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Single nucleotide polymorphisms (SNPs) involved in insulin resistance, weight regulation, lipid metabolism and inflammation in relation to metabolic syndrome: an epidemiological study

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Abstract

Background: Mechanisms involved in metabolic syndrome (MetS) development include insulin resistance, weight regulation, inflammation and lipid metabolism. Aim of this study is to investigate the association of single nucleotide polymorphisms (SNPs) involved in these mechanisms with MetS.

Methods: In a random sample of the EPIC-NL study (n = 1886), 38 SNPs associated with waist circumference, insulin resistance, triglycerides, HDL cholesterol and inflammation in genome wide association studies (GWAS) were selected from the 50K IBC array and one additional SNP was measured with KASPar chemistry. The five groups of SNPs, each belonging to one of the metabolic endpoints mentioned above, were associated with MetS and MetS-score using Goeman’s global test. For groups of SNPs significantly associated with the presence of MetS or MetS-score, further analyses were conducted.

Results: The group of waist circumference SNPs was associated with waist circumference (P=0.03) and presence of MetS (P=0.03). Furthermore, the group of SNPs related to insulin resistance was associated with MetS score (P<0.01), HDL cholesterol (P<0.01), triglycerides (P<0.01) and HbA1C (P=0.04). Subsequent analyses showed that MC4R rs17782312, involved in weight regulation, and IRS1 rs2943634, related to insulin resistance were associated with MetS (OR 1.16, 95%CI 1.02-1.32 and OR 0.88, 95% CI 0.79; 0.97, respectively). The groups of inflammation and lipid SNPs were neither associated with presence of MetS nor with MetS score.

Conclusions: In this study we found support for the hypothesis that weight regulation and insulin metabolism are involved in MetS development. MC4R rs17782312 and IRS1 rs2943634 may explain part of the genetic variation in MetS.

Keywords: Metabolic syndrome, Genetics, MC4R, IRS1

Background

The metabolic syndrome (MetS) is a common multi-component condition consisting of abdominal obesity, dyslipidaemia, hypertension and hyperglycaemia. It is associated with an increased risk of CVD (cardiovascular diseases) and T2D(type 2 diabetes) [1]. A central question in understanding MetS is why its underlying traits cluster together.

Several mechanisms, including insulin resistance [1], abdominal obesity [1], and inflammation [2,3] have been proposed to underlie the clustering of MetS features. However, the etiology of MetS has not been unravelled completely yet. Genetic association studies may help to better understand MetS etiology.

A systematic review of genetic association studies on MetS showed that until now most SNPs for which an association with MetS has been found were involved in
lipid metabolism [4]. In a recent genome-wide association study (GWAS) even all single nucleotide polymorphisms (SNPs) associated with MetS were involved in lipid metabolism [5]. If effect sizes of SNPs involved in weight regulation and insulin resistance, pathways for which strong pathophysiological evidence exists [1], are small, these SNPs would not have been detected in GWAS, which have a low power due to adjustment for the large number of associations tested.

The SNPs for which an association with MetS has been established explain only a small part of the genetic variation in MetS [4-6]. Therefore, many more genetic variants remain to be discovered. SNPs associated with insulin resistance, abdominal obesity, inflammation and lipid levels in GWAS are likely candidates for an association with MetS itself. For several of these SNPs, however, an association with MetS has not been investigated with a candidate gene approach.

A common feature of genetic association studies is that the power to detect associations is low, because of small effect sizes and the small alpha caused by adjustment for multiple testing. A way to account for this problem is to increase the effect size and reduce the number of tests, by studying the joint effect of a group of SNPs [7]. To the best of our knowledge such an approach has not been undertaken in relation to MetS.

Therefore the aim of this study is to get more insight in the etiology of MetS, by studying associations between MetS and groups of SNPs that were found to be related to insulin resistance, abdominal obesity, inflammation or lipid levels in GWAS. For those groups of SNPs associated with MetS, we also study the association with the individual SNPs in this group.

Methods

EPIC-NL: Study design

In the EPIC-NL cohort the two Dutch contributions to the European Investigation into Cancer and Nutrition (EPIC) project are combined: the Prospect-EPIC and the MORGEN-EPIC (Monitoring Project on Risk Factors for Chronic Diseases) cohorts. Both cohorts were initiated in 1993. The study design of the combined cohort is described in detail elsewhere [8]. In brief, Prospect is a prospective cohort study among 17 357 women aged 70 who participated in a breast cancer screening program between 1993 and 1997. The MORGEN-project consists of 22 654 men and women aged 20–59 years recruited from three Dutch towns (Amsterdam, Doetinchem, and Maastricht). From 1993 to 1997, each year a new random sample of approximately 5000 individuals were examined.

