Unraveling the molecular mechanisms of CVD's: the PROMIS model

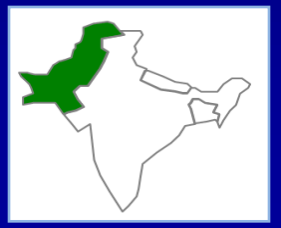
13 March 2015

Philippe M. Frossard
Vice-Provost Research, Nazarbayev University

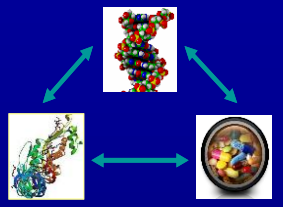
Focus of talk

CVD's in South Asians

Pakistan-based studies

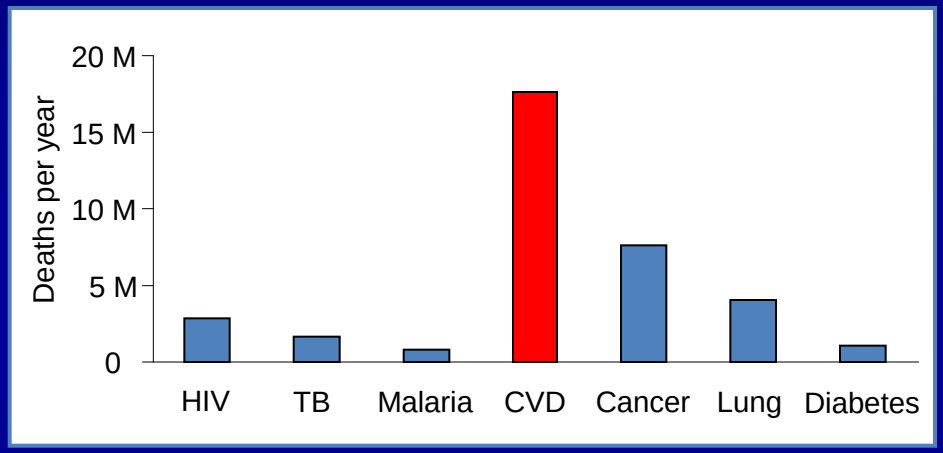


Genetic, biomarker and lifestyle investigations



Future Directions

Why does global cardiovascular health matter?



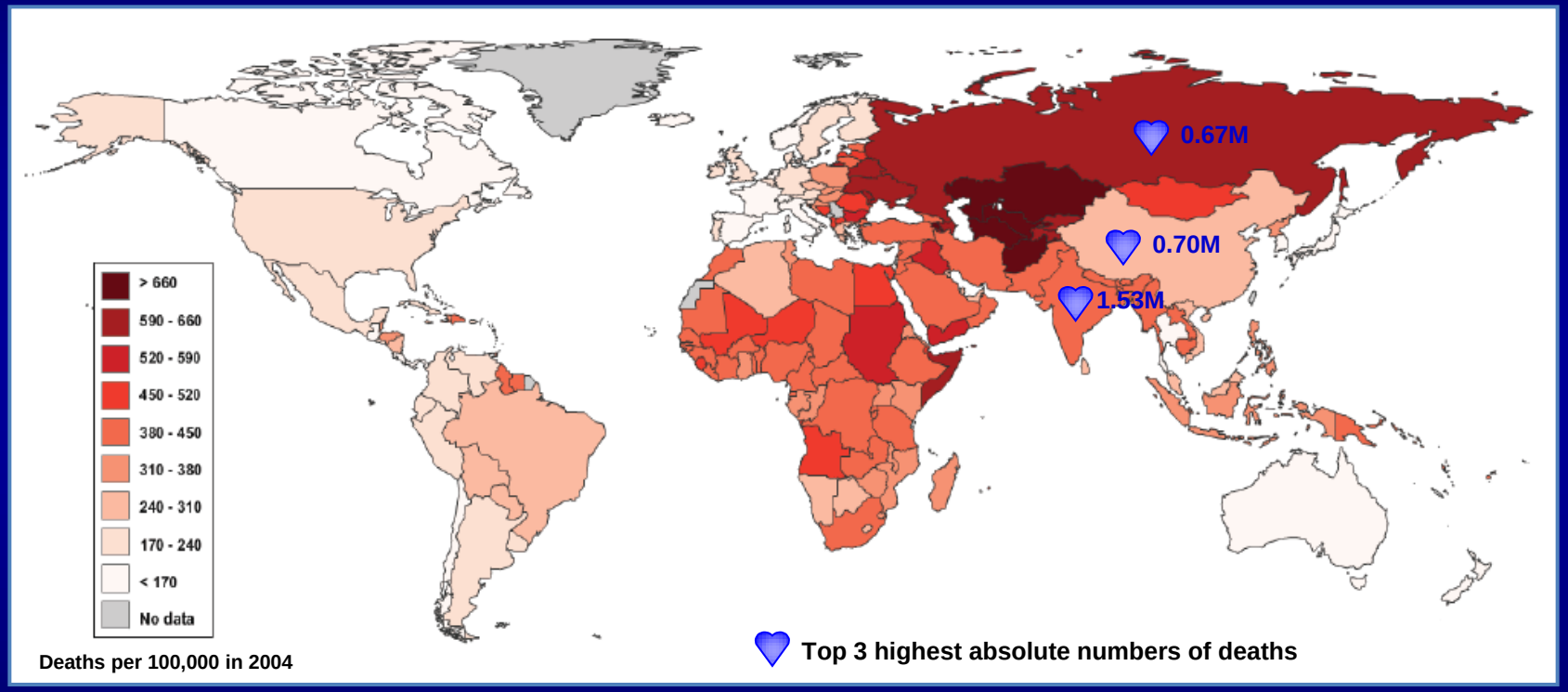
Cardiovascular disease accounts for 1 in 2 adult deaths worldwide

> 80% of cardiovascular deaths occur in developing countries

A leading cause of disability

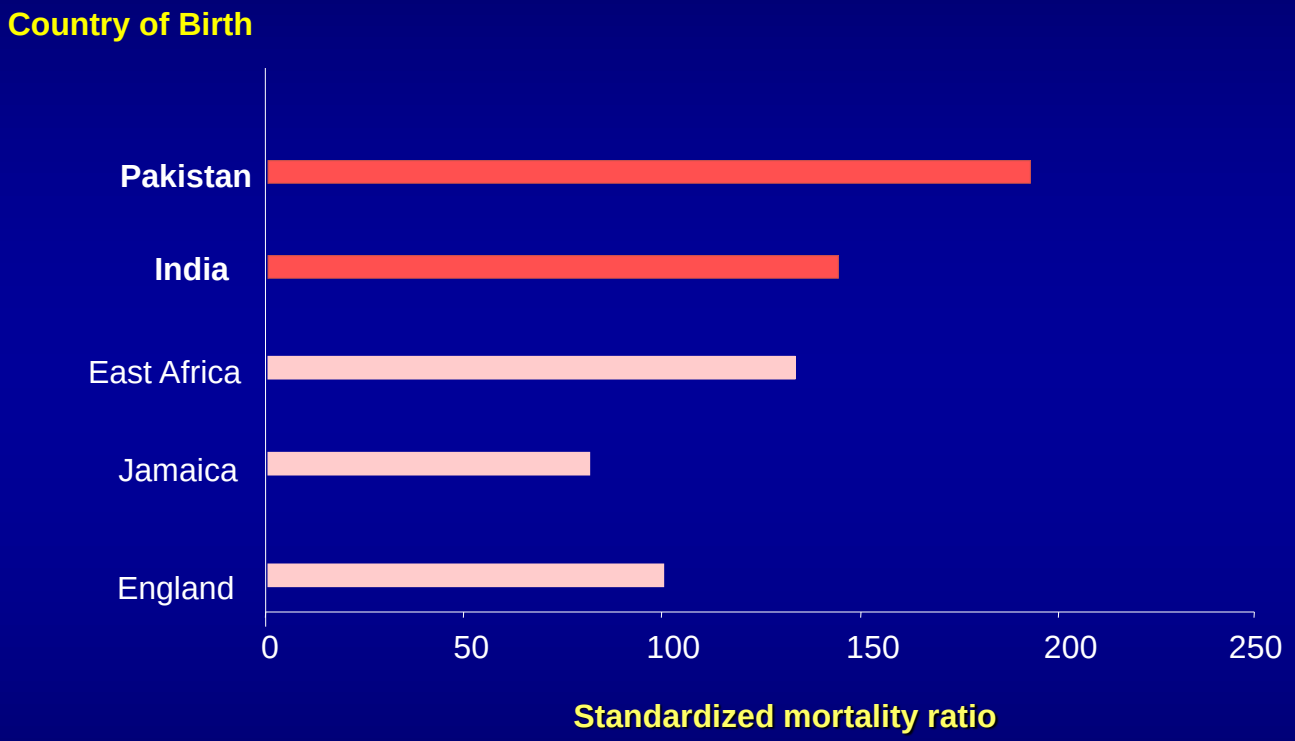
WHO Chronic Diseases Report 2005
Lopez *et al.*, Lancet 2006

Uneven global distribution of age-adjusted CVD mortality



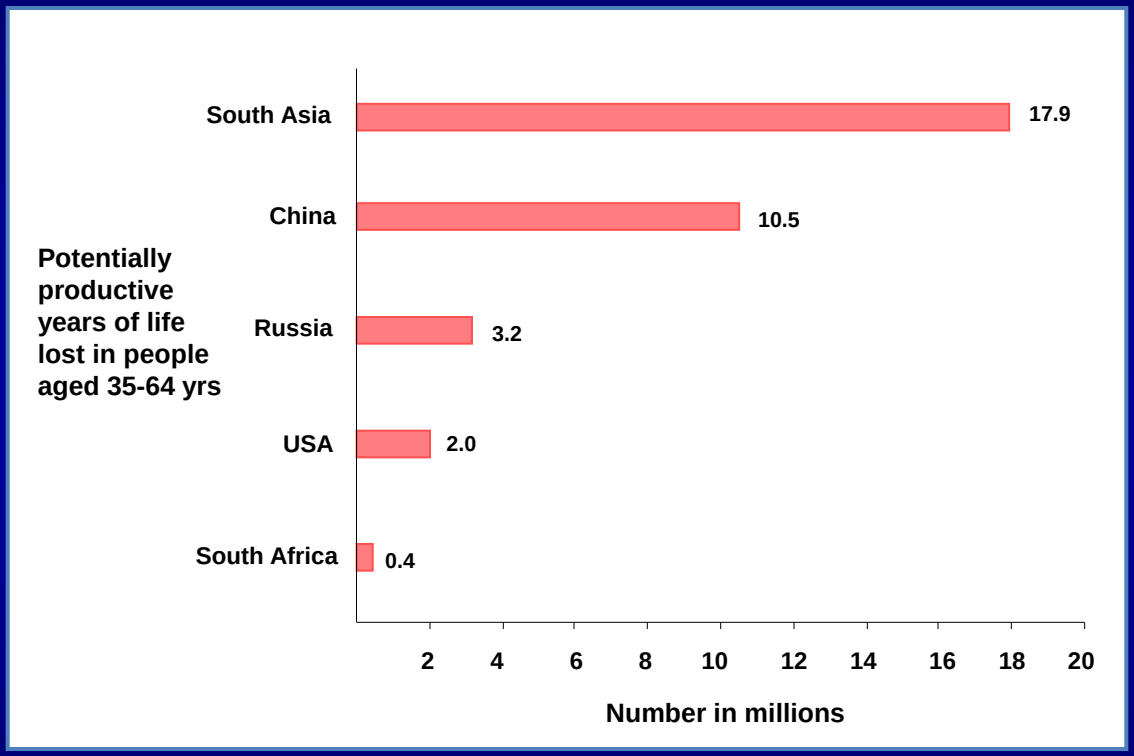
National Academy of Sciences, 2010

CVD mortality in the UK is highest in South Asian immigrants



Harding S *et al.*, 2008

Projected burden of CVD in 2030

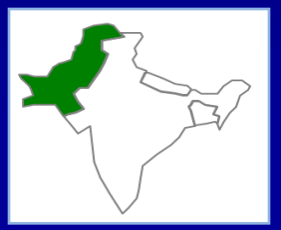


Adapted from Reddy *et al.*, Lancet 2005 & JACC 2007

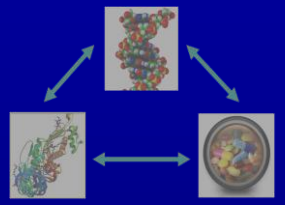
Focus of talk

CVD's in South Asians

Pakistan-based and led studies



Genetic, biomarker and lifestyle investigations



Future Directions

AKU medical students, Juma Labs, AKU, Karachi, Pakistan2003

PROMIS

Pakistan Risk Of Myocardial Infarction Study

This project aims at identifying the genetic, biomarker and lifestyle determinants of acute **myocardial infarction** in the Pakistani population.

