Defining the Landscape of HLA Risk Alleles in Primary Nephrotic Syndrome and Post Kidney Transplant Recurrence 1U01AI152585-01

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Disclosure

We have no conflict of interest associated with this talk

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BACKGROUND

- Childhood nephrotic syndrome (NS) is a rare immune-mediated kidney disease
- Prevalence:16/100,000 children
- NS classification: Initial response to corticosteroid RX
  - Steroid sensitive nephrotic syndrome (SSNS 80% ~MCD)
  - Steroid resistant nephrotic syndrome (SRNS 20% ~FSGS)
- SRNS major cause of CKD/ESKD (Cost/year $120B)
- Available treatment unsatisfactory
- Recurrence post transplant major cause of kidney allograft failure

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BACKGROUND

- Pathogenesis not completely known
- Evidence converge on common theme of T and B lymphocytes dysregulation
  - Alterations in T lymphocyte subsets during relapse of NS
  - Induction of remission by infections that can suppress T lymphocytes
  - Therapeutic agents used in RX are modulators of T cell function
  - B cells: B cell depletion with rituximab (monoclonal antibody against CD20 on B lymphocytes) can induce NS remission
  - HLA is essential in facilitating T and B lymphocyte interactions and may be an important mechanistic link in the pathogenesis of NS
  - Recent GWAS identified variants in HLA genes as risk loci for NS

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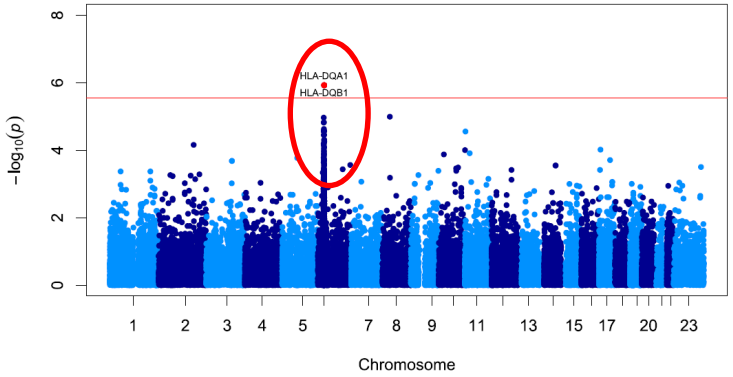
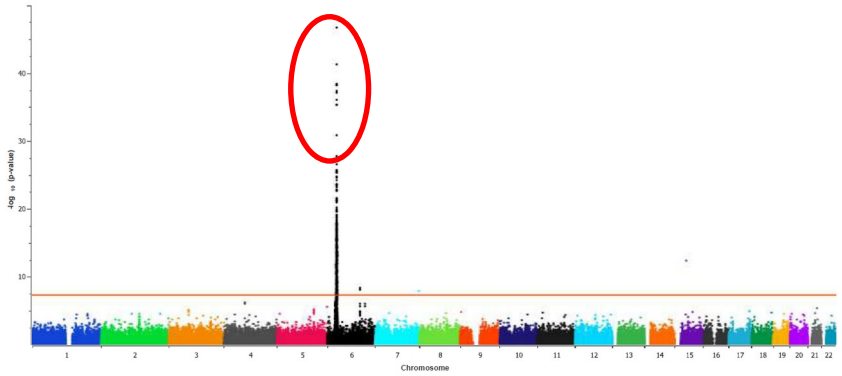
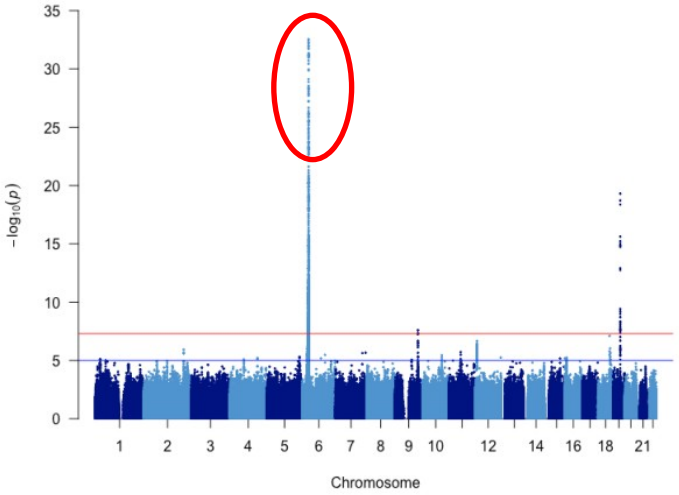
BACKGROUND