Laboratory and genetic analyses were performed in a 6.5% random sample of the EPIC-NL study, in all incident T2D cases and in all incident CVD cases. In our study we only used the random sample. After exclusion of participants with missing blood samples (n = 157), missing values for haemoglobin A1c (HbA1C), waist circumference, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, diastolic blood pressure, triglycerides or C-reactive protein (CRP) (n = 128), or with missing SNP data (n = 433) the study population consisted of 1886 participants. All participants signed informed consent before study inclusion. Both studies complied with the Declaration of Helsinki. The Prospect-EPIC study was approved by the Institutional Review Board of the University Medical Center Utrecht and the MORGEN project was approved by the Medical Ethical Committee of TNO, The Netherlands.

Baseline measurements

At baseline, a physical examination was performed and non-fasting blood samples were drawn. During the physical examination, systolic and diastolic blood pressure measurements were performed twice in the supine position on the right arm using a BosoOscillomat (Bosch & Son, Jungingen, Germany) (Prospect) or on the left arm using a random zero sphygmomanometer (MORGEN). The mean of both measurements was taken. Waist circumference and height were measured to the nearest 0.5 cm. Body weight was measured with light indoor clothing without shoes on, to the nearest 100 gr.

Biomarker measurements

HbA1c was measured with a homogeneous assay with enzymatic endpoint in erythrocytes. Triglycerides were measured in EDTA plasma using enzymatic methods, whereas hsCRP was measured with a turbidimetric method [8]. MetS was defined according to an adapted version of the AHA/NHLBI MetS definition as having at least 3 of the following 5 MetS features [9]: abdominal obesity (waist circumference \( \geq 102 \) cm; \( \geq 88 \) cm); low HDL cholesterol (\( \leq 1.0; \leq 1.3 \) mmol/L); hypertriglycerideremia (\( \geq 1.7 \) mmol/L); hypertension (\( \geq 130/85 \) mm Hg or use of hypertensive medication); hyperglycemia (HbA1C \( \geq 5.7\% \) or glucose lowering medication) [10,11]. MetS-score was calculated by summing the number of MetS features present in each participant.

Genotyping

Genomic DNA was extracted in different batches using standard methods, such as salting out, QIAamp® Blood Kit (Qiagen Inc., Valencia, CA, USA). The participants were genotyped using a gene-centric 50K iSelect chip array, further referred to as IBC array [12]. The design and coverage of the IBC array compared to conventional genome-wide genotyping arrays has been described in detail elsewhere [12]. Additionally, the MC4R rs17782313SNP was genotyped in 853 women of the
random sample with the KASPar chemistry, an allele-
specific PCR SNP genotyping that uses FRET quencher
cassette oligos [13]. For this SNP genomic DNA was
extracted with an in-house developed extraction method
at Kbiosciences (Hoddesdon Herts, UK).

From the available SNPs, we selected those signifi-
cantly associated (P ≤ 1.0*10⁻⁵) with waist circumference,
inflammatory markers, triglycerides or HDL cholesterol
or homeostasis model assessment insulin resistance
(HOMA-IR) in published GWAS until 01-01-2011. As
only a few GWAS on HOMA-IR are conducted, we add-
tionally included SNPs both associated with a glucose
related traits in GWAS (P ≤ 1.0*10⁻⁷) and with HOMA-IR
(P ≤ 0.05). Highly correlated proxy SNPs were included in
case the original SNP from the GWAS was not available
on the IBC array (I² EU 1000 Genome Pilot 1 ≥ 0.80). If SNPs
were only found in one GWAS, without a replication sam-
ple, they were excluded. In total we included 39 SNPs: 2
SNPs associated with waist circumference, 5 SNPs asso-
ciated with insulin resistance, 6 SNPs associated with in-
flammation, 16 SNPs associated with triglycerides and 16
SNPs associated with HDL cholesterol (Table 1). Sixteen
SNPs which were associated in GWAS with waist circum-
ference, inflammatory biomarkers and lipid levels were
not on the IBC CVD array (Appendix I).

Statistics

Distributions of genotypes were tested for deviation
from hardy-weinberg equilibrium (HWE) by chi-square
analyses. Triglycerides and hsCRP were log-transformed
to improve normality. Participants on blood pressure
medication were excluded from the analyses on blood
pressure, participants on glucose lowering medication
from the analysis on HbA1C, and participants with acute
inflammation (hsCRP > 10 mmol/L) from the analyses
from hardy-weinberg equilibrium (HWE) by chi-square
Statistics

P-values for the association of all groups of SNPs with
MetS or MetS-score are shown in Table 3. The group of
SNPs known for their association with insulin resistance,
was borderline significantly associated with MetS
(P = 0.06) and statistical significantly associated with
MetS-score (P = 0.003). This group of SNPs was also sig-
nificantly associated with HbA1C, triglycerides and HDL
cholesterol (Table 3). The associations of this group of
SNPs with MetS-score and MetS features weakened
slightly after adjustment for HbA1C (Table 3). Of the
five insulin resistance SNPs included in the group IRS1
rs2943634 was the only SNP individually associated
with MetS or MetS-score (Table 4). These associations
remained after adjustment for HbA1C (data not shown).
IRS1 rs2943634 was also associated with HbA1C (per al-
le difference -0.034, 95% CI -0.070; 0.002), triglyceri-
des (per allele difference -0.051, 95% CI -0.085; -0.017)
and HDL cholesterol (per allele difference 0.029, 95% CI
0.008; 0.052).

