Identifying Disease Susceptibility Genes in Familial IgAN

York Pei, MD, FRCP(C), FACP, FASN
Professor, Department of Medicine
Division of Nephrology
University of Toronto

ICCN Hong Kong 12-12-2015

Lecture Outline

- To review the rationales for mapping susceptibility genes for complex diseases
- To highlight current tools/approaches for mapping susceptibility genes for complex diseases
- To present preliminary whole exome sequencing results for familial IgAN

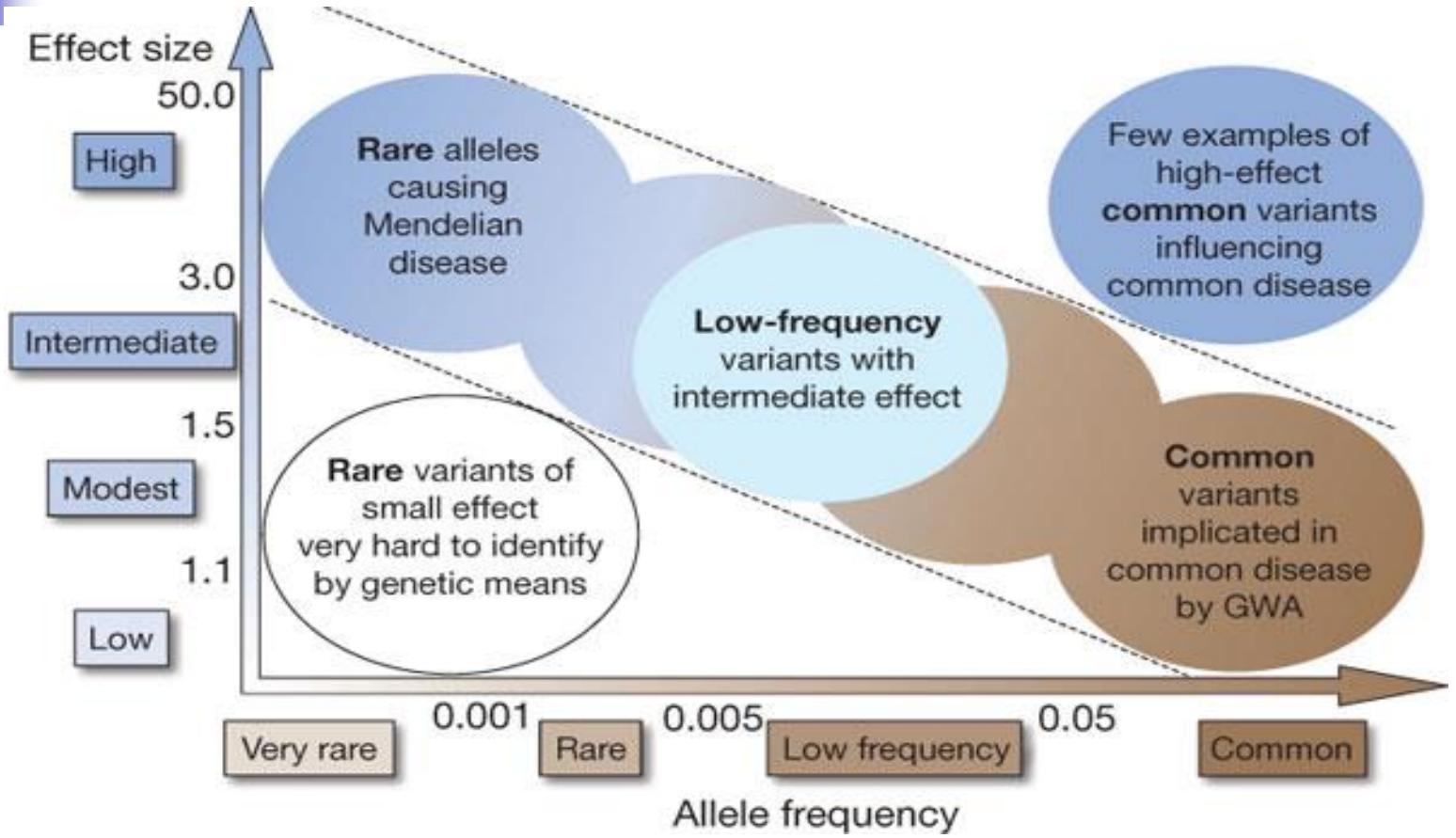
Identifying Susceptibility Genes in Complex Diseases

Why do it?

- To improve diagnosis and prognosis at the level of the individual patients
- To identify the primary disease pathway and molecular targets for biomarkers and drug Rx

Mapping Susceptibility Genes

What have We Learned?



