

Mechanisms underlying Diabetes and Cardiovascular disease in Indian Asians

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The clinical problem ...



- Indian Asians ~15% of local population
- Compared to Europeans, increased
 - MI admission rates x2
 - CVD mortality x1.5
 - T2D prevalence x3
 - ESRD x5

Indian Asians

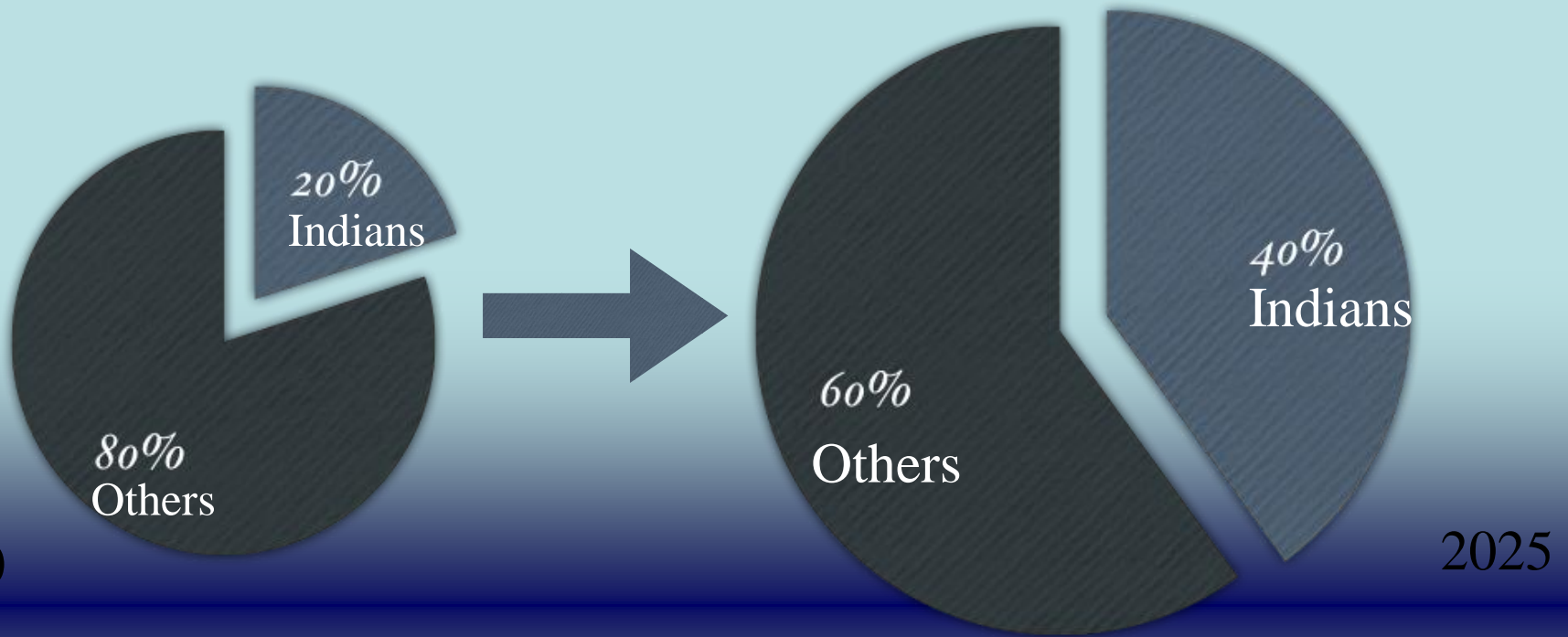
- Definition:
 - India
 - Pakistan
 - Sri Lanka
 - Bangladesh
- Together >1.5 billion people
- Largest migrant population in UK (~3million)



Burden of T2D in India

- Largest numbers with T2D for any country worldwide
 - 2007: 40.8M
 - 2025: 69.9M
- Accounted for ~908,000 deaths in 2007
- Huge economic burden
 - 200: 3.4 *billion* USD
 - 2025: 5.4 *billion* USD

Global burden of CVD



1990

2025

14 million CVD deaths

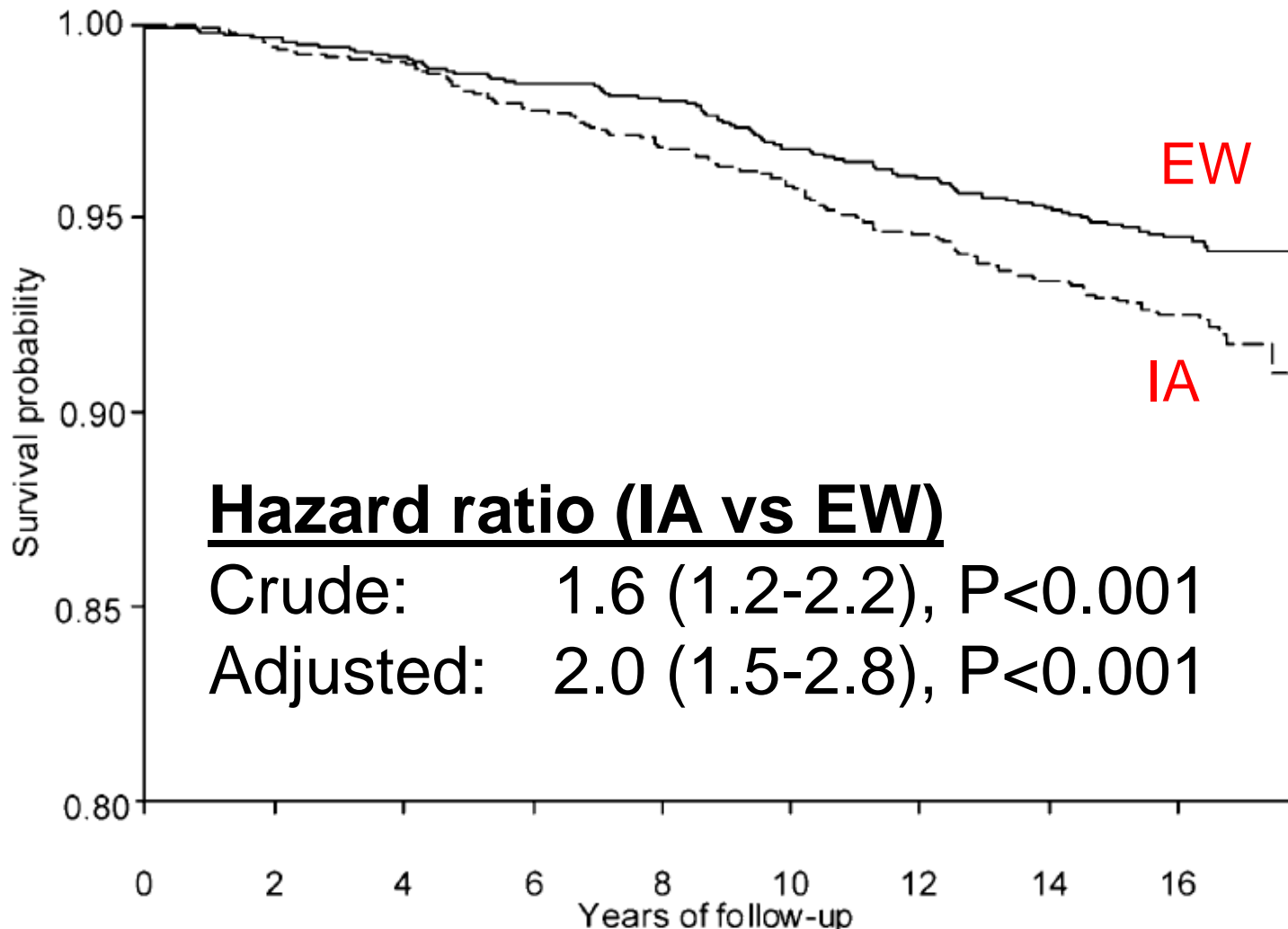
30 + million CVD deaths

CHD risk factors in Indian Asian sub-groups

West London sample 1420 men & women, 1987-1990

	Sikh	Hindu	Muslim
Smoking	↓	↑	→
Blood pressure	↑	↑	→
Total cholesterol	→	↓	→
Diabetes	↑	↑	↑
Waist-hip ratio	↑	↑	↑
2hr insulin	↑	↑	↑
Fasting triglycerides	↑	↑	↑
HDL cholesterol	↓	↓	↓

16 year follow-up



London Life Sciences Population (LOLIPOP) Study

Primary aim

Identify the environmental and genetic factors that contribute to cardiovascular disease, diabetes, obesity & chronic kidney disease in UK Indian Asians compared to Europeans.

LOLIPOP – A Community Study



2002 – 2008
– M+F, 35-75 years
– 17,606 Indian Asians
– 9,766 Europeans

Coronary Research Nurses

GP surgeries

GP surgeries

GP surgeries

5 patients / nurse / day



LOLIPOP – Assessment

- Questionnaire
 - Past medical and family history; drug , smoking and alcohol use; physical activity
- Examination
 - blood pressure; height, weight and BMI; W-H ratio; body fat (bioimpedence); 12 lead ECG
- Analysis
 - Routine haematology and biochemistry
Fasting glucose, insulin and lipids, and HbA1c.
Urine microalbuminuria

Follow-up: 2010-2012

Aim

- Develop and validate new CVD risk functions

Follow-up

- Clinical (face-face) follow-up
- The Office for National Statistics (ONS)
- Routinely collected NHS data

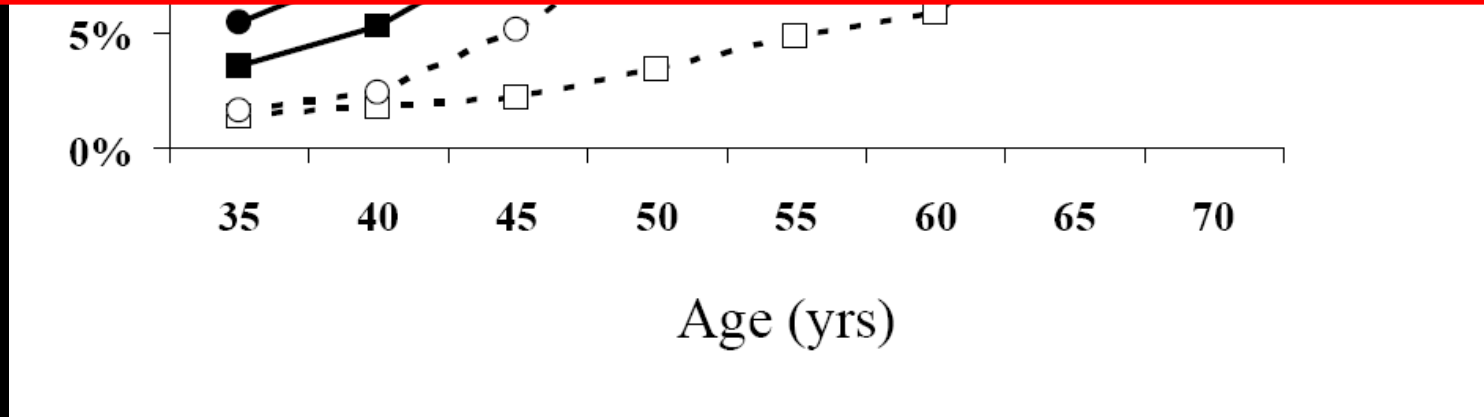
Primary CVD end-point

- Composite of CHD mortality, non-fatal myocardial infarction, coronary revascularisation (PCI, CABG), stroke, and peripheral vascular disease