The group of SNPs known for their association with
waist circumference, was statistical significantly associated
with MetS (P = 0.03) and tended to be associated with
Table 1 SNPs included in the analyses of random sample of EPIC-NL (n=1886)

| Gene               | SNP (literature) | SNP (dataset) | MAF (dataset) | ref | r² SNPs |
|--------------------|------------------|---------------|---------------|-----|--------|
| Insulin resistance |                  |               |               |     |        |
| PPARG              | rs1801282        | rs1801282     | G: 0.13       | [14] | -      |
| IRS1               | rs2943634        | rs2943634     | A: 0.35       | [15] | -      |
| GCKR               | rs780094         | rs780094      | T: 0.37       | [14] | -      |
| IGF1               | rs35767          | rs35767       | A: 0.16       | [14] | -      |
| GCK                | rs4607517        | rs1799884     | T: 0.18       | [14] | 1      |
| Abdominal obesity  |                  |               |               |     |        |
| FTO                | rs1421085        | rs1421085     | C: 0.40       | [16] | -      |
| MC4R²              | rs17782313       | rs17782313    | C: 0.25       | [16] | -      |
| Inflammation       |                  |               |               |     |        |
| IL6R               | rs4537545        | rs4537545     | T: 0.39       | [17] | -      |
| LEPR               | rs6700896        | rs1805096     | A: 0.38       | [17] | 0.89   |
| CRP                | rs7553007        | rs1341665     | A: 0.32       | [17] | 1      |
| ADIPOQ             | rs1648707        | rs182052      | A: 0.34       | [18] | 1      |
| IL18               | rs1834481        | rs5744256     | G: 0.26       | [19] | 1      |
| GCKR               | rs780094         | rs780094      | T: 0.37       | [20] | -      |
| Triglycerides      |                  |               |               |     |        |
| AFF1               | rs442177         | rs3775214     | G: 0.43       | [21] | 0.96   |
| APOB               | rs673548         | rs673548      | A: 0.22       | [22] | -      |
| APOB               | rs693            | rs693         | G: 0.50       | [23] | -      |
| APOAS-A4-C3-A1     | rs12286037       | rs12286037    | T: 0.08       | [24] | -      |
| APOAS              | rs6589566        | rs2075290     | C: 0.06       | [25] | 1      |
| FADS1              | rs174548         | rs174548      | G: 0.29       | [21] | -      |
| FADS1-2-3          | rs174547         | rs174577      | A: 0.35       | [26] | 1      |
| GALNT2             | rs4846914        | rs4846914     | G: 0.41       | [23] | -      |
| LPD                | rs328            | rs328         | G: 0.10       | [23] | -      |
| MLXIPL             | rs17145738       | rs17145750    | T: 0.16       | [24] | 0.86   |
| PLTP               | rs7679           | rs6073952     | A: 0.20       | [26] | 0.82   |
| TRIB1              | rs2954029        | rs2954029     | T: 0.47       | [26] | -      |
| CLIP2              | rs16996148       | rs16996148    | T: 0.10       | [24] | -      |
| GCKR               | rs780094         | rs780094      | T: 0.37       | [27] | -      |
| ANGPTL3-DOCK7      | rs1748195        | rs1748197     | A: 0.35       | [24] | 1      |
| ANGPTL3-DOCK7      | rs12130333       | rs12130333    | T: 0.24       | [23] | -      |
| HDL cholesterol    |                  |               |               |     |        |
| ABCA1              | rs1883025        | rs1883025     | T: 0.24       | [26] | -      |
| ABCA1              | rs3890182        | rs3890182     | A: 0.10       | [21] | -      |
| APOB               | rs11902417       | rs11902417    | A: 0.24       | [21] | -      |
| CETP               | rs1800775        | rs1800775     | A: 0.46       | [28] | -      |
| CETP               | rs3764261        | rs3764261     | A: 0.31       | [29] | -      |
| FADS1              | rs174548         | rs174548      | G: 0.29       | [21] | -      |
| FADS1-2-3          | rs174547         | rs174577      | A: 0.35       | [26] | -      |
| GALNT2             | rs4846914        | rs4846914     | G: 0.41       | [23] | -      |
| LCAT               | rs255052         | rs255052      | A: 0.17       | [24] | -      |
| LCAT               | rs12449157       | rs1109166     | C: 0.18       | [21] | 0.94   |
MetS-score (P=0.08). The association with MetS and the suggested association with MetS-score disappeared after adjustment for waist circumference (Table 3). Furthermore, no association was found with any individual MetS feature except for waist circumference (Table 3). Of the two abdominal obesity SNPs only *MC4R* rs17782313 was individually associated with MetS (Table 4). This association remained after adjustment for waist circumference. *MC4R* rs17782313 was not associated with any individual MetS feature, including waist circumference itself (data not shown). The groups of SNPs linked in GWAS with inflammation, triglycerides or HDL cholesterol were neither associated with MetS nor with MetS-score (all P-values ≥ 0.15). Therefore no further data-analyses were done for these groups of SNPs.

