QTL-based Association Analyses Reveal Novel Genes Influencing Pleiotropy of Metabolic Syndrome (MetS)

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Objective: Metabolic Syndrome (MetS) is a phenotype cluster predisposing to type 2 diabetes and cardiovascular disease. We conducted a study to elucidate the genetic basis underlying linkage signals for multiple representative traits of MetS that we had previously identified at two significant QTLs on chromosomes 3q27 and 17p12.

Design and Methods: We performed QTL-specific genomic and transcriptomic analyses in 1,137 individuals from 85 extended families that contributed to the original linkage. We tested in SOLAR association of MetS phenotypes with QTL-specific haplotype-tagging SNPs as well as transcriptional profiles of peripheral blood mononuclear cells (PBMCs).

Results: SNPs significantly associated with MetS phenotypes under the prior hypothesis of linkage mapped to seven genes at 3q27 and seven at 17p12. Prioritization based on biologic relevance, SNP association, and expression analyses identified two genes: insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) at 3q27 and tumor necrosis factor receptor 13B (TNFRSF13B) at 17p12. Prioritized genes could influence cell-cell adhesion and adipocyte differentiation, insulin/glucose responsiveness, cytokine effectiveness, plasma lipid levels, and lipoprotein densities.

Conclusions: Using an approach combining genomic, transcriptomic, and bioinformatic data we identified novel candidate genes for MetS.
These QTLs were also found to be epistatically interactive and pleiotropic, influencing a broad spectrum of the MetS phenotypes (11).

The present study was undertaken in 1,137 individuals in 352 nuclear families (85 extended families) of Northern European descent from the cohort that contributed to the QTL linkage. We conducted an in-depth phenotyping procedure by measuring 42 MetS traits that included both clinical outcome and biologic precursor phenotypes. In addition, we obtained dense haplotype-tagging SNP genotypes and transcriptional profiles of peripheral blood mononuclear cells (PBMCs) for a subset of these individuals. Gene prioritization was based upon biologic relevance as well as SNP and PBMC expression associations with the MetS phenotypes.

Methods and Procedures
Subjects and phenotypes
The study cohort consists of 1,137 individuals representing 85 extended nuclear families. Analysis of distribution of pairwise relationships showed the majority of the number of pairs were highest of parent-offspring \((n = 1,300)\) and between siblings \((n = 1,494)\). Details of recruitment and phenotyping procedures have been described previously (11). Briefly, each family was recruited through an obese proband (BMI \(\geq 30\)) with the minimal requirement of the availability of one obese sibling and one never-obese (BMI \(<27\)) and availability of at least one, preferably both, of the parents. Clinical phenotypes included weight, height, BMI, waist circumference (WC), hip circumference (HC), waist to hip ratio (WHR), fasting glucose (FG), fasting insulin (FI), insulin to glucose ratio (IGR), homeostasis model assessment (HOMA), plasma triglycerides (TG), total cholesterol (TC), LDL-cholesterol (LDL-c) and calculated LDL-cholesterol levels (cal. LDL-c), HDL-cholesterol (HDL-c), systolic and diastolic blood pressure (sBP and dBP) and pulse. Biological phenotypes were determined according to standard published procedures, included measurement of total fat mass in kilogram and percentage (Fatkg and Fatpct) and lean mass in kilogram and percentage (Leankg and Leanpct) by Dual-emission X-ray absorptiometry (DEXA) (14); total abdominal, visceral and subcutaneous fat sizes (TAF, VF and SubQF) by computed tomography (CT/MRI) scans average of three sections at the fourth lumbar spine (15); resting energy expenditure (REE) and respiratory quotient (RQ) were measured in resting subjects using a Deltatrac Indirect Calorimeter (Sensor Medics, VIASYS Healthcare, Conshohocken, PA) after a 10-h fast; insulin/glucose responsiveness indices: insulin sensitivity (SI), glucose effectiveness (SG), acute insulin response to glucose (AIRg) and disposition index (DI) by Minimal Model (16); lipids/fibroprotein sizing (HDL median diameter (HMD), LDL-cholesterol median diameter (LMEDn), LDL-cholesterol dominant peak diameter (LDLpdp) and apoB-containing non-HDL median diameter (BMED) which includes VLDL, IDLD, LP\(_2\) and LDL) were measured by polyacrylamide gradient gel electrophoresis (17); circulating levels of adiponectin, leptin by a double antibody, equilibrium RIA (Millipore Corporation, Billerica, MA); and TNF-alpha, interleukin-1beta (IL-1\(\beta\)) and interleukin-6 (IL-6) as described (18). All study procedures for adults and children were approved by the Institutional Review Boards of the Medical College of Wisconsin and Children’s Hospital of Wisconsin, respectively.

SNP genotyping and data cleaning
Genomic DNA was extracted and prepared from whole blood using commercial kits (Puregene, Minneapolis, MN). Genome-wide SNP genotyping was performed using Affymetrix Genome-Wide Human SNP 6.0 arrays and SNP calls were generated by Genotype Console 3.2. Individuals with fewer than 95% of available markers called were excluded. 869,222 autosomal SNPs were prepared by Preswalk and checked for Mendelian consistency with SimWalk2. A SNP was eliminated if: (1) fewer than 95% of the cohort were typed successfully; (2) the SNP was monoallelic; (3) the SNP had more than two alleles; (4) fewer than five copies of the SNP existed in the study cohort. Hardy-Weinberg equilibrium (HWE) was tested for each SNP using SOLAR (19); SNPs with excessive deviation from HWE \((P < 10^{-5})\) were excluded. Missing SNP data were imputed with MERLIN (20).

Transcriptional profiling
Genome-wide transcriptional profiles of a subset of the SNP genotyping cohort (369 individuals from 55 nuclear families) were obtained as previously described (21) with modifications. Briefly, for each individual 2.5-ml blood was collected into a PAXgene\textsuperscript® Blood RNA Tube (BD, Franklin Lakes, NJ) following an overnight fast. Total RNA was isolated from each tube using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA) and anti-sense RNA (aRNA) was synthesized using the MessageAmp II-Biotin aRNA kit (Ambion, Austin, TX). A total of 1.5 \(\mu\)g aRNA was hybridized to Illumina HumanWG-6 version 2 or version 3 chips (Illumina, San Diego, CA) and expression detected on the Illumina\textsuperscript® BeadArray\textsuperscript™ 500XG Reader. Illumina GenomeStudio software (version 2010.3) was used for preliminary data analysis with standard background normalization.

Statistical analysis
Variance-component-based pedigree analysis. The heritability of each of the 42 MetS traits was first established, then a bivariate analysis in SOLAR was used to partition phenotypic correlations between the traits into presumed environmental and additive genetic components, as described previously (22).

Measured genotype analysis. In SOLAR, each SNP genotype was converted to a covariate measure equal to 0, 1, or 2 copies of the minor allele (or, for missing genotypes, a weighted covariate based on imputation). These covariates were included in variance-components mixed models for measured genotype analyses (23) versus null models that incorporated the random effect of kinship and fixed effects such as age, age\(^2\), sex and their interactions. Individual scores from a principal components analysis of representative SNPs were also included to correct for possible population stratification (24).

Within chromosomal regions showing the strongest prior evidence of linkage (as logarithm of odds, LOD, score) with MetS traits in our MRC-OB cohort, we selected all available SNPs on the Affymetrics 6.0 array that mapped within a 1-LOD confidence interval of the maximum LOD score in each region. Each SNP covariate was tested independently in a 1 degree of freedom likelihood ratio test. To account for the linkage disequilibrium between SNPs, we calculated the effective number of independent SNPs \((N_{eff})\) using the method of Moskvina and Schmidt (25). Critical \(P\) values were calculated using Bonferroni/Šidák correction for each linkage region based on its \(N_{eff}\).

