Two Major QTLs and Several Others Relate to Factors of Metabolic Syndrome in the Family Blood Pressure Program

Aldi T. Kraja, Dabeeru C. Rao, Alan B. Weder, Richard Cooper, J. David Curb, Craig L. Hanis, Stephen T. Turner, Mariza de Andrade, Chao Agnes Hsiung, Thomas Quertermous, Xiaofeng Zhu, Michael A. Province

Abstract—Genome-wide variance components linkage analysis was performed on 4 latent factors underlying metabolic syndrome derived from 10 risk factors. The latent factors represent obesity and insulin, blood pressure, lipids and insulin, and central obesity. The metabolic syndrome factor scores were derived in 4 ethnic groups recruited in 3 Networks of the Family Blood Pressure Program: GENOA (blacks, Hispanics, and whites), HyperGEN (blacks and whites), SAPPHIRE (Asians). Heritabilities of metabolic syndrome factors ranged from 66% for obesity and insulin to 11% for blood pressure factor. We observed higher heritabilities for obesity and insulin, and lipids and insulin, whereas those for blood pressure and central obesity were smaller. Linkage analysis detected two major quantitative trait loci. One of them linked to the obesity and insulin factor with a lod score of 3.94 ($P=0.00001$, marker GATA11A06, D18S53, 41.24 cM) at marker positions linkage (lod 4.71, at 46.84 cM at 1-cM-apart distances linkage), located on chromosome 18p11.21 in GENOA black. The other linked to the blood pressure factor with a lod score of 3.22 ($P=0.000059$, marker GATA49C09, D17S1290, 82 cM) at marker positions linkage (lod 3.56, at 84.63 cM for 1 cM apart distances linkage) located on chromosome 17q23.1 in Hispanics. These quantitative trait loci, together with 4 additional ones with lod scores $>2.5$, and 30 additional ones with lod score $>1.7$, offer hope for dissecting the genetic architecture of metabolic syndrome with beneficial implications for molecular diagnosis, prognosis, and in potential medical intervention. (Hypertension. 2005;46:751-758.)

Key Words: blood pressure ■ insulin ■ lipids ■ metabolic syndrome ■ obesity

Metabolic syndrome (MetS), comprising a constellation of obesity (OBS), insulin (INS) resistance, hypertension (HT), dyslipidemia, and prothrombotic and proinflammatory states, is an important public health problem because the component risk factors contribute considerably to morbidity and mortality from cardiovascular diseases (CVD).1–4 Therefore, identifying genetic causes of MetS is of paramount significance. Epidemiological studies have contributed a more accurate definition of MetS, especially emphasizing the fact that MetS includes, but is not the same as, insulin resistance.1,13–14 Multivariate analysis such as factor analysis of the MetS risk factors has identified important latent factors. Investigations of MetS through factor analysis have varied in terms of the number of risk factors considered and different studies have reported a group of factor domains15–22 or a single (primary) MetS factor.23–29 A number of clinical studies have been performed for attenuating the compound effect of MetS components. For example, angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors, activation of receptor y of peroxisome proliferators, and statins therapy have shown individually protective effects against CVD.30–32 It has also been shown that interventions on MetS components and type 2 diabetes (T2D) with lifestyle changes can significantly alter the risk of MetS in animals and humans.33–34

The preceding work has emphasized many important aspects of MetS, but finding genetic causes of MetS is vital, because knowing the genetic causes of MetS can pave the way to control the risk for coronary heart disease and T2D. Although the genetic analysis of MetS is still in its early stages, recently a group of studies have reported quantitative trait loci (QTLs) for MetS or its components.28,35–38 In continuation of these efforts, we attempt to shed some light
on putative QTLs and genes for MetS latent factors based on one of the largest ethnically diverse family studies known as the Family Blood Pressure Program (FBPP) supported by the National Heart, Lung, and Blood Institute (NHLBI). The FBPP represents a consortium of 4 large Networks funded by the NHLBI, whose main goal is to study the causes of high blood pressure.

