

Association of the Ghrelin Receptor Gene Region With Left Ventricular Hypertrophy in the General Population

Results of the MONICA/KORA Augsburg Echocardiographic Substudy

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Abstract—Growth hormone (GH) can influence left ventricular myocardial growth, structure, and function. The GH secretagogue receptor (GHSR, ghrelin receptor) is known to be involved in GH release and is expressed in the myocardium. We hypothesized that genetic variants within the *GHSR* are associated with parameters of left ventricular mass (LVM) and geometry. Ten single-nucleotide polymorphisms (SNPs) covering the gene region were genotyped in 1230 members of the general population (Monitoring Trends and Determinants on Cardiovascular Diseases Augsburg Echocardiographic Substudy). Linkage disequilibrium analysis revealed a linkage disequilibrium block consisting of 5 SNPs forming 2 common haplotypes. One haplotype was found significantly more often in subjects without left ventricular hypertrophy ([LVH] 69% versus 59%; permuted $P=0.0015$), whereas the second haplotype was significantly more frequent in individuals with LVH (32% versus 26%; $P=0.019$). Homozygous subjects presented with an increase of risk with respect to all heart size parameters. A significantly increasing frequency of the risk haplotype could be observed from the lowest (20.9%) to the highest quintile (31.0%) of gender-specific LVM distributions ($P=0.0096$). We found association of the minor alleles of individual single nucleotide polymorphisms contributing to the haplotypes with higher LVM indices, septal wall thickness, and different LVH criteria consistent in men and women in matched cases and controls (LVM, women: 144.8 ± 30.9 [noncarrier] versus 171.3 ± 36.0 [homozygous], $P=0.001$; men: 186.7 ± 42.4 versus 236.3 ± 64.5 , $P=0.002$). These data suggest that common variants in the *GHSR* region are associated with parameters of LVM and geometry independent of blood pressure and body mass in the general population and, thus, may be involved in the pathogenesis of LVH. (*Hypertension*. 2006;47:920-927.)

Key Words: ventricular function, left ■ genetics ■ growth substances ■ hypertrophy

Left ventricular (LV) hypertrophy (LVH) constitutes one of the most powerful independent risk factors for cardiovascular morbidity and mortality in high-risk patients and in the general population.¹⁻³ Its heterogeneous etiology results from the complex interaction among genetic, environmental, and lifestyle factors with hypertension and obesity as principal determinants for this major cardiovascular condition.⁴ There is evidence that LV mass (LVM) displays considerable variance in subjects, despite well-comparable environmental and predisposing factors, suggesting a substantial heritable component to the development of LVH. Specifically, 60% of the blood pressure (BP)-independent variation in cardiac mass may be attributable to genetic factors.⁵ Moreover, neuroendocrine factors, such as growth hormone (GH), contribute to the determination of cardiac mass and the development of cardiac hypertrophy.² In fact, it has been demonstrated in animals and in humans⁶⁻¹⁰ that GH influences LV

myocardial growth, structure, and function. GH primarily stimulates the local production of insulin-like growth factor 1, which, in turn, promotes tissue growth by paracrine and autocrine mechanisms.^{6,7,11,12} The GH secretagogue receptor (ghrelin receptor, GHSR) is known to be involved in the control of GH release by mediating the strong stimulatory effect of the endogenous ligand, ghrelin, on GH secretion. Furthermore, *GHSR* is expressed in the myocardium, suggesting cardiovascular actions of the receptor and its ligand.¹³⁻¹⁶

In a previous study, we systematically explored the linkage disequilibrium (LD) and haplotype structure of the genomic region encompassing the *GHSR* gene and demonstrated a significant relationship between common genetic variants of the *GHSR* and body mass.¹⁷

Here, we comprehensively analyzed whether *GHSR* also affects LVM and heart size, independent of body mass. We tested the relationship between common single nucleotide poly-

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morphisms (SNPs) and haplotypes of the *GHSR* and parameters of LV geometry and structure. We report significant association of the two most common haplotypes with multiple parameters of LVM and LVH in a well-characterized sample of the general population. With respect to the biological function of the *GHSR*, these consistent findings may be plausible and of potential clinical and scientific relevance.

