Genetic Variants Within the *LPIN1* Gene, Encoding Lipin, Are Influencing Phenotypes of the Metabolic Syndrome in Humans

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OBJECTIVE—Lipin, a novel molecular protein expressed by adipocytes, has marked effects on adipose tissue mass, insulin sensitivity, and glucose homeostasis. Thus, we hypothesized that genetic variants within *LPIN1* are associated with traits of the metabolic syndrome.

RESEARCH DESIGN AND METHODS—A total of 15 single nucleotide polymorphisms (SNPs) covering the *LPIN1* gene region were genotyped in an age- and sex-stratified sample of the general population (Monitoring Trends and Determinants on Cardiovascular Diseases Study Augsburg; DNA and phenotypes of 1,416 Caucasians). Ten SNPs were also genotyped for replication in an independent sample of 1,030 subjects recruited throughout Germany. The metabolic syndrome was defined via the sum of its core components and, additionally, by a factor score derived from factor analysis. Permutation-based methods were used to test the association between genetic *LPIN1* variants and metabolic traits for empirical significance.

RESULTS—Linkage disequilibrium (LD) analysis revealed three LD blocks encompassing *LPIN1*. We identified three associated three-marker haplotypes: one common haplotype (26.8% frequency) increases the risk for the metabolic syndrome (odds ratio 1.6 [95% CI 1.2–2.2]), while the other two, being less common (5.7 and 4.0%), are strongly associated with lower blood pressure levels (systolic blood pressure 127 ± 18 vs. 135 ± 20 mmHg; $P = 0.0001$), a lower BMI (24.6 ± 3.6 vs. 26.9 ± 4.1 kg/m^2; $P = 3.7 \times 10^{-7}$) and waist circumference (82 ± 12 vs. 90 ± 12 cm; $P = 3.2 \times 10^{-8}$), lower A1C levels (5.1 ± 0.7 vs. 5.3 ± 0.9%; $P = 0.0002$), as well as a lower metabolic syndrome factor score (−0.67 ± 1.00 vs. 0.04 ± 1.24; $P = 1.4 \times 10^{-7}$). Furthermore, the frequencies of arterial hypertension (23.7 vs. 46.4%; $P = 0.00001$), obesity (12.9 vs. 30.8%; $P = 0.0003$), diabetes (2.2 vs. 8.2%; $P = 0.041$), and the presence of three or more metabolic syndrome components (3.3 vs. 13.7%; $P = 0.002$) were significantly lower than in subjects not carrying one of these protective haplotypes. Strong associations were also observed in the replication sample using the same haplotypes but with effects in the opposite direction.

CONCLUSIONS—These data suggest that allelic variants of the *LPIN1* gene have significant effects in human metabolic traits and thus implicate lipin in the pathophysiology of the metabolic syndrome. ***Diabetes*** **57:209–217, 2008**

Obesity has been described as the central causative component in the development of the metabolic syndrome. In fact, increased adiposity is associated with many metabolic alterations, including insulin resistance, dyslipidemia, and hypertension, all key components of the metabolic syndrome. It is now evident that adipose cells secrete multiple bioactive molecules collectively referred to as adipokines, and many of these have been implicated in the association between central adiposity and cardiovascular pathology. From the wide range of adipokines identified over the past few years, it is apparent that adipose tissue is a secretory organ of considerable complexity that is closely integrated into overall physiological and metabolic control. Both in humans and in animal models, it has been shown that either increased adipose tissue mass, as seen in obesity, or abnormally low amounts of adipose tissue, as seen in lipodystrophy, lead to metabolic dysregulation and insulin resistance (1–3). Thus, factors that influence adipose tissue mass and function exert important effects on metabolic homeostasis (4).

Human and rodent data provide evidence that lipin is one crucial factor involved in the development and function of adipose tissue (5,6). Lipin is a novel protein that is prominently expressed in peripheral tissues, namely adipose tissue and skeletal muscle. It has been identified through positional cloning of the mutated gene (*LPIN1*) in a mouse model of lipodystrophy (fatty liver dystrophy mouse [fld]), which is characterized by lipin deficiency, reduction of adipose tissue mass, mild hyperglycemia, and insulin resistance (5,7). In contrast, enhanced lipin expression in adipose tissue of lipin transgenic mice results in increased expression of lipogenic genes, increased lipid storage, and accelerated diet-induced obesity (8). These findings suggest that lipin promotes lipodystrophy in its absence as well as obesity when present at high levels (8). Thus, lipin might be critical for adipocyte differentiation and the maintenance of mature adipocyte function and lipogenesis and thereby may have profound effects in the pathogenesis of obesity, adipocyte gene expression, and metabolic alterations associated with obesity (9).

Recent findings (10,11) report on a strong negative

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LD, linkage disequilibrium; MI, myocardial infarction; MONICA, Monitoring Trends and Determinants on Cardiovascular Diseases; SNP, single nucleotide polymorphism.

TABLE 1
Clinicial and anthropometric characteristics of the MONICA study population according to sex

	Men	Women
n	721	690
Age (years)	52 ± 14	52 ± 14
BMI (kg/m^2)	27.0 ± 3.6	26.4 ± 4.7
Waist circumference (cm)	95 ± 10	83 ± 11
Obesity (%)*	25.8	33.5
HDL cholesterol (mg/dl)	48 ± 14	60 ± 17
Dyslipidemia (%)*	29.0	29.0
Systolic blood pressure (mmHg)	137 ± 19	131 ± 21
Diastolic blood pressure (mmHg)	83 ± 12	79 ± 11
Arterial hypertension (%)*	49.2	40.4
A1C (%)	5.2 ± 0.9	5.3 ± 0.8
Diabetes (%)*	7.3	8.4
Three or more metabolic syndrome components (%)*	10.3	12.5

Data are means ± SD unless otherwise indicated. *Definitions are as defined in the text.

correlation between lipin mRNA expression, fasting glucose and insulin levels, and insulin resistance in both human and mouse adipose tissues. Moreover, intragenic polymorphisms and haplotypes exhibited associations with serum insulin levels and BMI, highlighting the importance of lipin in glucose homeostasis and obesity (10), both critical contributors to the metabolic syndrome. Interestingly, the gene encoding lipin, *LPIN1*, is localized on chr2p, a genomic region that has previously been linked to fat mass and plasma leptin levels (12–15).