T2D prevalence: Indian Asians v European whites

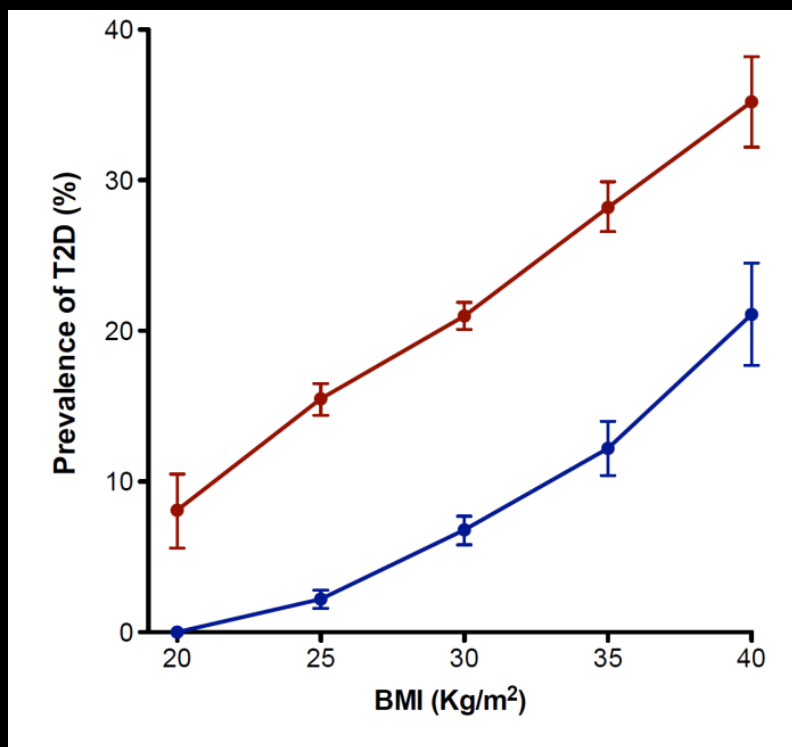


	OR for T2D in IA vs EW	P=
Age / gender	3.26 (2.93 to 3.62)	1.3×10^{-104}
+ BMI, WHR and physical inactivity	3.06 (2.73 to 3.42)	3.6×10^{-85}

