

Epigenome-wide association identifies DNA methylation markers in peripheral blood that predict incident Type-2 diabetes amongst Indian Asians and Europeans.

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Abstract

Background: Indian Asians, one-quarter of the world's population, are at high risk of type-2 diabetes (T2D). We investigated whether DNA methylation is associated with future T2D in Indian Asians, and identifies their excess risk of T2D compared to Europeans.

Methods: We completed the 8 year follow-up of 25,372 UK Indian Asian and European participants of the London Life Sciences Prospective Population (LOLIPOP) study. We carried out epigenome-wide association in a nested case-control study comprising 1,074 Indian Asians with incident T2D and 1,590 age-sex matched controls, followed by replication testing of 7 top-ranking signals in 1,141 Europeans (377 with incident T2D). For both discovery and replication, DNA methylation was measured in the baseline blood sample, collected prior to T2D onset. Epigenome-wide significance was inferred at $P < 10^{-7}$. We compared methylation levels between Indian Asian and European controls free from T2D, to estimate the potential contribution of DNA methylation to increased risk of future T2D amongst Asians.

Findings: The incidence of T2D was 3.1 fold (95%CI 2.8-3.6, $P < 0.0001$) higher amongst Indian Asians than Europeans, and was not explained by differences in adiposity, physical activity, family history of T2D or baseline glycaemic measures. The absolute difference in methylation level between T2D cases and controls ranged from $0.5 \pm 0.1\%$ to $1.1 \pm 0.2\%$. Methylation markers at 5 loci were associated with future T2D (relative risk [95%CI] per 1% increase in methylation - *ABCG1*: 1.09 [1.07-1.11], $P = 1.3 \times 10^{-17}$; *PHOSPHO1*: 0.94 [0.92-0.95], $P = 4.2 \times 10^{-11}$; *SOCS3*: 0.94 [0.92-0.96], $P = 1.4 \times 10^{-9}$, *SREBF1*: 1.07 [1.04-1.09], $P = 2.1 \times 10^{-10}$; *TXNIP* 0.92 [0.90-0.94], $P = 1.2 \times 10^{-17}$). A methylation score integrating results from the 5 loci is strongly associated with future T2D (relative risk top vs bottom quartile: 3.51, 95%CI 2.79-4.42, $P = 1.3 \times 10^{-26}$), and is independent of established risk factors. Methylation score is higher amongst Indian Asians compared to Europeans ($P = 10^{-34}$), suggesting that DNA methylation may help identify the high risk of T2D in Asians.

Interpretation: DNA methylation in blood is strongly and independently associated with future T2D. The 5 methylation markers identified help identify the increased risk of T2D amongst Indian Asians compared to Europeans. DNA methylation may provide new insights into the pathways underlying T2D, and new opportunities for risk stratification and prevention of T2D amongst Indian Asians.

Background

Type-2 diabetes (T2D) is a major public health problem in all regions of the world, particularly amongst rapidly urbanising countries such as India.^{1, 2} Indian Asians (people originating from India, Pakistan, Bangladesh or Sri Lanka), who comprise one-quarter of the world's population, are at high risk of T2D compared to North Americans and Europeans.²⁻⁴ T2D will affect more than 100 million people in India alone by 2030.¹

Diet, obesity and physical inactivity are major risk factors for T2D in Indian Asians, as they are in other populations, but do not appear to account for their increased risk of T2D compared to Europeans.^{5, 6} Genome-wide association studies amongst Indian Asians and Europeans identify common genetic variants at ~80 genetic loci influencing risk of T2D,^{4, 7-10} although these only explain ~5% of T2D risk in both populations.^{11, 12} Improved understanding of the mechanisms underlying the high incidence of T2D amongst Indian Asians is urgently needed to help stem the epidemic of T2D in this population.

DNA methylation at CpG sites (cytosine-guanine nucleotide pairs) influences gene expression, cellular differentiation and molecular response to environmental stressors.¹³⁻¹⁶

Methylation at the *FTO* locus and other loci containing genetic variants linked to T2D, has been reported to be associated with prevalent T2D in Ashkenazi Jews, and methylation of *FTO* identified individuals who progressed from normal to impaired glucose metabolism in a follow-up study.¹⁷ In addition disturbances of methylation at the *PPARG*, *KCNQ1*, *TCF7L2* and *IRS1* loci have been reported in adipose and pancreatic tissue from people with prevalent T2D.¹⁸⁻²⁰ These observations raise the possibility that alterations in DNA methylation may be involved in the biological pathways underlying development of T2D.

In the present study we carried out epigenome-wide association of DNA methylation in a nested case-control study of Indian Asians and Europeans with incident T2D, identified from the 8 year follow-up of 25,372 participants of the London Life Sciences Prospective Population (LOLIPOP) study. Our aim was to investigate whether variations in DNA methylation are associated with future T2D, and help identify the high risk of T2D amongst Indian Asians.

Methods and materials

LOLIPOP is a prospective population study of Indian Asian (N=17,606) and European (N=7,766) men and women, recruited at age 35-75 years from the lists of 58 General Practitioners in West London, United Kingdom between 2003 and 2008. Indian Asians had all 4 grandparents born on the Indian subcontinent (India, Pakistan, Sri Lanka or Bangladesh), Europeans were of self-reported white ancestry.

