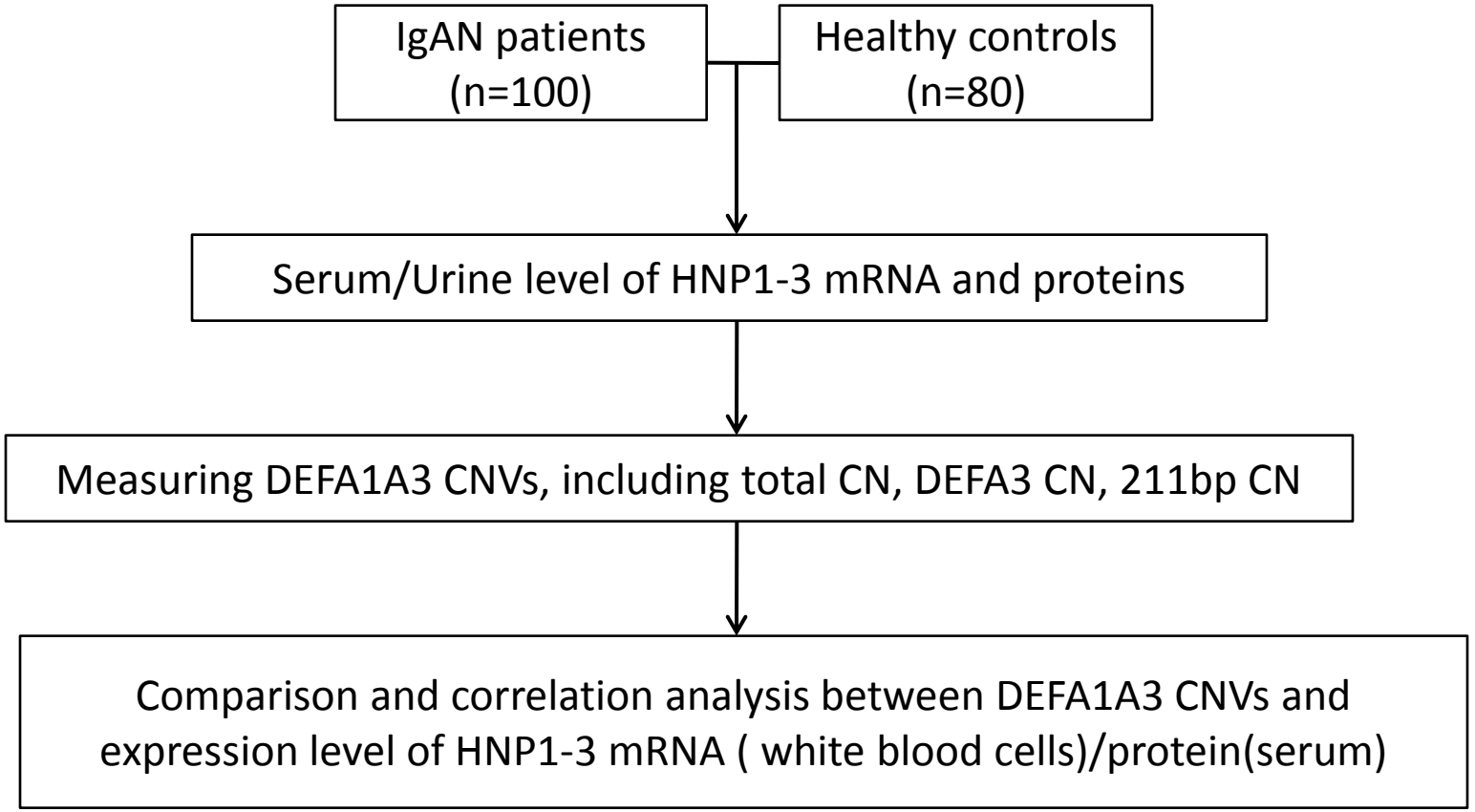
CN Distribution in MN cases and controls

Gene/ alleles	**Cases**		**Control**		*P* value
	Mean	Interquartile range	Mean	Interquartile range	
DEFA1A3	7.00	(6.00,8.00)	7.00	(6.00,8.00)	3.41×10^{-1}
DEFA1	5.00	(4.00,7.00)	5.00	(4.00,7.00)	6.09×10^{-1}
DEFA3	2.00	(1.00,2.00)	1.00	(1.00,2.00)	1.00×10^{-2}
215bp	6.00	(5.00,7.00)	5.00	(4.00,6.00)	1.03×10^{-5}
211bp	1.00	(0.00,2.00)	2.00	(1.00,2.00)	2.82×10^{-7}

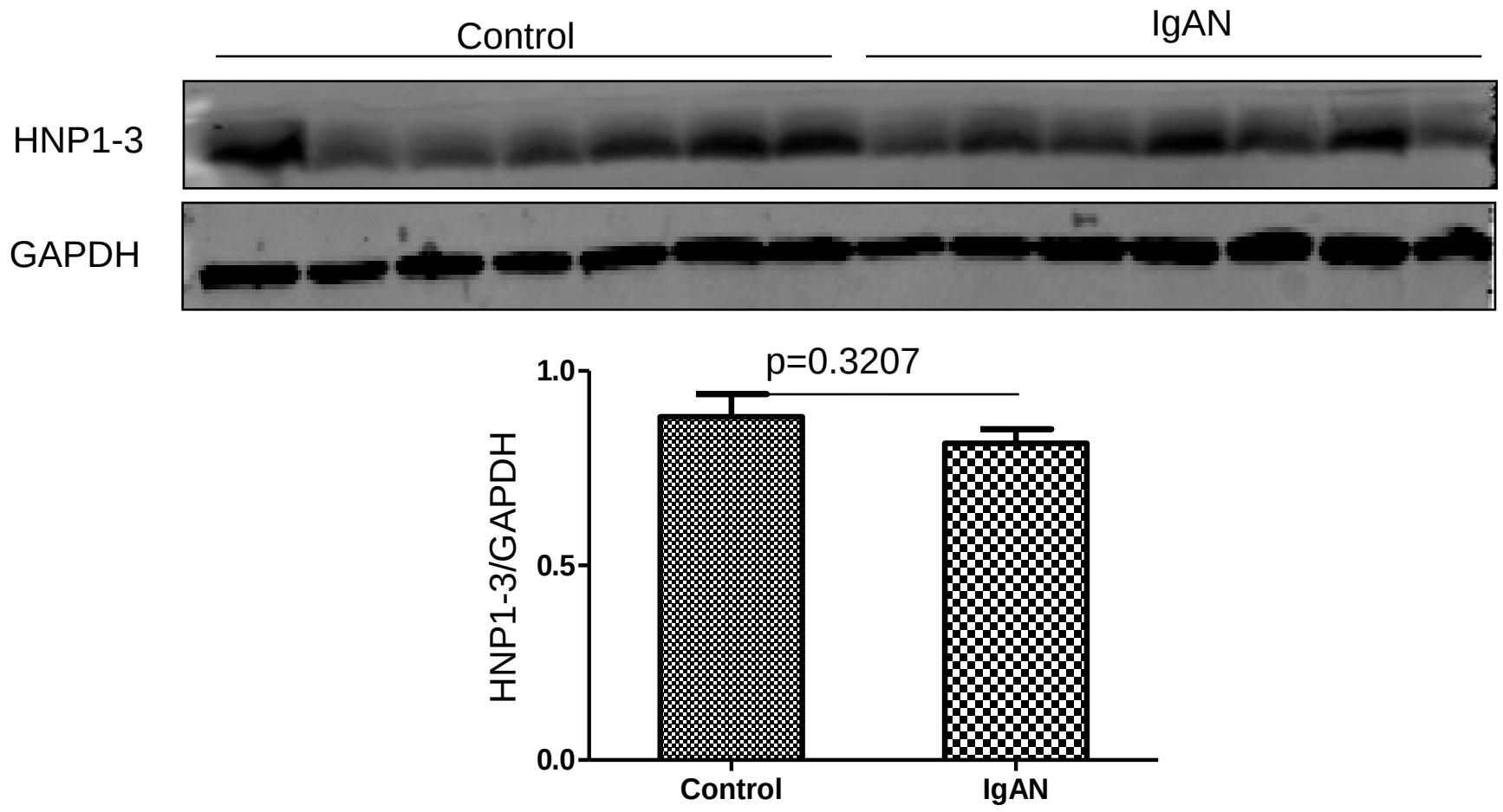
Association analysis in MN adjusted by age and sex

