INSPIRED
INdia-Scotland Partnership for
pRecision mEdicine in Diabetes

Heterogeneity in genetic architecture for HDL- Cholesterol between
Scottish and South Indian population with type 2 diabetes

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Funding Acknowledgement

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What we know??

- Type-2 diabetes mellitus (T2DM) patients have a substantially higher risk of cardiovascular morbidity compared to the non-diabetics and are disproportionately affected in LIC compared with MIC and HIC and by ethnicity such as Asian and Caucasian. [1,2]

- High-density lipoprotein cholesterol (HDL-c) has a protective effect against cardiovascular diseases. [3]

- A number of genetic polymorphisms influencing HDL-c levels such as *CETP, PCSK9* and *LPL*. [4]

- Preliminary data suggested that HDL-c profile between the two populations [Scottish (1.20±0.33) and South Indian (1.04±0.23)] were significantly different (p value <0.001). [5]

1. Anjana RM, Mohan V, Rangarajan S, et al. Contrasting Associations Between Diabetes and Cardiovascular Mortality Rates in Low-, Middle-, and High-Income Countries: Cohort Study Data From 143,567 Individuals in 21 Countries in the PURE Study. *Diabetes Care* 2020; dc200886.
2. Einarson TR, Acs A, Ludwig C, Panton UH. Prevalence of cardiovascular disease in type 2 diabetes: a systematic literature review of scientific evidence from across the world in 2007-2017. Cardiovasc Diabetol. 2018;17:83.
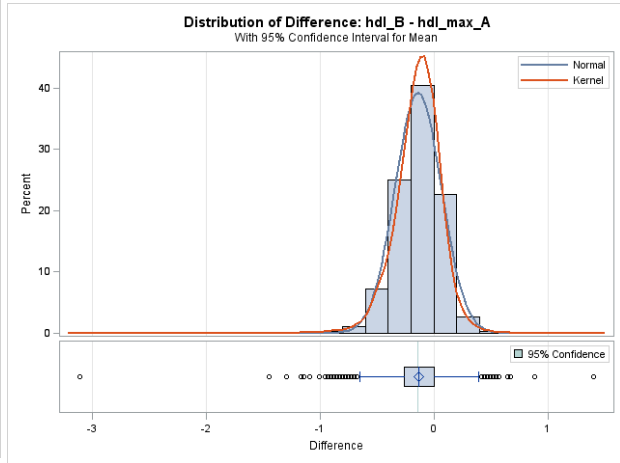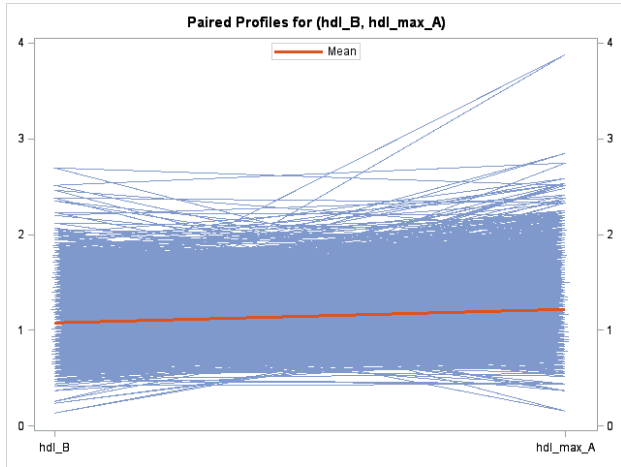3. Kontush A. HDL-mediated mechanisms of protection in cardiovascular disease. Cardiovasc Res. 2014;103:341–9.
4. Liu DJ, Peloso GM, Yu H, Butterworth AS, Wang X, Mahajan A, et al. Exome-wide association study of plasma lipids in 300,000 individuals. Nat Genet.2017;49:1758.
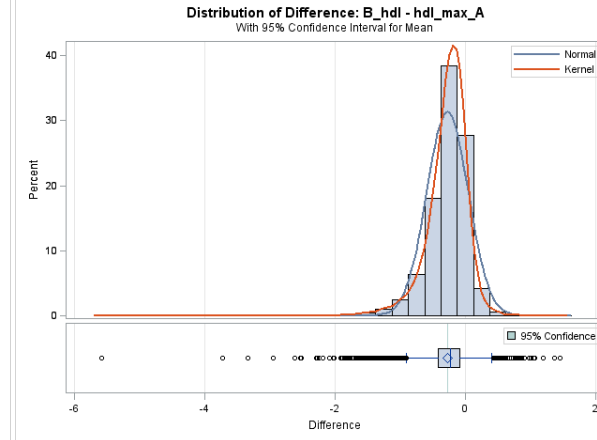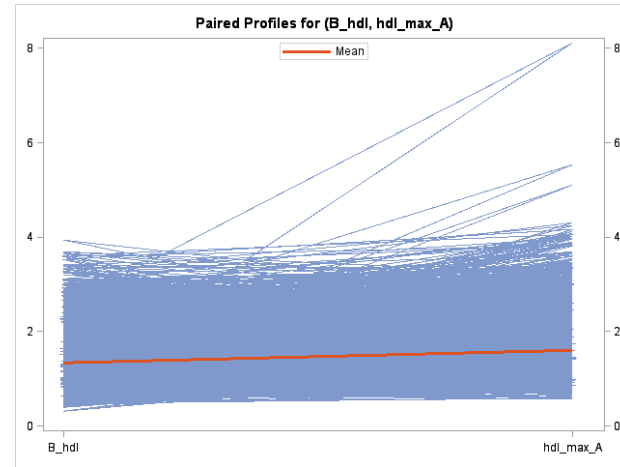5. INSPIRED WP1 – Unpublished data provided by MK Siddiqui

What we saw earlier??

South Indian

Scottish

HDL-c description (n =4,315); Male = 2615 (60.6%)					
Variable	Mean	Std Dev	Median	Minimum	Maximum
HDL (Baseline)	1.08	0.25	1.06	0.13	2.69
HDL (After)	1.21	0.29	1.16	0.16	3.88

HDL-c description (n= 10,633), Male = 5958(56.03%)					
Variable	Mean	Std Dev	Median	Minimum	Maximum
Hdl (Baseline)	1.33	0.409	1.26	0.31	3.93
Hdl (After)	1.60	0.48	1.51	0.58	8.11

Difference between Before and After HDL value (Paired T test)						
N	Mean	Std Dev	Min	Max	t Value	P value
4,315	-0.14	0.20	-3.10	1.40	-43.83	<.0001

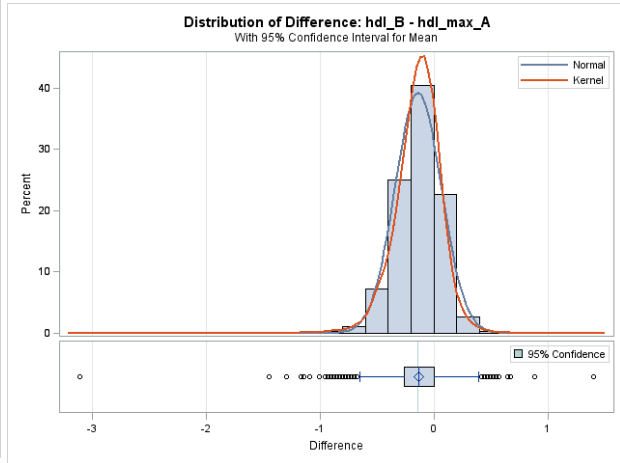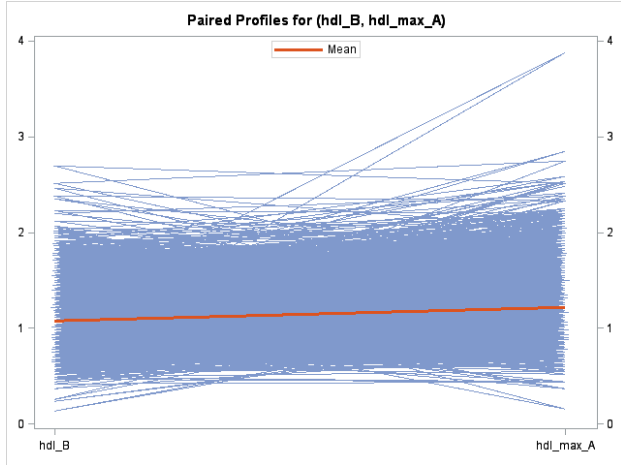| Difference between Before and After HDL value (Paired T test)						
N	Mean	Std Dev	Min	Max	t Value	P value
10,633	-0.27	0.32	-5.58	1.46	-87.97	<.0001

12.6 % increase in HDL-c after statin initiation

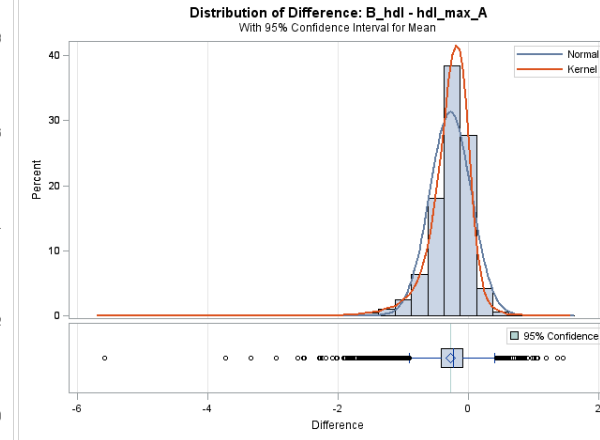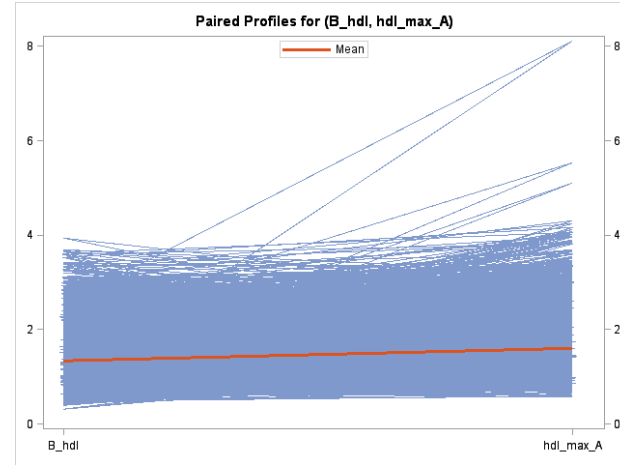20.3 % increase in HDL-c after statin initiation

*Unit mmol/l

What we find earlier??