Methods

Study Subjects

Subjects participated in the Monitoring trends and determinants ON Cardiovascular Diseases (MONICA) Augsburg Echocardiographic Substudy, as part of the third MONICA Augsburg Survey, which is now continued in the framework of Cooperative Health Research in the Augsburg Area (KORA). The study population of the LVH substudy was sampled from the general population of the city of Augsburg, Germany, in 1994–1995, which originated from a sex- and age-stratified cluster sample of all German residents of the Augsburg study area. The Augsburg project is part of the international collaborative World Health Organization MONICA Study.¹⁸ The study design, sampling frame, and data collection have been described in detail elsewhere.¹⁸ The third survey represents individuals aged 25 to 74 years (mean: 51.8 ± 13.8), including 851 (50.7%) women and 827 (49.3%) men and ≈ 300 subjects for each 10-year increment (total $n=1674$). More details are described in an online supplement section available at <http://www.hypertensionaha.org>.

From the 1678 subjects, both DNA samples and high-quality echocardiographic data were available in 1230 subjects (603 men and 627 women). The study was approved by the local ethics committee, and all of the participants gave written informed consent.

Echocardiographic Measurements

Two-dimensional guided M-mode echocardiograms from standard left parasternal and apical windows, derived M-mode echocardiograms, and Doppler recordings were performed by 2 expert sonographers on a commercially available echocardiograph (Hewlett Packard Inc, Sonos 1500) with a 2.5- or 3.5-Mhz transducer. M-mode tracings were recorded on stripchart paper at a speed of 50 mm/s. To reduce interobserver variability, all of the M-mode tracings were analyzed by a single cardiologist who was unaware of the clinical data. Measurements for M-mode-guided calculations of LV mass were taken just below the tip of the mitral valve. LV internal end-diastolic (LVEDD) and end-systolic diameters and septal (SWT) and posterior wall thickness (PWT) were performed according to the guidelines of the American Society of Echocardiography. LVM was calculated according to the formula described by Devereux and Reichek¹⁹ as:

$$\text{LVM (g)} = 1.04[(\text{LVEDD} + \text{SWT} + \text{PWT})^3 - \text{LVEDD}^3] - 13.6$$

The rank correlation for 144 duplicate measurements by the 2 sonographers was 0.91 for the determination of LVM.

Definitions

When defining the range of normal LVM and the status of LVH, individual body size and composition must be taken into consideration, because metabolic demand and perfusion needs vary with stature and determine the physiological adaptation of heart size. Because the most appropriate method of adjusting LVM for body size still remains controversial and to demonstrate independence of the type of indexation, LVM was indexed to height^{2.7} ($h^{2.7}$) as $\text{LVM}_h^{2.7}$, body surface area (BSA) as LVM_{BSA} , and lean body mass (LBM) as LVM_{LBM} .

Regarding the definition of LVH, we used all 3 indexations, described above. Because exponentials of body height have been evoked to better account for the nonlinear association of body size with LVM,^{19–22} LVH was first defined as $\text{LVM}_h^{2.7} > 50 \text{ g/m}^{2.7}$ in men and $> 47 \text{ g/m}^{2.7}$ in women. Secondly, LVH was defined as $\text{LVM}_{\text{BSA}} > 131 \text{ g/m}^2$ in men and $> 100 \text{ g/m}^2$ in women, which was suspected to underestimate the prevalence of LVH associated with obesity. Recent work suggests that accounting for LBM is the optimal

method of LVM indexation, thereby eliminating sex-specific differences in LVM, indicating that heart size in men and women might reflect the metabolic demand of the fat-free body compartment rather than hormonal or genetic differences.²³ Thus, the third LVH criteria was based on $\text{LVM}_{\text{LBM}} > 4.1 \text{ g/kg}$ in men and women.²⁴

Hypertension was considered as a BP of $\geq 140/90 \text{ mm Hg}$, current intake of antihypertensive medication, or both. Body mass index was computed as weight divided by height squared (kg/m^2). Obesity was defined as a body mass index $> 30 \text{ kg/m}^2$ in men and women. Diabetes mellitus was defined as a history of diabetes or intake of antidiabetic medication.

