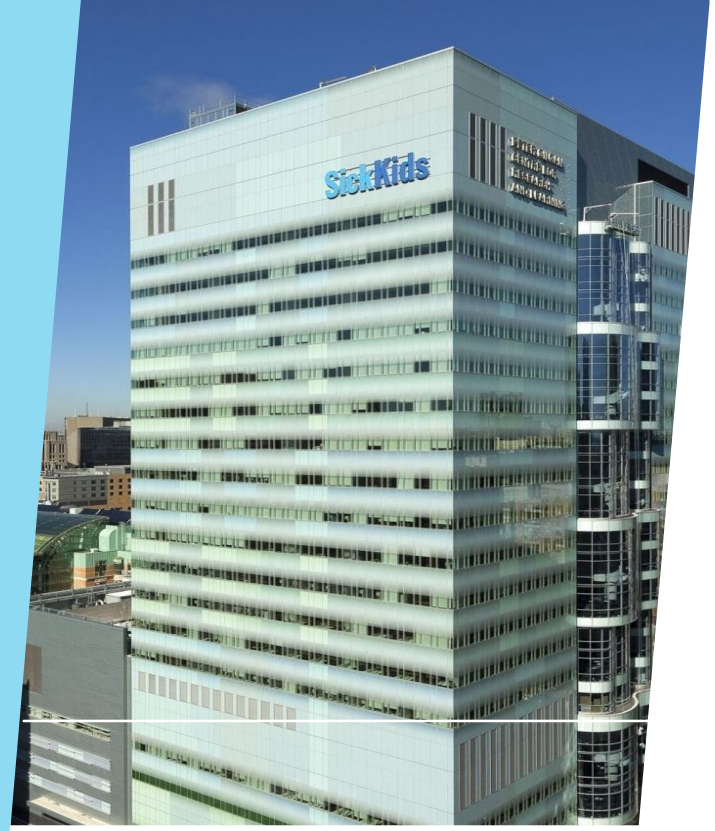
Phenome-wide Association Study of Cystic Fibrosis Modifier Genes

Supervisor: Dr. Lisa Strug

Faizan Khalid Mohsin



The Hospital for Sick Children

SickKids®
RESEARCH INSTITUTE

Strug Lab

Overview

- Background: Cystic Fibrosis and Modifier Genes
- Research Question
- What is a PheWAS?
- UK Biobank Database
- Statistical Methodology and Results
- Limitations and Challenges
- Future Work

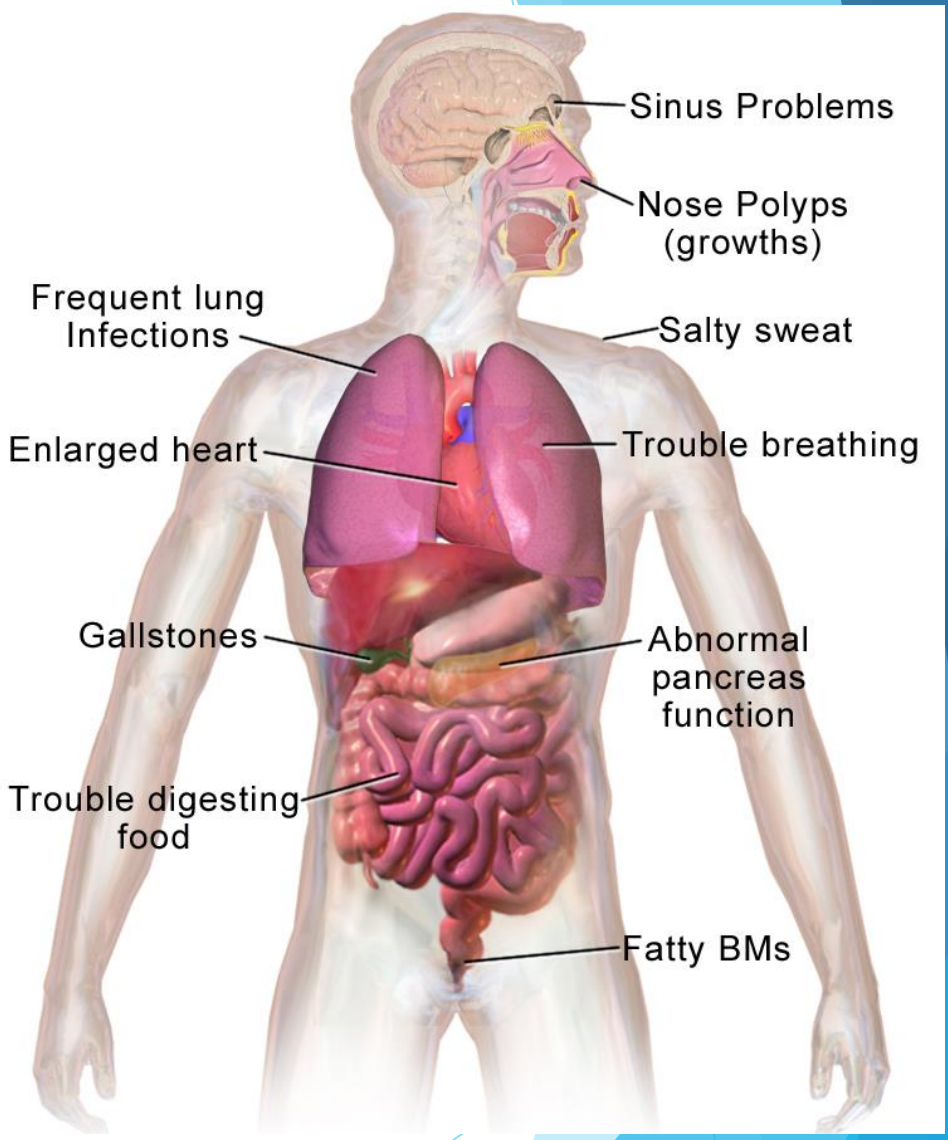
BACKGROUND

Cystic Fibrosis

- Cystic fibrosis (CF) is the most common fatal genetic disease affecting Canadian children and young adults. At present, there is no cure.
- Commonly suffer from lung disease.

Phenotype

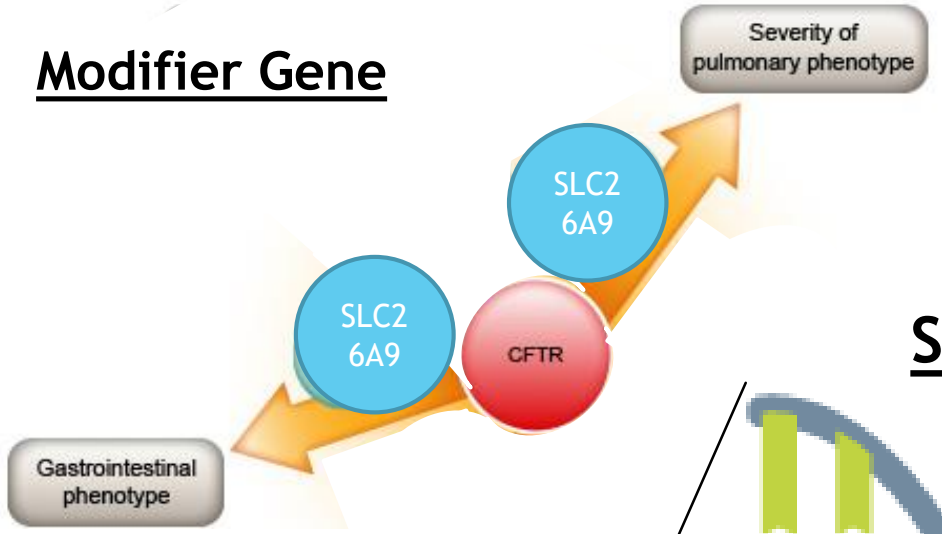
- All physical and observable traits.
- E.g. Height, hair color, white blood cell count, and diseases you may have (diabetes, cystic fibrosis, etc.).