GWAS findings emphasize the role of adaptive immunity in the pathogenesis of SSNS







Gbadegesin et al JASN 2015
Population: South Asian, N: 214
Loci: *HLA-DQA1, HLA-DQB1*

Dufek et al JASN 2019
Population: European, N: 422
Loci: *HLA-DR/DQ,* ***CALHM6***

Jia et al KI 2020
Population: Japanese, N: 987
Loci: *HLA-DR/DQ*, ***NPHS1-KIRREL2, TNFS15***

BACKGROUND

- Limitations of current GWAS studies
  - GWAS chips unable to infer precise allelic association because of high levels of polymorphism, gene duplications, and high levels of LD in HLA region
  - Use of small mono-ethnic cohorts in the majority of studies
  - Exclusion of children with SRNS
  - The HLA loci identified account for a very small % of the disease phenotype: there are other disease loci in and outside of the HLA genes yet to be identified

- Deep NGS sequencing of all 11 HLA genes in a multiethnic cohort will identify new haplotypes and determine shared regulatory motifs that provide insight to molecular mechanisms.

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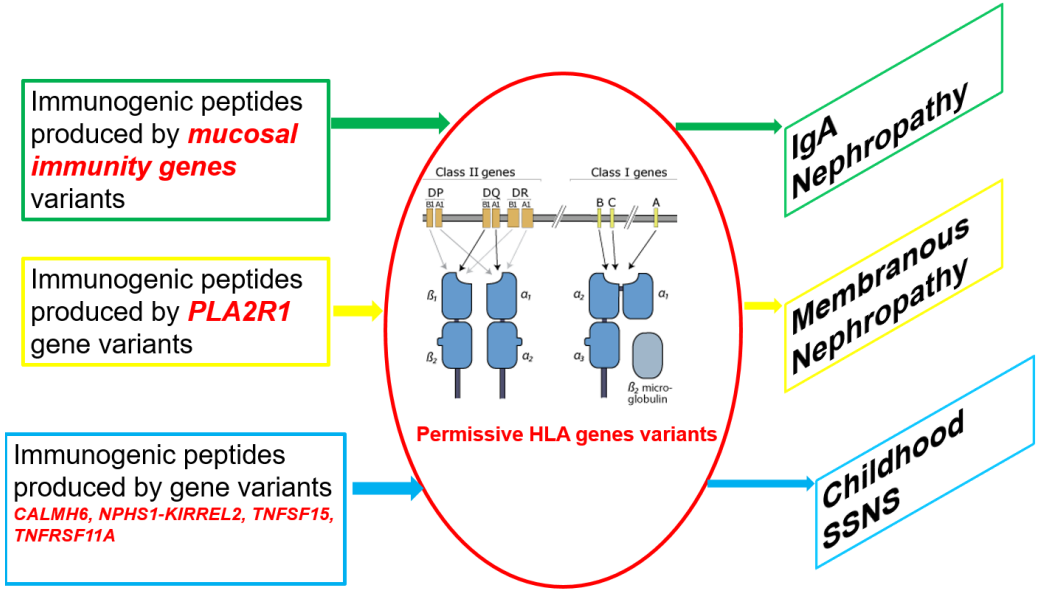
BACKGROUND

- HLA region pleotropic for multiple immune mediated glomerular diseases (GD) that can cause secondary NS
- Multiple HLA genes loci associated with IgAN and MN (2^0 NS)
- Both 1^0 & 2^0 NS associated with loci outside of HLA region
- Unified disease model for GD: At least 2 genetic hits



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BACKGROUND

- Incomplete coverage of the HLA regions by GWAS preclude understanding of overlap between 1^0 & 2^0 NS
- Defining the HLA risk overlap may lead to ID of unified pathways important in the pathogenesis of 1^0 & 2^0 NS
- Unclear if haplotype driven HLA expression in immune cells and kidney underlies disease mechanisms
- The role of NS HLA alleles/haplotypes in NS recurrence following kidney transplantation unknown

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PROJECT GOALS

- ***Broad hypothesis:*** Certain HLA alleles/haplotypes associated with NS can predict pattern of corticosteroid response in NS and risk of disease recurrence following kidney transplantation