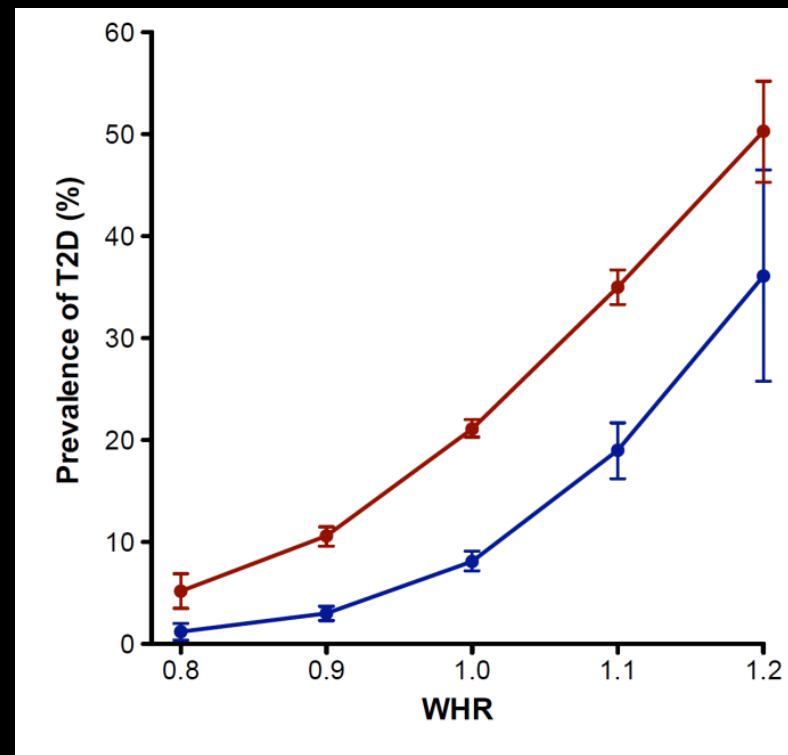


Adiposity does not explain increased risk of T2D in South Asians vs Europeans

Body mass index

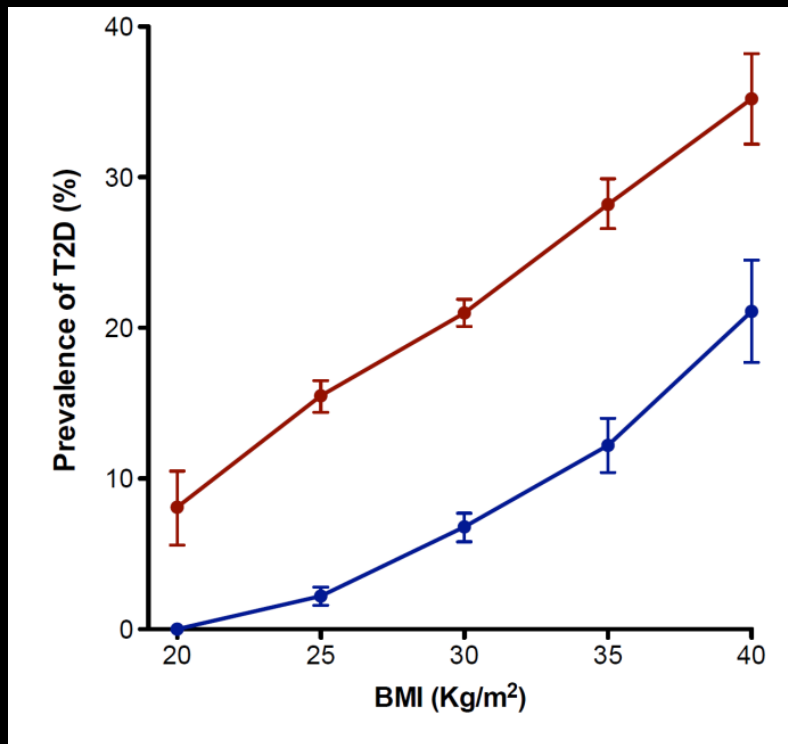


Waist-hip ratio

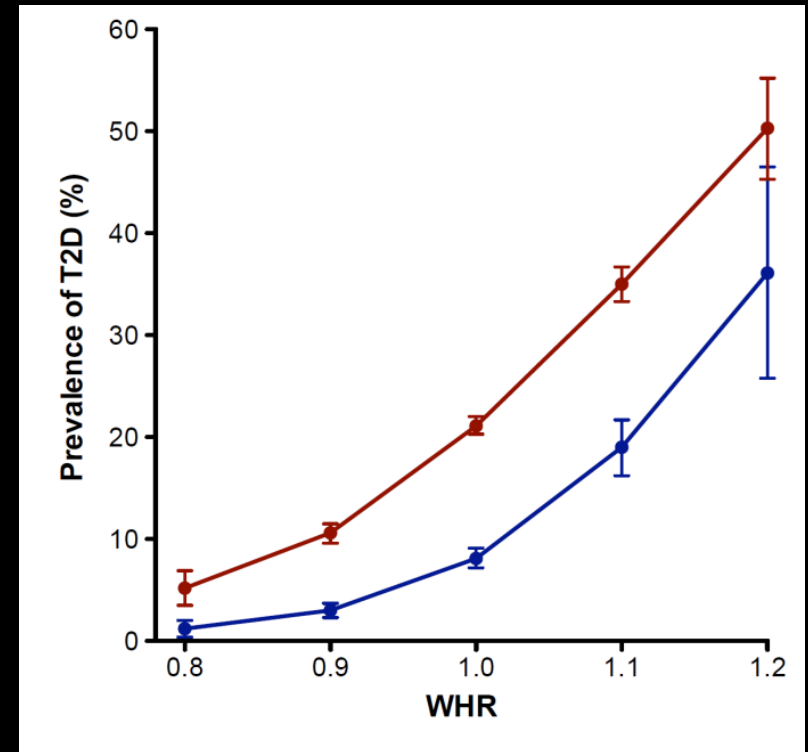


Adiposity and increased risk of T2D in migrant vs non-migrant Indians

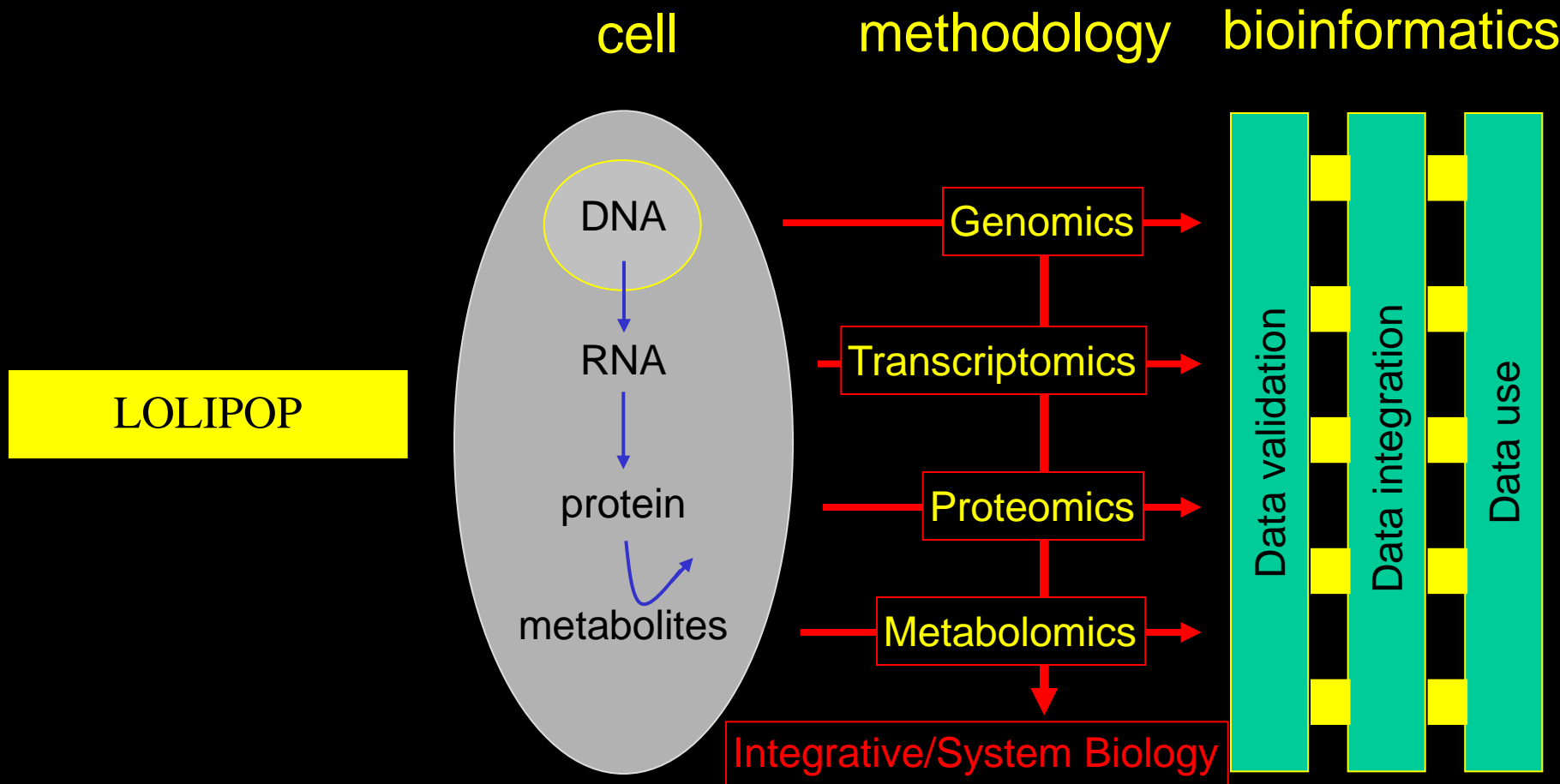
Body mass index



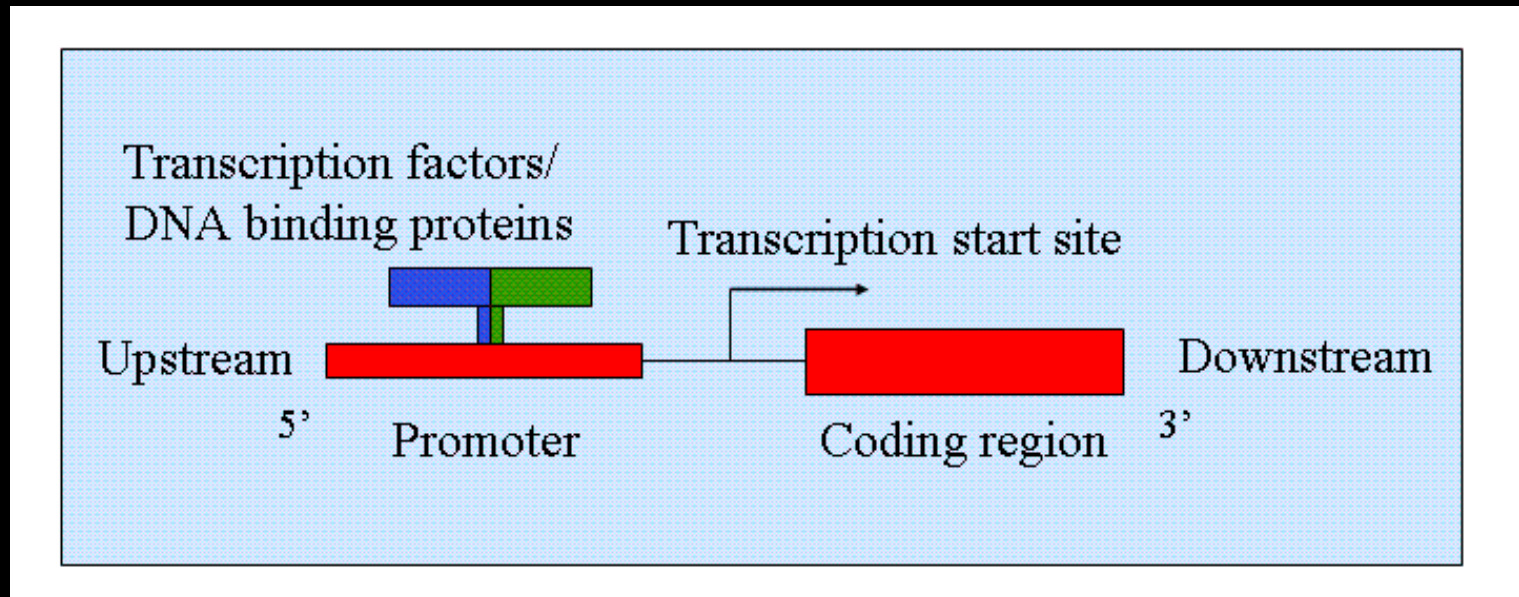
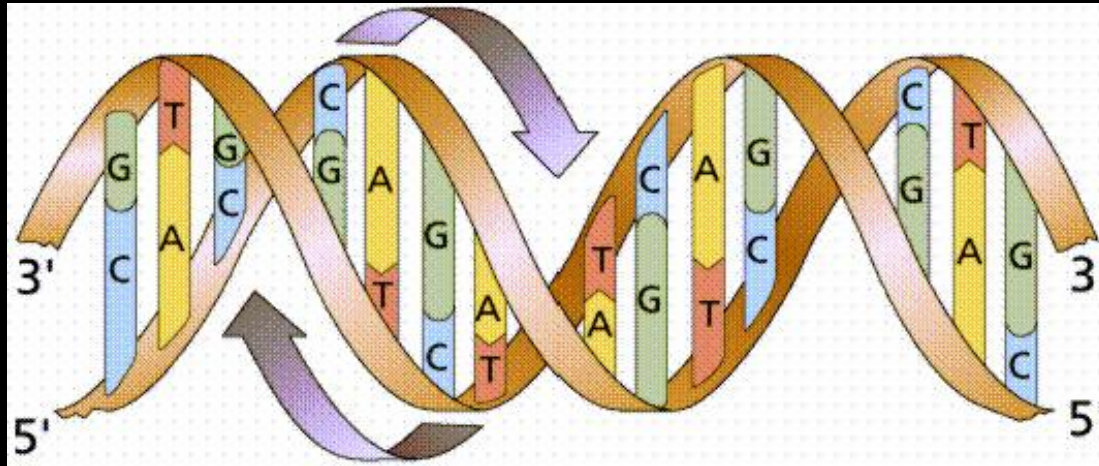
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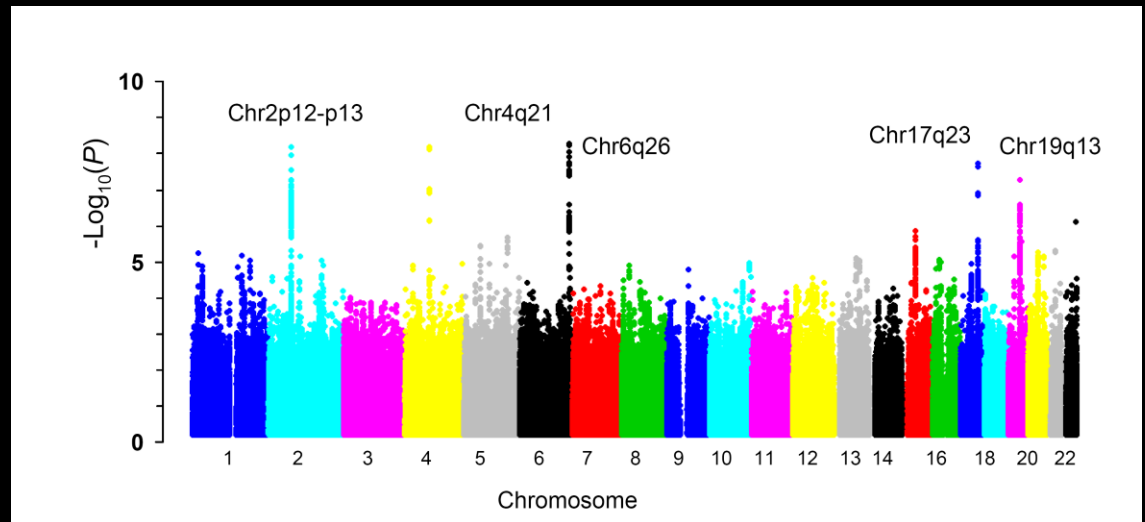
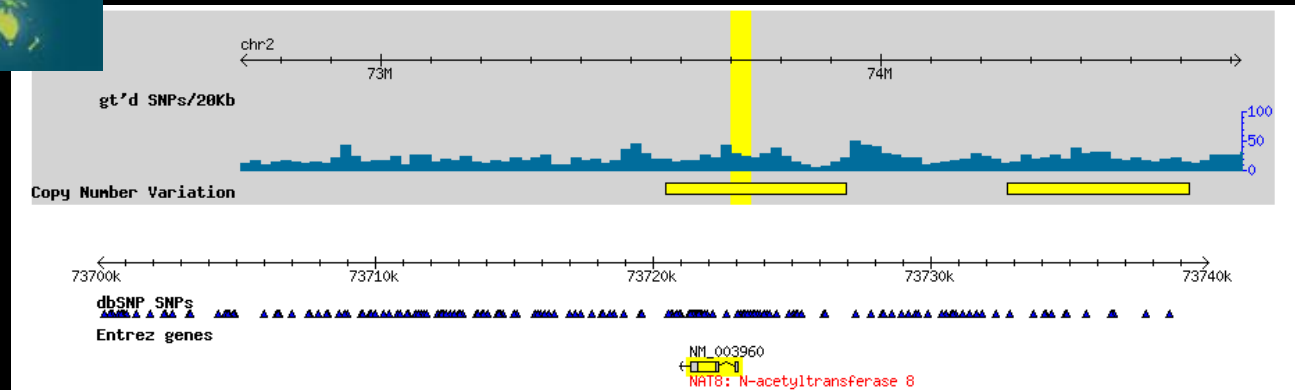
LOLIPOP - Discovery