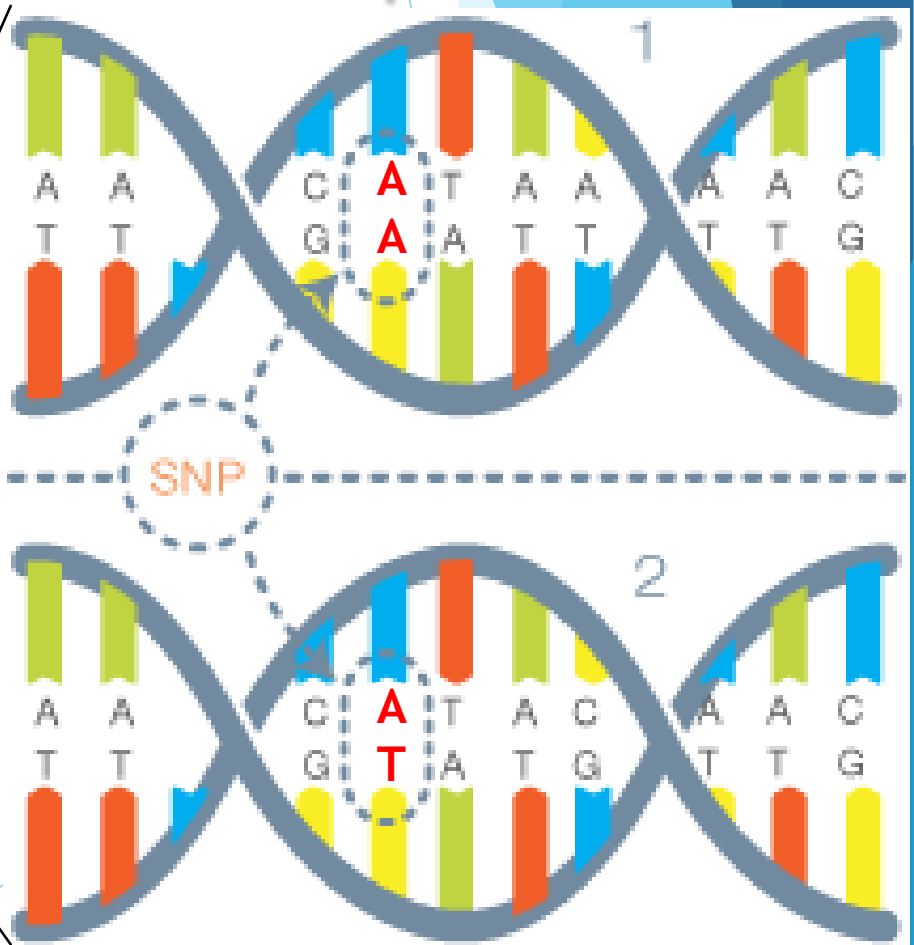


Typically: genotype (G) + environment (E) → phenotype (P)

Modifier Genes

- **Cystic Fibrosis (CF) Genetic modifiers** are SNPs that affect the severity of the disease.
- Modifier gene: SLC26A9
- Affects lung function for people with CF.
- SNP: RS4077468
- SNP Variation:
  - AA
  - AT
  - TT





Research Question

In the general public what is the impact of variation in the three modifier genes on a person's phenotypes.

- We will answer this question through a Phenome-wide Association Study (PheWAS).

The 3 modifier genes of interest:

1) SLC26A9 (Chromosome 1 - SNP rs4077468 Substitute: rs4077469; r = 1)
2) SLC6A14 (Chromosome X – SNP rs3788766 Substitute: rs5905176; r = 0.770)
3) SLC9A3 (Chromosome 5 – SNP rs57221529 Substitute: rs17497684; r = 0.821)

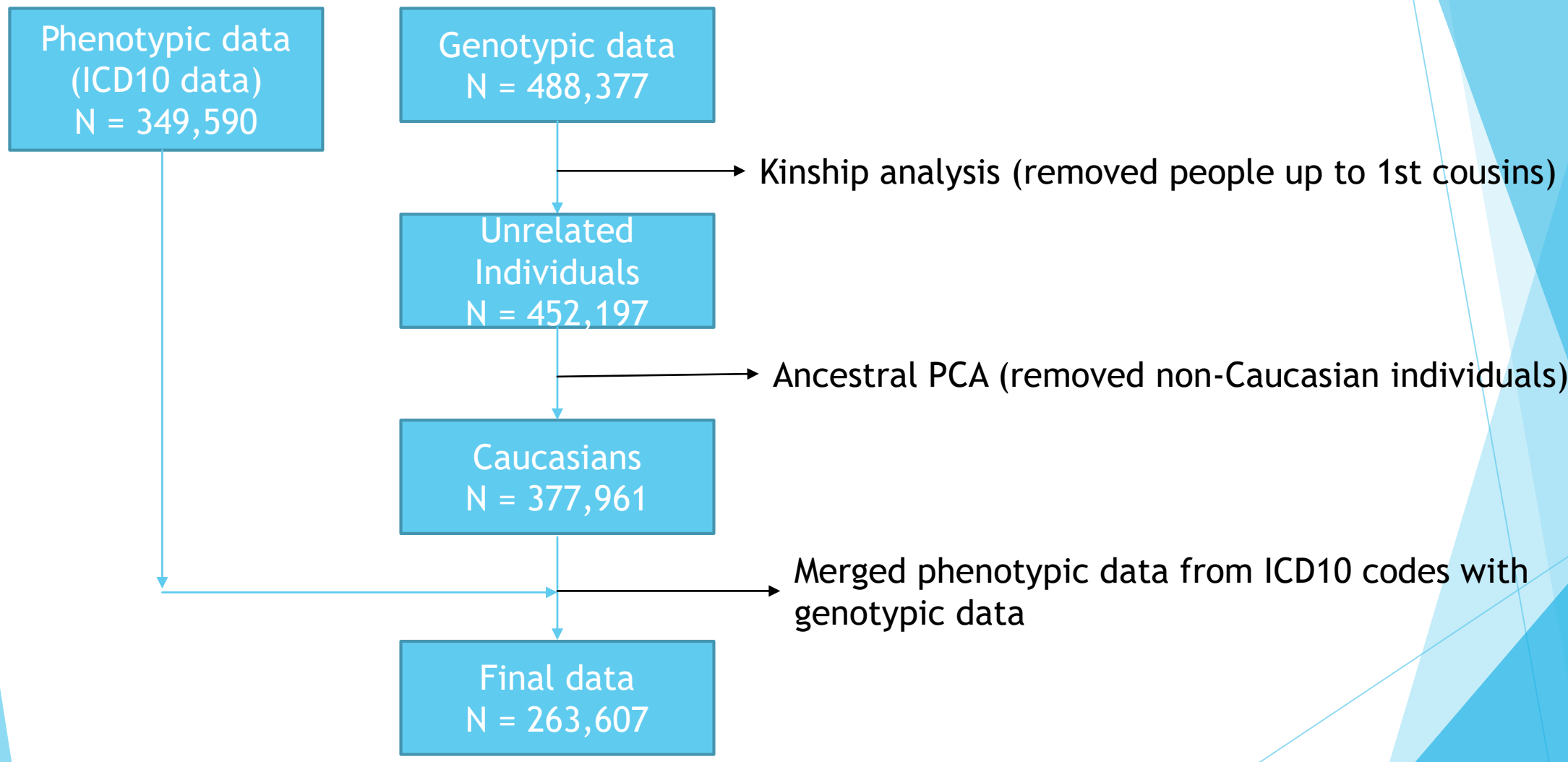
UK Biobank

Cohort

- Approximately 500,000 people aged between 40-69 years in 2006-2010 from across the country (UK).
- Volunteers recruited from England, Scotland and Wales.
- 1511 phenotypes obtained from ICD10 codes (30GB).
- Genotype data (100GB).
  Micro arrays: between 500,000 to 1 million SNPs per person.