Nature 461: 749, 2009

Molecular Genetics of fIgAN

- Familial clustering consistent with autosomal dominant inheritance with reduced penetrance
- A genome scan of multiplex families

  $\rightarrow$ a major locus on chr. 6q22 (IGAN1)[1]
- A second genome scan of multiplex families showed suggestive linkage to two additional loci (chr.4q22-31 and 17q12-22)[2]
- No disease genes identified to date

[1] Nature Genet 26:354-7, 2000
[2] Am J Hum Genet 79:1130-7, 2006

Identifying Disease Susceptibility Genes in Familial IgAN

Xuewen Song[1], Nicole M. Roslin[2], Meng yi Xu[1], Kairong Wang[1], Jannel Liu[1], Bushra Joarder[1], Amirreza Haghighi[1], Melody Ren[1], Mitchell Li Cheong Man[1], Joseph Leung[3], Sydney Tang[3], K.N. Lai[3], Andrew D. Paterson[2], Florent Soubrier[4], and York Pei[1].

[1]Division of Nephrology, University Health Network, Toronto, Ontario, Canada;
[2]Program in Genetics and Genomic Biology, Hospital for Sick Children, Toronto, Canada;
[3]Division of Nephrology, Hong Kong University; [4]Genetics Department, Hospital Pitié-Salpêtrière and INSERM, Université Pierre et Marie Curie Paris 06 (UPMC), Paris, France.

ICCN Hong Kong 12-12-2015

Study Patients

In total, 109 Pts from 54 families with fIgAN (1 family with 3 exomes, 53 families each with 2 exomes)

- Canadian IgAN families (n=5):
  - IgAN3*, 7, 8, & 9* each with 2 exomes
  - IgAN6* with 3 exomes
- Chinese IgAN families (n=24):
  - each with 2 exomes (HK1-24)

Data set 1 (59 exomes): SSV4/SSV5 capture kits HiSeq2500

- French IgAN families (n=25)
  - Each with 2 exomes (FR1-25)

Data set 2 (50 exomes): SSV5 capture kit HiSeq2500

* Three multiplex families with linkage data

Mean target reads: Median: 86.2x; Mean: 88x

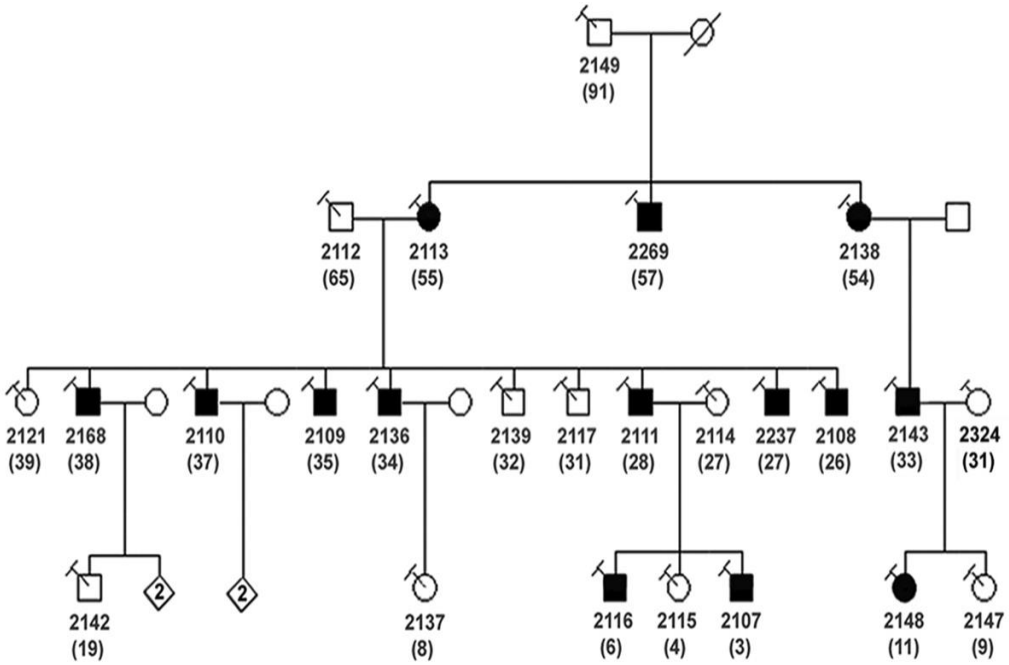
% exons with no coverage: Median: 0.12%; Mean: 0.14%