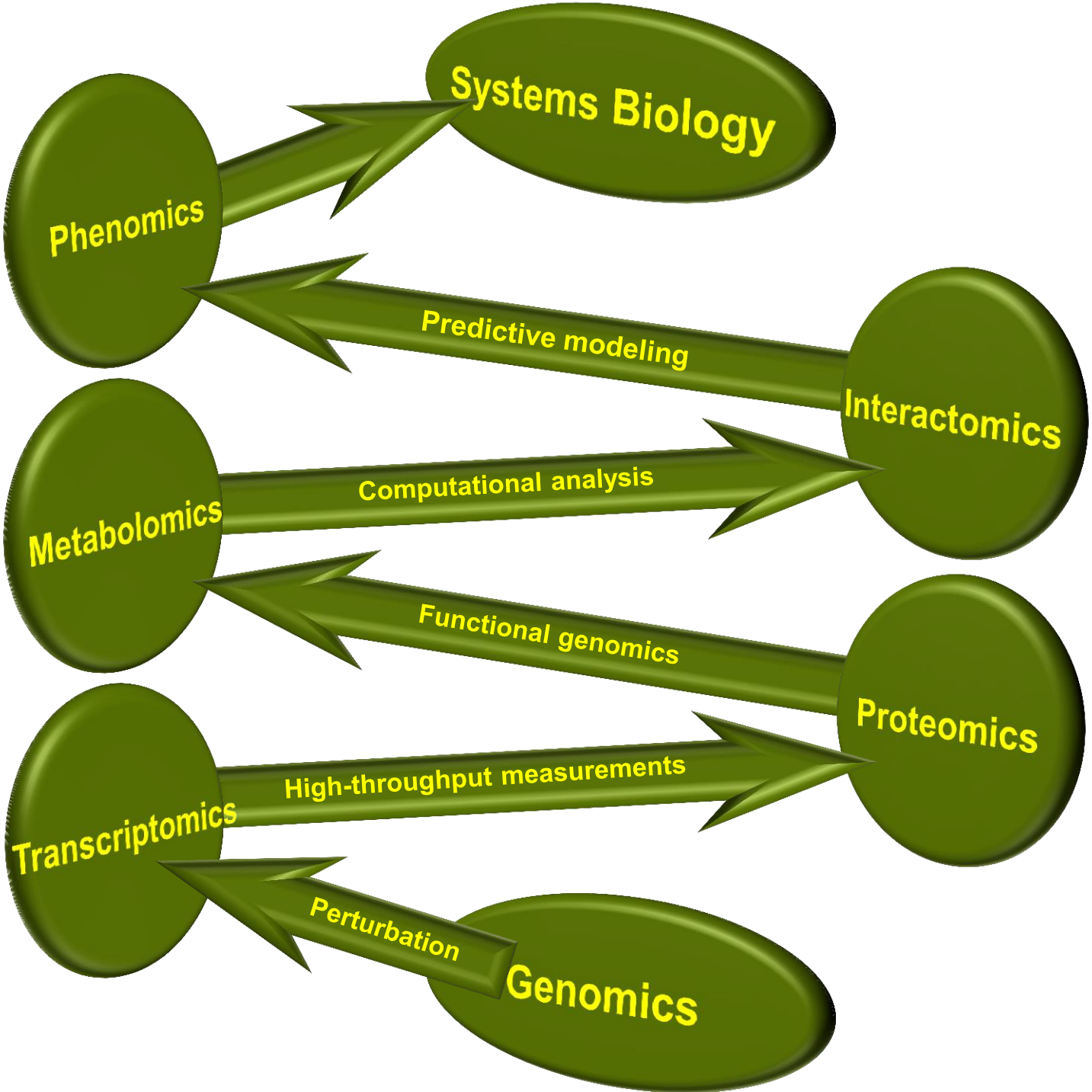
RACE

Risk Assessment of Cerebrovascular Events

This project aims at identifying the genetic, biomarker and lifestyle determinants of **stroke** in the Pakistani population.

Pakistan Type 2-Diabetes Study

This project aims at identifying the genetic, biomarker and lifestyle determinants of **type 2-diabetes** in the Pakistani population.


Systems Biology
Phenomics
Predictive modeling
Interactomics
Metabolomics
Computational analysis
Functional genomics
Proteomics
Transcriptomics
High-throughput measurements
Perturbation
Genomics

PROMIS, RACE, Type-2 Diabetes Association Study Design

Compare genome, epigenome, transcriptome, proteome, metabolome, interactome, etc., in groups of

MI/Stroke/NIDDM Patients

vs.

Matched Controls (comparison group)

Pakistan Risk of MI Study (PROMIS)
Risk Assessment of Cerebrovascular Events Study (RACE)
Pakistan type-2 Diabetes Study

Outcomes recorded

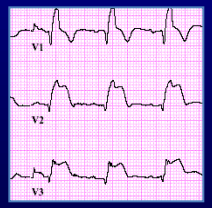
18K MI cases
troponin + ECG confirmed
Mean age of onset : 54y

10K type 2 diabetes cases
HbA1c confirmed

6K stroke cases
CT/MRI confirmed
Mean age of onset: 61y

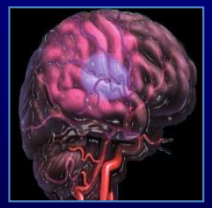
25K controls
No history of CVD

Multiple disease outcomes



18K MI cases

10K T2D cases



6K stroke cases

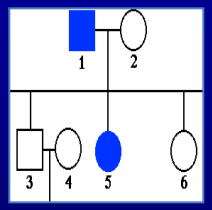
25K controls

Saleheen et al., Eur. J. Epid. *2010*

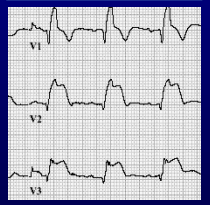
Pakistan-based studies

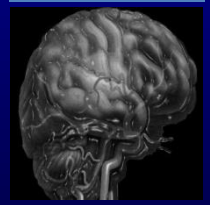
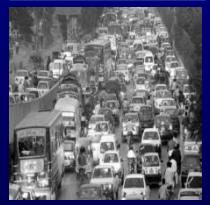
Clinical & lifestyle information

Multiple disease outcomes





18K MI cases | **10K T2D cases** | **6K stroke cases** | **25K controls**

Examples

Diet
200 item locally validated FFQ

Medical history
Personal and family
Medication usage

Anthropometry
Height, weight, waist, hip

Consanguinity
Parental and personal
1st degree cousin marriages

Pakistan-based studies

Examples

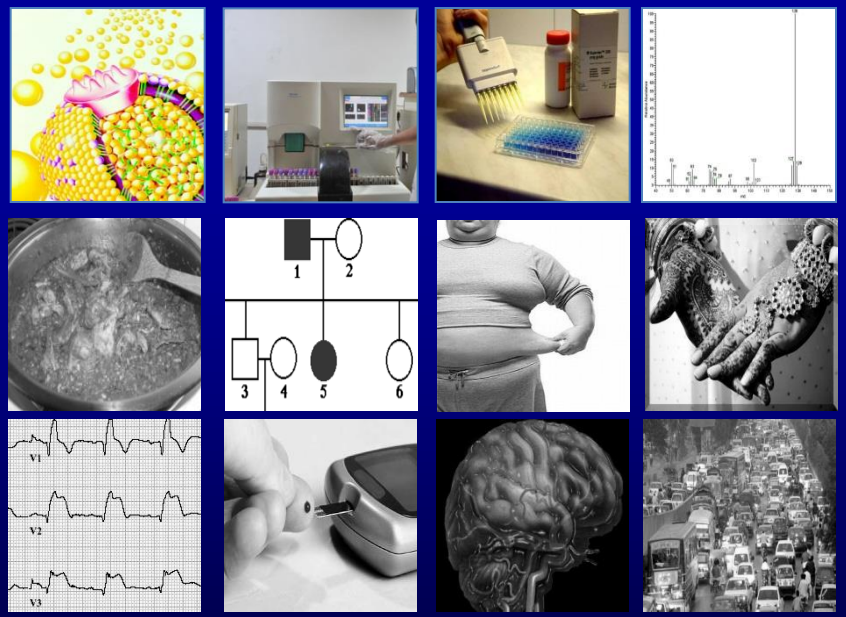
>50 soluble biomarkers

NMR and MS metabonomics

Multiple intermediate phenotypes

Clinical & lifestyle information

Multiple disease outcomes



18K MI cases | **10K T2D cases** | **6K stroke cases** | **25K controls**

Pakistan-based studies

Selected soluble biomarkers being measured in 15K participants

ADAMTS7
Adiponectin
ALT
AST
ANGPTL3
ANGPTL4*
apoA-I
apoA-II
apoA-IV
apoA-V
apoB48
apoCIII
apoE
AST
Calcium
Ceruloplasmin
CETP mass
CETP activity*
Cholesterol efflux*
c-peptide
Creatinine
CXCL12*
IL-6
Insulin
intact PTH
Ion mobility assays*
Iron
Leptin
Lipoprotein subfractions
Lp(a)
LpPLA2 Activity*
LpPLA2 Mass
MMP9
MPO*
NEFA
oxPL and others*
PCSK9
PDGF-D
PON activity*
Potassium
P-selectin
Resistin
Serum amyloid A
sICAM-1
Sodium
E-selectin
Ferritin
Fetuin-A
FGF-21
FGF-23
Fractalkine
sVCAM-1
TSH
Vitamin D

* Functional assays

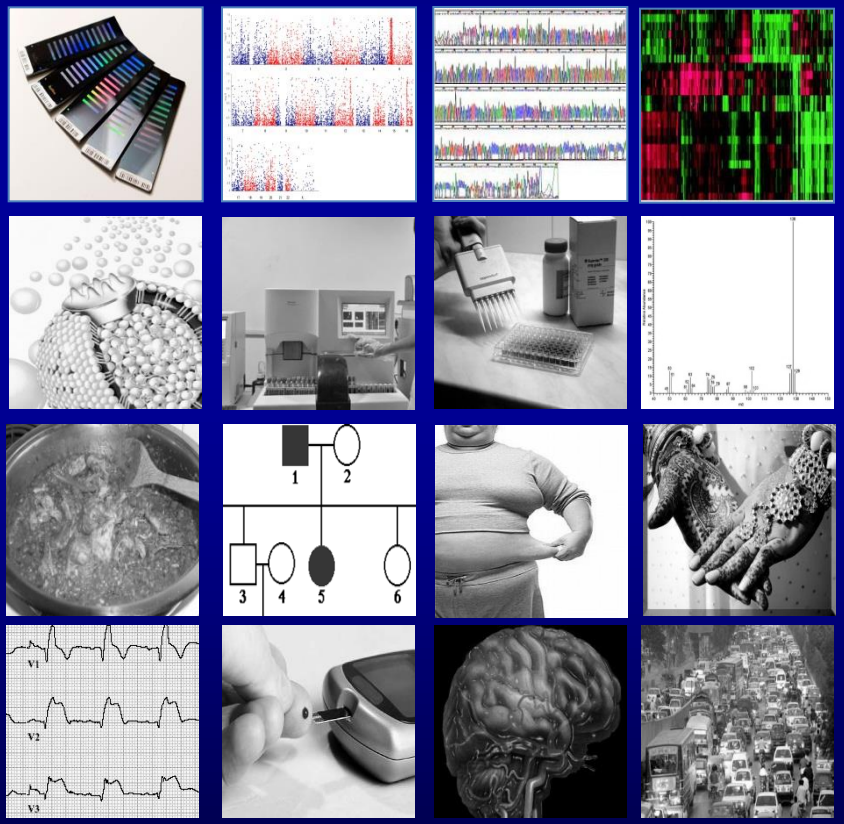
Pakistan-based studies

Genetic information

Multiple intermediate phenotypes

Clinical & lifestyle information

Multiple disease outcomes



18K MI cases | **10K T2D cases** | **6K stroke cases** | **25K controls**

Examples

Array	No. of participants
GWAS	22K
Metabochip	18K
IBC-Cardiochip	4K
Exome-Sequencing	14K
Lymphocyte & Monocyte RNA	300
Exome-chip	22K