SNPs and Genotyping Methods

SNPs

Initially, 10 SNPs covering the *GHSR* gene (4.3 kb in size) and its flanking regions were selected from public databases (dbSNP, <http://www.ncbi.nlm.nih.gov/SNP/>). Of the 10 selected SNPs, 1 SNP (rs572169) was located in exon 1 (796 bp), 1 (rs509035) was in the intron (which spans 2.2 kb), 3 spanned 41.5 kb past the 3' end of the gene, and 5 covered a region of 53.5 kb past the 5' end of the gene. The 8 SNPs located beyond the boundaries of the gene 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 total, a region of 99.3 kb was covered with SNPs with an average resolution of 1 SNP per 10 kb. The coding SNP (rs572169) resulted in a synonymous amino acid substitution, according to dbSNP. The minor allele frequencies were $> 5\%$.

Genotyping

SNPs were genotyped using the 5'-exonuclease activity (TaqMan) assay on a HT7900 (Applied Biosystems). SNP assays were ordered from Applied Biosystems through an assay-by-design service. Of all genotypes, 10% were repeated in independent PCR reactions to check for consistency and to ensure intraplate and interplate genotype quality control. No genotyping discrepancies were detected between the repeated samples. The overall failure rate of $< 0.5\%$ was attributed to insufficient PCR amplification.

Statistical Analysis

For each of the 10 SNPs, we tested whether the observed allele frequencies departed from Hardy–Weinberg proportion. No deviations from the expected genotype proportions were detected for any of the SNPs used in the analyses. We assessed LD between all pairs of SNPs, applying the standard definition of r^2 .²⁵ A haplotype block was defined as a region in which all pairwise r^2 values were > 0.45 .¹⁷ Haplotype frequencies were estimated using the expectation-maximization algorithm.²⁶ Statistically inferred 5-marker haplotypes were tested for association with the discrete trait LVH defined by all 3 of the indexation types using the haplotype trend regression method.²⁷ Moreover, we analyzed association between the number of copies of the haplotypes 1 and 2 with quantitative traits of LVM and heart size applying the same method.²⁷ Permutation tests (50 000 permutations) were used to test for empirical significance. In addition to this haplotype analysis, a single-locus analysis was performed in the entire study sample as well as in a subset of matched cases and controls to determine the effect of individual SNPs. By use of an automated, randomized selection of control subjects, all of the available subjects with LVH defined as $\text{LVM}_h^{2.7} > 50 \text{ g/m}^{2.7}$ in men and $> 47 \text{ g/m}^{2.7}$ in women ($n=109$ case subjects) were carefully matched by age (± 10 years), gender, hypertension, and obesity, whereby 1 case subject was matched with ≤ 4 control subjects ($n=355$ control subjects). The $\text{LVH}_h^{2.7}$ criteria were used because of the higher prevalence of LVH as compared with the LVH_{BSA} or LVH_{LBM} criteria. Frequencies of genotypes in the total study sample, as well as in cases and controls, were compared by the Armitage test for trend, and odds ratios with their 95% confidence intervals were reported.²⁸ Additionally, we compared the genotype-specific means of parameters of LVM and heart size using a multiple regression model. Finally, we divided the population into quintiles of respective phenotype distributions and calculated adjusted quintile 5 ($> 80\%$ percentile)/quintile 1 ($\leq 20\%$ percentile) risk ratios using multiple regression

TABLE 1. Echocardiographic Characteristics of Male and Female Probands With Complete Genotyping and High-Quality Echocardiography of the MONICA Augsburg LVH Substudy