Factor analysis of 10 risk variables in 4 major ethnic groups (blacks, whites, Hispanics, and Asians) has characterized MetS in terms of 4 latent factors (see expanded Methods at http://hyper.ahajournals.org). Here we report genome-wide linkage analyses that identified important putative QTLs for the latent factors underlying MetS.

### Populations Sampled and Methods

The FBPP pooled database is described in detail elsewhere. All studies included in the FBPP were approved by the corresponding institutional review committees and subjects gave their informed consent. In short, the FBPP includes large samples on blacks, whites, Hispanics, and Asians in 4 multicenter Networks funded by the NHLBI for identifying genetic causes of hypertension. One of the 4 Networks, GenNet, was excluded from our analysis for not having available the lipid data. We have performed factor analysis on the following 10 risk variables in the GENOA, HyperGEN, and SAPPHIRE Networks: body mass index (BMI) (kg/m²), waist circumference (WAIST) (cm), waist-to-hip ratio (WHR), fasting INS (mU/dL), fasting glucose (GLUC) (mg/dL), systolic blood pressure (SBP), diastolic blood pressure (DBP) (mm Hg), low-density lipoprotein (LDL) cholesterol (mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), and fasting triglycerides (TG) (mg/dL).

All participants with missing values for any of the 10 risk factors were excluded. Data on INS, GLUC, and TG were excluded if the fasting time was <8 hours. Each of the risk factors was adjusted for age, age², and age³, and for the field center when appropriate, within gender, race, and Network. Factor analysis of the 10 risk factors, using the maximum likelihood method, yielded 4 factors: obesity and insulin factor (Obesity-INS), blood pressure factor (BP), lipids and insulin factor (Lipids-INS) and central obesity factor (Central-OBS) (see Table 1 at http://hyper.ahajournals.org). Factor analysis was performed both with and without rotation. In the linkage analyses we used factor scores produced by factor analysis with Varimax rotation.

More details, as well as asserting normal distribution of each risk factor and any transformations, are provided online.

DNA was extracted from whole blood by standard methods at each of the 4 networks. Approximately 400 microsatellite markers were genotyped by the NHLBI Mammalian Genotyping Service (Marshfield, Wis), for an average spacing of 10 cM, which covers ~95% of the human genome. Screening Set 8 of markers was applied for all 4 networks. Extensive quality control operations yielded complete data on 370 autosomal markers. The identity by descent coefficients were estimated in nuclear families with the MAPMAKER/SIBS. Linkage analysis was performed in nuclear families by applying at marker positions (and for 2 most highest lod score results also at 1-cM-apart distances), the multipoint variance components linkage analysis using SEGPATH. The variance components methods are well-known and have been explained extensively in the literature and consequently are only briefly described here. In general, the phenotypic variance (Vp) is partitioned into several familial and nonfamilial components of variance. The familial components include additive genetic and shared environmental variances (Vg), whereas the nonfamilial component is described as a nonshared or residual environmental variance (Vr). For linkage analysis, this model is extended by dividing the total genetic variance into an additive variance component at a measured trait locus (Vg) and a residual polygenic variance component (Vr). The proportion of the total phenotypic variance that is because of the additive polygenic component (Vg) is the residual genetic heritability (h²g = Vg / Vp), whereas that caused by the trait locus represents the QTL heritability (h²l = Vl / Vp).

### Results

The number of nuclear families and sib pairs analyzed in each network were, respectively, as follows: GENOA blacks, 696 and 1312; GENOA Hispanics, 442 and 2670; GENOA whites, 509 and 1510; HyperGEN blacks, 1202 and 1724; HyperGEN whites, 649 and 1180; SAPPHIRE Chinese, 407 and 2072; and SAPPHIRE Japanese, 158 and 596.