Heredity

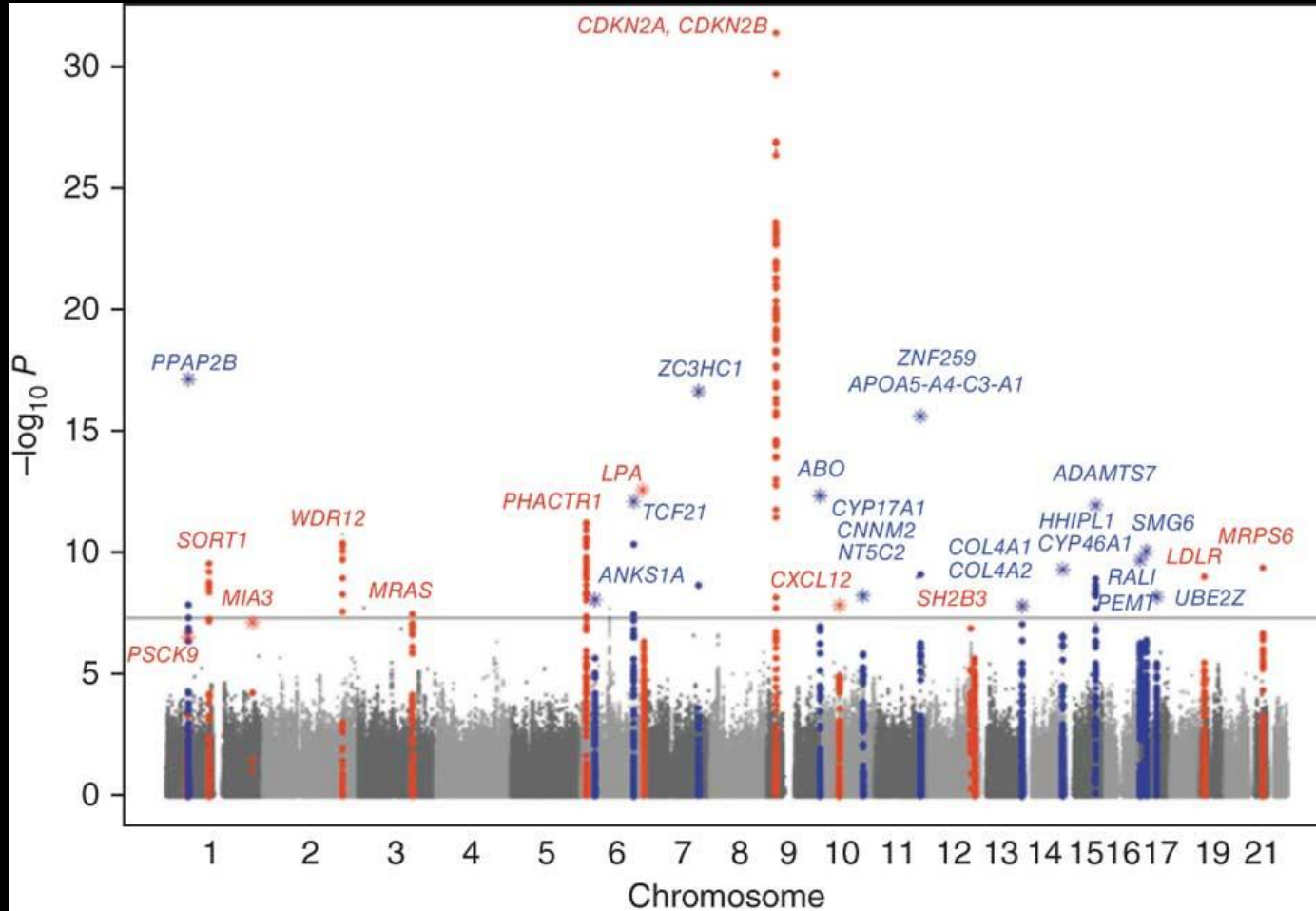


Discovery: Genome-wide association



CARDIOGRAM

22,233 CAD cases and 64,762 controls;
replication in 56,682 people



HUNDREDS of new loci for CVD / risk factors

New opportunities for

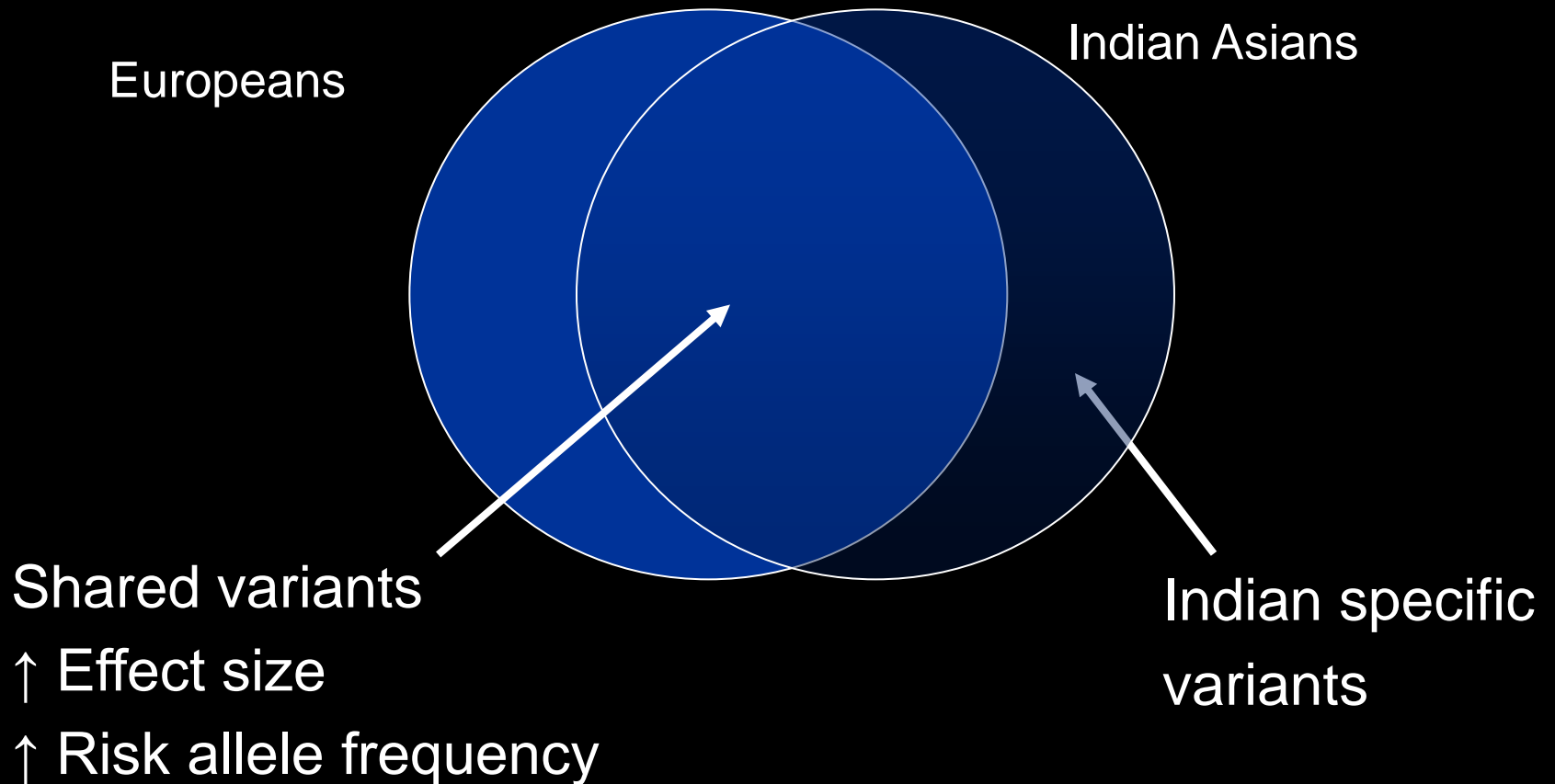
- Biological mechanisms
- Diagnostic tools
- Risk stratification
- Therapeutic strategies

CHD, Diabetes and related metabolic traits are heritable in Indian Asians

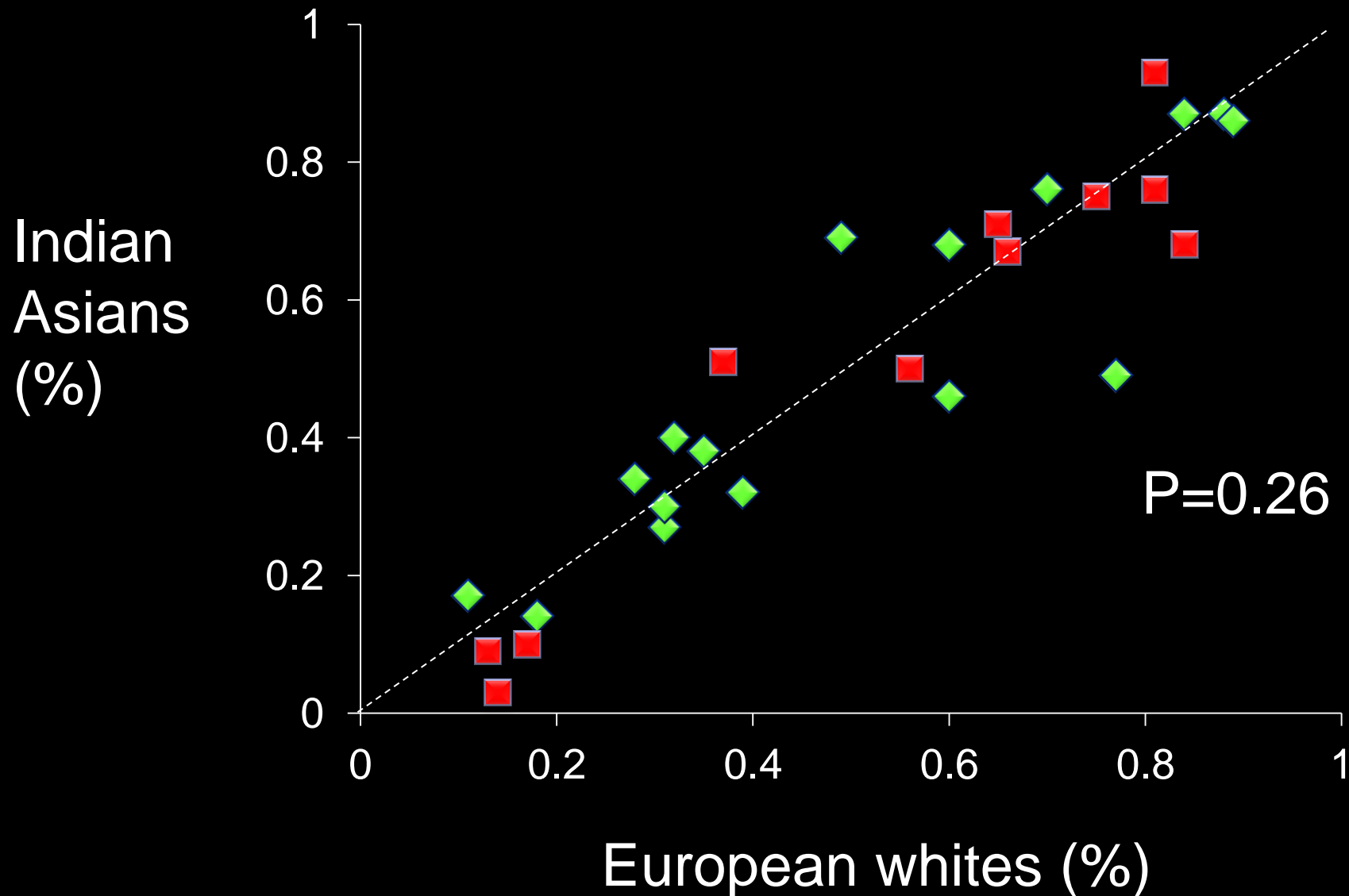
1634 relatives from 180 Indian Asian families

	Heritabilities, h^2 (SE)	Common environment, c^2 (SE)
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Log _e HOMA-IR	0.22 (0.04)**	0.10 (0.03)*

