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ARTICLE

The search for putative unifying genetic factors for components of the metabolic syndrome

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Abstract
Aims/hypothesis The metabolic syndrome is a cluster of factors contributing to increased risk of cardiovascular disease and type 2 diabetes but unifying mechanisms have not been identified. Our aim was to study whether common variations in 17 genes previously associated with type 2 diabetes or components of the metabolic syndrome and variants in nine genes with inconsistent association with at least two components of the metabolic syndrome would also predict future development of components of the metabolic syndrome, individually or in combination.
Methods Genetic variants were studied in a large prospective study of 16,143 non-diabetic individuals (mean follow-up time 23 years) from the Malmö Preventive Project. In this study, development of at least three of obesity (BMI≥ 30 kg/m^2), dyslipidaemia (triacylglycerol≥1.7 mmol/l and/or lipid-lowering treatment), hypertension (blood pressure≥ 140/90 mmHg and/or antihypertensive medication) and hyperglycaemia (fasting plasma glucose≥5.6 mmol/l and/or known diabetes) was defined as development of the metabolic syndrome. The risk of developing at least three components of the metabolic syndrome or the individual components was calculated by logistic regression adjusted for age at baseline, follow-up time and sex.
Results Polymorphisms in *TCF7L2* (rs7903146, OR 1.10, 95% CI 1.04–1.17, p=0.00097), *FTO* (rs9939609, OR 1.08, 95% CI 1.02–1.14, p=0.0065), *WFS1* (rs10010131, OR 1.07, 95% CI 1.02–1.13, p=0.0078) and *IGF2BP2* (rs4402960, OR 1.07, 95% CI 1.01–1.13, p=0.021) predicted the development of at least three components of the metabolic syndrome in both univariate and multivariate analysis; in the case of *TCF7L2*, *WFS1* and *IGF2BP* this was due to their association with hyperglycaemia (p< 0.00001, p=0.0033 and p=0.027, respectively) and for *FTO* it was due to its association with obesity (p=0.004). A polymorphism in the *GCKR* gene predicted dyslipidaemia (rs1260326, OR 1.15, 95% CI 1.09–1.22, p<0.00001) but not the metabolic syndrome. None of the studied polymorphisms was associated with more than two components of the metabolic syndrome. A composite genotype score of the 17 polymorphisms associated with type 2 diabetes predicted the development of at least three components of the metabolic syndrome (OR 1.04, p<0.00001) and the development of hyperglycaemia (OR 1.06, p<0.00001). Carriers of ≥19 risk alleles had 51 and 72% increased risk of developing at least three components of the metabolic syndrome and hyperglycaemia, respectively, compared with carriers of ≤12 risk alleles (p<0.00001 for both).
Conclusions/interpretation Polymorphisms in susceptibility genes for type 2 diabetes (*TCF7L2*, *WFS1*, *IGF2BP2*) and obesity (*FTO*) predispose to the metabolic syndrome by

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increasing the risk of one specific component of the metabolic syndrome. The findings argue against a unifying genetic component for the metabolic syndrome.

Keywords Common polymorphism · Genetics · Metabolic syndrome · Prospective study · Type 2 diabetes

Abbreviations

CVD	cardiovascular disease
GWAS	genome-wide association study
IDF	International Diabetes Federation
MMP	Malmö Preventive Project
NCEP-ATPIII	National Cholesterol Education Program Adult Treatment Panel III
SNP	single nucleotide polymorphism

Introduction

The metabolic syndrome is defined as a cluster of factors that increase the risk of type 2 diabetes and cardiovascular disease (CVD) [1]. In addition to environmental factors, such as a sedentary lifestyle and an energy-rich diet, substantial evidence exists for a genetic component of the syndrome. Environmental triggers may thus play a variable role depending on the genetic background of the individual. The metabolic syndrome clusters in families [2] and its prevalence differs between ethnic groups [3]. The heritability of the metabolic syndrome has been estimated to be about 24% [4] and that of components of the metabolic syndrome ranges from ~16% for systolic blood pressure to ~60% for HDL-cholesterol levels [4–7].

It is not established whether the metabolic syndrome has a common pathogenic background. One way to address this question is to study whether the different components of the metabolic syndrome share associated genetic factors.