### Discussion

In this population based study of 1886 participants, we studied the relation between MetS and groups of SNPs associated in GWAS with waist circumference, insulin resistance, inflammation, triglycerides or HDL cholesterol. Only the group of waist circumference SNPs and the group of insulin resistance SNPs were associated with MetS or MetS-score.

**Waist circumference SNPs**

In our study the group of SNPs which were associated with waist circumference in GWAS (*MC4R* rs17782313 and *FTO* rs1421085) was associated with waist circumference, as well as with MetS. The association with MetS disappeared after adjustment for waist circumference, indicating that the association with MetS is driven by the association with waist circumference. Furthermore, the association with MetS seemed mainly to be driven by *MC4R* rs17782313. In the KORA study among 7888 adults, an association between *MC4R* rs2229616 (*r^2*=1 with rs17782313) and MetS was found [30], supporting our findings. Unfortunately, in our study, data on *MC4R* rs17782313 were available for women only. However, since in the KORA study [30] the association between *MC4R* rs2229616 and MetS was not dependent on sex, we expect that this did not influence our findings. In our study the association between *MC4R* rs17782313 and MetS remained after adjustment for waist circumference. Furthermore, we found no association between *MC4R* rs17782313 and any individual MetS feature, including waist circumference. This suggests that the association between *MC4R* rs17782313 and MetS, is at least in part, independent of body weight. In both human and animal studies *MC4R* rs17782313 had an effect on insulin...
Table 2 Characteristics of the random sample of EPIC-NL (n=1886)

|                          | Total (n=1886) | Men (n=465) | Women (n=1421) |
|--------------------------|----------------|-------------|----------------|
| Sex (% men)              | 24.6 (465)     |             |                |
| Age (yr)                 | 50.1 (11.7)    | 49.0 (11.4) | 52.2 (11.2)    |
| Waist circumference (cm) | 85.5 (11.6)    | 92.0 (11.4) | 82.9 (10.5)    |
| Abdominal obesity(%)\*   | 27.7 (522)     | 21.9 (10.2) | 29.6 (420)     |
| HbA1C (%)                | 5.46 (0.69)    | 5.27 (0.61) | 5.53 (0.71)    |
| Hyperglycemia\*          | 28.3 (534)     | 17.8 (83)   | 31.7 (451)     |
| Diabetic medication(%)   | 1.2 (22)       | 0.2 (1)     | 1.5 (21)       |
| HDL-cholesterol (mmol/L) | 1.27 (0.35)    | 1.14 (0.28) | 1.31 (0.36)    |
| Low HDL-cholesterol(%)\* | 47.8 (902)     | 29.2 (136)  | 53.9 (766)     |
| Triglyceride (mmol/L)\*  | 1.32 (1.72)    | 1.22        |                |
| Hypertension(%)\*        | 45.6 (860)     | 45.1 (641)  |                |
| Blood pressure lowering medication (%) | 10.7 (202) | 5.6 (26) | 12.4 (176) |
| High sensitive CRP (mmol/L) | 1.41 (1.20) | 1.49        |                |
| MetS-score (number of features) | 1.8 (1.4) | 1.7 (1.3) | 1.9 (1.4) |
| MetS prevalence(%)\*     | 30.3 (572)     | 25.2 (117)  | 32.0 (455)     |

Data are presented as means (standard deviation), median with inter-quartile range or % (n); HbA1C haemoglobin A1c, MetS metabolic syndrome, hsCRP high sensitive C-reactive protein.

\*Abdominal obesity, low HDL cholesterol, hypertension, hypertriglyceridemia and MetS are defined according to the criteria of AHA-NHLBI and MetS-score that remained after adjustment for HbA1c, indicating that this association was not driven by HbA1C. However, since HbA1C is not an optimal marker of insulin resistance ($r^2$ between HOMA-IR – HbA1C =0.50 [34]), we can not rule out that insulin resistance mediates this association. Out of the group of five insulin resistance SNPs, IRS1 rs2943634 was the only SNP significantly associated with MetS and MetS score. It was also associated with HbA1C, triglycerides and HDL cholesterol. Accordingly an IRS1 knock-out mouse model displayed a MetS like phenotype with insulin resistance, increased blood pressure, increased triglycerides, decreased HDL cholesterol and decreased LPL activity [35]. Furthermore, in human studies IRS1 rs2943634 has been associate with glucose related [15] and lipid traits [36]. In contrast, in a study among 1126 non-Hispanic whites, 898 non-Hispanic blacks and 906 Mexican Americans, IRS1 rs7578326 ($r^2$ with rs2943634=0.82) was not associated with MetS, neither in the overall population, nor in specific ethnic groups [37]. However, as the number of Caucasian participants and MetS prevalence were lower than in our study, the former study may have been underpowered in Caucasian. Besides IRS1 rs2943634 the group of insulin resistance SNPs consisted of PPARG rs1801282, GCKR rs780094, GCKRs1799884, and IGF1rs35767. In line with other studies, none of these SNPs were associated with MetS in our data [4,37,38]. This may be explained by the relatively weak effect on HOMA-IR of PPARG rs1801282, GCK rs1799884, and IGF1 rs35767 [14,15] or by pleiotropic effects of PPARG rs1801282 and GCK rs780094. The 12pro allele of PPARG rs1801282 has opposite effects on insulin resistance and BMI in Caucasian subjects [39,40], whereas GCKR rs780094 has opposite effects on insulin resistance and lipid levels [27]. These opposite effects may result in a zero association with MetS, as observed by for example Passaro et al. [40].