At baseline all participants completed a structured assessment of cardiovascular and metabolic health, including personal and family history, leisure time physical activity, and anthropometry. Participants were seen between 8am and mid-day, after an overnight fast (8 hours), allowing collection of fasting blood samples for complete blood count and measurement of glucose, insulin, HbA1c, lipid profile. Amino acid concentrations were measured by ^1H Nuclear Magnetic Resonance.²¹ T2D was defined as physician diagnosis, fasting glucose $\geq 7\text{mmol/L}$ or HbA1c $\geq 6.5\%$.²² Physical activity was defined as engaging in ≥ 90 mins of at least moderately vigorous leisure time physical activity (≥ 3 METS) per week. Homeostasis model assessment of insulin resistance (HOMA-IR) and beta cell function (HOMA-B) were calculated.²³ Aliquots of whole blood were stored at -80°C before extraction of genomic DNA. The LOLIPOP study is approved by the National Research Ethics Service (07/H0712/150) and all participants gave written informed consent.

At follow-up, electronic health records from primary care practitioners were extracted for each participant, and structured queries used to identify individuals with new onset T2D. In addition 7,640 participants attended clinical evaluation, including questionnaire and fasting blood samples for glucose and HbA1c, enabling identification of people confirmed to be free from T2D at the end of follow-up (not on treatment for T2D and fasting glucose $< 7\text{mmol/L}$ and HbA1c $< 6.5\%$). Incident T2D cases were defined as free from T2D at baseline, but who developed T2D during follow-up. Controls were free from T2D both at baseline and follow-up.

Epigenome-wide association was done amongst 1,074 Indian Asian cases of incident T2D and 1,590 Indian Asian controls. Controls were matched to cases by age (5 year groups) and sex. DNA methylation was quantified in the baseline DNA samples collected at study enrolment. Samples were analysed in random order, blind to case-control status. Bisulfite conversion of genomic DNA from peripheral blood was performed using the EZ DNA methylation kit (Zymo Research, Orange, CA). Epigenome-wide association was carried out using the Illumina HumanMethylation450 array (450K array) according to manufacturer's instructions (further details in the **Supplementary Appendix**). Replication testing was done amongst 1,141 Europeans from the LOLIPOP study (N=181 incident

T2D cases, N=568 controls) and KORA S3 and S4 studies (N=196 incident T2D cases, 196 controls, **Supplementary Appendix**). DNA methylation was quantified in the baseline DNA samples collected at study enrolment, when all participants were free from T2D. Samples were matched for age and gender. Replication testing in samples from the LOLIPOP study was carried out by pyrosequencing of bisulfite-treated DNA (**Supplementary Appendix, Supplementary Table 1**), and in KORA samples by 450K array. Levels of DNA methylation at the CpG sites of interest were compared between a representative sample of 186 Indian Asian and 192 European controls (free from T2D) in the LOLIPOP study using pyrosequencing.

The association of the identified DNA methylation markers with adiposity phenotypes quantified by dual-energy x-ray absorptiometry was studied amongst 972 participants of the Avon Longitudinal Study of Parents and Children (ALSPAC) study with DNA methylation measured by 450K array (**Supplementary Appendix**).

Fine mapping by targeted resequencing of the TXNIP locus

To better understand the relationship between DNA methylation markers and T2D, we carried out fine-mapping of one of the identified loci (*TXNIP*: chr 1, bp 145,436,694-145,446,572) in 172 samples. We used a combination of next generation sequencing and pyrosequencing (further details are provided in the **Supplementary Appendix**). We carried forward the 8 CpG sites showing $r > 0.5$ with the sentinel marker for pyrosequencing amongst 238 Indian Asian incident T2D cases and 382 Indian Asians controls, to quantify their association with T2D both as single markers and in aggregate (mean methylation across the 8 sites assayed).

Cross-tissue patterns of DNA methylation, and associations with gene expression

We examined cross tissue patterns of methylation paired peripheral blood and liver samples from 175 individuals in the ABOS study (**Supplementary Appendix**). We separately investigated the relationship of methylation with gene expression, using peripheral blood leucocytes from Indian Asians and Europeans (N=2,201), and in liver (N=116), **Supplementary Table 2** and **Supplementary Appendix**.

Statistical and bioinformatic analyses

Epigenome-wide data were analysed in R version 2.15 using minfi and other R scripts.²⁴ Marker intensities were quantile normalised. A differential white blood cell count (lymphocyte, monocyte and granulocyte counts) was available for all participants, and the

epigenome-wide methylation scores were used to impute a further 4 lymphocyte subsets (CD4, CD8, NK and B cells).²⁵ A principal components analysis was carried out to quantify latent structure in the data, including batch effects.

We performed single marker tests using logistic regression to examine the association of each autosomal CpG site with T2D, adjusted for age and gender. We included intensity values from Infinium 450K control probes, bisulfite conversion batch, measured white cells and imputed white cell subsets, and the first 5 principal components, as covariates in the regression models (**Supplementary Table 3**), as these progressively reduced test statistic inflation.²⁶⁻²⁸ We corrected the association results for the genomic control inflation factor. Comparisons between 36 samples measured in duplicate confirmed high reproducibility for quantification of DNA methylation, with no evidence for confounding by batch effect (**Supplementary Figure 1**).

We also used logistic regression to test the association of DNA methylation with T2D in the replication stage. Results were combined across the discovery and replication stages by inverse variance meta-analysis. Epigenome wide significance was set at $P < 1 \times 10^{-7}$ providing Bonferroni correction for the 466,186 autosomal markers tested. Our choice of threshold was supported by the results of permutation testing (**Supplementary Figure 2** and **Supplementary Appendix**). Markers on the sex chromosomes were tested similarly for association with T2D, but separately in men and women.