To accomplish this, we first investigated whether common variants in genes that have previously been associated with type 2 diabetes in candidate gene or linkage studies and convincingly replicated (*TCF7L2*, *PPARG*, *KCNJ11* and *WFS1*) or have been identified in genome-wide association studies (GWAS) or meta-analysis of GWAS with genome-wide significant *p* values (*SLC30A8*, *HHEX*, *IGF2BP2*, *CDKAL1*, *CDKN2A*, *FTO*, *JAZF1, CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, *NOTCH2* and *GCKR*) [8–18] also predict the development of components of the metabolic syndrome, individually or in combination, in 16,143 individuals from the Malmö Preventive Project (MPP) followed for a mean period of 23 years. Second, we investigated ten polymorphisms in nine genes (*ADRB1*, *ADRB2*, *ADRB3*, *CAPN10*, *IRS1*, *UCP2*, *PPARGC1A*, *PTPN1* and *ENPP1*), each of which has been associated with at least two components contributing to the metabolic syndrome [19–32].

Methods

In the MPP, 33,346 Swedish participants (22,444 men and 10,902 women, mean age 49 years, 24.5% with IFG and/or IGT from the city of Malmö in southern Sweden participated in health screening during 1974–1992 (attendance rate 71%). All individuals underwent a physical examination and measurements of fasting blood glucose and triacylglycerol concentrations were performed. In addition, 18,900 consecutive individuals also underwent an OGTT. Information on lifestyle factors and medical history was obtained from a questionnaire. Of individuals participating in the initial screening, 4931 have died and 551 were lost from follow-up for other reasons. Twenty-five thousand of the eligible individuals were invited to a re-screening visit during 2002–2006, including a physical examination and fasting blood samples for measurements of glucose, triacylglycerol and HDL-cholesterol concentrations. Of the invited individuals, 17,284 participated in the re-screening; 1,141 of them were excluded from the present study because of the lack of DNA or crucial clinical information or because of type 2 diabetes at baseline. Thus, 16,143 non-diabetic participants, 2,063 of whom developed type 2 diabetes, were included in the follow-up study [33]. The diagnosis of diabetes was confirmed from patient records or was based on a fasting plasma glucose concentration greater than 7.0 mmol/l. The clinical characteristics of individuals included in the study both at baseline and at follow-up are summarised in Table 1.

Definition of the metabolic syndrome As waist and HDL-cholesterol measurements were not available for most participants at baseline, we were unable to construct the definitions of the metabolic syndrome according to any of the common definitions (National Cholesterol Education Program Adult Treatment Panel III [NCEP-ATPIII] [34], WHO [35], International Diabetes Federation [IDF] [36]). We therefore defined the components of the metabolic syndrome as follows: (1) obesity (BMI≥30 kg/m^2); (2) dyslipidaemia (triacylglycerol≥1.7 mmol/l and/or lipid-lowering treatment); (3) hypertension (blood pressure≥ 140/90 mmHg and/or antihypertensive medication); and (4) hyperglycaemia (fasting plasma glucose≥5.6 mmol/l and/or overt diabetes). The presence of at least three of these components defined the metabolic syndrome and occurred in 6.7% individuals at baseline; 35% had none of the components.

Table 1 Clinical characteristics of MPP participants at baseline and after follow-up

Characteristic	Data available (*n*)	Baseline characteristic	Data available (*n*)	Re-examination characteristic
Number of participants	16,143		16,143	
Men, women	10,455, 5688		10,455, 5688	
Age (years)	16,143	46±7	16,143	68±6***
BMI (kg/m^2)	16,136	24.3±3.3	16,055	27.1±4.1***
Waist (cm)	827	78.6±9.8	16,051	94.8±12.2
Systolic BP (mmHg)	16,140	127±14	16,099	145±20***
Diastolic BP (mmHg)	16140	84±9	16,099	84±11
Fasting plasma glucose (mmol/l)	16,083	5.4±0.6	16,111	5.8±1.3***
Triacylglycerol (mmol/l)	16,104	1.26±0.77	16,118	1.26±0.80
HDL-cholesterol (mmol/l)	289	1.54±0.37	16,112	1.40±0.42
Smoking (%)[a]	15,736	37.3	0	NA
Antihypertensive medication (%)	16,119	4.2	16,141	37.7***
Lipid-lowering medication (%)	0	NA	16,141	20.0
Type 2 diabetes (%)	16,143	0	16,122	13.2***
Obesity (%)	16,135	5.1	16,042	21.2***
Dyslipidaemia (%)	16,104	18.2	16,118	34.5***
Hypertension (%)	16,124	34.2	16,099	84.5***
Hyperglycaemia (%)	16,143	39.9	16,117	47.2***
Metabolic syndrome	16,131	6.7	16,080	28.3***
Metabolic syndrome, NCEP-ATPIII	0	NA	16,080	30.2