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PROJECT GOALS

AIM 1: Identify HLA alleles for NS by NGS
Cases: 1,500 multi-ethnic NS patients
Controls: 1,500 ethnic matched BM donor controls
Results: Identification of NS HLA alleles
- Identification of ethnic specific HLA alleles
- Identification of predictors of steroid response

AIM 2: Investigate the association between primary NS HLA alleles and secondary NS (IgAN and Mn) by NGS (35X WGS Data)
Cases: 1,200 patients with IgAN or MN
Controls: 1,200 ethnic matched controls
Results: Identification of alleles shared between NS, IgAN, and MN and alleles unique to each.

IMPACT
- Improved stratification of disease in NS
- Tailored immunosuppression pre and post transplant

Determine the role of variations in 11 major HLA genes in clinical course of NS pre and post transplant

IMPACT
- Unified treatment strategies for glomerular diseases
- Slowing of NS progression to ESKD

AIM 3: Determine structural and functional motifs within NS HLA risk alleles and non-risk alleles, and protein expression of risk and non-risk alleles in immune cells and kidneys of patients with NS
Methods: RNA seq and RNAscope®
Results: Role of HLA expression in disease mechanisms

AIM 4: Determine the ability of NS HLA risk alleles to predict disease recurrence following kidney transplantation.
Cases: 100 patients with NS recurrence
Controls: 150 patients with no recurrence
Results: Identification of predictors of disease recurrence in kidney transplant

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SUMMARY OF EXPERIMENTS

- ***Aim 1:*** Identify NS HLA risk alleles/haplotypes using high resolution HLA gene sequencing in a cohort of multi-ethnic patients and determine the relationship between genotypes and therapy response

- ***Approach***
  - NGS of the 11 major HLA genes and additional MHC genes using an ***innovative, high throughput typing strategy that utilizes hybrid probe capture technology*** (CareDx, Brisbane CA) on 1,500 patients with NS and 1,500 ancestry matched controls.
  - Replicate the top 20 variants identified in this aim in the 35x whole genome sequencing (WGS) data from the 1,200 patients and controls with MCD or FSGS enrolled in the CureGN Study

- ***Expected outcomes***
  - Identification of new HLA risk alleles/haplotypes for NS in a large multi-ethnic cohort of patients with NS and confirmation of previously reported HLA loci.
  - Identification of ancestry specific loci.
  - Identification of variants that predict pattern of response to corticosteroids.

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SUMMARY OF EXPERIMENTS

- ***Aim 2:*** Investigate the association between primary NS HLA risk alleles/haplotypes and secondary causes of immune-mediated NS, such as IgA and membranous nephropathy.

- ***Approach***
  - HLA typing of 1,200 patients with IgAN or MN and ancestry controls from the CureGN study for which 35X WGS is available
  - We will compare allele frequency of variants that are significant in Aim 1 (Primary NS) in 1,200 patients with IgA or MN and controls

- ***Expected outcomes***
  - Confirmation of HLA loci previously associated with IgAN and MN and identification of new disease loci.
  - Identification of alleles shared between primary NS, IgAN, and MN and alleles unique to each group.

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SUMMARY OF EXPERIMENTS

- ***Aim 3:*** Determine common structural and functional motifs within NS HLA risk alleles/haplotypes and non-risk alleles by in-silico modeling and compare gene and protein expression of these alleles in B lymphocytes and kidneys of patients with primary NS

- ***Approach***
  - Evaluate the structural, functional and peptide binding differences between risk and non-risk alleles by in-silico modeling tools: (I-TASSER server, PolyPhen, Sift, MutationTaster) and epitope binding: IEDB T Cell Epitope Prediction Tools
  - Examine HLA gene expression, surface protein expression, and intrarenal expression by bulk RNAseq, and flowcytometry in lymphocyte and kidney biopsy samples obtained from patients with high and low risk haplotypes during relapse and remission of NS

- ***Expected outcomes***
  - NS HLA risk alleles will exhibit higher HLA gene and/or protein expression during relapse than non-risk alleles
  - SSNS patients, who respond to corticosteroid treatment, will have decreased HLA expression at time of remission
  - Differential expression of HLA will provide direct evidence for an HLA intrinsic role in NS and RNAScope® intrarenal cell-specific HLA expression patterns will focus and inform our understanding of NS pathways

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SUMMARY OF EXPERIMENTS

- ***Aim 4:*** Determine the ability of known and novel NS HLA risk alleles/haplotypes to predict disease recurrence following kidney transplantation.