Lipid SNPs

We did not observe an association between groups of SNPs known for their association with triglycerides or HDL cholesterol and MetS. On the contrary, in a GWAS [5] and a systematic review of genetic association studies [4], the majority of SNPs associated with MetS was involved in lipid metabolism. The lack of an association with MetS may be a power issue, because the association between lipid SNPs and lipid levels was relatively weak in EPIC-NL. Subgroup analyses revealed that these weak associations were consistent for all lipid SNPs and could not be explained by medication use, sex or a difference between the MORGEN and Prospect study. Furthermore, it is unlikely that the non-fasting state of our samples gives an explanation, as in a GWAS, the association with lipid levels was independent of the fasting state for most SNPs [41].
Table 3 P-values for Goeman’s global test, testing the statistical significance of associations of waist circumference and insulin resistance SNPs with metabolic syndrome and related features

| Group of SNPs | MetS | MetS-score | WC (cm) | HbA1C (%) | Log (TG) (mmol/L) | HDL (mmol/L) | SBP (mm HG) | DBP (mm HG) | Log (CRP) (mmol/L) |
|--------------|------|------------|---------|-----------|------------------|------------|------------|-----------|------------------|
| n            | 1886 | 1886       | 1886    | 1864      | 1886             | 1886       | 1684      | 1684      | 1683             |
| WC³          | P=0.03 | P=0.08   | P=0.01  | P=0.73    | P=0.81           | P=0.36     | P=0.29    | P=0.11    | P=0.22           |
| Adj WC       | P=0.16 | P=0.80   | -       | P=0.47    | P=0.55           | P=0.09     | P=0.36    | P=0.34    | P=0.68           |
| IR           | P=0.06 | P=0.003  | P=0.45  | P=0.04    | P=0.0003         | P=0.0005   | P=0.07    | P=0.16    | P=0.16           |
| Adj HbA1C    | P=0.12 | P=0.01   | P=0.70  | -         | P=0.0005         | P=0.0008   | P=0.10    | P=0.22    | P=0.17           |

All analyses are adjusted for age, sex and cohort; Mets Metabolic syndrome, WC waist circumference, TG triglycerides, HDL HDL-cholesterol, HbA1C haemoglobin A1C, SBP systolic blood pressure, DBP diastolic blood pressure, Adj adjusted, IR insulin resistance.

1Subjects which are using glucose lowering medication are excluded.
2Subjects with CRP > 10 mmol/L are excluded.
3Subjects which are using blood pressure lowering medication are excluded.
4Data available in 853 women.

Inflammation SNPs

We found no significant association between a group of inflammation SNPs and MetS. Accordingly, in a study among 4286 British women, a CRP haplotype was not associated with the individual features of MetS [42]. Furthermore, Rafiq et al. could not detect an association between T2D, an endpoint of MetS and 8 SNPs known to alter circulating levels of inflammatory proteins, which were located in the IL-18, IL1RN, IL6R, MIF, PAI1 and CRP genes [43]. Overall, this evidence may suggest that genetic variants in inflammatory genes do not play a causal role in MetS development. However, for several reasons it cannot be ruled out that some SNPs in inflammatory pathways are causally related to MetS. First, for some inflammatory proteins SNPs-MetS associations have not been investigated yet. Second, as the global test gives a combined result for all SNPs, the global test may be not significant despite the presence of an association between one of the single SNPs and MetS.

Table 4 Individual SNPs associated with waist circumference or insulin resistance in GWAS in relation to MetS and MetS-score

|       | MetS | MetS-score |
|-------|------|------------|
| n     | 1886 | 1886       |
| WC    | 1.02(0.93; 1.12) | 0.05(−0.03; 0.14) |
| IRS1 rs2943634 | 1.04(0.91; 1.19) | 0.10(−0.02; 0.23) |
| PPAR1 rs1801082 | 0.88(0.79; 0.97) | −0.14(−0.23;−0.06) |
| GCKR rs780094 | 0.99(0.89; 1.09) | 0.05(−0.04; 0.13) |
| GCRF rs35767 | 1.03(0.91; 1.16) | 0.03(−0.08; 0.14) |
| GCK rs1798984 | 1.07(0.95; 1.20) | 0.08(−0.03; 0.22) |

Data are presented as PR per minor allele for MetS and as minor allele change for MetS Score; Mets Metabolic syndrome, WC waist circumference, IR insulin resistance.