Variants	MN cohort (493 cases/ 500 controls)	
	P	OR[a](95%CI)
DEFA1A3	8.76×10^{-1}	1.00(0.94,1.07)
DEFA1	5.93×10^{-1}	0.98(0.93,1.04)
DEFA3	6.50×10^{-2}	1.16(0.99,1.35)
215bp	6.63×10^{-4}	1.12(1.05,1.20)
211bp	1.11×10^{-7}	0.74(0.67,0.83)

Function study of DEFA1A3 CNVs

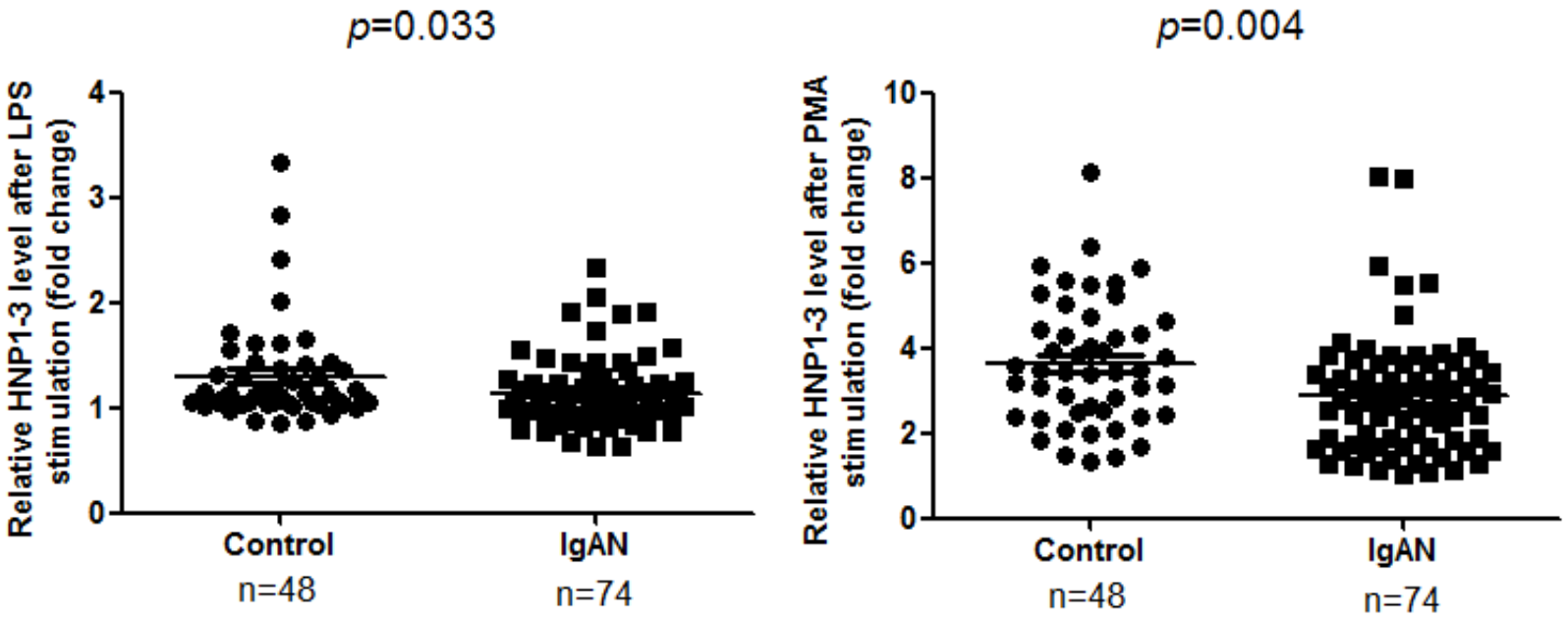


HNP1-3 expression level in neutrophils isolated from IgAN patients and controls



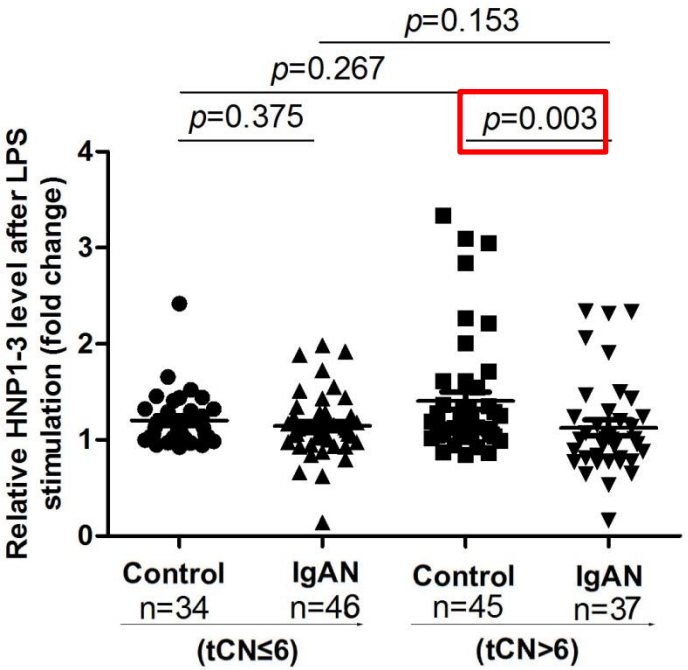
Western Blot: Total HNP1-3 expression showed no difference in neutrophils isolated from IgAN patients and controls.