Tables 1 and 2 show the heritabilities for each factor. The Obesity-INS factor heritability estimates suggested relatively large genetic influences in blacks 0.61 ± 0.06 (GENOA), 0.55 ± 0.05 (HyperGEN); in whites 0.47 ± 0.05 (GENOA), 0.62 ± 0.05 (HyperGEN); in Hispanics 0.58 ± 0.05; and in

### Table 1. Heritability Coefficients for the Latent Factors of MetS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>GENOA</th>
<th>HyperGEN</th>
<th>SAPPHIREChinese</th>
<th>SAPPHIREJapanese</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blacks</td>
<td>Blacks</td>
<td>Chinese</td>
<td>Japanese</td>
</tr>
<tr>
<td>Obesity_INS</td>
<td>0.66</td>
<td>0.55</td>
<td>0.65</td>
<td>0.55</td>
</tr>
<tr>
<td>BP</td>
<td>0.35</td>
<td>0.35</td>
<td>0.18</td>
<td>0.33</td>
</tr>
<tr>
<td>Lipids_INS</td>
<td>0.47</td>
<td>0.35</td>
<td>0.47</td>
<td>0.43</td>
</tr>
<tr>
<td>Central_OBS</td>
<td>0.30</td>
<td>0.36</td>
<td>0.49</td>
<td>0.38</td>
</tr>
</tbody>
</table>

### Table 2. Heritability Coefficients for the Latent Factors of MetS

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>GENOA</th>
<th>HyperGEN</th>
<th>GENOA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whites</td>
<td>Whites</td>
<td>Hispanics</td>
</tr>
<tr>
<td>Obesity_INS</td>
<td>0.47</td>
<td>0.60</td>
<td>0.58</td>
</tr>
<tr>
<td>BP</td>
<td>0.11</td>
<td>0.31</td>
<td>0.37</td>
</tr>
<tr>
<td>Lipids_INS</td>
<td>0.54</td>
<td>0.43</td>
<td>0.43</td>
</tr>
<tr>
<td>Central_OBS</td>
<td>0.26</td>
<td>0.24</td>
<td>0.33</td>
</tr>
</tbody>
</table>
Japanese 0.55±0.11 and 0.65±0.07 for the Chinese sample. Lower genetic influences were found for the BP factor, with the highest values 0.37±0.04 in the Hispanics and with lowest values in the whites (GENOA) 0.11±0.02 and Chinese 0.18±0.03. For Lipids-INS factor, the heritabilities were highest in whites (GENOA) 0.54±0.04 and the lowest in blacks (HyperGEN) 0.35±0.04. Central-OBS had a lower heritability compared with Obesity-INS factors.

The most prominent linkage evidence was obtained for the Obesity-INS factor with a lod score of 3.94 (P=0.00001, GENOA blacks; marker GATA11A06, on chromosome 18, 41.24 cM) when linkage analysis was applied at marker locations (Table II). When linkage analysis was applied at 1-cM distances, the 1-lod score interval was located within 38 to 55 cM, by reaching its maximum of 4.71 lod score at 46.84 cM. Other Obesity-INS factor lod score results were 2.59 (P=0.00028, GENOA whites, marker GATA81D12, on chromosome 16, 87.62 cM), 2.48 (P=0.00036, SAPPHiRe Chinese, marker AFM044XG3, on chromosome 17, 116.86 cM), 2.40 (P=0.00044, HyperGEN whites, marker GATA30E06, on chromosome 2, 210.43 cM), 2.39 (P=0.00046, GENOA whites, marker COS14OD4, on chromosome 8, 43.96 cM), 2.35 (P=0.00050, GENOA whites, marker GGAA9D03, on chromosome 17, 50.74 cM) and 1.95 (P=0.00138, HyperGEN blacks, marker GATA23C03, on chromosome 13, 8.87 cM) (Tables II to VIII).

A lod score of 3.22 was found for the BP factor on chromosome 17 in Hispanics (P=0.000059, with the maximum reached at marker GATA28011, 100.02 cM). A lod score of 1.95 was found for the BP factor on chromosome 4 (P=0.00138, marker GATA10G07, 88.35 cM). Around the same region, much smaller peaks were found for the BP factor in GENOA blacks and whites, and also in Chinese (Tables II to VIII).