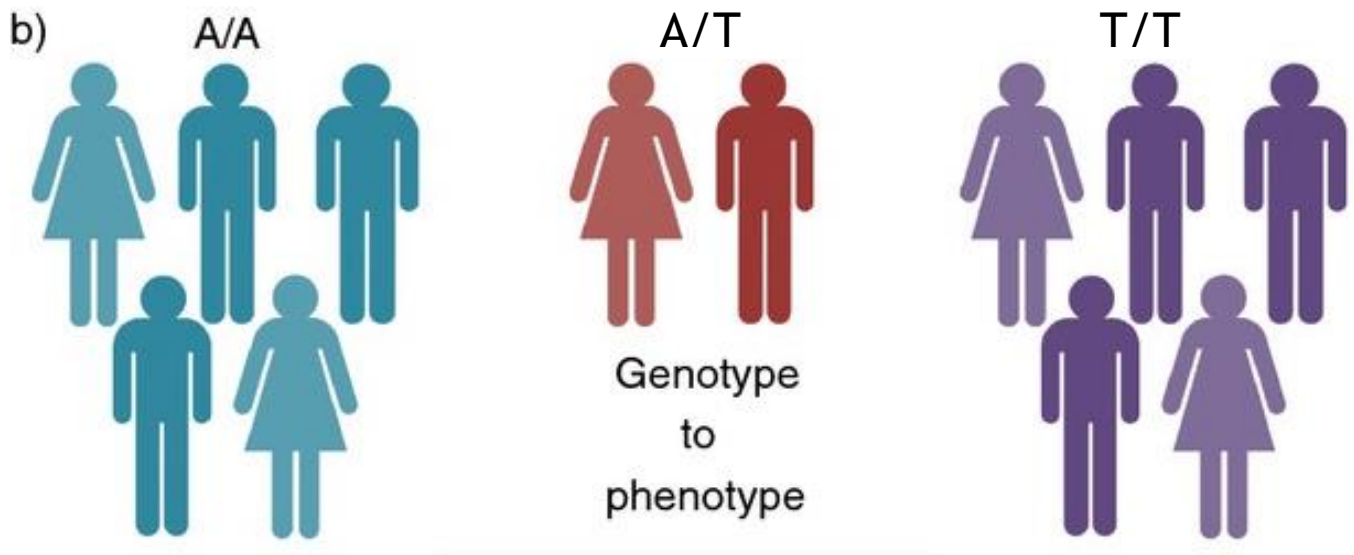
Significant time spent data cleaning (formatting, merging, etc.).

Data Flow Chart for SLC26A9 - SNP rs4077468)



What is a PheWAS?

- PheWAS: Phenome-Wide Association Study
- Tests the association between genetic variants of interest with every phenotype measured.



Association: genotype (G) → all phenotypes (P's)

Phenome-Wide Association Study (PheWAS)

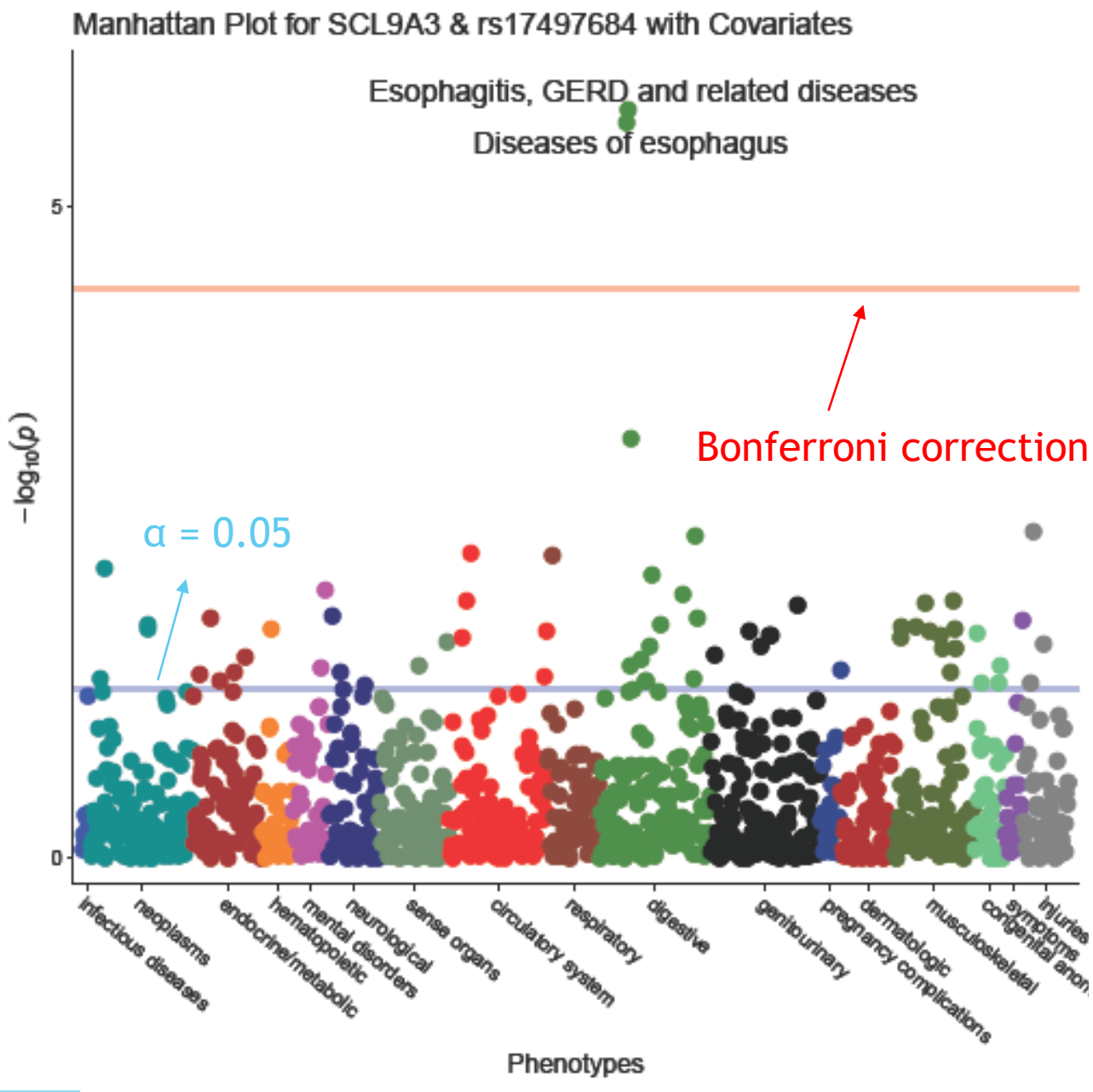
- **Statistical Method: Additive Model for performing PheWAS:**
  - Logit(Phenotype_i) = SLC26A9 + covariates i=1, …,1511

