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

13 March 2015

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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

Top 3 highest absolute numbers of deaths

Deaths per 100,000 in 2004

National Academy of Sciences, 2010
CVD mortality in the UK is highest in South Asian immigrants

Country of Birth

- Pakistan
- India
- East Africa
- Jamaica
- England

Standardized mortality ratio

Harding S et al., 2008
Projected burden of CVD in 2030

Number in millions

Potentially productive years of life lost in people aged 35-64 yrs

South Asia

China

Russia

USA

South Africa

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, Pakistan 2003
This project aims at identifying the genetic, biomarker and lifestyle determinants of acute myocardial infarction in the Pakistani population.
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.
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

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

Clinical & lifestyle information

- Multiple disease outcomes

- **18K MI cases**
- **10K T2D cases**
- **6K stroke cases**
- **25K controls**
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 | sVCAM-1 | TSH | Fractalkine | FGF-21 | FGF-23 | Vitamin D |

* Functional assays
Pakistan-based studies

Genetic information

Multiple intermediate phenotypes

Clinical & lifestyle information

Multiple disease outcomes

Examples

<table>
<thead>
<tr>
<th>Array</th>
<th>No. of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>GWAS</td>
<td>22K</td>
</tr>
<tr>
<td>Metabochip</td>
<td>18K</td>
</tr>
<tr>
<td>IBC-Cardiochip</td>
<td>4K</td>
</tr>
<tr>
<td>Exome-Sequencing</td>
<td>14K</td>
</tr>
<tr>
<td>Lymphocyte &amp; Monocyte RNA</td>
<td>300</td>
</tr>
<tr>
<td>Exome-chip</td>
<td>22K</td>
</tr>
</tbody>
</table>
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

Assuming 1:1 ratio of cases:controls, type I error = 0.001, and power = 80%
Value of large sample sizes – Gene * Environment interactions

![Graph showing power against number of cases for different values of R_{ge}.]
Figure 5: Actual and projected rate of recruitment in the coming years
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

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Odds ratio (95% CI) for MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never smoker</td>
<td>0.9</td>
</tr>
<tr>
<td>Snuff</td>
<td></td>
</tr>
<tr>
<td>Chewer</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
</tr>
</tbody>
</table>
Certain behavioural risk factors can be ethnically distinctive

South Asian patterns of tobacco consumption

Cooking fat consumption

- Never smoker
- Smoker
- Snuff
- Chewer

<table>
<thead>
<tr>
<th>Cooking Fat Consumption</th>
<th>Odds Ratio (95% CI) for MI</th>
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</thead>
<tbody>
<tr>
<td>Oil only</td>
<td>0.9</td>
</tr>
<tr>
<td>Oil &amp; ghee</td>
<td>1.0</td>
</tr>
<tr>
<td>Ghee only</td>
<td>1.5</td>
</tr>
</tbody>
</table>

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

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
Pakistanis have on average 5-fold higher levels of homozygosity than Europeans.
Consanguinity in Pakistan

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

**Parental Consanguinity**
- Myocardial infarction
- Hypertension
- Ischemic stroke

**Spousal Consanguinity**
- Myocardial infarction
- Hypertension
- Ischemic stroke

Odds Ratio (95% CI)

"negative control"

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Genes</th>
<th>P value</th>
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<tr>
<td>9q31.1</td>
<td>ABCA1</td>
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<td>19q13.32</td>
<td>TOMM40, PVRL2</td>
<td>4.5e-06</td>
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<td>AP006216.3, AP006216.4, ZNF259</td>
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<td>15q21.3</td>
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<td>8p21.3</td>
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<td>16q13</td>
<td>CETP</td>
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<td>1p13.3</td>
<td>PSRC1, CELSR2</td>
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<td>2p24.1</td>
<td>APOB</td>
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<td>11q12.2</td>
<td>FADS1, FADS2</td>
<td>5.5e-05</td>
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<tr>
<td>11q23.3</td>
<td>ZNF259</td>
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<td>13q34</td>
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<tr>
<td>8p21.3</td>
<td>Intergenic</td>
<td>3.2e-09</td>
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<tr>
<td>7q11.23</td>
<td>TBL2</td>
<td>8.8e-05</td>
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<tr>
<td>1p31.3</td>
<td>DOCK7</td>
<td>9.2e-10</td>
</tr>
<tr>
<td>2p24.1</td>
<td>Intergenic</td>
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<tr>
<td>19q13.32</td>
<td>APOC1, APOE</td>
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<td>2p23.3</td>
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<td>11q23.3</td>
<td>ZNF259</td>
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</table>

Lipid level (mmol/L)