Based on these data, we hypothesized that genetic variations across *LPIN1* contribute to the expression of phenotypes related to the metabolic syndrome and carried out a comprehensive genetic association analysis. We systematically explored the linkage disequilibrium (LD) and haplotype structures of the *LPIN1* genomic region with single nucleotide polymorphisms (SNPs) and assessed the role of common sequence variants and haplotypes in the metabolic syndrome and its components in a representative sample of the general population. In addition, we sought to replicate our results in an independent cohort recruited throughout Germany.

RESEARCH DESIGN AND METHODS

General population. The subjects participated in the echocardiographic substudy (total n = 1,674) of the third Monitoring Trends and Determinants on Cardiovascular Diseases (MONICA) Augsburg survey 1994/1995. The third survey represents a sex- and age-stratified random sample of all German residents of the Augsburg area and consists of individuals aged 25–74 years ([means ± SD] 51.8 ± 13.8), including 851 (50.7%) women and 827 (49.3%) men and ~300 subjects for each 10-year increment. The study design, sampling frame, and data collection have been described in detail elsewhere (16). Briefly, all the participants completed a detailed questionnaire on demography, medication, history of concomitant diseases, cardiovascular risk factors, lifestyle, health behavior, and psychological factors. Anthropometric data were obtained by detailed physical examination. The clinical characteristics of this study population are summarized in Table 1. Unfortunately, fasting insulin, fasting glucose, and fasting triglyceride levels were not available in this MONICA study sample. The study was approved by the local ethics committee, and all participants gave written informed consent.

Replication cohort. Our replication cohort consisted of spouses, brothers-in-law, and sisters-in-law of members of the German Myocardial Infarction Family Study. The ascertainment strategy and study design have been described in detail elsewhere (17). Briefly, myocardial infarction (MI) kindreds were ascertained through index patients, who were identified by screening >200,000 patient charts in 14 cardiac in-hospital rehabilitation centers distributed throughout Germany. Index patients had all suffered from MI before the age of 60 years. If at least one sibling had suffered from MI or had severe coronary artery disease (percutaneous transluminal coronary angioplasty or bypass surgery before the age of 70 years), the nuclear family (index patient, available parents, all siblings, and all spouses) was contacted and invited to participate in the study. The study protocol was approved by the ethics committee of the University of Regensburg, Germany, and all participants gave informed consent.

TABLE 2
Clinical and anthropometric characteristics of subjects in the replication sample

	Men	Women
n	380	649
Age (years)	60 ± 10	56 ± 9
BMI (kg/m^2)	27.3 ± 3.8	27.0 ± 4.5
Obesity (%)*	16.8	19.1
HDL cholesterol (mg/dl)	54 ± 12	66 ± 15
Fasting triglycerides (mg/dl)	157 ± 130	125 ± 68
Dyslipidemia (%)*	15.6	17.0
Arterial Hypertension (%)*	45.5	40.3
A1C (%)	5.6 ± 0.8	5.5 ± 0.6
Diabetes (%)*	11.7	7.5
Three of more metabolic syndrome components (%)*	8.9	8.3

Data are means ± SD unless otherwise indicated. *Definitions are as defined in the text.

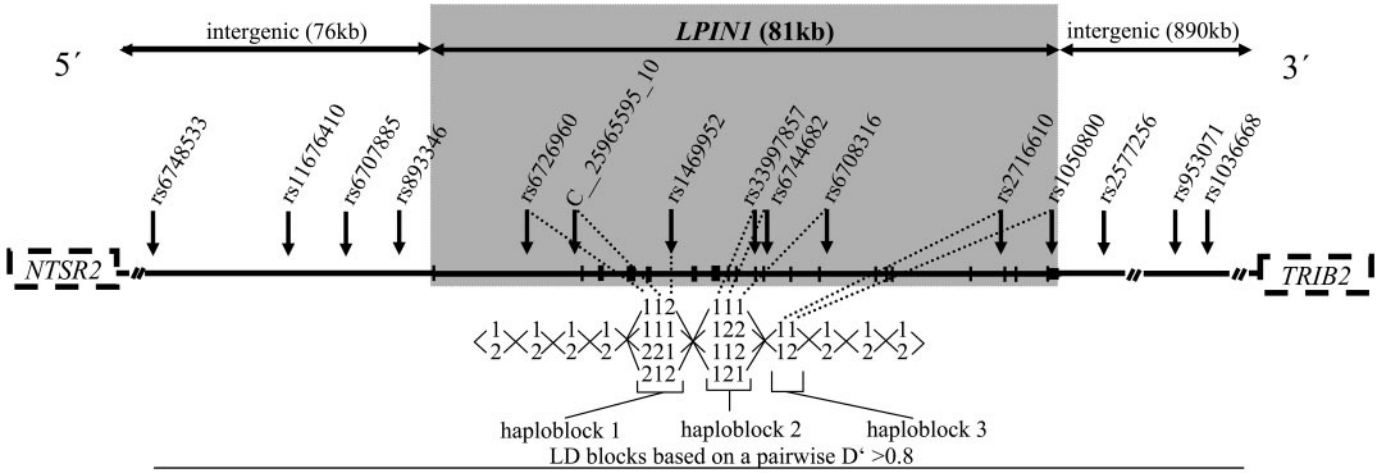
The cohort for the present replication study consisted of unrelated Caucasian individuals (n = 1,029, n = 380 men, and n = 649 women) who were examined by the same protocol as the affected family members and had no evidence of coronary artery disease by history and physical examination. Descriptive statistics for this sample are presented in Table 2. Contrary to the MONICA study sample, fasting triglyceride levels were available in this sample.

