Components of the Metabolic Syndrome and Risk of Type 2 Diabetes

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Context: The metabolic syndrome (MetS) is associated with type 2 diabetes (T2D). However, whether each of the 5 components of the MetS individually is causally associated with T2D is unknown.

Objective: We tested the hypothesis that each component is causally associated with T2D.

Design: Mendelian randomization using genetic variations that alter levels of the MetS components are randomly assorted at gamete formation and free of confounding and reverse causation, which allows us to infer causality.

Setting: General community.

Study Participants: A total of 95 756 individuals from the prospective Copenhagen General Population Study.

Main Outcome Measure: Type 2 diabetes.

Results: A 1-cm larger waist circumference was associated with an observational 5% (95% confidence interval, 4%–5%) and a causal genetic 5% (1%–10%) higher risk of T2D. In contrast, although a 1-unit higher level of triglycerides and blood pressure and a 1-unit lower level of high-density lipoprotein cholesterol were associated with higher observational T2D risk, the corresponding causal genetic risks were not. As expected, a 1 mmol/L higher glucose level was associated with an observational 32% (30%–34%) and a causal genetic 82% (21%–173%) higher T2D risk.

Conclusions: In conclusion, larger waist circumference and higher glucose levels were each causally associated with higher risk of T2D. Findings like these may change clinical thinking so that waist circumference control will be prioritized to the same extend as control of blood pressure, lipids, and glucose levels in T2D prevention. **(*J Clin Endocrinol Metab* 101: 3212–3221, 2016)**

The metabolic syndrome (MetS) is a complex of interrelated cardiovascular risk factors, is associated with increased risk of type 2 diabetes (T2D) and cardiovascular disease, and is according to the Joint Scientific Statement: "Harmonizing the metabolic syndrome" defined as the presence of 3 or more of the following 5 components: large waist circumference, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol level, hypertension, and hyperglycemia (1). Prospective studies of the association between the MetS and T2D risk have consistently

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Abbreviations: BP, blood pressure; CI, confidence interval; Dkr, Danish kroner; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HR, hazard ratio; MetS, metabolic syndrome; SNP, Single Nucleotide Polymorphism; T2D, type 2 diabetes.

shown an increased T2D risk in a range of populations (2). However, the clinical utility of the MetS has been questioned because all risk factors should be individually and aggressively treated regardless of whether the MetS is present or not. Therefore, knowledge about potential causal relationships between the individual MetS components and T2D may be more useful than the actual MetS diagnosis, that is, if the aim is to prevent T2D.

Observational associations between the individual MetS components and T2D have been demonstrated previously (3, 4) but as observational association studies often suffer from confounding and reverse causation, such studies cannot confer causality. To circumvent confounding and reverse causation, a Mendelian randomization approach can be used (5). This approach uses the fact that genetic variants that alter levels of the 5 MetS components are randomly assorted during gamete formation, like patients are randomized to placebo or active treatment in intervention trials; and, if genotypes associated with levels of each of the 5 components are also associated with higher T2D risk compared with genotypes associated with normal levels, it follows that this likely is a causal association. To use this approach, we genotyped variants associated with each of the 5 MetS components and not previously associated with T2D. Furthermore, the selected genetic variants have no major effect on other known cardiovascular risk factors and, therefore, can be used to study the impact of longstanding changes in levels of each of the 5 components without known pleiotropic effects.

We tested the hypothesis that large waist circumference, hypertriglyceridemia, low HDL cholesterol (HDL-C) level, hypertension, and hyperglycemia are associated observationally and causally with T2D using a Mendelian randomization approach. First, the associations between the MetS components (X) and T2D (Y) risk were examined (Figure 1). Second, the associations between genetic variants (Z) and the MetS components (X) were examined. Third, the associations between genetic variants (Z) and T2D (Y) risk were examined to identify potentially causal associations.

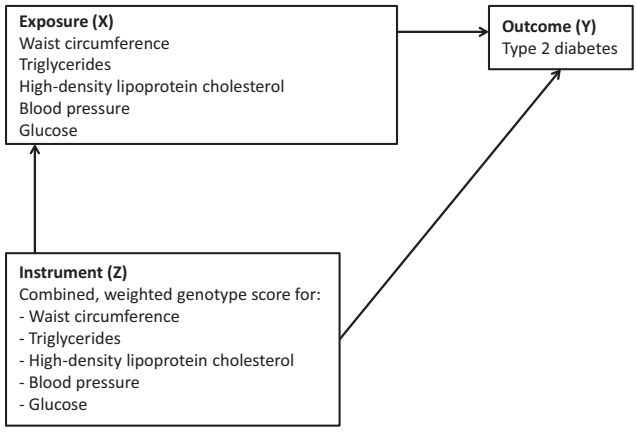

Figure 1. Mendelian randomization triangulation design with exposure (X), outcome (Y), and genetic instrument (Z). Study of association between 1) X and Y, 2) Z and X, and 3) Z and Y.

Materials and Methods

Study population

The Copenhagen General Population Study is a prospective study initiated in 2003 with on-going inclusion (6). Participants were selected based on the national Danish Civil Registration System to reflect the Danish population aged 20–100 years. Individuals with T2D at baseline were excluded from analyses. Median follow-up time was 6.0 (range, 0–10.9) years.