- ***Approach***
  - We will determine the association of NS recurrence in kidney transplant in 250 patients with NS who are post transplant with NS HLA risk haplotypes
  - We will determine whether NS recurrence is increased when donor genotypes include NS HLA risk allele/haplotype
  - Develop a clinical and genomic prediction model for NS recurrence

- ***Expected outcome***
  - Identification of HLA haplotypes/risk alleles for NS recurrence
  - Identification of phenotypic and genotypic risk calculator of NS recurrence that will form the basis for risk stratification pre and post-transplantation

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DATA COLLECTED AND KEY FINDINGS

- Regulatories
  - IRB approval Duke as a center
  - IRB in progress Duke as a coordinating center

- Case record forms (CRF)
  - CRF for all studies created and validated

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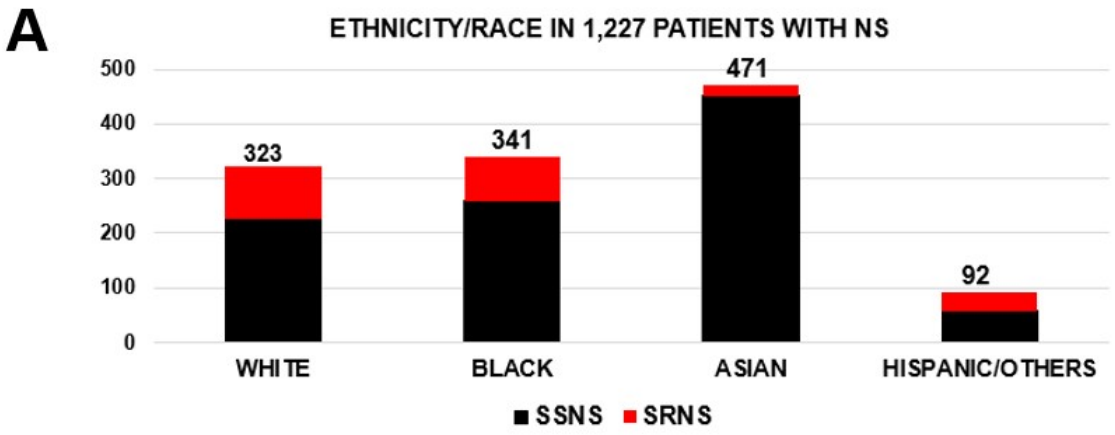
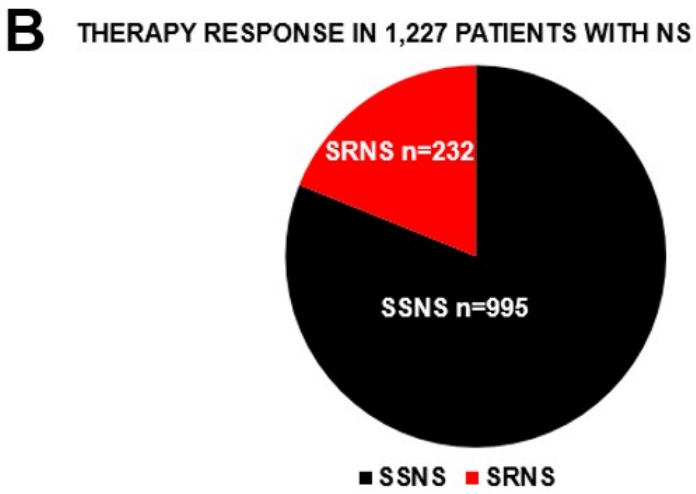
DATA COLLECTED AND KEY FINDINGS