MONICA Trait	Men (n=603)	Women (n=627)
LVM, g	181.8±45.3†	137.1±36.2
SWT, mm	11.2±2.2†	10.0±2.0
PWT, mm	9.2±1.4†	8.3±1.4
LVEDD, mm	50.2±4.5†	45.9±4.1
LVM _{BSA}	92.2±21.3†	79.1±18.3
LVM _h ^{2,7}	40.4±11.0†	37.7±11.0
LVM _{LBM}	3.0±0.7	3.1±0.8
LVH _{BSA} , %	5.4*	10.9
LVH _h ^{2,7} , %	15.7	17.9
LVH _{LBM} , %	9.1*	11.2

Data are expressed as mean±SD or %.

* <0.01 ; † <0.001 .

models. In analyses in which permutation procedures were not feasible, *P* values were corrected for multiple testing by a method that specifically considers SNPs being in LD with each other and that is based on the spectral decomposition of matrices of pairwise LD between SNPs.²⁹

Results

Phenotypic Characteristics

The mean values of body size and BP, as well as the proportion of individuals presenting with obesity, diabetes, or hypertension, including the intake of antihypertensive medication, are displayed in Table I (available online). Whereas the prevalence of hypertension and diabetes was higher in men ($P\leq 0.05$), obesity was more frequently observed in women ($P\leq 0.05$). Echocardiographic characteristics are shown in Table 1. With the exception of LVM indexed to LBM, parameters of LVM and heart size were, as expected, higher in men than in women. In contrast, the prevalence of LVH was slightly higher in women than in men with respect to all of the definitions used. Moreover, substantial differences regarding the prevalence of LVH according to the different indexations could be observed. The prevalence of LVH was markedly higher when using indexations to $h^{2,7}$, particularly in men.

Linkage Disequilibrium Evaluation and Haplotype Structure in the General Population

Figure 1 depicts the gene structure and all of the SNPs used in this study, including their position and general characteristics based on the Golden Path Genome Browser (accessed at <http://www.genome.ucsc.edu>). The pairwise LD block structure, defined by the 10 SNPs covering a 99.3-kb region, and the haplotype construction, have been published previously.¹⁷ Briefly, a region of strong LD spanning 11.63 kb and encompassing most of the intron, exon 1, and the 5' adjacent region of the *GHSR* gene was detected between 5 SNPs (rs509035, rs572169, rs519384, rs512692, and rs863441). The 2 most frequently occurring haplotypes, derived from this high-LD block, comprised 94% of total chromosomes in subjects of the MONICA LVH substudy (Table 2). For further association analysis, we mainly focused on the 5 SNP haplotypes, as

well as on the single markers contributing to this haplotype block. We tested the hypothesis of a relation between these 5-marker haplotypes and/or individual SNPs and LVM phenotypes. To ensure that the adjacent SNPs, which were not included in the high LD block, were not associated with those phenotypes, we additionally performed association analysis for these SNPs. In essence, none of the surrounding SNPs showed any evidence for association with LVM related phenotypes (Table II, available online).

Haplotype Association Analysis

Initially, we tested for association of the 2 most common haplotypes with the LVH affection status. The 2 most common haplotypes showed significant association with LVH (Table 2), independent of the indexation type used (Table III, available online). Haplotype 1 was more frequently present in individuals without LVH, corresponding to a "nonsusceptible" haplotype, whereas haplotype 2 was more often found in individuals with LVH, corresponding to a "susceptible" or "risk" haplotype. Moreover, we observed a significant relation between the number of copies of haplotype 1 and 2 with LVM in the entire study sample (Figure 2a and 2b). Individuals homozygous for the susceptible haplotype 2 or lacking the nonsusceptible haplotype 1 presented with higher LVM than individuals with 1 or no copy of the respective haplotype. Analogously, testing for association between the 2 haplotypes and additional parameters of LVM and heart size, as well as different LVM indexations, such as LVM, LVH_{BSA}, LVH_h^{2,7}, LVH_{LBM}, SWT, and LVEDD, revealed similar relationships with significant probability values (Figure I, available online). Notably, individuals with 0, 1, or 2 copies of the risk haplotype did not significantly differ with respect to age (51.8 ± 14.1 versus 52.7 ± 13.4 versus 50.6 ± 12.8 years of age), BP levels (systolic 134 ± 20 versus 135 ± 20 versus 135 ± 20 mm Hg; diastolic 80 ± 12 versus 81 ± 12 versus 81 ± 13 mm Hg), the prevalence of hypertension (43.7% versus 47.2% versus 41.3%) and gender (49.4% versus 53.7% versus 50.0% men).