Pakistan-based studies

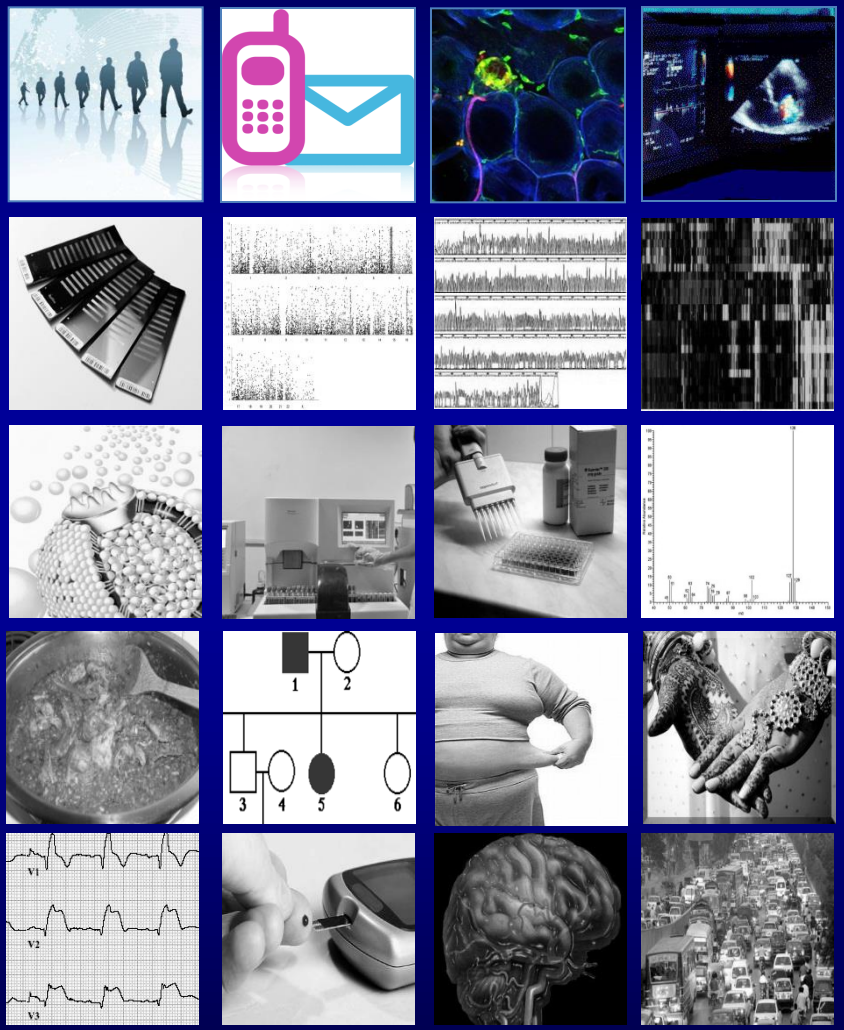
Flexibility in recruitment

Genetic information

Multiple intermediate phenotypes

Clinical & lifestyle information

Multiple disease outcomes



18K MI cases | **10K T2D cases** | **6K stroke cases** | **25K controls**

Examples

Continuing recruitment

~10K new participants per year

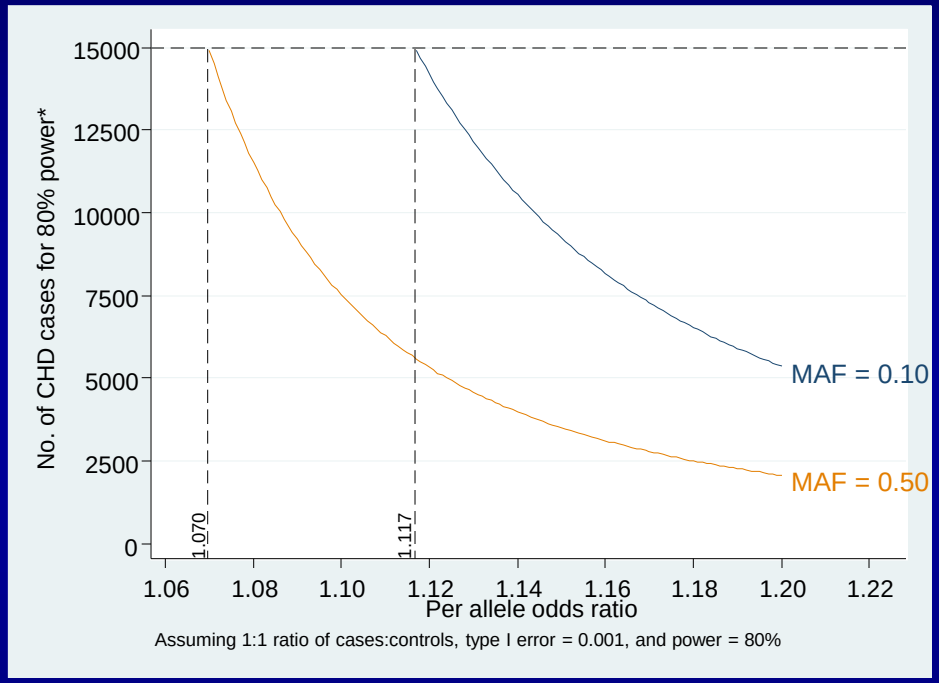
Recall of participants

eg, based on genotype

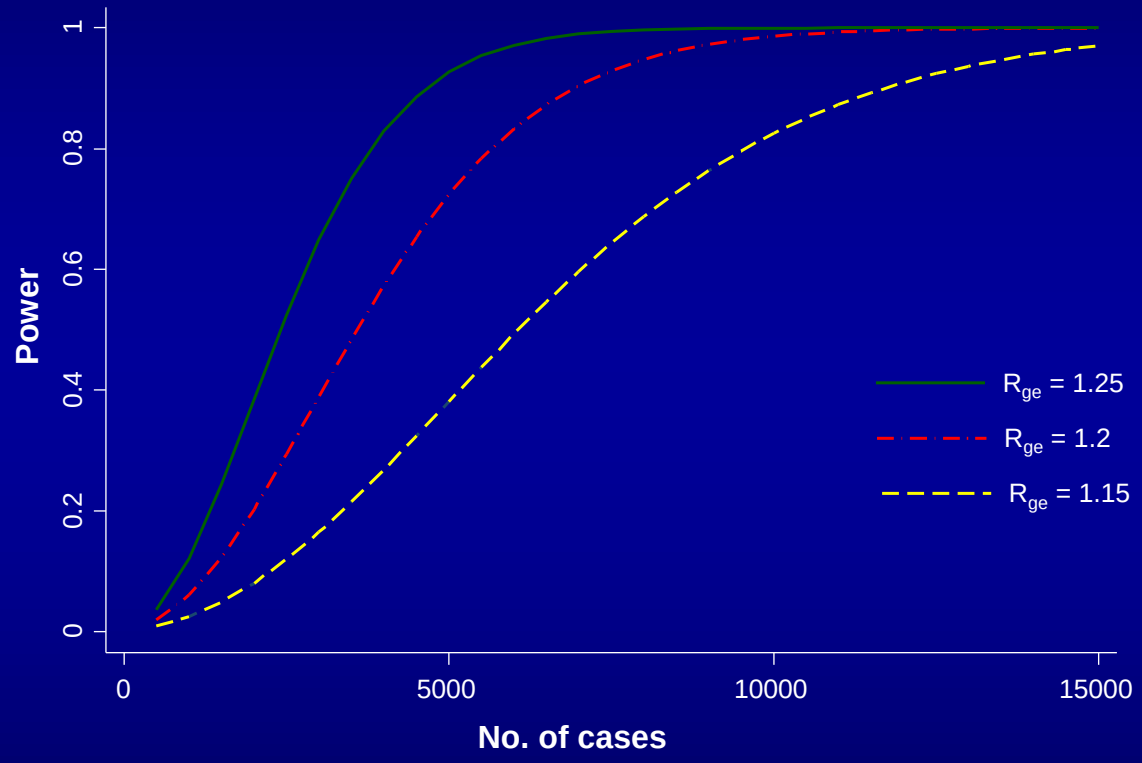
Potential for substudies

eg, tissue sampling, physiological measurements, imaging

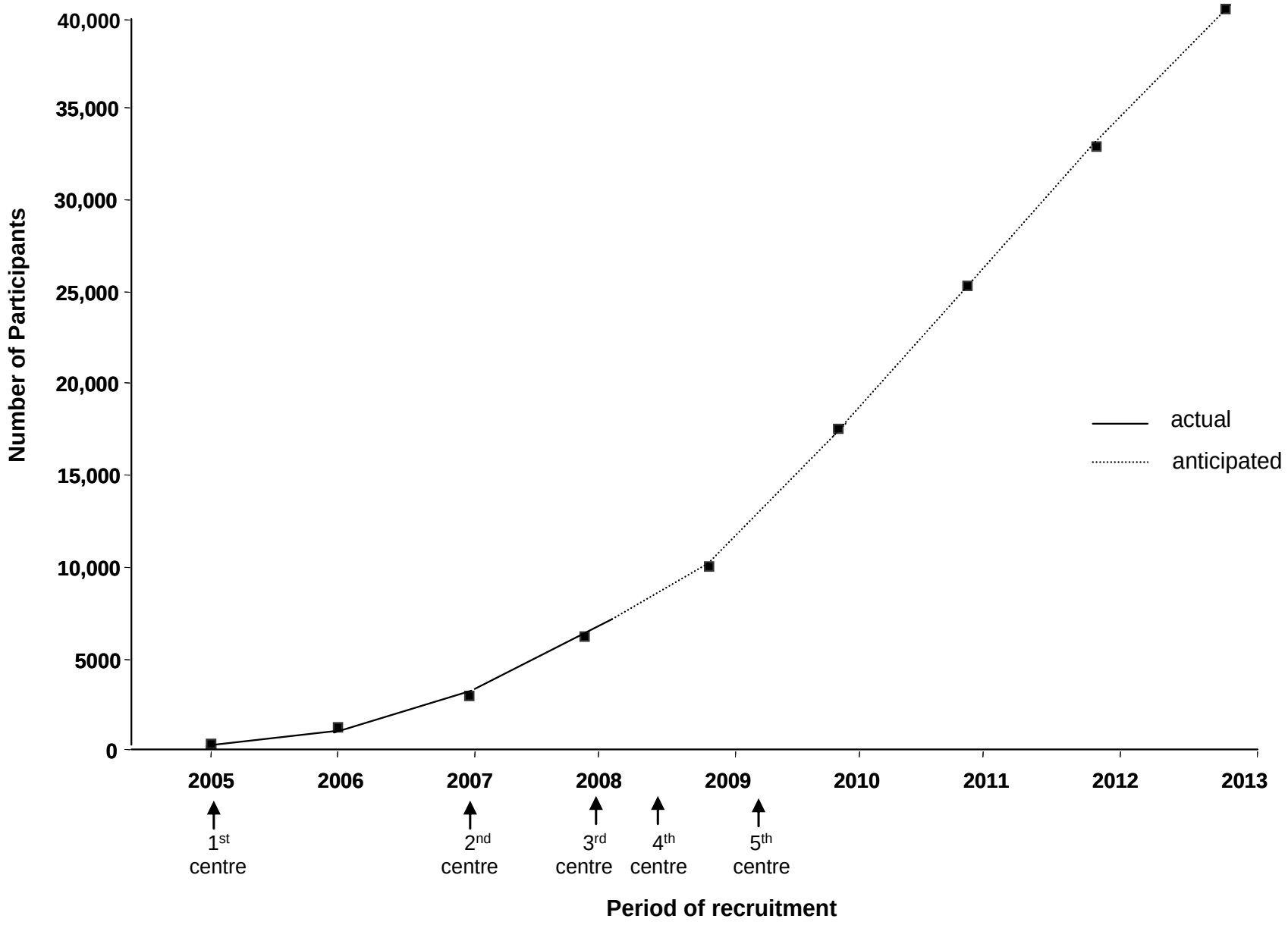
Value of large sample sizes – Gene-disease associations



Value of large sample sizes – Gene * Environment interactions



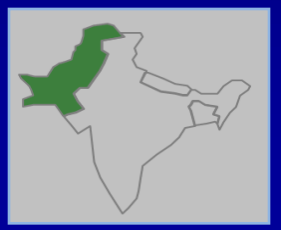
Actual and projected rate of recruitment



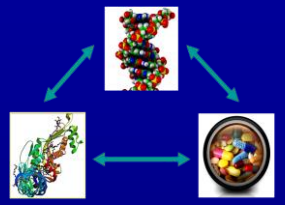
Focus of talk

CVD's in South Asians

Pakistan based studies



Genetic, biomarker and lifestyle investigations

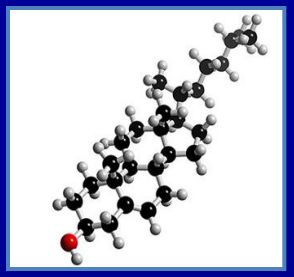


Future Directions

Pakistan based studies – powerful platforms for discovery

Genetic factors

Intermediate biological pathways

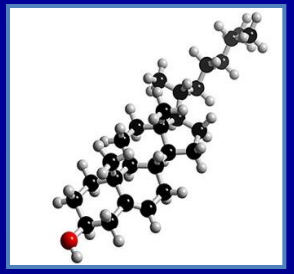


Lifestyle and Behavioural

Pakistan based studies –powerful platforms for discovery

Genetic factors

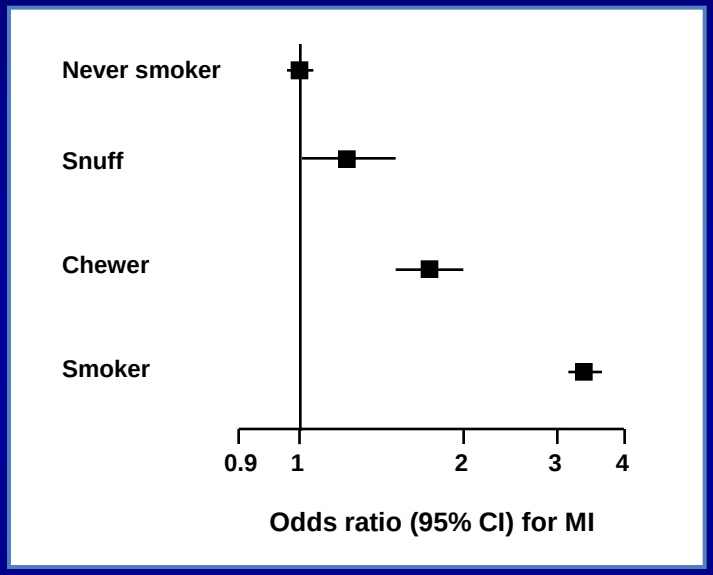
Intermediate biological pathways



Lifestyle and Behavioural

Certain behavioural risk factors can be ethnically distinctive

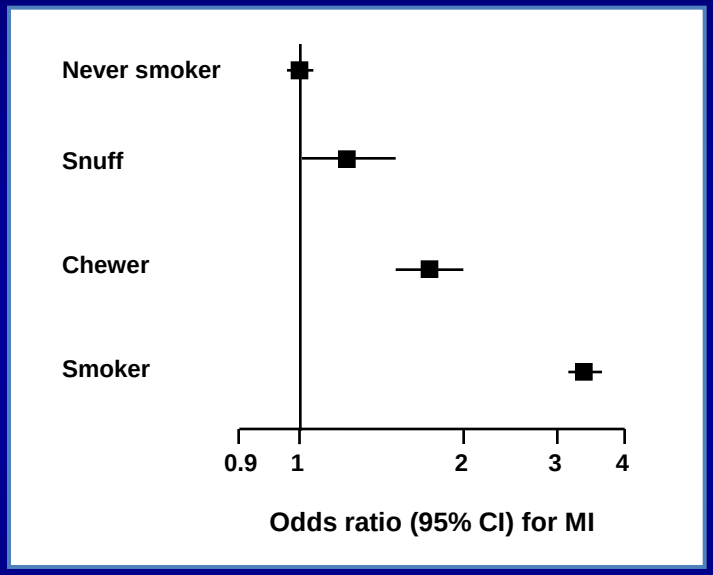
South Asian patterns of tobacco consumption