In this study we have explored the biomarkers involved in MetS development, by studying SNPs related to these biomarkers. Advantage of this approach is that according to the principles of Mendelian randomization the associations we investigated are neither affected by reverse causality nor by socioeconomic and behavioural confounders [44]. Furthermore, as all participants were Caucasian, it is unlikely that our study results have been affected by population stratification. However, for some SNPs we measured, like IRS1 rs2943634 [45], allele frequencies are very heterogeneous among different populations. Therefore, our results warrant replication in other study populations. Sixteen SNPs which were associated in GWAS with MetS related traits were not on the IBC CVD array. Inclusion of the three waist circumference SNPs, which were not on the array, would probably have increased the possibility to find associations with several MetS features. As the global test of inflammation SNPs on hsCRP was already highly significant (P=7.3*10⁻⁶), inclusion of additional SNPs, which were absent on the IBC CVD array, would not have changed our results for the global test, but may have revealed additional individual SNPs. The total number of lipid SNPs in our study was relatively large and relatively few lipid SNPs were missing. Therefore we believe that inclusion of additional lipid SNPs would not have changed our results considerably on the group level. The IBC CVD array covered all insulin resistance SNPs discovered in GWAS. However, up till now for only three SNPs a genome wide association with HOMA-IR has been found and replicated. To increase power we also included those SNPs associated with glucose related traits in GWAS, which were also associated with HOMA-IR (P≤0.05). However, as the association between the group of insulin resistance SNPs and HbA1C was just significant, the power to detect associations with MetS and its features was still low.

In conclusion, we found that SNPs associated with waist circumference or insulin resistance in GWAS were
also associated with MetS. These results are in line with the hypotheses that weight regulation and insulin metabolism are causative factors for MetS.

Individual SNPs for which we found an association with MetS were MC4R rs17782312 which is involved in weight regulation and IRS1 rs2943634 which is involved in insulin resistance.

**Appendix I**

Loci related to waist circumference, insulin resistance, inflammatory biomarkers, triglycerides and HDL cholesterol in genome wide association studies till 01-01-2011 which are not on the IBC CVD array.

**Waist circumference**

NRXN3 – rs10146997 [46]
TFAP2B – rs987237 [16]
MSRA – rs7826222 [16]

**Insulin resistance**

**Inflammatory biomarkers**

HNF1A – rs1183910 [20]
ARL15 – rs4311394 [18]
APOE, APOC1, APOCII – rs2943634 which is involved in insulin resistance.

**Triglycerides**

LPL – rs326 [49]
AP0A1 – rs2075292 [49]
AP0A1, AP0C3, AP0A4, AP0A5 – rs10892151 [50]
AP0A1, AP0C3, AP0A4, AP0A5 – rs4938303 [21]
CLIP2 – rs7557067 [26]

**HDL cholesterol**

CETP – rs9989419 [21]
LIPC – rs10468017 [26]
CLIP2 – rs2304130 [21]
MAB,MVK – rs9943753 [21]

**Abbreviations**

CVD: Cardiovascular diseases; GWAS: Genome-wide association study; HbA1C: Haemoglobin A1c; HDL: High-density lipoprotein; HWE: Hardy-Weinberg equilibrium; HOMA-IR: Homeostasis model assessment insulin resistance; hsCRP: High sensitive C-reactive protein; MetS: Metabolic syndrome; SNP: Single nucleotide polymorphism; T2D: Type 2 diabetes.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contribution**

CMP analyzed the data, contributed to the discussion and wrote the manuscript. JMA8 contributed to the discussion and reviewed the manuscript. NCO contributed to the discussion and reviewed the manuscript. MET contributed to the discussion and reviewed the manuscript. EJMF researched the data, contributed to the discussion and reviewed the manuscript. All authors read and approved the final manuscript.