Data are mean±SD
For the definition of the metabolic syndrome used in this study, please refer to the Methods ***$p<0.001$
[a] Refers both to current and previous smoking
NA, not available

SNP selection and genotyping We selected 17 polymorphisms associated with type 2 diabetes to be investigated in this study. Four of the selected single nucleotide polymorphisms (SNPs) had been identified earlier in candidate gene or linkage studies and convincingly replicated (*TCF7L2*, *PPARG*, *KCNJ11* and *WFS1*) [9, 11–13]. Seven of the SNPs were identified by GWAS and were genome-wide significant (*SLC30A8*, *HHEX*, *IGF2BP2*, *CDKAL1*, *CDKN2A*, *FTO* and *GCKR*) [14–17]. The remaining six SNPs were identified in a meta-analysis of GWAS (*JAZF1*, *CDC123*/*CAMK1D*, *TSPAN8*/*LGR5*, *THADA*, *ADAMTS9* and *NOTCH2*) [8] at a genome-wide-significant level. Of them *FTO* has been associated with obesity and type 2 diabetes, whereas *GCKR* has been associated with increased triacylglycerol and decreased glucose levels [10, 14, 18, 37–39]. We additionally selected ten SNPs in nine genes (*ADRB1*, *ADRB2*, *ADRB3*, *CAPN10*, *IRS1*, *UCP2*, *PPARGC1A*, *PTPN1* and *ENPP1*) that have been associated with at least two components of the metabolic syndrome, such as type 2 diabetes (*ADRB2*, *ADRB3*, *CAPN10*, *IRS1*, *UCP2*, *PPARGC1A* and *ENPP1*) [19, 23, 25, 29, 30, 40], obesity (*ADRB1*, *ADRB2*, *ADRB3*, *CAPN10*, *IRS1*, *UCP2*, *PTPN1* and *ENPP1* [21, 22, 26–29, 32, 41]), hypertension (*ADRB1*, *PPARGC1A* and *PTPN1*) [20, 27, 31] or the metabolic syndrome (*ADRB1* and *ADRB3*) [42].

DNA was extracted from whole blood using Qiagen Maxipreps (Qiagen, Valencia, CA, USA). Genotyping was performed using matrix-assisted laser desorption ionisation-time of flight mass spectrometry on the MassARRAY platform (Sequenom, San Diego, CA, USA) (rs7903146, rs1801282, rs5219, rs7754840, rs10811661, rs1044498, rs3787348, rs8192678, rs1801278, rs659366, rs2975760 and rs3792267), by an allelic discrimination assay-by-design method using an ABI 7900 PCR system (Applied Biosystems, Foster City, CA, USA) (rs4402960, rs9939609, rs10010131, rs1111875, rs864745, rs12779790, rs7961581, rs7578597, rs4607103, rs10923931, rs1260326, rs1801253, rs1042714 and rs4994) and using a Kaspar allelic discrimination method (KBioscience, Hoddesdon, UK) (rs13266634).

To ascertain genotype quality, random samples of 5% were re-genotyped and the concordance rate was >99%. All SNPs were in Hardy–Weinberg equilibrium ($p>0.01$) with the exception of rs864745 in the *JAZF1* gene ($p=0.001$). Genotyping errors are an unlikely explanation for this as 2416 samples (15%) were re-genotyped with two methods (MassARRAY and ABI 7900) with a concordance of 98.7%.

Statistical analyses The OR for the risk of developing the metabolic syndrome or its components was calculated using

Table 2 Risk of developing at least three components of the metabolic syndrome

Gene symbol	SNP	OR (95% CI)	*p* value
TCF7L2	rs7903146	1.10 (1.04–1.17)	0.00097
PPARG	rs1801282	0.97 (0.90–1.05)	0.50
KCNJ11	rs5219	1.01 (0.96–1.07)	0.66
HHEX	rs1111875	1.05 (1.00–1.11)	0.069
IGF2BP2	rs4402960	1.07 (1.01–1.13)	0.021
CDKAL1	rs7754840	0.97 (0.91–1.03)	0.33
FTO	rs9939609	1.08 (1.02–1.14)	0.0065
CDKN2A	rs10811661	1.07 (1.00–1.15)	0.059
SLC30A8	rs13266634	1.04 (0.98–1.10)	0.22
WFS1	rs10010131	1.07 (1.02–1.13)	0.0078
GCKR	rs1260326	1.03 (0.97–1.09)	0.33
JAZF1	rs864745	1.00 (0.95–1.06)	0.88
ADAMTS9	rs4607103	1.00 (0.94–1.06)	0.87
CAMK1D	rs12779790	1.00 (0.93–1.07)	0.89
TSPAN8/LGR5	rs7961581	0.98 (0.93–1.04)	0.57
NOTCH2	rs10923931	0.96 (0.88–1.06)	0.45
THADA	rs7578597	1.05 (0.96–1.14)	0.33