Methods

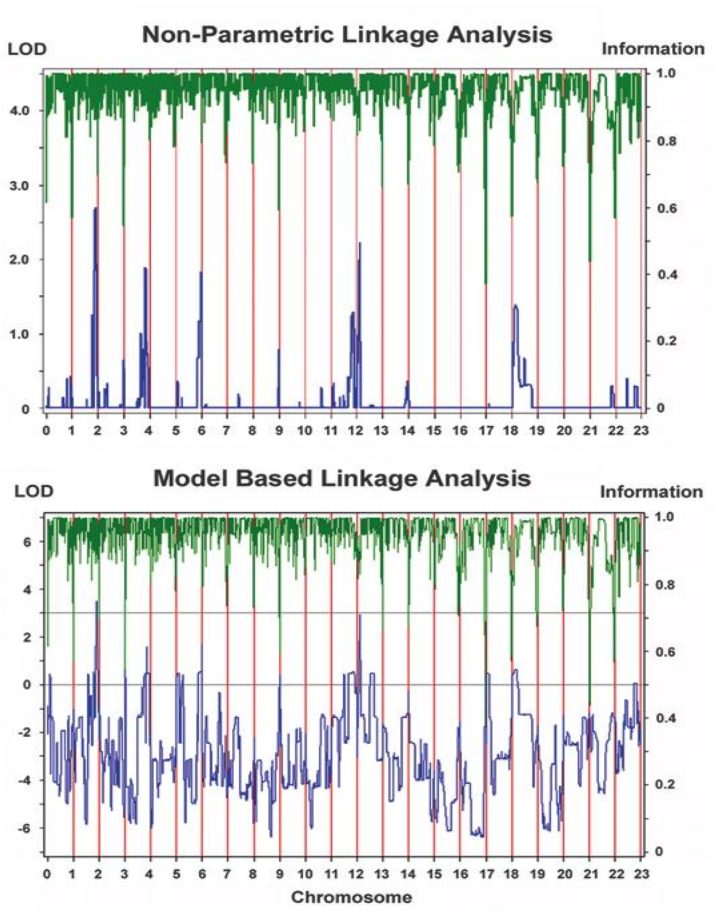
- **Linkage analysis in 3 large multiplex families**
- **Exome sequencing (2-3 patients/family)**
  - Agilent SureSelect V4/V5 kit for exon capture
  - Illumina HiSeq2500 for sequencing
- **Identify rare heterozygous deleterious variants**
  - MAF <= 1% and 5% (1000G, ESP, Complete Genomics)
  - High impact (i.e. nonsense, frameshift, splicing, stop codon)
  - Moderate impact (i.e. inframe indel, *non-synonymous missense variant)
- **Follow-up studies**
  - Validation by Sanger sequencing
  - Within-family segregation
  - Additional mutations of the same gene in unrelated families

*Predicted by at least 2/6 algorithms:
- SIFT <= 0.05
- Polyphen2 >= 0.95
- Mutation Assessor >= 2
- PhylopPMam_avg >= 2.5
- PhylopVert100_avg >= 4
- CADD_phred >= 15

Combined Linkage and WES Analysis in IgAN6

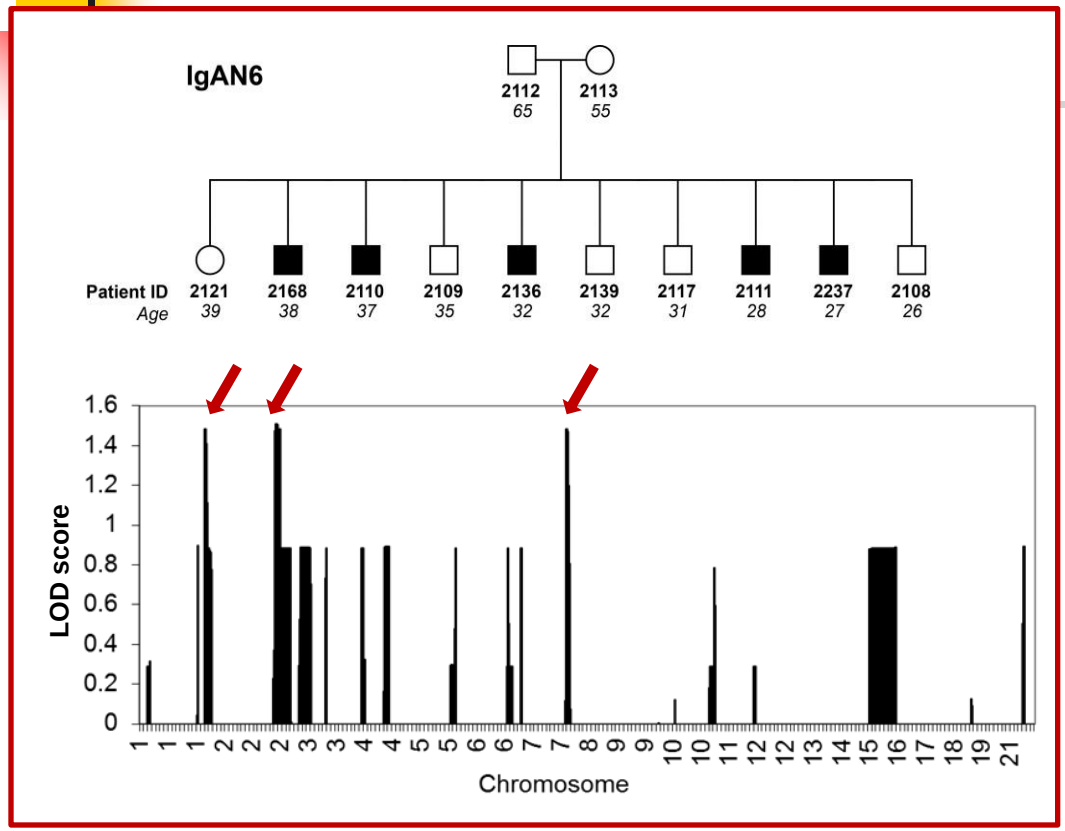


COL4A3 (p.G695R, CM040407) co-segregated with 14 affected



JASN 18: 2408-15, 2007

Repeat Affected Only Linkage Analysis in IgAN6
(with patients with +ve Bx or ESRD)