Gene expression analysis. Microarray data were available in two batches, one of Version 2 arrays (48,701 probes, 307 samples) and the other of Version 3 (48,803 probes, 230 samples). To guard
against possible batch effects and probe differences, each batch was analyzed separately: The number of probe transcripts detectable at $P \leq 0.05$ by BeadStudio software was counted, a false discovery rate (FDR) was computed across all probes, and transcripts detectable at 5% FDR were retained. Expression levels were log$_2$ transformed and inverse-quartile normalized. Transformed and normalized expression levels for probes that mapped to the 1-LOD QTL regions were tested for association with each phenotype in models that included the random effect of kinship. Gene-centric $P$ values were calculated by combining independent $P$ values from the two microarray batches and multiple probes using Stouffer’s weighted $Z$ score method (26) implemented in R.

Results
Heritability and genetic intercorrelation
A total of 1,137 individuals, representing extended families that contributed to the linkage on chromosomes 3 and 17 (11), were recruited and measured for the clinical phenotypes. Nearly 503 of those also underwent the biologic phenotyping procedures and 369 individuals also provided blood PBMCs for the transcriptional profiling. Their mean (± SD) age was 40.29 years ± SD 17.68 years and ranged from 6 to 90 years, 11.7% of the subjects were younger than age 18 and 58.3% of the subjects were female. Table 1 shows means (± SD) of the 42 phenotypes and their levels of heritability. All phenotypes measured were significantly heritable ($P < 0.05$), justifying further genetic analysis.

Figure 1 illustrates the traits that significantly inter-correlated genetically. BMI, the trait most commonly used in GWA analyses as the phenotype representing the degree of obesity, was correlated with the other phenotypes of body composition (WC and HC), insulin responsiveness (FI, IGR, and HOMA), and lipid panel (TG and HDL-c). BMI was also significantly correlated with both total body fat and lean masses (Fatkg, Fatpct, Leankg and Leanpct). WC, another commonly used phenotype to represent central body or visceral fat distribution, was significantly correlated with clinical phenotypes of insulin responsiveness (IGR and HOMA), plasma lipids (TG and HDL-c) and cardiovascular performance measures (resting pulse and sBP). As with BMI, it was correlated both with visceral and subcutaneous fat masses (VF and SubQF). WHR, another clinical phenotype of body fat distribution, on the other hand was correlated with VF, REE, lipoprotein density profile and circulating levels of TNF-alpha. Of the biologic phenotypes, Fatkg was genetically correlated with REE, phenotypes of insulin responsiveness (SI, SG, and AIRG), LDL peak size (LDLppd), and plasma leptin. VF was also genetically correlated with measures of body composition, REE, insulin sensitivity (SI), and plasma of IL-6. AIRG was correlated with Leankg, insulin responsiveness (DI), and leptin. Finally, SI, a direct marker of insulin sensitivity, was genetically correlated with all biologic measures of body composition (Fatkg, Leankg, Fatpct, and Leanpct) and clinical parameters of insulin response.

SNP genotype–phenotype association
Tables 2 and 3 show the highest levels of association of SNPs bordered by 1 LOD score drop from the linkage peak at 3q27 QTL (LOD$_{BMI} = 3.3$) and 17p12 (LOD$_{Leptin} = 5.0$), respectively. The strength of the association is indicated by the negative logarithm of $P$ values. The genes representative of this association annotated from those SNPs (hg18/NCBI36 human genome assembly) are also shown. The regional threshold for associations was determined by Bonferroni/Sidak correction for the effective number of independent SNPs given their linkage disequilibrium ($n_{SNPs} = 6,305$, $n_{effective} = 3881.47$, $P_{cor} = 3.2 \times 10^{-5}$, 3q27; $n_{SNPs} = 1,629$, $n_{effective} = 1412.93$, $P_{cor} = 3.6 \times 10^{-5}$, 17p12) (27).

QTL3q27
Body composition and insulin responsiveness. Table 2 shows four biologically relevant genes identified in this region to be associated with body composition and insulin/glucose responsiveness. Transducing (beta)-like 1 X-linked receptor 1 (TBLIXR1) (rs6443434 and rs11710431), a signal transducer influencing multiple biological functions including cytoskeletal assembly, was associated with both clinical (weight and BMI) and biologic (Leanpct, REE, TAF and SG) traits. Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) (rs12639517), known to regulate IGF2 mRNA translation, was associated with the identifier phenotype WHR. Neuroligin 1 (NLGN1) (rs569221), a brain synapses protein, was associated with Leankg. Lipoma-preferred partner (LPP) (rs6785028), a cell adhesion and growth signal communicator, was associated with AIRG. Within this region, there were two associations that exceeded the regional significance threshold. SNP rs7433602 mapped to N-acetylated alpha-linked acidic dipeptidase-like 2 (NAALADL2), a dipeptidase that lacks a functional domain, was associated with VF. SNP rs10933727 was associated with HOMA and was mapped to a hypothetical gene LOC100131551.

Lipids/lipoprotein and cardiovascular performance. Of the lipids/lipoprotein phenotypes, there are two biologically relevant genes disclosed by SNP association. ATP11B (rs2700839) in association with LDLppd is an ATPase functioning in ion transport in cell membrane. Another gene, Rho family guanine nucleotide exchange factor (MCF2L2), referred by rs7631640 was associated with pulse. It was identified as a gene associated with type 2 diabetes (28). Only one association in this category was above the regional significance threshold, that is, rs10937009 with BMED. This SNP is near the gene NADH dehydrogenase (ubiquinone) 1 beta (NDUFB5), which transfers electrons from NADH to the respiratory chain.

Adipokine/cytokines. In the adipokine/cytokines phenotype category, we identified a biologically relevant association between rs6807480 and circulating IL-6 that is annotated to gene FGF12, a fibroblast growth factor. Another association above the threshold was between rs13070418 and TNF-alpha. This SNP was annotated to the gene SOX2, a transcription factor functioning in embryonic development.

QTL17p12
Body composition and insulin responsiveness. There are seven biologically relevant genes annotated to SNP-phenotype associations (Table 3). Three of those were identified by multiple associations, including TNFRSF13B (eight associations), H535T3A1 (eight associations) and MYOC (four associations). Of those, TNFRSF13B, a pro-inflammatory macrophage receptor, was associated with VFA and TAF at levels above regional significance threshold. H535T3A1 [heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1], a heparan sulfate biosynthesis enzyme, was associated with HC, DEXA measures of body fatness and leanness, and several of the other biological phenotypes which index insulin responsiveness (SG and AIRG).
**MYOC (myocardin)**, a cardiogenesis regulator, was associated with clinical phenotypes of body weight, BMI, and WC. There were four associations annotated to potentially important biological regulator genes relevant to MetS. **RICH2**, a Rho-GTPase activator, was associated with Fatpct. **TRPV2** (transient receptor potential cation channel), a heat responsive channel, was associated with RQ. **COX10** (cytochrome c oxidase component 10), an essential component of the mitochondrial respiratory chain, was associated with FG. **PMP22** (peripheral myelin protein 22) associated with SI, is known to function in the peripheral nervous system.