When dividing the MONICA LVH population into quintiles of LVM_h^{2,7} distribution, a significantly increasing frequency of the risk haplotype from the lowest (Q1) to the highest (Q5) quintile could be observed (Figure 3). Similar relationships were found for additional LVM indexations (LVM_{BSA} and LVM_{LBM}, data not shown).

In addition, we calculated multivariate adjusted Q5/Q1 risk ratios for individuals carrying 1 or 2 copies of the risk haplotype versus individuals carrying no copy of this haplotype (Figure 4). There was a substantial increase of risk with the number of haplotype copies for LVM, indexed LVM_h^{2,7}, and other parameters of heart size (SWT, PWT, and LVEDD). Individuals carrying 2 copies of the risk haplotype presented with a significantly increased risk with respect to all of the parameters. Even heterozygous individuals had a significantly increased risk compared with individuals without the risk haplotype with respect to most LVM and heart size parameters, except SWT and LVEDD. Results for additionally indexed LVM parameters (LVM_{BSA} and LVM_{LBM}) revealed even stronger associations with increasing number of copies of the risk haplotype (data not shown).

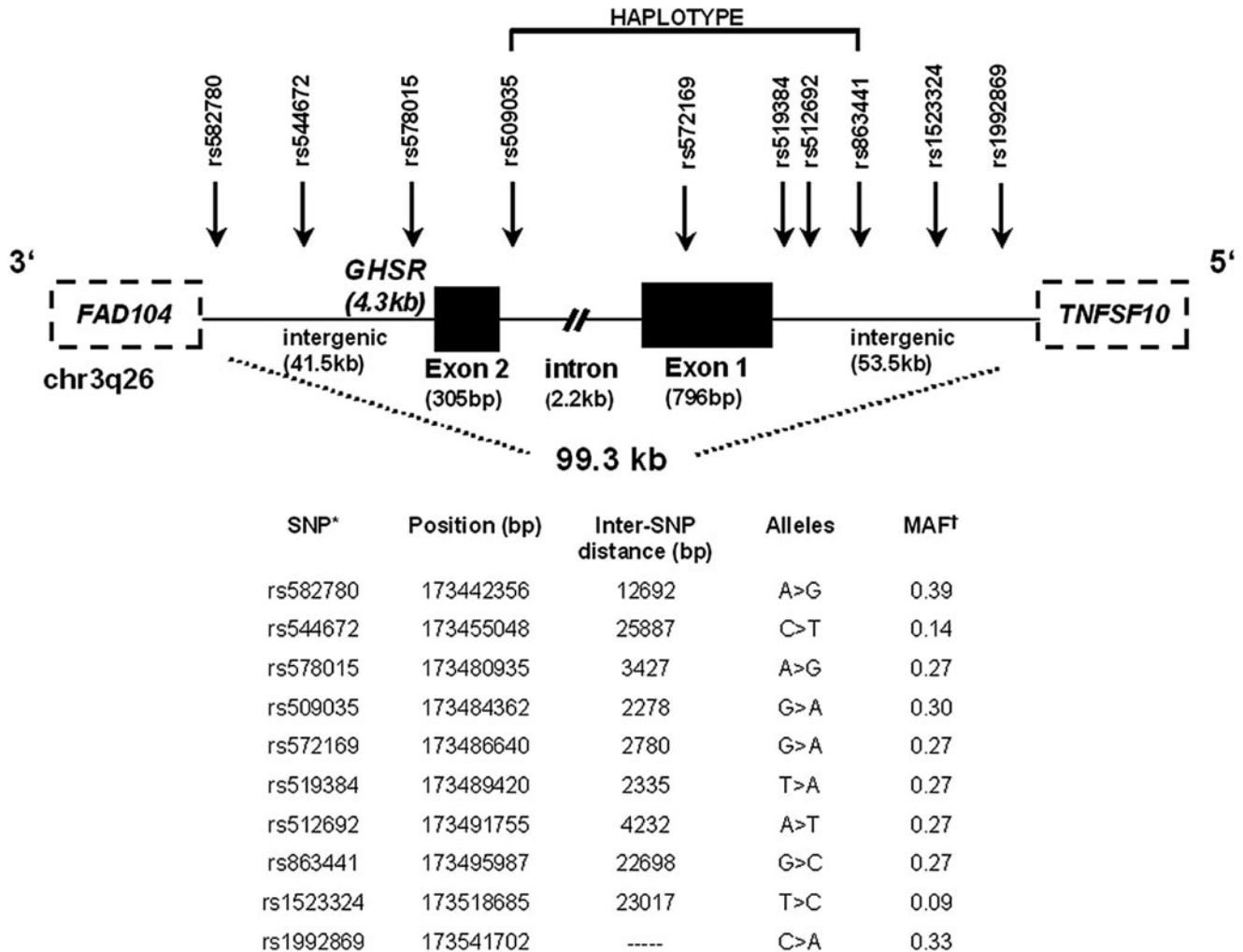


Figure 1. Structure of the GHSR 1a isoform and position of the 10 selected SNPs covering the GHSR gene region including general SNP characteristics as given in the dbSNP database and the Golden Path Genome Browser (July 2003 release). There is no publicly available SNP in exon 2. SNPs are shown as rs numbers from the dbSNP database. MAF indicates minor allele frequency.

SNP Association Analysis