Role of the funding source

The funding source had no role in the collection, analysis, or interpretation of the data and the writing of the report. ML, BL, AD and JC had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Baseline characteristics of the 13,535 Indian Asians and 7,066 Europeans free from T2D at enrolment to the LOLIPOP study are shown (**Supplementary Table 4**). Despite lower body mass index and younger age, Indian Asians had higher waist circumference, waist-hip-ratio, glucose, insulin, HbA1c and triglycerides, and lower HDL cholesterol, compared to Europeans (all $P < 0.001$). Family history of T2D was more common, and physical activity lower, amongst Indian Asians compared to Europeans ($P < 0.001$).

We identified 1,608 Indian Asians and 306 Europeans with new-onset, incident T2D. Indian Asians who developed T2D during follow-up had higher baseline body mass index, waist circumference and waist-hip ratio, and higher fasting glucose, insulin, HOMA-IR and HbA1c compared to controls ($P = 1.3 \times 10^{-77}$ to $P = 1.3 \times 10^{-125}$, **Table 1**).

The incidence of new-onset T2D was 11.9% amongst Indian Asians and 4.3% amongst Europeans in the LOLIPOP cohort over mean 8.5 years follow-up. The age, sex adjusted risk of T2D was 3.1 fold (95%CI 2.8-3.6, $P = 9.4 \times 10^{-68}$) higher amongst Indian Asians than Europeans, and remained 2.5 fold (95%CI 2.1-2.9, $P = 1.1 \times 10^{-28}$) higher after further adjustment for major T2D risk factors including waist-hip ratio, physical activity, family history of T2D glucose, insulin and HbA1c (**Table 2**).

Epigenome-wide association and replication

Characteristics of the 1,074 Indian Asians with incident T2D and 1,590 Indian Asian controls investigated by epigenome-wide association are shown in **Supplementary Table 5**. Epigenome-wide association revealed an excess of association across a range of P-value thresholds (**Supplementary Table 6, Supplementary Figure 3-4**). Methylation markers at 7 genetic regions were associated with incident T2D at $P < 5 \times 10^{-7}$ (**Table 3, Supplementary Table 7**); these markers were carried forward to replication testing among 1,141 Europeans (**Supplementary Table 8**). Five of the 7 methylation markers were associated with incident T2D at $P < 0.05$ (**Supplementary Tables 7 and 9**) in the replication samples. In combined analysis of epigenome-wide discovery and replication data, all 5 markers reached epigenome-wide significance ($P < 10^{-7}$) for association with T2D ($P = 4.7 \times 10^{-10}$ to $P = 1.5 \times 10^{-18}$, **Table 3**). Regional plots are shown in **Supplementary Figure 5**; the 5 loci are identified by nearest gene (*ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1* and *TXNIP*). Current knowledge concerning function of these genes is summarised in **Supplementary Table 10**.

The absolute difference in methylation level between T2D cases and controls ranged from $0.5 \pm 0.1\%$ to $1.1 \pm 0.2\%$ (**Supplementary Table 7**). The relative risk for incident T2D

per 1% increase in methylation were: *ABCG1*: 1.09 [1.07-1.11], $P=1.3\times 10^{-17}$; *PHOSPHO1*: 0.94 [0.92-0.95], $P=4.2\times 10^{-11}$; *SOCS3*: 0.94 [0.92-0.96], $P=1.4\times 10^{-9}$, *SREBF1*: 1.07 [1.04-1.09], $P=2.1\times 10^{-10}$; *TXNIP* 0.92 [0.90-0.94], $P=1.2\times 10^{-17}$). Relative risk of T2D between the top and bottom quartiles of methylation at the 5 identified markers ranged from 1.73 (95%CI 1.39-2.16, $P=1.2\times 10^{-6}$) to 2.41 (95%CI 1.93-3.02, $P=1.2\times 10^{-14}$, **Figure 1**) in Indian Asians, and 1.77 (95%CI 1.45-2.15, $P=1.7\times 10^{-8}$) to 2.14 (95%CI 1.76-2.61, $P=3.5\times 10^{-14}$) in combined analysis with Europeans (**Supplementary Table 11**).

Relationships of DNA methylation with conventional risk factors for T2D

DNA methylation at the 5 identified loci was associated with body mass index, waist-hip ratio, glucose, HOMA-IR and other metabolic measures of insulin resistance (**Supplementary Table 12**). Methylation at *SREBF1*, *PHOSPHO1* and *ABCG1* was also associated ($P<0.05$) with quantitative measures of total and regional body fat distribution, and with lean mass, as assessed by DEXA amongst participants of the ALSPAC study (**Supplementary Tables 13-15**). The association of *PHOSPHO1* with lean mass remained after adjustment for BMI.

In multivariable analysis, the relationship between methylation at the *TXNIP* locus and incident T2D remained significant at $P<10^{-7}$ after adjustment for measured known risk factors for T2D, including baseline body mass index, waist-hip ratio, HOMA-IR, HOMA-B, branched-chain and aromatic amino acid concentrations (**Supplementary Tables 16**). In contrast, associations of markers at *ABCG1*, *PHOSPHO1*, *SOCS3* and *SREBF1* with T2D were weaker and no longer reached $P<10^{-7}$ after adjustment for adiposity or HOMA-IR.