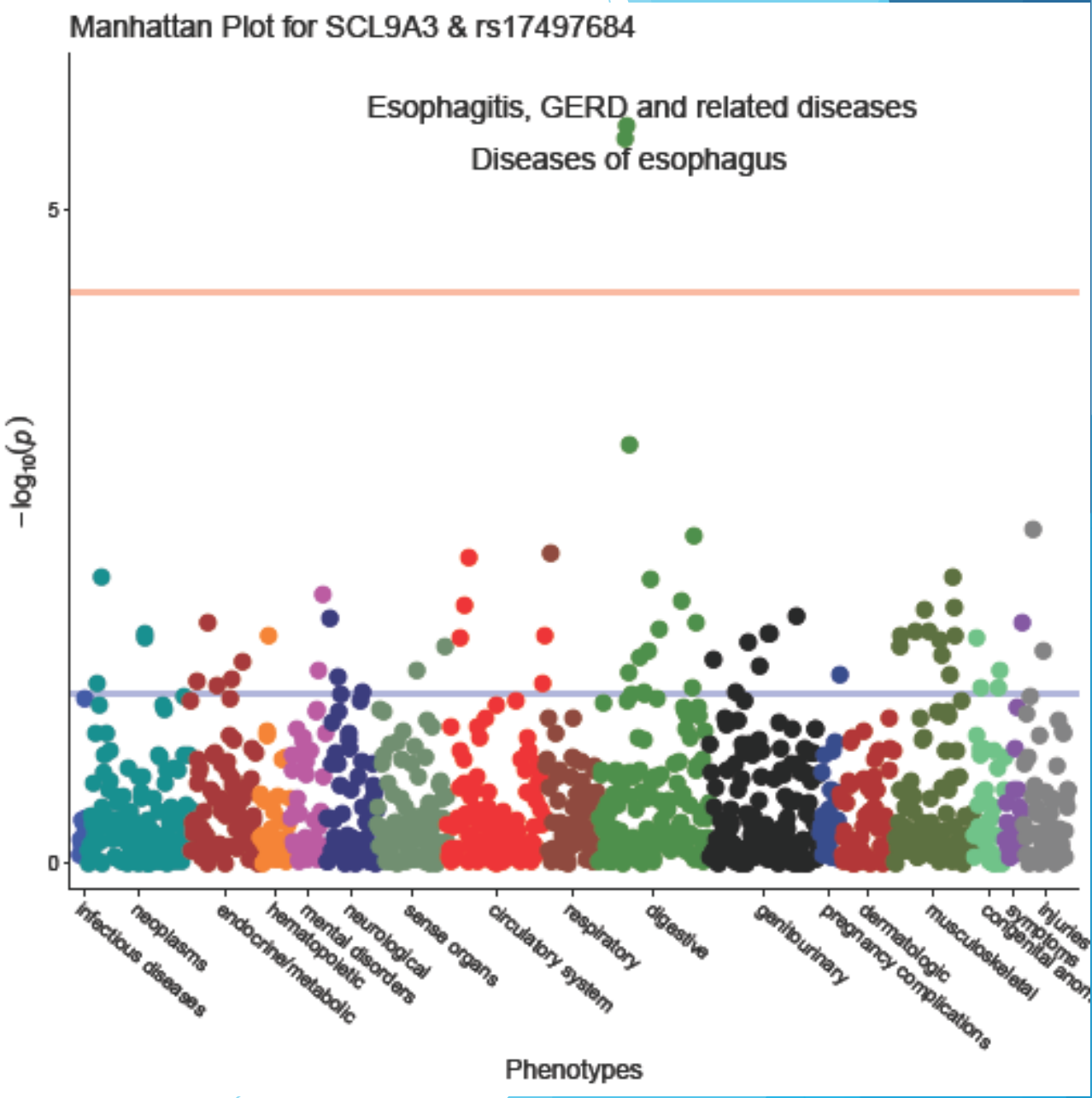
$$\text{Phenotype\_i} = \begin{cases} 0 & \text{if do not have phenotype i} \\ 1 & \text{if have phenotype i} \end{cases}$$

$$\text{SLC26A9} = \begin{cases} 0 & \text{if RS4077468\_AA} \\ 1 & \text{if RS4077468\_AT} \\ 2 & \text{if RS4077468\_TT} \end{cases}$$

  - Perform adjusted and unadjusted logistic regression.
  - Adjusted for covariates: Age, age-squared and sex.
- **Software:**
  - R ("PheWAS/PheWAS" package from github) and PLINK.
  - Linux environment for high performance computing.

SLC9A3 (Chromosome 5 – SNP rs57221529 Substitute: rs17497684; r = 0.821)





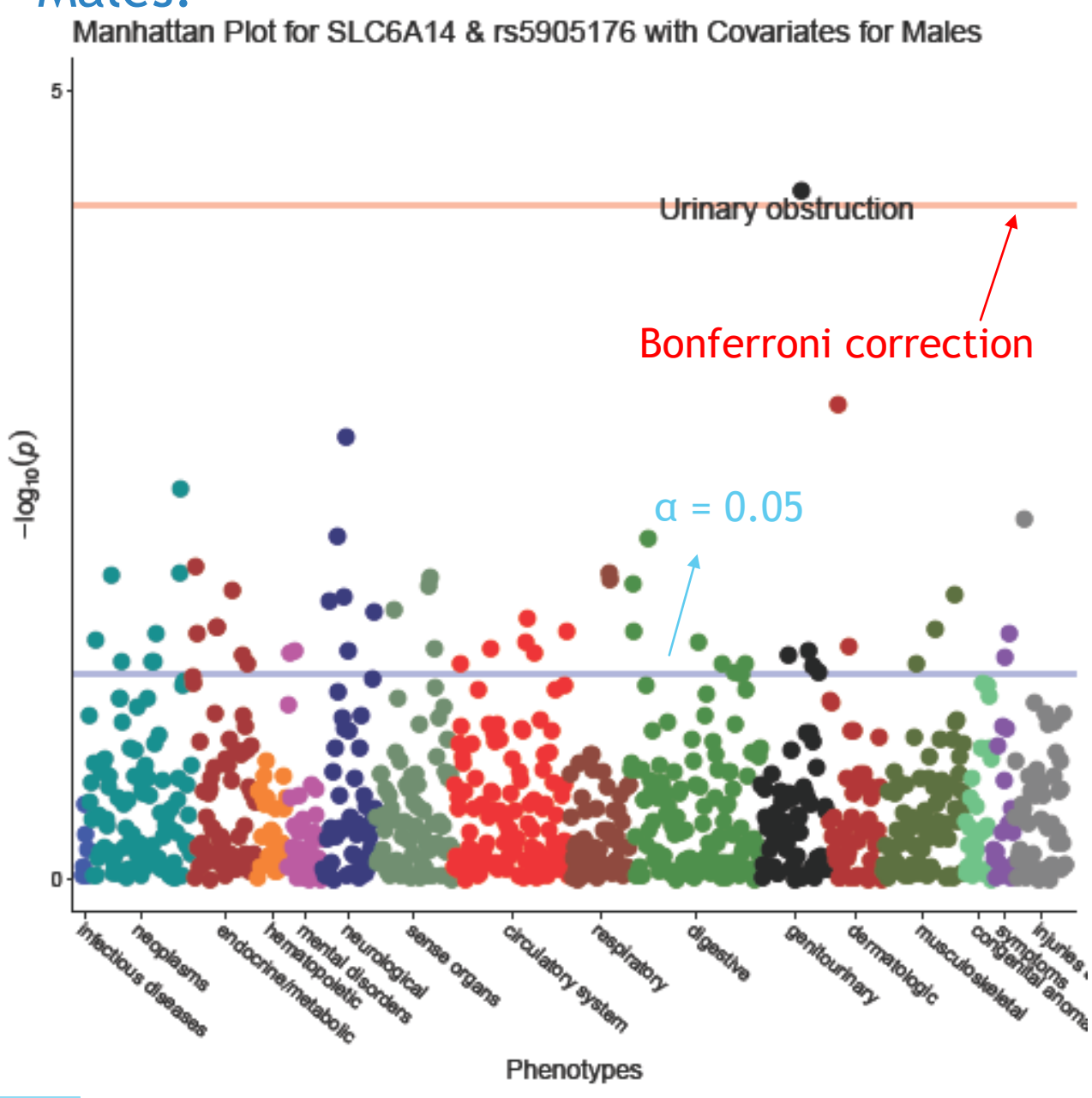
SLC9A3 (Chromosome 5 – SNP rs57221529 Substitute: rs17497684; r = 0.821)