All participants were white and of Danish descent. Follow-up was 100% complete, that is, we did not lose track of even a single individual. The study was approved by Herlev and Gentofte Hospital, Copenhagen University Hospital, and Danish ethical committees (KF-100.2039/91 and H-KF-01-144/01), and was conducted according to the Declaration of Helsinki. Informed consent was obtained from participants.

Type 2 diabetes

Information on a diagnosis of T2D according to World Health Organization, International Classification of Diseases, eighth edition code 250 and 10th edition codes E11, E13, and E14 was collected from 1977 through November 2014 by reviewing all hospital admissions and diagnoses entered in the national Danish Patient Registry and all causes of death in the national Danish Causes of Death Registry. A diagnosis of T2D required a plasma glucose level according to changing diagnostic criteria over time (7–9). Some participants of the Copenhagen General Population Study reported taking antidiabetic medication when asked about this in the questionnaire from the Copenhagen General Population Study but did not have a diagnosis in the national Danish Patient Registry. However, to ensure validity of the diagnosis, only T2D patients with a diagnosis in the national Danish Patient Registry were included as diabetes medication may be prescribed for other indications.

Components of the MetS

Trained technicians measured waist circumference from the midpoint between the iliac crest and the lower rib while standing. Blood pressure (BP) was measured using an automatic Digital Blood Pressure Monitor (Kivex). Measured BP was adjusted for use of antihypertensive medication by adding a constant value of 10 mm Hg for systolic BP and 5 mm Hg for diastolic BP (10). Plasma levels of nonfasting glucose, triglycerides, and HDL-C were measured using standard hospital assays. Measured glucose level was adjusted for use of antidiabetic medication by adding a constant value of 1 mmol/L (11). MetS was defined according to the Joint Scientific Statement: "Harmonizing the metabolic syndrome" as fulfilling 3 of the following 5 criteria: 1) waist circumference more than or equal to 88 cm for women and more than or equal to 102 cm for men, 2) triglyceride levels more than or equal to 1.7 mmol/L, 3) HDL-C level less than 1.3 mmol/L in women and less than 1.0 mmol/L in men, 4) systolic BP more than or equal to 130 mm Hg and/or diastolic BP more

than or equal to 85 mm Hg, and 5) glucose level more than or equal to 5.7 mmol/L (1).

Covariates

Physical activity was categorized from self-reported data as low, moderate, and high; smoking was ever vs never smoker and number of pack years was recorded in smokers; alcohol consumption was U/wk (1 U = 12 g alcohol); education was schooling for less than 10, 10–12, and more than or equal to 13 years; income was less than 100 000 Danish kroners (Dkr), 1–400 000 Dkr, 4–600 000 Dkr, and more than 600 000 Dkr. Information on antihypertensive and antidiabetic medication and menopause status was self-reported.

Genetic variants

We genotyped variants in *FTO* (rs9939609), *MC4R* (rs17782313), *TMEM18* (rs6548238), *BDNF* (rs10767664), and *GNPDA2* (rs10938397) influencing waist circumference and body mass index (BMI) (12, 13) in 96 813 participants; *TRIB1* (rs2954029), *APOA5* (rs651821), *LPL* (rs328), and *LPL* (rs118204057) influencing triglyceride levels (14–16) in 97 199 participants; *ABCA1* N1800H (rs146292819), *CETP* (rs708272), and *CETP* (rs1800775) influencing HDL-C levels (17) in 93 097 participants; *ATP2B1* (rs2681472), *CYP17A1* (rs11191548), *ADRB2* (rs1042713), *ADRB2* (rs1042714), *GNAS* (rs6015450), and *FGF5* (rs1458038) influencing BP (18) in 65 154 participants; and *G6PC2* (rs560887), *GCK* (rs4607517), *DGKB* (rs2191349), *ADRA2A* (rs10885122), and *ADCY5* (rs11708067) influencing glucose levels (19–22) in 105 461 participants. We excluded genetic variants previously associated with T2D. Genetic variants known to be associated with both glucose level and one of the other components of the MetS were also excluded in order to be able to study the impact of longstanding changes in levels of each of the 5 components without known pleiotropic effects (Supplemental Table 1). Genotyping was by the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems. Inc). Genotypes were verified by sequencing 60 randomly selected samples (20 wild types, 20 heterozygotes, and 20 homozygotes) of each variant by Sanger sequencing on an Applied Biosystems 3730 system. Reproducibility were 100% and call rates for genotypes were more than 99% for all assays.

For each individual, a combined, weighted genotype score was calculated by summation of scores across the genetic variants for each component. The combined, weighted genotype scores were for each component of the MetS divided into 6 groups of appropriate sizes to ensure sufficient statistical power and with a reasonably large reference group representing the background population, and to also ensure linearity of the continuous traits across groups.