DNA Methylation score and risk of future T2D

In combined analysis the 5 methylation markers identified were each associated with incident T2D amongst Indian Asians (**Supplementary Table 17**). A methylation score combining results for the 5 markers (weighted by effect size) was strongly associated with risk of future T2D amongst Indian Asians (relative risk for Q4 vs Q1: 3.51 [95%CI 2.79-4.42], $P=1.3\times 10^{-26}$; per 1SD: 1.68 [95%CI 1.55-1.83], $P=1.1\times 10^{-33}$; **Supplementary Table 11**) and was not accounted for by known T2D risk factors including adiposity and HOMA-IR (**Supplementary Table 18**). Methylation score replicated in the independent sample of Europeans with incident T2D (relative risk for Q4 vs Q1: 2.49 [95%CI 1.50-4.15], $P=4.6\times 10^{-4}$; per 1SD: 1.88 [95%CI 1.56-2.26], $P=2.5\times 10^{-11}$; **Supplementary Tables 9**) with no evidence for heterogeneity of effect with Indian Asians ($P>0.1$).

Sensitivity and interaction analyses

As a sensitivity analysis we excluded Indian Asians with pre-diabetes at baseline (HbA1c \geq 6% or fasting glucose \geq 6mmol/l); DNA methylation score remained independently associated with future T2D (relative risk for Q4 vs Q1: 3.1 [95%CI 2.3-4.1], $P=6.1\times 10^{-14}$; per 1SD: 1.65 [95%CI 1.48-1.84], $P=2\times 10^{-19}$; **Supplementary Tables 19 and 20**).

We found evidence for an interaction between adiposity and DNA methylation. Amongst the 1,932 normoglycaemic Indian Asians without prediabetes, future risk of T2D was up to 4-fold higher in the highest quartile vs lowest quartile of methylation amongst obese and overweight Indian Asians, but not amongst normal-weight individuals ($P_{\text{int}}=3.0\times 10^{-4}$, **Figure 2**). DNA methylation score identifies a subgroup of obese normoglycaemic Indian Asians at high risk of future T2D.

Methylation markers and the increased risk of T2D in Indian Asians

Methylation at the *ABCG1* and *SREBF1* loci was higher, and at *PHOSPHO1* and *SOCS3* lower, amongst Indian Asian compared to European controls (**Supplementary Table 21**). At each locus the direction for differential methylation amongst Indian Asians was concordant with the direction associated with an increased risk of T2D.

In multivariable analysis DNA methylation score is 0.86 (95%CI 0.74-0.98, $P<0.0001$) SD higher amongst Indian Asians than Europeans after adjustment for age, gender, body mass index, waist-hip ratio, glucose and insulin. Based on the relationship of methylation score with T2D (relative risk of T2D 1.41 per 1SD change in methylation score), an 0.86 SD higher methylation score is associated with a 1.34 [ie $\exp(\ln(1.41)*0.86)$] increased risk of future T2D amongst Indian Asians. Higher methylation score amongst Indian Asians thus identifies ~32% [$\ln(1.34)/\ln(2.5)$] of their unexplained excess 2.5-fold risk of T2D compared to Europeans.

Fine-mapping of the TXNIP locus

Resequencing of the *TXNIP* locus reveals a cluster of 8 CpG sites in the 3'UTR of *TXNIP* which show methylation that correlates closely with methylation at the sentinel marker ($r>0.5$, **Figure 3**). Average methylation across these 8 CpG sites is closely associated with risk of future T2D, and this regional association is stronger than for any individual CpG site (relative risk per 1SD change - discovery marker [cg19693031]: 1.29, 95%CI 1.15-1.43, $P=5.2\times 10^{-3}$; regional score 1.38, 95%CI 1.24-1.52, $P=7.9\times 10^{-4}$; **Figure 3**).

Functional genomics and bioinformatic analyses

To test whether DNA methylation in blood correlates with methylation in a metabolically relevant tissue, we compared methylation at the 5 sentinel CpG sites in blood and liver using paired samples from 175 obese individuals. Our results **suggest** a relationship between methylation in peripheral blood with methylation in liver at the *TXNIP* and *SOCS3* loci (**Supplementary Table 22**). We also investigated the relationships between the methylation at the 5 loci associated with T2D, with expression of nearest gene in blood and liver. In blood, methylation is strongly associated with expression of *ABCG1* and *SREBF1* amongst both Indian Asians and Europeans ($P=3.8 \times 10^{-3}$ to 3.8×10^{-21}), and also shows evidence for association with expression of *PHOSPHO1* and *SOCS3*. In liver, methylation is associated with *TXNIP* expression (**Supplementary Tables 23-24**).

Discussion

We report a large, prospective, cohort study of incident T2D amongst UK Indian Asians and Europeans. We show a 3-fold higher risk of T2D amongst Indian Asians that is not accounted for by differences in adiposity, glycaemic measures or physical activity, compared to Europeans. Using epigenome-wide association, we show that differential methylation of genomic DNA at five genetic loci is associated with incident T2D in both Indian Asians and Europeans. Fine-mapping of the top-ranking locus reveals multiple additional methylation markers that are associated with T2D. Methylation levels at the 5 genetic loci are unfavourable amongst Indian Asians, and may help identify their unexplained increased risk of T2D, compared to Europeans.

Methylation of DNA at CpG sites regulates gene expression and mediates the biological response to environmental exposures.¹³⁻¹⁶ Although previous studies have investigated the association of methylation with T2D, these have been largely limited to the study of patients with established disease.^{17-20, 29, 30} Here we identify a strong association between differential methylation at 5 genetic loci (*ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1* and *TXNIP*) and risk of future T2D amongst Indian Asians and Europeans. We find an ~four-fold higher risk of future T2D between upper and lower quartiles of a DNA methylation score, which is independent of known major risk factors for T2D. The association of DNA methylation score with risk of T2D is particularly evident in normoglycaemic Indian Asians, amongst whom methylation enables identification of a subset of metabolically unhealthy obese individuals, at high risk of future T2D. Although further validation of our findings will be needed to confirm generalizability to non-migrant Indian Asians, our findings raise the possibility that DNA methylation may help identify Indian Asians who would benefit from early pharmacologic or lifestyle intervention to prevent T2D.