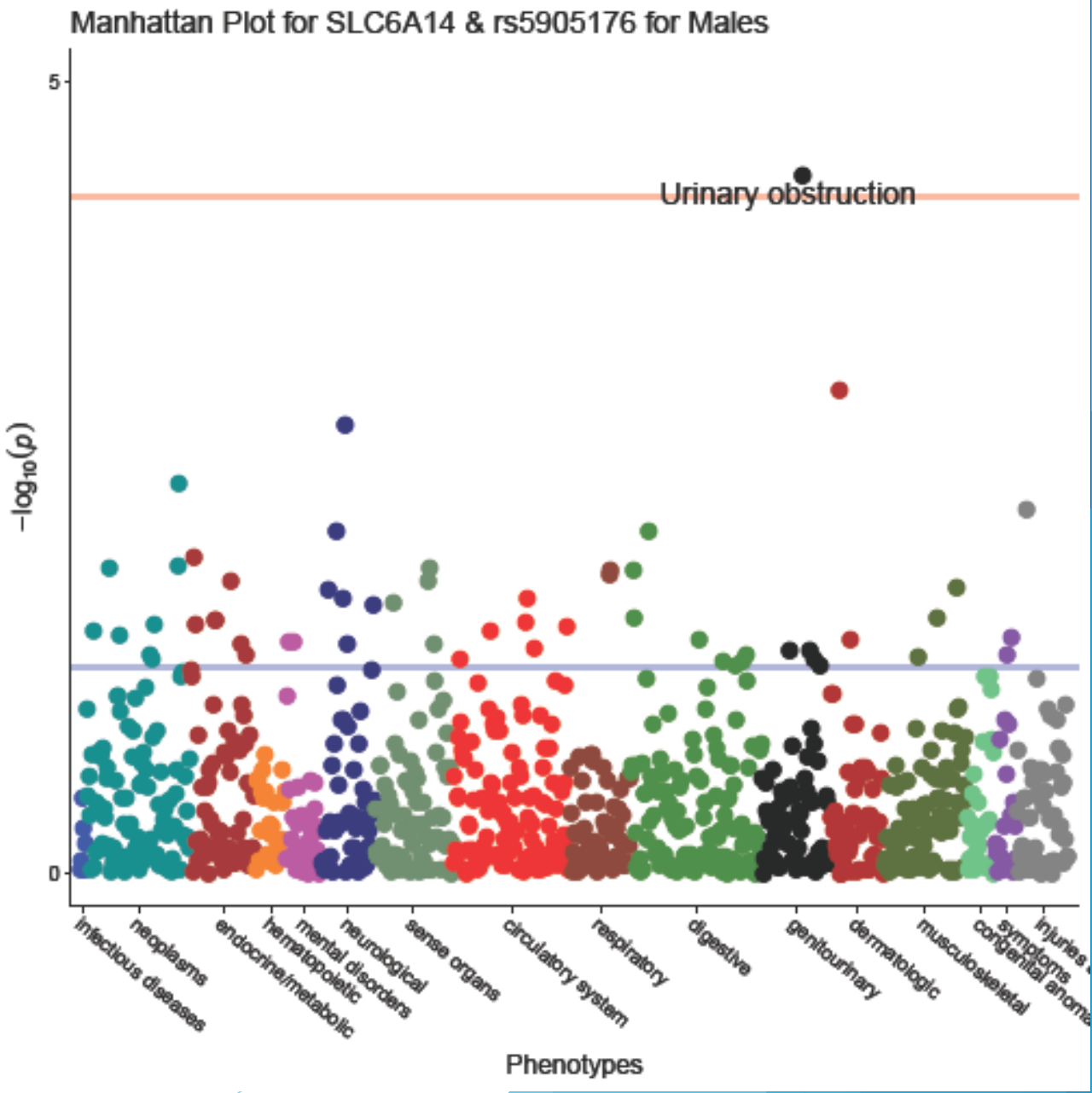
Esophagitis, GERD and related diseases

OR = 1.064
P-value = 1.79E-06
Cases = 19,687
Controls = 243,236

		C Allele Count		
	level	**AA**	**AC**	**CC**
	C Allele Count	**0**	**1**	**2**
n		170050	82784	10089
Esophagitis, GERD and related diseases	Controlls	157647 (92.7)	76284 (92.1)	9305 (92.2)
phecode: 530.1 (%)	Case	12403 (7.3)	6500 (7.9)	784 (7.8)
Diseases of esophagus	Controlls	156593 (92.1)	75759 (91.5)	9241 (91.6)
phecode: 530 (%)	Cases	13457 (7.9)	7025 (8.5)	848 (8.4)
age (mean (SD))		57.85 (7.78)	57.84 (7.79)	57.70 (7.77)
age2 (mean (SD))		3407.06 (871.43)	3406.22 (872.61)	3389.88 (869.40)
SEX (%)	0	94320 (55.5)	45716 (55.2)	5598 (55.5)
	1	75730 (44.5)	37068 (44.8)	4491 (44.5)

SLC6A14 (Chromosome X – SNP rs3788766 Substitute: rs5905176; r = 0.770) for Males.





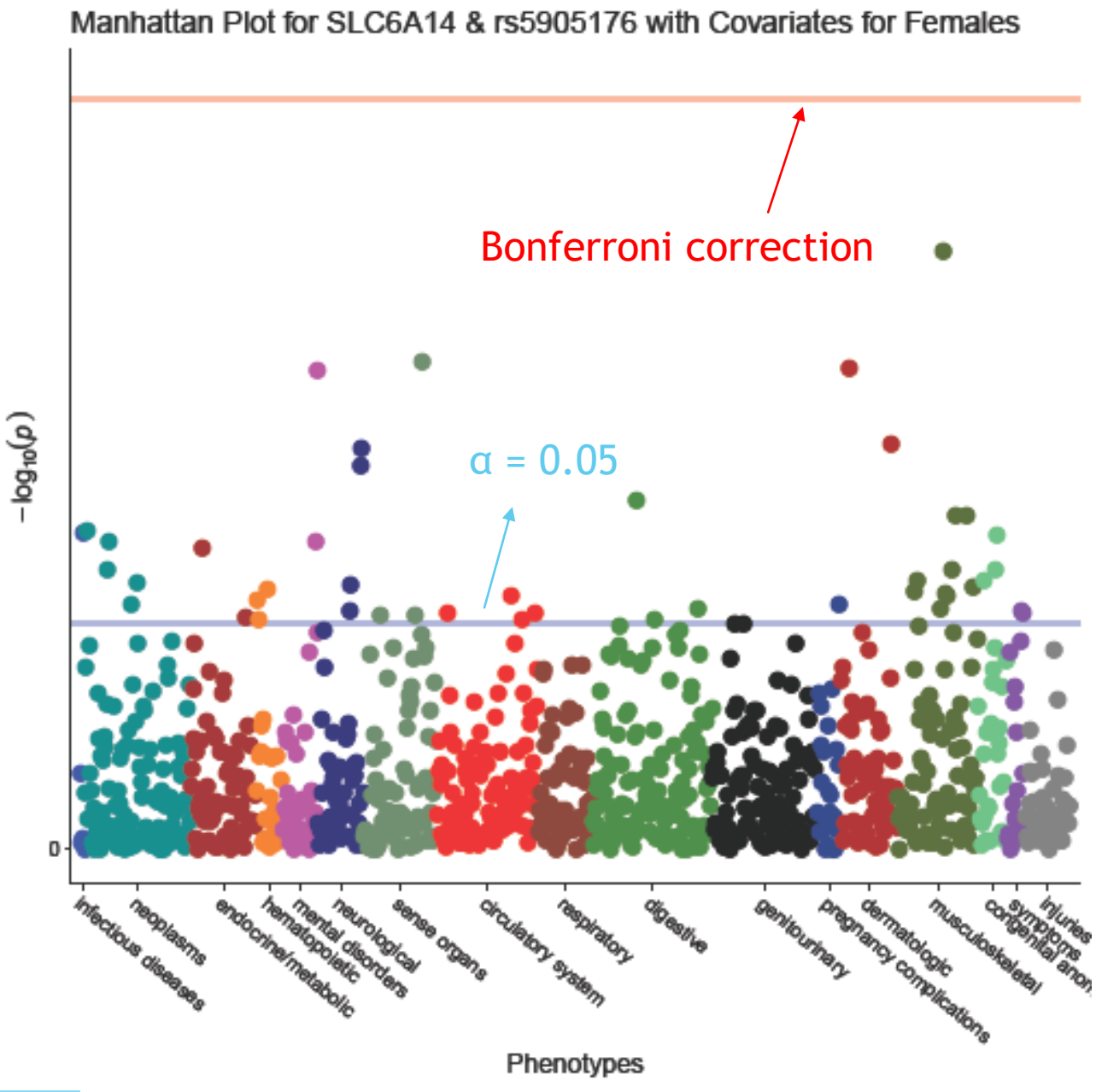
SLC6A14 (Chromosome X – SNP rs3788766 Substitute: rs5905176; r = 0.770) for Males.