Lipids-INS factor showed a linkage peak of 2.66 on chromosome 11 in the HyperGEN blacks (P=0.00023, marker AFM157XH6, at 131.26 cM), a lod score of 2.43 in the HyperGEN whites on chromosome 8 (P=0.00041, marker AFM143XD8, 0.73 cM), and 2.07 on chromosome 4 in the Chinese (P=0.001, marker GATA107, at 145.98 cM).

For Central-OBS, several moderate linkage peaks were found, especially a lod score of 2.67 in GENOA blacks (P=0.00023, marker ATA26D07, chromosome 13, 82.93 cM), 2.61 in Japanese (P=0.00027, marker GATA4A10, chromosome 3, 152.62 cM), and a 2.46 in HyperGEN blacks (P=0.00038, marker GATA81E09, chromosome 20, 32.94 cM) (Tables II to IV and V to VIII).
the online supplements). BMI, WAIST, LDL, and HDL had a median heritability $\geq 40\%$, whereas a median heritability close to 30% resulted for WHR, INS, GLUC, SBP, and DBP. Twin data generally had higher heritability estimates. Given these heritabilities, it is not surprising that we found smaller heritabilities for BP and Central-OBS as compared with the heritabilities of Obesity-INS and Lipids-INS. Similar trends have been reported in the literature for the MetS factors.\textsuperscript{17,44}

The most important findings in our analyses were 2 linkage peaks above a lod score of 3 (Figures 2 and 3). The QTL for Obesity-INS factor in GENOA blacks was located in chromosome 18p11.21 at GATA11A06 (D18S53) marker location, but within 38 to 55 cM 1-lod interval when linkage at 1-cM-apart distance was performed.

Although Soria et al\textsuperscript{45} have reported a lod score of 4.5 close to the D18S53 marker for “activated protein C (APC) resistance,” which, as a risk factor, accounted for 20% to 60% of the familial thrombophilia, we cannot provide any evidence that this trait is related to the Obesity-INS factor. It is well known the associations of the melanocortin 4 receptor (MC4R) with obesity.\textsuperscript{46–48} However, this gene is located on the q arm of the same chromosome. It is possible that the presence of another member of melanocortin receptors, melanocortin 5 receptor (MC5R), in the same region with our QTL

Figure 2. MetS factors linkage analysis—GENOA: blacks, chromosome 18.

Figure 3. MetS factors linkage analysis—GENOA: Hispanics, chromosome 17.
might provide a candidate gene that needs to be tested. Chagnon et al have shown that in the Quebec Family Study, MC5R was strongest in linkage and association with obesity phenotypes compared with MC4R.49 Parker et al have reported a QTL region for type 2 diabetes on chromosome 18p11, which improved the linkage signal when subsetting groups by age and BMI.50 Tilburg et al have replicated the Parker et al finding for the same QTL region in an independent sample of Dutch population.50–51 Although the Dutch study involves a larger region with a maximum peak more distant than ours, it shows a lod score of \( \approx 1.5 \) at our linkage peak on chromosome 18p11. Similar findings (lod \( \approx 1.3 \)) on chromosome 18 (D18S53, 41.24 cM) for multiple sclerosis is replicated in Australian sib-pairs.52

The QTL for the BP factor in Hispanics was located on chromosome 17q23.1 with a peak located at the marker GATA49C09 (D17S1290) location, but within 74 to 94 cM 1-lod score interval when linkage at 1-cM-apart distance was performed. The Chinese and Japanese as well as the whites in HyperGEN showed lower peaks for BP factor around that region. Searching in 1-lod score interval around the linkage peak for probable candidate genes, the most probable one is the ACE gene.53 ACE gene, located in a region between AFM268yd5 (89 cM) and UT9 (97 cM) microsatellite markers, encodes an enzyme involved in catalyzing the conversion of angiotensin I into a physiologically active peptide angiotensin II. Angiotensin II is a potent vasopressor that controls blood pressure. ACE is also able to inactivate bradykinin, a potent vasodilator. ACE enzyme plays a key role in the renin-angiotensin system. Other possible candidates exist, although they are more distant. For example, rare mutations in WNK4 gene, (located between 58 cM) and UT9 (97 cM) microsatellite markers, encodes an enzyme inferred by conserved synteny maps among mouse, rat, and human59–60) were found to be associated with hypertension. Therefore, further investigations in might bear some hidden correlation to body height. The list of coefficients of correlations to height (\( r, P \)) follows: BMI (\(-0.21688, <.00001\)), WAIST (\(-0.01758, 0.4489\)), INS (\(-0.00557, 0.8111\)), WHR (\(0.20410, <.00001\)), GLUC (\(0.02883, 0.2157\)), and HDL (\(-0.18550, <.0001\)). A QTL with a lod score of 2.59 for Obesity-INS factor in GENOA whites was located at marker GATA81D12 (D16S2624, 16q22). Jawaheer et al reported a QTL (\( P = 0.0339 \)) at the same location for rheumatoid arthritis.60 It is possible that inflammatory processes prevalent in obese people, as well as in those having rheumatoid arthritis, may point on similar inflammatory pathway(s).