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**References**

1. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005, 365(9468):1415–1428.
2. Hotamisligil GS: Inflammation and metabolic disorders. Nature 2006, 444 (7121):860–867.
3. Sutherland JP, McKinley B, Eckel RH: The metabolic syndrome and inflammation. Metab Syndr Relat Disord 2004, 2(2):82–104.
4. Povel CM, Boer JMA, Relling E, Feiskens EJM. Genetic variants and the metabolic syndrome: a systematic review. Obes Rev 2011, 12(11):952–967.
5. Kraja AT, Vaidya D, Pankow JS, Goodarzi MO, Assimes TL, Kullo IJ, Sovio U, Mathias RA, Sun YV, Franceschini N, et al: A Bivariate Genome-Wide Approach to Metabolic Syndrome: STAMPEED Consortium. Diabetes 2011, 60(6):1329–1339.
6. Bosy-Westphal A, Onur S, Geisler C, Wolf A, Korth O, Pleuflemer M, Schrezenmeier J, Krauwelk M, Muller M. Common familial influences on clustering of metabolic syndrome traits with central obesity and insulin resistance: the Kiel obesity prevention study. Int J Obes ( Lond) 2007, 31 (5):784–790.
7. Goeman JJ, van de Geer SA, de Kort F, van Houwelingen HC: A global test for groups of genes: testing association with a clinical outcome. Bioinformatics 2004, 20(1):93–99.
8. Beulens JW, Monninkhof EM, Verschuren WM, van der Schouw YT, Smit J, Ocke MC, Jansen EH, van Dieren S, Grobbee DE, Peeters PH, et al: Cohort profile: the EPIC-NL study, Int J Epidemiol 2010, 39(5):1170–1178.
9. Grundy SM, Cleeman J, Daniels SR, Donato KA, Eckel RH, et al: The metabolic syndrome in children and adolescents. JAMA 2004, 291(1):236–243.
10. Grundy SM, Cleeman J, Daniels SR, Donato KA, Eckel RH, et al: The metabolic syndrome in children and adolescents. JAMA 2004, 291(1):236–243.
11. American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes 2010, 60(1):S1–S66.
12. American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes 2010, 60(1):S1–S66.
13. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Galver L, Barrett JC, Grant SF, Farlow DN, et al: Concept, design and implementation of a cardiovascular gene-centric 50 k SNP array for large-scale genomic association studies. PLoS One 2008, 3(10):e3583.
14. Bauer F, Elbers CC, Adan RA, Loos RJ, Onland-Moret NC, Grobbee DE, van Viet-Oaptchouk JW, Wijmenga C, van der Schouw YT: Obesity genes identified in genome-wide association studies are associated with adiposity measures and potentially with nutrient-specific food preference. Am J Clin Nutr 2009, 90(4):951–959.
15. Dupuis J, Langenberg C, Prokopenko I, Saugera R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Glynis AL, et al: New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 2012, 10(2):105–116.
16. Rung J, Cauchi S, Albrechtsen A, Shen L, Rocheau G, Cavalli-Sforza E, Bocquet F, Balkau B, Belisle A, Borgh-Christensen K, et al: Genetic variant near IRS1 is associated with type 2 diabetes, insulin resistance and hyperinsulinaemia. Nat Genet 2009, 41(10):1110–1115. Epub 2009 Sep 11.
17. Lindgren CM, Heid IM, Randall JC, Lamina C, Steinhordtov V, Qin L, Speliotes EK, Thorlefssson G, Wiltbank CM, et al: Genome-wide association scan meta-analysis identifies three loci influencing adiposity and fat distribution. PLoS Genet 2009, 5(6):e1000508.
C-reactive protein levels and risk of coronary heart disease. *JAMA* 2009, 302(1):37–48.

18. Richards JB, Waterworth D, O’Rahilly S, Hsvrt MF, Loos RJ, Penny JR, Tanaka T, Timpson NJ, Semple RK, Sorsan P, et al. A genome-wide association study reveals variants in *ARL15* that influence adiponectin levels. *PLoS Genet* 2009, 5(11):e1000768.

19. He M, Cornelis MC, Kraft P, van Dam RM, Sun Q, Laurie CC, Mirel DB, Chesnain Di, Ridker PM, Hunter DJ, et al. Genomewide association study identifies variants at the IL18-BCO2 locus associated with interleukin-18 levels. *Anteisos* 2010, 30(4):885–890.

20. Ridker PM, Ware G, Parker A, Zee RY, Danik JS, Buring JE, Kwiatkowski D, Kaplan L, Bennett D, Li Y, Tanaka T, et al. Novel findings in myocardial infarction: Genomewide analysis among 18,245 nondiabetic individuals. *Diabetes Care* 2009, 32(5):912–919.

21. Sabatti C, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, Jones CG, Kayticas M, Aittokallio P, van der Beek P, et al. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. *Arterioscler Thromb Vasc Biol* 2010, 30(11):2264–2276.

22. Sahni A, Service SK, Hartikainen AL, Pouta A, Ripatti S, Brodsky J, Jones CG, Zaitlen NA, Aittokallio P, van der Beek P, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. *Am J Hum Genet* 2008, 82(1):139–149.

23. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet* 2009, 41(1):55–60.

24. Wallance C, Newhouse SJ, Braun P, Zhang F, Tobin M, Falchi M, Ahmad K, Dobson RJ, Marcano AC, Hajat C, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. *Am J Hum Genet* 2009, 85(1):47–55.

25. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet* 2009, 41(1):55–60.

26. Wallance C, Newhouse SJ, Braun P, Zhang F, Tobin M, Falchi M, Ahmad K, Dobson RJ, Marcano AC, Hajat C, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. *Am J Hum Genet* 2009, 85(1):47–55.

27. Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, Zaitlen NA, Varilo T, Kaakinen M, et al. Genetic variants influencing circulating lipid levels. *Nature* 2007, 447(7145):707–713.

28. Heid IM, Vollmert C, Kronenberg F, Huth C, Ankerst DP, Luchner A, Hinney A, et al. Genetic variation near RS1 associated with reduced adiposity and an impaired metabolic profile. *Nat Genet* 2011, 43(7):753–760. doi:10.1038/ng.1866.