All analyses are adjusted for age at baseline, follow-up time and sex
The *p* values given are two-sided, uncorrected for multiple testing
Proteins encoded by genes listed (in order of appearance in table): *TCF7L2*, transcription factor 7-like 2; *PPARG*, peroxisome proliferator-activated receptor-gamma; *KCNJ11*, potassium channel, inwardly rectifying, subfamily J, member 11, *HHEX*, hematopoetically expressed homeobox; IGF2BP2, insulin-like growth factor 2 mRNA-binding protein 2, *CDKAL1*, CDK5 regulatory subunit-associated protein 1-like 1; *FTO*, fat mass- and obesity-associated; *CDKN2A*, cyclin-dependent kinase inhibitor 2A; *SLC30A8*, solute carrier family 30 (zinc transporter) member 8; *WFS1*, Wolfram syndrome 1; *GCKR*, glucokinase regulatory protein; *JAZF1*, juxtaposted with another zinc finger gene 1; *ADAMTS9*, a disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 9; *CAMK1D*, calcium/calmodulin-dependent protein kinase 1-delta; *TSPAN8*, tetraspanin 8; *LGR5*, leucine-rich repeat-containing G protein-coupled receptor 5; *NOTCH2*, notch homolog 2; *THADA*, thyroid adenoma associated

logistic regression analyses assuming an additive model, adjusted for age at baseline, follow-up time and sex. Only participants free from the investigated component of the metabolic syndrome at baseline were included in the analysis. Multivariate logistic regression analyses were performed adjusting for age at baseline, follow-up time and sex and including all the 17 gene polymorphisms associated with type 2 diabetes in the model. In addition, we conducted backward elimination of SNPs with a retention threshold of $p<0.05$. The two methods gave similar results and only the backward elimination results are presented.

As all the individual SNPs were assumed to explain only a very small proportion of the individual or combined components of the metabolic syndrome, we constructed a composite genotype score on the basis of the number of unfavourable alleles (those associated with type 2 diabetes except in *GCKR*, where the unfavourable allele was that associated with higher triacylglycerol levels) that were carried by each individual for each of the 17 SNPs associated with type 2 diabetes. In addition, we constructed a composite risk score by including only SNPs significant for any components of the metabolic syndrome in the multivariate analysis (*TCF7L2*, *HHEX*, *FTO*, *IGF2BP2*, *CDKAL1*, *SLC30A8* and *GCKR*).

All statistical analyses were performed using the SPSS 14.0 statistical analysis software package (SPSS, Chicago, IL, USA), and nominal two-sided *p* values and corrected *p* values (Bonferroni, corrected for 27 SNPs included in this study) of $p<0.05$ were considered significant. Power calculations were performed with the genetic power calculator at http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html [43]. In our sample of 14,996 individuals with fewer than three components of the metabolic syndrome at baseline, 3843 had at least three components of the syndrome (cases) and 11,153 had fewer than three components of the metabolic syndrome (controls) after follow-up time. The risk allele frequencies ranged from 0.1 to 0.9 and at the significance level of 0.05 our power to detect association was 76–99% for OR 1.1–1.2.

Results

Components of the metabolic syndrome in MPP at baseline and after the follow-up At inclusion in the study, 6.7% of individuals had at least three components of the metabolic syndrome according to the criteria described above. Among individuals with fewer than three components of the metabolic syndrome at baseline, 25.6% had at least three components of the syndrome after the mean follow-up time of 23 years. Among individuals without a specific component of the metabolic syndrome at baseline, 18.0% developed obesity, 28.0% dyslipidaemia, 73.5% hypertension and 37.9% hyperglycaemia during the follow-up time. Compared with the baseline situation, in which 35.0% of the individuals had none of the four components of the metabolic syndrome, 8.7% had none at follow-up ($p<0.00001$), 30.1% had one (compared with 38.1% at baseline, $p<0.00001$), 32.0% had two (compared with 19.1% at baseline, $p<0.00001$), 20.9% had three (compared with 5.8% at baseline, $p<0.00001$) and 7.3% had four (compared with 0.8% at baseline, $p<0.00001$) (Table 1). The risk of developing at least three of four components of the metabolic syndrome was affected by age at baseline (OR 0.992, 95% CI 0.986–0.999, $p=0.016$), smoking (OR 1.36, 95% CI 1.26–1.47, $p<0.00001$) and male sex (OR 1.41, 95% CI 1.27–1.57, $p<0.00001$), after adjustment for follow-up time.