The reasons underlying the disturbances in methylation at the *ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1* and *TXNIP* loci prior to T2D onset are not known. The 5 methylation sites identify genes in key pathways underlying T2D and associated metabolic defects. *TXNIP* is recognised to be a key component of pancreatic beta cell biology, nutrient sensing, energy metabolism and regulation of cellular redox. *TXNIP* expression is highly induced by glucose through activation of the carbohydrate response element-binding protein which binds the *TXNIP* promoter.³¹ *TXNIP* downregulates GLUT1, the major transmembrane glucose transporter, thereby acting as a negative feedback loop to regulate glucose entry and mitochondrial oxidative stress. *TXNIP* is one of the most glucose responsive genes expressed in human islets; in animal models *Txnip* is a mediator of glucotoxic beta cell death, while *Txnip* downregulation protects against and

obesity-induced diabetes by preventing beta cell apoptosis and preserving beta cell mass.³² *TXNIP* may also contribute to regulation of adiposity and energy expenditure through hypothalamic pathways.³³ *ABCG1* is involved in cholesterol and phospholipid transport, and insulin secretion.³⁴ *Abcg1*^{-/-} mice have impaired glucose tolerance and insulin secretion with normal insulin sensitivity.³⁵ Methylation at *ABCG1* has been recently reported to be associated with fasting insulin and HOMA-IR.³⁶ *SREBF1* is a key transcriptional regulator of hepatic lipogenesis.³⁷ Insulin activates *SREBF1*, and *SREBF1* may contribute to the dyslipidaemia and hepatic steatosis seen in obesity, insulin resistance and T2D.³⁸ Our results pave the way for functional studies to define the pathways linking DNA methylation at these sites to adiposity, T2D and their related metabolic disturbances.

DNA methylation is influenced by both genetic and environmental factors, including adverse intra-uterine and early-life exposures, and may also show transgenerational inheritance.³⁹ *TXNIP* expression is highly sensitive to glucose concentration, consistent with abnormal *TXNIP* methylation being an early marker for impaired glucose homeostasis.³¹ In contrast, we show that methylation at *ABCG1*, *PHOSPHO1*, *SOCS3* and *SREBF1* is associated with body mass index, waist circumference, insulin and HOMA-IR; our findings suggest that DNA methylation at these loci provides additional information about T2D susceptibility beyond routine clinical measures of adiposity, and that DNA methylation may be a biomarker of metabolically unfavourable patterns of adiposity and insulin resistance.

We conclude that DNA methylation at the *ABCG1*, *PHOSPHO1*, *SOCS3*, *SREBF1* and *TXNIP* loci is strongly associated with future T2D amongst Indian Asians, and together these loci help identify their increased risk compared to Europeans. Our findings provide new insight into the disturbances underlying T2D, and the basis for development of DNA methylation signatures to enable risk stratification and personalised medicine approaches to tackle the emerging global epidemic of T2D.

Research in context panel

Systematic review

Indian Asian are at high risk of developing type-2 diabetes (T2D); this increased risk is not explained by adiposity, physical inactivity, adverse diet or known genetic susceptibility factors. DNA methylation is a major mechanism in genomic regulation, and has recently been implicated in adiposity and insulin resistance. We searched PubMed on Nov 27, 2014, with the terms (“epigenome-wide association study” OR “EWAS”) AND “Type 2 Diabetes”. We found five articles, of which none were epigenome-wide association study with incident T2D as the phenotype of interest. Three were environment-wide association studies (Patel CJ et al., PLoS One. 2010 May 20; 5(5):e10746; Patel CJ et al., Hum Genet. 2013 May; 132(5):495-508; Hall MA et al., Pac Symp Biocomput. 2014:200-11). One of the studies is an epigenome-wide association study with respect to fasting measures of glucose, insulin, and HOMA-IR among 837 nondiabetic participants (Hidalgo B et al., Diabetes. 2014 Feb;63(2):801-7), while the other was in peripheral blood samples from 48 obese and 48 lean African-American youth to examine the methylation profiles with obesity as the endpoint (Xu X et al., Epigenetics. 2013 May;8(5):522-33). Hypomethylation at *FTO* is reported to be associated with prevalent T2D, and also associated with progression to impaired glucose metabolism (Toperoff G et al., Hum Mol Genet. 2012 Jan 15;21(2):371-83). Other studies have investigated DNA methylation and T2D in adipose, muscle and pancreatic tissue from small numbers of people (Nilsson et al., Diabetes. 2014 Sep;63(9):2962-76; Ribel-Madsen R et al., PLoS One. 2012;7(12):e51302; Volkmar M et al., EMBO J. 2012 Mar 21;31(6):1405-26). We found no other epigenome-wide study investigating whether differences in DNA methylation in peripheral blood predict future T2D.

Interpretation

We report a large, prospective population study of Indian Asians and European. We show an ~3-fold higher risk of T2D amongst Indian Asians that is not explained by conventional risk factors. Using epigenome-wide association, we identify and replicate a strong and independent association of DNA methylation with future T2D. We find a four-fold higher risk of future T2D between upper and lower quartiles of methylation, and show that methylation helps identify the increased risk of T2D amongst Indian Asians. Our findings of differences in DNA methylation underlying T2D will be of major interest to researchers and

clinicians worldwide, and may provide the basis for development of new strategies for risk stratification and personalised approaches to prediction and prevention of T2D.