- Phenotyping of 1,000+ patients with NS





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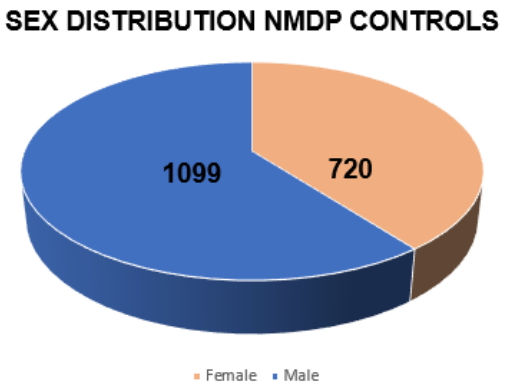
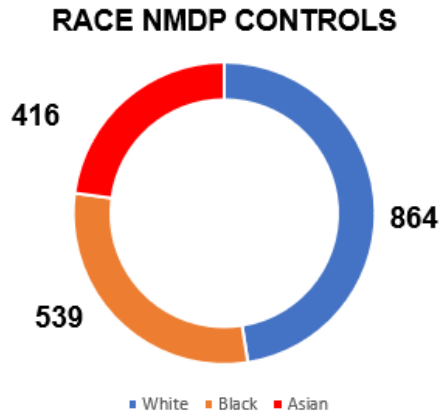
DATA COLLECTED AND KEY FINDINGS

- IN-HOUSE CONTROLS

- NMDP CONTROLS





KEY ISSUES, CHALLENGES, AND SOLUTION

- COVID 19 disruption
- Solutions
  - E-consenting
  - Engaging more collaborators

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MOVING FORWARD

- Genotyping of all cases and controls
- Start NGS of HLA genes
- Ramp up enrollment

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- AFTERNOON SESSION

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GENETIC ANALYSIS

- Data:
  - HLA sequencing for 1500 NS cases, 1500 (BM) controls
  - Genotyping array data: >1.7 million SNPs
    - TOPMed Imputation Server
  - 35x WGS, 1200 indiv. from CureGN (IgAN+MN+controls)
  - Binary traits: NS vs no-NS, SSNS vs SRNS, post-transplant recurrence
- Association models for multiethnic (structured) populations:
  - HapQTL for haplotype association, Mixed-effect model, Firth logistic regression with PCA, LIGERA (developed by Dr. Ochoa)
  - Condition on known variants, local ancestry
  - Evaluate test statistic inflation, power, in simulations

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GENETIC ANALYSIS

- Annotations:
  - NCBI and ensemble genome browsers
  - Regulatory regions: ENCODE, RoadMap Epigenomics
- Ancestry:
  - Subanalysis: single-ancestry tests, for identifying weaker ancestry-specific effects
  - Global ancestry estimation with ALStructure
  - Efficient Local Ancestry Inference (ELAI)

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GENETIC ANALYSIS

- Preliminary analysis: 750 SSNS cases only, array
  - Not imputed (first pass)
  - Control samples: practically any adults
    - 1000 Genomes Project: WGS, includes Sri Lankan (STU)!
    - Duke CATHGEN: whole-exome sequencing, greater number of African-American samples
- Problem: array bias perfectly confounded with case/control status!
  - Partial fix: more aggressive filtering, including double Hardy-Weinberg Equilibrium (HWE) test, before and after merging
  - Plan: Develop a population genetics approach for cross-platform QC