SNPs and genotyping

SNPs. Fifteen SNPs covering the *LPIN1* gene and intergenic regions were genotyped in the MONICA study sample. Of 1,674 subjects, both DNA samples and high-quality phenotypic data were available in 1,411 subjects. Information for SNPs (rs number, polymorphic site, and localization within the gene) was taken from SNP public databases (dbSNP [http://www.ncbi.nlm.nih.gov/SNP], GeneWindow [http://genewindow.nci.nih.gov], and Applied Biosystems [www.appliedbiosystems.com]). SNPs were selected with priority to HapMap SNPs (http://www.hapmap.org), tagging the gene based on an r^2 cutoff = 0.8 and a minor allele frequency >5%. However, if no predesigned Taqman assay for these tagSNPs was availabe, we selected alternative SNPs using the following selection criteria: validated SNPs with minor allele frequency >5%, submitted multiple times or discovered by The SNP Consortium, location in conserved coding or noncoding sequences that are in high LD and as close as possible to the respective HapMap tagSNP. Of 15 selected SNPs, 4 covered a region of 76 kb past the 5′ end of the gene, 1 was located in exon 10, 6 were in the introns, 1 was in the untranslated region 3′, and 3 were within 890 kb past the 3′ end of the gene (Fig. 1). In total, a region of 281 kb was covered with SNPs. The coding SNP (rs33997857) led to a nonsynonymous amino acid substitution (V494M). The seven intergenic SNPs were picked to determine the extent of LD and to explore the impact of sequence variations in noncoding and intergenic regions on the disease. In the replication sample, a reduced set of 10 SNPs were genotyped due to the LD structure (rs6748533, rs6707885, rs893346, rs2577256, and rs1036668 were not genotyped).

Genotyping. SNPs were genotyped using a 5′-exonuclease activity (TaqMan) assay on an HT7900 (Applied Biosystems, Darmstadt, Germany). SNP assays were ordered from Applied Biosystems either as Custom TaqMan SNP genotyping assays or predesigned TaqMan SNP genotyping assays. Probes were labeled with the fluorophores FAM or VIC. Genotyping was done on 384-well plates prepared with the GENESIS Freedom pipetting robot from Tecan (Crailsheim, Germany). The Universal PCR Master Mix from Applied Biosystems was used in a 5-μl total reaction volume with 10 ng DNA per reaction. Allelic discrimination was measured automatically on the ABI Prism HT7900 (Applied Biosystems) using the Sequence Detection Systems 2.1 software (autocaller confidence level 95%). Ten percent of all genotypes were repeated in independent PCR to check for consistency and to ensure intra- and interplate genotype quality control. No genotyping discrepancies were detected between the repeated samples. Call rates were ≥98.0%.

Definitions. Despite increasing literature about the metabolic syndrome, it is not completely understood why its components cluster in individuals. In an



SNP no.	rs- or Assay-ID-number of SNPs	Position	MAF	Call rate [%]	Gene region	Polymorphism (Codon change)
1	rs6748533	11,769,325	0.14	99.4	Intergenic 5′	C/G
2	rs11676410	11,787,218	0.17	98.9	Intergenic 5′	G/A
3	rs6707885	11,794,067	0.16	99.5	Intergenic 5′	A/G
4	rs893346	11,800,212	0.08	99.3	Intergenic 5′	G/A
5	rs6726960	11,816,382	0.22	99.7	Intron 1	T/C
6	C__25965595_10	11,825,293	0.20	99.7	Intron 1	T/C
7	rs1469952	11,834,970	0.46	99.1	Intron 5	G/A
8	rs33997857	11,844,689	0.03	98.7	exon 10	A/G (ATG,GTG)
9	rs6744682	11,846,779	0.31	99.1	Intron 11	T/A
10	rs6708316	11,854,609	0.33	98.7	Intron 13	C/G
11	rs2716610	11,876,833	0.16	98.0	Intron 17	T/C
12	rs1050800	11,883,265	0.17	99.0	UTR3′	T/C
13	rs2577256	11,889,941	0.49	98.9	Intergenic 3′	C/T
14	rs953071	11,924,066	0.40	99.2	Intergenic 3′	C/T
15	rs1036668	12,050,089	0.23	98.0	Intergenic 3′	G/T

FIG. 1. Structure of the *LPIN1* region and position of the 15 genotyped SNPs, including general SNP characteristics as given in the dbSNP database and the University of California Santa Cruz Genome Browser (March 2006 assembly). *LPIN1* gene region deposited gray. Information for SNP C_25965595_10 is taken from Applied Biosystems (www.appliedbiosystems.com) and GeneWindow (http://genewindow.nci.nih.gov). *Minor allele frequency. Haplotypes were inferred using PHASE 2.0 (27), and haploblocks were derived from the Haploblockfinder (28) genetical analysis software. Only haplotypes with frequencies ≥2% are shown.