**TABLE 1** MetS Cohort phenotypic characteristics and heritability levels

| Trait                                    | Mean       | Heritability ± SE |
|------------------------------------------|------------|-------------------|
| **Body composition and insulin responsiveness** |            |                   |
| Weight, kg                               | 87.57 ± 27.36 | 0.47 ± 0.06       |
| Height, cm                               | 167.7 ± 12.45 | 0.56 ± 0.05       |
| BMI, kg/m²                                | 30.94 ± 9.31  | 0.44 ± 0.06       |
| Waist circumference (WC), cm             | 95.56 ± 21    | 0.40 ± 0.06       |
| Hip circumference (HC), cm               | 107.94 ± 22.04| 0.57 ± 0.05       |
| Waist to Hip ratio (WHR)                 | 0.89 ± 0.1    | 0.31 ± 0.06       |
| Total Fat Mass (Fatkg), kg               | 32.36 ± 16.47 | 0.50 ± 0.1        |
| Total Fat Mass (Fatpct), %               | 36.61 ± 12.14 | 0.48 ± 0.1        |
| Total Lean Mass (Leankg), kg             | 51.87 ± 13.56 | 0.66 ± 0.09       |
| Total Lean Mass (Leanpct), %             | 65.58 ± 31.75 | 0.44 ± 0.1        |
| Subcutaneous Fat (SubQF), g              | 311.9 ± 176.7 | 0.57 ± 0.1        |
| Visceral Fat (VF), g                     | 177.9 ± 111.3 | 0.32 ± 0.07       |
| Total Abdominal Fat (TAF), g             | 489.8 ± 248.8 | 0.51 ± 0.1        |
| Respiratory Quotient (RQ)                | 0.83 ± 0.08   | 0.35 ± 0.09       |
| Resting Energy Expenditure (REE), kcal/24 hrs | 1740.5 ± 342.21 | 0.31 ± 0.09     |
| REE/weight, kcal/24hrs/kg                | 19.26 ± 3.47  | 0.32 ± 0.11       |
| REE/Lean mass (REE/LM), kcal/24hrs/kg    | 33.48 ± 6.18  | 0.36 ± 0.1        |
| Fasting Glucose (FG), mmol/l             | 4.86 ± 1.64   | 0.38 ± 0.05       |
| Fasting Insulin (FI), pmol/l             | 107.5 ± 105.6 | 0.30 ± 0.05       |
| Insulin/glucose (IGR)                    | 0.25 ± 0.62   | 0.25 ± 0.05       |
| Homeostasis model assessment (HOMA)      | 4.31 ± 8.5    | 0.30 ± 0.05       |
| Insulin Sensitivity (SI), (× 10⁻⁴/min/μU/ml) | 3.05 ± 2.83   | 0.15 ± 0.09       |
| Glucose Effectiveness (SG), min⁻¹        | 0.02 ± 0.01   | 0.13 ± 0.08       |
| Acute Insulin Response to glucose (AIRg), μU/ml × 10min | 467.02 ± 400.51 | 0.52 ± 0.08     |
| Disposition Index (DI), AUC (Insulin₀–₁₀ min)/(Insulin₀–₉ min) | 1191.18 ± 1081.76 | 0.26 ± 0.09     |
| **Lipids/lipoprotein profiles and cardiovascular performance** |            |                   |
| Triglycerides (TG), mmol/l               | 1.33 ± 1.29   | 0.38 ± 0.06       |
| Total Cholesterol (TC), mmol/l           | 4.88 ± 1.16   | 0.34 ± 0.05       |
| LDL-cholesterol (LDL-c), mmol/l          | 3.19 ± 0.98   | 0.28 ± 0.05       |
| Calculated LDL-C (cal. LDL-c), mmol/l    | 3.25 ± 1.16   | 0.35 ± 0.05       |
| HDL-cholesterol (HDL-c), mmol/l          | 1.06 ± 0.37   | 0.64 ± 0.06       |
| HMED, nm                                 | 8.93 ± 0.55   | 0.53 ± 0.07       |
| LMEdn, nm                                | 27.03 ± 0.78  | 0.59 ± 0.07       |
| LDLpdp, nm                               | 27.35 ± 1.2   | 0.65 ± 0.07       |
| BMED, nm                                 | 27.55 ± 1.17  | 0.63 ± 0.07       |
| Systolic Blood Pressure (sBP), mmHg      | 124.58 ± 18.57 | 0.24 ± 0.05     |
| Diastolic Blood Pressure (dBP), mmHg     | 77.13 ± 11.27 | 0.24 ± 0.06       |
| Pulse, beats/min                         | 71.17 ± 14.14 | 0.20 ± 0.05       |
| Adiponectin, ng/ml                       | 9.51 ± 5.6    | 0.48 ± 0.06       |
| Leptin, ng/ml                            | 19.28 ± 17.3  | 0.24 ± 0.06       |
| TNF-alpha, pg/ml                         | 4.44 ± 6.28   | 0.32 ± 0.06       |
| Interleukin-1 beta (IL-1β), pg/ml        | 1.33 ± 18.42  | 0.16 ± 0.06       |
| Interleukin-6 (IL-6), pg/ml              | 7.68 ± 53.48  | 0.25 ± 0.07       |
was associated with LDL-c. PMP22 was associated with HMED (above the threshold). TNFRSF13B was associated with sBP. Finally MPRIP, which is a myosin phosphatase Rho interacting protein, was associated with three of the lipoprotein sizing measures (LMEDn, LDLppd, and BMED).

Adipokine/cytokines. Associations with adiponectin and TNF-alpha were mapped to HS3ST3A1, which was also associated with body composition and insulin responsiveness. COPS3 (COP9 constitutive photomorphogenic homolog subunit 3), a kinase regulating signal transduction, was associated with IL-6.

PBMC mRNA expression. Of the biologically relevant genes identified by SNP association at 3q27 and 17p12, Tables 4 and 5 show the association (as in \(P\) values) of transcript levels in relation to each of the 42 phenotypes. At 3q27, expression of three biologically relevant genes, LPP, IGF2BP2, and TBL1XR1, were strongly associated with \(P < 0.01\) MetS phenotypes. ATP11B, MCF2L2, and NLGN1 had nominal associations with MetS phenotypes \((P < 0.05)\). LPP was found associated with both biologic and clinical phenotypes (REE/weight, IL-1\(\beta\), FI, TG, and HDL-c). IGF2BP2 was associated with body weight, BMI, WC, HC, Fatkg, Fatpct, Leankg and Leanpct, RQ, REE, SI, DI, LDL-c, HMED, LDLppd, and leptin. TBL1XR1 was associated with Leankg and resting pulse. ATP11B was associated with WHR, MCF2L2 with SI, LDLppd, and BMED, and NLGN1 with FG. Of the seven genes identified by SNP-phenotype association as biological relevant, the transcript levels of only one gene, FGF12, did not pass any significant level. At 17p12, of the eight biologically relevant genes, seven had transcription levels significantly associated with related MetS phenotypes: TNFRSF13B, COX10, RICH2, TRPV2, MPRIP, COPS3, and PMP22. TNFRSF13B was associated with WHR, RQ, LDLppd and BMED. COX10 was associated with weight, BMI, SG and TG. PMP22 was associated with TG and HMED, and RICH2 with Leankg, REE, AIRg, and leptin. TRPV2 was associated with fat mass, lean mass and REE/leanmass, and MPRIP with TG, HMED and pulse. HS3ST3B1 was not represented in our transcriptomic profile. However, because of its extensive SNP association with multiple biologic phenotypes, and because of its known relevant function, we prioritized it as a significant candidate.

Summary of candidate gene prioritization. Table 6 summarizes the prioritized genes identified by SNP association in the two QTL regions, their identifier phenotype and the genetic correlates of this identifier. The steps of this prioritizing strategy included the level of significance of the SNP associations, function of identified gene in relation to the MetS and level of PBMC gene expression in association with the MetS phenotypes. Figure 2A and 2B show the final four prioritized genes located within the QTL peaks at 3q27 and 17p12, together with their identifier phenotype(s).
The present study focused on identification of genes situated in previously-determined QTLs at chr3q27 and chr17p12. Both targeted SNP genotype association and mRNA expression analyses were performed. The results provided for the first time clarification of the differential informativeness of the clinical phenotypes vs. concurrently measured biologic traits. Although BMI and waist circumferences, the commonly used phenotypes in the GWAS meta-analyses, were genetically intercorrelated with other clinical phenotypes of MetS, they were not reflecting their biologically targeted traits. BMI was intercorrelated with both total body fat and lean mass. Waist circumferences were also intercorrelated with subcutaneous and visceral fat sizes. Within each of the QTLs a number of genes were differently associated with specific clinical phenotypes.