South Indian

Scottish

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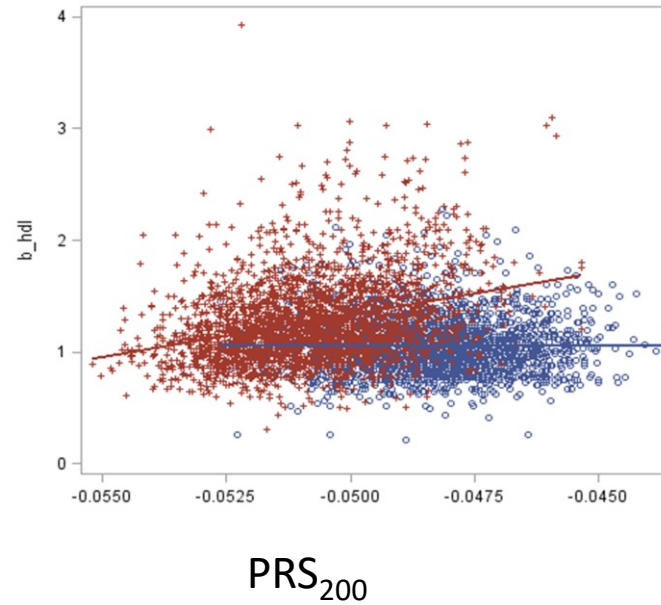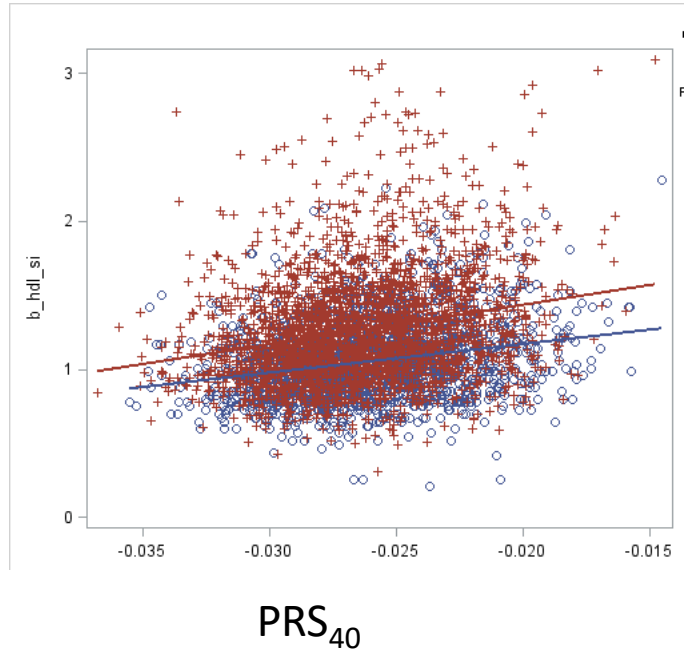| N	Mean	Std Dev	Min	Max	t Value	P value
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12.6 % increase in HDL-c after statin initiation

20.3 % increase in HDL-c after statin initiation

*Unit mmol/l

Also... from earlier work !!!!
Regression of Baseline HDL-c (a) with Polygenic risk score (PRS_{40} and PRS_{200})

Population 1 – South Indian (India)
Population 2- Scottish (UK)

PRS_{40}

PRS_{200}

- Genetic loci discovered for HDL-c in Scottish populations are present at similar frequency in South Indian populations, however the level of HDL-c modulated by these variants is lower.

- This Preliminary results suggests that additional population-specific genetic variation and selective resistance may exist for low HDL-c levels and lower HDL-c response observed in the South Indians.

Our objectives….

- To identify genetic variants for HDL-c levels among South Indian and Scottish Population.

- To study the heterogeneity of effects of genetic variants and Genes for HDL-c among the study population

- To estimate and compare the polygenic risk score (PRS) for HDL-c among the study population

Study Methodology

| Sections	Description
Study design:	Retrospective cohort study
Study site:	Dundee(UK) and Chennai (India)
Study data:	For Epidemiology : Cohort of Tayside(UK) and MDRF[*] (India) For Genetics : GoDARTS (UK), GoSHARE(UK), and MDRF (India)
Data Source:	Electronic Medical Records
Study population :	Lipid lowering (Statins) drug users in the cohort

MDRF- Madras Diabetes Research Foundation, Chennai, India

Study tools

Order	Analysis	Software used
1	GWAS	snptest , BOLT-LMM
2	Conditional analysis	snptest, GCTA
3	Meta analysis	GWAMA
4	Heterogeneity Analysis (Q , I^2)	GWAMA, MANTRA
5	Annotation, Visualisation, and functional consequences of genes	FUMA, LocusZoom
6	Gene based test / gene set analysis	MAGMA (provided by FUMA)
7	Polygenic risk score (PRS)	PRSice-2

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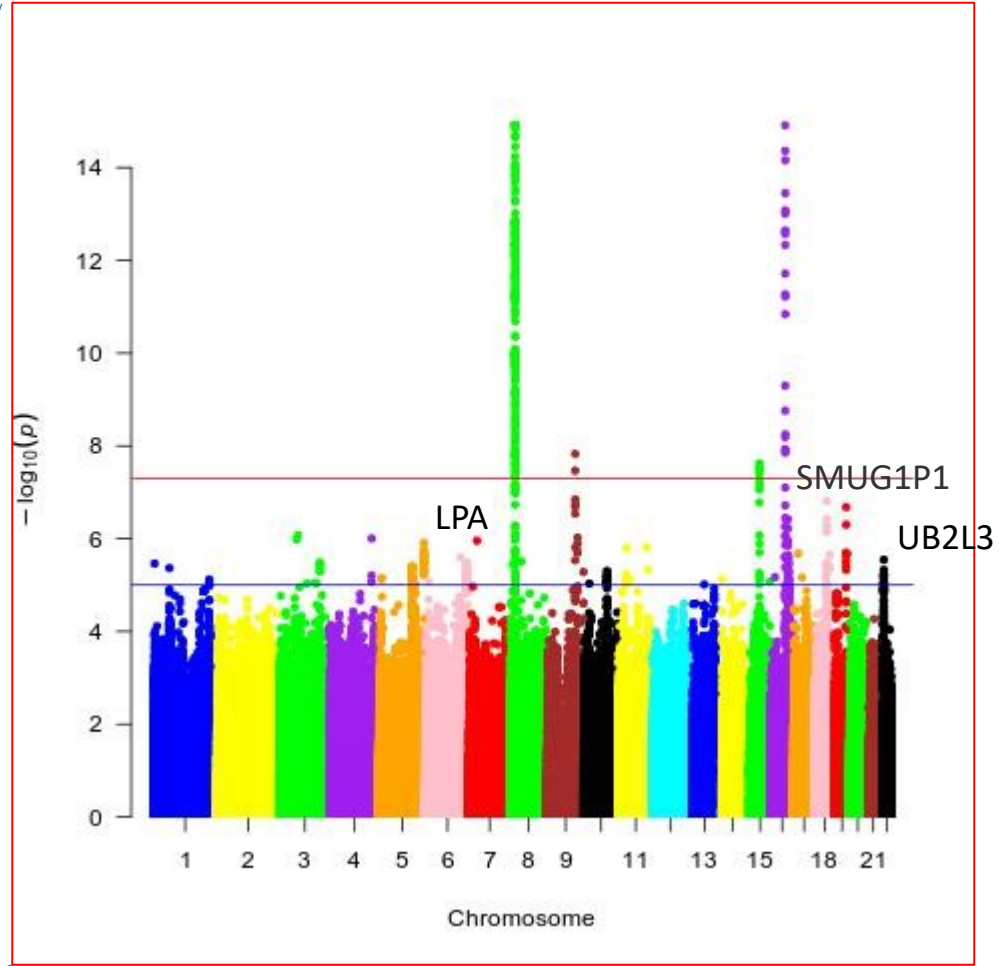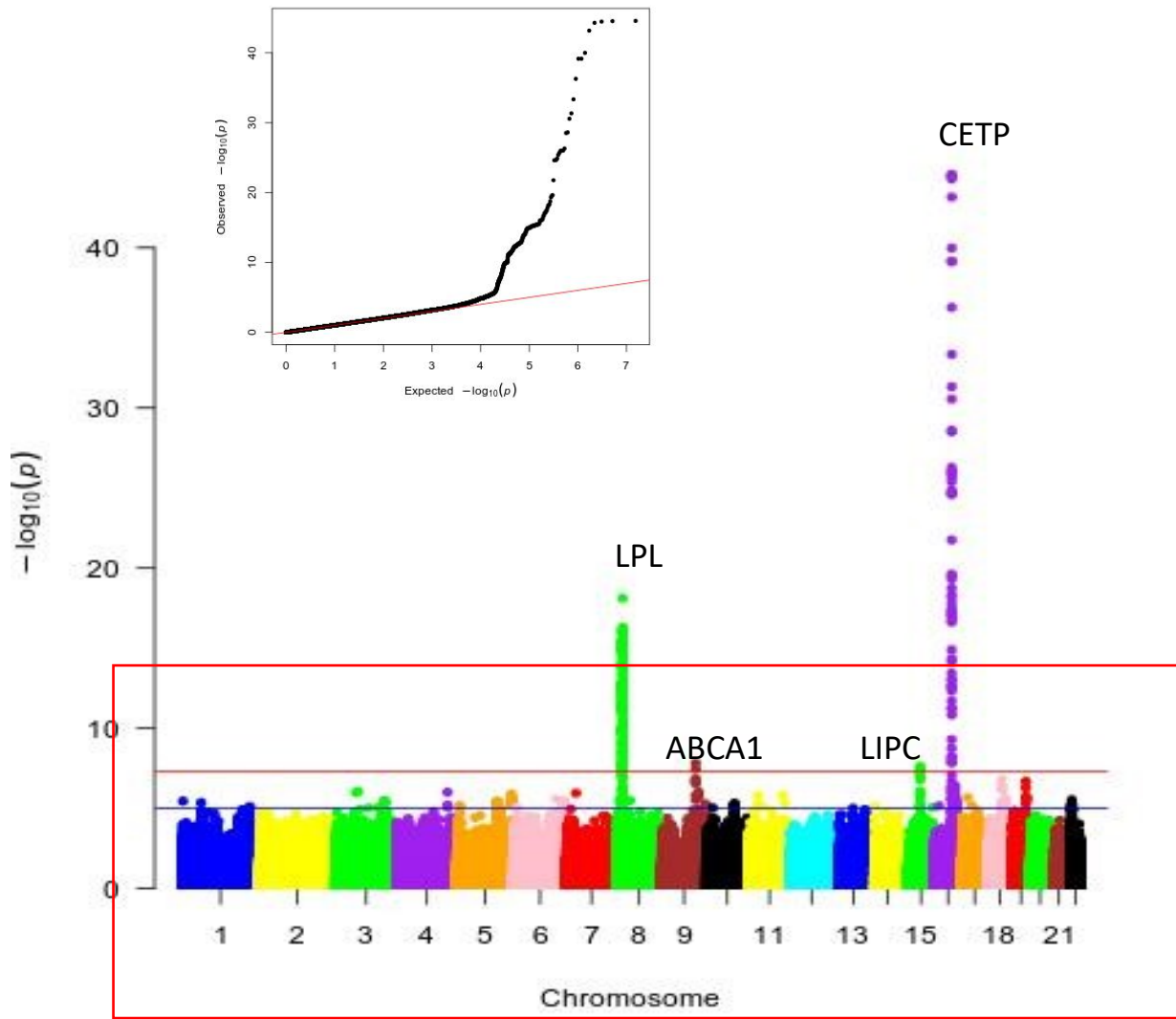- To estimate and compare the polygenic risk score (PRS) for HDL-c among the study population