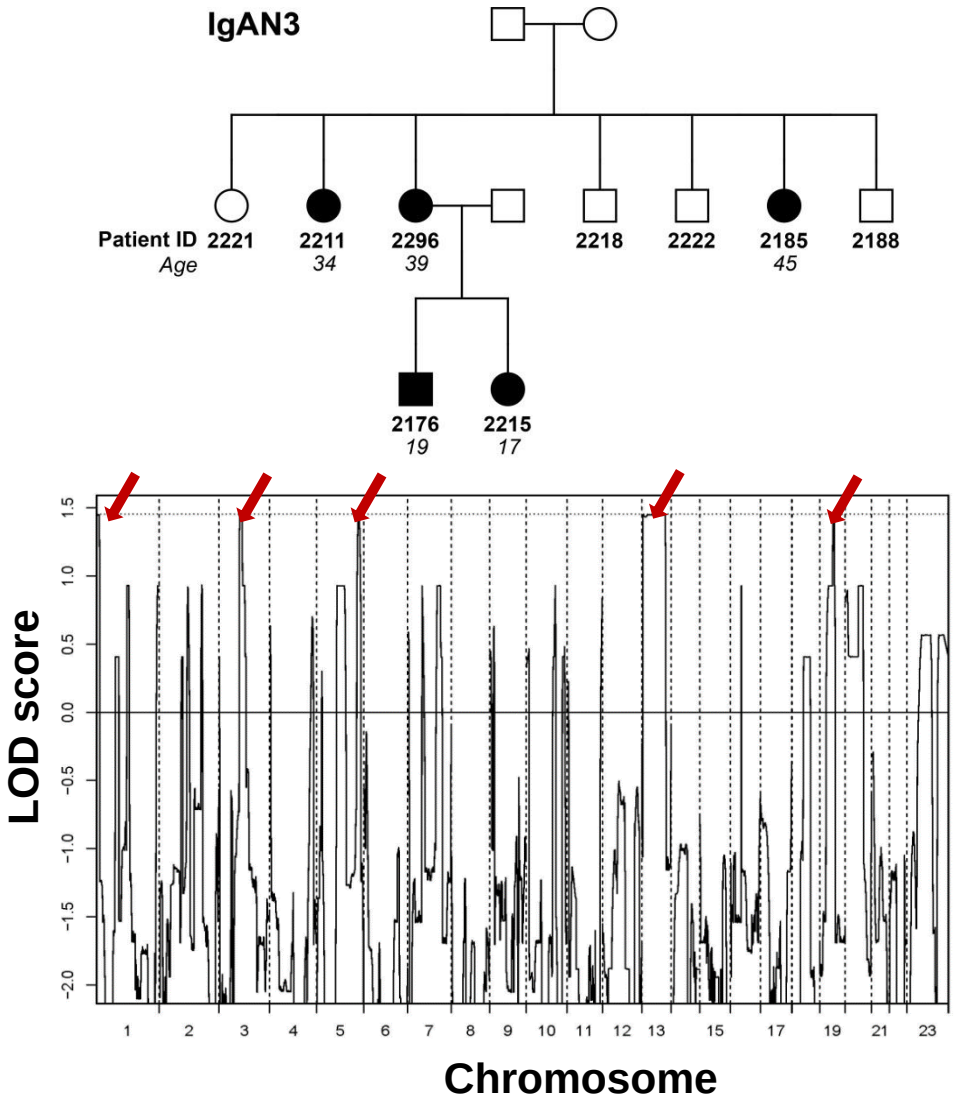
Identified 3 regions with LOD score ~1.45:
Chr 1q42-44 (137 genes)
Chr 2q36-37 (76 genes)
Chr 7q33-36 (222 genes)

Model under autosomal dominant heritance

- 75% penetrance
- a disease allele frequency of 0.001
- phenocopy rate of 0.01

No pathogenic mutation was identified in three regions of suggestive linkage. However, non-canonical splice variants and CNVs have not been excluded.