### TABLE 2 MetS SNP association with phenotypes and their gene annotation at 3q27 QTL

| Variant | Minor Allele | MAF  | -Log10(p) | Associated phenotype | Nearest gene | Location | Annotation |
|---------|--------------|------|-----------|----------------------|--------------|----------|-----------|
| rs6443434 | A            | 0.43 | 3.76      | Weight               | TBL1XR1      | upstream | signal transduction |
| rs11710431 | A            | 0.19 | 4.55      | Height               | TBL1XR1      | intergenic | signal transduction |
| rs6443434 | A            | 0.43 | 3.49      | BMI                  | TBL1XR1      | upstream | signal transduction |
| rs6772734 | A            | 0.02 | 3.82      | WC                   | LOC131583    | intergenic | unknown function |
| rs13068101 | T            | 0.28 | 3.06      | HC                   | C3orf59      | intergenic | unknown function |
| rs12639517 | T            | 0.33 | 4.03      | WHR                  | SF2BP2       | intergenic | controls IGF translation |
| rs11708240 | G            | 0.14 | 3.54      | Fatkg                | NAALADL2     | intergenic | dipeptidase |
| rs13098487 | G            | 0.22 | 3.59      | Fatpct               | Nelgn1       | intronic | brain synapses |
| rs669221 | A            | 0.19 | 4.47      | Leankg               | Nelgn1       | intronic | brain synapses |
| rs301177  | C            | 0.23 | 3.39      | Leanpct              | TBL1XR1      | intergenic | signal transduction |
| rs4074869 | C            | 0.44 | 3.70      | SubQF                | EPHB3        | intergenic | ephrinB receptor |
| rs7433602 | T            | 0.29 | 5.03      | VF                   | NAALADL2     | intergenic | dipeptidase |
| rs12496403 | A            | 0.01 | 3.76      | TAF                  | TBL1XR1      | intergenic | signal transduction |
| rs13324849 | T            | 0.09 | 4.39      | RO                   | NAALADL2     | intergenic | dipeptidase |
| rs6443434 | A            | 0.43 | 3.51      | REE                  | TBL1XR1      | upstream | signal transduction |
| rs4074869 | C            | 0.44 | 4.47      | REE/weight           | EPHB3        | intergenic | ephrinB receptor |
| rs13321995 | A            | 0.40 | 4.89      | REE/Leanmass         | C3orf21      | intronic | unknown function |
| rs6807927 | A            | 0.27 | 3.96      | FG                   | SENP2        | intronic | SUMO protease |
| rs10933727 | G            | 0.32 | 4.43      | Fl                   | LOC100131551 | intergenic | unknown function |
| rs2513  | A            | 0.10 | 3.57      | IGR                  | AK091265     | downstream | unknown function |
| rs10933727 | G            | 0.32 | 5.93      | HOMA                 | LOC100131551 | intergenic | unknown function |
| rs9814142 | T            | 0.29 | 5.03      | SI                   | HRG          | 5’UTR     | thrombosis |
| rs1463332 | C            | 0.32 | 3.29      | SG                   | TBL1XR1      | intergenic | signal transduction |
| rs6785028 | T            | 0.26 | 3.87      | AIR                  | PP           | intronic | cell adhesion |
| rs937589  | T            | 0.02 | 3.70      | DI                   | C3orf59      | intronic | unknown function |
| rs10804866 | A            | 0.26 | 3.74      | TG                   | TBL1XR1      | intergenic | signal transduction |
| rs1895070 | A            | 0.10 | 4.66      | TC                   | mRNA FLJ31690 | downstream | unknown function |
| rs1895070 | A            | 0.10 | 4.25      | LDL-c                | mRNA FLJ31690 | downstream | unknown function |
| rs1895070 | A            | 0.10 | 4.57      | cal-LDL-c            | mRNA FLJ31690 | downstream | unknown function |
| rs4452373 | G            | 0.00 | 3.90      | HDL-c                | mRNA HD448050 | intergenic | unknown function |
| rs9716  | C            | 0.24 | 3.55      | HMED                 | ST6GAL1      | downstream | glyceroltransferase |
| rs6793161 | T            | 0.01 | 3.62      | LMEDn                | mRNA AF338197 | intergenic | unknown function |
| rs7631640 | C            | 0.12 | 3.70      | LDLppd               | ATP11B       | intergenic | ATPase |
| rs10937009 | G            | 0.00 | 5.14      | BMED                 | NDUF5        | intergenic | respiratory chain |
| rs260589 | G            | 0.33 | 3.27      | aSB                  | TPRG1        | intergenic | tumor protein regulated |
| rs6444517 | G            | 0.10 | 4.64      | dBp                  | OSTN         | intergenic | osteoblastic differentiation |
| rs57631640 | C            | 0.09 | 4.51      | Pulse                | MCF2L2       | downstream | guanine exchange factor |
| rs17427594 | C            | 0.10 | 3.60      | Adiponectin          | CLDN1        | intergenic | cell junction |
| rs7727334 | A            | 0.02 | 3.45      | Leptin               | FAM43A       | intergenic | unknown function |
| rs13070418 | T            | 0.36 | 5.06      | TNFalpha             | SOX2         | intergenic | transcription factor |
| rs9877500 | C            | 0.22 | 4.11      | IL-1β                | IOCG         | intronic | unknown function |
| rs6807480 | A            | 0.36 | 3.60      | IL-6                 | FGF12        | intronic | fibroblast growth factor |

### Discussion

The present study focused on identification of genes situated in previously-determined QTLs at chr3q27 and chr17p12. Both targeted SNP genotype association and mRNA expression analyses were performed. The results provided for the first time clarification of the differential informativeness of the clinical phenotypes vs. concomitantly measured biologic traits. Although BMI and waist circumferences, the commonly used phenotypes in the GWAS meta-analyses, were genetically intercorrelated with other clinical phenotypes of MetS, they were not reflecting their biologically targeted traits. BMI was intercorrelated with both total body fat and lean mass. Waist circumferences were also intercorrelated with subcutaneous and visceral fat sizes. Within each of the QTLs a number of genes were differently associated with specific clinical phenotypes.
| Variant        | Minor Allele | MAF  | \(-\log_{10}(p)\) | Associated phenotype | Nearest gene       | Location       | Annotation                  |
|----------------|--------------|------|-------------------|----------------------|-------------------|-----------------|------------------------------|
| rs9889434      | T            | 0.46 | 2.56              | Weight               | MYOCD             | intergenic      | Cardiogenesis               |
| rs12943502     | C            | 0.49 | 3.15              | Height               | NM_001001684      | intergenic      | unknown function            |
| rs2052003      | T            | 0.33 | 2.77              | BMI                   | MYOCD             | intergenic      | Cardiogenesis               |
| rs2052003      | T            | 0.33 | 2.99              | WC                    | MYOCD             | intergenic      | Cardiogenesis               |
| rs4792371      | G            | 0.46 | 3.35              | WC                    | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs7219760      | T            | 0.09 | 2.96              | WHR                   | NM_001001684      | intergenic      | unknown function            |
| rs893022       | A            | 0.03 | 2.75              | Fatkg                 | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs2190699      | G            | 0.34 | 2.95              | Fatpct                | RICH2             | intergenic      | GTPase activator            |
| rs3944086      | C            | 0.11 | 2.80              | Leankg                | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs9907078      | C            | 0.33 | 3.02              | Leanpct               | CDRT15            | upstream        | unknown function            |
| rs12949155     | G            | 0.43 | 3.47              | SubQF                 | NM_001001684      | intergenic      | unknown function            |
| rs4343329      | C            | 0.26 | 6.54              | VF                    | TNFRSF13B         | intronic        | pro-inflammation            |
| rs7225344      | C            | 0.26 | 5.68              | VF                    | TNFRSF13B         | intronic        | pro-inflammation            |
| rs4985700      | C            | 0.40 | 5.13              | VF                    | TNFRSF13B         | intronic        | pro-inflammation            |
| rs7226097      | C            | 0.41 | 4.22              | VF                    | TNFRSF13B         | intronic        | pro-inflammation            |
| rs11651352     | A            | 0.23 | 4.17              | VF                    | TNFRSF13B         | downstream      | pro-inflammation            |
| rs4343329      | C            | 0.26 | 5.23              | TAF                   | TNFRSF13B         | intronic        | pro-inflammation            |
| rs8081054      | A            | 0.33 | 4.74              | TAF                   | NM_001001684      | intergenic      | unknown function            |
| rs7225344      | C            | 0.26 | 4.51              | TAF                   | TNFRSF13B         | intronic        | pro-inflammation            |
| rs8079010      | A            | 0.34 | 3.98              | RQ                    | TRP2              | intronic        | pro-inflammation            |
| rs4985700      | C            | 0.40 | 4.01              | REE                   | TNFRSF13B         | intronic        | pro-inflammation            |
| rs9303119      | G            | 0.45 | 2.81              | REE/weight            | NM_001001684      | intergenic      | unknown function            |
| rs7222088      | G            | 0.43 | 3.53              | REE/Leanmass          | AK123100          | intergenic      | unknown function            |
| rs2108683      | A            | 0.11 | 3.48              | FG                    | SOX10             | intronic        | heparan sulfate             |
| rs3744342      | G            | 0.48 | 3.04              | R1                    | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs3744342      | G            | 0.48 | 2.91              | V1                    | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs6502302      | A            | 0.01 | 2.87              | VOMA                  | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs9908356      | G            | 0.21 | 3.19              | SI                    | PMP2              | intronic        | Peripheral nervous system   |
| rs2106795      | T            | 0.14 | 3.79              | SG                    | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs2996033      | T            | 0.25 | 2.95              | AIR                   | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs12453295     | A            | 0.01 | 3.02              | DI                    | MYOCD             | intronic        | Cardiogenesis               |
| rs4792303      | A            | 0.05 | 2.80              | TG                    | RICH2             | intronic        | GTPase activator            |
| rs173160       | C            | 0.34 | 2.72              | cal. LDL-c            | HS3ST3B1          | intronic        | heparan sulfate             |
| rs11870163     | T            | 0.42 | 3.11              | LDL-c                 | HS3ST3A1          | intronic        | Cardiogenesis               |
| rs173160       | C            | 0.34 | 2.72              | cal. LDL-c            | MYOCD             | intronic        | Cardiogenesis               |
| rs8077611      | A            | 0.01 | 3.54              | HDL-c                 | NM_001001684      | intergenic      | unknown function            |
| rs230922       | T            | 0.30 | 5.21              | LMED                  | PMP2              | intronic        | Peripheral nervous system   |
| rs7214429      | G            | 0.45 | 3.64              | UMDn                  | MPRIp              | intronic        | Myosin phosphatase          |
| rs7214429      | G            | 0.45 | 3.20              | LDLppd                | MPRIp              | intronic        | Myosin phosphatase          |
| rs7214429      | G            | 0.45 | 3.58              | uMED                  | MPRIp              | intronic        | Myosin phosphatase          |
| rs7214091      | C            | 0.40 | 4.05              | sBP                   | TNFRSF13B         | intronic        | heparan sulfate             |
| rs1233861      | A            | 0.23 | 3.36              | gBP                   | MYOCD             | intronic        | Cardiogenesis               |
| rs12944789     | C            | 0.36 | 4.36              | Pulse                 | NM_001001684      | intergenic      | unknown function            |
| rs8070118      | G            | 0.41 | 2.22              | Adiponectin           | HS3ST3A1          | intergenic      | heparan sulfate             |
| rs2906985      | G            | 0.06 | 3.75              | Leptin                | CORT4             | intronic        | heparan sulfate             |
| rs9915627      | A            | 0.21 | 4.13              | TNF-alpha             | HS3ST3A1          | intergenic      | downstream                |
| rs9904641      | T            | 0.27 | 3.48              | IL-1β                 | CORT4             | intronic        | unknown function            |
| rs1736202      | C            | 0.41 | 3.60              | IL-6                  | CORT3             | intronic        | signal transduction         |