Manhattan plot and QQ plot of HDL-c: Meta analysis of Scottish T2D population* (n=12,043)
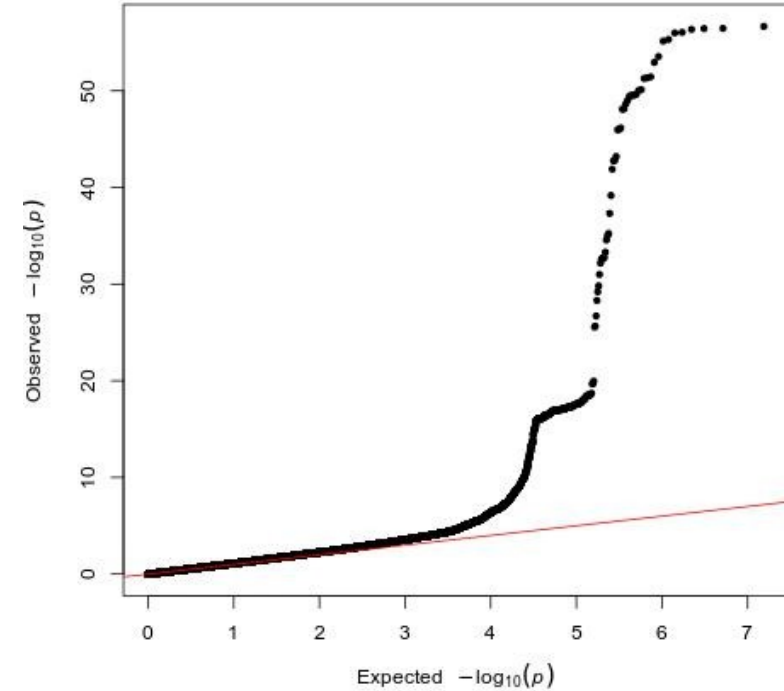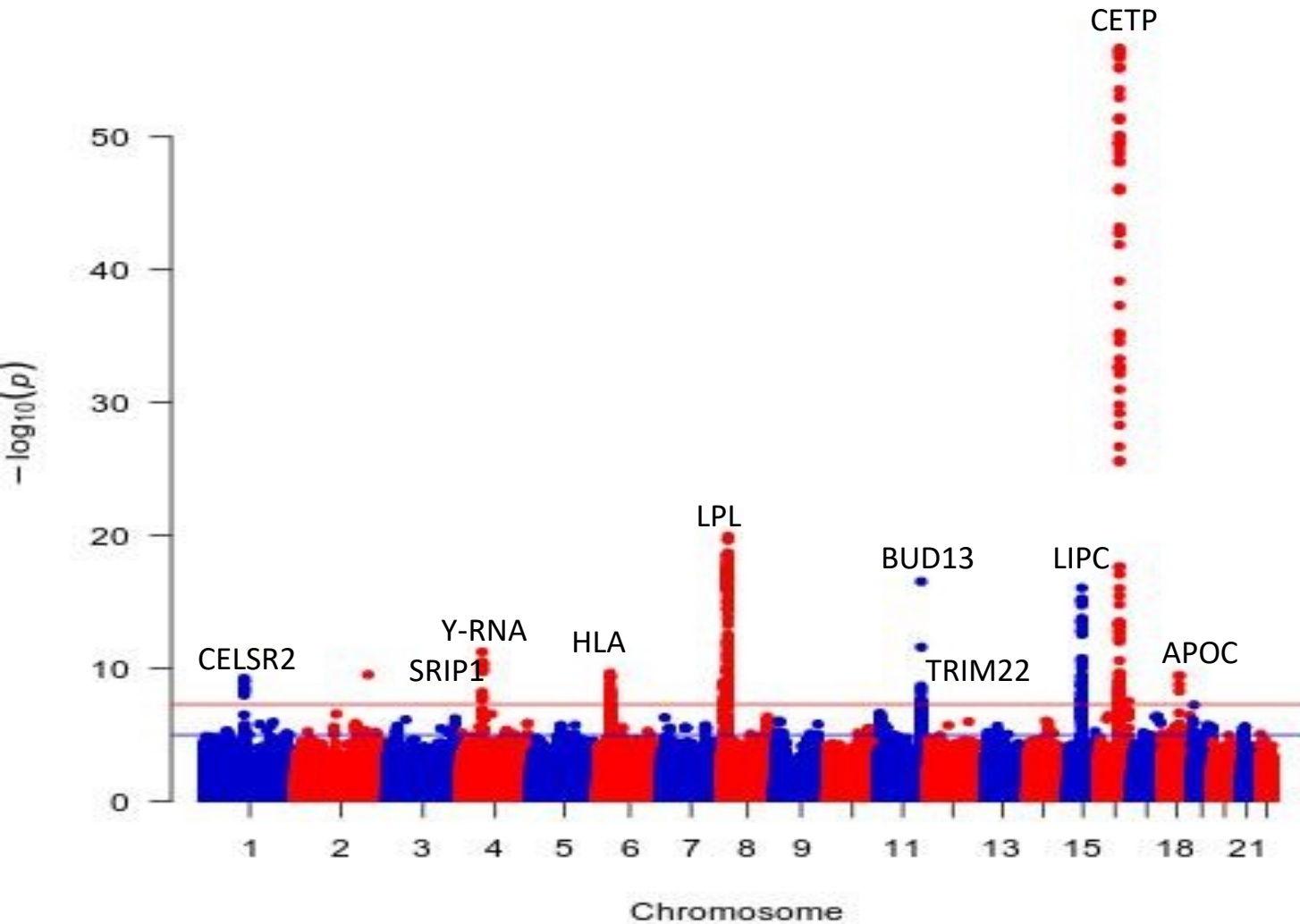
Adjusted for Age and Sex

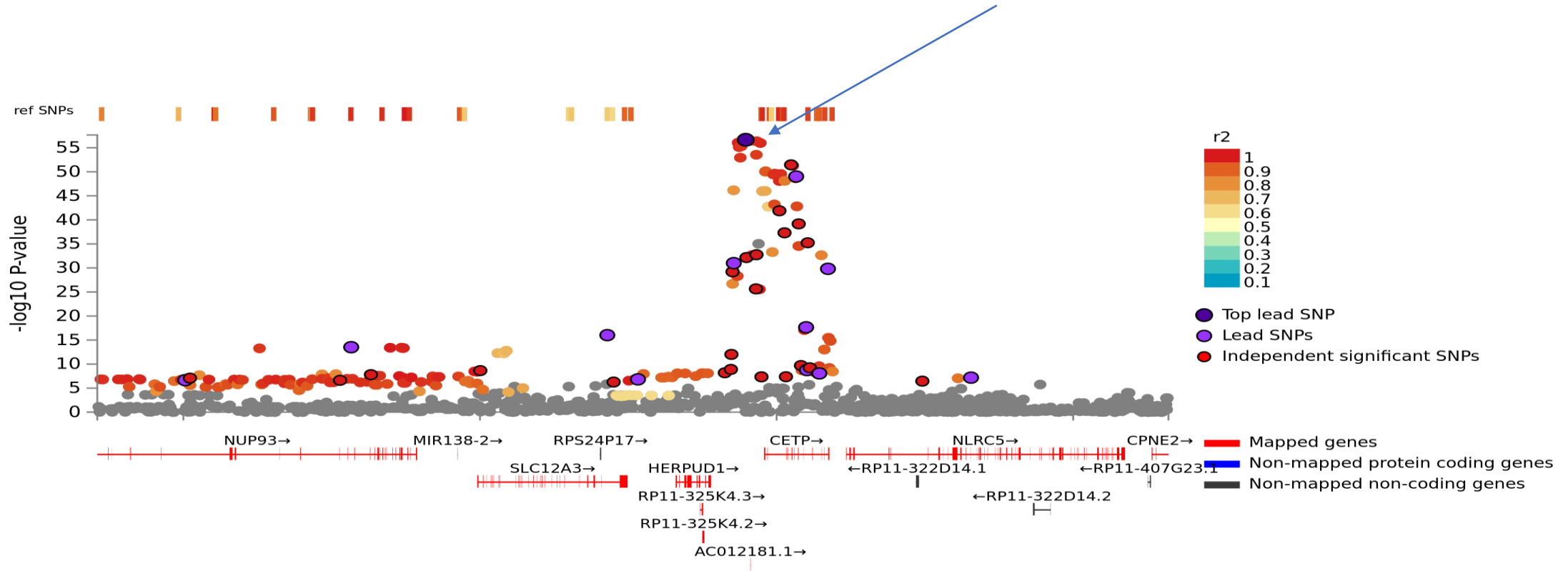# Manhattan plot and QQ plot of HDL-c: Meta Analysis of South Indian population*

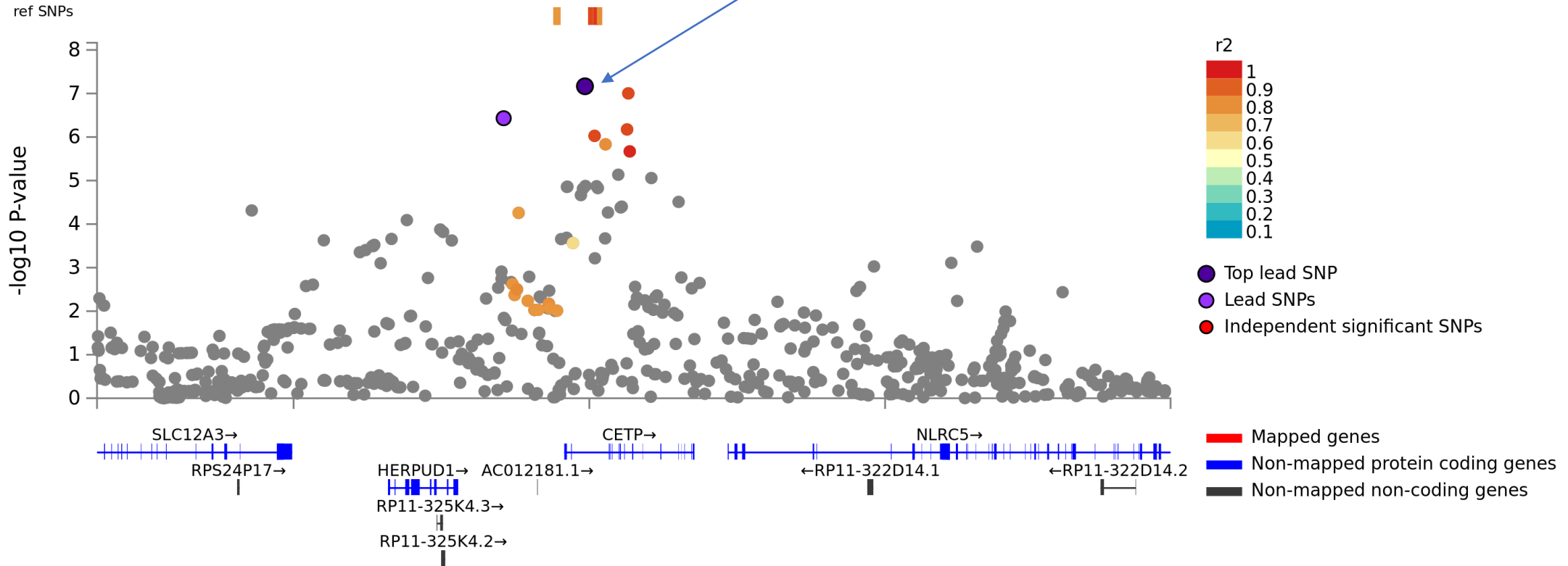*MDRF f1 and f2

Adjusted for Age and Sex

Chr16:56989590 rs247616

Population	EA	NEA	EAf	BETA	SE	P.value	n_studies	effects
Meta-UK	T	C	0.324	0.076	0.0047	2.19E-57	4	++++
MDRF	T	C	0.285	0.05	0.057	7.1E-29		

Population	EA	NEA	EAf	BETA	SE	P.value	n_studies	effects	Hetero (i2)	pvalue
Meta-UK	C	A	0.19	-0.078	0.005	6.3E-44	4	++++		
MDRF	C	A	0.24	-0.036	0.005	1.5E-11			0.96	6.8E-8

Ganesan M, Nizamuddin S, Katkam SK, et al. c.*84G>A Mutation in CETP Is Associated with Coronary Artery Disease in South Indians. *PLoS One*. 2016;11(10):e0164151. Published 2016 Oct 21. doi:10.1371/journal.pone.0164151

Our objectives….