An additional interesting QTL in our study was related to Central-OBS factor in Japanese located at marker GATA4A10 (D3S1764, 3q23 152.62 cM). Around the same location (marker D3S1764), Wu et al reported a BMI QTL with a lod score of 3.45 for the GENOA blacks.64 The strength of our study originates from the fact that we used large family samples, multivariate latent factors, 4 major ethnicities, and microsatellite markers with an average spacing of \( \approx 10 \) cM. Some of our findings replicate with single traits from other studies. However, we did not have a full evidence of replication about the QTLs for BP in Hispanics and Obesity-INS factor in blacks in other ethnicities/networks of FBPP. Hirshhorn et al have demonstrated through simulations that a modest QTL (explaining 20% of variance) can produce strong signal in one scan, but it can be undetectable in another scan, merely because of sampling variation.62 An added possibility is that a common causal genetic mutation in one population might be rare in another population. It is important to note that sampling criteria did vary among the Networks and/or ethnicities.39,40

Another problem that may arise in testing many markers is the problem of false discoveries. Rao and Gu suggested that to achieve a minimum of false results in a linkage analysis of 400 markers one may relax the threshold to a lod score of \( \approx 1.75 \), which corresponds to a tolerance of 1 false-positive per genome scan.65 It is expected that this threshold, used by us in reporting results, may achieve a better “balance” of the types of the statistical errors. However, clustering of risk factors into MetS factors reflects multiple interrelations among risk factors as is the case of Obesity-INS, Lipids-INS factors, or a manifestation of a dominant common factor, as is the case of BP and central OBS factors. This arrangement of the risk factors into MetS factors lessens multiple comparisons issues.38

Two appealing examples that implicate ACE one of our candidate genes show that age, environments, and the interaction of genes influence gene findings. Mashimo et al analyzing the influence of aging and salt-loading in modulating BP QTLs in rats reported 3 QTLs with peaks at 8 or 10 weeks of age.66 After a salt-loading stage, one of the previous peaks and a new high lod peak on chromosome 10 that included ACE gene were identified, showing that age and salt intake controlled gene-phenotype expression. Borecki et al in the Family Heart Study (FHS) found that the AGT gene, but not ACE, was a significant predictor of hypertension.67 However, the interaction of homozygous risk genotypes for AGT and ACE was strongly associated with hypertension. Therefore, further investigations in
understanding the genetic structure of MetS will have beneficial implications in molecular diagnosis, prognosis, and in medical intervention in addition to contributing to a better perception of the genetic design of the multifaceted traits.

Perspectives
In itself, MetS represents a combination of a group of abnormalities that have become a source of increased risk for CVD. It is possible that for the MetS constituent abnormalities different biochemical and physiological pathways exist. It is also expected that independently, as well as in interaction, they contribute in the development and in the excess expression of MetS. Consequently, identifying genes that contribute significantly in any of the MetS constituent abnormalities can provide important information in understanding, preventing, and treating MetS. The 2 major QTLs reported, together with several other QTLs identified, warrant finer association tests in discovering causative genes for the constituent abnormalities of MetS.

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