Individual and cumulative effect of type 2 diabetes genetic susceptibility variants on risk of coronary heart disease

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Abstract

Aims/hypothesis Type 2 diabetes is a major risk factor for CHD. We hypothesised that diabetes genetic susceptibility variants might be associated with increased CHD risk.

Methods We examined the individual and cumulative effect of 38 common genetic variants previously reported to be associated with type 2 diabetes on risk of incident CHD in 20,467 participants of the European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk Study who had been free of CHD at baseline.

Results During a mean follow-up of 10.7 years, 2,190 participants had a CHD event. Two individual variants next to the *TSPAN8* (HR 1.07, 95% CI 1.00–1.14) and the *CDKN2A/B* region (1.11, 1.04–1.17) were significantly associated with increased CHD risk. A genetic score based on the 38 diabetes variants was significantly associated with an increased risk of CHD (1.08, 1.01–1.14 per score tertile). Adjustment for prevalent and incident diabetes

attenuated the association of the *TSPAN8* variant (1.06, 0.99–1.13) and the genetic score (1.05, 0.99–1.12 per score tertile) with CHD risk, but not that of the *CDKN2A/B* variant (1.11, 1.05–1.18). Addition of the genetic score did not improve risk discrimination based on clinical risk factors.

Conclusions/interpretation The increased risk of CHD observed with genetic susceptibility to type 2 diabetes was at least partly mediated by its diabetes-predisposing effect and was not useful for clinical risk discrimination. The potential role of pathways associated with the variant *CDKN2A/B* in linking diabetes and CHD needs further exploration.

Keywords Coronary heart disease · Genetic score · Single nucleotide polymorphism · Type 2 diabetes

Abbreviations

EPIC European Prospective Investigation into Cancer and Nutrition
GWAS Genome-wide association studies
LD Linkage disequilibrium

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Introduction

A steadily increasing number of common genetic variants associated with type 2 diabetes mellitus has been reported (electronic supplementary material [ESM] Table 1). Several of these variants are within genes that are plausible candidates for regulation of gluco-metabolic processes, and thus provide insight into the pathogenesis of diabetes.

Diabetes is a major risk factor for CHD, but the underlying pathophysiological mechanisms are largely

unclear. Due to the strong and presumably causal association between diabetes and CHD, variants that are associated with risk of diabetes would be expected to also be associated with increased risk of CHD. If confirmed, this would have several implications. Diabetes variants that are more strongly related to CHD risk might point to associated pathways that mechanistically link diabetes to CHD. So far few type 2 diabetes variants have been examined for their association with cardiovascular outcome and the results are inconsistent [1–3]. Furthermore, although the effect of individual genetic variants on diabetes risk is small, an aggregate of many genetic variants combined to a score might substantially influence risk of diabetes and in consequence risk of CHD. The independent contribution of genetic diabetes susceptibility to CHD risk has not been examined.

We therefore examined the association between individual type 2 diabetes variants and risk of incident CHD events and the risk associated with a genetic predisposition score in participants of the European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk Study [4].

Methods

EPIC-Norfolk is a prospective population-based study of 25,663 men and women aged 40 to 79 years and living in Norfolk, UK; it has been described in detail elsewhere [4]. Baseline samples of DNA were available for genotyping in 21,323 participants. We excluded 165 participants with self-report of non-European descent and 691 participants with baseline history of myocardial infarction. Incident cases of diabetes were ascertained as previously described [5] using multiple sources of evidence, including self-report, and linkage to primary care registers, secondary care registers, hospital admissions and mortality data. Incident cases of CHD and cardiovascular disease were defined by the corresponding ‘International Classification of Disease’ codes on death certificates and hospital discharge data, which have proven to be accurate in an earlier validation study [6]. We report results with follow-up to March 2007. The study was approved by the Norwich District Health Authority Ethics Committee and all participants gave signed, informed consent.

We genotyped 42 autosomal type 2 diabetes variants reported in European-origin populations at a genome-wide association significance level of 5×10^{-8} , or with consistent replication in several cohorts or proxies in high linkage disequilibrium (LD) (ESM Table 1). Genotypes were obtained from the iPLEX (Sequenom, San Diego, CA, USA) platform or Custom TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). Four variants did not pass genotyping quality control criteria and were excluded from analyses.