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- **To study the heterogeneity of effects of genetic variants and Genes for HDL-c among the study population**

- To estimate and compare the polygenic risk score (PRS) for HDL-c among the study population

Heterogeneity analysis

- **Cochran's Q :** weighted sum of squared differences between individual study effects and the pooled effect across studies, with the weights being those used in the pooling method.

    Q is distributed as a chi-square statistic with k (number of studies) minus 1 degrees of freedom.

- **I^2 :** describes the percentage of variation across studies that is due to heterogeneity rather than chance
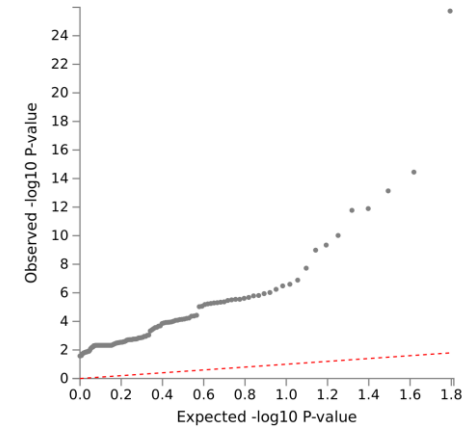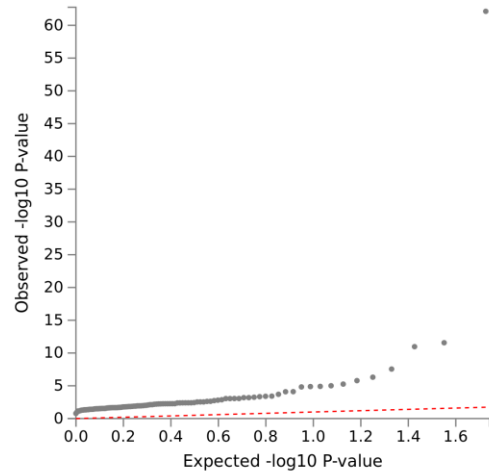
- **Posterior Probability > 0.5 :** Heterogeneity in allelic effect
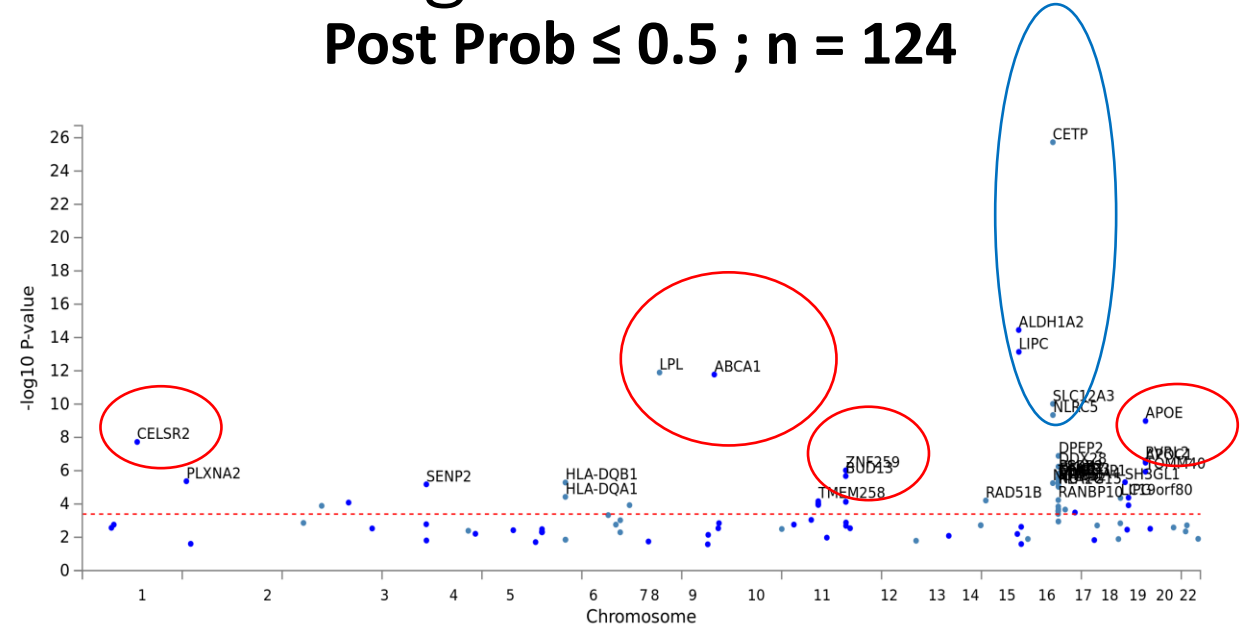
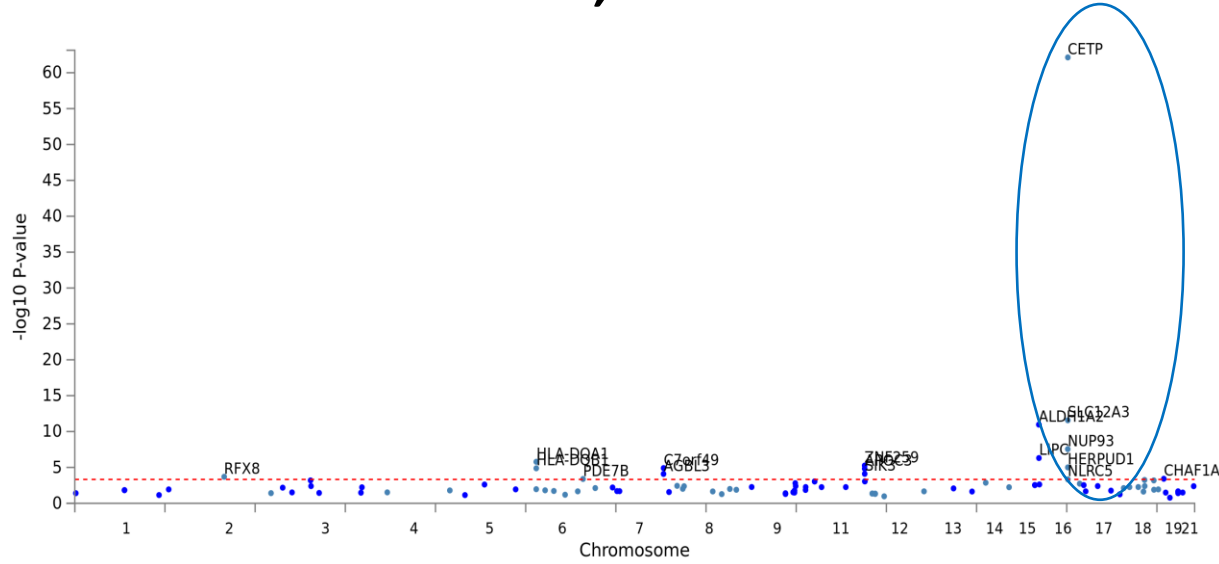# Comparison of genes Manhattan plot heterogenous Vs non heterogenous
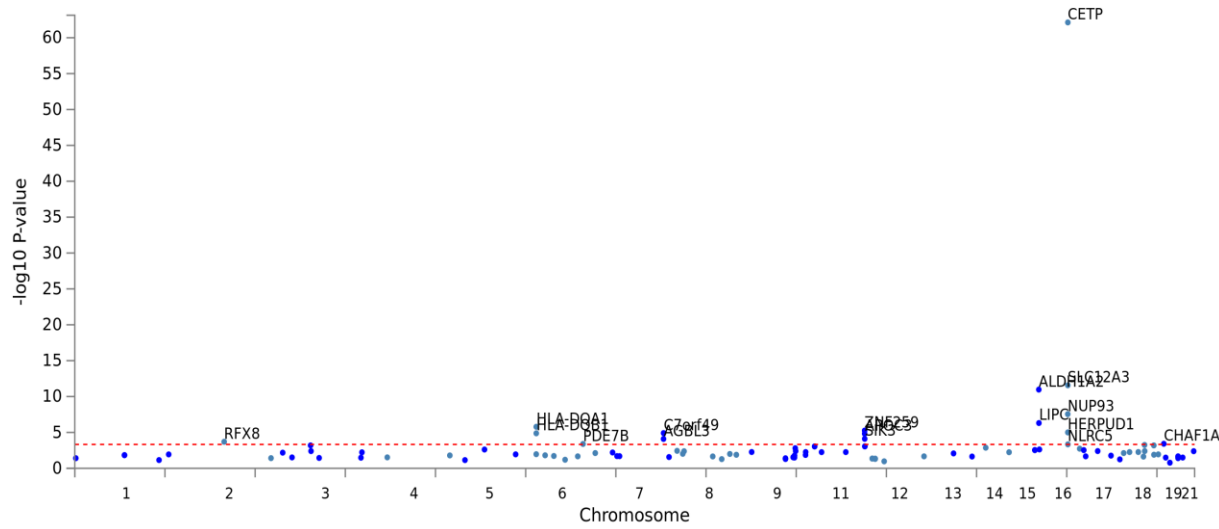
Post Prob > 0.5, n= 106