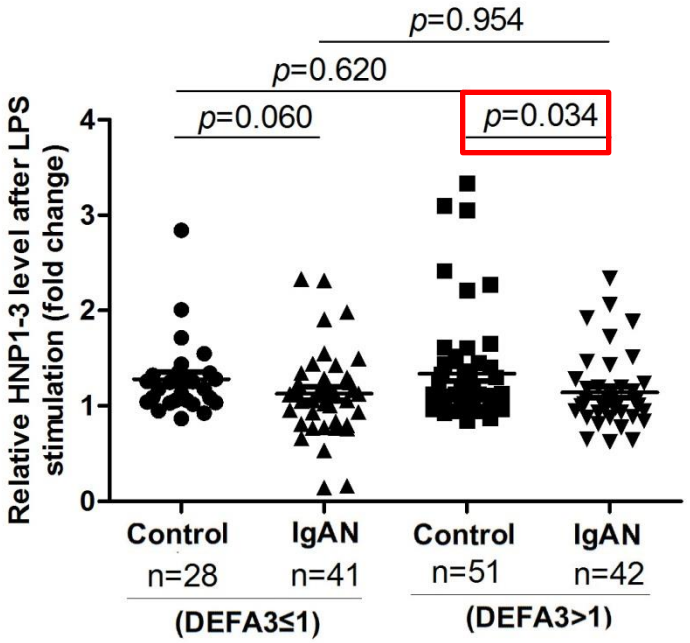
HNP1-3 secretion by neutrophils after stimulated by LPS or PMA

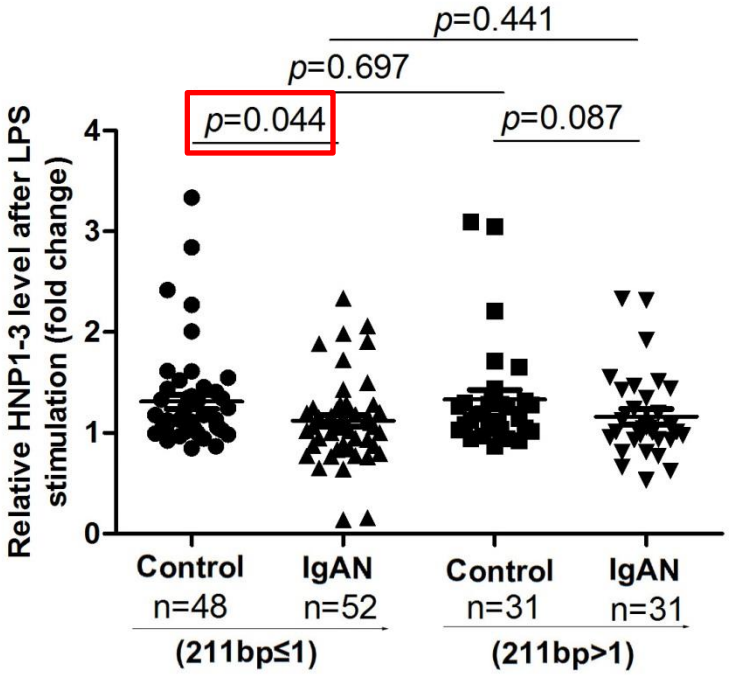


ELISA: After stimulation of LPS (100ng/ml, 6h) or PMA (20ng/ml, 6h), the extracellular HNP1-3 levels are significantly lower in neurophils isolated from IgAN patients than controls.

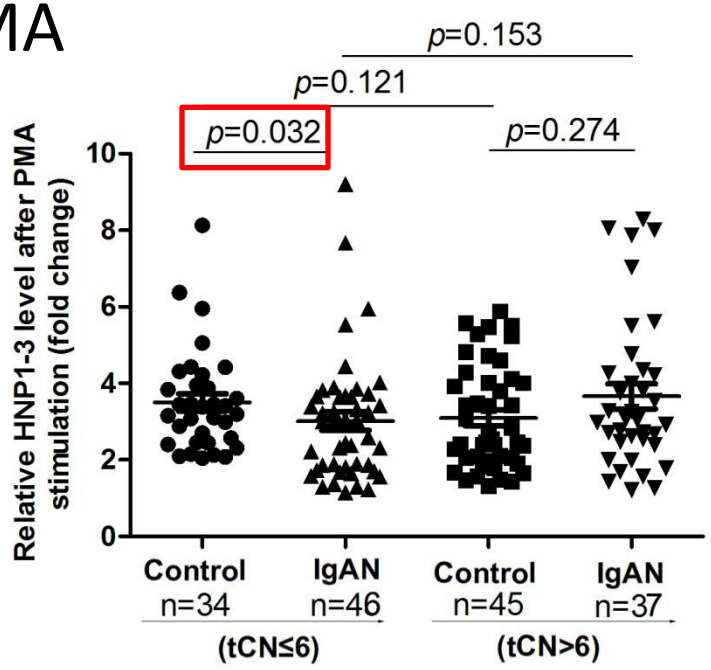
LPS

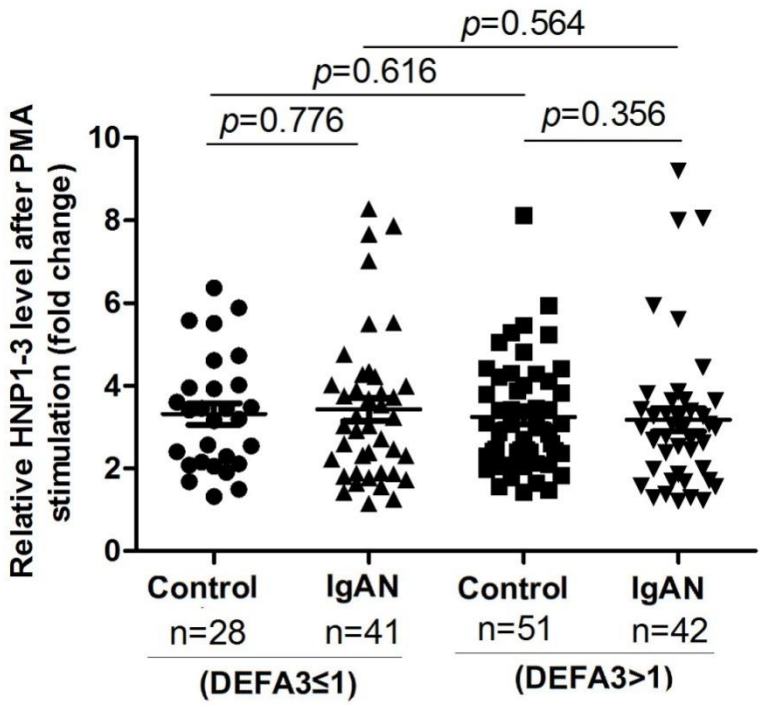

p=0.153
p=0.267
p=0.375
p=0.003
Relative HNP1-3 level after LPS stimulation (fold change)
0
1
2
3
4
Control
n=34
IgAN
n=46
Control
n=45
IgAN
n=37
(tCN≤6)
(tCN>6)