The diabetes-risk-increasing alleles were determined on the basis of recent literature (ESM Table 1). A genetic predisposition score was calculated for each participant by adding the diabetes risk-increasing alleles from all 38 variants. We also calculated a weighted score based on the effect size (OR) obtained from previous replication analyses (ESM Table 1). Logistic regression was used to calculate the risk (OR) of individual variants and the genetic score for combined prevalent and incident diabetes. Cox proportional hazard regression was used to examine the risk (HR) of incident CHD. All reported *p* values were not corrected for multiple testing unless indicated otherwise. Harrell’s *c*-statistics were calculated to estimate the risk discrimination of models with and without the genetic score. In sensitivity analyses, we calculated a genetic score by: (1) excluding the four variants with an OR for diabetes of less than 1 in our cohort; and (2) standardising the score in persons with <50% missing genotypes to those for persons with complete data. We observed similar results for both scores as in primary analysis (data not shown).

All analyses were performed using STATA version 10.1 (Statacorp, College Station, TX, USA). We had 96%/82% power to detect a relative risk of 1.2/1.15 of individual variants for incident CHD with an α level at $p=0.05$.

Results

During a mean follow-up of 10.7 years, 2,190 (10.7%) of 20,467 participants had an incident CHD event. Baseline characteristics are shown in Table 1.

Table 2 shows the associations of 38 type 2 diabetes variants with risk of prevalent and incident diabetes and

Table 1 Baseline characteristics according to incident CHD

Characteristic	With incident CHD	Without incident CHD	Total
<i>n</i>	2,190	18,277	20,467
Age (years)	64.5±8.0	58.0±9.2	58.7±9.3
Male sex (%)	65.7	46.1	48.2
BMI (kg/m ²)	27.2±3.8	26.2±3.8	26.3±3.8
Systolic BP (mmHg)	144±19	135±18	136±18
Cholesterol (mmol/l)	6.45±1.20	6.14±1.16	6.17±1.17
Smoking (%)	66.5	52.4	53.9
Family history			
Myocardial infarction (%)	43.5	35.3	36.2
Diabetes (%)	11.7	13.1	12.9
Prevalent diabetes (%)	8.1	2.5	3.1
Incident diabetes (%)	6.8	2.7	3.1

Data presented as mean ± SD or percentage

Table 2 Age- and sex-adjusted association of diabetes susceptibility variants with risk of type 2 diabetes mellitus and incident CHD in EPIC-Norfolk 1993–2007 (in ascending order of previously reported effect size on type 2 diabetes)