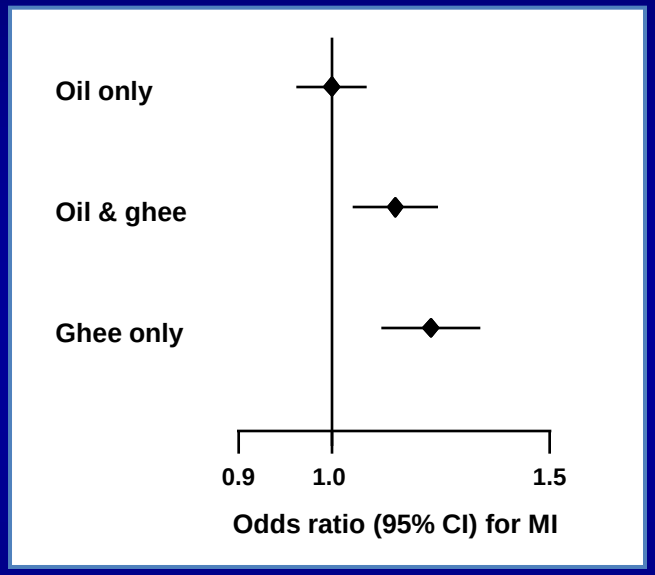
PROMIS, *in preparation*

Certain behavioural risk factors can be ethnically distinctive

South Asian patterns of tobacco consumption



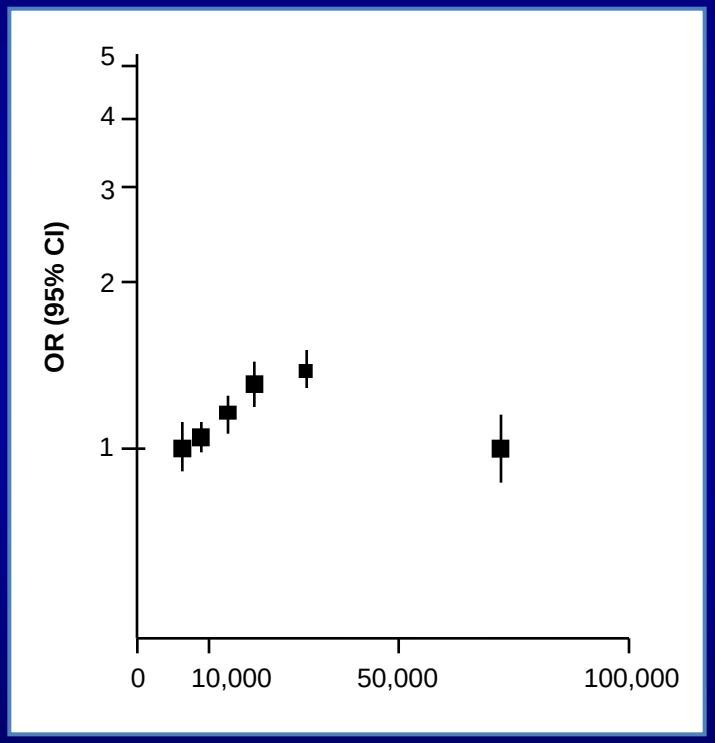
Cooking fat consumption



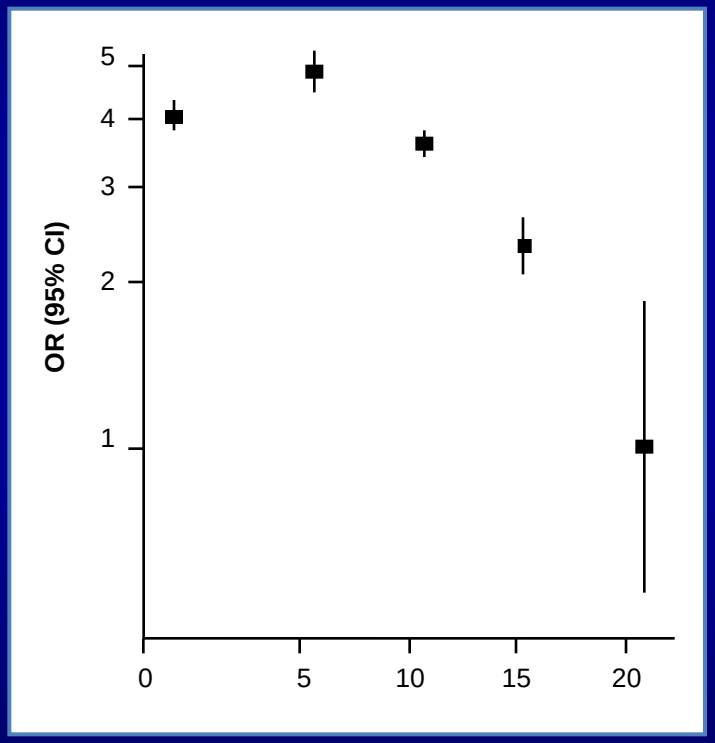
PROMIS, *in preparation*

Socioeconomic determinants of MI in Pakistan

Monthly income (Pak. rupees)



Formal education (years)

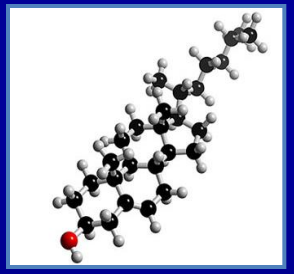


PROMIS, *in preparation*

Pakistan based studies –powerful platforms for discovery

Genetic factors

Intermediate biological pathways



Lifestyle and Behavioural

Study of South Asians should speed discovery of CVD risk factors / targets

Differences in genetic architecture

Enrichment for recessive alleles

Insights of universal relevance

More efficient genetic evaluation of drug targets

Study of South Asians should speed discovery of CVD risk factors / targets

Differences in genetic architecture

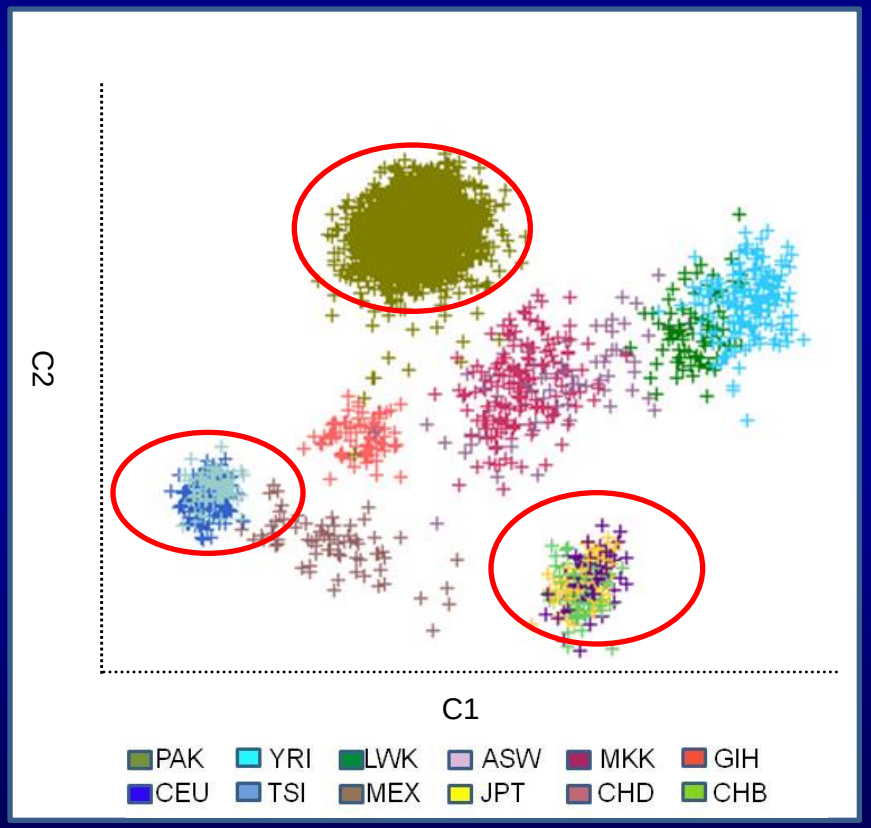
Enrichment for recessive alleles

Insights of universal relevance

More efficient genetic evaluation of drug targets

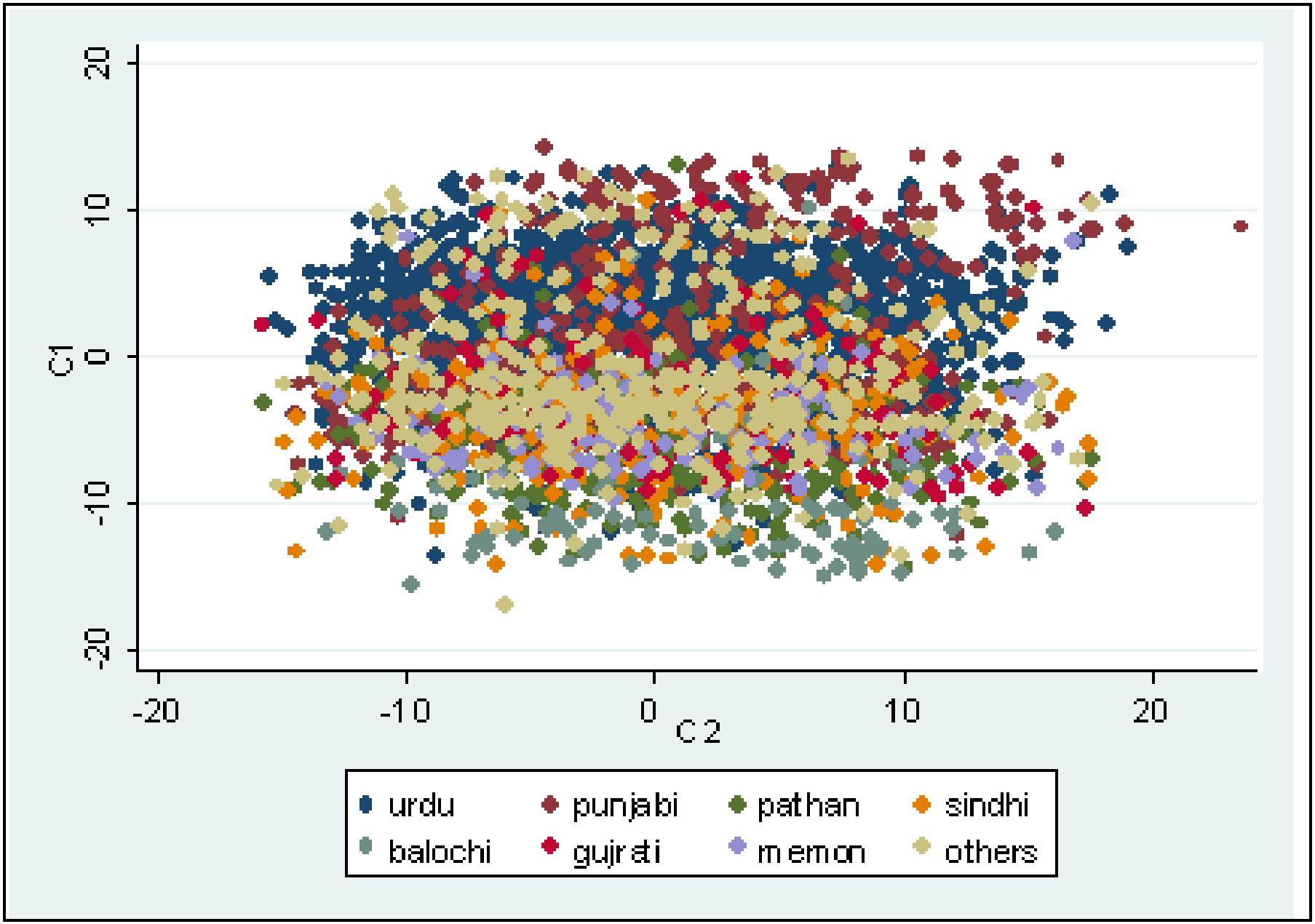
Investigation of population with diverse ancestries can help gene discovery