**Table 3** MetS SNP association with phenotypes and their gene annotation at 17p12 QTL
found to be associated with the multiple phenotypes of body composition, insulin responsiveness, lipoprotein density profiles and cytokines. Our results revealed two novel genes (\textit{IGF2BP2} and \textit{TNFRSF13B}), whose function could account for the biologic pathways influencing MetS phenotypes. \textit{IGF2BP2} is also known to mediate glucose/insulin response effectiveness. \textit{TNFRSF13B} is a mediator of cytokine activities like TNF-alpha and its influence on the proinflammatory response. Our results thus unraveled the genetic origin of the epistatic and pleiotropic effects exerted by the identified QTLs with their potential influences on MetS. It also emphasizes the rationale behind the extensive efforts and the cost encountered in measuring the biologic phenotypes to identify those genes participating in the evolution of MetS phenotypic clusters.

### TABLE 4 Association of lymphocyte expression levels of prioritized genes with MetS phenotypes at 3q27 QTL

| Phenotype                        | ATP11B | FGFI2 | IGF2BP2 | LPP | MCF2L2 | NLGN1 | TBL1XR1 |
|----------------------------------|--------|-------|---------|-----|--------|-------|---------|
| **Body composition and insulin responsiveness** |        |       |         |     |        |       |         |
| weight                           | 0.51   | 0.35  | 0.00    | 0.62| 0.96   | 0.18  | 1.00    |
| height                           | 0.17   | 0.14  | 0.12    | 0.97| 1.00   | 0.31  | 0.00    |
| BMI                              | 1.00   | 0.89  | 0.00    | 0.74| 0.36   | 0.21  | 0.34    |
| WC                               | 0.44   | 0.63  | 0.00    | 0.69| 0.73   | 0.68  | 0.34    |
| HC                               | 0.01   | 0.57  | 0.01    | 0.50| 0.63   | 0.80  | 0.28    |
| WHR                              | 0.02   | 0.39  | 0.12    | 0.37| 0.51   | 0.92  | 0.69    |
| Fatkg                            | 0.61   | 0.73  | 0.02    | 0.34| 0.84   | 0.16  | 0.09    |
| Fatpct                           | 0.93   | 0.96  | 0.27    | 0.48| 0.18   | 0.18  | 0.65    |
| Leankg                           | 0.14   | 0.70  | 0.00    | 0.86| 0.33   | 0.10  | 0.01    |
| Leanpct                          | 0.93   | 1.00  | 0.26    | 0.43| 0.25   | 0.16  | 0.55    |
| SubQF                            | 0.82   | 0.28  | 0.06    | 0.74| 0.84   | NA    | 0.96    |
| VF                               | 0.81   | 0.28  | 0.21    | 0.91| 0.83   | NA    | 0.89    |
| TAF                              | 0.86   | 0.24  | 0.11    | 0.80| 0.94   | NA    | 0.86    |
| RQ                               | 0.18   | 0.90  | 0.01    | 0.22| 0.06   | NA    | 0.84    |
| REE                              | 0.81   | 0.63  | 0.00    | 0.20| 0.45   | NA    | 0.52    |
| REE/weight                       | 0.24   | 0.99  | 0.09    | 0.03| 0.12   | NA    | 0.29    |
| REE/leanmass                     | 0.43   | 0.86  | 0.38    | 0.08| 0.93   | NA    | 0.31    |
| FG                               | 0.57   | 0.77  | 0.21    | 0.71| 0.74   | 0.04  | 0.83    |
| Fi                               | 0.73   | 0.12  | 0.68    | 0.05| 0.81   | 0.85  | 0.13    |
| IGR                              | 0.55   | 0.20  | 0.68    | 0.05| 0.99   | 0.58  | 0.13    |
| HOMA                             | 0.79   | 0.15  | 0.40    | 0.06| 0.67   | 0.41  | 0.16    |
| SI                               | 0.07   | 0.13  | 0.04    | 0.67| 0.04   | NA    | 0.64    |
| SG                               | 0.83   | 0.51  | 0.17    | 0.75| 0.37   | NA    | 0.62    |
| AIR                              | 0.19   | 0.15  | 0.45    | 0.94| 0.98   | NA    | 0.31    |
| DI                               | 0.18   | 0.26  | 0.02    | 0.49| 0.83   | 0.57  | 0.73    |
| **Lipids/lipoprotein profiles and cardiovascular performance** |        |       |         |     |        |       |         |
| TG                               | 0.46   | 0.35  | 0.90    | 0.01| 0.30   | 0.44  | 0.62    |
| TC                               | 0.64   | 0.91  | 0.12    | 0.94| 0.77   | 0.45  | 0.84    |
| LDL-c                            | 0.45   | 0.68  | 0.02    | 0.60| 0.35   | 0.86  | 0.17    |
| cal. LDL-c                       | 0.50   | 0.58  | 0.76    | 0.67| 0.62   | 0.75  | 0.95    |
| HDL-c                            | 0.20   | 0.35  | 0.08    | 0.02| 0.64   | 0.15  | 0.67    |
| HMED                             | 0.81   | 0.97  | 0.02    | 0.49| 0.60   | 0.08  | 0.56    |
| LMEdN                            | 0.81   | 0.93  | 0.10    | 0.67| 0.44   | 0.46  | 0.76    |
| LDLppd                           | 0.56   | 0.58  | 0.03    | 0.89| 0.01   | 0.31  | 0.35    |
| BMED                             | 0.88   | 0.50  | 0.19    | 0.90| 0.04   | 0.86  | 0.65    |
| sBP                              | 0.40   | 0.51  | 0.07    | 0.79| 0.45   | 0.45  | 0.83    |
| dBP                              | 0.79   | 0.25  | 0.71    | 0.62| 0.37   | 0.49  | 0.67    |
| Pulse                            | 0.67   | 0.70  | 0.56    | 0.36| 0.23   | 0.30  | 0.00    |
| Adiponectin                      | 0.22   | 0.30  | 0.05    | 0.37| 0.92   | 0.57  | 0.84    |
| Leptin                           | 0.76   | 0.61  | 0.05    | 0.73| 0.10   | 0.77  | 0.40    |
| TNF-alpha                        | 0.90   | 0.81  | 0.94    | 0.32| 0.95   | 0.45  | 0.63    |
| IL-1/β                           | 0.58   | 0.59  | 0.59    | 0.04| 1.00   | 0.57  | 0.81    |
| IL-6                             | 0.34   | 0.87  | 0.57    | 0.82| 0.42   | 0.84  | 0.79    |
To prioritize genes mapped to the associated SNPs, we stressed their biologic function, statistical level and association of selected genes’ expression levels with related MetS phenotypes. In this respect, our highest level of priority at 3q27 was *IGF2BP2* whose sequence polymorphism was associated with WHR, the only clinical phenotype that was genetically intercorrelated with visceral fat size (VF). WHR was also correlated with other biologic markers including REE, all lipoprotein sizes profile and cytokine TNF-alpha. The transcript levels of *IGF2BP2* were also significantly associated with biologic measures of body composition phenotypes (Fatkg, Leankg, RQ, and REE), insulin/glucose responsiveness (SI and DI), lipoprotein sizes profile (HMED and LDLppd) and plasma leptin. The protein product of *IGF2BP2* binds the mRNA of insulin-like growth factor 2 (IGF2) and therefore regulates its translation. IGF2 is a hormone that affects growth and development in neonatal stages (27).