Locus	SNP ^a	Effect allele ^b /other	RAF ^c (%)	OR for T2D ^d (95% CI)	<i>p</i> value	HR for CHD ^e (95% CI)	<i>p</i> value	HR for CHD ^{e,f} (95% CI)	<i>p</i> value
<i>VEGFA</i>	rs9472138	T/C	29.8	1.05 (0.96–1.14)	0.33	1.02 (0.96–1.09)	0.53	1.02 (0.95–1.09)	0.60
<i>OASL/HNF1A</i>	rs7957197	T/A	80.8	1.00 (0.90–1.11)	0.97	1.01 (0.94–1.09)	0.77	1.01 (0.94–1.09)	0.73
<i>ZFAND6</i>	rs11634397	G/A	66.6	0.99 (0.91–1.08)	0.86	0.99 (0.93–1.05)	0.73	0.99 (0.93–1.05)	0.73
<i>TP53INP1</i>	rs896854	T/C	49.7	1.08 (1.00–1.17)	0.05	0.96 (0.90–1.02)	0.17	0.96 (0.90–1.01)	0.13
<i>ADAMTS9</i>	rs4607103	C/T	76.3	0.98 (0.89–1.08)	0.68	1.00 (0.94–1.07)	0.94	1.01 (0.94–1.08)	0.88
<i>DCD</i>	rs1153188	A/T	73.4	1.07 (0.97–1.17)	0.18	1.02 (0.95–1.09)	0.62	1.01 (0.95–1.08)	0.72
<i>PRC1</i>	rs8042680	A/C	31.3	1.04 (0.96–1.14)	0.32	1.05 (0.98–1.12)	0.15	1.05 (0.98–1.12)	0.15
<i>SHBG</i>	rs1799941	G/A	74.1	1.10 (1.00–1.20)	0.06	1.01 (0.95–1.09)	0.68	1.01 (0.94–1.08)	0.85
<i>DGKB/TMEM195^g</i>	rs2191349	T/G	54.1	1.06 (0.97–1.15)	0.18	0.98 (0.92–1.04)	0.53	0.98 (0.92–1.04)	0.49
<i>GCKR</i>	rs780094	C/T	60.8	1.09 (1.01–1.19)	0.04	1.05 (0.99–1.12)	0.10	1.05 (0.99–1.12)	0.12
<i>KCNQ1</i>	rs231362	G/A	51.9	1.08 (1.00–1.17)	0.06	0.95 (0.90–1.01)	0.10	0.95 (0.89–1.01)	0.07
<i>GCK</i>	rs4607517	A/G	17.8	1.09 (0.98–1.20)	0.11	1.00 (0.93–1.08)	0.94	1.00 (0.92–1.08)	0.98
<i>HMGA2</i>	rs1531343	C/G	10.3	0.99 (0.87–1.13)	0.87	1.01 (0.92–1.11)	0.86	1.02 (0.92–1.12)	0.76
<i>TLE4/CHCHD9^h</i>	rs13292136	C/T	93.1	1.04 (0.88–1.22)	0.65	1.02 (0.91–1.15)	0.74	1.01 (0.90–1.14)	0.81
<i>BCL11A</i>	rs243021	A/G	46.5	1.12 (1.03–1.22)	0.01	0.98 (0.93–1.05)	0.62	0.98 (0.92–1.04)	0.42
<i>ADAM30</i>	rs2641348	G/A	10.5	1.07 (0.94–1.21)	0.32	1.02 (0.93–1.13)	0.65	1.02 (0.92–1.12)	0.74
<i>TSPAN8</i>	rs7961581	C/T	28.3	1.06 (0.97–1.16)	0.18	1.07 (1.00–1.14)	0.04	1.06 (0.99–1.13)	0.08
<i>JAZF1</i>	rs864745	T/C	49.4	1.09 (1.00–1.18)	0.04	1.03 (0.97–1.09)	0.37	1.02 (0.96–1.08)	0.56
<i>IRS1</i>	rs7578326	A/G	65.5	1.12 (1.02–1.22)	0.01	1.06 (1.00–1.13)	0.06	1.06 (0.99–1.13)	0.08
<i>SYN2/PPARG</i>	rs17036101	G/A	93.9	1.08 (0.90–1.28)	0.42	0.96 (0.85–1.09)	0.52	0.95 (0.84–1.08)	0.46
<i>MTNR1B</i>	rs10830963	G/C	27.7	1.05 (0.96–1.15)	0.26	0.97 (0.90–1.04)	0.35	0.96 (0.90–1.03)	0.22
<i>NOTCH2</i>	rs10923931	T/G	10.3	1.06 (0.93–1.21)	0.38	1.01 (0.92–1.11)	0.85	1.01 (0.91–1.11)	0.90
<i>WFS1</i>	rs10010131	G/A	59.2	1.10 (1.01–1.19)	0.03	1.02 (0.96–1.08)	0.53	1.01 (0.95–1.07)	0.77
<i>SLC30A8</i>	rs13266634	C/T	69.4	1.05 (0.96–1.15)	0.27	1.02 (0.95–1.09)	0.60	1.01 (0.94–1.08)	0.83
<i>CDKN2A/B</i>	rs564398	T/C	57.6	0.98 (0.91–1.07)	0.68	1.11 (1.04–1.17)	0.001	1.11 (1.05–1.18)	0.001
<i>THADA</i>	rs7578597	T/C	88.6	1.20 (1.05–1.37)	0.01	0.96 (0.87–1.05)	0.34	0.94 (0.86–1.03)	0.19
<i>ADCY5</i>	rs11708067	A/G	76.3	1.11 (1.01–1.23)	0.03	1.06 (0.99–1.14)	0.09	1.06 (0.99–1.13)	0.12
<i>API5</i>	rs9300039	C/A	89.4	1.04 (0.91–1.18)	0.59	0.94 (0.86–1.04)	0.22	0.94 (0.85–1.03)	0.18
<i>HHEX</i>	rs1111875	C/T	59.0	1.07 (0.99–1.17)	0.09	1.00 (0.95–1.07)	0.90	0.99 (0.93–1.05)	0.80
<i>KCNJ11</i>	rs5219	T/C	36.3	1.05 (0.96–1.14)	0.26	1.02 (0.96–1.08)	0.60	1.01 (0.95–1.08)	0.68
<i>PPARG</i>	rs1801282	C/G	88.0	1.15 (1.01–1.32)	0.03	0.93 (0.85–1.02)	0.11	0.92 (0.84–1.01)	0.07
<i>CENTD2ⁱ</i>	rs1552224	A/C	84.0	1.21 (1.08–1.36)	0.002	1.00 (0.92–1.08)	0.93	0.99 (0.91–1.07)	0.79
<i>NPPB</i>	rs198389	A/G	57.5	1.02 (0.94–1.11)	0.64	1.02 (0.96–1.09)	0.44	1.02 (0.96–1.09)	0.46
<i>CDKN2A/B</i>	rs10811661	T/C	82.1	1.12 (1.00–1.25)	0.04	1.05 (0.97–1.13)	0.26	1.04 (0.96–1.13)	0.33
<i>IGF2BP2</i>	rs4402960	T/G	31.4	1.11 (1.02–1.21)	0.02	0.99 (0.92–1.05)	0.67	0.97 (0.91–1.04)	0.38
<i>CDKAL1</i>	rs7756992	G/A	26.5	1.17 (1.06–1.21)	9.9×10^{-4}	1.06 (0.99–1.13)	0.11	1.05 (0.98–1.12)	0.19
<i>KCNQ1</i>	rs2237892	C/T	94.2	1.10 (0.92–1.31)	0.32	1.03 (0.91–1.18)	0.61	1.03 (0.90–1.17)	0.67
<i>TCF7L2</i>	rs7903146	T/C	29.2	1.64 (1.34–1.59)	9.5×10^{-18}	1.05 (0.98–1.12)	0.17	1.02 (0.95–1.09)	0.58
Gene score									
Tertile				1.42 (1.30–1.55)	1.9×10^{-15}	1.08 (1.01–1.14)	0.02	1.05 (0.99–1.12)	0.09
Tertile, weighted				1.50 (1.38–1.64)	2.5×10^{-20}	1.07 (1.01–1.13)	0.03	1.04 (0.98–1.10)	0.19