South Asians have a distinct genetic architecture



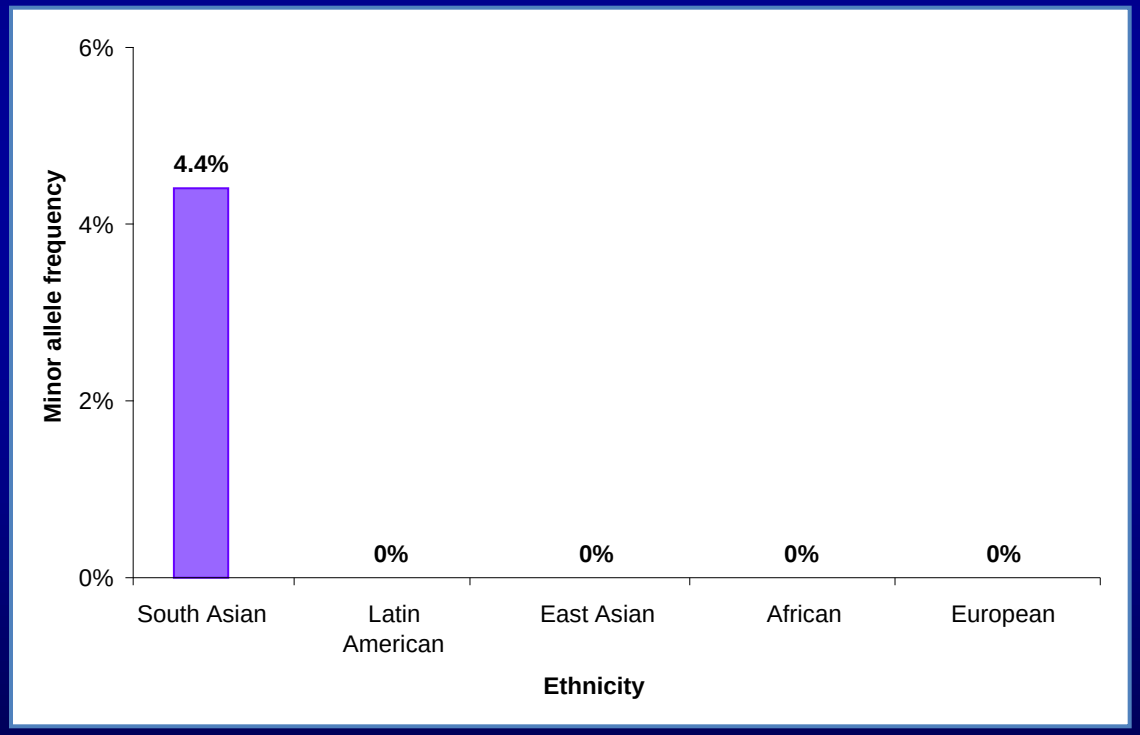
Saleheen et al., Circ Cardiovasc Genet. *2010*

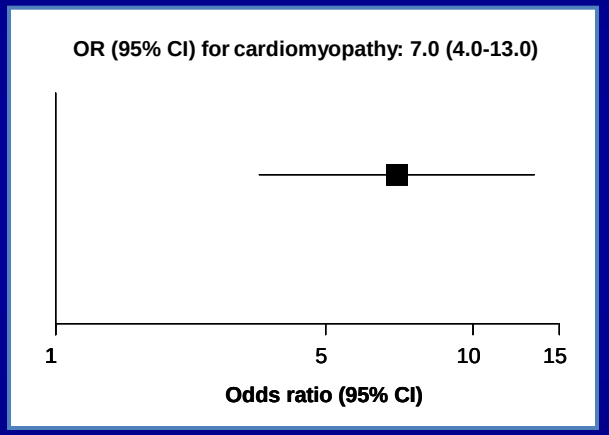
Scatter plot of the first two principle components generated through identity by state analysis



Ethnic-specific variants can provide insight into disease pathology

A South-Asia-specific 25-bp deletion in the *MYBPC3* gene is associated with heritable cardiomyopathies and heart failure





Dhandapany *et al.*, Nat Genet. 2009

Study of non-Europeans should speed discovery of CVD risk factors / targets

Differences in genetic architecture

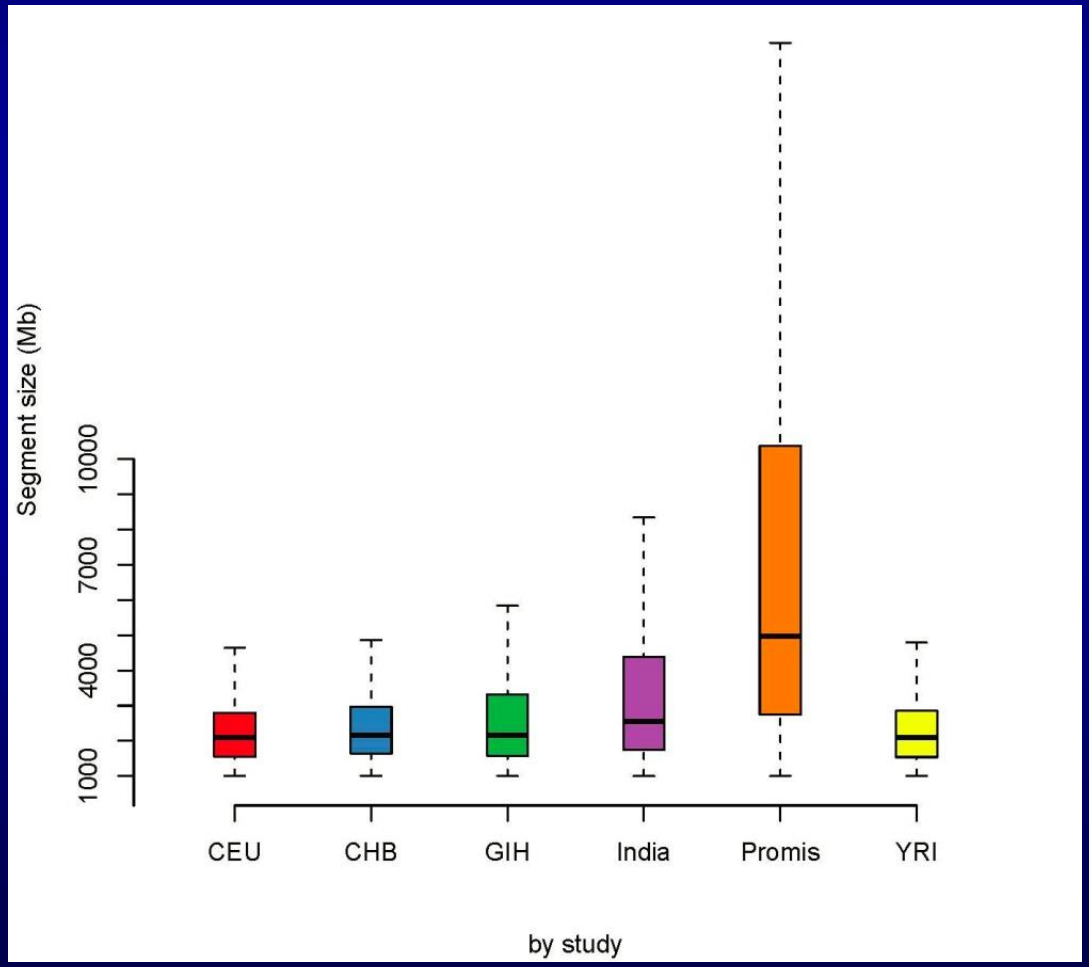
Enrichment for recessive alleles

Insights of universal relevance

More efficient genetic evaluation of drug targets

Homozygosity across different populations

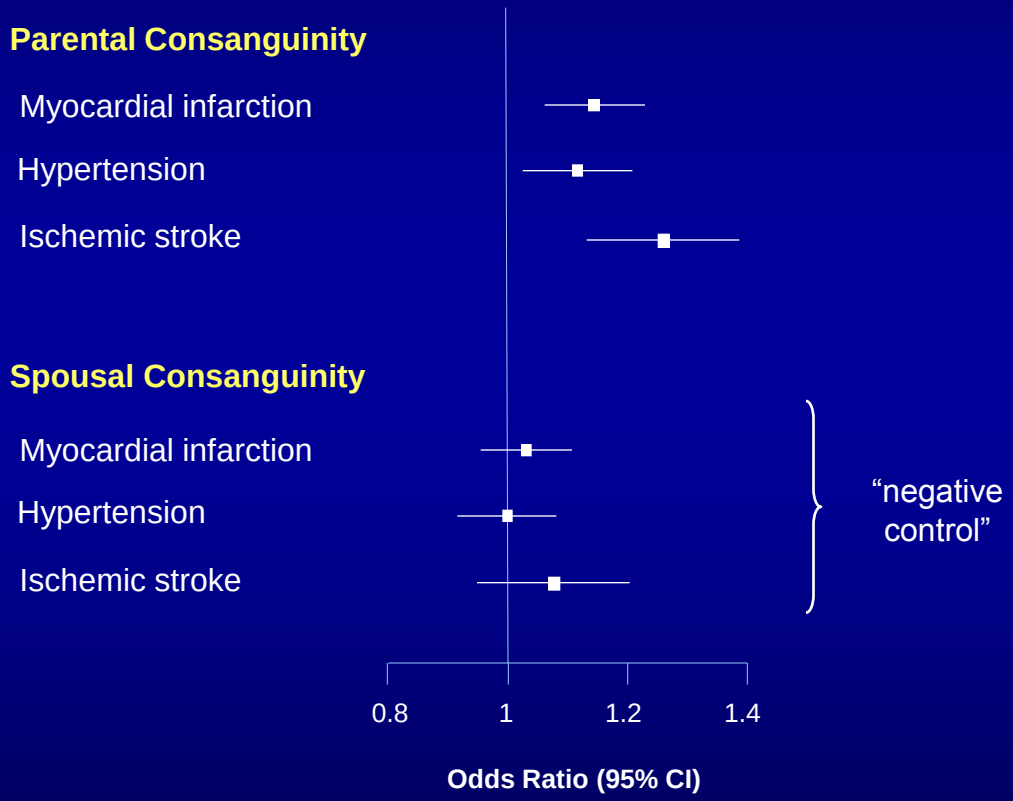
Pakistanis have on average 5-fold higher levels of homozygosity than Europeans