Post Prob ≤ 0.5 ; n = 124

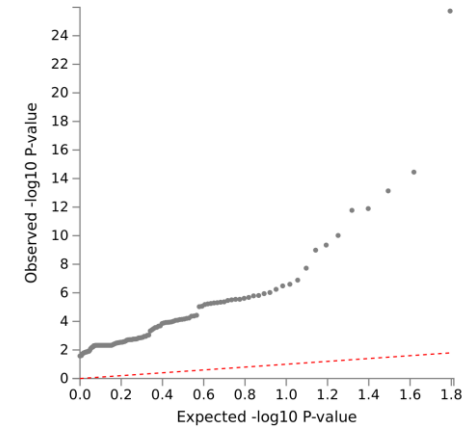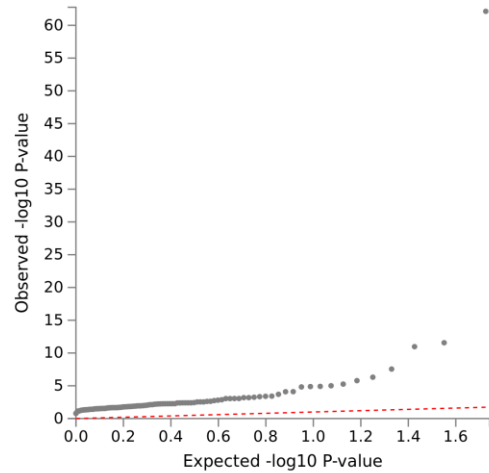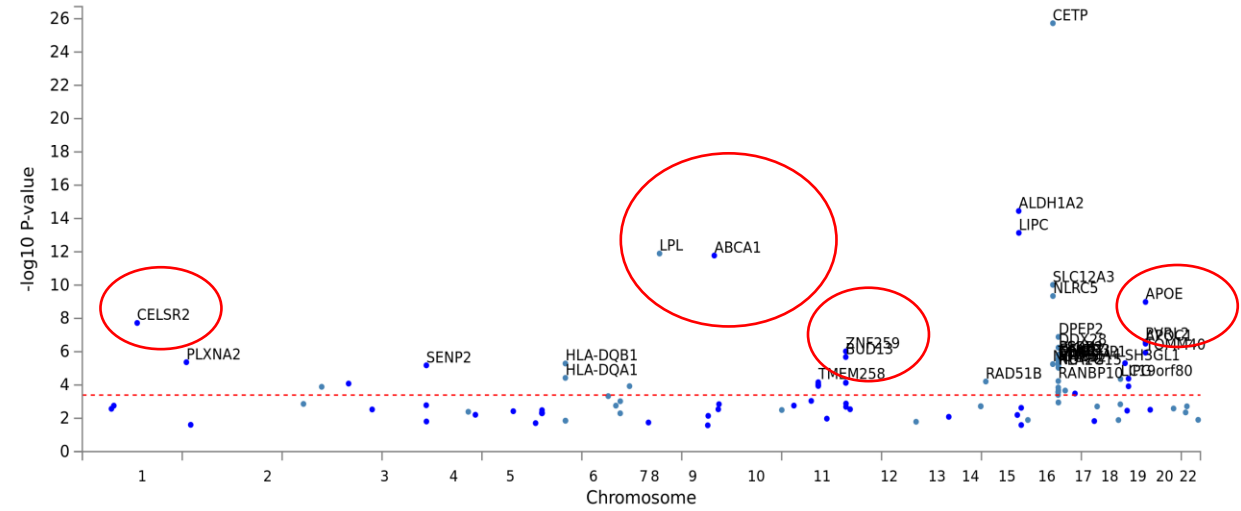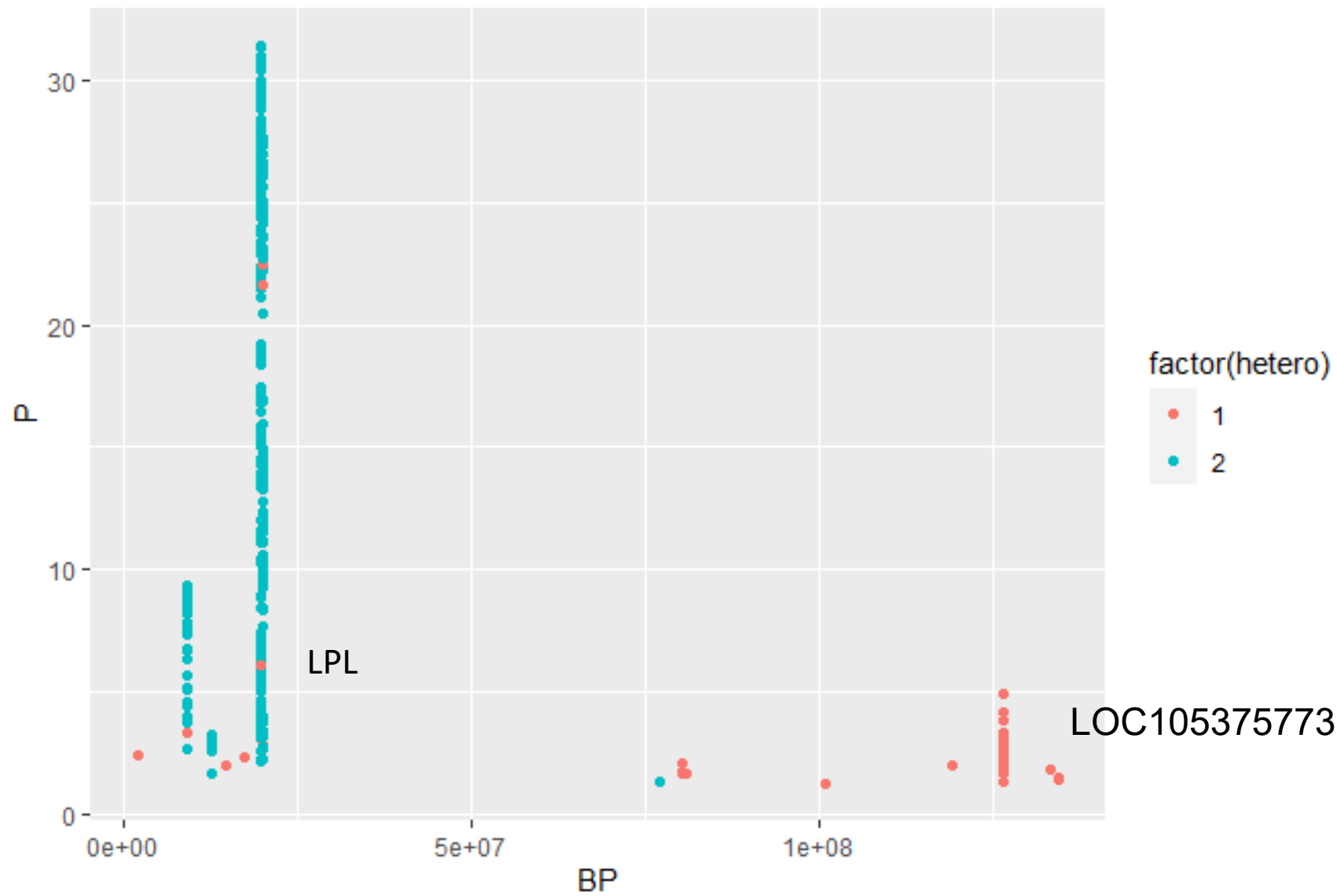# Comparison of genes Manhattan plot heterogenous Vs non heterogenous

Post Prob > 0.5, n= 106

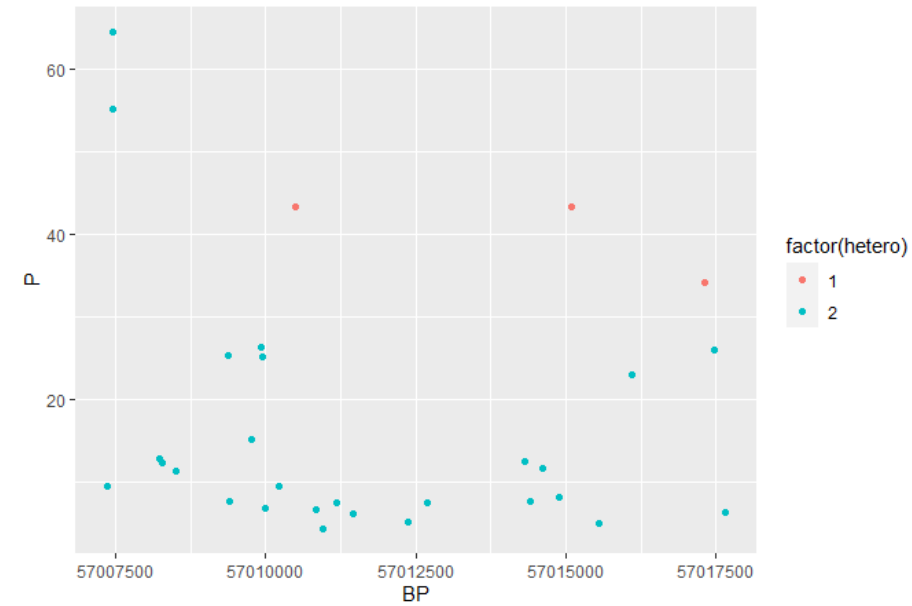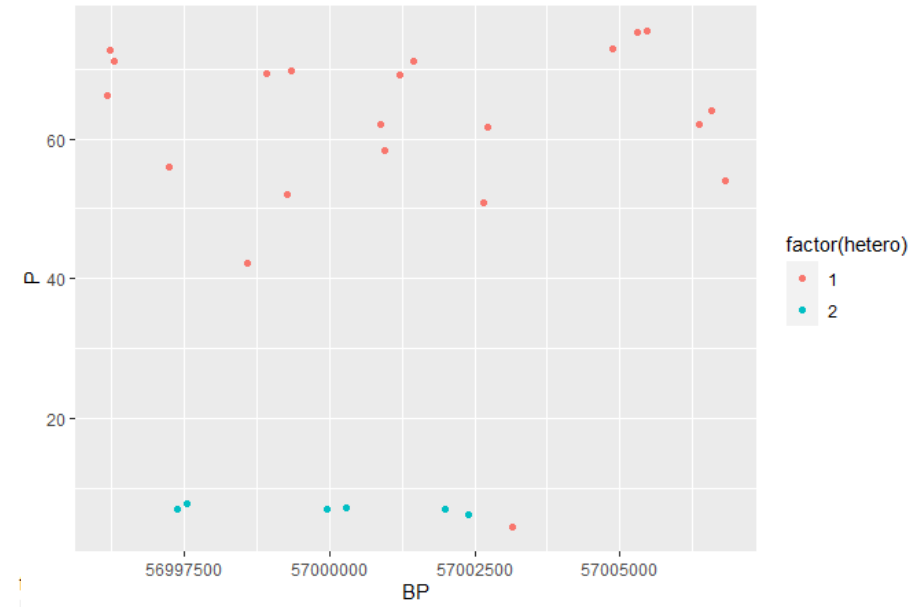**Post Prob ≤ 0.5 ; n = 124**