29. Vassy JL, Shadner P, Yang Q, Liu T, Yesupriya A, Chang MH, Dowling NF, Ned RM, Dupuis J, Florez JC, et al. Genetic associations with metabolic syndrome and its quantitative traits by race/ethnicity in the United States. *Metab Syndr Relat Disord* 2011, 9(6):475–482.

30. Bogren M, Lysenkov V, Jonsson A, Berglund G, Nilsson P, Groop L, Orho-Melander M. The search for putative unifying genetic factors for components of the metabolic syndrome. *Diabetologia* 2008, 51(12):2242–2251.

31. Tenjé A, Schulz M, Loeffer M, Stumvoll M. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with Pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. *Diabetes Care* 2006, 29(11):2489–2497.

32. Pesarro A, Dalla Nora E, Marcelli C, Di Vito F, Rinaldi P, Sani M, Bisi C, Fellin R, Zuliani G. PPARGamma Pro12Ala and ACE ID polymorphisms are associated with BMI and fat distribution, but not metabolic syndrome. *Cardiovasc Diabetol* 2011, 10:112.

33. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet* 2009, 41(1):55–60.

34. Wallance C, Newhouse SJ, Braun P, Zhang F, Tobin M, Falchi M, Ahmad K, Dobson RJ, Marcano AC, Hajat C, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. *Am J Hum Genet* 2009, 82(1):139–149.

35. Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet* 2009, 41(1):55–60.

36. Wallance C, Newhouse SJ, Braun P, Zhang F, Tobin M, Falchi M, Ahmad K, Dobson RJ, Marcano AC, Hajat C, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. *Am J Hum Genet* 2009, 85(1):47–55.

37. Vassy JL, Shadner P, Yang Q, Liu T, Yesupriya A, Chang MH, Dowling NF, Ned RM, Dupuis J, Florez JC, et al. Genetic associations with metabolic syndrome and its quantitative traits by race/ethnicity in the United States. *Metab Syndr Relat Disord* 2011, 9(6):475–482.

38. Bogren M, Lysenkov V, Jonsson A, Berglund G, Nilsson P, Groop L, Orho-Melander M. The search for putative unifying genetic factors for components of the metabolic syndrome. *Diabetologia* 2008, 51(12):2242–2251.

39. Tenjé A, Schulz M, Loeffer M, Stumvoll M. Association of Pro12Ala polymorphism in peroxisome proliferator-activated receptor gamma with Pre-diabetic phenotypes: meta-analysis of 57 studies on nondiabetic individuals. *Diabetes Care* 2006, 29(11):2489–2497.

40. Pesarro A, Dalla Nora E, Marcelli C, Di Vito F, Rinaldi P, Sani M, Bisi C, Fellin R, Zuliani G. PPARGamma Pro12Ala and ACE ID polymorphisms are associated with BMI and fat distribution, but not metabolic syndrome. *Cardiovasc Diabetol* 2011, 10:112.

41. Chasman DI, Pare G, Mora S, Hopewell JC, Peloso G, Clarke R, Cupples LA, Morris AD, McCarthy MI, Ferrucci L, et al. Gene variants influencing measures of inflammation or predisposing to autoimmune and inflammatory diseases are not associated with the risk of type 2 diabetes. *Diabetologia* 2008, 51(12):2205–2213.

42. Davey Smith G, Ebrahim S. ‘Mendelian randomization’: can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003, 32(1):1–22.

43. Yoshuichi I. Evidence of selection at insulin receptor substrate-1 gene loci. *Acta Diabetol* 2012, 51:15.

44. Heid IM, Vollmert C, Kronenberg F, Huth C, Ankerst DP, Luchner A, Hinney A, et al. Association of the MC4R V103I polymorphism with the metabolic syndrome: the KORA study. *Obesity (Silver Spring)* 2008, 16(2):369–376.

45. Butler AA, Cone RD: The melanocortin receptors: lessons from knockout models. *Neuropeptides* 2002, 36(3):177–84.

46. Chambers J, Elliott P, Zabaneh D, Zhang W, Li Y, Froqel P, Balding D, Scott J, Kooper JS. Common genetic variation near MC4R is associated with waist circumference and insulin resistance. *Nat Genet* 2008, 40(6):716–718.

47. Freathy RM, Timpson NJ, Lawlor DA, Pouta A, Ben-Shlomo Y, Ruckonken A, Ebrahim S, Shields B, Zeggini E, Weedon MN, et al. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. *Diabetologia* 2008, 51(7):1419–1426.

48. Koren JS, Chambers JC, Aguilar-Salinas CA, Hindis DA, Hyde CL, Wames GR, Gomez Perez FJ, Frazer KA, Elliott P, Scott J, et al. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. *Nat Genet* 2008, 40(2):149–151.

49. Pollin TI, Dancott CM, Shen H, Ott SH, Shelton J, Horemooty RB, Post W, McLennan JC, Bielak LF, Persay PA, et al. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science* 2008, 322(5908):1702–1705.