Saleheen *et al.*, in preparation

Consanguinity in Pakistan

1/3 of population report being offspring of first cousin marriages



PROMIS, in preparation

Study of non-Europeans should speed discovery of CVD risk factors / targets

Differences in genetic architecture

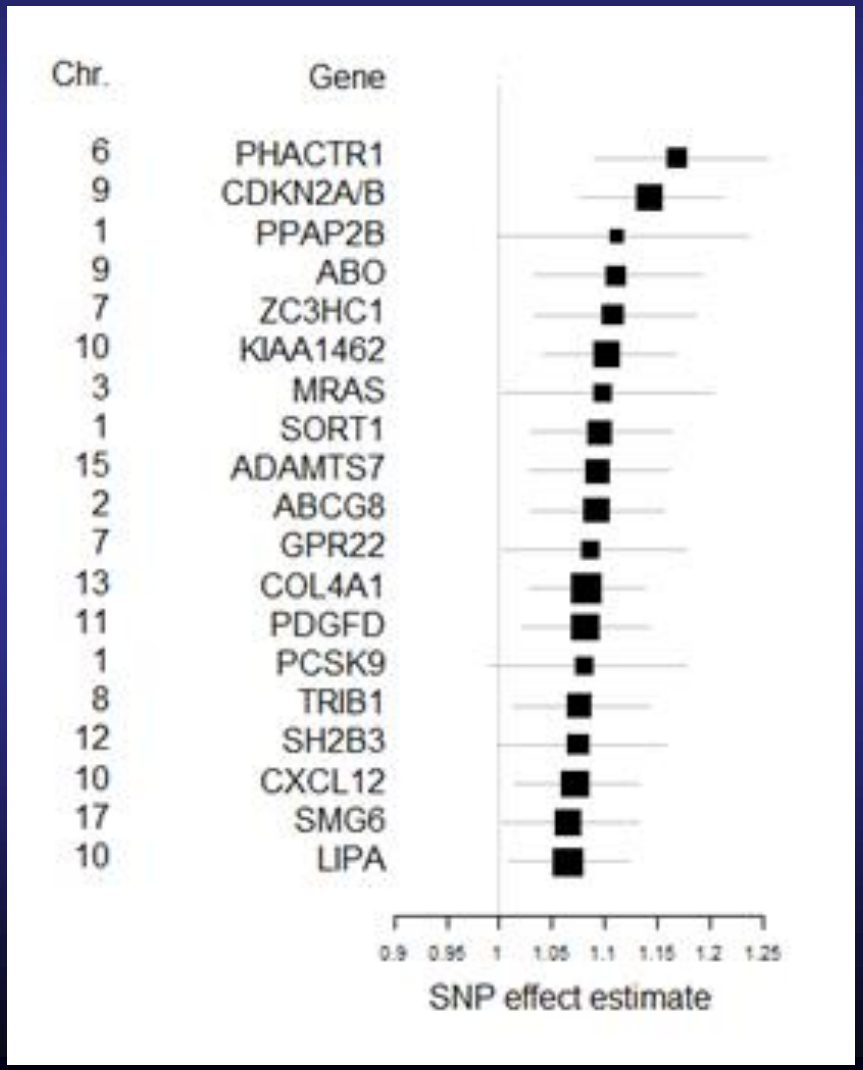
Enrichment for recessive alleles

Insights of universal relevance

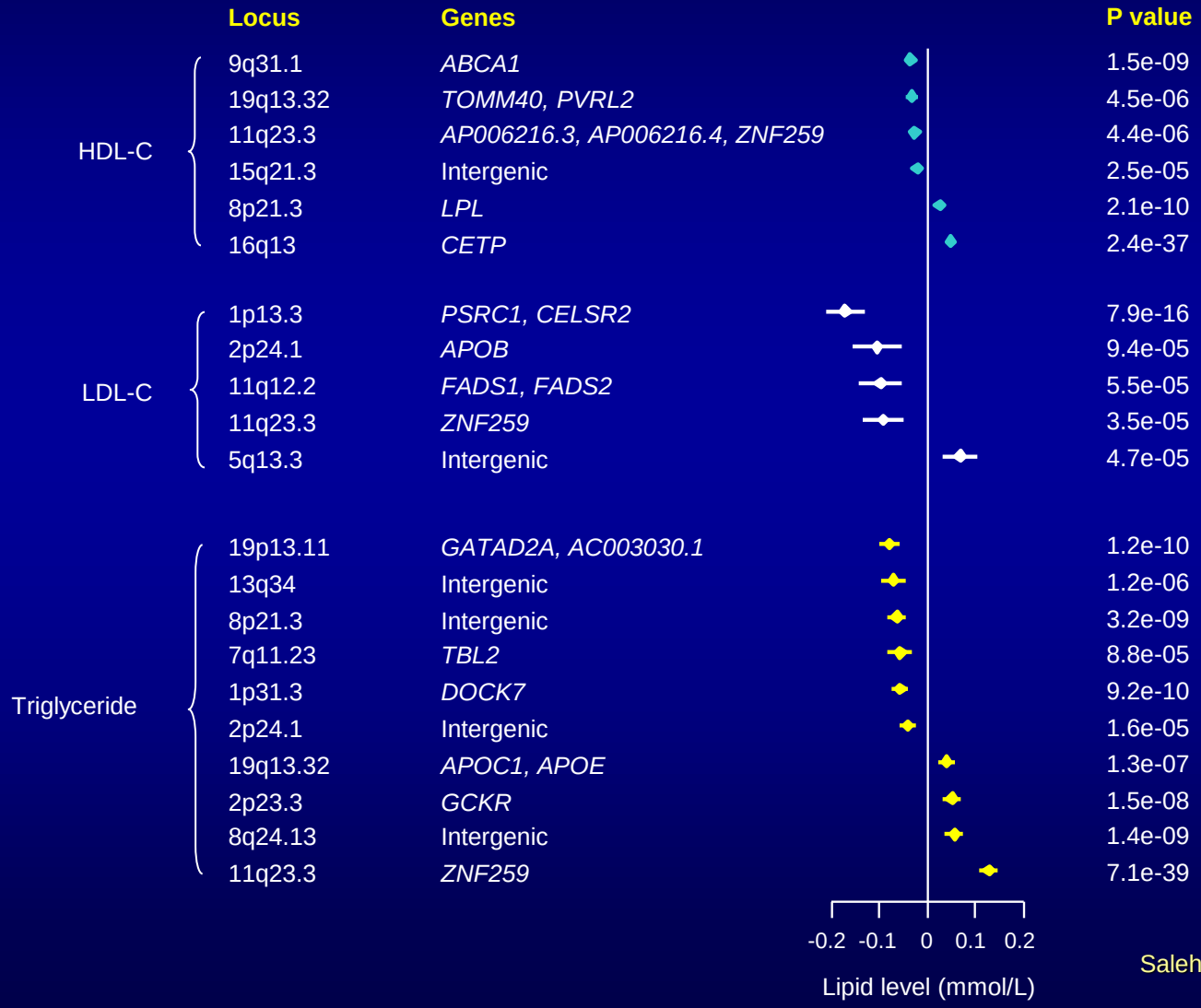
More efficient genetic evaluation of drug targets

Association of established CHD loci in PROMIS

7k MI cases & 7k controls



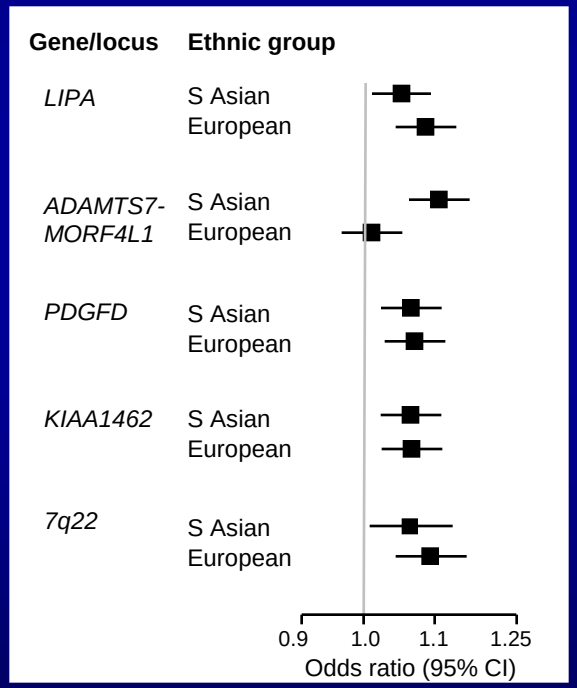
Variants associated with lipid levels in Europeans are also associated in Pakistan



Saleheen *et al.*, Circ. CVD Genet. 2010

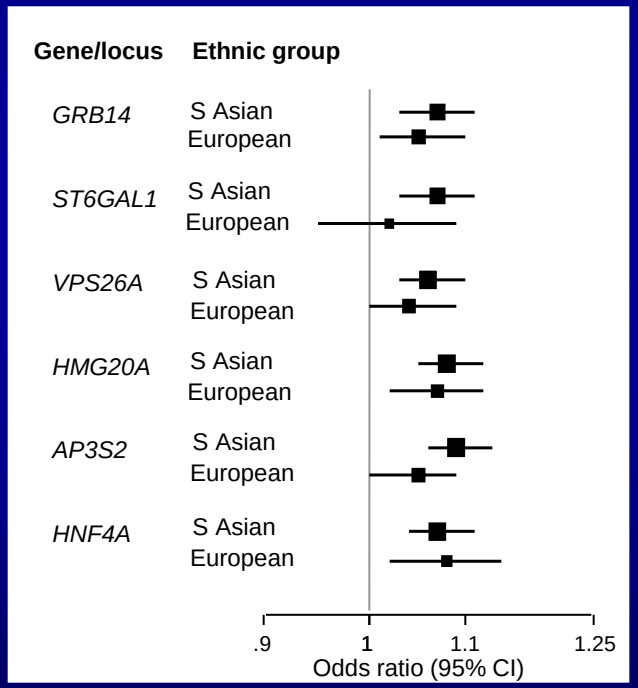
Pooling of Pakistani and European data has identified "cosmopolitan" loci for complex diseases

5 novel loci for CHD



C4D consortium, Nature Genetics 2011

6 novel loci for T2DM



Kooner *et al.*, Nature Genetics 2014

Study of non-Europeans should speed discovery of CVD risk factors / targets

Differences in genetic architecture

Enrichment for recessive alleles

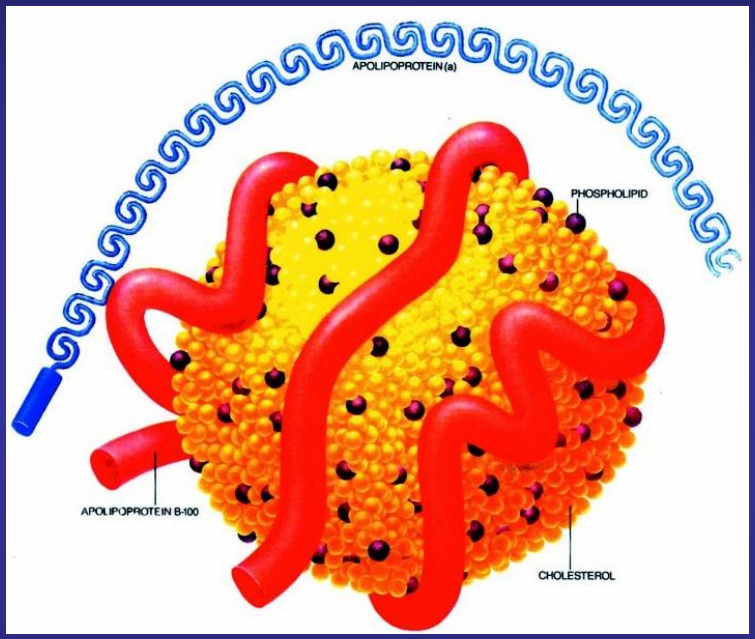
Insights of universal relevance

More efficient genetic evaluation of intermediate pathways (e.g., Mendelian Randomization experiments)

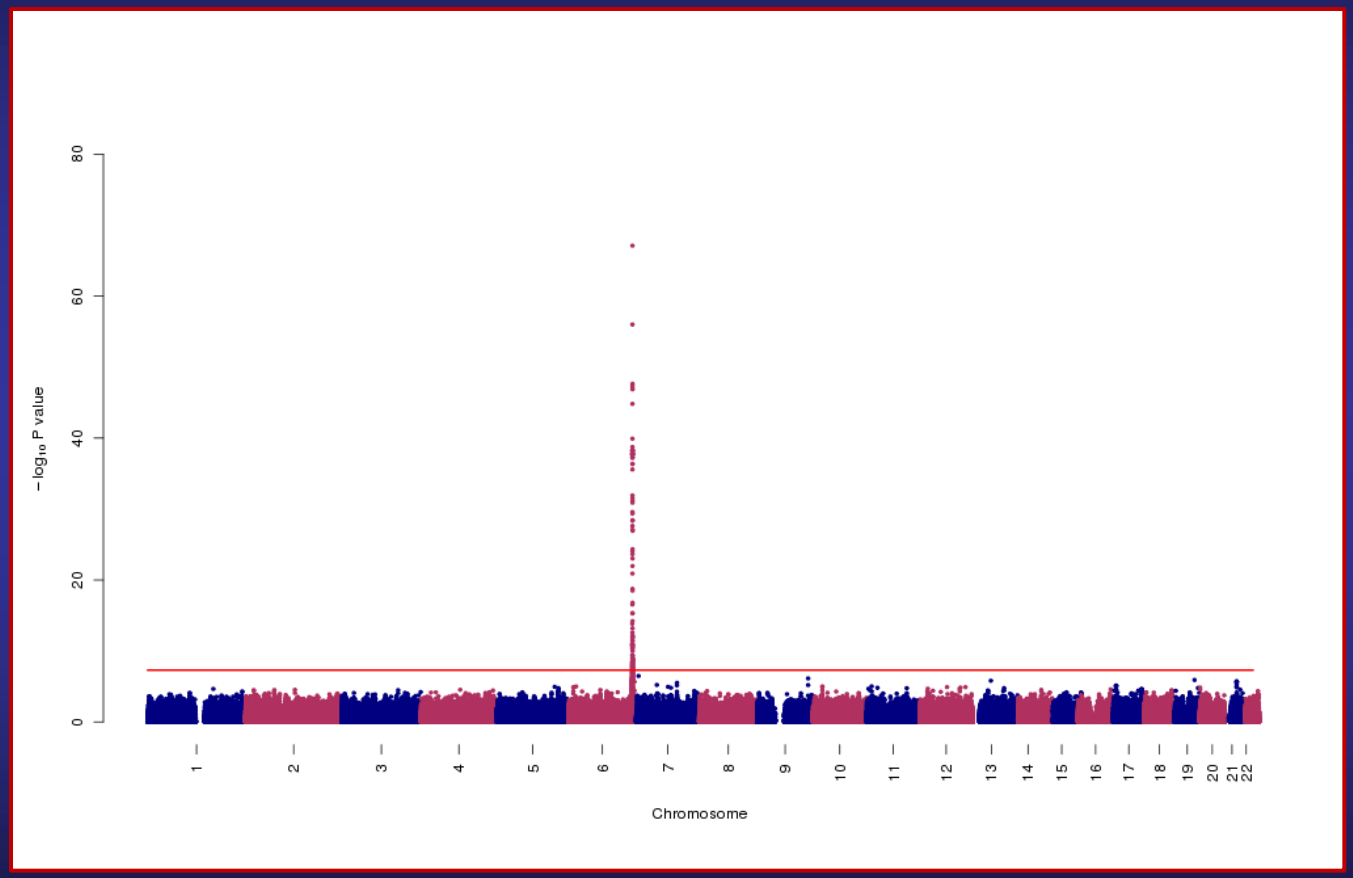
Lipoprotein(a)