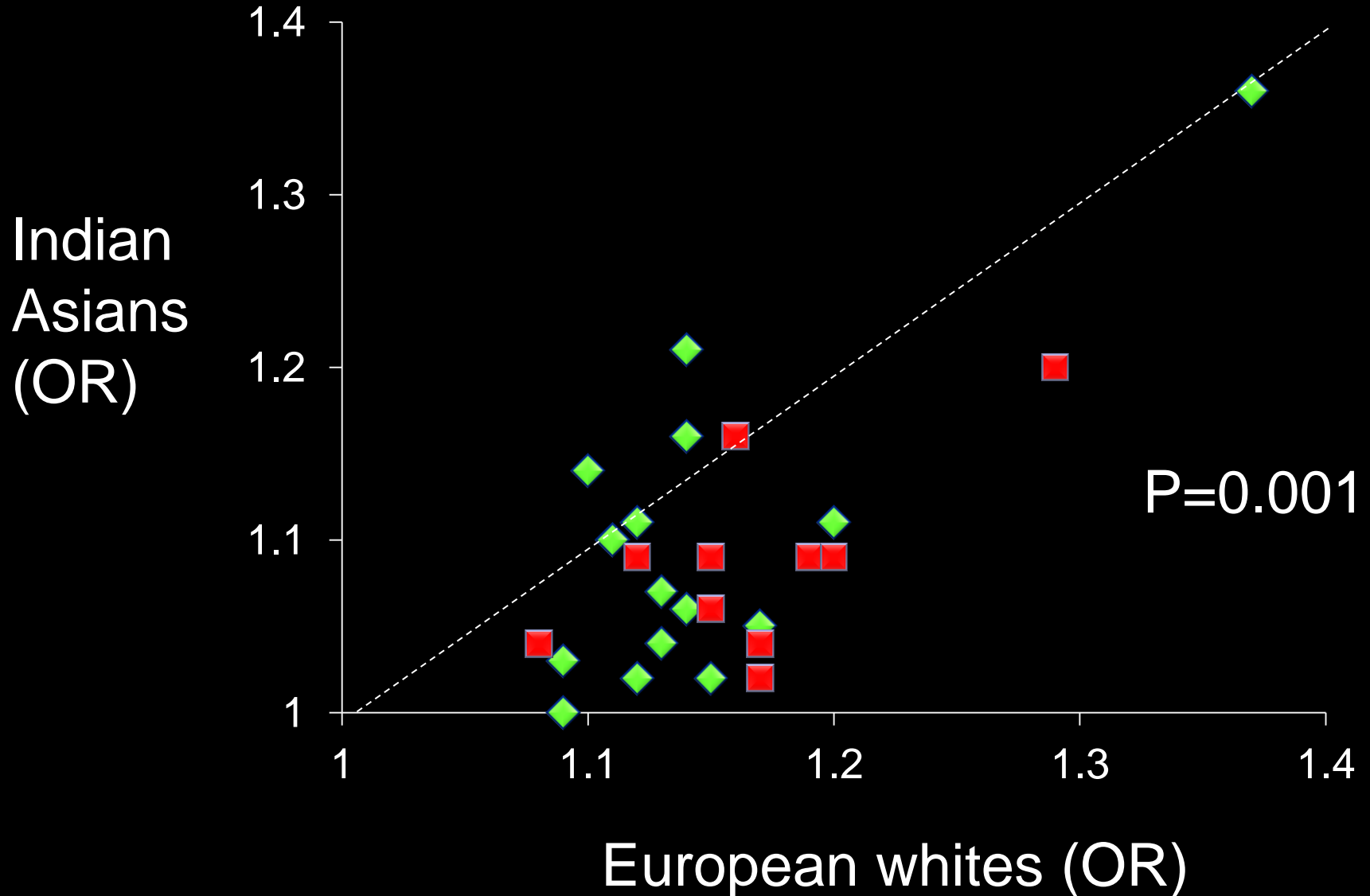
Genomic hypotheses



Risk allele frequencies at known genetic loci



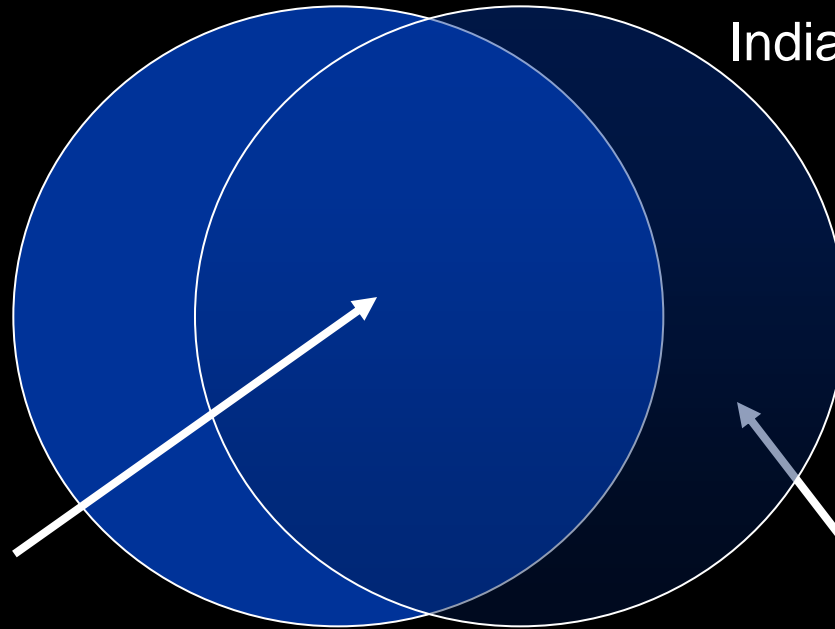
Effect sizes at known genetic loci



Genomic hypothesis

Europeans

Indian Asians



Shared variants

↑ Effect size

↑ Risk allele frequency

Indian specific variants

LOLIPOP Genomics

Resource

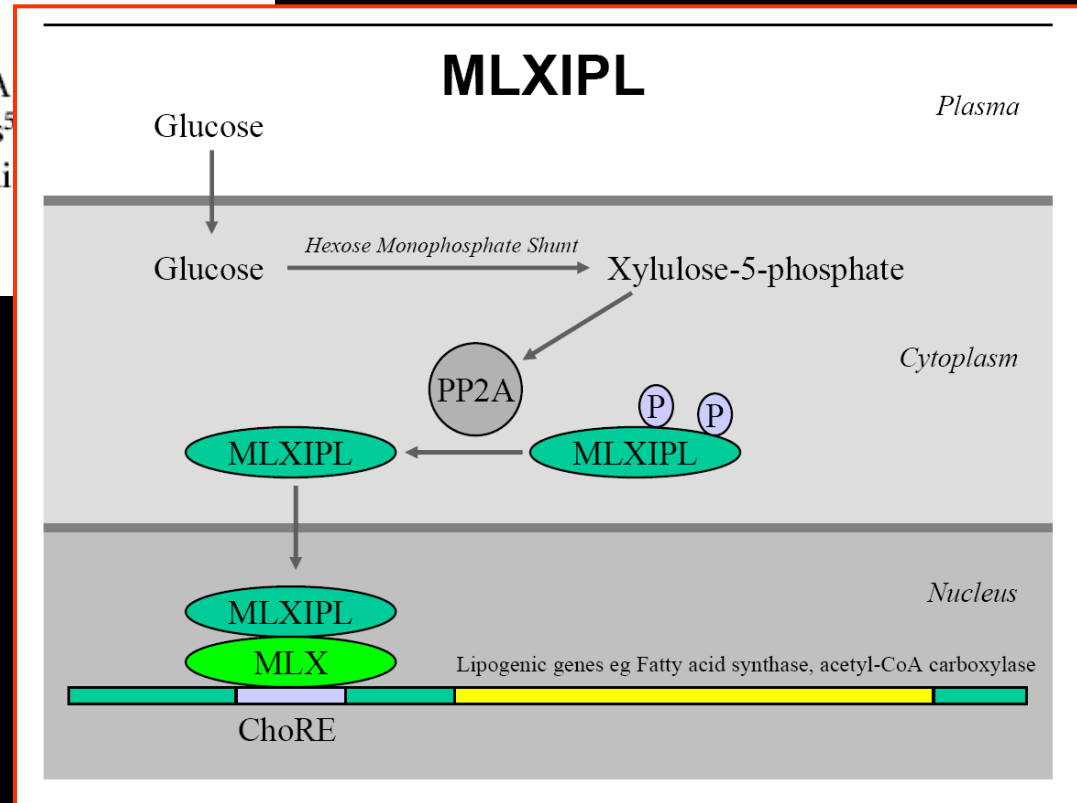
- LOLIPOP GWA scans = 13,502
- International collaborations to create studies of ~100K

Strategy

- Seek genetic factors underlying CHD, T2D and related metabolic disorders in Indian Asians
- Compare effects sizes / allele frequencies with Europeans to explain increased risk in Asians

Genome-wide scan identifies variation in *MLXIPL* associated with plasma triglycerides

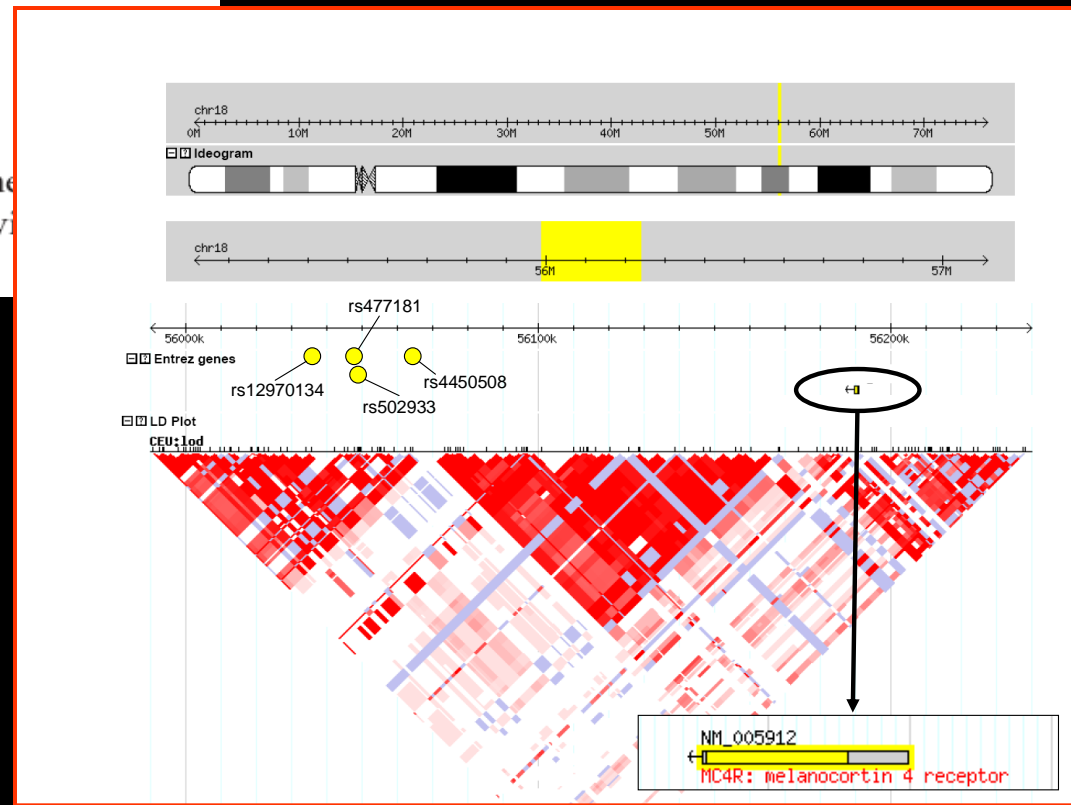
Jaspal S Kooner^{1,7}, John C Chambers^{2,7}, Carlos A A David A Hinds⁴, Craig L Hyde⁵, Gregory R Warnes⁵, Francisco J Gómez Pérez³, Kelly A Frazer⁴, Paul Elliott⁵, James Scott¹, Patrice M Milos^{5,6}, David R Cox⁴ & John F Thompson⁵



Common genetic variation near *MC4R* is associated with waist circumference and insulin resistance

John C Chambers^{1,6}, Paul Elliott^{1,6}, Delilah Zabaneh¹, Weihua Zhang¹, Yun Li², Philippe Froguel^{3,4}, David James Scott⁵ & Jaspal S Kooner⁵

~1cm increased waist circumference per allele copy



Genetic variation in *SCN10A* influences cardiac conduction

John C Chambers^{1,2,11}, Jing Zhao^{3,11}, Cesare M N Terracciano^{4,11}, Connie R Bezzina^{5,6,11}, Weihua Zhang^{1,2}, Riyaz Kaba⁴, Manoraj Navaratnarajah⁴, Amol Lotlikar³, Joban S Sehmi^{2,4}, Manraj K Kooner^{2,4}, Guohong Deng^{2,7}, Urszula Siedlecka⁴, Saurabh Parasramka², Ismail El-Hamamsy⁴, Mark N Wass⁸, Lukas R C Dekker⁶, Jonas S S G de Jong⁶, Michael J E Sternberg⁸, William McKenna⁹, Nicholas J Severs⁴, Ranil de Silva^{2,4}, Arthur A M Wilde^{5,6}, Praveen Anand¹⁰, Magdi Yacoub^{4,11}, James Scott^{4,11}, Paul Elliott^{1,11}, John N Wood^{3,11} & Jaspal S Kooner^{2,4,11}

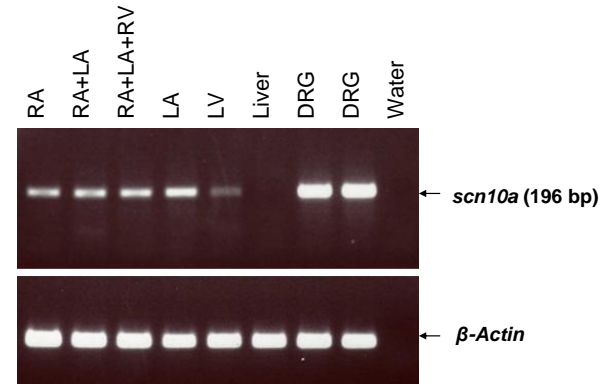
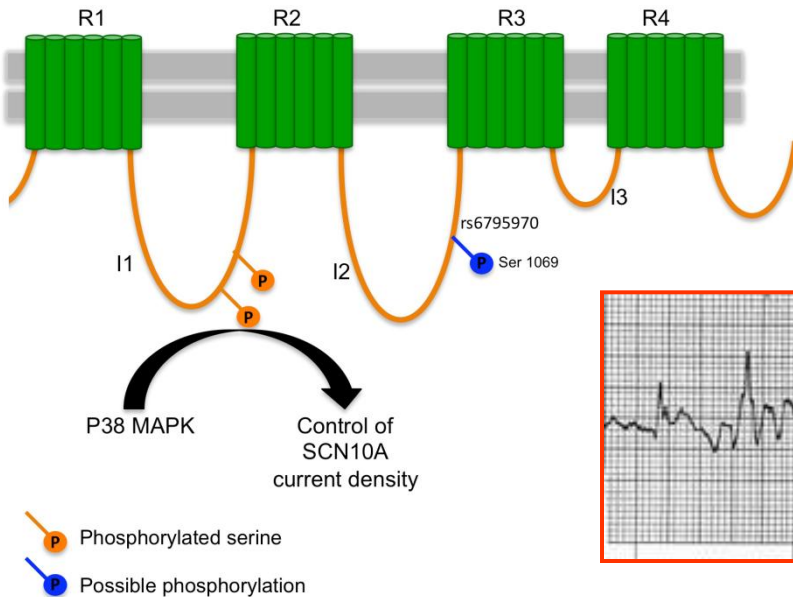