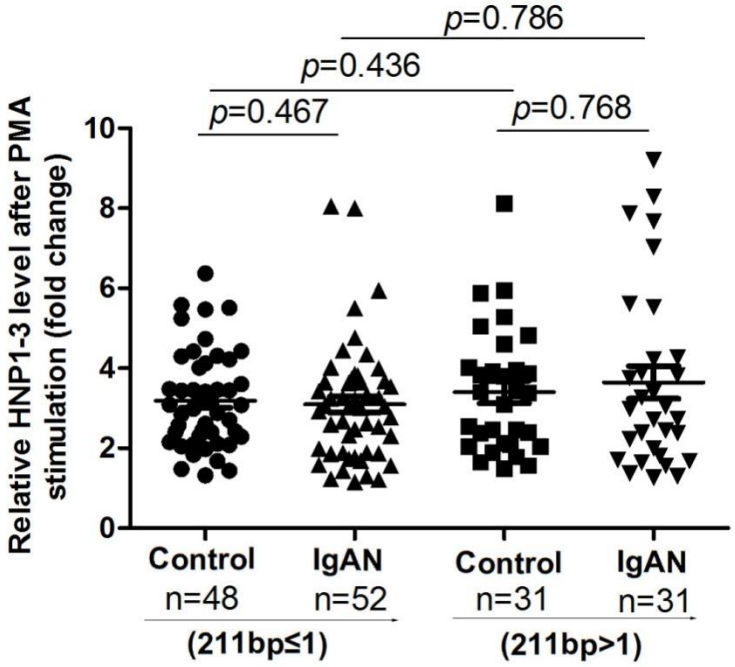

p=0.954
p=0.620
p=0.060
p=0.034
Relative HNP1-3 level after LPS stimulation (fold change)
0
1
2
3
4
Control
n=28
IgAN
n=41
Control
n=51
IgAN
n=42
(DEFA3≤1)
(DEFA3>1)


p=0.441
p=0.697
p=0.044
p=0.087
Relative HNP1-3 level after LPS stimulation (fold change)
0
1
2
3
4
Control
n=48
IgAN
n=52
Control
n=31
IgAN
n=31
(211bp≤1)
(211bp>1)

PMA


p=0.153
p=0.121
p=0.032
p=0.274
Relative HNP1-3 level after PMA stimulation (fold change)
0
2
4
6
8
10
Control
n=34
IgAN
n=46
Control
n=45
IgAN
n=37
(tCN≤6)
(tCN>6)


p=0.564
p=0.616
p=0.776
p=0.356
Relative HNP1-3 level after PMA stimulation (fold change)
0
2
4
6
8
10
Control
n=28
IgAN
n=41
Control
n=51
IgAN
n=42
(DEFA3≤1)
(DEFA3>1)

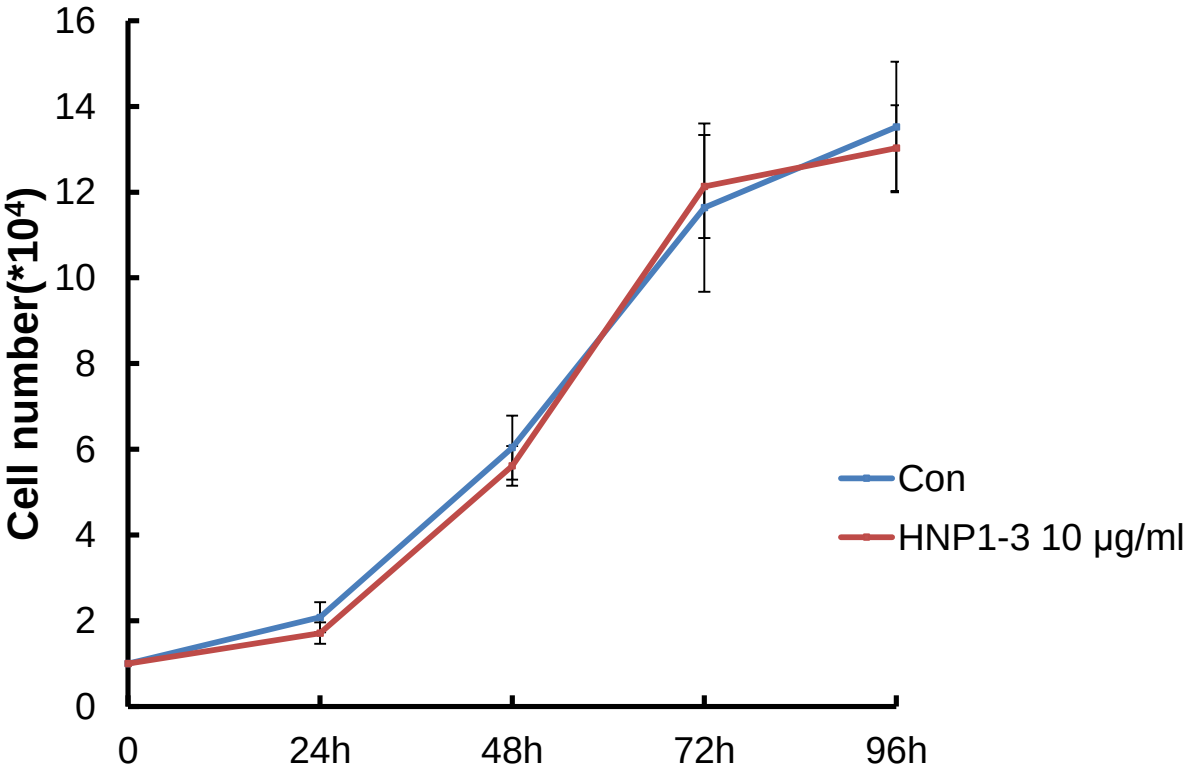

p=0.786
p=0.436
p=0.467
p=0.768
Relative HNP1-3 level after PMA stimulation (fold change)
0
2
4
6
8
10
Control
n=48
IgAN
n=52
Control
n=31
IgAN
n=31
(211bp≤1)
(211bp>1)