Statistical analyses

All analyses were performed using Stata SE version 13.1 (StataCorp). Two-sided $P < .05$ was considered significant. For genotypes, a deviation from Hardy-Weinberg equilibrium was tested using a Pearson χ^2 test. For trend tests, using Cuzick nonparametric extension of a Wilcoxon rank sum test, groups of subjects classified by each of the 5 MetS components or combined, weighted genotype scores representing these components were ranked according to changing levels and coded as 0, 1, 2, 3, 4, and 5. First, to test whether each of the 5 MetS components was associated with T2D risk, Kaplan-Meier curves were used to estimate cumulative incidence, and Cox regression models with age as time scale and left truncation (delayed entry at examination) were used to estimate hazard ratios (HRs) for T2D. Individuals were categorized according to the guideline the Joint Scientific Statement: "Harmonizing the metabolic syndrome" with: waist circumference less than 88 (women)/less than 102 cm (men), triglyceride levels less than 1.7 mmol/L, HDL-C level more than 1.3 (women)/more than 1.0 mmol/L (men), systolic BP less than 130 mm Hg, diastolic BP less than 85 mm Hg, and glucose level less than 5.7 mmol/L were used as reference groups for each of the 5 MetS components, respectively (1). Adjustment was for age (as time scale), gender, physical activity, pack-years in smokers, alcohol consumption, education, income, waist circumference, triglyceride levels, HDL-C level, systolic and diastolic BP, and glucose level with the MetS component studied excluded from the adjustment. For analyses of HDL-C levels on T2D risk, triglycerides were excluded from the adjustment, and for analyses of triglyceride levels on T2D risk, HDL-C was excluded from the adjustment, because these 2 parameters are highly correlated. Second, to test whether genotypes were associated with levels of the 5 MetS components one-way ANOVA was used to compare levels of the 5 components as a function of the combined, weighted genotype scores. Third, to test whether genotypes were associated with T2D risk, Cox regression analysis was used to estimate T2D risk as a function of the combined, weighted genotype scores associated with each of the 5 components of the MetS. Fourth, instrumental variable analysis was performed using a 2-stage least-squares regression approach with the Stata package *ivreg2* to assess potential causal relationships between altered levels of the exposure covariates, that is, the 5 MetS components and T2D risk using the combined, weighted genotype scores as instruments for the exposure covariates. In brief, 1) the first stage was a conventional linear regression assessing the association between the genetic instruments and levels of the exposure covariates. Strength of the combined, weighted genotype scores as instruments was evaluated by *F*-statistics from the first-stage regression, where $F > 10$ indicates sufficient statistical strength of the combined, weighted genotype score as a proxy of the exposure covariates, and R^2 in percent is a measure of percent contribution of genotype to the variation in levels of the exposure covariates (5). 2) The predicted value of the exposure covariates from the model was saved and used as an independent variable in the second stage, where the dependent variable was T2D. Causal odds ratios were estimated using the multiplicative generalized method of moments estimator implemented in the user-written Stata command *ivpois*. Fifth, as the main finding of this study is a causal relationship between waist circumference and T2D, the association between potentially confounding factors and waist circumference, T2D, and the combined, weighted waist circumference genotype score was examined.

Results

Of 95 756 participants, 823 women and 1000 men developed T2D during up to 10.9 years of follow-up. Baseline characteristics of participants from the Copenhagen General Population Study by T2D status are shown in Table 1.

Table 1. Baseline Characteristics of Participants From the Copenhagen General Population Study

	T2D		
	No	**Yes**	***P* Comparison**
Number of individuals	93 933	1823	
Age, y	57 (47–67)	65 (57–72)	<.001
Waist circumference, cm			
Women	83 (76–91)	97 (87–108)	<.001
Men	95 (89–103)	104 (97–113)	<.001
Triglycerides, mmol/L	1.37 (0.95–2.02)	2.15 (1.51–3.13)	<.001
HDL-C, mmol/L			
Women	1.75 (1.43–2.11)	1.44 (1.16–1.82)	<.001
Men	1.36 (1.10–1.68)	1.20 (0.97–1.51)	<.001
Systolic BP, mm Hg	138 (124–153)	150 (140–167)	<.001
Diastolic BP, mm Hg	80 (73–89)	86 (80–95)	<.001
Glucose, mmol/L	5.1 (4.7–5.6)	6.3 (5.3–7.7)	<.001
Alcohol, U/wk	7 (3–14)	7 (2–16)	.001
Smoking, ever	52%	65%	<.001
Number of pack years, in smokers	15 (5–30)	26 (11–42)	<.001
Activity			<.001
Low	33%	48%	
Moderate	64%	50%	
High	2.2%	1.4%	
Education			<.001
0–10 y	24%	48%	
≥10–12 y	58%	43%	
≥13 y	18%	8.7%	
Income			<.001
<100 000 Dkr/y	1.5%	3%	
100 000–400 000 Dkr/y	39%	61%	
400 000–600 000 Dkr/y	37%	29%	
>600 000 Dkr/y	23%	7%	
Menopause, women only	63%	88%	<.001

Continuous values are summarized as median and interquartile range. Categorical values are summarized in percent. *P* for comparison using Student's *t* test. Alcohol 1 U = 12 g; Dkr, 100 000 Dkr ~ 17 000 United States dollars/£10 500.

Age, waist circumference, triglyceride level, BP, glucose level, alcohol consumption, and pack-years in smokers were higher and HDL-C level was lower for individuals developing T2D during follow-up. Also, the percentage of individuals with a low physical activity level, a short education, and a low income were higher among individuals developing T2D. All genotypes were in Hardy-Weinberg equilibrium.