Fig 4a. Mouse heart

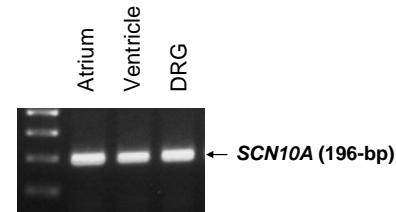


Fig 4b. Isolated mouse myocytes

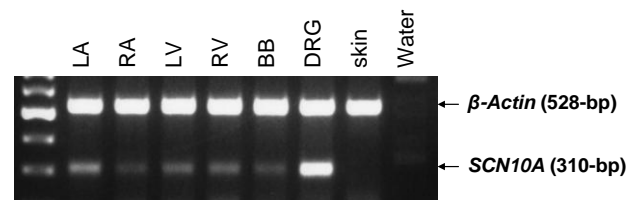
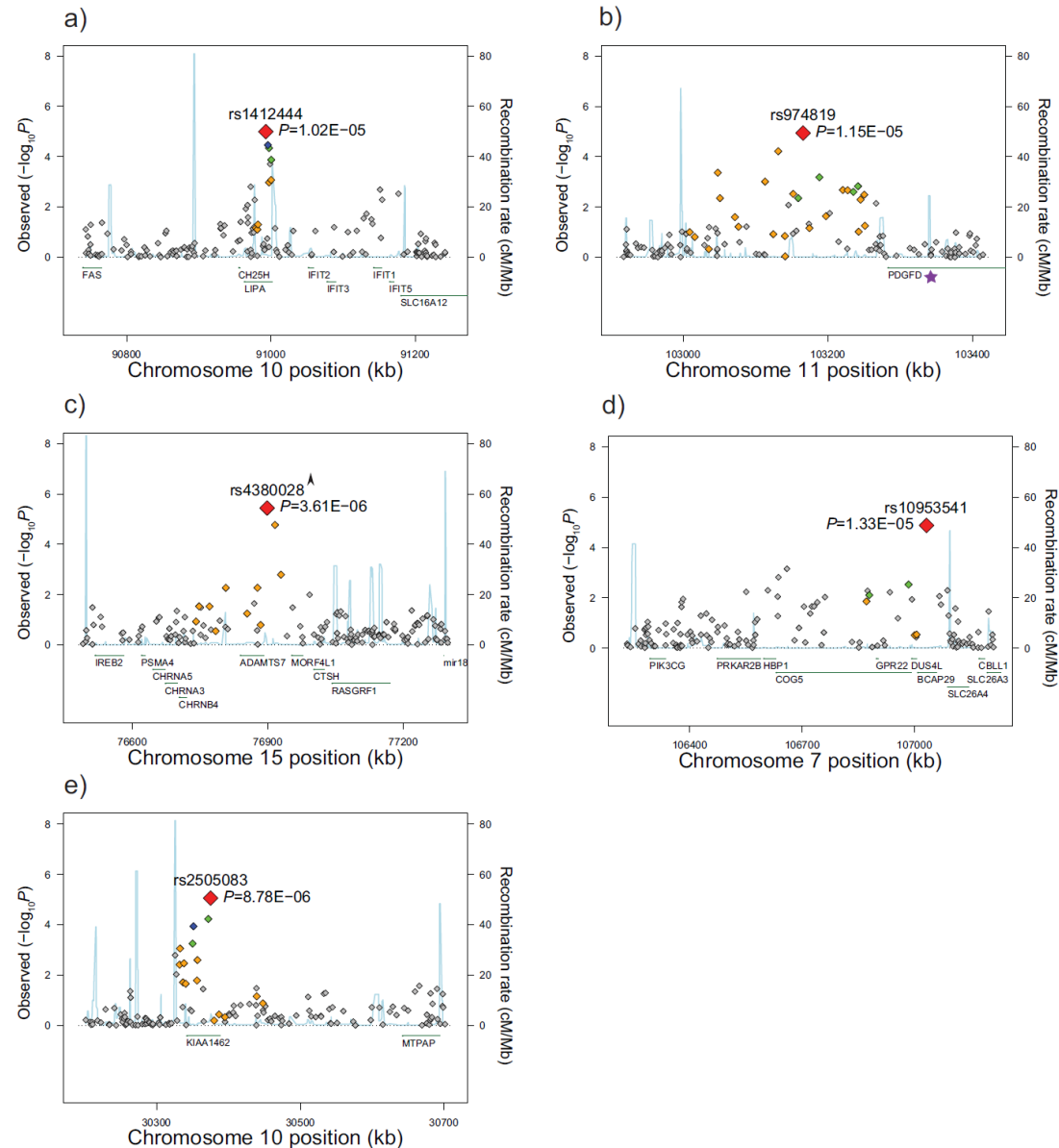


Fig 4c. Human heart

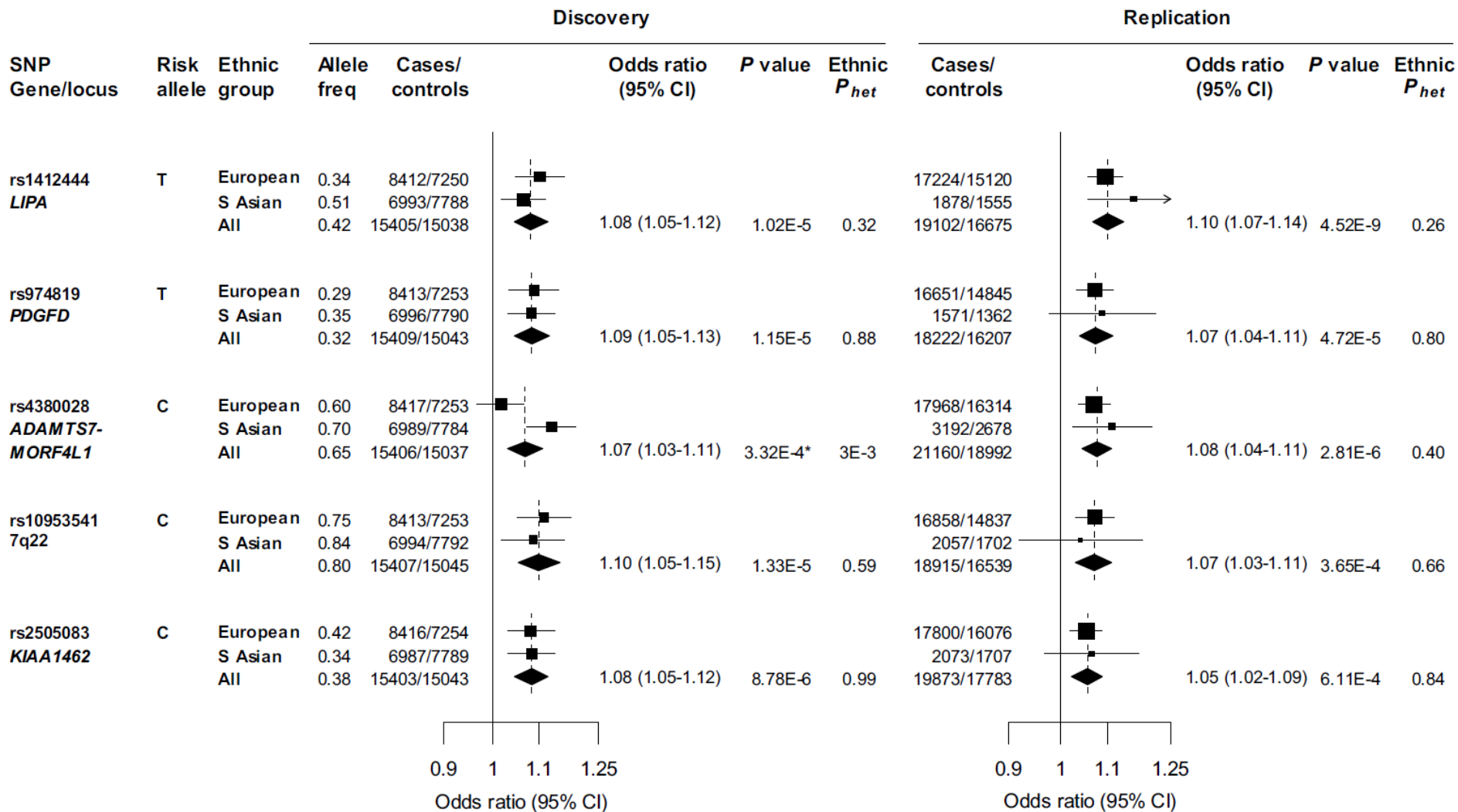


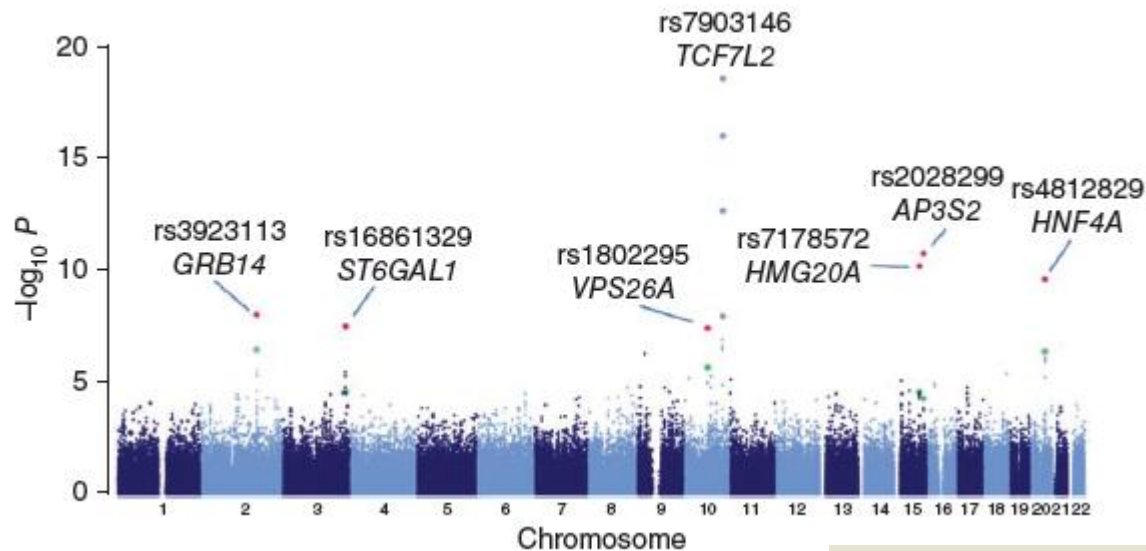
A Genome-wide association study in Europeans and South Asians reveals five novel loci for coronary artery disease.

Peden et al.
Nature Genetics
2011 (In Press)



Are these specific to South Asians?