Furthermore, we undertook a single locus analysis in the entire study sample, as well as in a subset of matched cases and controls to reduce the chance of a false-positive association because of confounding factors. We determined the association of the 5 individual SNPs contributing to the haplotype with LVH (defined as $LVM_h^{2,7}$; Table 3). The 2 SNPs located in the intron (rs509035) and exon 1 (rs572169) consistently showed nominally significant association with LVH in the entire study sample and the matched case and control subjects (entire study sample, best $P=0.0043$; matched sample, best $P=0.0025$). Additionally, a marginal association could be

observed for 1 of the 3 SNPs located in the 5' region (rs512692). When the results were corrected for multiple testing for SNPs in LD, most P values remained significant, with the exception of rs512692, which was only marginally significant in the first place. In the entire study sample, the increase of risk by the presence of the minor allele of these SNPs ranged between 31% ($P=0.020$) and 76% ($P=0.0025$).

The genotype-specific means of parameters of LVM and heart size of the intronic (rs509035) and the exonic (rs572169) SNP in the subset of matched cases and controls are summarized in Table IV (available online). A substantial increase in LVM, SWT, PWT, LVEDD, and LVM indices could be detected with

TABLE 2. Haplotype Structures of the LD Block and Their Frequencies and Association With LVH in the MONICA Study Population

ID	Intronic rs509035	Exonic rs572169	5' rs519384	5' rs512692	5' rs863441	Haplotype Frequency		Asymptotic <i>P</i> Value	Empirical ³ <i>P</i> Value
						LVH*	No LVH		
1	1 (G)	1 (G)	1 (T)	1 (A)	1 (G)	0.63	0.69	0.0119	0.0118
2	2 (A)	2 (A)	2 (A)	2 (T)	2 (C)	0.30	0.26	0.0310	0.0308

*Defined as $LVM_h^{2,7} > 50 \text{ g/m}^2$ in men and $> 47 \text{ g/m}^2$ in women; empirical P values are based on 50 000 permutations.