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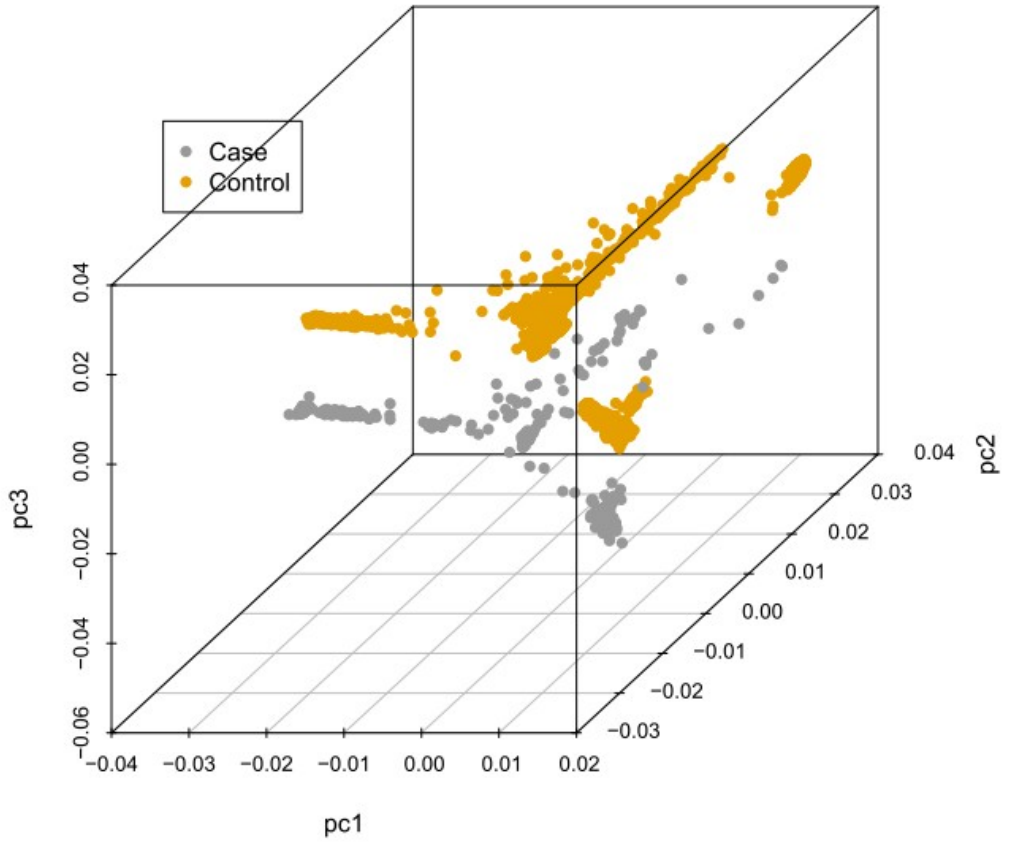
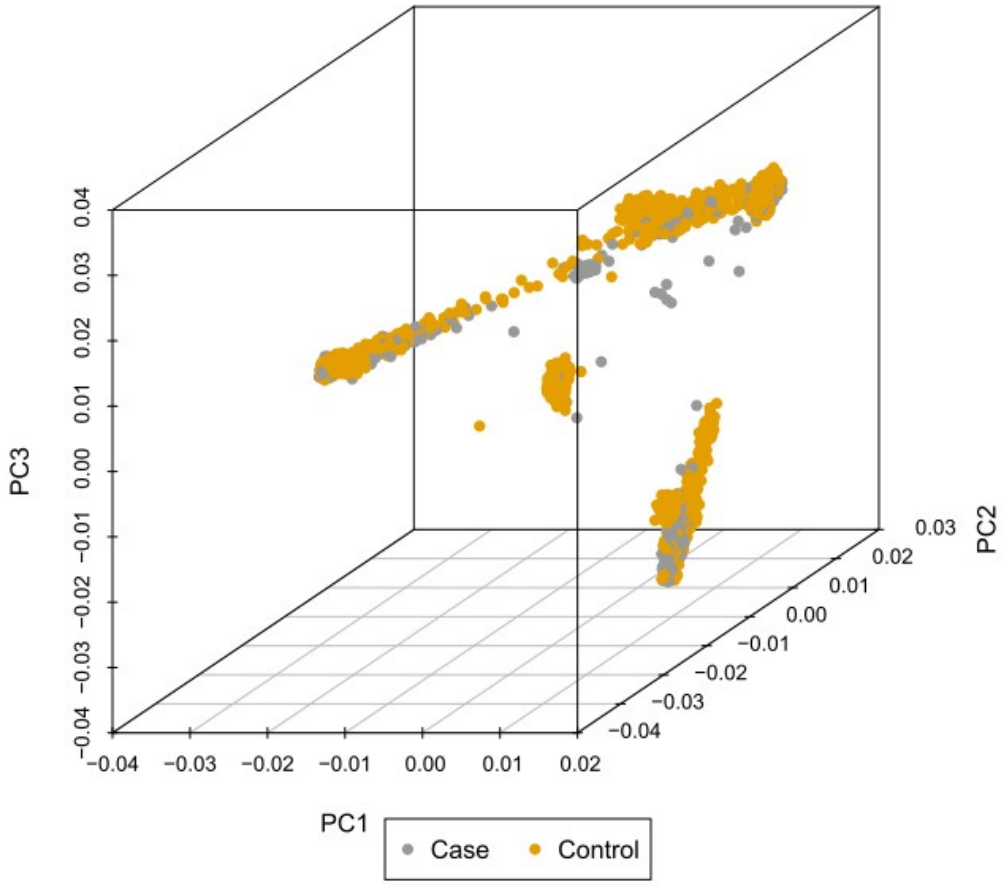
GENETIC ANALYSIS

Array (cases) + 1000 Genomes (controls)
simple merge: case/control separation!