LETTERS

nature
genetics

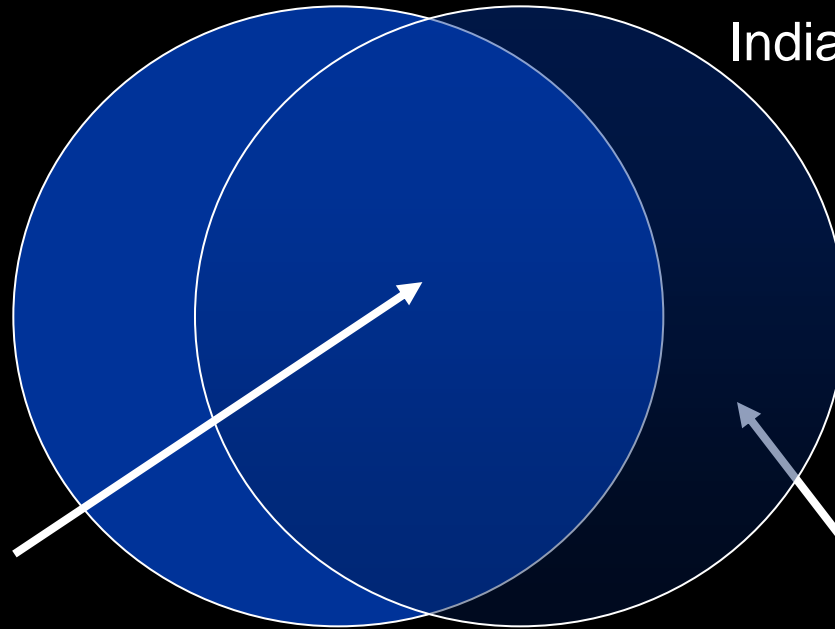
Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci

Jaspal S Kooner^{1-3,4,6}, Danish Saleheen^{4,5,46}, Xueling Sim^{6,46}, Joban Sehmi^{1,2,46}, Weihua Zhang^{7,46}, Philippe Frossard^{4,46}, Latonya F Been⁸, Kee-Seng Chia^{6,9}, Antigone S Dimas^{10,11}, Neelam Hassanali¹², Tazeen Jafar^{13,14}, Jeremy B M Jowett¹⁵, Xinzhong Li¹, Venkatesan Radha¹⁶, Simon D Rees^{17,18}, Fumihiko Takeuchi¹⁹, Robin Young⁵, Tin Aung^{20,21}, Abdul Basit²², Manickam Chidambaram¹⁶, Debashish Das², Elin Grundberg²³, Åsa K Hedman¹¹, Zafar I Hydrie²², Muhammed Islam¹³, Chiea-Chuen Khor^{6,21,24}, Sudhir Kowlessur²⁵, Malene M Kristensen¹⁵, Samuel Liju¹⁶, Wei-Yen Lim⁶, David R Matthews¹², Jianjun Liu²⁴, Andrew P Morris¹¹, Alexandra C Nica¹⁰, Janani M Pinidiyapathirage²⁶, Inga Prokopenko¹¹, Asif Rasheed⁴, Maria Samuel⁴, Nabi Shah⁴, A Samad Shera²⁷, Kerrin S Small^{23,28}, Chen Suo⁶, Ananda R Wickremasinghe²⁶, Tien Yin Wong^{20,21,29}, Mingyu Yang³⁰, Fan Zhang³⁰, DIAGRAM³¹, MuTHER³¹, Goncalo R Abecasis³², Anthony H Barnett^{17,18}, Mark Caulfield³³, Panos Deloukas³⁴, Timothy M Frayling³⁵, Philippe Froguel³⁶, Norihiro Kato¹⁹, Prasad Katulanda^{12,37}, M Ann Kelly^{17,18}, Junbin Liang³⁰, Viswanathan Mohan^{16,38}, Dharambir K Sanghera⁸, James Scott¹, Mark Seielstad³⁹, Paul Z Zimmet¹⁵, Paul Elliott^{7,40,46}, Yik Ying Teo^{6,9,24,41,42,46}, Mark I McCarthy^{11,12,43,46}, John Danesh^{5,46}, E Shyong Tai^{9,44-46} & John C Chambers^{2,3,7,46}

Genomic hypothesis

Europeans

Indian Asians



Shared variants

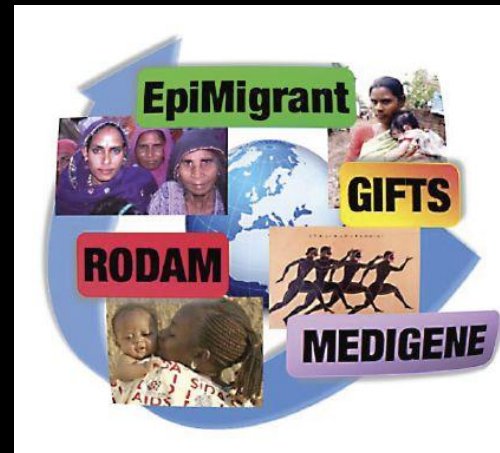
↑ Effect size

↑ Risk allele frequency

Indian specific variants

EpiMigrant

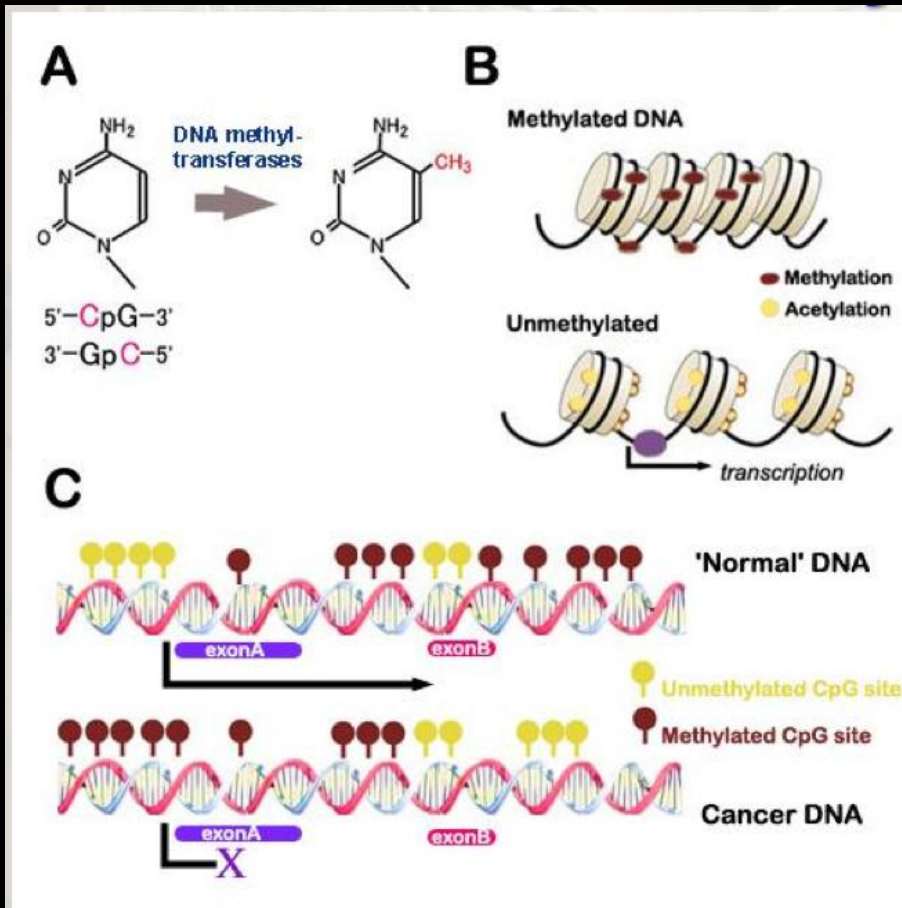
Identification of epigenetic markers underlying increased risk of Type-2 diabetes in South Asians



Epigenetics

- Epigenetics: “Heritable changes in gene expression not determined by change in DNA sequence”
- May contribute to ‘heritability’ seen in
 - Somatic cells: eg cellular differentiation
 - Germline cell: Transgenerational effects
- Mechanisms include:
 - DNA methylation
 - RNA methylation
 - Histone modification

DNA Methylation



- Most DNA methylation occurs at CpG sites.
- ~30 million CpG sites in human genome.
- Human DNA has about 80%-90% of CpG sites methylated.
- Hypermethylation of promoter CpGs leads to gene silencing

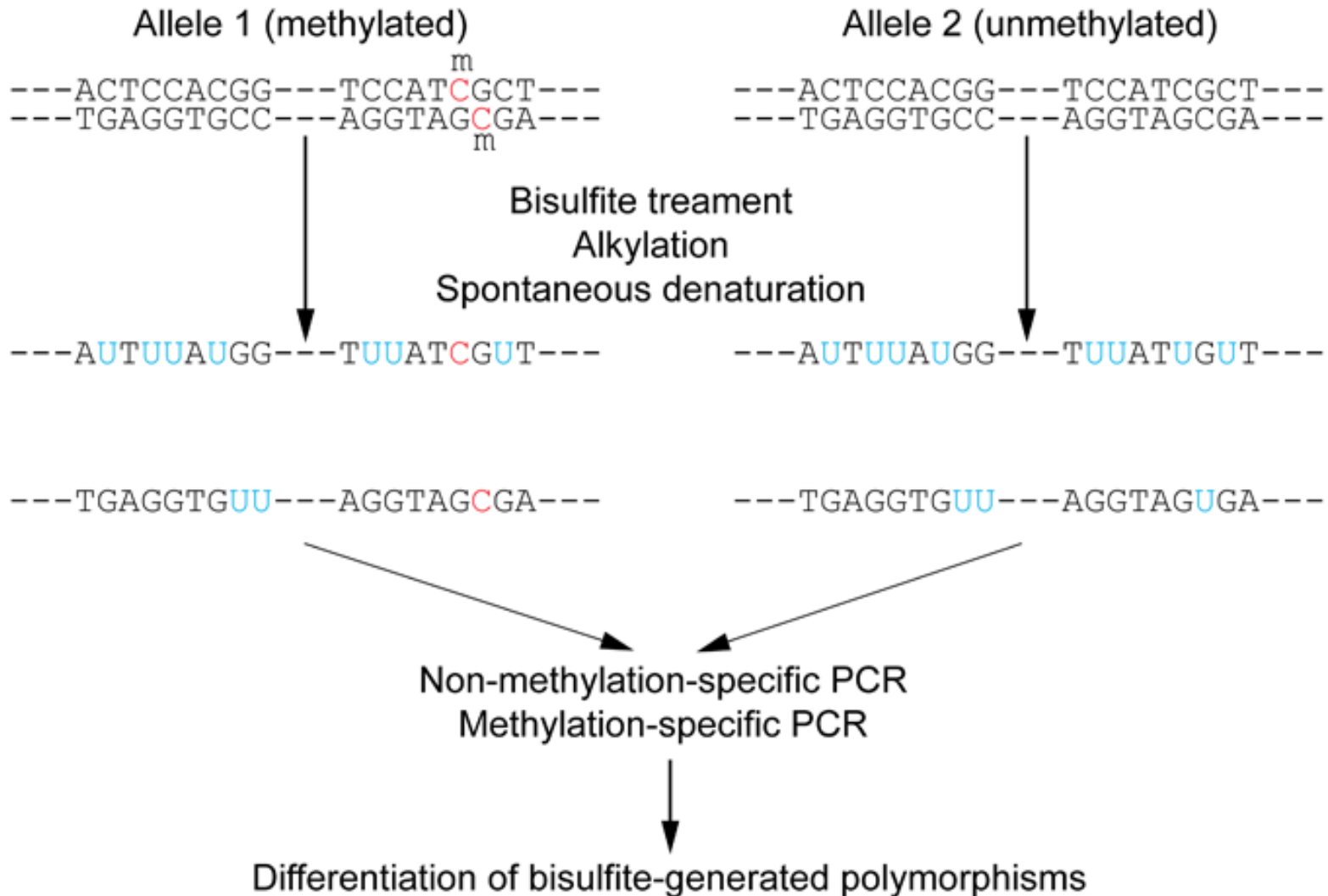
Agouti^{vy} mice



- Agouti protein influences hair colour, weight and risk of T2D
- Agouti^{vy} = promoter mutation → increased gene expression
- Agouti^{vy} carrier phenotype in isogenic colony
 - Variable between individuals
 - Influenced by dietary factors (methyl donors)
 - Offspring mirror maternal phenotype

How do we detect methylation?