attempt to answer this question, many investigators have used factor analysis to identify patterns underlying the co-occurrence of metabolic syndrome components (18–23). These analyses support the current clinical definitions of the metabolic syndrome in such that the core components central obesity, insulin resistance, blood pressure, and lipid measurements are linked by one single underlying factor (23). Moreover, in the present investigation the use of current proposed definitions for the metabolic syndrome (24) has been hampered by the fact that blood samples have not been taken routinely in the fasting state in the MONICA study. Accordingly, we used factor analysis to describe the metabolic syndrome by means of a factor score, computed for each subject derived from the following variables: waist circumference (cm), type 2 diabetes/pre-diabetes (yes/no), mean arterial blood pressure (mmHg), and HDL cholesterol (mg/dl). Here, type 2 diabetes/pre-diabetes was defined as a history of type 2 diabetes and/or serum A1C >6.0% (25). The factor score represents the degree of association between the original measured variables and the unmeasured underlying factor (metabolic syndrome factor); the higher the score the more pronounced is the expression of the metabolic syndrome. The resulting metabolic syndrome factor score is normally distributed with a mean of 0.0 ± 1.2 and a range between −4.9 and +5.8 (online appendix Fig. 1 [available at http://dx.doi.org/10.2337/db07-0083]). As a quantitative trait, it does not need an arbitrary cutoff to define affection status and can thus reduce phenotypic misclassification. As demonstrated in online appendix Fig. 1, the metabolic syndrome factor score strongly correlates with the number of the core components and was significantly higher in obese than in nonobese individuals ($P < 0.0001$), in hypertensive than in normotensive subjects ($P < 0.0001$), in diabetic than in nondiabetic subjects subjects ($P < 0.0001$), and in subjects with dyslipidemia compared with subjects without dyslipidemia ($P < 0.0001$).

Additionally, we used the presence of three or more metabolic syndrome components to define the metabolic syndrome. The following criteria were used in this regard: hypertension was defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg or intake of antihypertensive medication, type 2 diabetes/pre-diabetes as history of diabetes and/or serum A1C >6.0% (25), obesity as BMI >30 kg/m^2 (24), waist circumference as >102 cm in men and >88 cm in women (26), and dyslipidemia as HDL <40 mg/dl in men and <50 in women (26).

Statistical analysis. For each of the 15 SNPs, we tested whether the observed allele frequencies departed from Hardy-Weinberg proportions. Statistically, inferred haplotypes were derived using the PHASE software (27). Using the haplotype file format from PHASE output, we assessed LD between all pairs of SNPs using the HaploBlockFinder software (28), applying the definition of Lewontin's standardized disequilibrium coefficient (D′). A haplotype block was defined as a region in which all pairwise D′ values were >0.8 (29). Individual SNPs were tested for association using linear and logistic regression analyses. Haplotypes with frequencies ≥2% were tested using the haplotype trend regression method (30). This test fits a model of additive effects of haplotypes under the hypothesis of no haplotype effects and under the assumption of Hardy-Weinberg equilibrium. Permutation-based procedures, permuting the dependent variable, were performed to test for empirical significance. The permuted *P* value is the fraction of permuted tests that get a better *P* value than the original test.

Factor analysis was used to identify a specific cluster of metabolic

syndrome components on the basis of correlation between original measures. Factor extraction was conducted using the method of principle components. Eigenvalues were used to condense the variance in a correlation matrix and represent that amount of variance accounted for by a factor. Variables with eigenvalues of ≥1.0 are traditionally considered worth analyzing. Here, only one factor with eigenvalue >1.0 could be extracted based on the original measured variables included in this study. A factor score that is a combination of the original measured variables representing the predicted value of the underlying metabolic syndrome factor was computed for each individual. This score was then considered a dependent variable in quantitative trait regression analysis. Power calculations were computed over a range of parameters for the linear trend test (31,32), indicating that our sample size is sufficient to achieve 80% power for at least half of the genetic model parameter settings (online appendix Fig. 3). Additionally, we divided the study population into tertiles of metabolic syndrome factor score distribution and calculated tertile 3–to–tertile 1 risk ratios using crude and adjusted logistic regression models. A two-sided *P* value of <0.05 was considered significant. All *P* values reported represent nominal *P* values; empirical *P* values are reported when specifically indicated.

RESULTS

Phenotypic characteristics. The clinical and anthropometric characteristics of the MONICA study population are presented in Table 1. The participants present with the expected characteristics of our study sample of the general population. The metabolic syndrome defined by the presence of three or more components could be observed in 10.3% of men and 12.5% of women.

LD evaluation and haplotype structure in the study population. Figure 1 depicts the gene structure and all SNPs used in this study, including their position and general characteristics based on the March 2006 release of the University of California Santa Cruz Genome Browser (available at http://www.genome.ucsc.edu). No deviations from the expected Hardy-Weinberg proportions were detected for any of the SNPs. LD analysis revealed slight LD spanning the whole gene region and three high LD blocks (Fig. 1; online appendix Fig. 2). Specifically, two three-marker haploblocks each forming four common haplotypes and one two-marker haploblock forming two common haplotypes could be identified. The first three-marker haploblock (haploblock 1: rs6726960, C_25965595_10, and rs1469952) is located within 18.6 kb between intron 1 and intron 5 of the *LPIN1* gene and forms four haplotypes with frequencies ≥2.0%. The second three-marker haploblock (haploblock 2: rs33997857, rs6744682, and rs6708316) is located within 9.9 kb between exon 10 and intron 13 and forms four haplotypes with frequencies ≥4%. The two-marker haploblock (haploblock 3: rs2716610 and rs1050800) is positioned within 6.4 kb between intron 17 and the untranslated region 3′. Altogether, 15 SNPs spanning the *LPIN1* gene and intergenic regions formed three different haploblocks with 10 haplotypes with frequencies ≥2% in this study sample.

Association of individual SNPs with the metabolic syndrome and its components. We tested all individual SNPs for association with the metabolic syndrome and its single components. Single SNP association analyses are summarized in Fig. 2. Noticeable, yet inconsistent, association results were found between single SNPs and parameters of the metabolic syndrome, particularly in men with blood pressure and HDL cholesterol levels. In women, these associations were less pronounced.