^a SNP, single nucleotide polymorphism^b According to type 2 diabetes risk-increasing allele in original reports^c RAF, risk allele frequency in participants without known type 2 diabetes^d OR for type 2 diabetes (T2D) is for combined prevalent ($n=640$) and incident ($n=640$) cases per allele or gene score tertile^e HR is per type 2 diabetes risk allele or gene score tertile, minimum events $n=2,043$, total participants $n=19,201$ for individual variants, $n=1,573$ and $n=14,871$ respectively for the gene score^f Adjusted for prevalent and incident type 2 diabetes^g *TMEM195* (also known as *AGMO*); ^h *CHCHD9* (also known as *CHCHD2P9*); ⁱ *CENTD2* (also known as *ARAP1*)

incident CHD. In our population individual diabetes variants showed an effect direction for risk of diabetes that was consistent with the original reports in 34 of the 38 variants. The risk of incident CHD ranged from HR 0.93 to 1.11 per diabetes risk allele across variants. Two variants, one next to the *CDKN2A/B* locus and one next to the *TSPAN8* locus, showed a significant association with CHD, with the same direction of effect as reported for diabetes. When correcting for multiple testing, the association of the *CDKN2A/B* variant with CHD remained significant ($p=0.04$) but not that of the *TSPAN8* variant.