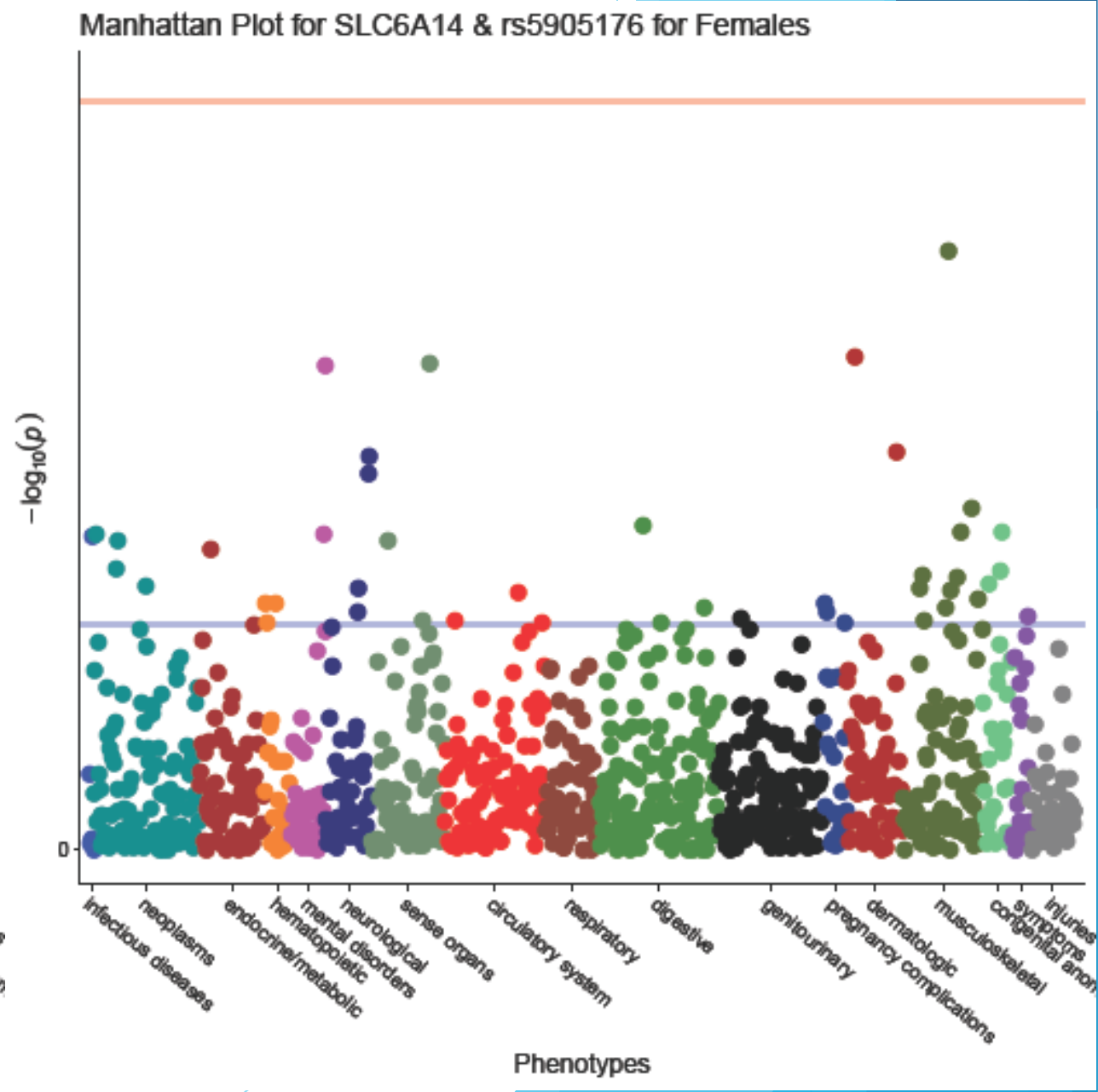
Esophagitis, GERD and related diseases

OR = 1.68
P-value = 4.24E-05
Cases = 64
Controls = 117,334

Phecode			Urinary obstruction		
		level	Controls	Cases	p
n			117334	64	
rs5905176_G (%)	AA	0	79076 (67.4)	27 (42.2)	<0.001
	GG	2	38258 (32.6)	37 (57.8)	
age (mean (SD))			58.44 (7.73)	62.19 (5.96)	<0.001
age2 (mean (SD))			3475.18 (868.88)	3902.25 (706.87)	<0.001

SLC6A14 (Chromosome X – SNP rs3788766 Substitute: rs5905176; r = 0.770) for Females.



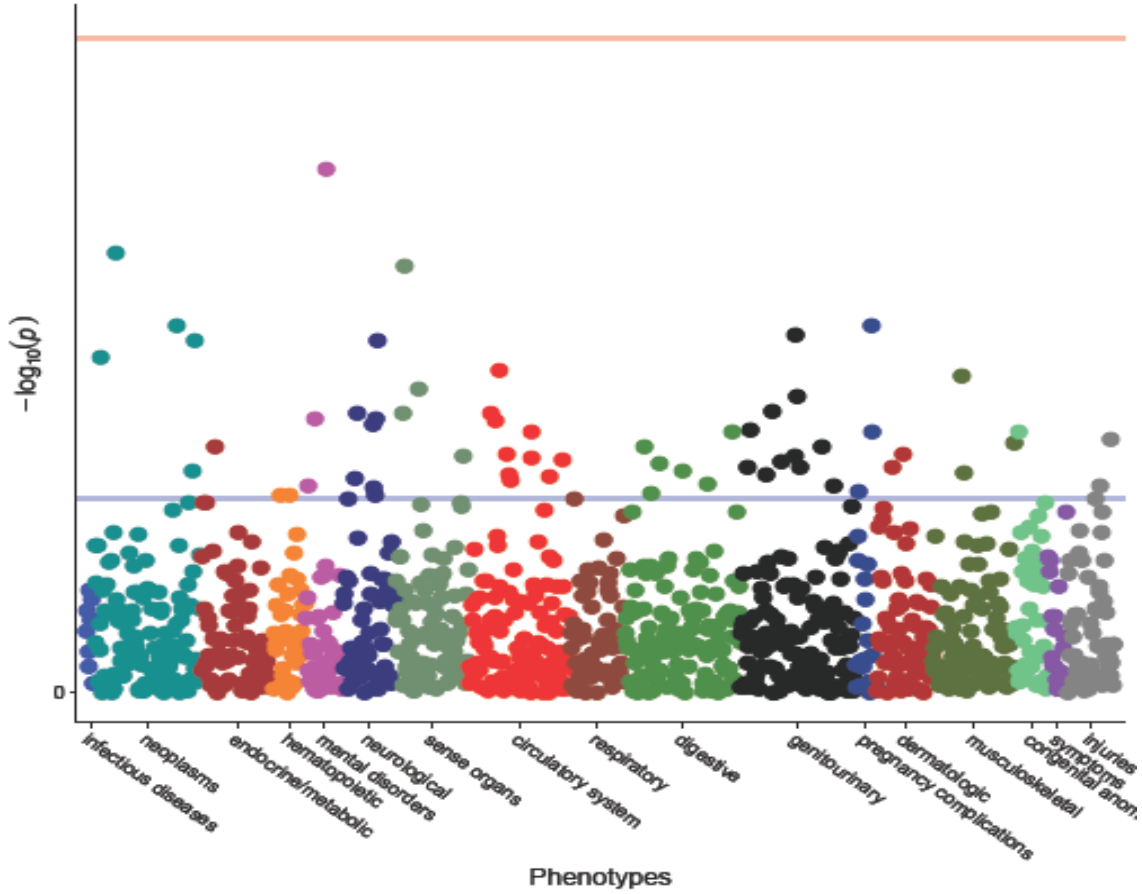


SLC6A14 (Chromosome X – SNP rs3788766 Substitute: rs5905176; r = 0.770) for Females.