Linkage Analysis in IgAN3



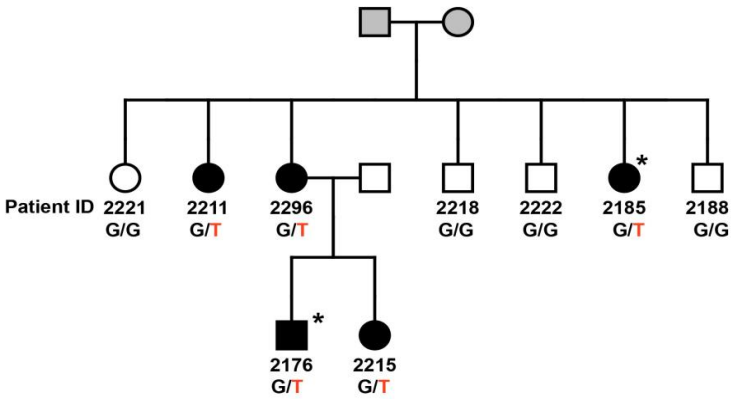
Autosomal dominant model

- **Penetrance: 70-90%**
- **disease allele of 0.005**
- **phenocopy rate of 0.001**

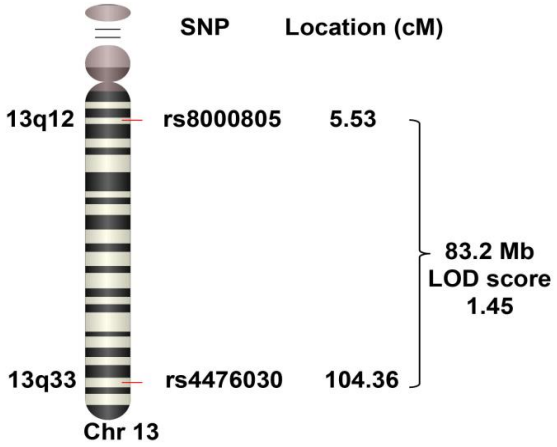
Chr. 1p36 (70 genes)
Chr. 3p14-q13 (132 genes)
Chr. 5q34-35 (55 genes)
Chr. 13q12-33 (347 genes)
Chr. 19p13-q13 (691 genes)

Combined Multipoint Linkage & WES Analysis in IgAN3 Family Identified a Novel G310V Mutation in LCP1 Gene

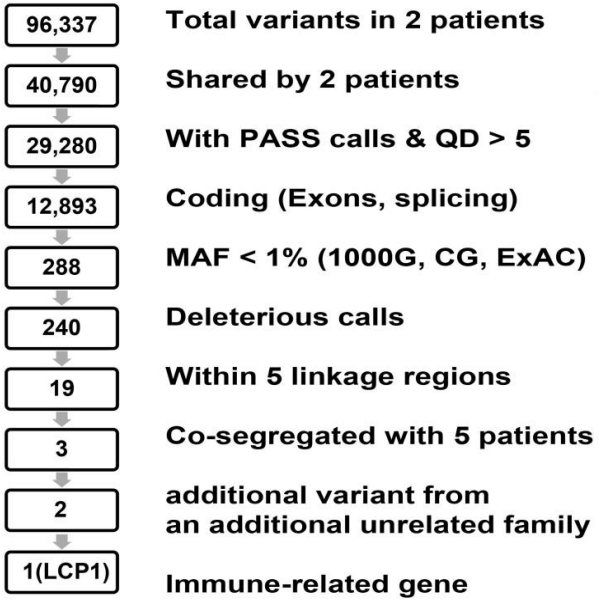
a



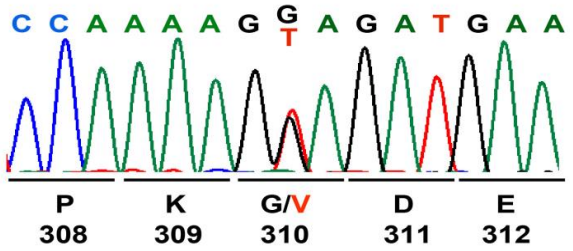
b



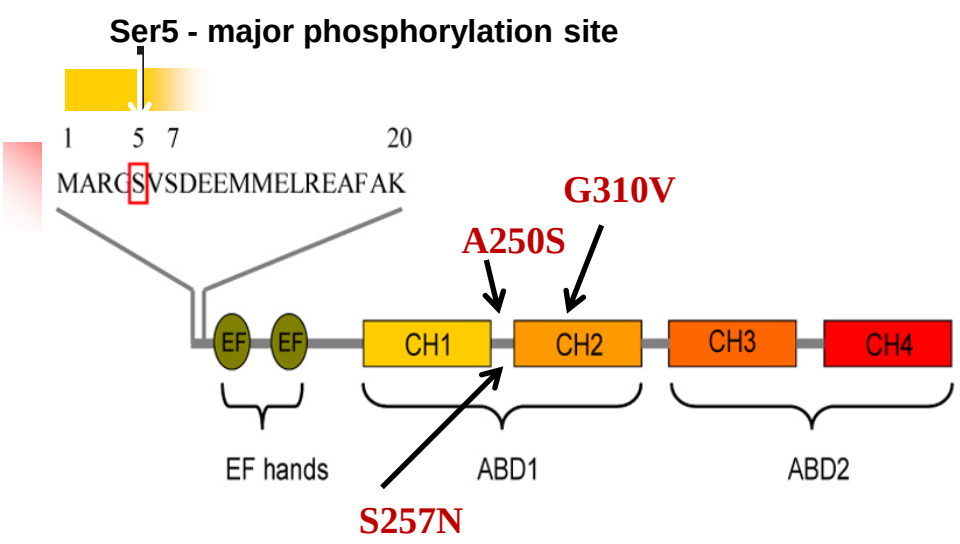
c



d



LCP1: Rare Deleterious Variants from 3 Unrelated IgAN Families



LCP1 (lymphocyte cytosolic protein 1, 627 AA):