Correlation between CNs and HNP1-3 secreted from neutrophils

IgAN	CN_TOTAL	CN_A1	CN_A3	CN_211	CN_215
LPS stimulated	-0.107	-0.069	-0.038	-0.105	0.028
PMA stimulated	0.165	0.171	-0.087	0.052	0.127
High CNV (LPS)	0.327 *	0.280	-0.081	0.039	0.328*
High CNV (PMA)	0.266	0.308	-0.231	0.161	0.157
Low CNV (LPS)	-0.196	-0.035	-0.054	-0.116	0.016
Low CNV (PMA)	-0.125	-0.095	0.053	-0.105	0.032

*p<0.05

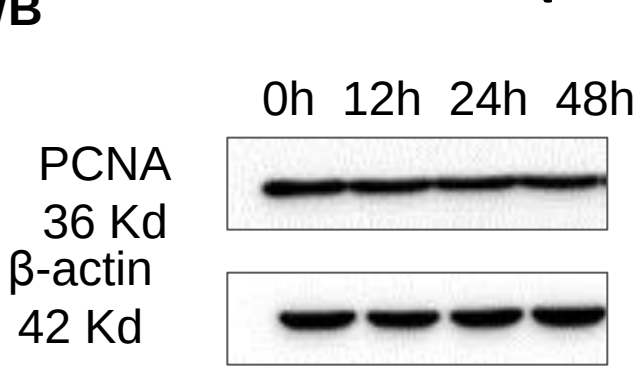
The Effect of HNPs on Cell Proliferation (time-dependent)

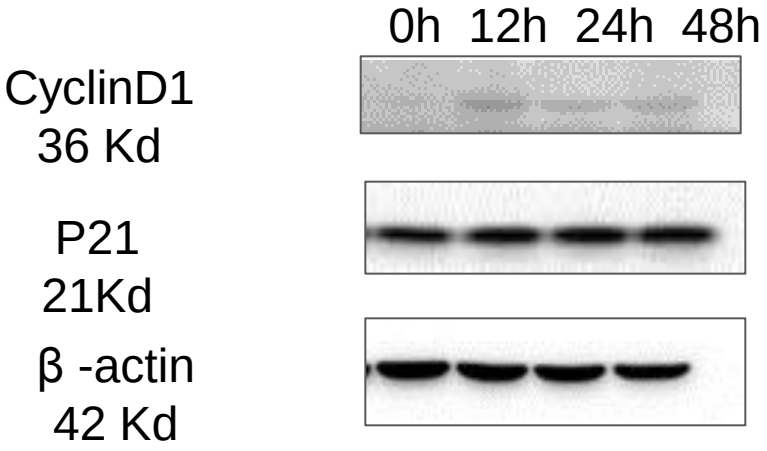


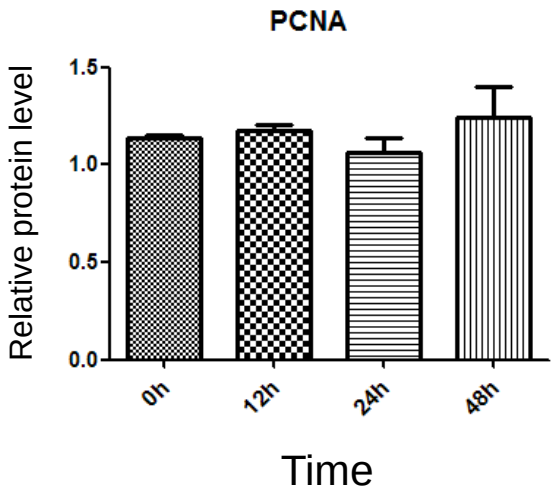
HNP1-3 10 μg/mL treated HMC for 0,24,48h,72h,96h in 48-well plates.
No significant differences.

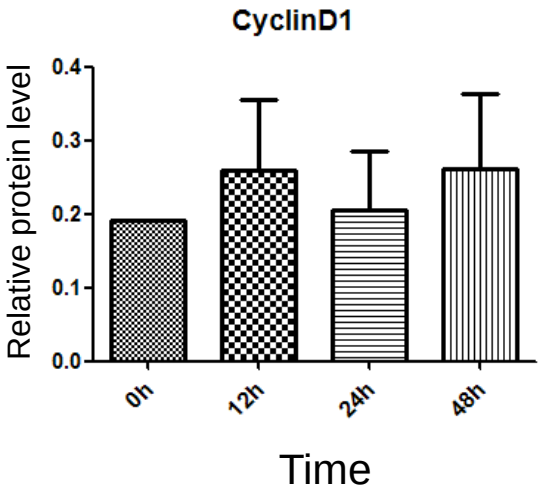
The Effect of HNPs on Cell Proliferation (time-dependent)