MetS components and observational T2D risk

Cumulative incidence of T2D was higher among individuals with large waist circumference, high systolic and diastolic BP, high triglyceride and glucose levels, and low HDL-C level compared with individuals with normal values of the 5 MetS components (Supplemental Figure 1). T2D risk increased stepwise with increasing levels of waist circumference, triglyceride level, systolic and diastolic BP, glucose level, and decreasing HDL-C level (Figure 2). The multifactorially adjusted HR was 5.6 (95% confidence interval [CI], 4.8–6.5) for individuals with waist circumference more than or equal to 108 (women)/more than or equal to 122 (men) cm vs less than 88(women)/less than 102 (men) cm, 2.1 (1.7–2.5) for individuals with triglyceride level more than or equal to 4.7 mmol/L vs less than 1.7 mmol/L, 2.1 (1.8–2.6) for individuals with HDL-C level less than 1.0 (women)/less than 0.7 (men) mmol/L vs more than or equal to 1.3 (women)/more than or equal to 1.0 (men) mmol/L, 1.6 (1.4–1.9) for individuals with systolic BP more than or equal to 150 mm Hg vs less than 130 mm Hg, 1.4 (1.2–1.7) for individuals with diastolic BP more than or equal to 105 mm Hg vs less than 85 mm Hg, and 18 (16–21) for individuals with glucose level more than or equal to 8.7 mmol/L vs less than 5.7 mmol/L. T2D risk increased stepwise with increasing number of MetS components (Figure 2). The multifactorially adjusted HR was 79 (50–127) for individuals with 5 components vs zero components.

Combined, weighted genotype scores, and the 5 MetS components

F values for the 5 genetic instruments representing the 5 MetS components were 56 for waist circumference, 211 for triglycerides, 478 for HDL, 17.6 for systolic BP, 14.1 for diastolic BP, and 56 for glucose. Genetic variants located on the same chromosome; the 2 *CETP* Single Nucleotide Polymorphisms (SNPs) (linkage r^2 = 81), the 2

Metabolic syndrome components (median and IQ range)		No. of participants	No. of events	Hazard ratio (95% CI)	P trend
Waist size, cm					<0.001
<88(w)/102(m)	84 (77-91)	64,667	537	1.0	
88-97(w)/102-111(m)	96 (91-105)	19,959	556	2.1 (1.8-2.3)	
98-107(w)/112-121(m)	104 (100-113)	7,914	408	3.2 (2.8-3.7)	
>=108(w)/122(m)	116 (111-124)	3,216	322	5.6 (4.8-6.5)	
Triglycerides, mmol/L					<0.001
<1.7	1.1 (0.8-1.3)	61,419	609	1.0	
1.7-2.69	2.1 (1.9-2.3)	22,069	591	1.5 (1.4-1.7)	
2.7-3.69	3.1 (2.9-3.3)	7,508	307	1.9 (1.6-2.1)	
3.7-4.69	4.1 (3.9-4.3)	2,695	146	2.1 (1.8-2.6)	
>=4.7	5.6 (5.1-6.6)	2,065	170	2.1 (1.7-2.5)	
HDL cholesterol, mmol/L					<0.001
>=1.3(w)/1.0(m)	1.7 (1.4-2.0)	80,192	1,183	1.0	
1.29-1.0(w)/0.99-0.7(m)	1.0 (0.9-1.2)	12,905	481	1.8 (1.6-2.0)	
<1.0(w)/0.7(m)	0.8 (0.7-0.9)	2,659	159	2.1 (1.8-2.6)	
Systolic blood pressure, mmHg					<0.001
<130	120 (110-124)	32,070	215	1.0	
130-139	133 (130-136)	17,208	230	1.1 (0.9-1.4)	
141-149	142 (140-145)	15,689	337	1.3 (1.1-1.6)	
≥150	162 (155-173)	30,789	1,041	1.6 (1.4-1.9)	
Diastolic blood pressure, mmHg					<0.001
<85	75 (70-80)	58,131	727	1.0	
85-94	88 (85-90)	23,825	637	1.3 (1.2-1.5)	
95-104	98 (95-100)	10,060	303	1.1 (0.9-1.2)	
>=105	109 (105-113)	3,740	156	1.4 (1.2-1.7)	
Glucose, mmol/L					<0.001
<5.7	4.9 (4.6-5.2)	72,746	619	1.0	
5.7-6.6	6 (5.8-6.2)	15,903	456	2.5 (2.2-2.8)	
6.7-7.6	7 (6.8-7.3)	4,437	279	4.8 (4.1-5.5)	
7.7-8.6	8 (7.8-8.3)	1,478	148	7.5 (6.2-9.0)	
>=8.7	9.7 (9-11.2)	1,192	321	18 (16-21)	
Metabolic syndrome components					<0.001
0		17,104	19	1.0	
1		30,978	141	2.8 (1.7-4.7)	
2		24,777	367	7.6 (4.8-12.1)	
3		14,847	522	17 (11-27)	
4		6,539	517	37 (24-59)	
5		1,511	257	79 (50-127)	