- **A250S (0.29% ASN, damaging 4/6): HK16_F (affected only)**
- **S257N (rs149807920, 0.17% EUR, damaging 2/6): FR4**
- **G310V (novel, damaging 4/6): IgAN3**

Mutation burden test*:

MAF ≤ 0.29% (M+H)

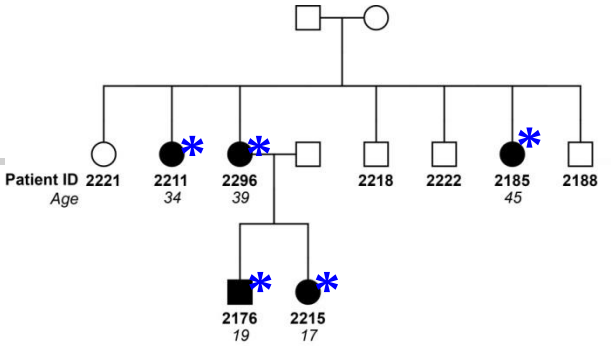
- **IgAN: 3/108 alleles**
- **ExAC: 965/121041**
- **p = 0.0372**

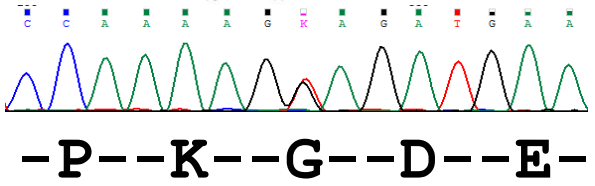
MAF ≤ 0.09673% (M only)

- **IgAN: 3/108 alleles**
- **ExAC: 721/121036**
- **p = 0.0103**

***One-tail Chi-square with Yates correction**

IgAN3 (5 patients with G310V)

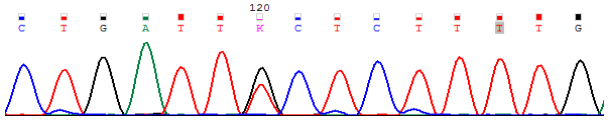




c.929G>T
p.G310V

-P--K--G--D--E-

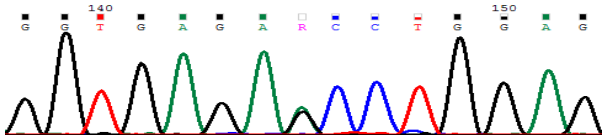
HK16_F (2 patients with A250S)