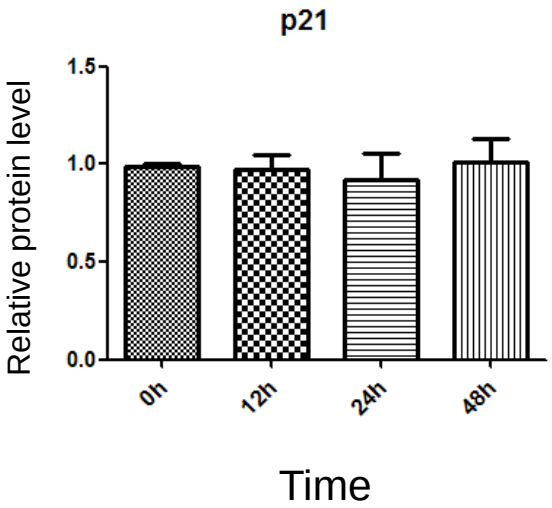
WB







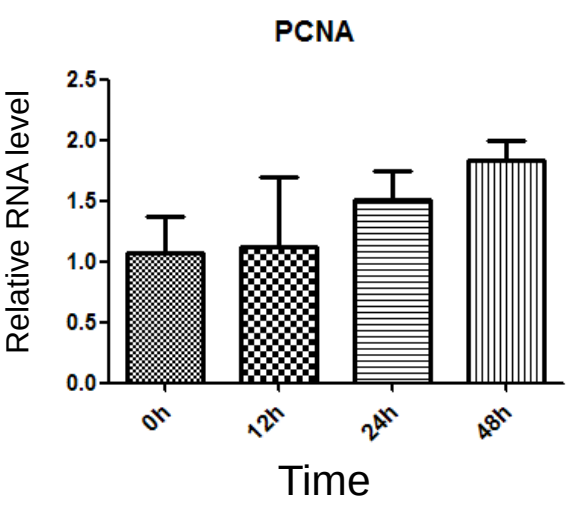


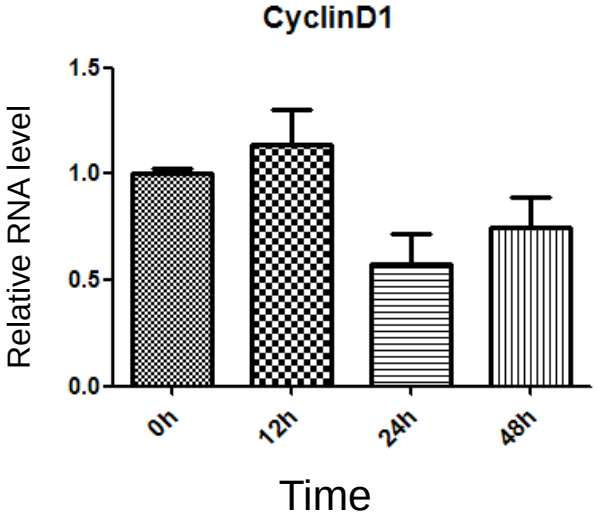


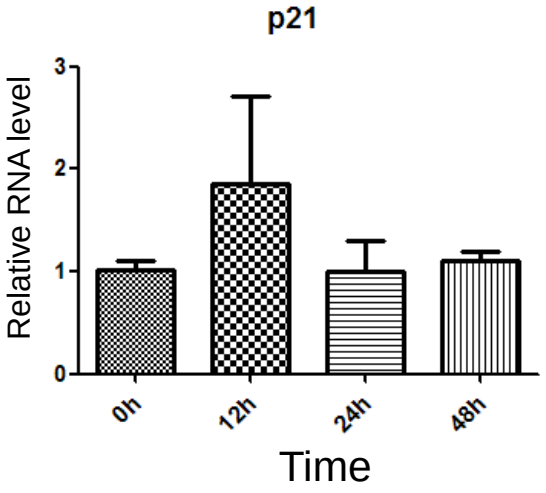
HNP1-3 10 μg/mL treated HMC for 0,12,24,48h, No significant differences.

The Effect of HNPs on Cell Proliferation (time-dependent)

QPCR







PCNA

CyclinD1

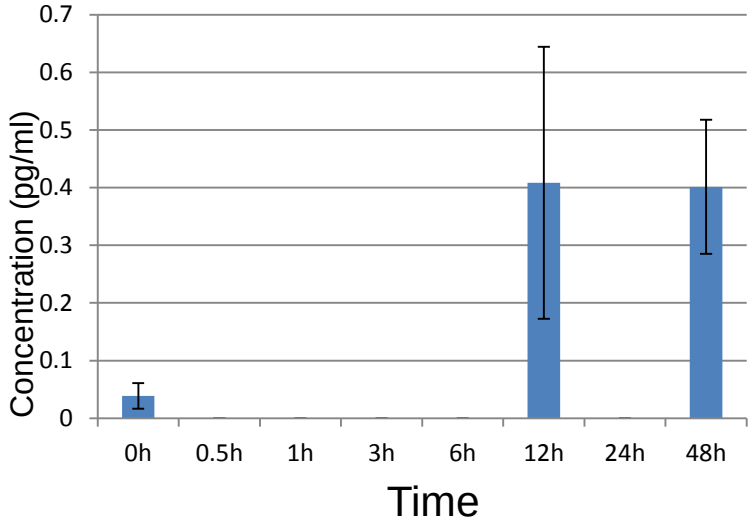
p21

HNP1-3 10 μg/mL treated HMC for 0,12,24,48h
No significant differences.

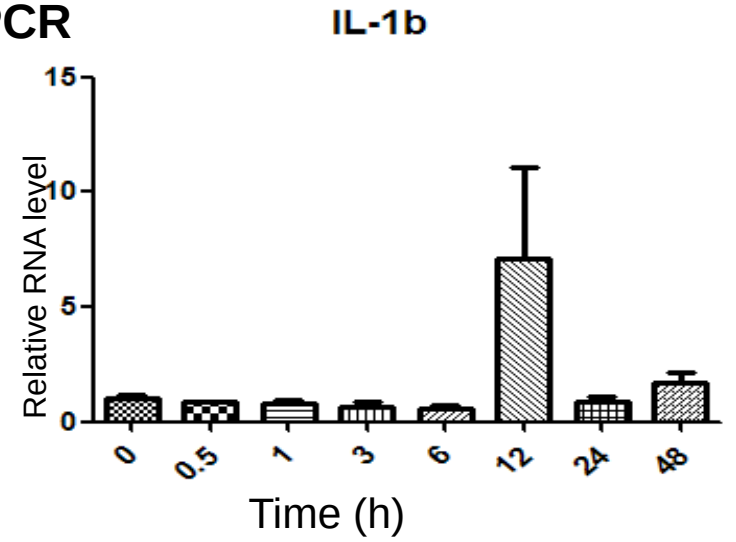
The Effect of HNP1-3 on Inflammatory Cytokines Expression