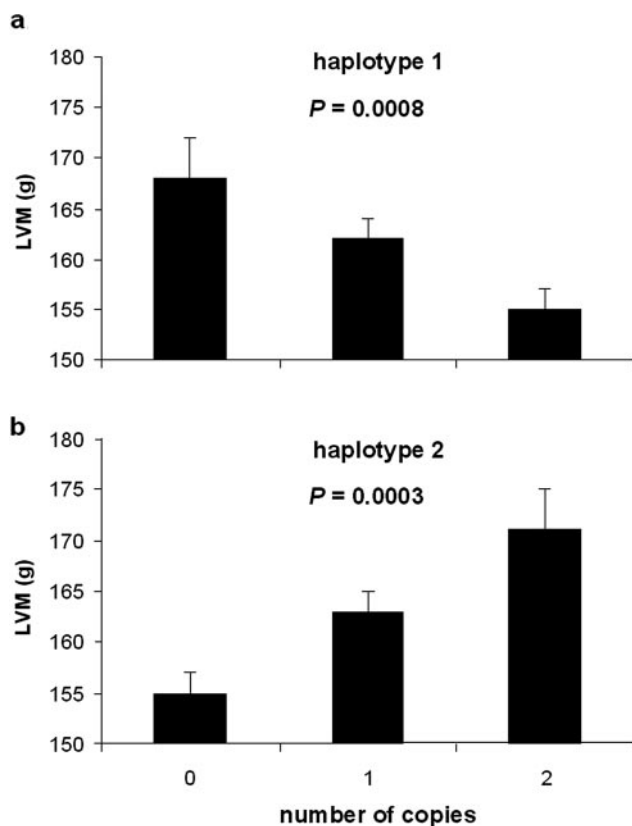


Figure 2. Relation between the number of copies of haplotype 1 (a) and 2 (b) with LVM in the entire study sample.

an increasing number of copies of the minor allele in men and in women.

Discussion

The present study offers a comprehensive analysis of the relation of genetic variants and haplotype structure across the

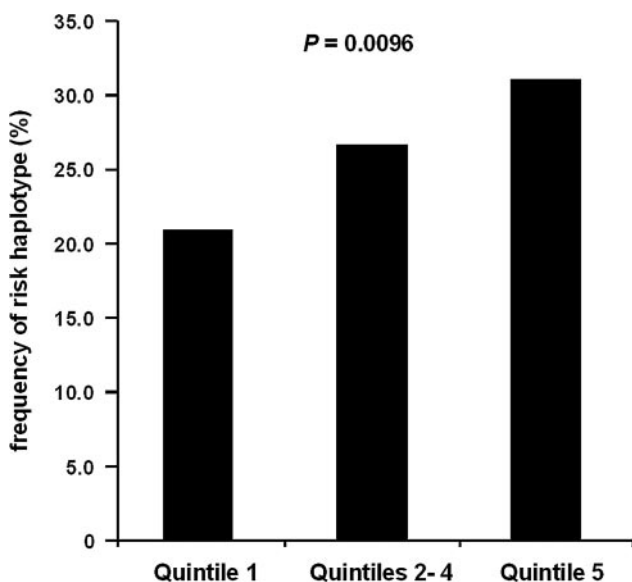


Figure 3. Increasing frequency of the risk haplotype from the lowest (Q1) to the highest (Q5) quintile of the $LVM_{h2.7}$ distribution. For this analysis individual affiliation to quintiles were determined for both genders separately. P for trend.