c.748G>T
p.A250S

-L--I--A--L--L-

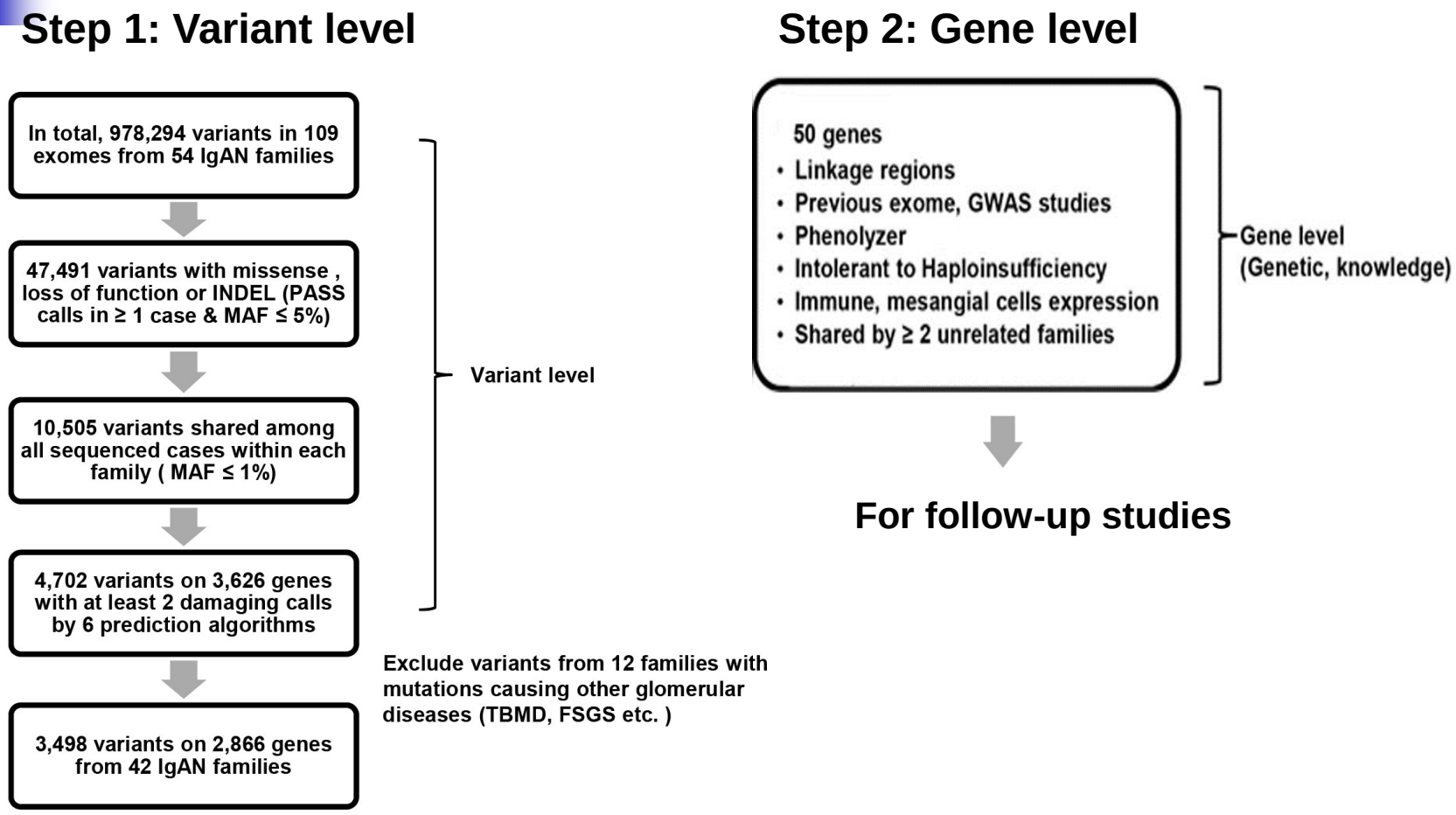
FR4 (2 patients with S257N)



c. 770G>A
p.S257N

-G--E--S--L--E-

Filtering Algorithm to Identify Rare Deleterious Variants In 54 Families and 109 Exomes



Identifying Mutations That May Cause Other Glomerular Diseases in 12 IgAN Families

	Family ID	Ethnicity	Patient ID (Gender)	Relationship	Gene	Exon: Nt and AA change (zygosity)	*Impact	SNP ID (MAF ExAC)
TBMD or Alport syndrome (n=9)	FR25	Caucasian	6401 (F) 6402 (F)	2nd degree	COL4A3	exon23: c.1504+1G>A (het)	LoF	NA (NA)
	FR12	Caucasian	6375 (F) 6376 (M)	full siblings	COL4A3	exon15: c.871G>A; p.G291R (het)	M6	NA (NA)
	IgAN6	Caucasian	2110 (M) 2111 (M) 2168 (M)	full siblings	COL4A3	exon28: c.2083G>A; p.G695R (het)	M6	rs200287952 (0.027%EUR)
	IgAN9	Caucasian	6524 (M) 6612 (M)	full siblings	COL4A3	exon37: c.3161G>A; p.G1054E (het)	M4	NA (NA)
	HK22	East Asian	5896 (M) 6900 (F)	full siblings	COL4A3	exon43: c.3856G>A; p.G1286R (het)	M5	NA (0.058%ASN)
	HK7	East Asian	5912 (F) 6320 (F)	mother daughter	COL4A4	exon47: c.4523G>C; p.G1508A (het)	M6	NA (NA)
	FR21	Caucasian	6393 (M) 6394 (M)	full siblings	COL4A4	exon32: c.2908C>T; p.Q970X (het)	LoF	NA (0.0015%EUR)
	FR14	Caucasian	6379 (M) 6380 (M)	2nd degree	COL4A5	exon3: c.142G>A; p.G48R (hemi)	M4	rs281874669 (NA)
	FR1	Caucasian	6353 (M) 6354 (M)	full siblings	COL4A5	exon17: c.973G>A; p.G325R (hemi)	M5	rs104886088 (NA)
FSGS (n=2)	HK16	East Asian	5891 (F) 6890 (M)	full siblings	ACTN4	exon4: c.398-2A>G (het)	LoF	NA (NA)
	HK8	East Asian	5911 (F) 6312 (M)	full siblings	ADCK4	exon9: c.G737A; p.S246N (hom)	M5	rs200841458 (0.13%ASN)
CFHR5 nephropathy (n=1)	FR13	Caucasian	6377 (M) 6378 (F)	full siblings	CFHR5	exon4: c.479_480insA; p.E163fs (het)	LoF	NA (NA)

* LoF - loss of function changes

M - missense changes, numbers 4 to 6 are the numbers of damaging calls by 6 prediction programs