Association of haplotypes with the metabolic syndrome and its components. Based on our LD analysis, we next screened for association between haplotypes and the metabolic syndrome factor score as well as with single components of the metabolic syndrome. Figure 3 depicts the results of these association tests. We found a highly significant association peak between the metabolic syndrome factor score, BMI, waist circumference, and blood pressure levels and allelic combinations of a three-marker haploblock across exon 10 and intron 13 of the *LPIN1* gene beginning at SNP marker rs33997857. This haploblock is consistent with haploblock 2 based on our LD analysis and is composed of the exonic SNP leading to a codon change (V494M) and two intronic SNPs. After performing 10,000 permutations, the metabolic syndrome factor score ($P < 0.0001$), the existence of three or more metabolic syndrome components ($P = 0.0004$), waist circumference ($P < 0.0001$), blood pressure levels ($P < 0.0001$), and A1C levels ($P < 0.0001$) remained significantly associated with these haplotypes (all *P* values based on permutation testing). We likewise tested for association between allelic variants and metabolic syndrome phenotypes across haploblock 1 and haploblock 3. In essence, we did not find any evidence of relationship here (data not shown).

As a consequence, we investigated each allelic variant of haploblock 2 in more detail. Association results of the respective haplotypes are shown in Table 3, with the exception of the most common haplotype consisting of all major SNP alleles. This haplotype did not show any evidence of association neither with the metabolic syndrome factor score nor with any of its components (data not shown). Subjects carrying the 122 haplotype (frequency 26.8%) were more often obese and diabetic and had higher A1C and LDL cholesterol levels than subjects not carrying this haplotype, implying susceptible characteristics of this haplotype. Moreover, the metabolic syndrome factor score as well as the frequency of having three or more metabolic syndrome core components were significantly higher in carriers of this susceptible haplotype.

In contrast, individuals carrying haplotype 121 (frequency 4.0%) or haplotype 112 (frequency 5.7%) had markedly lower blood pressure levels, a lower BMI, lower waist circumference, lower A1C and LDL cholesterol levels, as well as a lower metabolic syndrome factor score corresponding to nonsusceptible or protective effects of these haplotypes. Accordingly, the frequencies of arterial hypertension, obesity, diabetes, and the presence of three or more metabolic syndrome components were significantly lower in subjects carrying these protective haplotypes than in subjects without.

Figure 4 depicts the relative frequencies of the presence of the susceptible and protective haplotypes with and without metabolic syndrome. In this analysis, the protective haplotypes were pooled because of their low frequencies and similar impact on the metabolic phenotypes. Two traits of the metabolic syndrome with their corresponding odds ratios are present: tertile 3 versus tertile 1 of the metabolic syndrome factor score distribution as well as presence of three or more metabolic syndrome components versus two or fewer metabolic syndrome components. Using sex-adjusted logistic regression analysis, both the susceptible and the protective haplotypes showed significant association with the metabolic syndrome, independently of the definition used.

Replication of association in an independent cohort. To confirm our findings, we additionally tested for association in a cohort of healthy spouses of coronary artery disease patients. The characteristics of study participants were comparable with the sample from the MONICA study