Conflict-of-interest and author contribution statements

There are no conflicts of interest. All authors contributed to, read and approved the final version of the manuscript. Specific author contributions were as follows: i. Study concept and design: P.B., V.B., J.C.C., J.D., P.E., M-R.J., S. Jha, J.J., N. Kato, J.S. Kooner, M.I.M., J.P., D.S., J.S., E.T., J.T., A.R.W.; ii. Participant recruitment and characterisation: U.A., K.B., S.C., J.C.C., J.D., S.deL., H.R.E., P.E., P.F., C.G., H.G., T.R.G., C.H., T.I., S. Jha, S. Jones, A. Kasturiratne, J.S. Kooner, N. Kotea, S.K., J. Kriebel, P.P., C.L.R., R.C.R., J.S., W.R.S., S.T., B.T., P.V., A.R.W.; iii. Generation of molecular phenotype data: J.A., M.A-B., T.J.A., M.A-K., B.A., O.A., P.B., C.B., V.B., G.C., S.C., M.C-H., J.C.C., A.D., H.R.E., P.F., C.G., H.G., T.R.G., J.H., C.H., T.I., M-R.J., J.J., A.J. Kangas, J.S. Kooner, N. Kotea, S.K., S.A.K., M.L., Z.M., V.M., H.N., F.P., H.P., C.L.R., F.R., M.R., R.C.R., C.S., J.S., W.R.S., P.S., R.S., S.T., L.T., B.T., P.V., S.W., L.Y., W.Z.; iv. Data and statistical analyses: G.C., J.C.C., A.D., H.R.E., P.E., M-R.J., J.S. Kooner, J. Kriebel, B.L., M.L., M.I.M., J.P., C.L.R., R.C.R., S.W., L.Y., W.Z.; v. Manuscript drafting: V.B., J.C.C., A.D., P.E., C.G., H.G., M-R.J., S. Jha, N. Kato, J.S. Kooner, B.L., M.L., M.I.M., J.S., R.S., E.T., P.V., A.R.W.

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Figure legends

Figure 1. Relative risk of T2D by quartile of methylation marker; risk is in comparison to the quartile at the lowest end of risk for the respective methylation marker.

Figure 2. Incidence of T2D (8 year follow-up) by quartile of methylation score amongst normal weight (Body mass index [BMI] 18.5-24.9kg/m²), overweight (BMI 25.0-29.9kg/m²) and obese (BMI ≥30.0kg/m²) Indian Asians with normoglycaemia (HbA1c<6% and fasting glucose<6mmol/l). The P value is for the interaction between adiposity and DNA methylation on risk of T2D.

Figure 3. Targeted resequencing of the TXNIP locus by next generation sequencing. Bars present mean methylation at the CpG sites evaluated; the sentinel marker identified by EWAS is shown as a purple bar. The correlation track provides the correlation between methylation at each CpG site with the sentinel marker. The location and structure of the *TXNIP* gene are shown.

Inset: Relative risk for T2D for the methylation markers at the TXNIP locus identified by targeted resequencing. Results shown for: i. the 8 individual CpG sites assayed by pyrosequencing (blue; light blue for sentinel marker); ii. Sentinel marker by microarray (green); and iii. Regional methylation scores (orange). Results are as relative risk for T2D associated with 1SD reduction in methylation or methylation score. Score: sum of all 8 methylation markers.

Table 1. Baseline clinical characteristics of incident T2D cases and controls in the LOLIPOP cohort study. Results are presented as mean (SD) or % (N). P values are for comparison of T2D cases and controls in the respective ethnic groups.

	<u>Europeans</u>			<u>Indian Asians</u>		
	Controls	Incident T2D	P	Controls	Incident T2D	P
N	6,760	306		11,927	1,608	
Age (yrs)	51.8±11.2	58.9±9.5	<0.0001	48.7±10.8	52.2±10.0	<0.0001
Sex M (%)	58.8 (3,975)	75.2 (230)	<0.0001	57.9 (6,906)	67.3 (1,082)	<0.0001
Family history T2D (%)	17.7 (1197)	23.2 (71)	0.01	35.2 (4,198)	43.0 (691)	<0.0001
Physical activity (%)	52.9 (3,576)	39.9 (122)	<0.0001	32.5 (3,876)	26.2 (421)	<0.0001
Body mass index (kg/m ²)	27.0±4.8	31.1±5.4	<0.0001	26.8±4.3	28.9±4.6	<0.0001
Waist (cm)	93.5±13.0	105.6±13.0	<0.0001	26.8±4.3	28.9±4.6	<0.0001
Waist-hip ratio	0.91±0.08	0.97±0.07	<0.0001	0.93±0.08	0.97±0.07	<0.0001
Glucose (mmol/L)	5.0±0.5	5.7±0.6	<0.0001 ⁴	5.0±0.5	5.5±0.6	<0.0001
Insulin (IU/L)	8.8±7.4	15.5±10.7	<0.0001	11.2±8.2	16.0±10.4	<0.0001
HbA1c (%)	5.3±0.4	5.6±0.5	<0.0001	5.5±0.4	5.8±0.5	<0.0001
HOMA-IR	2.1±1.9	4.1±3.1	<0.0001	2.6±2.0	4.1±2.8	<0.0001
Impaired fasting glucose (%)	4.1 (277)	33.0 (101)	<0.0001	3.4 (406)	24.1 (388)	<0.0001

Table 2. Relative risk of new-onset T2D amongst Indian Asians compared to Europeans in the LOLIPOP study.