Glycoprotein attached to LDL particle

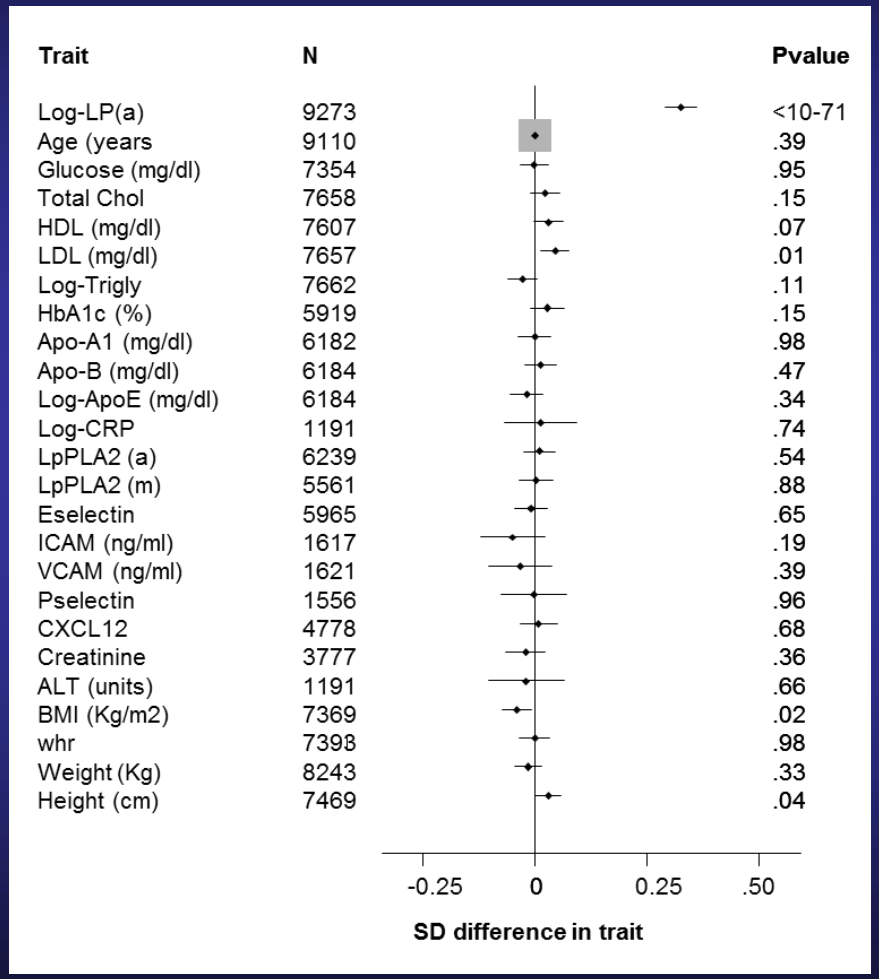
Homology with plasminogen

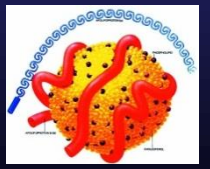


GWAS determinants of Lp(a)
10,000 PROMIS participants



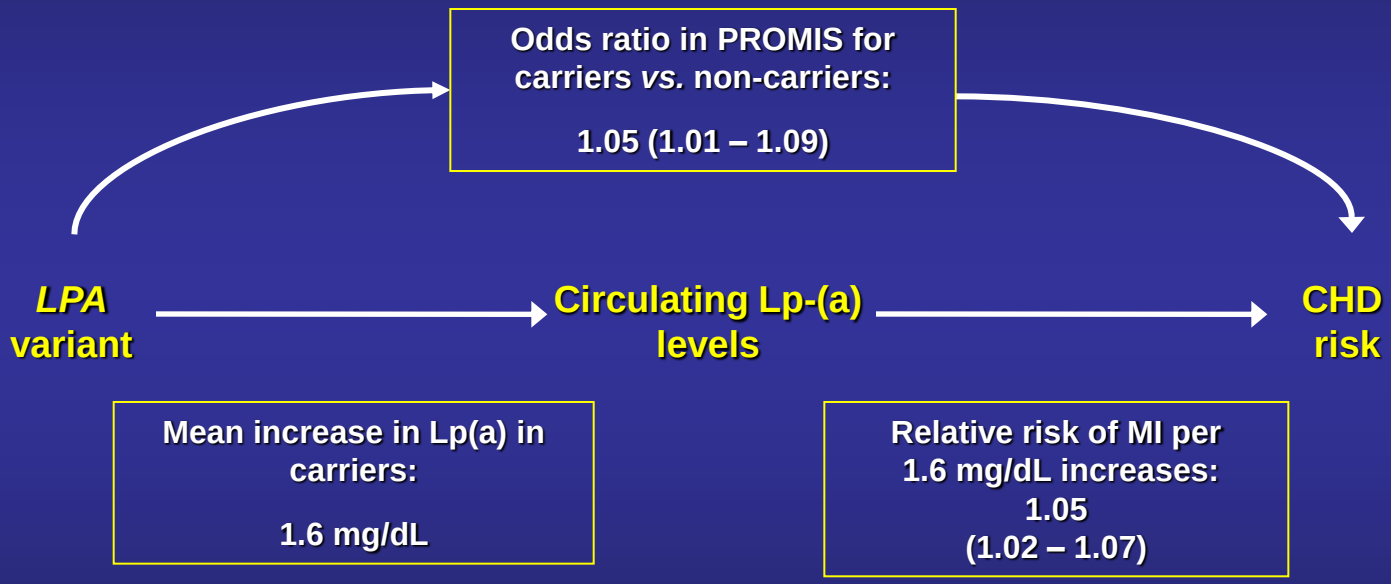
Association of Lp(a) related variant with intermediate pathways in PROMIS



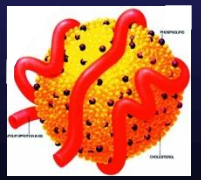


Saleheen *et al.*, in preparation

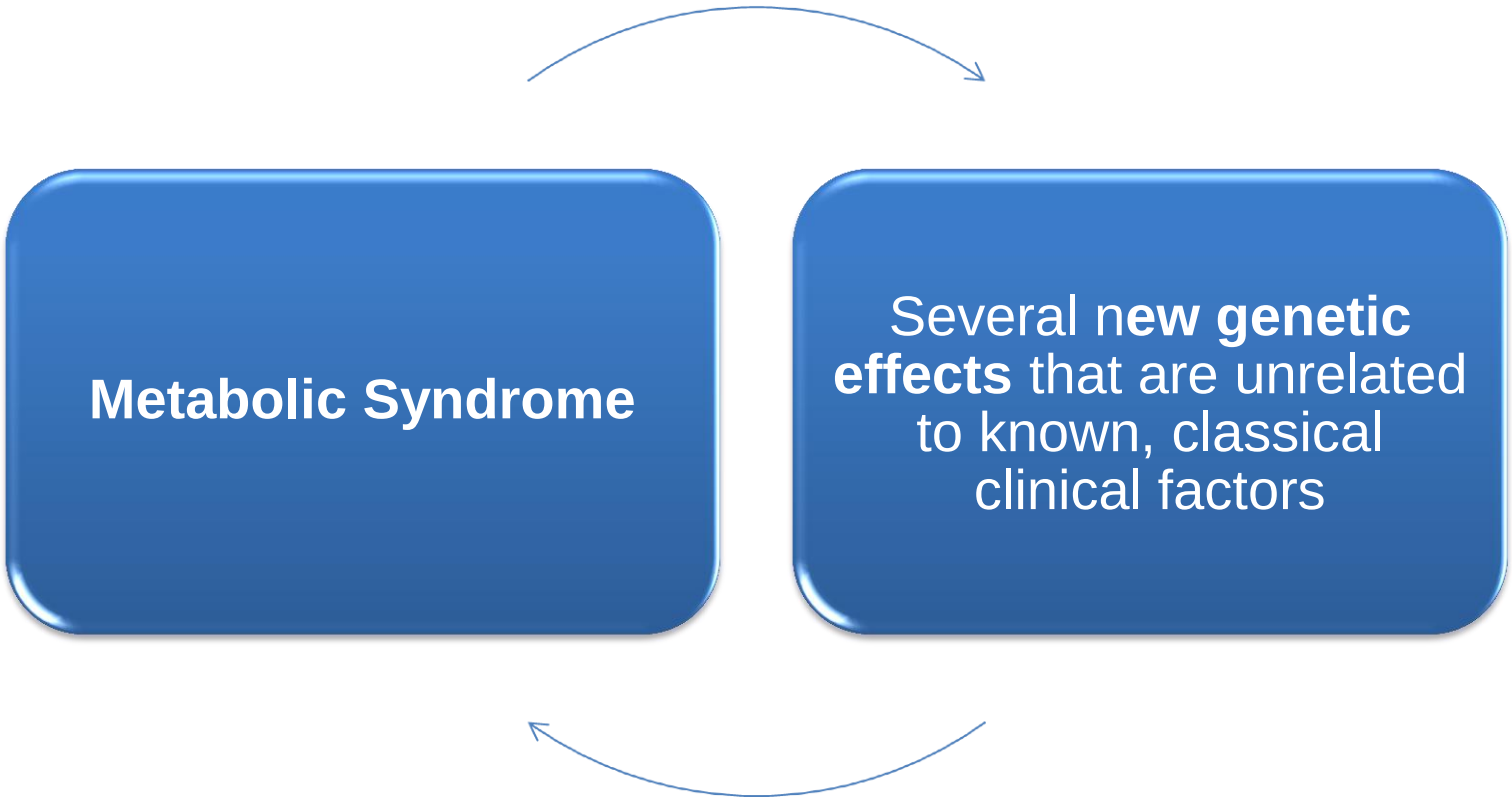
Integration of data involving Gene → Phenotype → CHD risk



Saleheen *et al.*, in preparation



Coronary Heart Disease in Pakistan



Metabolic Syndrome

Abdominal obesity (excessive fat tissue in and around the abdomen)

Atherogenic dyslipidemia (blood fat disorders - high triglycerides, **low HDL [hypo-alpha lipoproteinemia]** and high LDL - that foster plaque buildups in artery walls)

Hypertension (Elevated blood pressure)

Insulin resistance or glucose intolerance (the body cannot properly use insulin or blood sugar)

Prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor-1 in the blood)

Proinflammatory state (e.g., elevated C-reactive protein in the blood)

Are myocardial infarction-associated SNP’s associated with ischemic stroke?

- The major common loci associated with MI risk do not have effects (or effects of similar magnitude) on overall ischemic stroke.
- Different mechanisms are at play in the development of acute ischemic coronary and cerebrovascular events.

→ GWAS on RACE samples underway

Pakistan Risk of MI Study (PROMIS)

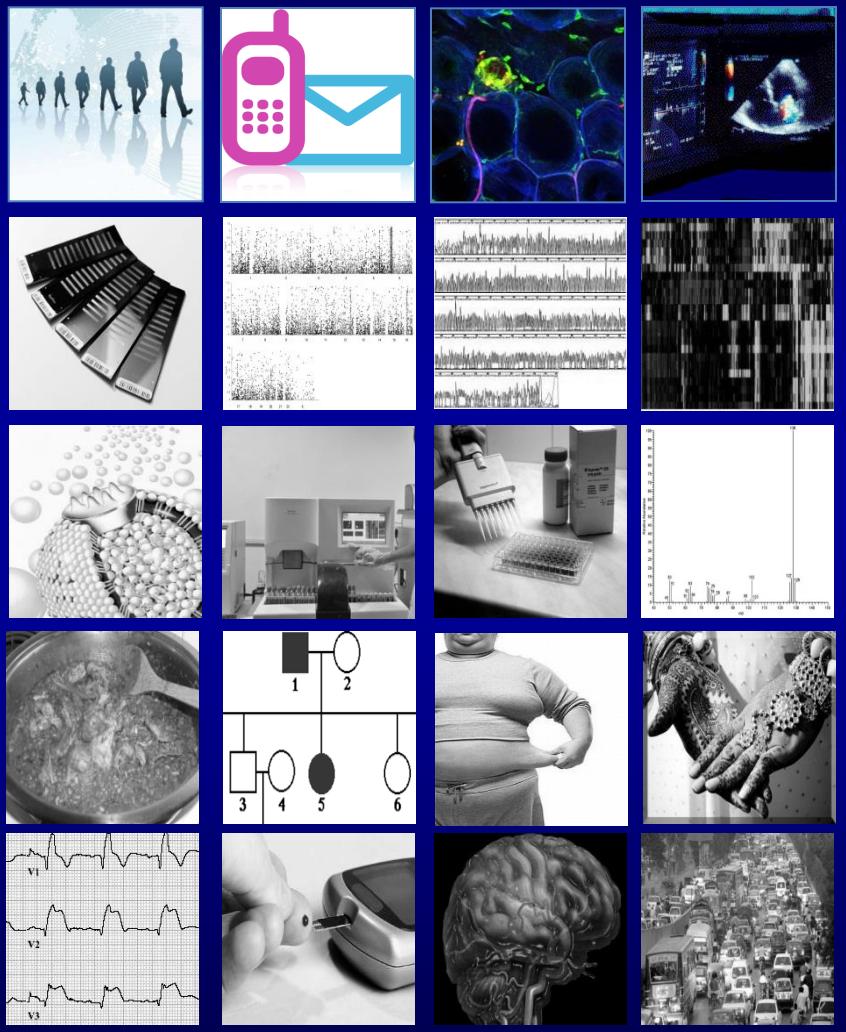
Flexibility in recruitment

Genetic information

Multiple intermediate phenotypes

Clinical & lifestyle information

Multiple disease outcomes



18K MI cases | **10K T2D cases** | **6K stroke cases** | **25K controls**

Examples

Continuing recruitment

~10K new participants per year.