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

<table>
<thead>
<tr>
<th>Gene/locus</th>
<th>Ethnic group</th>
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<tbody>
<tr>
<td>LIPA</td>
<td>S Asian, European</td>
</tr>
<tr>
<td>ADAMTS7-MORF4L1</td>
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<td>PDGFD</td>
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<td>KIAA1462</td>
<td>S Asian, European</td>
</tr>
<tr>
<td>7q22</td>
<td>S Asian, European</td>
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</table>

Odds ratio (95% CI)

6 novel loci for T2DM

<table>
<thead>
<tr>
<th>Gene/locus</th>
<th>Ethnic group</th>
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<tbody>
<tr>
<td>GRB14</td>
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<tr>
<td>ST6GAL1</td>
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<tr>
<td>VPS26A</td>
<td>S Asian, European</td>
</tr>
<tr>
<td>HMG20A</td>
<td>S Asian, European</td>
</tr>
<tr>
<td>AP3S2</td>
<td>S Asian, European</td>
</tr>
<tr>
<td>HNF4A</td>
<td>S Asian, European</td>
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</tbody>
</table>

Odds ratio (95% CI)

C4D consortium, Nature Genetics 2011

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

<table>
<thead>
<tr>
<th>Trait</th>
<th>N</th>
<th>Pvalue</th>
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<tbody>
<tr>
<td>Log-LP(a)</td>
<td>9273</td>
<td>&lt;10-71</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9110</td>
<td>.39</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>7354</td>
<td>.95</td>
</tr>
<tr>
<td>Total Chol</td>
<td>7658</td>
<td>.15</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>7607</td>
<td>.07</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>7657</td>
<td>.01</td>
</tr>
<tr>
<td>Log-Trigly</td>
<td>7662</td>
<td>.11</td>
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<tr>
<td>HbA1c (%)</td>
<td>5919</td>
<td>.15</td>
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<tr>
<td>Apo-A1 (mg/dl)</td>
<td>6182</td>
<td>.98</td>
</tr>
<tr>
<td>Apo-B (mg/dl)</td>
<td>6184</td>
<td>.47</td>
</tr>
<tr>
<td>Log-ApoE (mg/dl)</td>
<td>6184</td>
<td>.34</td>
</tr>
<tr>
<td>Log-CRP</td>
<td>1191</td>
<td>.74</td>
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<tr>
<td>LpPLA2 (a)</td>
<td>6239</td>
<td>.54</td>
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<tr>
<td>LpPLA2 (m)</td>
<td>5561</td>
<td>.88</td>
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<tr>
<td>Eselectin</td>
<td>5965</td>
<td>.65</td>
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<tr>
<td>ICAM (ng/ml)</td>
<td>1617</td>
<td>.19</td>
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<tr>
<td>VCAM (ng/ml)</td>
<td>1621</td>
<td>.39</td>
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<tr>
<td>Pselectin</td>
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<tr>
<td>CXCL12</td>
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<td>.68</td>
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<tr>
<td>Creatinine</td>
<td>3777</td>
<td>.36</td>
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<tr>
<td>ALT (units)</td>
<td>1191</td>
<td>.66</td>
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<tr>
<td>BMI (Kg/m2)</td>
<td>7369</td>
<td>.02</td>
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<tr>
<td>whr</td>
<td>7398</td>
<td>.98</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>8243</td>
<td>.33</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>7469</td>
<td>.04</td>
</tr>
</tbody>
</table>
Integration of data involving Gene $\rightarrow$ Phenotype $\rightarrow$ CHD risk

**LPA variant** $\rightarrow$ **Circulating Lp-(a) levels** $\rightarrow$ **CHD risk**

Odds ratio in PROMIS for carriers vs. non-carriers:
1.05 (1.01 – 1.09)

Mean increase in Lp(a) in carriers:
1.6 mg/dL

Relative risk of MI per 1.6 mg/dL increases:
1.05 (1.02 – 1.07)

Saleheen *et al.*, in preparation
Coronary Heart Disease in Pakistan

Metabolic Syndrome

Several new genetic effects that are unrelated to known, classical clinical factors
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

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

Genetic information

Multiple intermediate phenotypes

Clinical & lifestyle information

Multiple disease outcomes

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

Ideally-designed translational research programs

Participation of a uniquely structured population

Effective collaborations with leading hospitals

Network of international collaborators

Part of international consortia

Access to international grant-funding agencies

Training and development of researchers, clinicians, students, administrators
Involvement of Students in Research
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

Selected Publications over the Past 5 Years

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

**IBC 50K CAD Consortium.**

Funding Agencies

- National Institutes of Health
- Wellcome Trust
- Pfizer
- Fogarty
- British Heart Foundation
- MRC (Medical Research Council)
THANK YOU