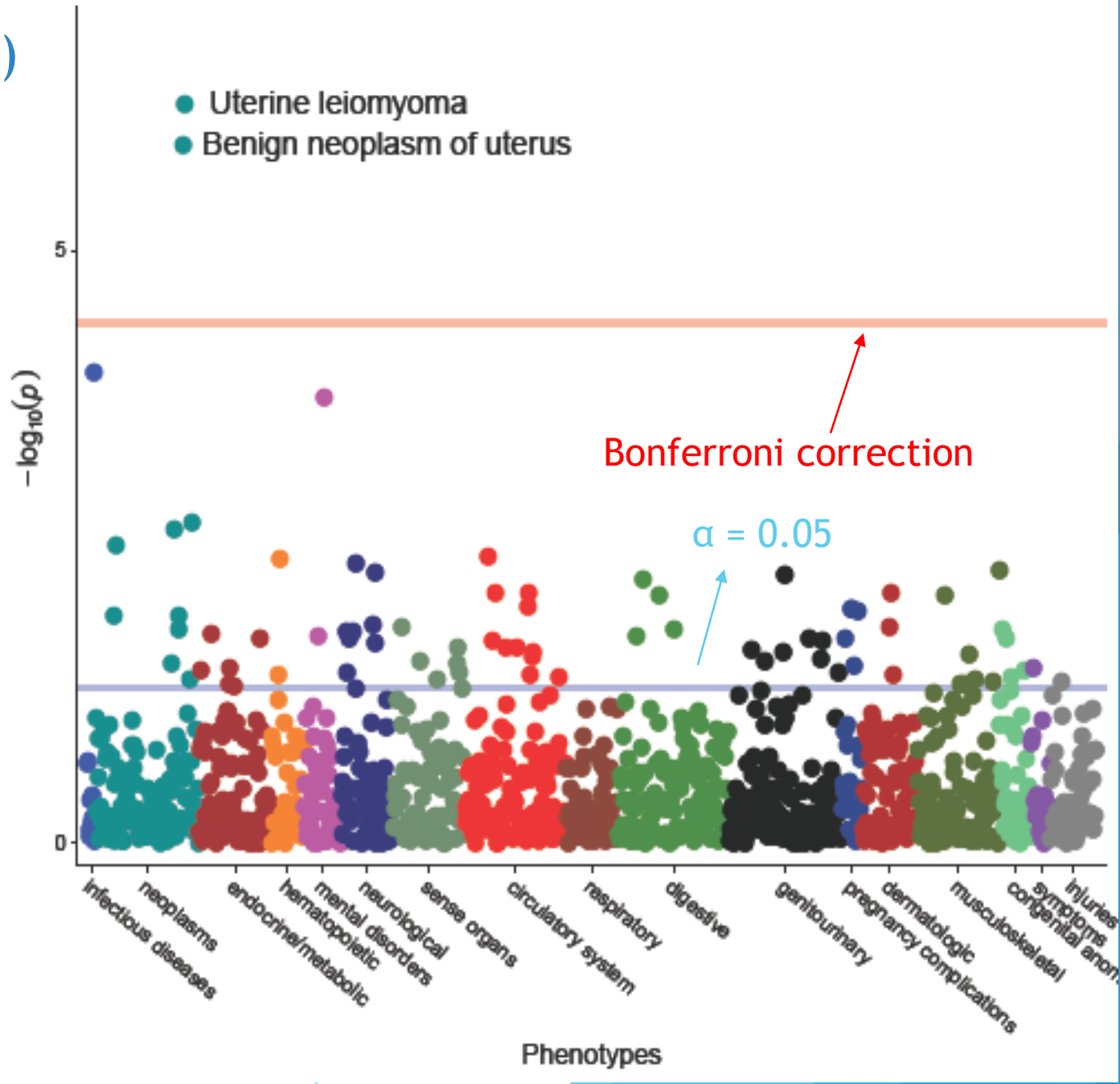
		G Allele Count		
	level	**AA**	**AG**	**GG**
	G Allele Count	**0**	**1**	**2**
n		66154	63693	15658
Urinary obstruction	Controls	66142 (100.0)	63668 (100.0)	15646 (99.9)
phecode: 733.6 (%)	Cases	12 (0.0)	25 (0.0)	12 (0.1)
age (mean (SD))		57.43 (7.77)	57.31 (7.83)	57.21 (7.80)
age2 (mean (SD))		3358.36 (867.71)	3345.77 (872.86)	3333.50 (868.15)

SLC26A9 (Chromosome 1 - SNP rs4077468 Substitute: rs4077469; r = 1)

Manhattan Plot for rs4077469 with Covariates



Manhattan Plot for rs4077469 without Covariates



Future Work

- Instead of using additive model to perform the PheWAS, use a genotypic model (treat allele count as categorical variable).
- Included interaction term between allele count and sex.
- Use curated phenotypic data
  - Lung function
- Validating UK biobank data set by replicating previously published PheWAS studies results.

Some Challenges and Limitations.

- Multiple testing.
  - 1511 phenotypes
  - Experimental wide α of 0·05
  - Bonferroni correction: $P < 2{\cdot}5E\text{-}5$
- Missing data (phenotype & genotype).
- Converting ICD10 codes to phenotypic codes.
- Relatedness, kinship analysis.
- Computing the ancestral PCA.
- Implementing all of this in a HPF.

Conclusion

- Results suggest that there maybe an association between gene SLC9A3 near SNP rs57221529, and having Esophagitis, GERD and related diseases. Further, every additional C allele increases the odds by about 6.4% of having the related diseases in an individual.
- However, further research work is required.

Questions?

Additional Slides

Phenome-Wide Association Study (PheWAS)

- **Statistical Method: "*Genotypic*" Model for performing PheWAS:**
  - Logit(Phenotype_i) = intercept + I(RS4077468_AT) + I(RS4077468_TT) + covariates

$$i=1, \ldots,1511 , \quad SLC26A9 = \begin{cases} 0 & \text{if RS4077468\_AA (reference)} \\ 1 & \text{if RS4077468\_AT} \\ 2 & \text{if RS4077468\_TT} \end{cases}$$

  - Perform adjusted and unadjusted logistic regression.
  - Adjusted for covariates: Age, age-squared and sex.

- **Software:**
  - R ("PheWAS/PheWAS" package from github) and PLINK.
  - Linux environment for high performance computing.