Selected Candidate Genes with Segregating Mutations in ≥2 Unrelated Families

Genes symbol	# of families	IgAN families with deleterious variants (*mutation impact)	Rank by Phenolyzer	Immune function	Mesangial cells (RPKM)**	Podocyte (RPKM)**	Comments
LCP1	2	IgAN3(M4), FR4(M2)	NA	yes	0.6	2.3	IgAN3 linkage region
GPALPP1	2	IgAN3(M6), HK1(M5)	NA	NA	7.3	5.2	IgAN3 linkage region
DEFA4	3	HK9 (LoF), HK15(LoF), HK23(LoF)	0.1%	yes	NA	NA	GWAS Loci
TLR1	2	FR19(LoF), IgAN8(M2)	2.0%	yes	0.1	0.1	Toll-like receptor
OAS1	2	IgAN8(M4), FR7(LoF)	7.4%	yes	NA	NA	Antiviral response
KLC3	2	HK10(LoF), HK19(M3)	7.3%	yes	0.3	0.1	MHC-II Antigen transport
KIF15	2	FR11(LoF), FR24(M4)	7.4%	yes	1.1	0.9	MHC-II Antigen transport
IFIH1	2	FR8(LoF), FR3(LoF)	8.0%	yes	10.4	1.7	Antiviral response
SIGLEC1	3	HK9(LoF), HK12(M3), FR10(M5)	8.7%	yes	0.1	0.0	Endocytosis/MΦ-restricted adhesion molecule
ERAP2	3	IgAN3(M3), FR11(LoF), Ita_IgAN6(M2)	11.6%	yes	NA	NA	MHC-I Antigen presentation
ASB4	2	HK13(LoF), HK19(M4)	11.9%	yes	0.0	0.0	Class I MHC mediated antigen processing
MARCO	2	HK2(LoF), FR8(M4)	15.8%	yes	0.0	0.0	Phagocytosis promoting R
LAMA5	4	HK6(M5), FR6(M4), FR16(M3), FR9(M5)	16.0%	NA	27.3	58.2	ECM protein in GBM
PCK2	4	HK20(LoF), FR2(M5), FR9(M6), IgAN7(M6)	20.5%	NA	15.7	4.3	Phosphoenolpyruvate carboxykinase
SLIT3	2	FR10(LoF), FR6(M5)	52.1%	NA	19.3	3.4	mesangial cells enriched
FAT1	3	F24(M2), FR11(M5), FR7(M4)	NA	NA	114.1	233.0	Mesangial cells/ podocyte enriched
MYOM2	3	HK14(LoF), FR16(M6), FR20(LoF)	NA	NA	0.1	125.9	podocyte enriched
CGNL1	3	HK3(M6), FR18(M5), IgAN7(M5)	NA	NA	5.1	88.1	podocyte enriched
PALLD	2	FR16(LoF), HK24(Indel)	NA	NA	7.2	24.0	podocyte enriched
SVEP1	4	HK21(M5), HK14(M4), FR5(M3), FR7(M3)	NA	NA	0.0	11.1	podocyte enriched
GJB2	3	IgAN7(M6), HK14(LoF), FR8(LoF)	78.8%	NA	1.8	0.1	Mucosal Barrier
ATP8B4	3	HK3(LoF), FR15(M5), FR18(M5)	79.8%	NA	0.0	0.2	With multiple families shared
PKP4	4	HK24(M4), IgAN7(LoF), HK4(M5), FR7(M3)	NA	NA	5.9	13.3	With multiple families shared
HMCN1	3	IgAN8(M5), FR15(M3), FR18(M2)	NA	yes	0.0	0.3	With multiple families shared
RYR3	4	HK4(M4), HK23(M5), FR16(M5), HK3(M4)	NA	NA	0.1	0.1	With multiple families shared
HEATR1	3	HK2(M5), HK10(M4), HK14(M5)	NA	NA	4.7	7.2	With multiple families shared

* LoF - loss of function changes
M - missense changes, numbers 4 to 6 are the numbers of damaging calls by 6 prediction programs

** RNAseq analysis on mouse purified mesangial cells, and podocyte (GEO ID: GSE64959)

Conclusions

- The genetics of familial IgAN is complex
- Presence of other glomerular diseases (e.g. TBMD) may confound the diagnosis of fIgAN in some putatively affected subjects ascertained based on urinary findings or even kidney biopsy
- Our data suggests that familial IgAN is underpinned by extensive genetic heterogeneity
- Exome sequencing combined with linkage analysis in multiplex families is a powerful approach to identify rare variants with high effect size

Future Directions

- Expanded sample size
- Examine rare synonymous exonic missense variants or intronic variants that may alter splicing in IgAN6
- Perform CNV analysis in IgAN6
- Identify gene(s) with deleterious variants in at least 5 additional familial and sporadic cases for follow-up functional studies

Acknowledgements

- Grant support by CIHR, PSI Foundation, and McLaughlin Centre for Molecular Medicine
- Exome sequencing by the Centre of Applied Genomics (TCAG)

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

PSI FOUNDATION

McLaughlin Centre
UNIVERSITY OF TORONTO

TCAG