The genetic score was significantly associated with risk of CHD, with each diabetes risk allele associated with a 1.5% (95% CI 0.2–2.8, $p=0.03$) risk increase for CHD in age- and sex-adjusted analysis. However, there was no significant association between the genetic score and risk of cardiovascular disease (HR 1.005, 95% CI 0.997–1.013, $p=0.22$).

Adjusting for prevalent and incident diabetes did not attenuate the association of the *CDKN2A/B* variant (HR 1.11, 95% CI 1.05–1.18, $p=0.001$), but did attenuate that of the *TSPAN8* variant (HR 1.06, 95% CI 0.99–1.13, $p=0.08$) and the genetic score (HR 1.05, 95% CI 0.99–1.12, $p=0.09$) with CHD risk (Table 2). Additional adjustment for BMI did not change results (data not shown).

The c -statistic for predicting a CHD event was unchanged by addition of the genetic score to a model including age and sex (0.73) or to a model including age, sex and traditional risk factors (0.76).

Discussion

We demonstrated a significant association of the *CDKN2A/B* and *TSPAN8* variants, and a genetic score built with 38 diabetes variants with risk of CHD. The association was mediated by the predisposition to diabetes for the *TSPAN8* variant and the genetic score. Addition of the genetic score did not improve clinical prediction of CHD.

A recent analysis of the Women's Health Study failed to show an improvement in cardiovascular risk prediction when adding a genetic score of variants associated with cardiovascular disease or intermediate traits [2]. However, the number of events was low ($n=777$) and the results are difficult to interpret, as different cardiovascular endpoints and genetic variants with contrary effects on distinct risk factors were lumped together. We observed a much stronger association of our diabetes-specific risk score with CHD than with cardiovascular disease. However, although the association between genetic diabetes susceptibility and CHD can be demonstrated, the magnitude of that association is too low to be of clinical use in the prediction of CHD.

The individual variants we identified to be associated with CHD were not those with the strongest association

with diabetes, suggesting that these variants or associated pathways might be of particular relevance to the development of CHD. However, the observed association of the *TSPAN8* variant with CHD was partly mediated by its association with diabetes and attenuated to borderline significance in the diabetes-adjusted model. Replication of our finding in a larger sample size may help to establish whether or not the *TSPAN8* locus is associated with CHD independently of diabetes.

The *CDKN2A/B* variant on chromosome 9p21 has been previously reported to be associated with CHD risk [7]. The 9p21 region is under intense research, since several variants within this region have been independently reported in genome-wide association studies (GWAS) for type 2 diabetes and CHD. Several of these variants, including our *CDKN2A/B* variant, were shown to disrupt transcription factor binding-sites and indeed one of the CHD variants had functional relevance for a pathway associated with atherosclerosis in human endothelial cells [8]. Our *CDKN2A/B* variant is in weak LD with one of the CHD variants ($r^2=0.24$ for rs10116277), which might explain the association with CHD risk. In support of this, we did not observe attenuation after adjustment for diabetes in our study. In contrast, in diabetic patients a significant interaction was found between a variant within the 9p21 region, which is in LD with our *CDKN2A/B* variant ($r^2=0.16$), and poor glycaemic control leading to an effect on risk of CHD [9]. This suggests not only separate effects on diabetes and CHD, but a mechanistic link between both diseases within this region.

The *IRS1* variant showed a borderline significant association with CHD here and is in high LD ($r^2=0.93$) with a variant reported in a GWAS of CHD [10]. Notably, the association between the *IRS1* variant and CHD here was not attenuated by adjustment for diabetes, suggesting that a potential common pathway for development of diabetes and CHD is associated with this variant.

In conclusion, the increased risk of CHD associated with aggregate genetic diabetes susceptibility was partly mediated by predisposition to diabetes and did not improve clinical risk prediction. The identification of individual variants associated with CHD risk independently of their diabetes predisposition might help to provide insight into the pathophysiological link between diabetes and CHD.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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