Chromosome 8

Chromosome 16
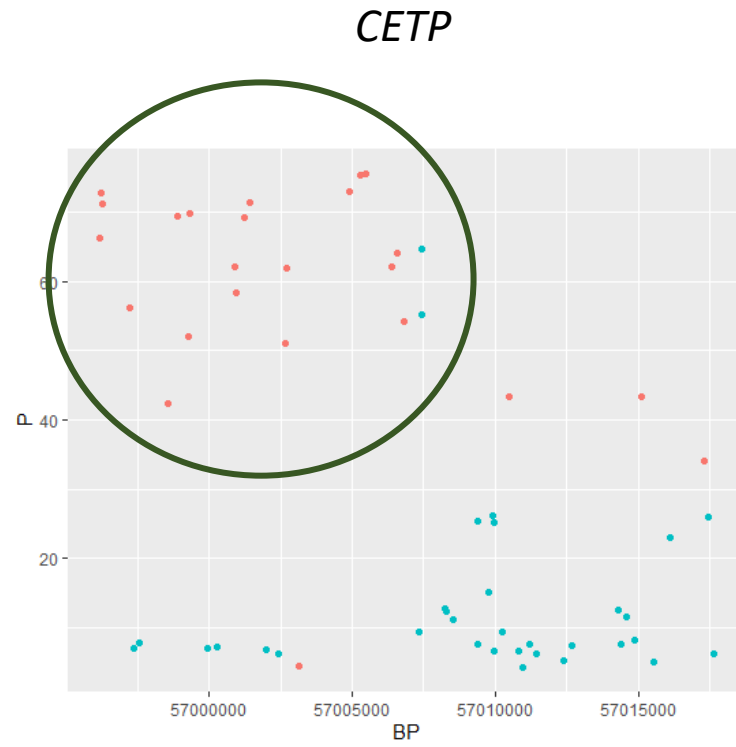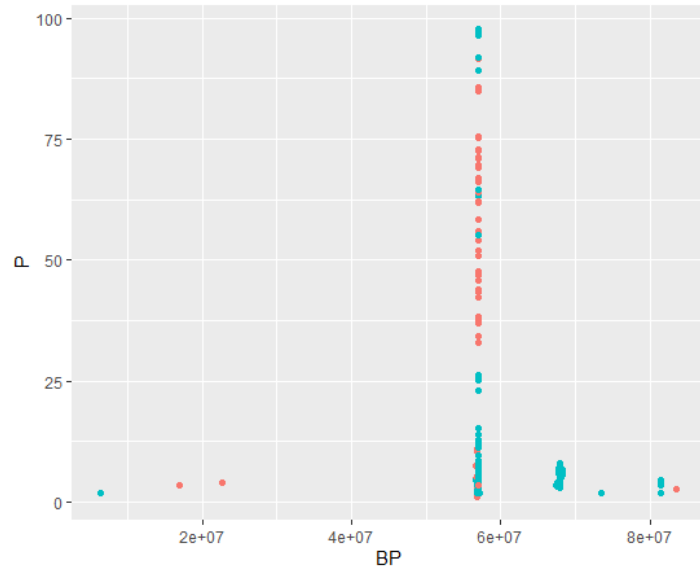
CETP
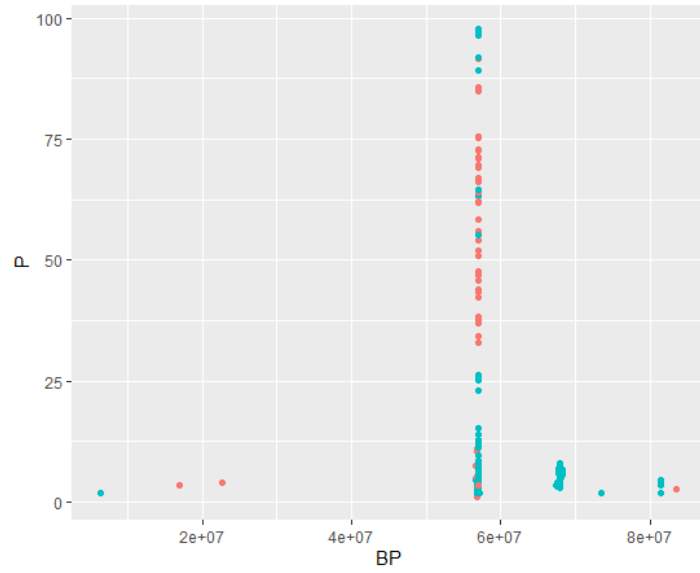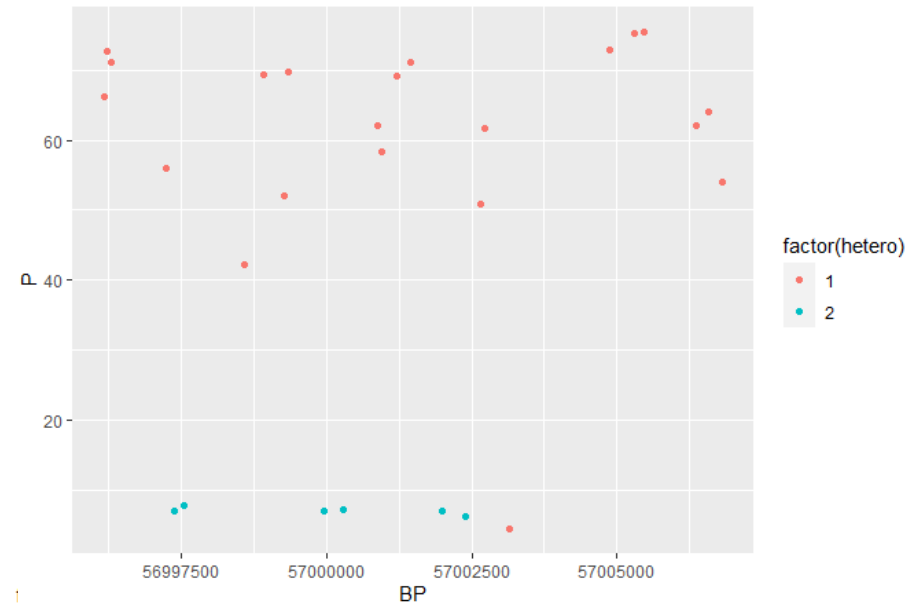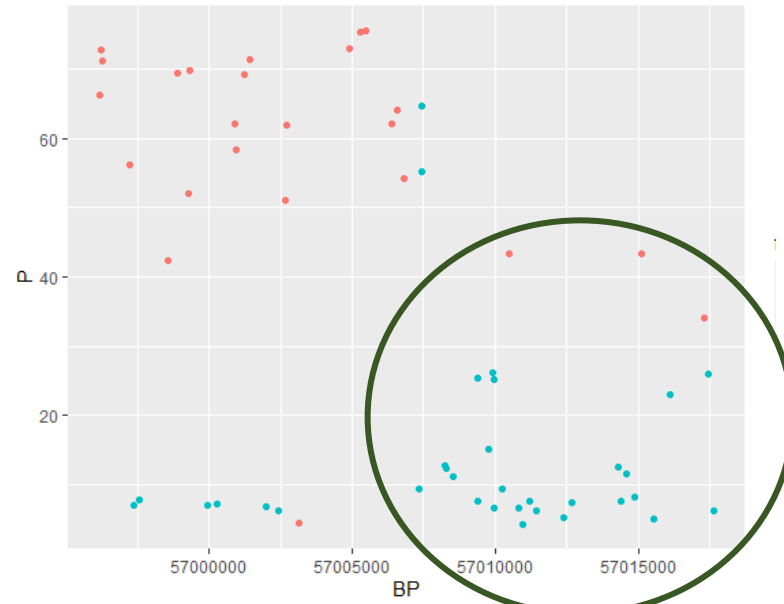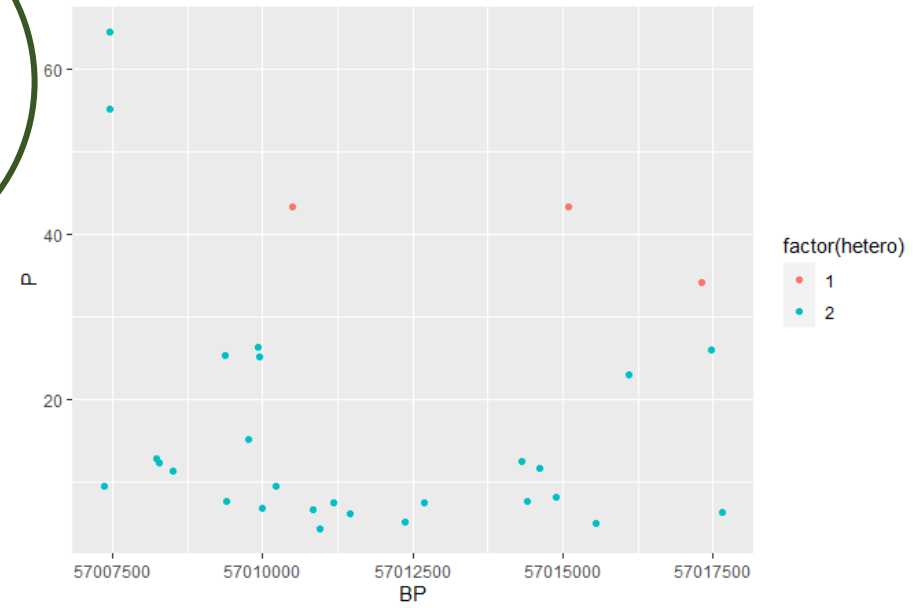
Chromosome 16

CETP

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Polygenic Risk Score (PRS)

- All reported SNPs ($p > 5*10^8$) associated with HDL-c were selected from a Trans-ethnic meta-analysis[#].

- Weighted PRS were constructed using PRSice - v 2 (Kings College, London).

- PRS were calculated for additive genetic models and on the basis of unfavourable allele (allele associated with lower HDL-c).