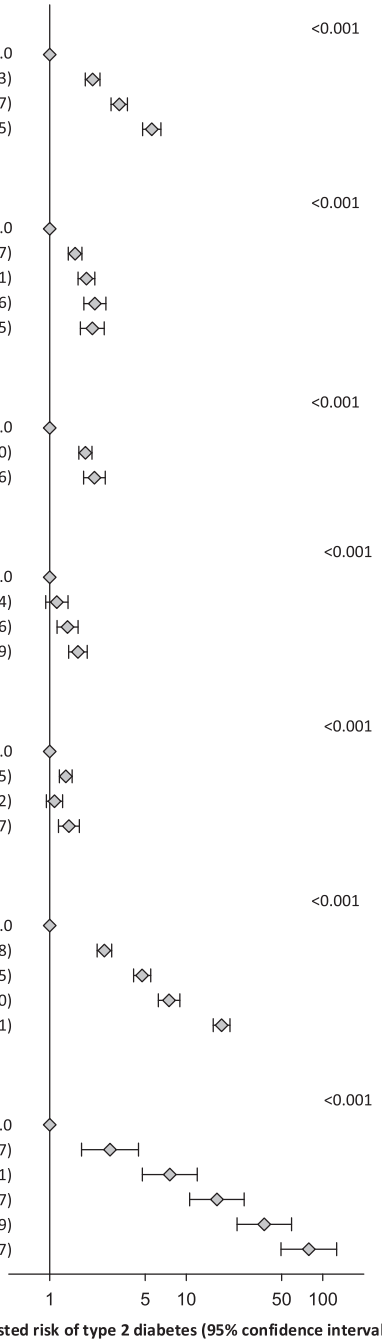

Figure 2. Risk of T2D in the Copenhagen General Population Study (n = 95 756) as a function of each of the 5 components of the MetS (waist circumference, triglyceride level, HDL-C level, systolic and diastolic BP level, and glucose level). HRs were adjusted for age, gender, physical activity, pack years in smokers, alcohol consumption, education, income, waist circumference, triglyceride level, HDL-C level, systolic and diastolic BP, and glucose level. The covariate being studied was excluded from the adjustment. For analyses of HDL-C levels on T2D risk, triglycerides were excluded from the adjustment, and for analyses of triglyceride levels on T2D risk, HDL-C was excluded from the adjustment, because these 2 parameters are highly correlated. w, women; m, men.

ADRB2 SNPs ($r^2 = 48$), and the 2 *LPL* SNPs ($r^2 = 0$) were only in modest linkage equilibrium. For the combined, weighted genotype scores, waist circumference score 6 vs score 1 was associated with a 2.4% larger waist circumference; triglyceride score 6 vs score 1 was associated with a 33% higher triglyceride level; HDL-C score 6 vs score 1 was associated with a 34% lower HDL-C level; BP score 6 vs score 1 was associated with a 1.9% higher systolic and a 1.6% higher diastolic BP; and glucose score 6 vs score 1 was associated with a 4.1% higher glucose level (Figure 3,

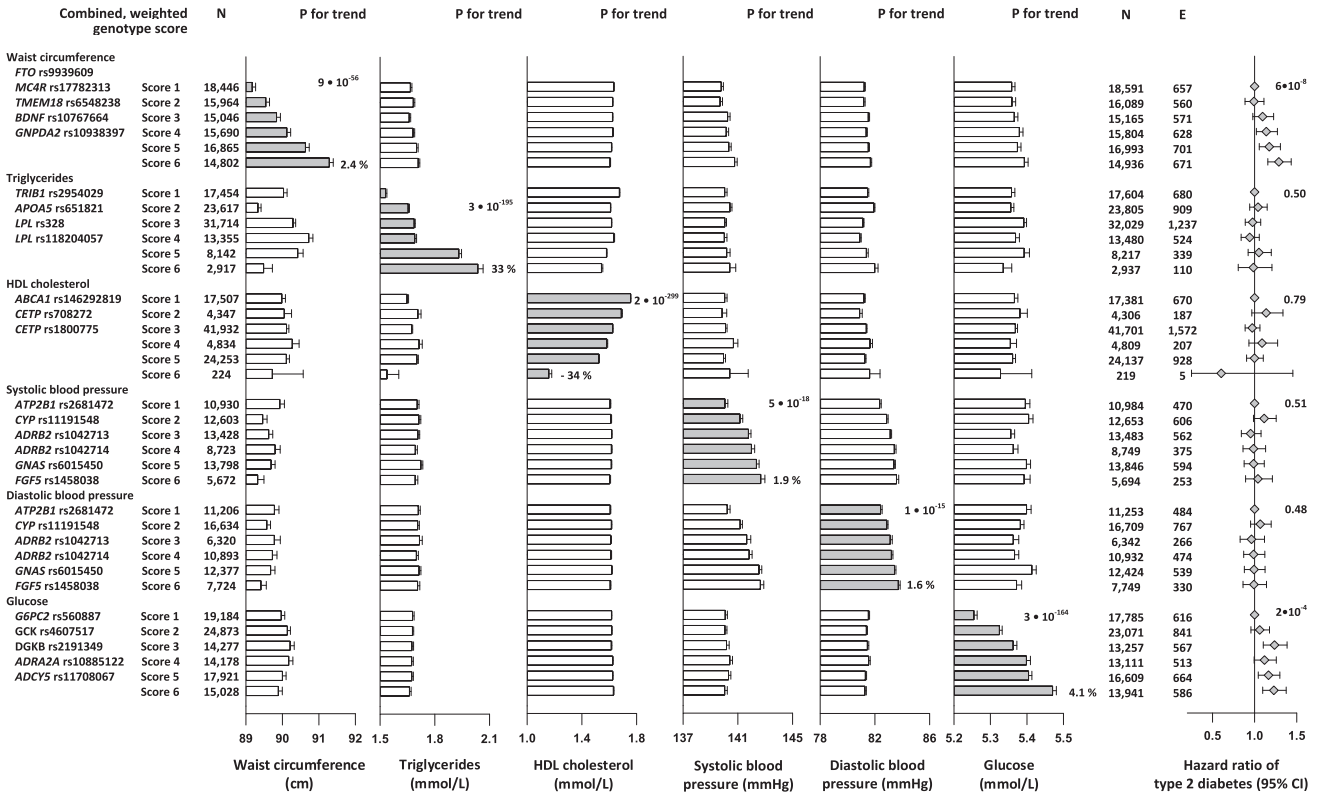

Figure 3. Left, The 5 MetS components waist circumference, triglyceride level, HDL-C level, systolic and diastolic BP level, and glucose level as a function of the combined, weighted genotype scores selected as genetic instruments representing each of the 5 components selectively. Right, Risk of T2D as a function of the combined, weighted genotype scores associated selectively with each of the 5 components of the MetS. Based on the Copenhagen General Population Study.

bar plots on left panels). Taken together, for the genetic instruments for the 5 MetS components, the largest statistical power was available for HDL-C and triglycerides, the second largest statistical power was for glucose and waist circumference, whereas systolic and diastolic BP had the least statistical power.