At follow-up, data were available to define the metabolic syndrome also using the NCEP-ATPIII criteria. In total, 30.2% of the study participants had the metabolic syndrome according to the NCEP-ATPIII definition compared with 28.8% having at least three of the four components of the metabolic syndrome available in this study, and 22.2% had the metabolic syndrome according to both definitions.

Genetic prediction of components of the metabolic syndrome in MPP Out of the 17 SNPs associated with type 2 diabetes, polymorphisms in *TCF7L2* (rs7903146, OR 1.10, 95% CI 1.04–1.17, $p=0.00097$), *FTO* (rs9939609, OR 1.08, 95% CI 1.02–1.14, $p=0.0065$), *WFS1* (rs10010131, OR 1.07, 95% CI 1.02–1.13, $p=0.0078$) and *IGF2BP2* (rs4402960, OR 1.07, 95% CI 1.01–1.13, $p=0.021$) predicted the presence of at least three of the four components of the metabolic syndrome after the follow-up time (Table 2). However, after correction for multiple testing, only the *TCF7L2* rs7903146 remained significant ($p_c=0.026$). In multivariate logistic regression analysis, the following three SNPs were retained as predictors of at least three components of the metabolic syndrome present in an individual after follow-up: rs7903146 in *TCF7L2* ($p=0.0012$), rs111875 in *HHEX* ($p=0.0052$) and rs9939609 in *FTO* ($p=0.018$).

Notably, for the variants in *TCF7L2* (rs7903146, OR 1.17, 95% CI 1.09–1.25, $p<0.00001$), *IGF2BP2* (rs4402960, OR 1.10, 95% CI 1.03–1.18, $p=0.0033$), *CDKAL1* (rs7754840, OR 1.12, 95% CI 1.04–1.20, $p=0.0017$), *SLC30A8* (rs13266634, OR 1.12, 95% CI 1.05–1.19, $p=0.00061$), *WFS1* (rs10010131, OR 1.07, 95% CI 1.01–1.14, $p=0.027$) the risk of development of at least three of the four components of the metabolic syndrome could be explained by their effect on hyperglycaemia. The same four SNPs were retained as predictors of hyperglycaemia after follow-up in multivariate regression analysis (rs7903146, $p<0.00001$; rs4402960, $p=0.025$; rs7754840, $p=0.0019$; and rs13266634, $p=0.0037$). Even after correction for multiple testing, variants of *TCF7L2* and *CDKAL1* remained significant predictors of hyperglycaemia ($p_c=0.00016$ and 0.032, respectively).

The risk of developing obesity was increased by the polymorphism in *FTO* (rs9939609, OR 1.09, 95% CI 1.03–1.16, $p=0.004$) but the SNP did not remain significant after correction for multiple testing. In multivariate regression analysis, none of the SNPs was retained as a significant predictor of obesity at follow-up.

The risk of developing dyslipidaemia increased in individuals carrying the T allele of *GCKR* rs1260326 both in univariate (OR 1.15, 95% CI 1.09–1.22, $p<0.00001$, $p_c=0.000053$) and multivariate analysis ($p<0.00001$ for *GCKR*); no other variant increased the risk of dyslipidaemia (Table 3).

In multivariate regression analysis the rs13266634 in *SLC30A8* ($p=0.014$) was retained as a predictor of hypertension at follow-up, the C allele previously associated with hyperglycaemia now showing modestly reduced risk of developing hypertension (OR 0.88, 95% CI 0.79–0.97).

Complete data for all 17 SNPs associated with type 2 diabetes were available for 9740 individuals and for the seven SNPs significant in multivariate regression analysis for 10,875 individuals. A composite genotype score of the 17 SNPs predicted the development of at least three components of the metabolic syndrome with 4% risk increase per allele (OR 1.04, 95% CI 1.02–1.06, $p<0.00001$) as well as the development of hyperglycaemia (OR 1.06, 95% CI 1.04–1.08, $p<0.00001$). Carriers of ≥19 risk alleles (9.7% of individuals) had 51% increased risk of developing at least three components of the metabolic syndrome (OR 1.51, 95% CI 1.26–1.82, $p=0.000012$) compared with individuals carrying ≤12 risk alleles (15% of individuals) as well as 72 and 47% increased risk of developing hyperglycaemia (OR 1.72, 95% CI 1.39–2.13, $p<0.00001$) and hypertension (OR 1.47, 95% CI 1.11–1.96, $p=0.0079$), respectively.