- **Weighted PRS formula**

- $\text{wPRS} = \sum_i (S_i \times G_i) / M$

- S- beta estimate, G- no of effective allele, M – no of SNPs

Descriptive statistics of study population

population	n	Variable	N	N Miss	Mean	Median	Std Dev
South Indian	6056	age	5268	788	48.02	48.9	11.8
		B hdl	5268	788	1.042	1.009	0.25
		PRS	6056	0	-15.82	-15.825	0.32
Scottish	7034	age	4738	2296	59.35	60.0	11.23
		B hdl	4738	2296	1.22	1.170	0.34
		PRS	7034	0	-1.87	-1.866	0.16

Frequency	
Population	**Sex (Male)**
South Indian (n = 5268)	3175(60.26%)
Scottish (n=4738)	2567(54.17%)

Regression of Baseline HDL-c (a) with Polygenic risk score $(PRS_{GLGC-new})$
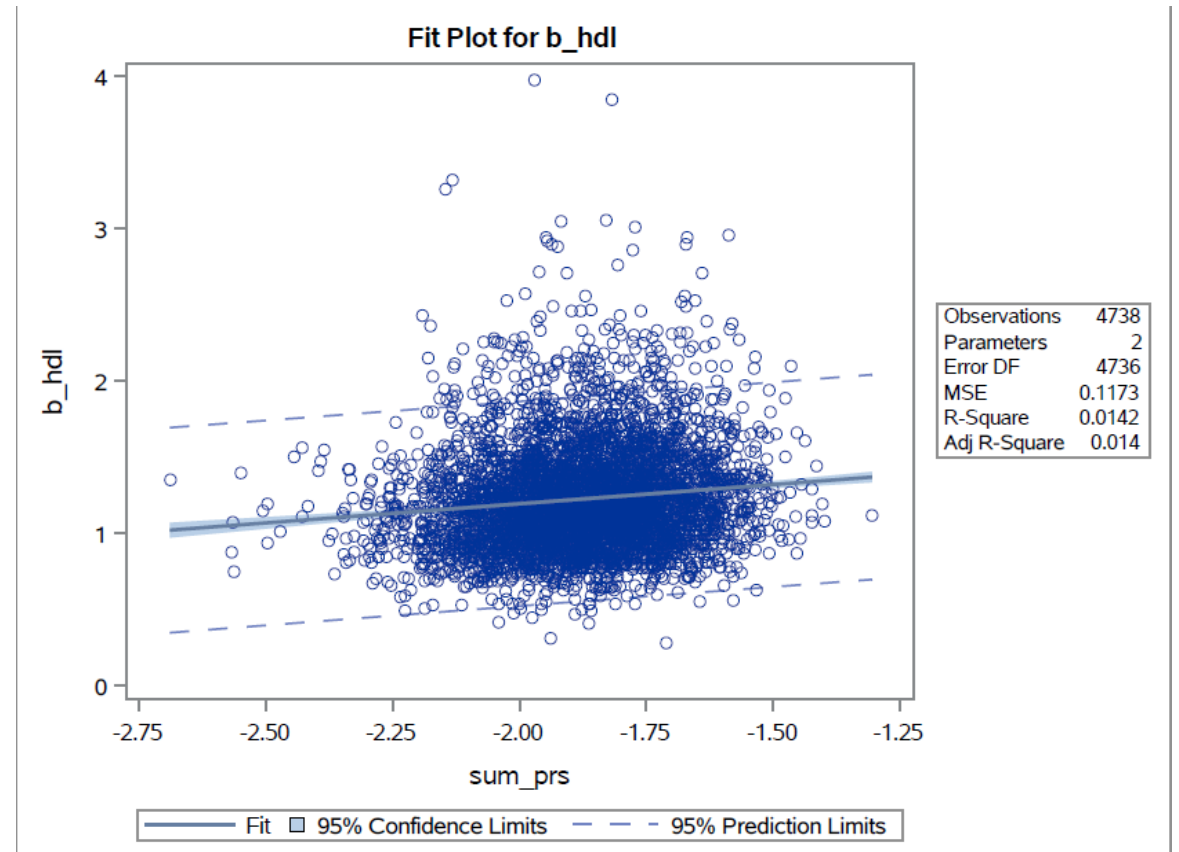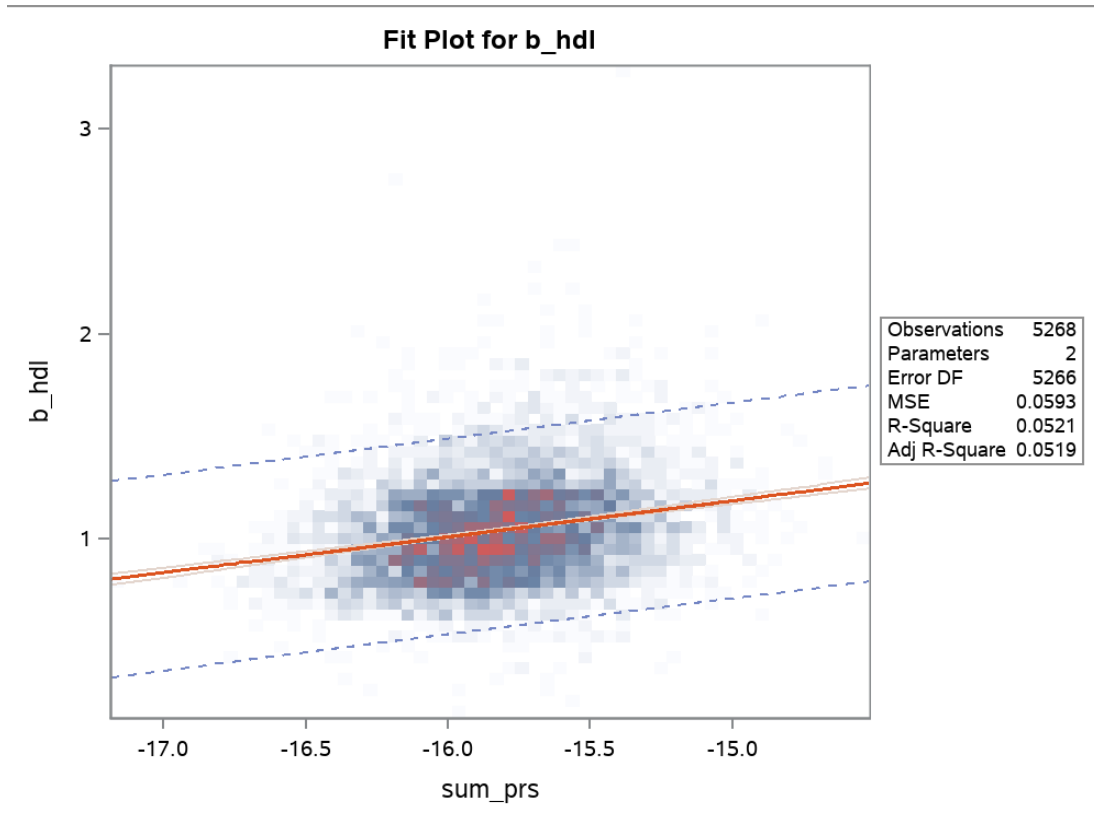
Fit Plot for b_hdl

Observations	5268
Parameters	2
Error DF	5266
MSE	0.0593
R-Square	0.0521
Adj R-Square	0.0519

Fit Plot for b_hdl

Observations	4738
Parameters	2
Error DF	4736
MSE	0.1173
R-Square	0.0142
Adj R-Square	0.014

Fit —— ■ 95% Confidence Limits — — 95% Prediction Limits

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	3.82467	0.16361	23.38	<.0001
sum_prs	sum_prs	1	0.17592	0.01034	17.01	<.0001

Parameter Estimates

Variable	Label	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	Intercept	1	1.69833	0.05735	29.62	<.0001
sum_prs	sum_prs	1	0.25178	0.03044	8.27	<.0001

Effect of PRS on baseline HDL-c between the population

Fit for b_hdl
With 95% Confidence and Prediction Limits

Fit computed at pop=1.474
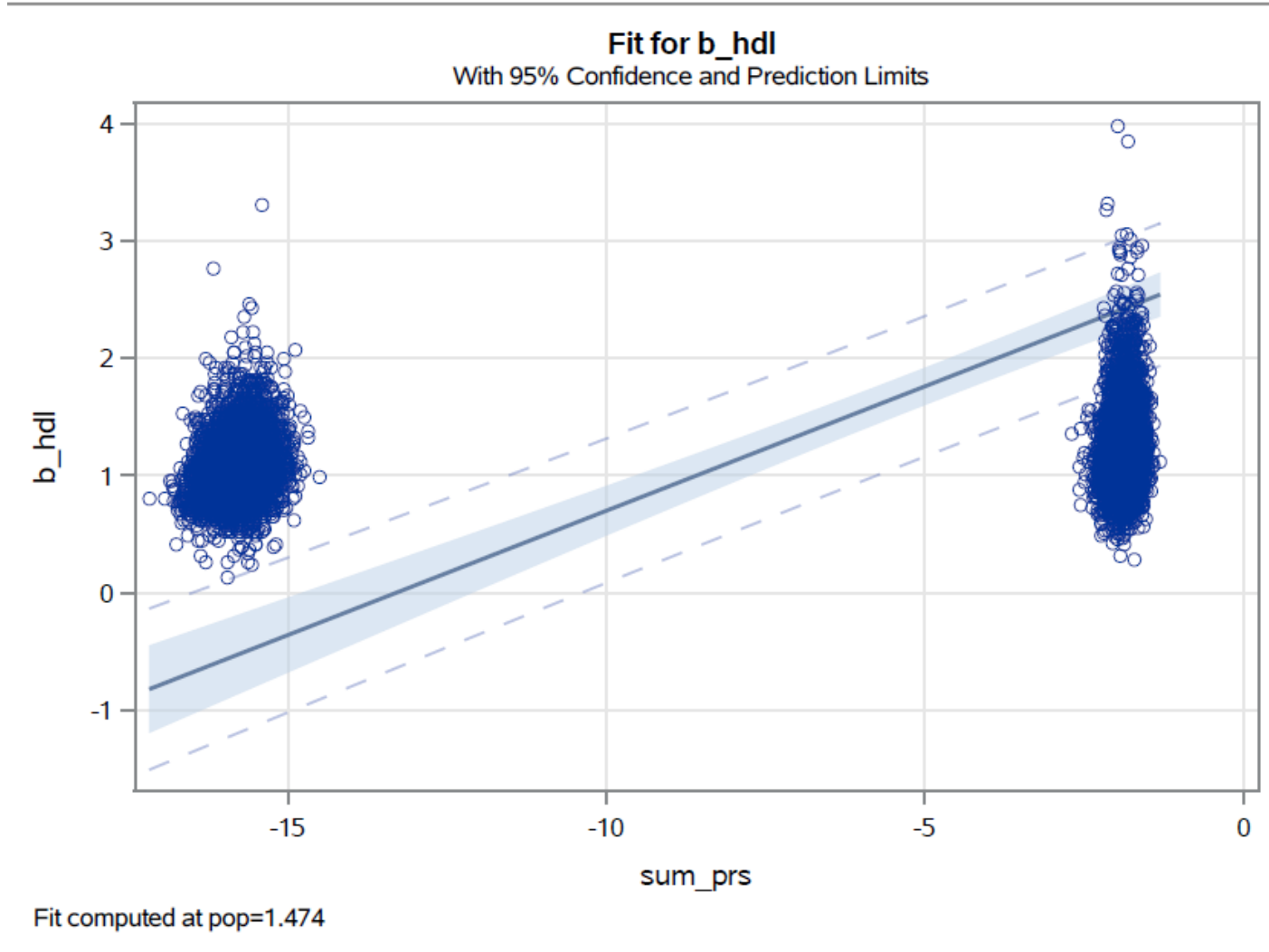
Dependent Variable: b_hdl

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	109.3230082	36.44100274	419.91	<.0001
Error	10002	868.0059319	0.0867832365		
Corrected Total	10005	977.3289401			

Root MSE	0.2945899464
R-Square	0.1118589696

Parameter	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	5.951009305069	0.398903217	14.92	<.0001
PRS	0.100067184963	0.0362155763	2.76	0.0057
Population	-2.126337842499	0.203973295	-10.42	<.0001
PRS*Population	0.075854744757	0.0290171557	2.61	0.0090