Adjustments made	Relative risk (95%CI) of T2D in Indian Asians vs Europeans	P
Model 1: Age, sex	3.1 (2.8-3.6)	<0.0001
Model 2: Model 1 + Family history of T2D, physical, activity	2.7 (2.4-3.1)	<0.0001
Model 3: Model 2 + body mass index, waist-hip ratio	2.9 (2.5-3.3)	<0.0001
Model 4: Model 3 + Glucose, Insulin, HbA1c	2.5 (2.1-2.9)	<0.0001
Model 5: Model 3 + HOMA-IR	2.9 (2.5-3.4)	<0.0001

Table 3. Association of methylation markers with future T2D. Results are presented as relative risk for T2D (95% confidence interval) associated with a 1% increase in respective methylation marker in the discovery phase (1,074 Indian Asians with incident T2D and 1,590 controls), in replication testing amongst 1,141 Europeans (377 with incident T2D), and in combined analysis. P_{het} is for heterogeneity of effect between discovery and replication.

Marker	Chr	Position	Locus	Discovery RR (95%CI)	P	Replication RR (95%CI)	P	Combined RR (95%CI)	P	P_{het}
cg19693031	1	145,441,552	<i>TXNIP</i>	0.92 (0.91 - 0.94)	1.0×10^{-13}	0.96 (0.94 - 0.98)	2.5×10^{-5}	0.92 (0.90 - 0.94)	1.5×10^{-18}	0.98
cg09152259	2	128,156,114	<i>PROC</i>	0.95 (0.93 - 0.97)	9.3×10^{-8}	0.99 (0.97 - 1.01)	0.32	0.95 (0.93 - 0.97)	4.8×10^{-7}	0.04
cg04999691	7	150,027,050	<i>C7orf29</i>	0.95 (0.93 - 0.96)	1.4×10^{-8}	1.00 (0.98 - 1.02)	0.71	0.96 (0.94 - 0.98)	4.8×10^{-5}	0.004
cg11024682	17	17,730,094	<i>SREBF1</i>	1.06 (1.04 - 1.08)	8.4×10^{-9}	1.03 (1.01 - 1.05)	5.4×10^{-3}	1.07 (1.04 - 1.09)	3.0×10^{-10}	0.07
cg02650017	17	47,301,614	<i>PHOSPHO1</i>	0.94 (0.92 - 0.96)	2.1×10^{-9}	0.97 (0.95 - 0.99)	1.2×10^{-3}	0.94 (0.92 - 0.95)	4.1×10^{-12}	0.48
cg18181703	17	76,354,621	<i>SOCS3</i>	0.95 (0.93 - 0.97)	2.1×10^{-7}	0.97 (0.95 - 0.99)	1.6×10^{-3}	0.94 (0.92 - 0.96)	4.7×10^{-10}	0.76
cg06500161	21	43,656,587	<i>ABCG1</i>	1.08 (1.06 - 1.10)	2.2×10^{-13}	1.04 (1.02 - 1.06)	1.2×10^{-4}	1.09 (1.07 - 1.11)	1.1×10^{-17}	0.32