| Phenotype                                      | COPS3 | COX10 | PMP22 | RICH2 | TNFRSF13B | TRPV2 | HS3ST3B1 |
|-----------------------------------------------|-------|-------|-------|-------|------------|-------|----------|
| Body composition and insulin responsiveness   |       |       |       |       |            |       |          |
| weight                                       | 0.30  | 0.04  | 0.58  | 0.26  | 0.43       | 0.92  | 0.58     |
| height                                       | 0.85  | 0.18  | 0.73  | 0.57  | 0.86       | 0.67  | 0.21     |
| BMI                                           | 0.19  | 0.01  | 0.71  | 0.41  | 0.24       | 0.95  | 0.26     |
| WC                                            | 0.67  | 0.23  | 0.46  | 0.45  | 0.64       | 0.94  | 0.76     |
| HC                                            | 0.59  | 0.09  | 0.14  | 0.35  | 0.24       | 1.00  | 0.41     |
| WHR                                           | 0.66  | 0.13  | 0.54  | 0.95  | 0.03       | 0.37  | 0.26     |
| Fatkg                                         | 0.99  | 0.22  | 0.41  | 0.14  | 0.65       | 0.03  | 0.46     |
| Fatpct                                        | 0.93  | 0.16  | 0.62  | 0.19  | 0.36       | 0.03  | 0.19     |
| Leankg                                        | 1.00  | 0.23  | 0.61  | 0.03  | 0.58       | 0.81  | 0.49     |
| Leanpct                                       | 0.89  | 0.17  | 0.53  | 0.17  | 0.46       | 0.03  | 0.19     |
| SubQF                                         | 0.55  | 0.23  | 0.67  | 0.39  | 0.23       | 0.04  | NA       |
| VF                                            | 0.88  | 0.40  | 0.59  | 0.44  | 0.58       | 0.80  | NA       |
| TAF                                           | 0.75  | 0.24  | 0.65  | 0.49  | 0.24       | 0.10  | NA       |
| RQ                                            | 0.83  | 0.20  | 0.84  | 0.16  | 0.01       | 0.39  | NA       |
| REE                                           | 0.45  | 0.94  | 0.59  | 0.02  | 0.94       | 0.75  | NA       |
| REE/weight                                    | 0.85  | 0.97  | 0.99  | 0.00  | 0.83       | 0.99  | NA       |
| REE/leanmass                                  | 0.07  | 1.00  | 0.93  | 0.16  | 0.94       | 0.00  | NA       |
| FG                                            | 0.33  | 0.17  | 1.00  | 0.64  | 0.11       | 0.39  | 0.66     |
| Fl                                            | 0.68  | 0.07  | 0.07  | 0.64  | 0.14       | 0.53  | 0.81     |
| IGR                                           | 0.26  | 0.20  | 0.15  | 0.34  | 0.20       | 0.58  | 0.95     |
| HOMA                                          | 0.90  | 0.06  | 0.15  | 0.81  | 0.23       | 0.48  | 0.73     |
| SI                                            | 0.41  | 0.81  | 0.84  | 0.05  | 0.12       | 0.16  | NA       |
| SG                                            | 0.10  | 0.05  | 0.88  | 0.62  | 0.13       | 0.49  | NA       |
| AIR                                           | 0.28  | 0.38  | 0.65  | 0.03  | 0.60       | 0.42  | NA       |
| DI                                            | 1.00  | 0.59  | 0.54  | 0.07  | 0.34       | 0.35  | 0.34     |
| Lipids/lipoprotein profiles and cardiovascular performance |       |       |       |       |            |       |          |
| TG                                            | 0.70  | 0.04  | 0.01  | 0.79  | 0.91       | 0.19  | 0.47     |
| TC                                            | 0.91  | 0.63  | 0.68  | 0.69  | 0.21       | 0.40  | 0.90     |
| LDL-c                                         | 0.83  | 0.28  | 0.32  | 0.27  | 0.75       | 0.45  | 0.99     |
| cal. LDL-c                                    | 0.99  | 0.91  | 0.26  | 0.45  | 0.26       | 0.40  | 0.84     |
| HDL-c                                         | 0.31  | 0.46  | 0.42  | 0.19  | 0.26       | 0.29  | 0.97     |
| HMED                                          | 0.26  | 0.22  | 0.04  | 0.78  | 0.03       | 0.19  | 0.21     |
| LMEDn                                         | 0.71  | 0.55  | 0.16  | 0.82  | 0.06       | 0.63  | 0.28     |
| LDLppd                                        | 0.74  | 0.81  | 0.03  | 0.08  | 0.00       | 0.15  | 0.90     |
| BMED                                          | 0.96  | 0.95  | 0.46  | 0.27  | 0.00       | 0.34  | 0.43     |
| sBP                                           | 0.96  | 0.47  | 0.54  | 0.41  | 0.20       | 0.30  | 0.89     |
| dBP                                           | 0.54  | 0.89  | 0.09  | 0.95  | 0.33       | 0.22  | 0.43     |
| pulse                                         | 0.50  | 0.94  | 0.36  | 0.81  | 0.34       | 0.07  | 0.33     |
| Adipokine and cytokines                       |       |       |       |       |            |       |          |
| adiponectin                                   | 0.95  | 0.76  | 0.77  | 0.47  | 0.29       | 0.45  | 0.34     |
| leptin                                        | 0.55  | 0.69  | 0.45  | 0.05  | 0.08       | 0.99  | 0.14     |
| TNF-alpha                                     | 0.02  | 0.17  | 0.70  | 0.60  | 0.81       | 0.46  | 0.21     |
| IL-1/β                                        | 0.39  | 0.53  | 0.21  | 0.48  | 0.40       | 0.53  | 0.40     |
| IL-6                                          | 0.66  | 0.76  | 0.31  | 0.05  | 0.55       | 0.34  | 0.36     |