Combined, weighted genotype scores, and T2D risk

For the combined, weighted genotype scores, higher waist circumference for score 6 vs score 1 was associated with a HR of 1.29 (95% CI, 1.16–1.44) for T2D; higher triglyceride for score 6 vs score 1 was associated with a HR of 0.98 (0.81–1.21) for T2D; lower HDL-C for score 6 vs score 1 was associated with a HR of 0.60 (0.25–1.45) for T2D; higher systolic BP for score 6 vs score 1 was associated with a HR of 1.04 (0.89–1.21) for T2D; higher diastolic BP for score 6 vs score 1 was associated with a HR of 0.99 (0.86–1.14) for T2D; and higher glucose for score 6 vs score 1 was associated with a HR of 1.23 (1.10–1.38) for T2D (Figure 3, forest plot on right panel).

Observational and causal T2D risk

A 1-cm larger waist circumference was associated with an observational 5% (95% CI, 4%–5%) higher and with a causal genetic 5% (1%–10%) higher risk of T2D (Figure 4). In contrast, although a 1-U higher level of triglycerides and BP and a 1-U lower level of HDL-C were associated with higher observational risk of T2D, the corresponding causal genetic risks were not. For systolic and diastolic BP the genetic risk was slightly lower and the observational and genetic risk estimates for T2D are in opposite directions. As expected, a 1 mmol/L higher glucose level was associated with an observational 32% (30%–34%) and a causal, genetic 82% (21%–173%) higher risk of T2D.

Associations with confounding factors

For a potential confounder to mediate part of an association, the confounder in question needs to be associated both with the exposure and the outcome. We therefore examined both the association between potentially confounding factors and waist circumference, T2D, and our genetic waist circumference instrument. In the observational design, several of the potential confounders were associated with both waist circumference and T2D (Figure 5, left and middle panels). In contrast, the combined, weighted waist circumference genotype score was not robustly associated with the potential confounders (Figure 5, right panel). This suggest that several factors likely