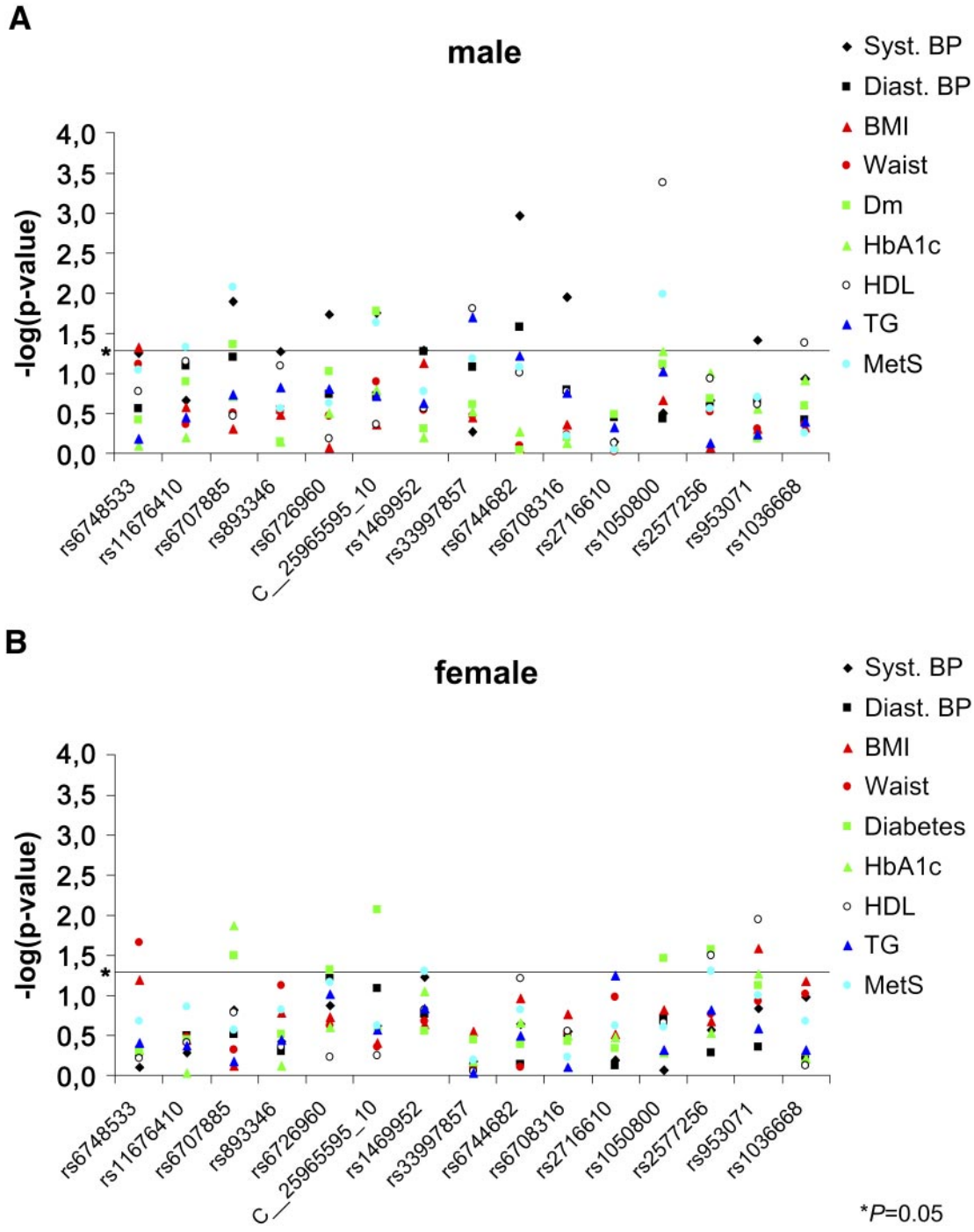


FIG. 2. Association between individual SNPs and traits of the metabolic syndrome in men (*A*) and women (*B*). The strength of association is represented by –log(*P* values).

general population, with the exception of a lower frequency of obese subjects and a higher proportion of women (Table 2). LD structure and haplotype frequencies were also comparable (111 [59.4%], 122 [23.3%], 112 [8.2%], and 121 [6.7%]). As depicted in Fig. 5 haploblock 2 was also significantly associated with traits of the metabolic syndrome, with the strongest effect on fasting triglycerides (which were not available in the MONICA study sample) and HDL cholesterol levels. The metabolic syndrome factor score and the trait diabetes also showed a significant association with alleles of haploblock 2. However, fasting triglycerides were higher in subjects presenting haplotype 112, and HDL cholesterol levels were lower in subjects with haplotypes 121 or 112 (Table 4). Moreover, type 2 diabetes was also more frequently found in subjects with haplotpye 121. As a consequence, the metabolic syndrome factor score was higher in subjects with these rare haplotypes, and, thus, the effects are in the opposite direction compared with those in the MONICA sample.

DISCUSSION

The present study offers a comprehensive analysis of common genetic variants across the *LPIN1* gene region

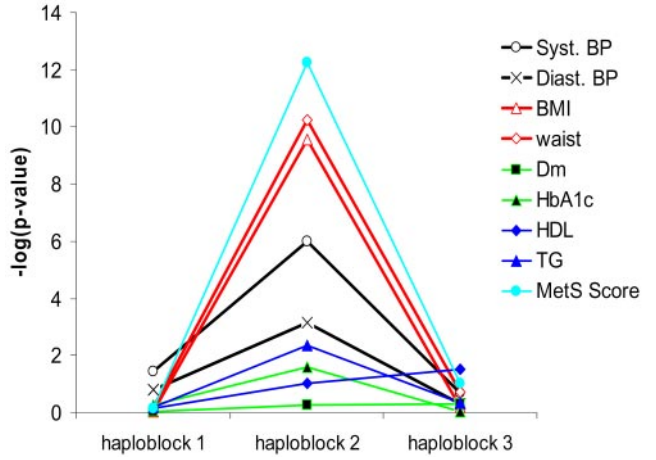


FIG. 3. −log(*P* value) plot by the haplotype trend regression method. Meaningful haplotype block structures based on LD analysis are indicated (haploblock 1, haploblock 2, and haplotype 3). Three-marker haplotypes from haploblock 2 (encompassing SNPs rs33997857, rs6744682, and rs6708316) show highly significant associations with traits of the metabolic syndrome.

with the metabolic syndrome and its individual components. We have identified *LPIN1* as a gene that is significantly associated with traits of the metabolic syndrome in the general population of the Augsburg area in Germany and have found association of the same haplotypes in a second sample recruited throughout Germany, however, with effects in the opposite direction. The similarity in the minor allele frequencies in both studies and the fact that the cluster of adjacent SNPs show the same pattern suggests that this discrepancy may not be due to technical issues. We cannot exclude spurious associations in the two studies; however, similar observations of associations with odds ratios in opposite direction have been made previously (33). Recently, Lin et al. (33) have used theoretical modeling to demonstrate that these associations may indeed represent confirmations of true associations and that multilocus effects and variation in interlocus correlations contribute to this "flip-flop phenomenon." Thus, considering also previous reports about *LPIN1*, our results may still demonstrate that the *LPIN1* gene plays a critical role in molecular pathways affecting human metabolic traits.

We were led to pursue *LPIN1* because, first, the physiological role of the *LPIN1* gene appears to be in the regulation of fat mass and body weight (5–8). Second, recent findings report on strong negative correlations between lipin mRNA expression and parameters of insulin sensitivity in both human (10,11) and mouse adipose tissues (10). These findings are supported by two recently released reports showing subcutaneous lipin mRNA levels to be decreased in subjects with either obesity (6,11), impaired glucose tolerance (11), or in women with the metabolic syndrome (6). In contrast, lipin mRNA levels were positively correlated with an increasing insulin sensitivity index and were upregulated following weight reduction in obese (6).

Suviolahti et al. (10) have recently identified for the first time an association between SNPs across *LPIN1* and insulin levels as well as between BMI in Finnish families and dyslipidemia and in a Finnish obesity case-control study, respectively (10). A comparison of our data with those of Suviolahti et al. reveals that we have genotyped three identical SNPs (rs893346, rs2716610, and rs1050800).