When constructing a composite risk score by including only SNPs significant in the multivariate analysis (*TCF7L2*, *HHEX*, *FTO*, *IGF2BP2*, *CDKAL1*, *SLC30A8* and *GCKR*) the carriers of ≥9 risk alleles (6.7% of individuals) had increased risk of developing at least three components of the metabolic syndrome (OR 1.73, 95% CI 1.39–2.15, $p<0.00001$), hyperglycaemia (OR 1.97, 95% CI 1.52–2.55, $p<0.00001$) and dyslipidaemia (OR 1.46, 95% CI 1.16–1.83, $p=0.0011$) compared with individuals carrying ≤3 risk alleles (8.9% of individuals).

Although none of the ten SNPs in nine genes previously associated with components of the metabolic syndrome significantly predicted the development of at least three components of the syndrome in this study (see Electronic supplementary material Table 1), the Trp64Arg polymorphism in *ADRB3* nominally predicted the development of obesity (rs4994, OR 1.13, 95% CI 1.01–1.26, $p=0.034$), whereas the SNP rs1044498 in *ENPP1* nominally predicted the development of hyperglycaemia (OR 1.10, 95% CI 1.01–1.19, $p=0.037$). However, these results would not stand correction for multiple testing but replicate previous similar findings.

None of the studied polymorphisms was associated with more than two components of the metabolic syndrome.

Discussion

Our study addressed the question whether previously reported susceptibility genes for type 2 diabetes (*TCF7L2*,

Table 3 Risk of developing components of the metabolic syndrome in individuals without the phenotype at baseline

Gene	SNP	Obesity		Dyslipidaemia		Hypertension		Hyperglycaemia	
		OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
TCF7L2	rs7903146	0.96 (0.90–1.03)	0.25	1.03 (0.96–1.09)	0.43	1.00 (0.92–1.10)	0.92	1.17 (1.09–1.25)	<0.00001
PPARG	rs1801282	1.02 (0.93–1.11)	0.73	0.93 (0.86–1.01)	0.087	0.99 (0.88–1.10)	0.81	0.94 (0.86–1.02)	0.16
KCNJ11	rs5219	0.95 (0.89–1.01)	0.099	1.00 (0.94–1.06)	0.99	1.07 (0.97–1.16)	0.11	1.06 (0.99–1.13)	0.084
HHEX	rs1111875	0.96 (0.90–1.02)	0.17	1.01 (0.96–1.07)	0.62	1.04 (1.00–1.13)	0.27	1.06 (0.99–1.12)	0.087
IGF2BP2	rs4402960	0.94 (0.88–1.00)	0.043	1.02 (0.96–1.08)	0.61	1.07 (0.99–1.17)	0.10	1.10 (1.03–1.18)	0.0033
CDKAL1	rs7754840	0.97 (0.91–1.04)	0.40	1.04 (0.98–1.11)	0.21	1.02 (0.93–1.11)	0.69	1.12 (1.04–1.20)	0.0017
FTO	rs9939609	1.09 (1.03–1.16)	0.0039	1.03 (0.98–1.09)	0.25	1.03 (0.95–1.11)	0.50	1.04 (0.98–1.11)	0.16
CDKN2A	rs10811661	1.00 (0.92–1.08)	0.97	1.00 (0.93–1.08)	0.96	1.09 (0.98–1.21)	0.13	1.06 (0.98–1.15)	0.16
SLC30A8	rs13266634	1.03 (0.97–1.10)	0.32	0.99 (0.93–1.05)	0.68	0.97 (0.89–1.05)	0.44	1.12 (1.05–1.19)	0.00061
WFS1	rs10010131	1.00 (0.94–1.06)	0.87	1.04 (0.98–1.09)	0.20	1.08 (1.00–1.17)	0.050	1.07 (1.01–1.14)	0.027
GCKR	rs1260326	1.00 (0.94–1.07)	0.97	1.15 (1.09–1.22)	<0.00001	0.93 (0.86–1.01)	0.076	1.00 (0.93–1.06)	0.89
JAZF1	rs864745	0.96 (0.90–1.01)	0.14	0.98 (0.93–1.03)	0.45	0.99 (0.91–1.06)	0.71	1.05 (0.99–1.11)	0.14
ADAMTS9	rs4607103	0.95 (0.88–1.01)	0.12	1.00 (0.93–1.06)	0.89	1.01 (0.92–1.11)	0.82	1.01 (0.94–1.08)	0.86
CAMK1D	rs12779790	0.96 (0.88–1.03)	0.25	0.99 (0.92–1.06)	0.70	1.02 (0.92–1.13)	0.69	1.03 (0.95–1.11)	0.54
TSPAN8/LGR5	rs7961581	0.96 (0.89–1.03)	0.21	0.98 (0.93–1.05)	0.62	1.05 (0.96–1.15)	0.30	1.05 (0.99–1.13)	0.13
NOTCH2	rs10923931	1.00 (0.90–1.12)	1.00	1.01 (0.91–1.12)	0.87	0.93 (0.81–1.07)	0.32	0.93 (0.83–1.04)	0.22
THADA	rs7578597	0.98 (0.89–1.08)	0.73	1.10 (1.00–1.20)	0.047	0.90 (0.79–1.03)	0.12	1.01 (0.92–1.12)	0.82