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Figure 1

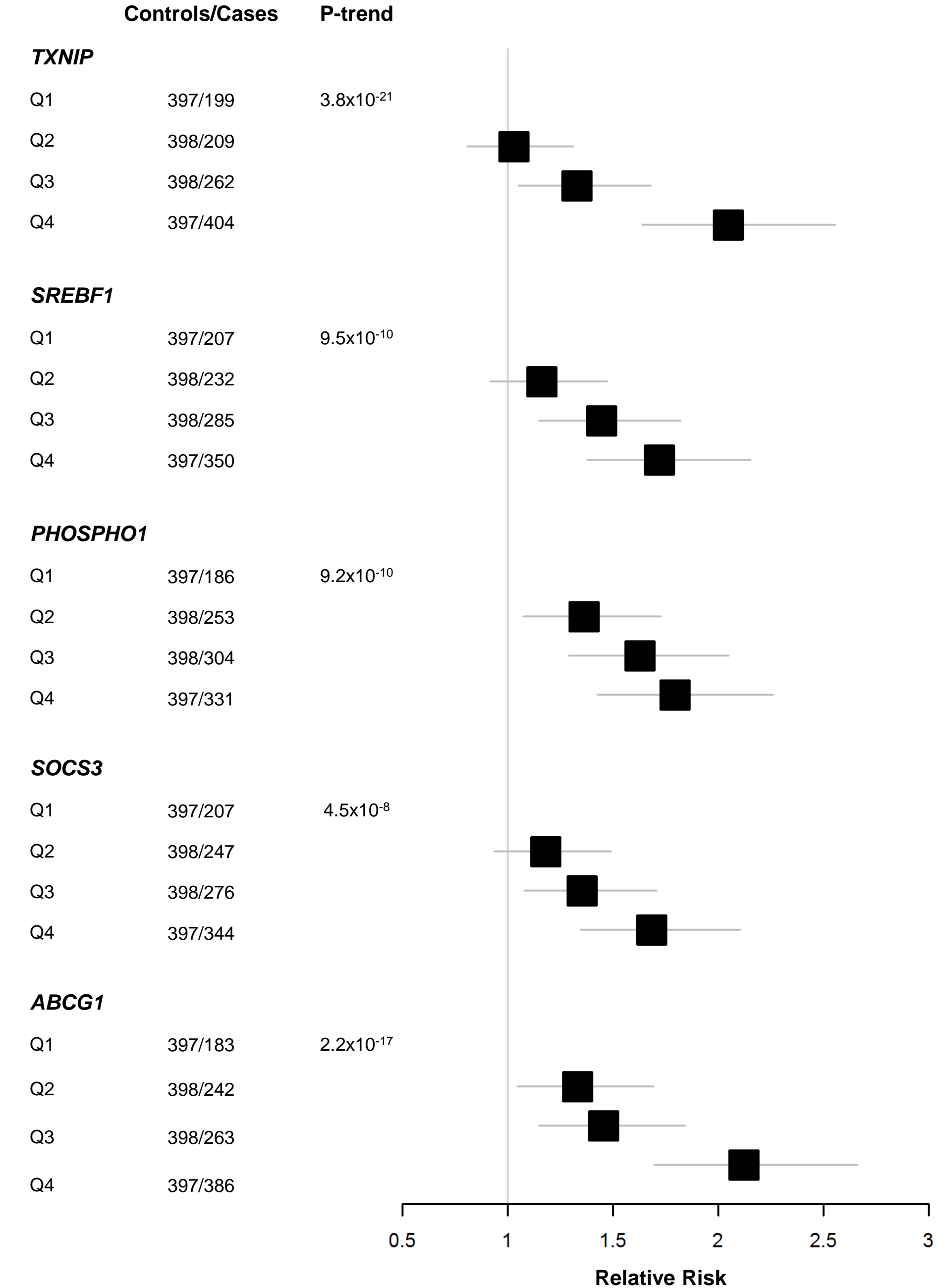


Figure 2

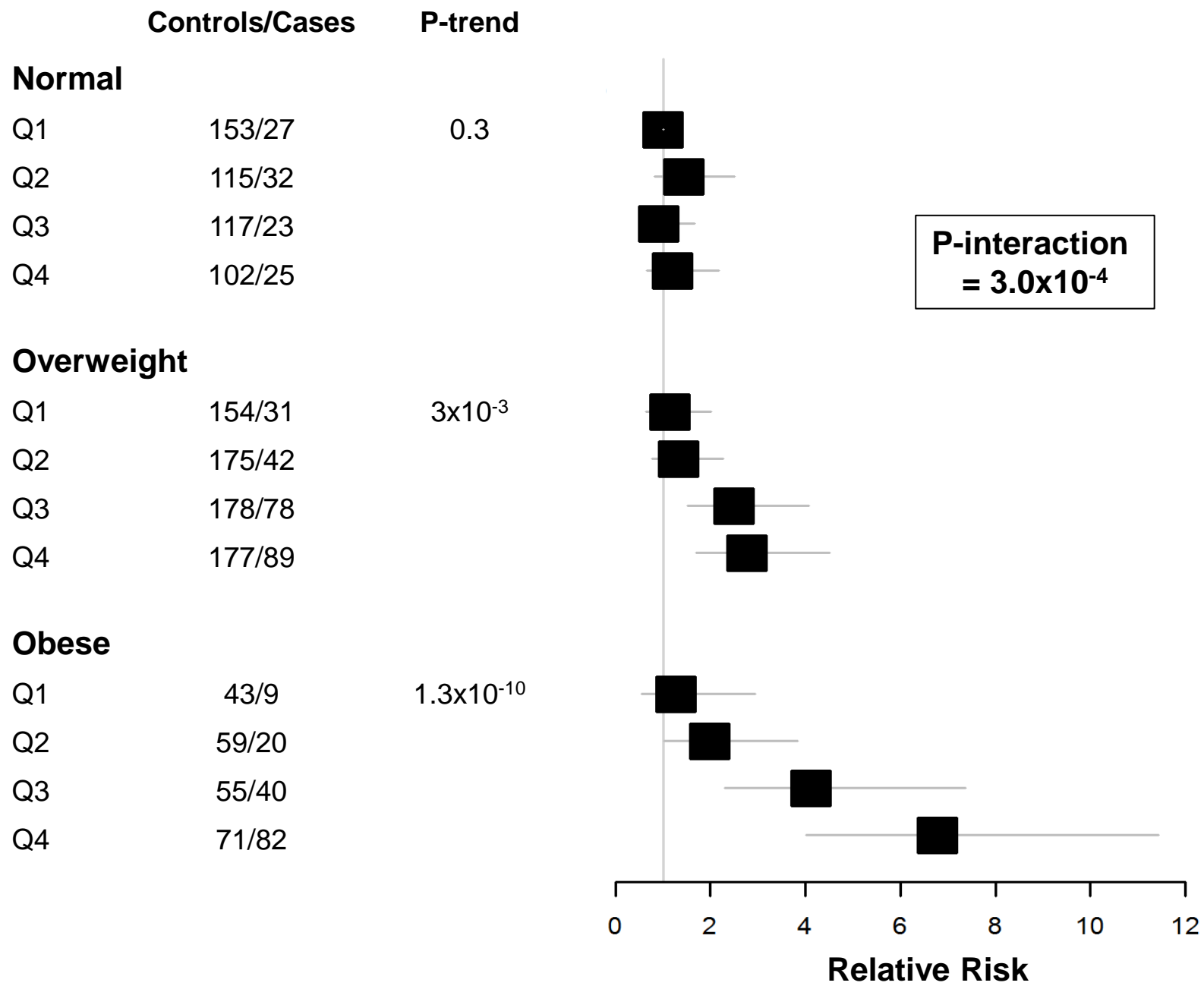


Figure 3