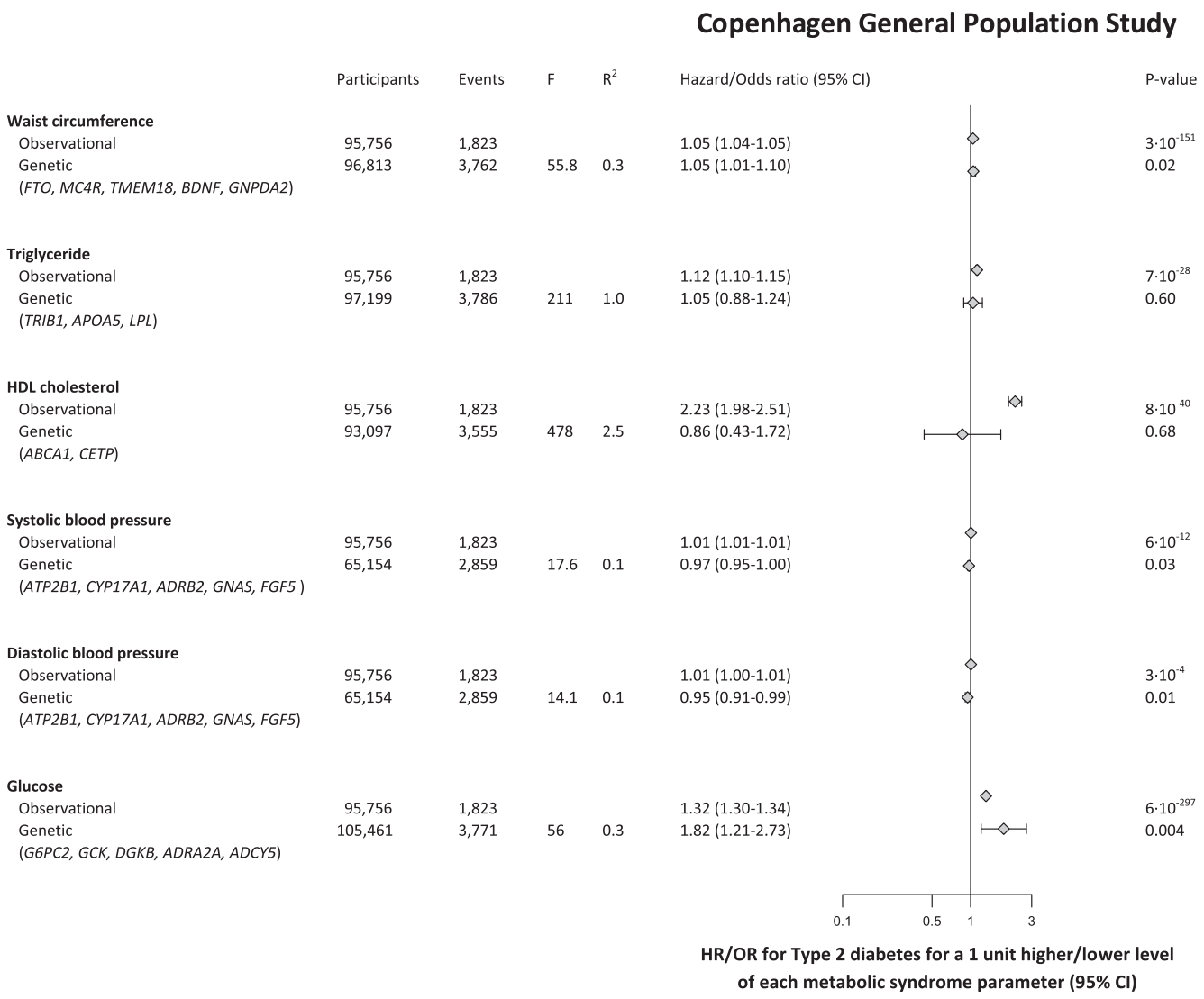

Figure 4. Observational and causal genetic risk of T2D for the 5 components of the MetS. The causal, genetic risk is compared with the observational prospective risk of T2D for a 1-U change in each of the 5 components and is given as an odds ratio (OR) with a 95% CI.

could confound the observational association between waist circumference and T2D, but it is unlikely that the same factors should confound the genetic instrumental variable analysis assessing the causal association between waist circumference and T2D, because these factors were not associated with genotype.

Discussion

The main findings of this study of a general population are that for every 1-cm larger waist circumference both observational and causal, genetic risk of T2D increased by 5%, and for every 1 mmol/L higher glucose level observational and causal, genetic risk of T2D increased by 32% and 82%, respectively. The finding of a causal association between glucose level and T2D was expected because a diagnosis of T2D is defined by a high glucose level. The finding of a causal association between waist circumference and T2D is compatible with a large waist circumference per se being causally related to the development of T2D and is a novel observation.

Our results are comparable with previous Mendelian randomization studies showing no causal association between high triglyceride levels and T2D (23–25), no causal association between low HDL-C levels and T2D (26), and a causal association between BMI and T2D where a 1-kg/m^2 genetically elevated BMI increased the odds of T2D by 27% (18%–36%) (27); the latter is compatible with previous results in our own study cohorts (28, 29). To our knowledge, no Mendelian randomization study of BP and T2D has been performed previously but observational studies have found an association between BP and T2D (30). In the present study, we find an observational higher risk of T2D and a genetically lower risk of T2D associated