All analyses are logistic regression analyses adjusted for age at baseline, follow-up time and sex
For the definition of the metabolic syndrome used in this study, please refer to the "Methods" section

SLC30A8, *HHEX*, *PPARG*, *KCNJ11*, *IGF2BP2*, *CDKAL1*, *CDKN2A*, *FTO*, *WFS1*, *JAZF1, CDC123/CAMK1D*, *TSPAN8/LGR5*, *THADA*, *ADAMTS9*, *NOTCH2* and *GCKR*), obesity (*FTO*) or dyslipidaemia (*GCKR*) predict the development of components of the metabolic syndrome, individually or in combination, in a large prospective study with >400,000 follow-up years. Another key question was whether the different components would share a unique genetic factor. This was clearly not the case. Although a number of variants increased future risk of the metabolic syndrome, this could be explained by their effect on hyperglycaemia (*TCF7L2*, *IGF2BP2* and *WFS1*) or obesity (*FTO*). In Danish population-based twin studies, Benyamin et al. found only modest genetic correlations between the different components of the metabolic syndrome [44]. This led the authors to conclude that components of the metabolic syndrome did not share a major common genetic background, supporting our results.

The T allele of *TCF7L2* rs7903146 was a strong predictor of the development of hyperglycaemia but was not associated with any of the other metabolic syndrome components. This is compatible with previous findings that variation in *TCF7L2* influences insulin secretion but not insulin action [45].

Somewhat surprisingly, several of the variants (*HHEX*, *PPARG*, *KCNJ11*, *FTO*, *NOTCH2* and *JAZF1*) which predict future type 2 diabetes in this cohort [33] did not predict the development of hyperglycaemia. This could be due to the low cut-off value used to define hyperglycaemia in this study as well as in the IDF definition of the metabolic syndrome, i.e. fasting plasma glucose ≥5.6 mmol/l, or reduced power, as fewer individuals were included in the analysis than when analysing the whole cohort for prediction of type 2 diabetes [36, 46].

Although none of the polymorphisms previously inconsistently associated with components of the metabolic syndrome predicted the development of at least three components of the syndrome in our study, some results are in line with earlier reports; for example, the SNP rs4994 in the *ADRB3* gene was weakly associated with the risk of developing obesity ($p=0.034$) and the *ENPP1* rs1044498 was associated with a modestly increased risk of developing hyperglycaemia ($p=0.037$). In our previous family-based study and in a large meta-analysis the C-allele (Arg) carriers of rs4994 had higher BMI than non-carriers [22, 32]. Association between *ENPP1* and hyperglycaemia was supported by a recent meta-analysis indicating that the minor C allele increased the risk of future type 2 diabetes [25].

Two of the investigated polymorphisms, namely those in *FTO* and *GCKR*, have previously been convincingly associated with two components of the metabolic syndrome [10, 14, 18, 37–39]. Common variants in the *FTO* gene have been associated with both BMI and type 2 diabetes; the predisposition to type 2 diabetes can be fully explained by the weight-increasing effect [10]. In keeping with these findings, we observed that *FTO* predisposes to the metabolic syndrome predominantly via its effect on obesity. Although the mechanisms by which variants in *FTO* increase the risk of obesity are not known, they could involve effects on appetite regulation as *FTO* is strongly expressed in the hypothalamus [47, 48]. In this study we did not observe the *FTO* polymorphism as a significant risk factor for hyperglycaemia. This might again be due to reduced power when analysing fewer individuals or the relatively low cut-off value used to define hyperglycaemia.