PheWAS Manhattan Plot

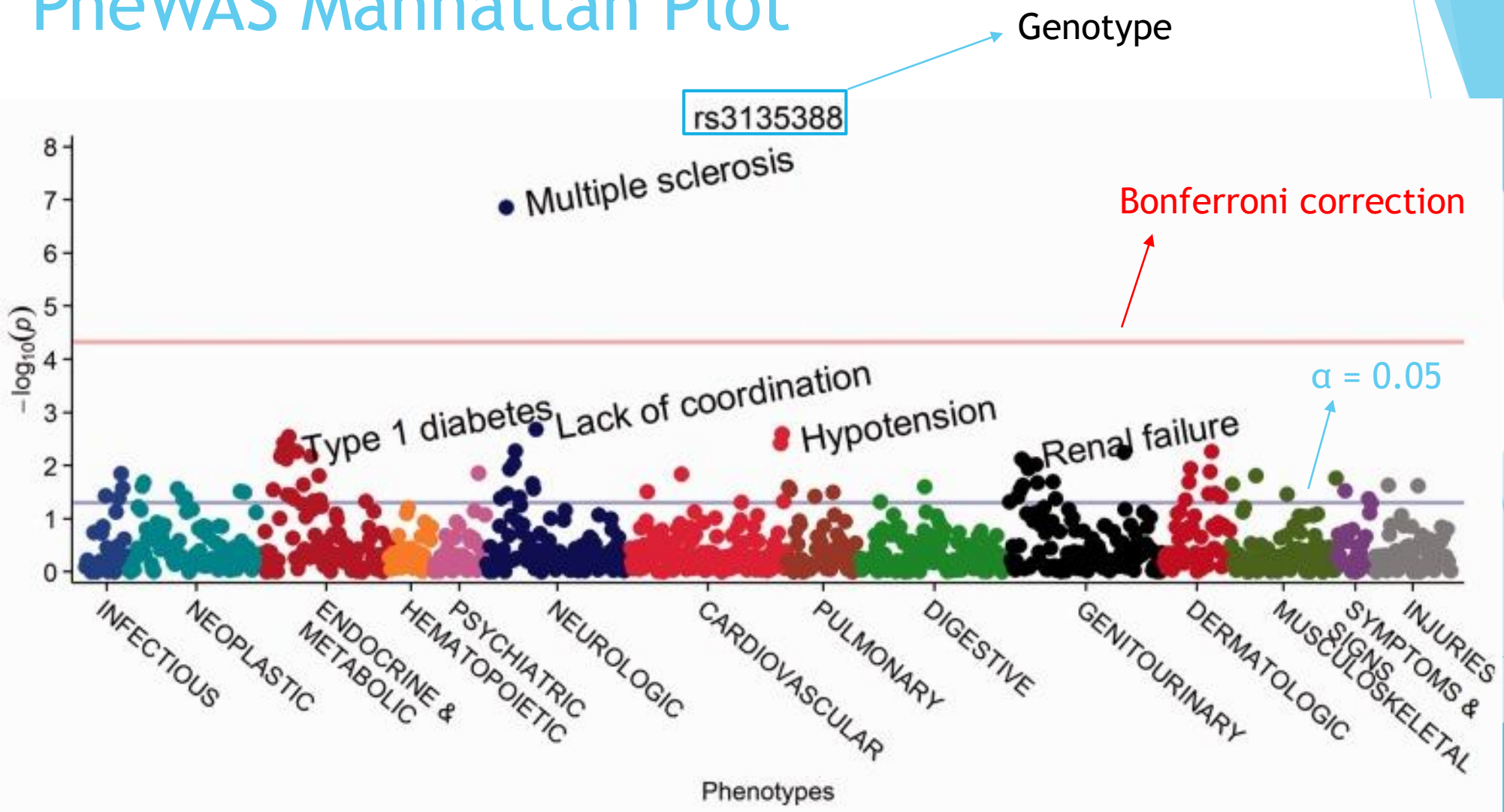


Figure 5. PheWAS Manhattan plot for rs3135388, with phenotypes ordered by PheWAS code. (Carrol et al. 2014)

Detailed Results for Multivariable Analysis.

SLC9A3 (Chromosome 5 – SNP rs57221529 Substitute: rs17497684; r = 0.821)

phecode	description	group	snp	beta	SE	OR	p	type	n_total	n_cases	n_controls	HWE_p	allele_freq	n_no_snp	bonferroni
530.1	Esophagitis, GERD and related diseases	digestive	rs17497684_C	0.062196	0.013023	1.064171	1.79E-06	logistic	262923	19687	243236	0.087651	0.195803	684	TRUE
530	Diseases of esophagus	digestive	rs17497684_C	0.059461	0.012569	1.061265	2.23E-06	logistic	262923	21330	241593	0.087651	0.195803	684	TRUE

		C Allele Count		
	level	AA	AC	CC
	C Allele Count	0	1	2
n		170050	82784	10089
Esophagitis, GERD and related diseases	Controlls	157647 (92.7)	76284 (92.1)	9305 (92.2)
phecode: 530.1 (%)	Case	12403 (7.3)	6500 (7.9)	784 (7.8)
Diseases of esophagus	Controlls	156593 (92.1)	75759 (91.5)	9241 (91.6)
phecode: 530 (%)	Cases	13457 (7.9)	7025 (8.5)	848 (8.4)
age (mean (SD))		57.85 (7.78)	57.84 (7.79)	57.70 (7.77)
age2 (mean (SD))		3407.06 (871.43)	3406.22 (872.61)	3389.88 (869.40)
SEX (%)	0	94320 (55.5)	45716 (55.2)	5598 (55.5)
	1	75730 (44.5)	37068 (44.8)	4491 (44.5)

SLC6A14 (Chromosome X – SNP rs3788766 Substitute: rs5905176; r = 0.770) for Males.

phecode	description	group	snp	beta	SE	OR	p	type	n_total	n_cases	n_controls	HWE_p	allele_freq	n_no_snp	bonferroni
599.1	Urinary obstruction	genitourinary	rs5905176_G	0.518311	0.126602	1.679189	4.24E-05	logistic	117398	64	117334	1	0.326198	194	TRUE

Phecode		Urinary obstruction		
	level	Controls	Cases	p
n		117334	64	
rs5905176_G (%)	0	79076 (67.4)	27 (42.2)	<0.001
	2	38258 (32.6)	37 (57.8)	
Urinary obstruction		117334 (100.0)	0 (0.0)	<0.001
phecode: 599.1 (%)	TRUE	0 (0.0)	64 (100.0)	
Disorder of skin and subcutaneous tissue NO	FALSE	115583 (98.5)	64 (100.0)	0.639
phecode: 689 (%)	TRUE	1751 (1.5)	0 (0.0)	
age (mean (SD))		58.44 (7.73)	62.19 (5.96)	<0.001
age2 (mean (SD))		3475.18 (868.88)	3902.25 (706.87)	<0.001

Diseases

- **Gastroesophageal reflux disease**, or **GERD**, is a digestive disorder that affects the lower esophageal sphincter (LES), the ring of muscle between the esophagus and stomach. Many people, including pregnant women, suffer from heartburn or acid indigestion caused by **GERD**.
- **Esophagitis** (uh-sof-uh-JIE-tis) is inflammation that may damage tissues of the esophagus, the muscular tube that delivers food from your mouth to your stomach.**Esophagitis** can cause painful, difficult swallowing and chest pain.

UK Biobank (Variables)

- Baseline characteristics
- Blood count
- Blood count processing
- Blood pressure
- Blood sample collection
- Body size measures
- Bone-densitometry of heel
- Bread/pasta/rice yesterday
- Breathing
- Cancer register
- Cancer screening
- Cannabis use
- Cereal yesterday
- Chest pain
- Claudication and peripheral artery disease
- Consent
- Consent timings and usage
- Death register
- Depression
- Diet
- Diet by 24-hour recall

Baseline characteristics

Field ID	Field title
21022	Age at recruitment
52	Month of birth
31	Sex
189	Townsend deprivation ind
34	Year of birth

- Blood count
- Blood count processing
- Blood pressure
- Blood sample collection
- Body size measures
- Bone-densitometry of heel
- Bread/pasta/rice yesterday
- Breathing
- Cancer register