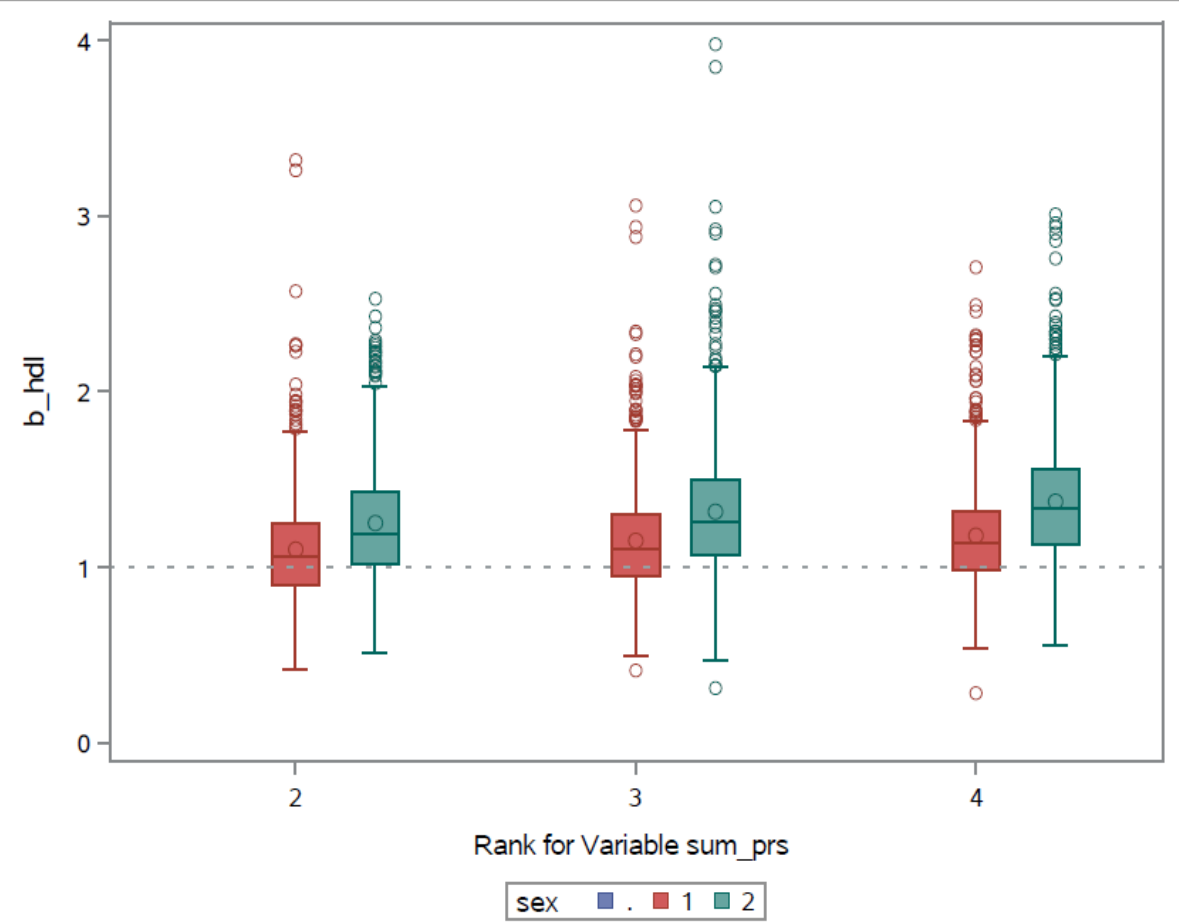# Effect of PRS (Quintile) on HDL-c levels
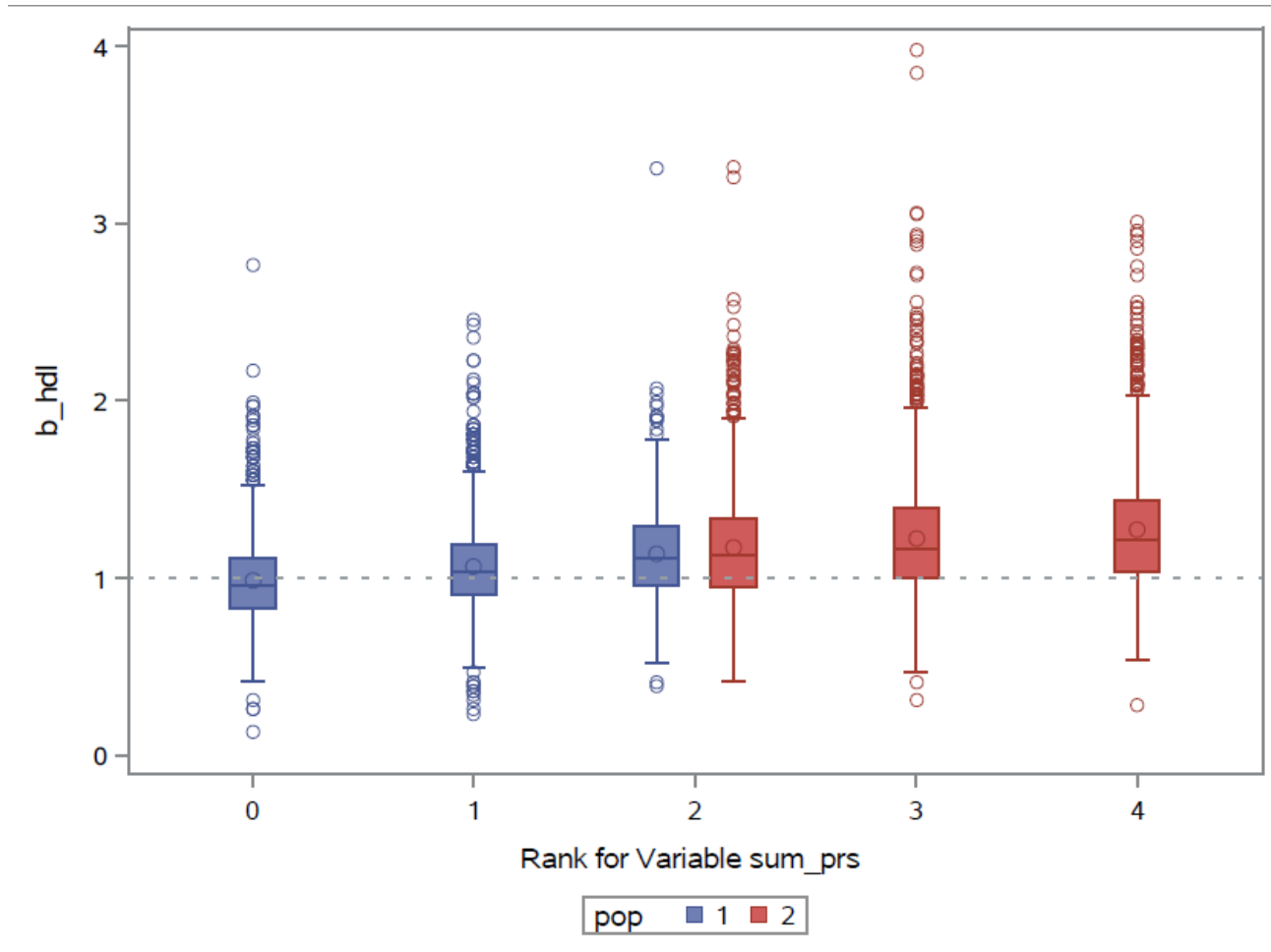
Pop 1 – South Indian
Pop 2 - Scottish

Effect of PRS on HDL-c levels (Gender wise)
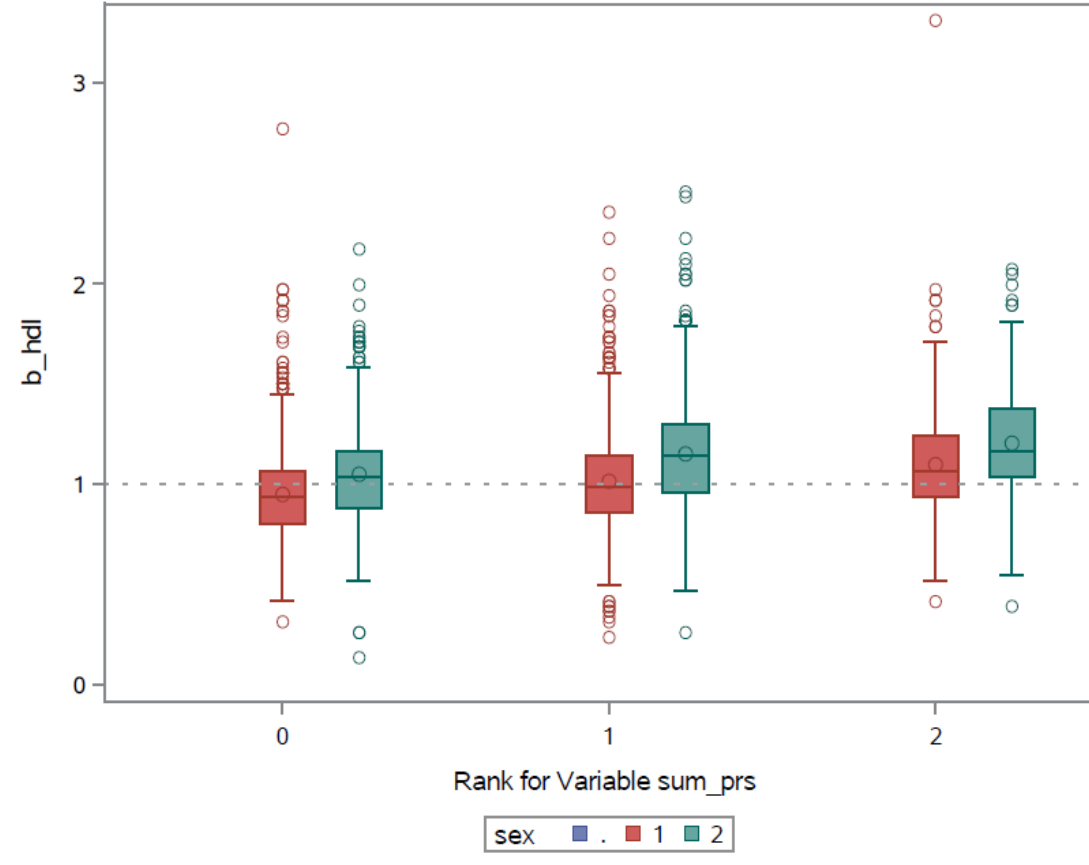
Pop 1 – South Indian

Pop 2 - Scottish

Conclusion

- Heterogeneity in genetic architecture for HDL- Cholesterol exist such as *CETP* region between the study population.

- PRS suggests that genetic loci for HDL-c in Scottish populations are present at both similar and varied frequency compared with South Indian populations, which modulate the HDL-c at certain levels

- Higher HDL-c levels was observed among females with increase in PRS within the population shows an effect of gender on PRS and HDL-c levels.

Acknowledgement

My supervisors

Dundee

- Prof Colin NA Palmer

- Prof Ewan R Pearson

MDRF

- Dr Guha Pradeepa

- Dr Radha Venkatesan

INSPIRED team

Dr Fred, Ms Isobel, Dr Simona, Dr Sundar, Dr Moneeza,

Anand, Aravind, Charvi, and all other INSPIRD colleagues

&

My family, my wife 'Aradhana' and my son '**Shrihaan**'

Thank you!

Any Questions?