The *GCKR* polymorphism has been associated primarily with triacylglycerol levels [14, 37, 38]. Interestingly, the same allele that has been associated with higher triacylglycerol levels has been associated with lower levels of fasting glucose [37–39]. In this study the *GCKR* variant did not predict hyperglycaemia or a combination of at least three components of the metabolic syndrome. The lack of association with hyperglycaemia might be due to reduced power, as we, in line with commonly used definitions of the metabolic syndrome, dichotomised the individuals as either being or not being hyperglycaemic. Indeed, the fasting plasma glucose levels were significantly lower in carriers of the T allele ($p=0.0025$) at follow-up. The lack of association of the *GCKR* variant with at least three components of the metabolic syndrome is not surprising as the T allele is associated with higher triacylglycerol levels but also with lower glucose, a favourable metabolic marker. The risk of developing the metabolic syndrome might thus be balanced by these opposing associations.

The SNP rs13266634 in *SLC30A8* was the only polymorphism associated with more than one component of the metabolic syndrome in multivariate logistic regression. As expected, association was found with hyperglycaemia, but the same allele was found to be slightly protective against the development of hypertension ($p=0.014$). The mechanism for this observation is unclear and the modest p value could imply a chance finding and needs confirmation in other studies.

Several studies have tried to identify underlying genetic risk factors for the metabolic syndrome [7], but no study has been able to identify genetic variants that would be shared by most components of the syndrome. Our relatively well-powered study showed that none of the genetic variants was shared by more than two of the components, thereby challenging the view that the metabolic syndrome has a common genetic background.

Despite its large size and long follow-up period, some limitations of the study should be mentioned. First, the definition of the metabolic syndrome is clearly known to influence both prevalence estimates and possible genetic

associations [49]. The definition of obesity in most of the metabolic syndrome definitions uses measures of abdominal obesity, i.e. waist or waist-to-hip ratio instead of BMI. Although BMI and waist circumference correlate strongly, BMI captures only about 80% of the variance in waist circumference. Second, we lack data on HDL-cholesterol at baseline. At follow-up the prevalence of the metabolic syndrome in this cohort was slightly higher using the NCEP-ATPIII definition than using our modified definition without HDL-cholesterol (30.2% vs 28.3%, $p=0.00022$). Including HDL-cholesterol in the definition of the metabolic syndrome classified another 1586 individuals as being dyslipidaemic at follow-up. Third, it is likely that we have underestimated the number of individuals who developed the metabolic syndrome as we can assume over-representation of the syndrome among participants who had died from cardiovascular disease.

One further caveat is that we restricted the analysis to known candidate genes. We cannot exclude the possibility that the different components would share some unknown genetic factor, which could only be identified by GWAS. It needs to be kept in mind that despite the recent success in finding genes contributing to complex metabolic traits defined as components of the syndrome (type 2 diabetes, obesity and lipid levels), no GWAS analysis for the metabolic syndrome has been reported. In addition, in GWAS for type 2 diabetes and obesity, the case groups have been selected mainly or solely on the basis of glucose tolerance status and BMI, respectively, and in none of the studies have individuals carrying other components of the metabolic syndrome than type 2 diabetes or obesity been excluded among controls, lowering the chance of identifying genes important for several of the components of the metabolic syndrome.

Although only seven of the 17 genes previously associated with type 2 diabetes predicted one of the components of the metabolic syndrome and only four predicted the development of at least three components of the syndrome in our study, a composite score including all 17 genes was significantly associated with the development of at least three components of the metabolic syndrome, though with a modest OR of 1.04 per allele. Nevertheless, individuals carrying at least 19 risk alleles had a more than 50% increased risk of having at least three components of the metabolic syndrome at follow-up compared with individuals with fewer than 13 risk alleles. However, as our study mainly focused on genes identified and verified as type 2 diabetes genes, it is obvious that the specific set of SNPs used to define the composite score for prediction of the metabolic syndrome in this study needs refinement. Because of the rapid progress of genome wide association and sequencing approaches, the flow of novel discoveries is expected to continue. In addition to the obvious need for GWAS for the metabolic syndrome, new genes robustly associating with components of the metabolic syndrome are constantly emerging, such as *MC4R* associated with obesity [50, 51] and the many novel genes regulating lipid levels [52–54]. We cannot exclude the possibility that one or several of these new candidate genes might provide a unifying genetic component for the metabolic syndrome.

We conclude that polymorphisms in candidate genes for type 2 diabetes (*TCF7L2*, *WFS1*, *IGF2BP2*) and obesity (*FTO*) predict the development of at least three components of the metabolic syndrome by increasing the risk of development of the component of the metabolic syndrome for which the original association was reported. These data do not support the view that the different components of the metabolic syndrome share a common genetic background.

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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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