Double HWE filter (before and after merge):
cases and controls overlap!



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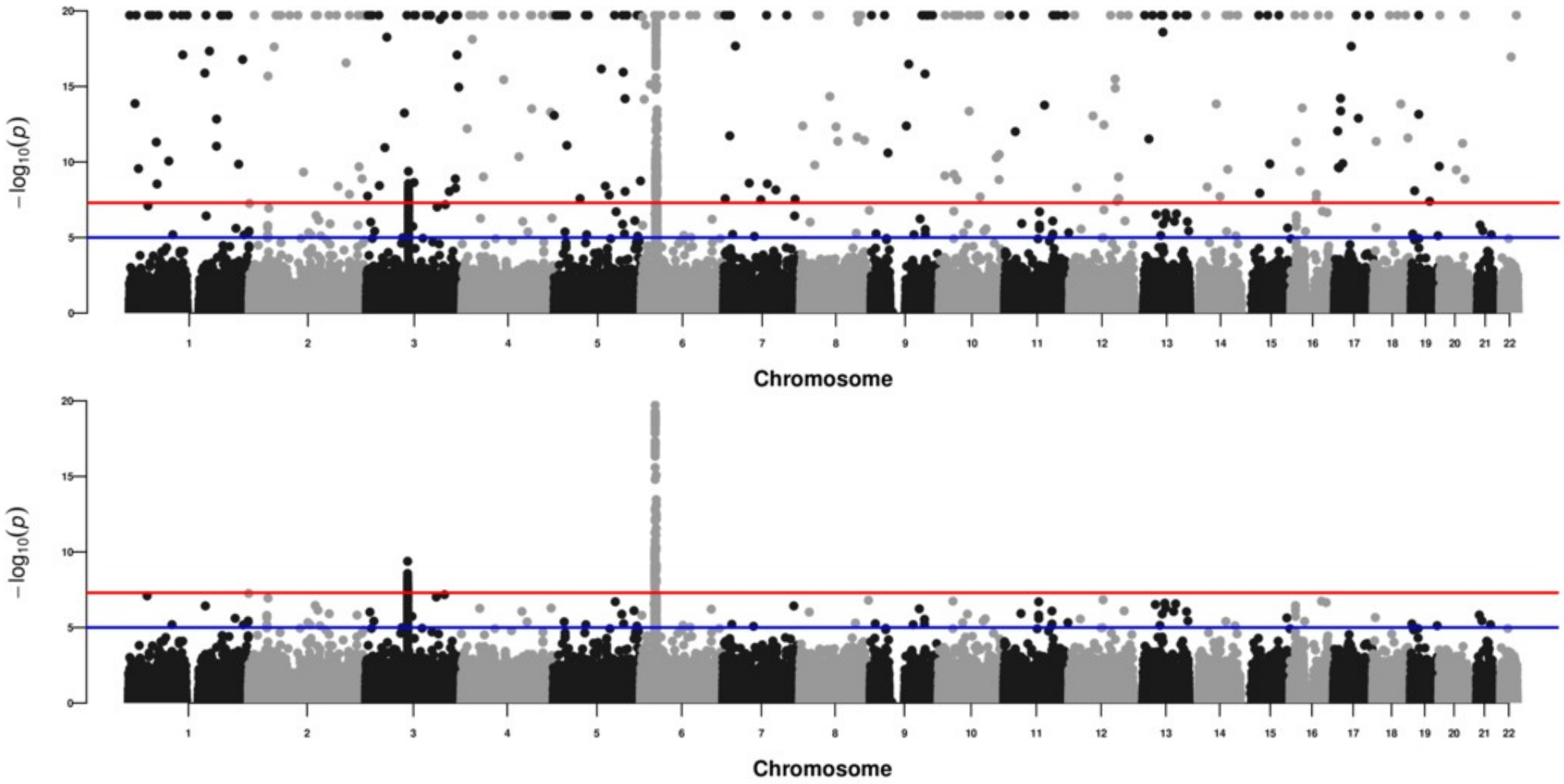
GENETIC ANALYSIS

- Top: association statistics remain inflated!
- Bottom: significant cases without significant neighbors removed (false positives)