Elisa

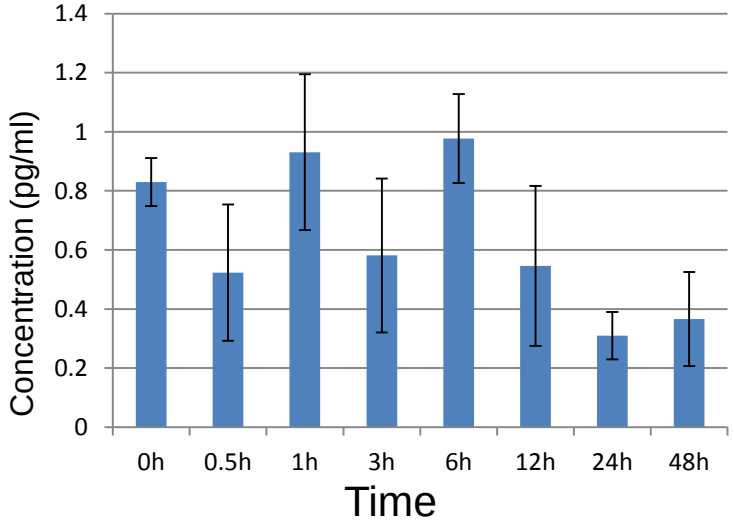
IL-1 β

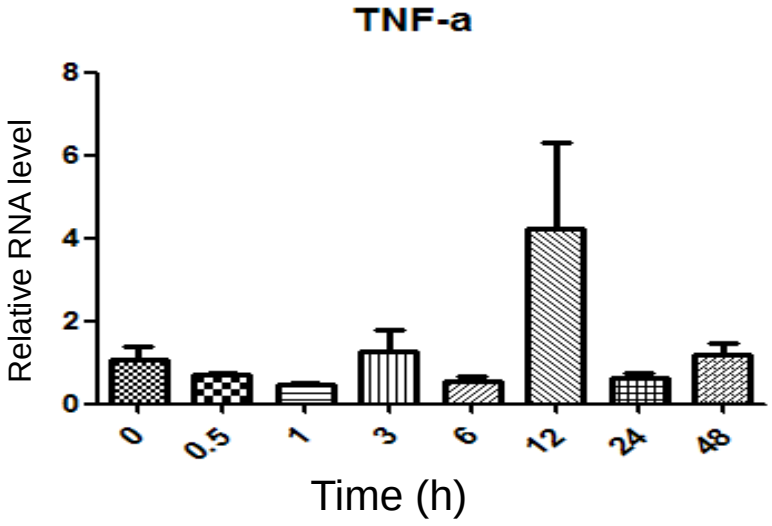


QPCR

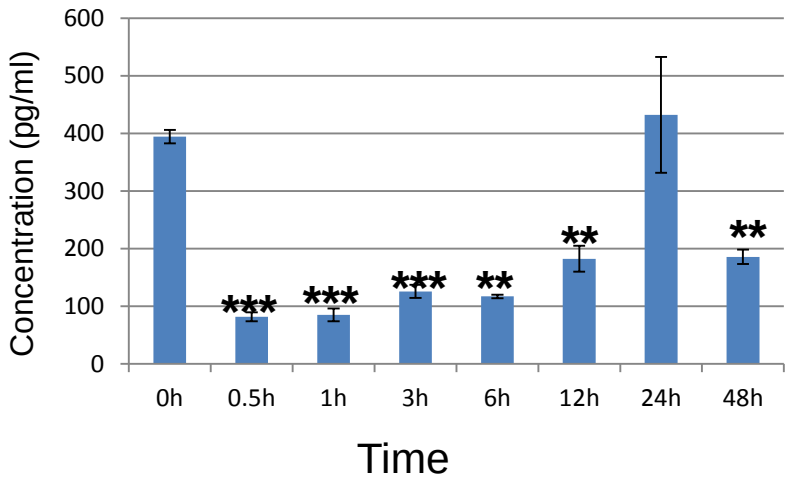


TNF-α

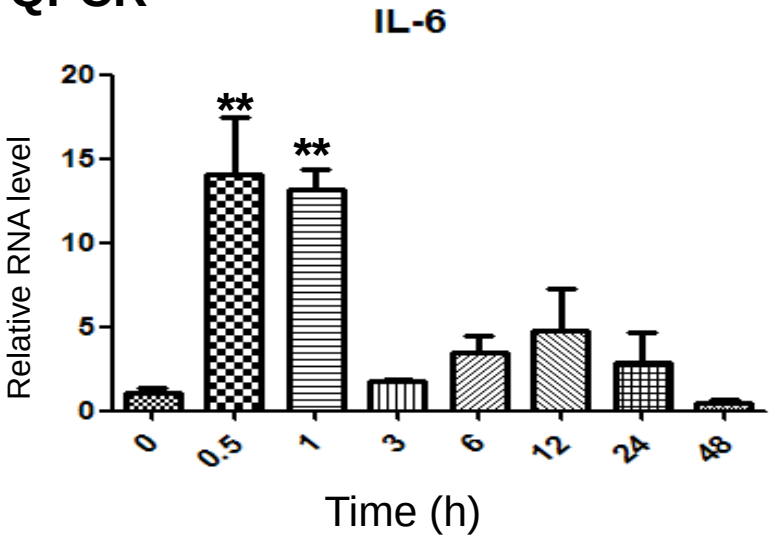




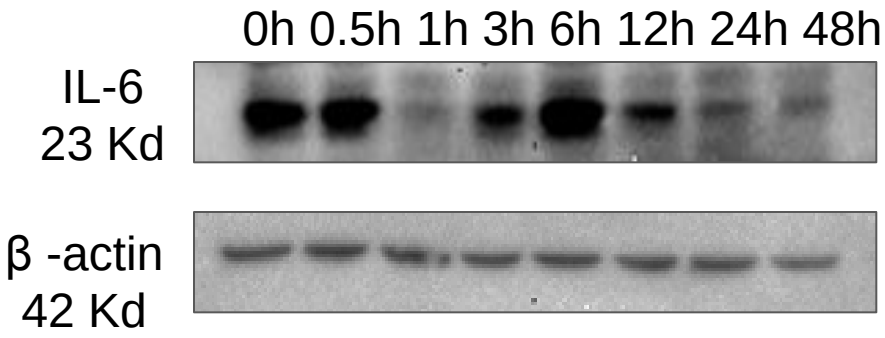
Elisa **IL-6 (cell supernatant)**

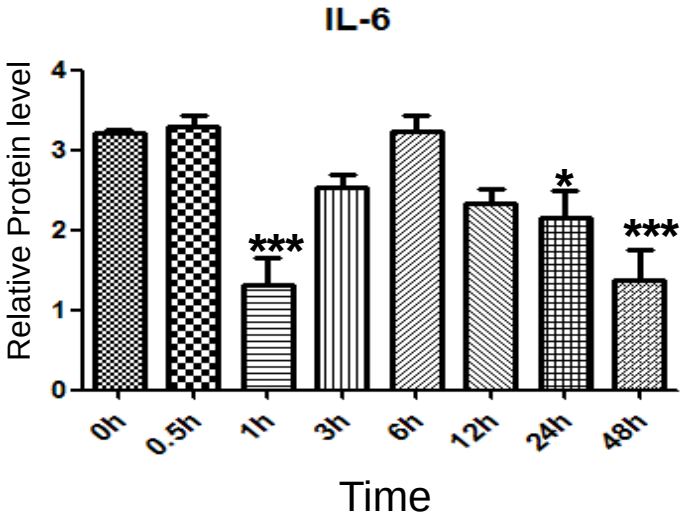


QPCR



Whole cell lyses

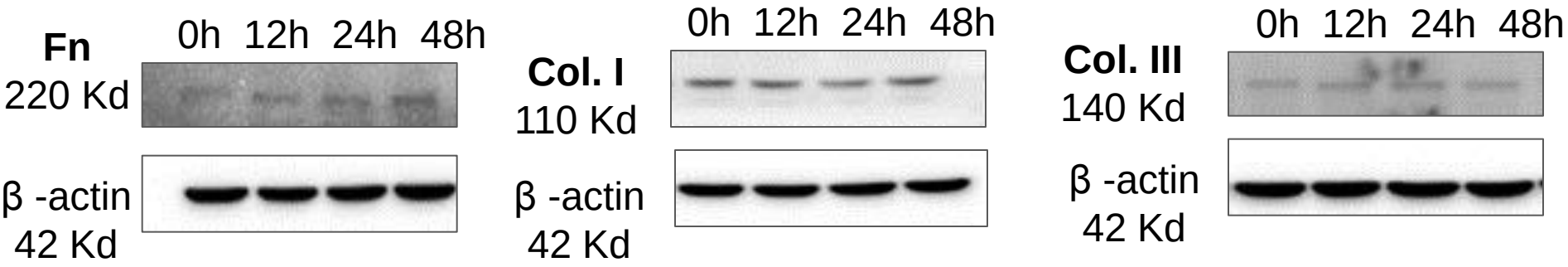


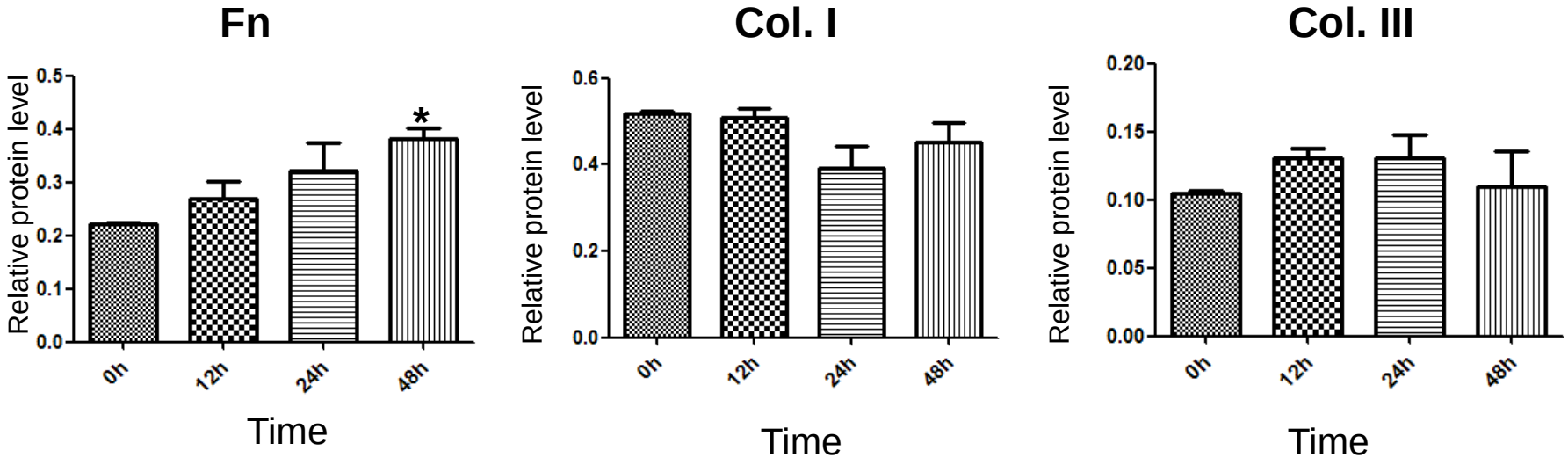


HNP1-3 treated HMC for 0,0.5,1,3,6,12,24,48h. *p<0.05, ***p<0.001 vs 0h

The Effect of HNP1-3 on ECM Accumulation

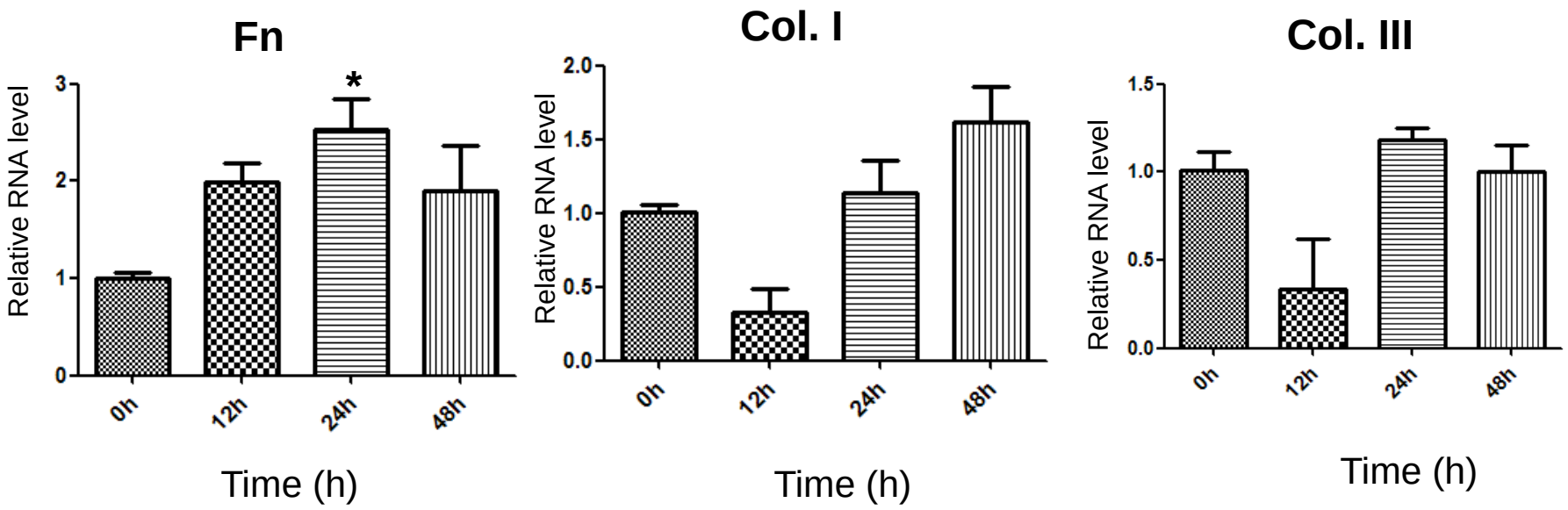
WB





HNP1-3 10 μg/mL treated HMC for 0,12,24,48h, *p<0.05, vs 0 h

QPCR



HNP1-3 10 μg/mL treated HMC for 0,12,24,48h. *p<0.05, vs 0 h

Summary

- Serum and Urine levels of HNP1-3 are both increased in IgAN patients. Urine level of HNP1-3 is negatively correlated with *DEFA1* CNs (p=0.008) and total *DEFA1A3* CNs (p=0.019) in all subjects.

- HAA binding IgA1 was negatively correlated with total *DEFA1A3* CNs (r=-0.37, p=0.01) and *DEFA1* CNs (r=-0.31, p=0.04) in IgAN patients with higher CNs (CN>6).

- Neutrophils isolated from IgAN patients had lower secretion capability of HNP1-3 as stimulated.

- The expression of interleukin-6 (IL-6) was significantly inhibited as co-cultures with HNP1-3, indicating the potential protective effect of HNP1-3 in IgAN.

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Thank you
Welcome to Guangzhou, China