Recall of participants

eg, based on genotype

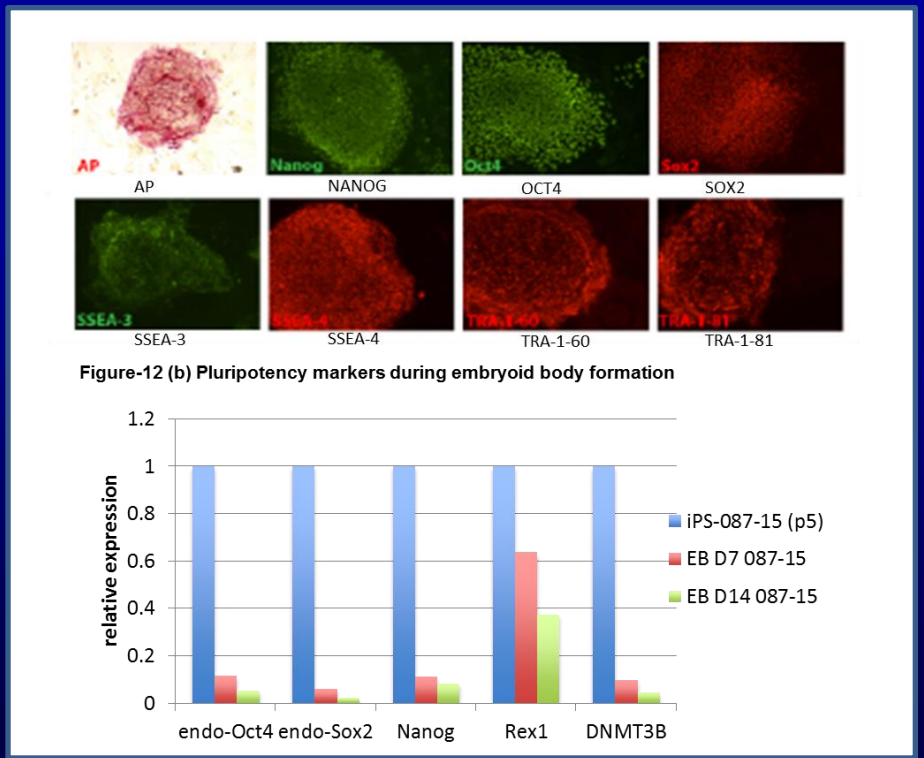
Potential for sub-studies

eg, tissue sampling, physiological measurements, imaging

Mechanistic studies in Humans

- Recall of PROMIS participants for genotypes most strongly associated with CHD risk and biomarker levels
- Isolation of M1 macrophages and adipose tissue for iPS cell transformation by Morrisey's method (UPenn).

Morrisey's method is two orders of magnitude more efficient than the standard Yamanaka protocol



Research Management and Process

Involvement of Students in Research

PLoS ONE 2007 Mar 14; 2:e280

Links

Factors associated with adherence to anti-hypertensive treatment in Pakistan

Hashmi SK, Afridi MB, Abbas K, Sajwani RA, Saleheen D, Ishaq M, Ambreen A, Ahmad U, Frossard PM

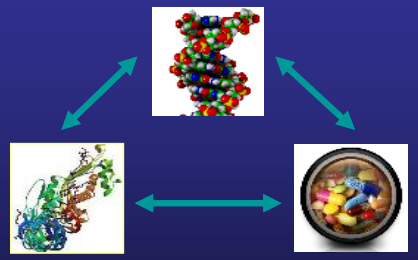
Medical College, Aga Khan University, Karachi, Pakistan

Adherence to anti-hypertensive treatment Research Group

General Conclusion

Genetic epidemiology has considerable potential to prioritize or de-prioritize targets for therapeutic modulation.

Combination of detailed genotyping with dense phenotyping in large studies (such as PROMIS) opens major opportunities for target identification and validation.



Selected Publications over the Past 5 Years

- DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium *et al.* (332 collaborators). *Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility*. **Nature Genetics** 2014; 46(3): 234-244.
- Saxena R, *et al. Genome-Wide Association Study Identifies a Novel Locus Contributing to Type 2 Diabetes Susceptibility in Sikhs of Punjabi Origin From India.* **Diabetes** 2013; 62(5): 1746-1755.
- Voight BF, *et al. Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomization study.* **The Lancet** 2012**;** 380(9841): 572-580.
- Cheng YC, *et al*. *Are myocardial infarction-associated single-nucleotide polymorphisms associated with ischemic stroke?* **Stroke** 2012; 43(4): 980-986.
- Kooner JS *et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci.* **Nature Genetics** 2011; 43(10): 984-989.
- C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC). *Association between C reactive protein and coronary heart disease: Mendelian randomization analysis based on individual participant data*. **British Medical Journal** 2011; 342: d548.
- The Coronary Artery Disease (C4D) Genetics Consortium. *A genome-wide association study in Europeans and South Asians reveals five novel loci for coronary artery disease.* **Nature Genetics** 2011; 43(4): 339-344.

Selected Publications over the Past 5 Years

- Saleheen D *et al*. *Association of the 9p21.3 locus with risk of first-ever myocardial infarction in Pakistan*. **Arteriosclerosis, Thrombosis and Vascular Biology** 2010; 30(7): 1467-1473.
- Saleheen D *et al*. *Genetic determinants of major blood lipids in Pakistan is compared with Europeans*. **Circulation: Cardiovascular Genetics** 2010; 3(4): 348-357.
- Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration. *Triglyceride-mediated pathways and coronary disease: collaborative analysis of 101 studies*. **The Lancet** 2010; 375(9726): 1634-1639.
- The International Stroke Genetics Consortium and the Wellcome Trust Case-Control Consortium. *Failure to validate association between variants on 12p13 and ischemic stroke*. **New England Journal of Medicine** 2010; 362(16): 1547-1550.
- CRP CHD Genetics Collaboration. *Bayesian methods for meta-analysis of causal relationships estimated using genetic instrumental variables.* **Statistics in Medicine** 2010; 29(12): 1298-1311.
- Saleheen D, Hashmi SK, Zaidi M, Rasheed A, Murtaza M, Abbas A, Nasim S, Hameed MQ, Shuja F, Sethi MJ, Hussain I, Shahid K, Khalid H, Ahmad U, Kastelein JJ, Frossard PM, Ishaq M. *Evaluation of therapeutic control in a Pakistani population with hypertension*. **Journal of Evaluation in Clinical Practice** 2010; 16(6): 1081-1084.

PLoS Genet. 2011 Sep;7(9):e1002260. Epub 2011 Sep 22.

Large-scale gene-centric analysis identifies novel variants for coronary artery disease.

IBC 50K CAD Consortium.

Butterworth AS, Braund PS, Farrall M, Hardwick RJ, Saleheen D, Peden JF, Soranzo N, Chambers JC, Sivapalaratnam S, Kleber ME, Keating B, Qasim A, Klopp N, Erdmann J, Assimes TL, Ball SG, Balmforth AJ, Barnes TA, Basart H, Baumert J, Bezzina CR, Boerwinkle E, Boehm BO, Brocheton J, Bugert P, Cambien F, Clarke R, Codd V, Collins R, Couper D, Cupples LA, de Jong JS, Diemert P, Ejebe K, Elbers CC, Elliott P, Fornage M, Franzosi MG, Frossard P, Garner S, Goel A, Goodall AH, Hengstenberg C, Hunt SE, Kastelein JJ, Klungel OH, Klüter H, Koch K, König IR, Kooner AS, Laaksonen R, Lathrop M, Li M, Liu K, McPherson R, Musameh MD, Musani S, Nelson CP, O'Donnell CJ, Ongen H, Papanicolaou G, Peters A, Peters BJ, Potter S, Psaty BM, Qu L, Rader DJ, Rasheed A, Rice C, Scott J, Seedorf U, Sehmi JS, Sotoodehnia N, Stark K, Stephens J, van der Schoot CE, van der Schouw YT, Thorsteinsdottir U, Tomaszewski M, van der Harst P, Vasan RS, Wilde AA, Willenborg C, Winkelmann BR, Zaidi M, Zhang W, Ziegler A, de Bakker PI, Koenig W, Mätz W, Trip MD, Reilly MP, Kathiresan S, Schunkert H, Hamsten A, Hall AS, Kooner JS, Thompson SG, Thompson JR, Deloukas P, Ouwehand WH, Watkins H, Danesh J, Samani NJ, Barnes T, Rafelt S, Codd V, Tomaszewski M, Ouwehand WH, Bruinsma N, Dekker LR, Henriques JP, Koch KT, de Winter RJ, Alings M, Allaart CF, Gorgels AP, Verheugt FW, Braund PS, Thompson JR, Samani NJ, Mueller M, Meisinger C, DerOhannessian S, Mehta NN, Ferguson J, Hakonarson H, Matthai W, Wilensky R, Hopewell JC, Parish S, Linksted P, Notman J, Gonzalez H, Young A, Ostley T, Munday A, Goodwin N, Verdon V, Shah S, Cobb L, Edwards C, Mathews C, Gunter R, Benham J, Davies C, Cobb M, Cobb L, Crowther J, Richards A, Silver M, Tochlin S, Mozley S, Clark S, Radley M, Kourellias K, Silveira A, Söderholm B, Olsson P, Barlera S, Tognoni G, Rust S, Assmann G, Heath S, Zelenika D, Gut I, Green F, Farrall M, Peden J, Goel A, Ongen H, Franzosi MG, Lathrop M, Seedorf U, Clarke R, Collins R, Hamsten A, Watkins H, Aly A, Anner K, Björklund K, Blomgren G, Cederschiöld B, Danell-Toverud K, Eriksson P, Grundstedt U, Hamsten A, Heinonen M, Hellénius ML, van't Hooft F, Husman K, Lagercrantz J, Larsson A, Larsson M, Mossfeldt M, Mälarstig A, Olsson G, Sabater-Lleal M, Sennblad B, Silveira A, Strawbridge R, Söderholm B, Öhrvik J, Zaman KS, Mallick NH, Azhar M, Samad A, Ishaq M, Shah N, Samuel M, Schunkert H, König IR, Kathiresan S, Reilly M, Assimes TL, Holm H, Preuss M, Stewart AF, Barbalic M, Gieger C, Absher D, Aherrahrou Z, Allayee H, Altshuler D, Anand S, Andersen K, Anderson JL, Ardissino D, Ball SG, Balmforth AJ, Barnes TA, Becker LC, Becker DM, Berger K, Bis JC, Boekholdt SM, Boerwinkle E, Braund PS, Brown MJ, Burnett MS, Buysschaert I, Carlquist JF, Chen L, Codd V, Davies RW, Dedoussis G, Dehghan A, Demissie S, Devaney J, Do R, Doering A, El Mokhtari NE, Ellis SG, Elosua R, Engert JC, Epstein S, de Faire U, Fischer M, Folsom AR, Freyer J, Gigante B, Girelli D, Gretarsdottir S, Gudnason V, Gulcher JR, Tennstedt S, Halperin E, Hammond N, Hazen SL, Hofman A, Horne BD, Illig T, Iribarren C, Jones GT, Jukema JW, Kaiser MA, Kaplan LM, Kastelein JJ, Khaw KT, Knowles JW, Kolovou G, Kong A, Laaksonen R, Lambrechts D, Leander K, Li M, Lieb W, Diemert P, Lettre G, Loley C, Lotery AJ, Mannucci PM, Maouche S, Martinelli N, McKeown PP, Meisinger C, Meitinger T, Melander O, Merlini PA, Mooser V, Morgan T, Mühleisen TW, Muhlestein JB, Musunuru K, Nahrstaedt J, Nelson CP, Nöthen MM, Olivieri O, Peyvandi F, Patel RS, Patterson CC, Peters A, Qu L, Quyyumi AA, Rader DJ, Rallidis LS, Rice C, Roosendaal FR, Rubin D, Salomaa V, Sampietro ML, Sandhu MS, Schadt E, Schäfer A, Schillert A, Schreiber S, Schrezenmeir J, Schwartz SM, Siscovick DS, Sivananthan M, Sivapalaratnam S, Smith AV, Smith TB, Snoep JD, Soranzo N, Spertus JA, Stark K, Stefansson K, Stirrups K, Stoll M, Tang WH, Thorgeirsson G, Thorleifsson G, Tomaszewski M, Uitterlinden AG, van Rij AM, Voight BF, Wareham NJ, AWells G, Wichmann HE, Willenborg C, Witteman JC, Wright BJ, Ye S, Ziegler A, Cambien F, Goodall AH, Cupples LA, Quertermous T, März W, Hengstenberg C, Blankenberg S, Ouwehand WH, Hall AS, Deloukas P, Thorsteinsdottir U, Roberts R, Thompson JR, O'Donnell CJ, McPherson R, Erdmann J, Samani NJ, Onland-Moret NC, van Setten J, de Bakker PI, Verschuren WM, Boer JM, Wijmenga C, Hofker MH, Maitland-van der Zee AH, de Boer A, Grobbee DE, Attwood T, Belz S, Braund P, Cambien F, Cooper J, Crisp-Hihn A, Diemert P, Deloukas P, Foad N, Erdmann J, Goodall AH, Gracey J, Gray E, Gwilliams R, Heimerl S, Hengstenberg C, Jolley J, Krishnan U, Lloyd-Jones H, Lugauer I, Lundmark P, Maouche S, Moore JS, Muir D, Murray E, Nelson CP, Neudert J, Niblett D, O'Leary K, Ouwehand WH, Pollard H, Rankin A, Rice CM, Sager H, Samani NJ, Sambrook J, Schmitz G, Scholz M, Schroeder L, Schunkert H, Syvannen AC, Tennstedt S, Wallace C.

Funding Agencies

THANK YOU

The WT Sanger Sequencing Laboratory, Hinxton, UK