TABLE 3
Association of three-marker haplotypes from haploblock 2 with traits of the metabolic syndrome

	Haplotype 122			Haplotype 121			Haplotype 112		
	Present	Not present	*P* value	Present	Not present	*P* value	Present	Not present	*P* value
n	647	769		93	1,323		143	1,273	
Hypertension traits									
Systolic blood pressure (mmHg)	135 ± 21	133 ± 19	NS	127 ± 18	135 ± 20	0.0001	128 ± 18	135 ± 20	0.00008
Diastolic blood pressure (mmHg)	81 ± 12	80 ± 12	NS	78 ± 12	81 ± 12	0.032	78 ± 11	81 ± 12	0.0016
Hypertension (%)*	47.1	43.0	NS	23.7	46.4	0.00001	32.2	46.4	0.001
Obesity traits									
BMI (kg/m^2)	26.8 ± 4.2	26.6 ± 4.1	NS	24.6 ± 3.6	26.9 ± 4.1	3.7×10^{-7}	25.3 ± 3.8	26.9 ± 4.2	0.00002
Waist circumference (cm)	90 ± 12	89 ± 13	NS	82 ± 12	90 ± 12	3.2×10^{-8}	85 ± 12	90 ± 12	0.00001
Obesity (%)*	32.8	27.0	0.019	12.9	30.8	0.0003	16.9	31.1	0.0005
Diabetes traits									
Diabetes (%)*	9.3	6.6	0.059	2.2	8.2	0.041	6.5	8.0	NS
A1C (%)	5.3 ± 0.9	5.2 ± 0.8	0.012	5.1 ± 0.7	5.3 ± 0.9	0.0002	5.1 ± 0.6	5.3 ± 0.9	0.013
Dyslipidemia traits									
LDL cholesterol (mg/dl)	147 ± 43	142 ± 43	0.021	135 ± 47	145 ± 43	0.032	130 ± 41	146 ± 43	0.00002
HDL cholesterol (mg/dl)	54 ± 16	54 ± 17	NS	56 ± 19	54 ± 16	NS	53 ± 18	54 ± 16	NS
Dyslipidemia (%)*	30.4	27.9	NS	30.4	28.9	NS	33.1	28.6	NS
Metabolic syndrome									
Factor score	0.10 ± 1.23	−0.11 ± 1.24	0.002	−0.67 ± 1.00	0.04 ± 1.24	1.4×10^{-7}	−0.42 ± 1.28	0.03 ± 1.22	0.00005
Three or more components* (%)	16.0	10.5	0.007	3.3	13.7	0.002	8.6	13.5	NS

Data are means ± SD. *Definitions are as defined in the text. NS, not significant.

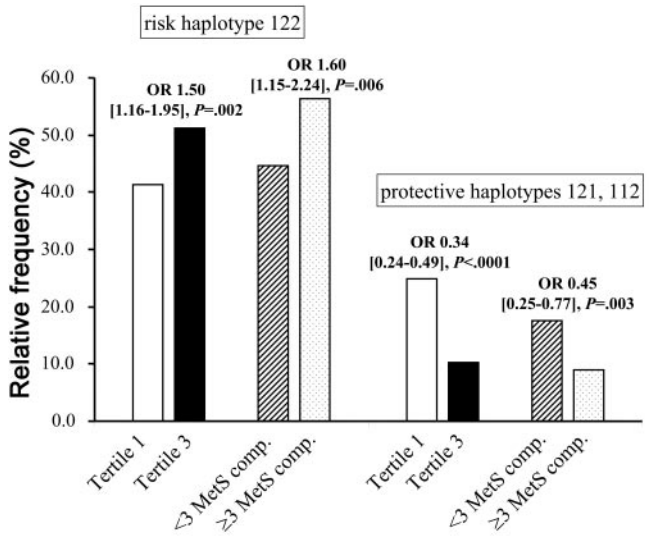


FIG. 4. Relative frequencies of the susceptible and the protective haplotypes in subjects within the first and third tertile of the metabolic syndrome factor score distribution (the first tertile implies individuals with the lowest evidence of a metabolic syndrome, while the third tertile encloses those with the highest evidence for a metabolic syndrome) as well as in subjects with three or more and less than three metabolic syndrome components (arterial hypertension, insulin resistance, abdominal obesity, and dyslipidemia). The corresponding odds ratios for susceptible and protective haplotype carriers with respect to these metabolic syndrome criteria are mentioned.

Suviolahti et al. reported moderate differences in allele frequencies in obese versus lean subjects for SNP rs2716610 and moderate differences in fasting insulin levels for SNP rs893346. None of these SNPs showed reliable associations with any of our phenotypes. However, one of the Suviolahti SNPs that was associated with BMI is located in the immediate 3′ vicinity of our haploblock, which analogously is associated with BMI in the general population of the MONICA study. According to the HapMap data, these markers are in strong LD, implying a possible replication of the findings.

While in our replication sample HDL cholesterol and triglyceride levels as well as the presence of type 2

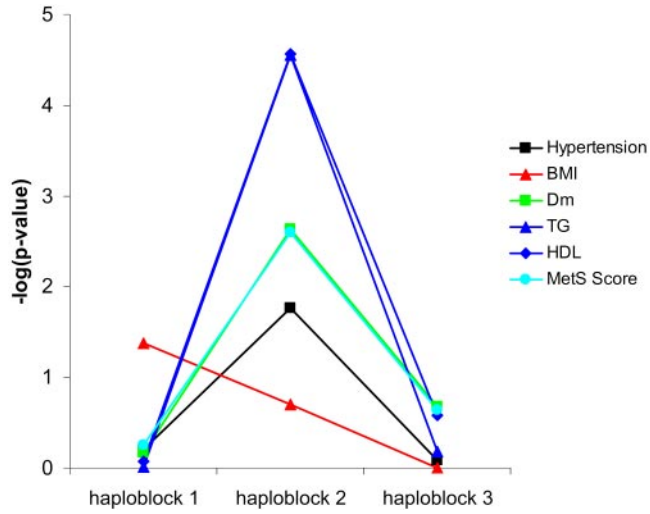


FIG. 5. −log(*P* value) plot by the haplotype trend regression method in the replication cohort. Using the same three-marker haploblock as in the MONICA study sample, the significant associations between three-marker alleles from haploblock 2 (bold, beginning with SNP rs33997857) and traits of the metabolic syndrome could be confirmed.