Bisulphite Sequencing



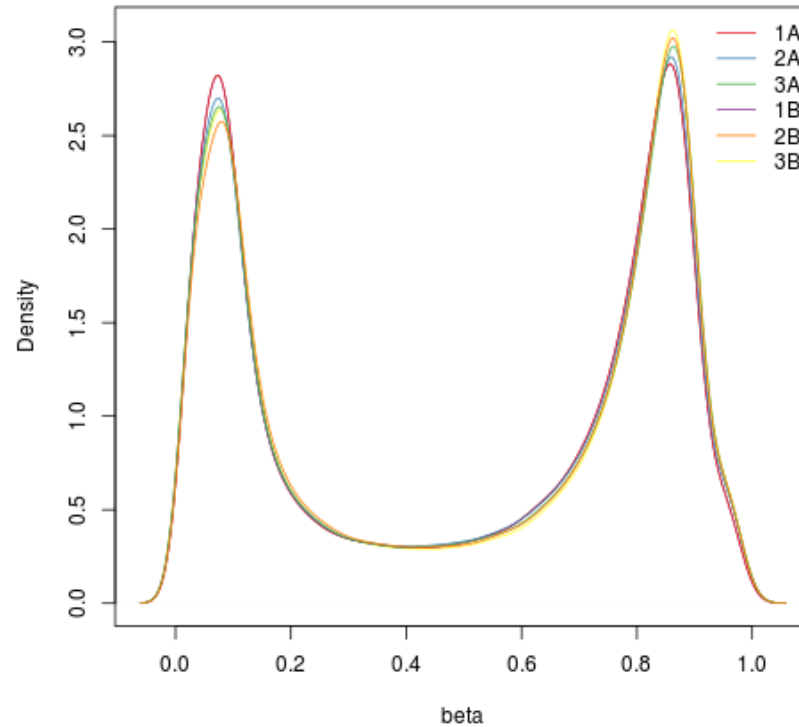
Illumina 450k

- Introduced November 2010
- 485,000+ methylation sites, content selected based on international consortium



Methylation score

$$\textit{beta} = \frac{I_{\textit{methylated}}}{I_{\textit{methylated}} + I_{\textit{unmethylated}}}$$

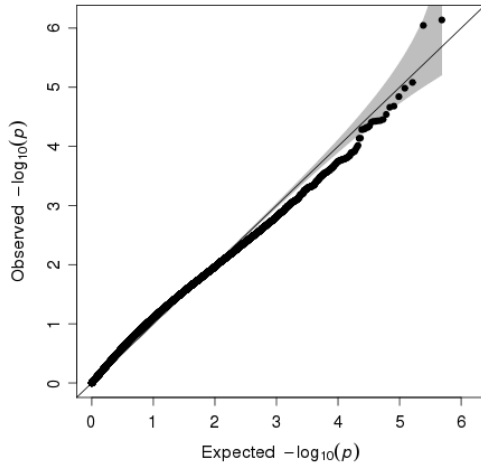


Pilot study

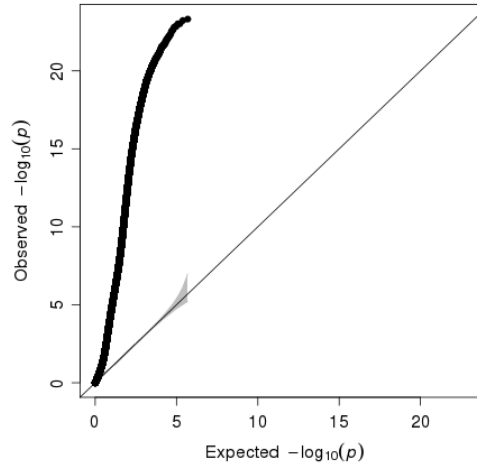
- Investigate for reverse causation
 - retrospective vs prospective cases
- Identify potential confounders
 - White cell count / subsets
 - Age, BMI
 - Other clinical factors

White blood cell count / subtypes

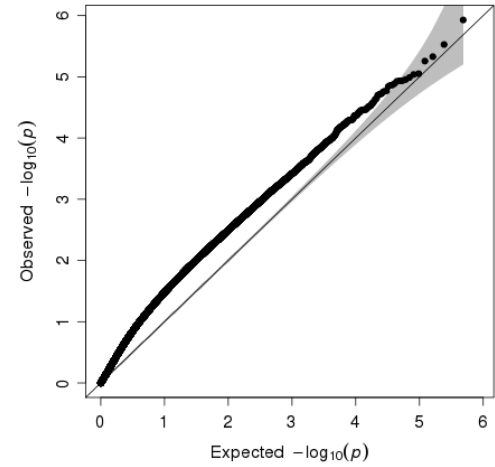
WBC



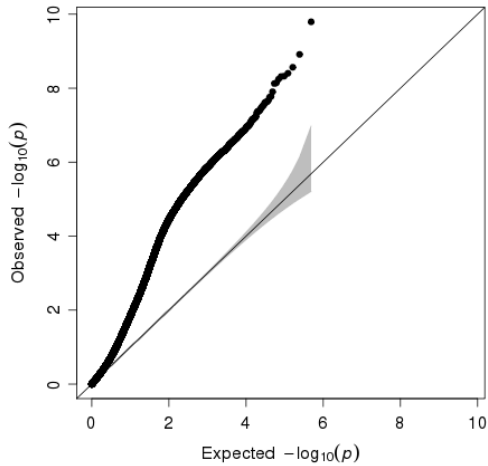
% Lymphocytes



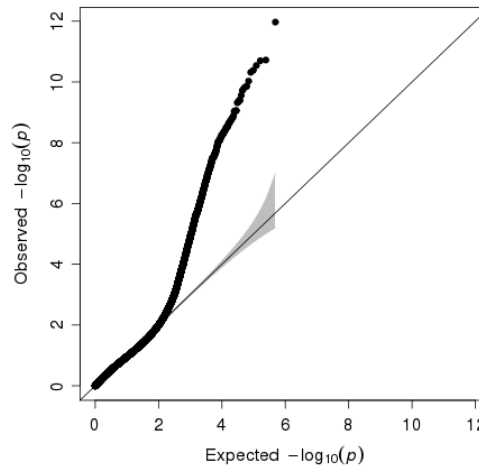
% Monocytes



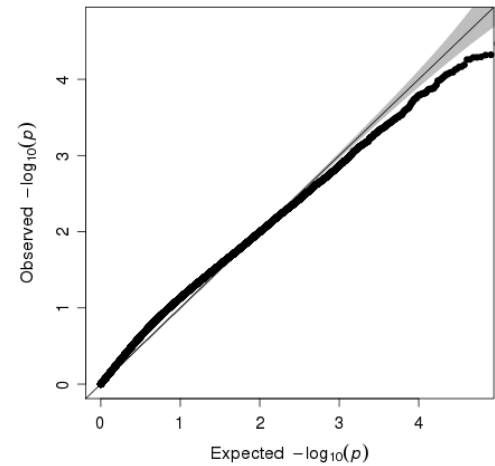
% Neutrophils



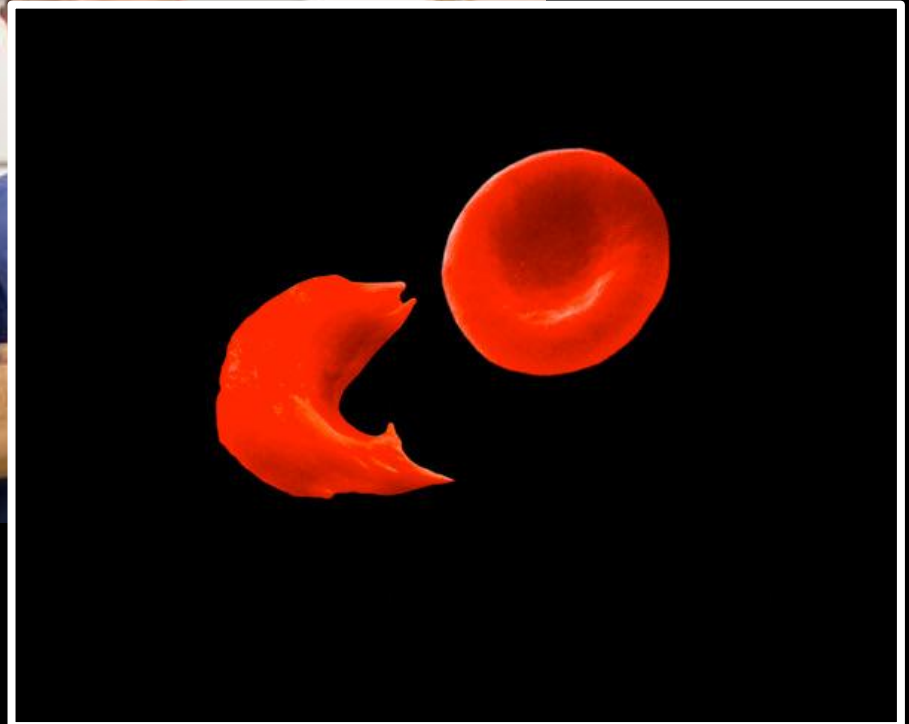
% Eosinophils



% Basophils



Genetic factors play a key role in phenotype



Heritability

- Definition: proportion of the difference between individuals that is due to genetic variation
- Phenotype (P) = Genotype (G) + Environment (E)
- Heritability = $\text{Var}(G) / \text{Var}(P)$

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1634 relatives from 180 Indian Asian families

	Heritabilities, h^2 (SE)	Common environment, c^2 (SE)
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HbA _{1c}	0.60 (0.04)**	0.03 (0.02)
Log _e HOMA-IR	0.22 (0.04)**	0.10 (0.03)*

Beware!

Phenotype	Untreated group	All participants ^b
	Heritabilities, h^2 (SE)	Heritabilities, h^2 (SE)
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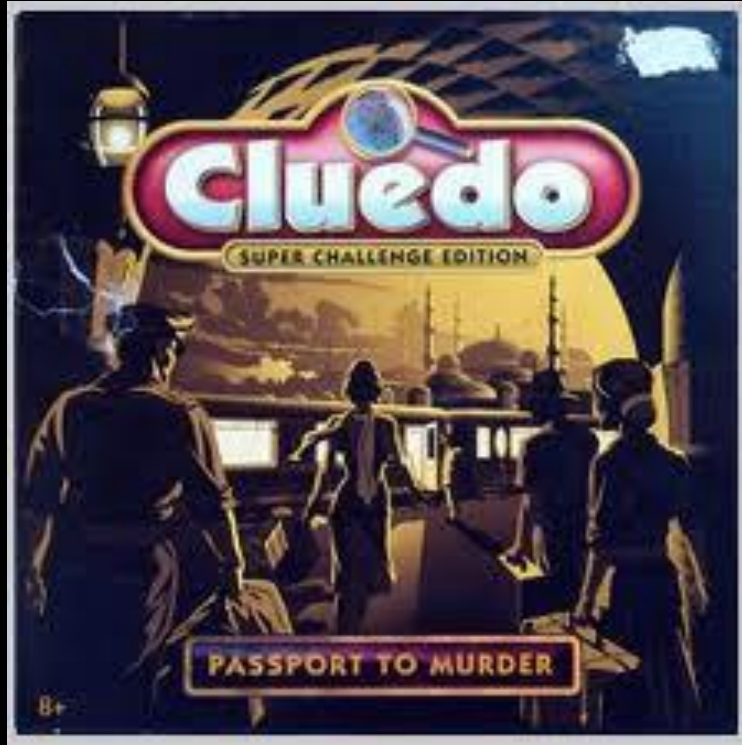
$$\text{Heritability} = \text{Var}(G) / \text{Var}(P)$$

Summary

- Indian Asians are at increased risk of CVD, T2D and related metabolic disorders
- Increased risk not accounted for by measured environmental factors
- Heritability in family studies suggests the presence of genetic susceptibility

Summary

- Our genetic association studies have provided a wealth of new discovery, all shared with Europeans.
- Strong evidence for major differences in genetic architecture in Asians - characterisation of Indian specific variation is the next strategic goal.
- We also need to consider novel mechanisms underlying heritable phenomena



Acknowledgments

- Jaspal S Kooner (Lead)
- Paul Elliott
- James Scott
- Weihua Zhang

And numerous local, national and international collaborators