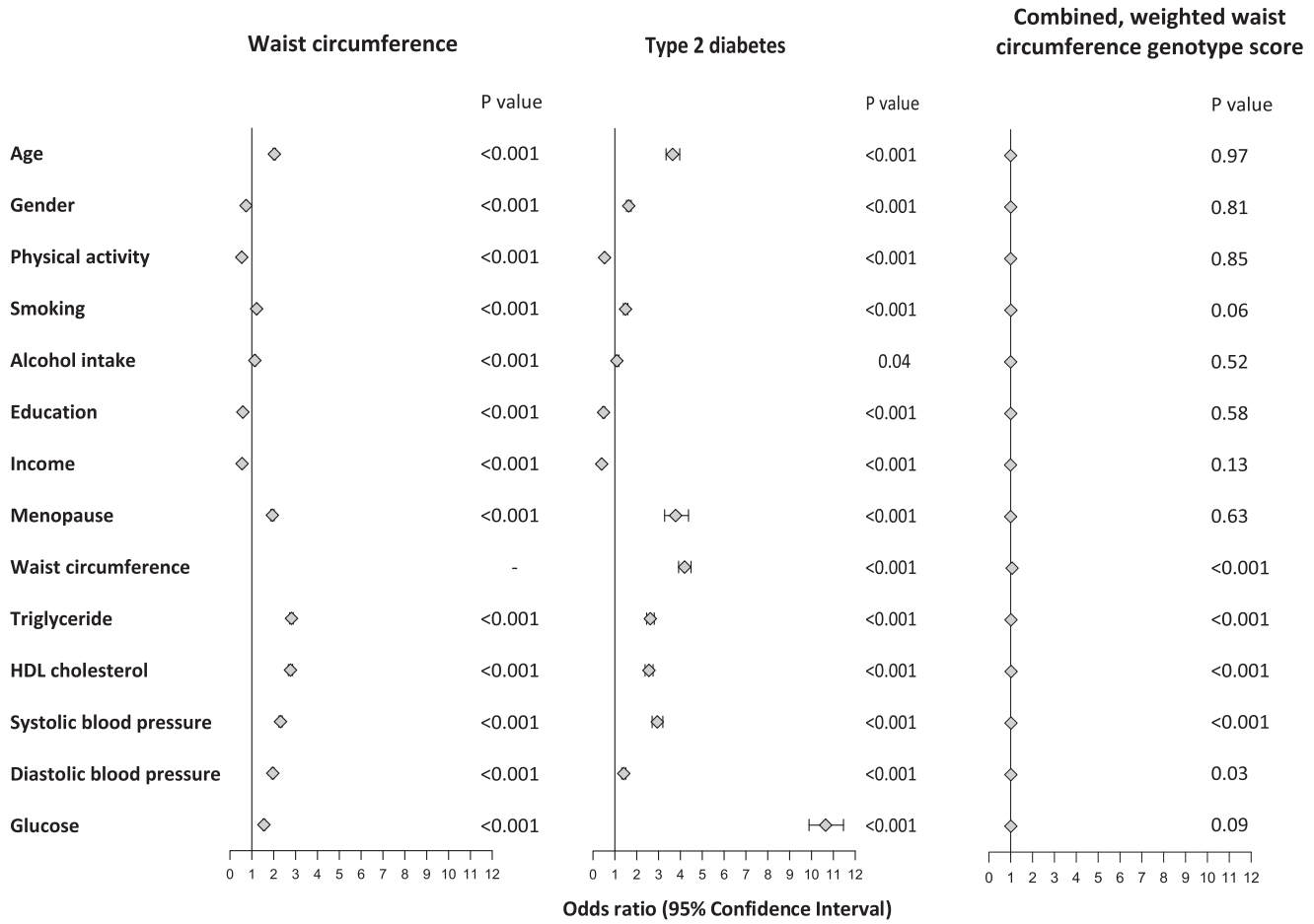

Figure 5. Association between potentially confounding factors (age, gender, physical activity, smoking status, alcohol intake, education status, income, menopause status, triglyceride level, HDL-C level, systolic BP, diastolic BP, and glucose level) and waist circumference, T2D, and combined, weighted waist circumference genotype score. Based on the Copenhagen General Population Study.

with higher BP. Thus, the observational and the genetic risk of T2D for systolic and diastolic BP are oppositely directed and we therefore cannot infer causality based on these results. Importantly, this is the first study to examine all 5 components simultaneously within the same study population, thus allowing head-to-head comparison with no other differences than those between the 5 components of the MetS.

Waist circumference has been shown to be the best anthropometric correlate of the amount of visceral adipose tissue with an approximately linear correlation (31). Mechanistically, it seems plausible that a large waist circumference can lead to T2D because increased visceral fat is associated with a shift in the normal balance of the adipokines resulting in an proinflammatory state (32). Activation of inflammatory pathways in hepatocytes can cause both local as well as systemic insulin resistance due to the drainage of inflammatory substances directly into the portal circulation (33, 34). Systemic inflammation has been proposed to have an important role in the pathogenesis of obesity-related insulin resistance (35); however, to believe that inflammation is the only factor responsible for the link between obesity and insulin resistance is clearly simplistic. Multiple endocrine and neural pathways are simultaneously disturbed leading to changed activity in various metabolic tissues including fat, liver, and muscle, in addition to the immune systems (36).

A metaanalysis of 15 studies comparing the strength of association with future T2D risk among anthropometric obesity indicators found waist circumference to be the best predictor of T2D with a pooled relative risk of 1.63 (1.49–1.79) for a 1-SD increase (37). Using a Mendelian randomization approach free from reverse causation and seemingly unconfounded, comparable with a randomized clinical intervention trial, we here show that both an observational and a genetic larger waist circumference were associated with increased T2D risk, suggesting that waist circumference is causally related to the development of T2D. An early intervention reducing waist circumference in women with a waist circumference more than or equal to 88 cm and in men with a waist circumference more than or equal to 102 cm would presumably reduce the incidence of T2D regardless of whether the MetS is present or not. The aim of our study was to investigate the individual

causal contribution of each MetS component to risk of T2D. In contrast to the growing success in the identification of genetic variants associated with the individual MetS components, little progress has been made in the identification of genetic variants underlying the syndromic clustering of MetS components (38, 39). However, investigating the risk of T2D associated with MetS as a whole using for example the 42 loci for the MetS at $P < 5 \times 10^{-8}$ reported by the genetic variants NHGRI-EBI Catalog as an instrument variable for the MetS would be an interesting future project.

Potential limitations of the study include the fact that we use nonfasting glucose levels, although fasting glucose levels are needed for the definition of the MetS. However, several studies have shown that postchallenge and postprandial glucose values correlate better with glycated hemoglobin levels than do fasting/preprandial glucose values (40–42). We may categorize more individuals with the MetS when using nonfasting values than if fasting values had been used; however, the study still has the ability to reflect a true relative association, because nonfasting glucose levels were used for all individuals, ie, both individuals not developing T2D and individuals developing T2D. Also, most of a day is spent in a nonfasting state and nonfasting values may better reflect the true risk. Another potential limitation of the study include the fact that only in- and outpatients hospital contacts with T2D were included in this study, excluding T2D cases treated solely by general practitioners. Further, misclassification may have occurred because some individuals reported taking antidiabetic medication, but did not have a diagnosis in the national Danish Patient Registry. Also, in general a large percentage of T2D cases are presumably undiagnosed as undiagnosed cases previously have been estimated to represent 46% of all T2D cases (43). Other potential limitations of the Mendelian randomization design include pleitrophy, selection bias, and population stratification. However, by using multiple genetic polymorphisms acting independently and via different pathways to change levels of the MetS components in all available individuals from an ethnically homogenous white population, these potential limitations are likely to have been avoided or minimized. Strengths of the study include the size of the study population and the validity of the included T2D cases.

In conclusion plasma glucose level was causally associated with diabetes as expected. Plasma triglyceride and HDL-C levels and BP were not causally associated with risk of T2D. However, for every 1-cm larger waist circumference, both observational and causal genetic risk of T2D was increased by 5%.

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