TABLE 4
Association of three-marker haplotypes from haploblock 2 with traits of the metabolic syndrome in the replication study sample

	Haplotype 122			Haplotype 121			Haplotype 112		
	Present	Not present	*P* value	Present	Not present	*P* value	Present	Not present	*P* value
n	429	601		102	928		136	894	
Systolic blood pressure (mmHg)	133.5 ± 16.8	135.3 ± 17.5	NS	136 ± 17	134.4 ± 17.3	NS	139 ± 18	134 ± 17	0.04
Diastolic blood pressure (mmHg)	80.4 ± 9.7	81.7 ± 9.9	NS	80 ± 8	81 ± 10	NS	82 ± 12	81 ± 9	NS
Hypertension (%)*	40.6	43.4	NS	46.1	41.8	NS	47.8	41.4	NS
BMI (kg/m^2)	26.7 ± 4.3	26.6 ± 4.1	NS	27.5 ± 4.2	26.6 ± 4.2	0.037	27.3 ± 3.2	26.6 ± 4.3	NS
Diabetes (%)*	8.9	9.1	NS	19.8	7.9	0.00007	8.6	12.0	NS
Triglycerides (mg/dl)	133 ± 76	140 ± 109	NS	145 ± 102	136 ± 96	NS	175 ± 182	131 ± 75	0.00008
HDL cholesterol (mg/dl)	63 ± 15	60 ± 15	0.003	57 ± 12	62 ± 15	0.008	56 ± 14	62 ± 15	0.00008
Factor score	−0.66 ± 1.09	−0.52 ± 1.15	NS	−0.12 ± 1.33	−0.63 ± 1.09	0.002	−0.32 ± 1.16	−0.62 ± 1.12	0.044

Data are means ± SD unless otherwise indicated. Asymptotic *P* values are given. *Definitions are as defined in the text. The most frequent hapotype, haplotype 111, did not show any evidence of association with these phenotypes and is thus not shown in the Table. NS, not significant.

diabetes and the metabolic syndrome factor itself were clearly associated with alleles of the same haploblock, the continuous traits BMI and blood pressure levels only showed weak evidence of association with these haplotypes in this population sample. This lack may possibly be due to unrecognized phenotypic differences across study cohorts. However, population stratification was not explicitly tested in our study. Gene-environmental interactions might be different in addition to the lower prevalence of obesity as well as the higher percentage of female individuals in the replication sample.

Furthermore, the proportional contribution of each of the single components to the metabolic syndrome may differ individually and in populations. In addition, multiple susceptibility genes may exist (genetic heterogeneity), of which only a subset are required for disease. Nevertheless, we find metabolic phenotypes that show association in two independent populations with *P* values far below the recently revised recommendation of the experiment-wise significance level of $P < 0.001$ quoted to be sensible in genetic association studies (34). Moreover, considering the findings of Suviolahti et al. (10), the association could be shown in multiple independent populations making false-positive findings unlikely.

Evidence for a role of lipin in traits of glucose metabolism accumulated with the recent reports of association of lipin mRNA levels and glucose transport and GLUT4 mRNA expression levels (6). As we do not have fasting glucose and insulin levels in our study samples, we were not able to approve these findings in our study.

The biological mechanism underlying this genetic association may be due to an altered gene expression. Indeed, strong negative correlations were observed between human lipin mRNA levels and glucose ($r = -0.81$), insulin ($r = -0.74$), triglyceride levels ($r = -0.64$), and the homeostasis model assessment of insulin resistance index ($r = -0.82$) (10). Moreover, lipin mRNA levels were strongly correlated with GLUT4 mRNA levels ($r = 0.69$) (6). Interestingly, our haplotype comprised one coding SNP leading to a nonsynonymous amino acid substitution and two intronic SNPs. However, with respect to the coding SNP, the same "1" allele occurred in all the haplotypes, indicating that this coding SNP is not responsible for the observed associations. This observation supports the hypothesis that the proper causal mutation is located elsewhere in this LD block or is in LD with it. Additional functional studies will be needed to clarify whether individuals carrying these different haplotypes show differences in the expression of lipin.

As we do not have fasting glucose, insulin, and triglyceride levels in our MONICA study cohort, we were not able to define the metabolic syndrome according to one of the currently proposed definitions. This, as well as the fact that study participants were recruited >10 years ago, might explain the low prevalence of the metabolic syndrome in our study population. However, several metabolic syndrome definitions are in use: the World Health Organization (1999), the European Group for the Study of Insulin Resistance (1999), the Adult Treatment Panel III (2001), as well as the most recently encouraged International Diabetes Federation criteria (2005). These definitions differ in their measures and cutoffs, and it is still a matter of debate which of them best represents the metabolic syndrome. Additionally, it is still unclear why its components cluster in individuals. Several recently performed factor analyses (18–23) support the current clinical definitions of the metabolic syndrome in such that the core components central obesity, insulin resistance, blood pressure, and lipid measurements are linked by one single underlying factor (23). Accordingly, we used factor analysis to describe the metabolic syndrome by means of a factor score, computed for each subject. As a quantitative trait, it does not need an arbitrary cutoff to define affection status and can thus reduce phenotypic misclassification. Together with the confirmatory association findings using individual components and their sum, we are confident that our metabolic syndrome factor score represents an adequate measure of the metabolic state in our study participants.

In summary, our work offers a comprehensive analysis of the LD structure, common genetic variants, and haplotypes within the *LPIN1* gene region. We suggest an association of common genetic variants of the *LPIN1* gene with the metabolic syndrome and its associated phenotypes, thus raising the possibility of lipin contributing to the pathophysiology of the metabolic syndrome.

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