To prioritize genes mapped to the associated SNPs, we stressed their biologic function, statistical level and association of selected genes’ expression levels with related MetS phenotypes. In this respect, our highest level of priority at 3q27 was *IGF2BP2* whose sequence polymorphism was associated with WHR, the only clinical phenotype that was genetically intercorrelated with visceral fat size (VF). WHR was also correlated with other biologic markers including REE, all lipoprotein sizes profile and cytokine TNF-alpha. The transcript levels of *IGF2BP2* were also significantly associated with biologic measures of body composition phenotypes (Fatkg, Leankg, RQ, and REE), insulin/glucose responsiveness (SI and DI), lipoprotein sizes profile (HMED and LDLppd) and plasma leptin. The protein product of *IGF2BP2* binds the mRNA of insulin-like growth factor 2 (IGF2) and therefore regulates its translation. IGF2 is a hormone that affects growth and development in neonatal stages (27). In adults, IGF2 is known to influence regulation of lipids/lipoprotein...
| Annotated associated genes | Original QTL region | Identifier phenotype | Correlated biological phenotypes | Biological function |
|----------------------------|---------------------|----------------------|----------------------------------|---------------------|
| **3q27** | | | | |
| LPPa,b | 3q27 | AIR | Fatkg, DI, leptin | signal transduction to cell adhesion sites; cell to cell communication |
| IGF2BP2a,b | 3q27 | WHR | VF, TAF, REE, HMED, LMEDn, LDLppd, BMED, TNF-alpha | neonatal development, body fat regulation and lipid metabolism |
| TBL1XR1a | 3q27 | BMI, weight, Leanpct, TAF, REE, SG, TG | WHR, Fatkg, Fapct, Leankg, Leanpct, SubQF, VF, REE/weight, REE/Leanmass, SI, AIR, DI, HMED, leptin, IL-6 | member of WD40 protein family known to regulate signal transduction, RNA processing, vesicular trafficking, cytoskeletal assembly and cytotic differentiation. |
| NLGN1a | 3q27 | Leanpct | Fatkg, SubQF, VF, TAF, REE/weight, REE/leanmass, SI, AIR, HMED | member of a family of neuronal cell surface proteins, known to act as splice site-specific ligands for beta-neurexins that could be involved in the formation and remodeling of central nervous system member of the ATPase family that participate the uphill transport of ions across cell membranes, including H+, Na+, K+ and Ca++ |
| ATP11Ba | 3q27 | LDLppd | WHR, Fatkg, HMED, BMED, TNF-alpha | member of the guanine exchange factor family. Together with ADIPQ, SOX2 polymorphism influence diabetic complications. |
| MCF2L2a | 3q27 | Pulse | Leanpct, SubQF, TAF, REE, SI, adiponectin, leptin | Member of the fibroblast growth factor family possess broad mitogen and cell survival activities, involved in several biological functions. |
| FGF12 | 3q27 | IL-6 | VF | A lymphocyte-specific member of the tumor necrosis factor (TNF) receptor superfamily, which plays a crucial role in humoral immunity. |
| TNFRSF13Bab | 17p12 | VF, TAF, REE, sBP | weight, BMI, WC, HC, WHR, Fatkg, Fapct, Leankg, Leanpct, SubQ-F, REE/weight, FI, IGR, HOMA, SI, SG, DI, LDL-c, dBP, pulse, leptin, IL-6 | A biosynthesis enzyme of heparan sulfate find structures, whose substrates are important in the cellular uptake and biological effects of the lipoproteins that carry out multiple biologic activities. |
| HS3ST3A1ab | 17p12 | HC, Fatkg, Leanpct, FI, IGR, HOMA, SG, AIR, Adiponectin, TNF-alpha | weight, height, BMI, WC, WHR, SubQF, VF, TAF, REE/weight, REE/leanmass, FG, SI, DI, TG, TC, LDL-c, cal. LDL-c, HDL-c, HMED, LMEDn, LDLppd, BMED, sBP, pulse, leptin, IL-1β, IL-6 | A nuclear protein, a transcriptional co-activator that modulates the function of cardiac and smooth-muscle cells. Important in cardiogenesis and smooth muscle cell differentiation. |
| MYOCDa | 17p12 | Weight, BMI, WC, DI, TC, cal. LDL-c, dBP | height, hips, WHR, Fatkg, Fapct, Leankg, Leanpct, SubQF, VF, TAF, REE/weight, REE/leanmass, FI, IGR, HOMA, SI, SG, AIR, TG, LDL-c, HDL-c, HMED, sBP, pulse, leptin, IL-6 | A GTPase activator protein, which activate CDC42 and Rac-1, known to interfere with platelet growth factor Bβ-induced membrane ruffling. |
| RICH2a | 17p12 | Fatpct, TG | weight, BMI, WC, HC, WHR, Fatkg, Leanpct, SubQF, VF, TAF, REE/weight, REE/leanmass, FI, IGR, HOMA, SI, LG, TG, TC, HOMA, SI, SG, DI, LDL-c, HDL-c, HMED, sBP, pulse, leptin, IL-6 | A channel protein in response to environmental stresses. |
| TRPV2a | 17p12 | RO | Pulse | The terminal component of the mitochondrial respiratory chain, which catalyzes the electron transfer from reduced cytochrome c to oxygen. Mutations were linked to cytochrome c function deficiency. |
| COX10a | 17p12 | FG | FI, HOMA, AIR, leptin | The major component of the myelin structure in the peripheral nervous system that might function in growth regulation, and in myelinization in the peripheral nervous system |
| PMP22a | 17p12 | SI, HMED | weight, BMI, WC, HC, WHR, Fatkg, Fapct, Leanpct, Leanpct, SubQF, VF, TAF, REE/weight, FI, IGR, HOMA, TG, HDL-c, LMEDn, LDLppd, BMED, pulse | Target myosin phosphatases interacting with RhoA and ROCK1 to regulate actin cytoskeleton. |
| MPRPa | 17p12 | LMEDn, LDLppd, BMED | WHR, Fatkg, FI, IGR, HOMA, TG, HDL-c, HMED, TNF-alpha | The protein encoded by this gene possesses kinase activity that phosphorylates regulators involved in signal transduction and protects proteins from ubiquitin-dependent degradation. |
| COPS3 | 17p12 | IL-6 | BMI, VF | A biosynthesis enzyme of heparan sulfate find structures, whose substrates are important in the cellular uptake and biological effects of the lipoproteins that carry out multiple biologic activities. |

*Candidate genes prioritized from SNP association analysis whose transcript levels are associated with MetS phenotypes

*Biologic candidate genes with the highest prioritization
metabolism and body weight (29). Lean type 2 diabetic men and women whose weight gain within the next 5 years can be predicted by circulating levels of IGF2 (30). In a number of genome-wide association studies, IGF2BP2 has also been found to be associated with the onset of type 2 diabetes (31,32).

At 17p12 QTL, our first priority gene is TNFRSF13B, a member of a well-characterized receptor family for tumor necrosis factors. Polymorphisms in the proximity of TNFRSF13B were associated with four MetS phenotypes. High levels of associations were observed for multiple variants near or within TNFRSF13B with VF and TAF, two of the most informative biological phenotypes of body fat distribution. Plasma leptin levels, the identifier phenotype of this linkage region have been recognized to be a surrogate of adverse adiposity between VF and SubQF (33). In our cohort leptin levels were genetically correlated with SubQF and TAF. We also observed association of the transcript levels of TNFRSF13B with WHR, RQ, LDLppd, and BMED. TNFRSF13B, sometimes referred to as TACI

![Plots of SNP associations with MetS phenotypes within QTLs at 3q27 (panel A) and 17p12 (panel B). Dots depict levels of association of identifier phenotypes with all SNPs in the QTL region. Vertical axis represents minus logarithm of the P values and horizontal represents the chromosomal position (kb). In panel A, LPP and IGF2BP2 are the highly prioritized genes in the 3q27 QTL region. In panel B, TNFRSF13B and HS3ST3A1 are the highly prioritized genes in the 17p12 QTL region.](image-url)
(transmembrane activator) or CAML (calcium-modulating cyclophilin ligand interactor), is a receptor shared by two members of the tumor necrosis factor superfamily, APRIL (TNFSF13) and BAFF (the B-cell activation factor of the TNF family). APRIL and BAFF can activate macrophages via shared receptors such as TACI and initiate inflammatory changes leading to the induction of mediators of proinflammatory cytokines and matrix degrading enzymes (34,35). Adverse adiposity distribution particularly accumulation of visceral fat mass is linked to insulin resistance. One possible hypothesis is that chronic state of inflammation largely attributed to macrophage infiltration of visceral adipose tissue could play a major causative role in the pathogenesis of MetS. Our results suggest a role for TNFRSF13B in the etiology of several phenotypes that may be genetically linked via a network through inflammatory pathways.