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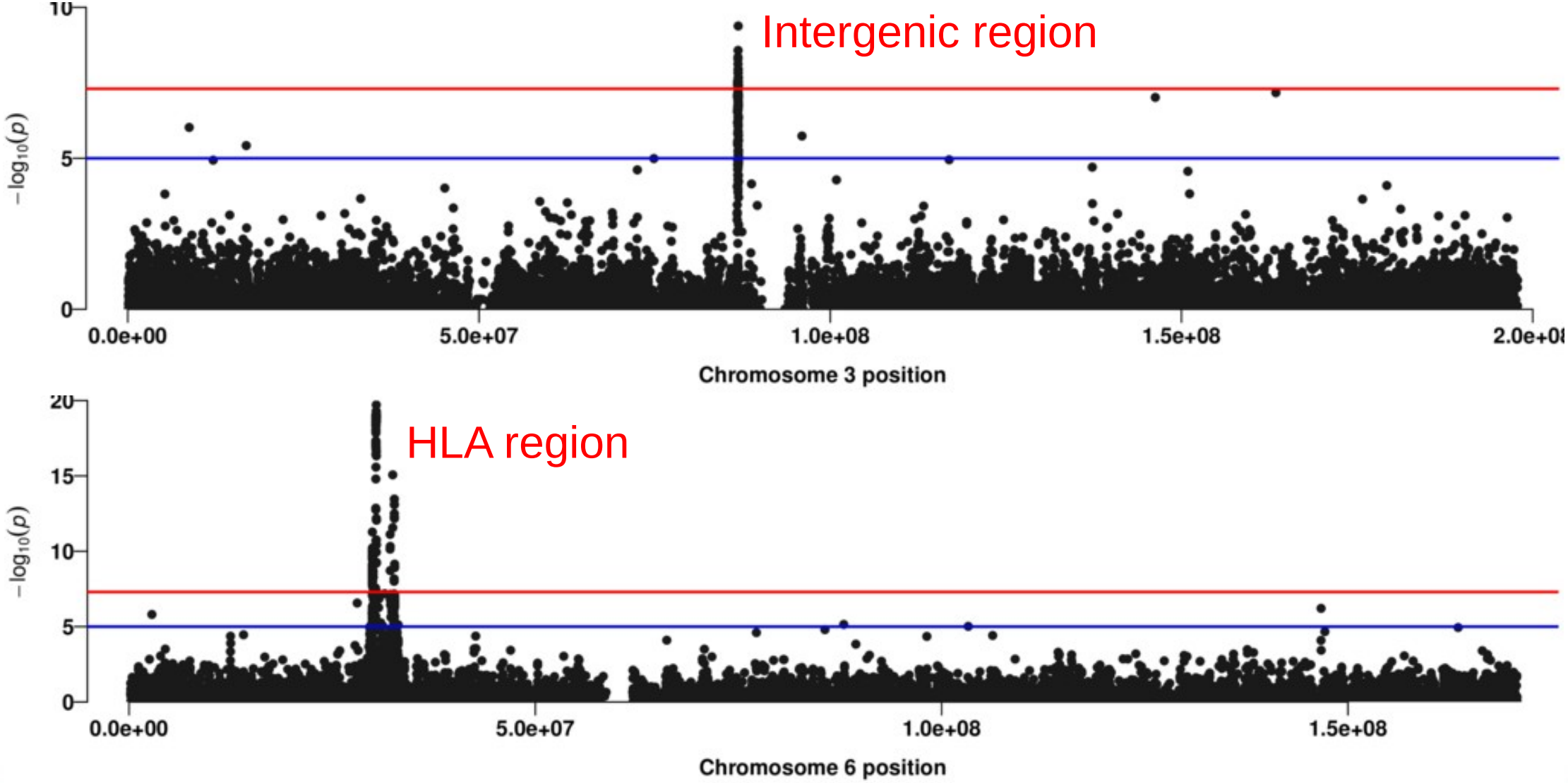
GENETIC ANALYSIS



DATA COLLECTION

- Data collection, storing, and data sharing with other members of the consortium.

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New analytic tools and how these could be leveraged by other members of the consortium

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- Areas seeking feedback from consortium members

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GENOMICS OF NS TEAM: DUKE

PEDIATRICS
Rasheed Gbadegesin
Eileen Chambers
Rachel Cason
Megan Chryst-Stangl

SURGERY AND IMMUNOLOGY
Annette Jackson
Brian Shaw

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