These include VF, TAF, REE, WHR, and some lipid densities subclasses.

On the basis of our filtering criteria focusing on the biology of MetS, our other prioritized genes include LPP, MCF2L2, TBL1XR1, ATP8B1, and NLG1 at 3q27 and HSD3T3A1, COX10, PMP22, RICH2, TRPV2 at 17p12. Some of these genes have been implicated in functions that may potentially be related to the pathogenesis of MetS but more research is needed to elucidate their precise biological role. We also noticed that none of the SNPs we found significantly associated with MetS traits in our cohort have been identified in previous GWAS for related phenotypes. The genes some of these SNPs are mapped to, including IGF2BP2 and TNFRSF13B (31,32,36), have been implicated in diabetes related phenotypes. In conclusion, our QTL-based association and PBMC expression genetic analyses guided by biology-focused approach revealed novel genes that could account for the complexity of MetS phenotypes both in relation to their informativeness as well as the epistatic and pleiotropic influences of its identified QTLs. They also account at least in part for potential biologic pathways underlying the MetS phenotypes. Whereas IGF2BP2 could relate to aberrations in glucose/insulin responsiveness, TNFRSF13B could mediate its association with adverse adiposity as well as aberrations of plasma lipids/lipoprotein sizes profile.

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References

1. Hetherington MM, Cecil JE. Gene-environment interactions in obesity. Forum Nutr 2010;63:195-203.
2. Catenacci VA, Hill JO, Wyatt HR. The obesity epidemic. Clin Chest Med 2009;30:415-441.
3. Day C. Metabolic syndrome, or what you will: definitions and epidemiology. Diab Vasc Dis Res 2007;4:32-38.
4. Carmelli D, Cardon LR, Fabbris R. Clustering of hypertension, diabetes, and obesity in adult male twins: same genes or same environments? Am J Hum Genet 1994;55:566-573.
5. Gibson F, Fregoul P. Genetics of the APM1 locus and its contribution to type 2 diabetes susceptibility in French Caucasians. Diabetes 2004;53:2977-2983.
6. Curran JE, Jowett JB, Elliott KS, et al. Genetic variation in seleneoprotein S influences inflammatory response. J Nat Genet 2005;37:1234-1241.
7. Kraja AT, Vaidya D, Pankow JS, et al. A bivariate genome-wide approach to metabolic syndrome: STAMPED consortium. Diabetes 2010;60:1329-1339.
8. Zabaneh D, Balking DJ. A genome-wide association study of the metabolic syndrome in Indian Asian men. PLoS One 2010;5:e11961.
9. Wang X, Li WD, Zhang CK, et al. A genome-wide association study on obesity and obesity-related traits. PLoS One 2011;6:e18939.
10. Liu YZ, Pei YF, Liu JF, et al. Powerful bivariate genome-wide association analyses suggest the SOX6 gene influencing both obesity and osteoporosis phenotypes in males. PLoS One 2009;4:e6827.
11. Kissebah AH, Sonnenberg GE, Myklebust J, et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci USA 2000;97:14478-14483.
12. Grayson BL, Wang L, Aune TM. Peripheral blood gene expression profiles in metabolic syndrome, coronary artery disease and type 2 diabetes. Genes Immun 2011;12:341-351.
13. Chyssac C, Dina C, Leprêtre F, et al.EIF4A2 is a positional candidate gene at the 3q27 locus linked to type 2 diabetes in French families. Diabetes 2006;55:1171-1176.
14. Svendsen OL, Haarbo J, Heitmann BL, et al. Measurement of body fat in elderly subjects by dual-energy X-ray absorptiometry, bioelectrical impedance, and anthropometry. Am J Clin Nutr 1991;53:1117-1123.
15. Peiris AN, Hennes MI, Evans DJ, et al. Relationship of anthropometric measures of body fat distribution to metabolic profile in premenopausal women. Acta Med Scand Suppl 1988;723:179-188.
16. Bergman RN. Toward physiological understanding of glucose tolerance. Minimal model approach. Diabetes 1989;38:1512-1527.
17. Rainwater DL, Moore PH Jr, Shelledy WR, et al. Characterization of a composite gradient gel for the electrophoretic separation of lipoproteins. J Lipid Res 1997;38:1261-1266.
18. Dupont NC, Wang K, Wadhwa PD, et al. Validation and comparison of luminex multiplex cytokine analysis kits with ELISA: determinations of a panel of nine cytokines in clinical sample culture supernatants. J Reprod Immunol 2006;66:175-191.
19. Almasy L, Blangero J. Multisport quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198-1211.
20. Burdick JT, Chen W-M, Abecasis GR, et al. In silico method for inferring missing genotypes in pedigrees. Nat Genet 2006;38:1002-1004.
21. Görög HH, Curran JE, Johnson MP, et al. Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat Genet 2007;39:1208-1216.
22. Kent JW Jr, Comuzzie AG, Mahaney MC, et al. Intercellular adhesion molecule-1 concentration is genetically correlated with insulin resistance, obesity, and HDL concentration in Mexican Americans. Diabetes 2004;53:2691-2695.
23. Boerwinkle E, Chakraborty R, Sing CF. The use of measured genotype information in the analysis of quantitative phenotypes in man. I. Models and analytical methods. Ann Hum Genet 1986;50 (Part 2):181-194.
24. Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006;38:904-909.
25. Moskvina V, Schmidt KM. On multiple testing correction in genome-wide association studies. *Genet Epidemiol* 2008;32:567-573.
26. Whitlock MC. Combining probability from independent tests: the weighted Z-method is superior to Fisher’s approach. *J Evol Biol* 2005;18:1368-1373.
27. Nielsen J, Christiansen J, Lykke-Andersen J, et al. A family of insulin-like growth factor II mRNA-binding proteins represses translation in late development. *Mol Cell Biol* 1999;19:1262-1270.
28. Ochiai Y, Serizawa M, Yanai K, et al. Search for type 2 diabetes susceptibility genes on chromosomes 1q, 3q and 12q. *J Hum Genet* 2008;53:314-324.
29. Li X, Cui H, Sandstedt B, et al. Expression levels of the insulin-like growth factor- II gene (IGF2) in the human liver: developmental relationships of the four promoters. *J Endocrinol* 1996;149:117-124.
30. Heald AH, Kärvestedt L, Anderson SG, et al. Low insulin-like growth factor-II levels predict weight gain in normal weight subjects with type 2 diabetes. *Am J Med* 2006;119:167.e9-15.
31. Voight BF, Scott LJ, Steinhorsdottir V, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. *Nat Genet* 2010;42:579-589.
32. Zeggini E, Scott LJ, Saxena R, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. *Nat Genet* 2008;40:638-645.
33. Cnop M, Landchild MJ, Vidal J, et al. The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. *Diabetes* 2002;51:1005-1015.
34. Lee SM, Kim EJ, Suk K, et al. BAFF and APRIL induce inflammatory activation of THP-1 cells through interaction with their conventional receptors and activation of MAPK and NF-xB. *Inflamm Res* 2011;60:807-815.
35. Lee SM, Kim WJ, Suk K, et al. Cell to cell interaction can activate membrane-bound APRIL, which are expressed on inflammatory macrophages. *Immune Netw* 2010;10:173-180.
36. Grassi MA, Tikhomirov A, Ramalingam S, et al. Genome-wide meta-analysis for severe diabetic retinopathy. *Hum Mol Genet* 2011;20:2472-2481.