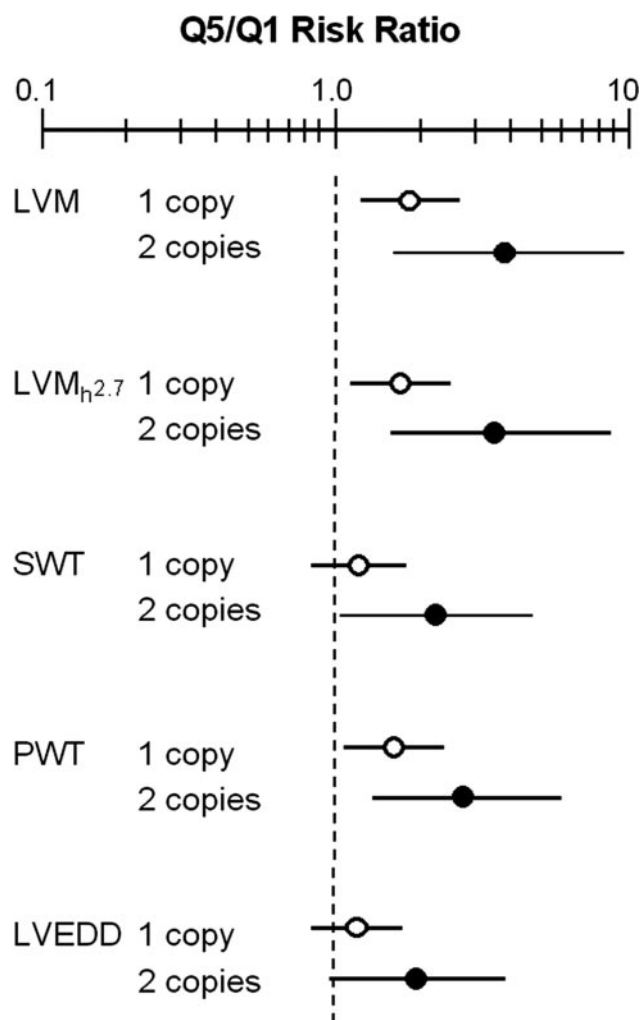


Figure 4. Multivariate adjusted Q5/Q1 risk ratios for subjects carrying 1 or 2 copies of the risk haplotype versus subjects carrying no copy. Age, gender, body mass index, BP >140/90 mm Hg, and antihypertensive medication were included as covariates.

entire *GHSR* gene region in the general population with parameters of LVM and heart size. Here, we focus on the 2 most common *GHSR* haplotypes, derived from a high LD block, and the 5 contributing SNPs. We report significant association of both *GHSR* haplotypes, as well as an intronic (rs509035) and an exonic (rs572169) SNP with multiple indexed and nonindexed parameters of LVM and heart size and LVH in the general population. Multivariate adjustments and the consistent findings for different types of LVM indexation indicate that the effects are independent of BP levels and body mass. To our knowledge, these data are the first to demonstrate that genetic variants within the *GHSR* gene region are associated with LVM and geometry parameters, as well as LVH in the general population and, thus, may be involved in the pathogenesis of LVH. Because we focused on common SNPs and haplotypes, the conclusions should be of great importance for a significant proportion of the population.

Cardiac mass is highly variable, and multiple pathophysiological mechanisms and states have been implicated in cardiac growth and the development of LVH. Specifically,

TABLE 3. Summary of the Association of SNPs With LVH* in the Total MONICA Echocardiographic Substudy Sample and in Matched Cases and Controls

SNP†	Genotypes																
	Cases				Controls				Allele 2 vs Allele 1			Genotype 22 vs 11			Armitage's Trend Test		
	11	12	22	MAF	11	12	22	MAF	OR (95% CI)‡	P	P _(corr.) §	OR (95% CI)‡	P	P _(corr.) §	OR (95% CI)‡	P	P _(corr.) §
rs509035																	
Matched	34	53	14	0.40	159	159	20	0.29	1.61 (1.16–2.22)	0.0043	0.0063	3.27 (1.51–7.20)	0.0019	0.0028	1.76	0.0025	0.0037
Total study	69	90	21	0.37	441	391	68	0.29	1.40 (1.10–1.77)	0.0054	0.0079	1.97 (1.14–3.43)	0.0142	0.0207	1.42	0.0043	0.0063
rs572169																	
Matched	39	52	15	0.39	165	157	23	0.29	1.51 (1.10–2.08)	0.0112	0.0164	2.76 (1.32–5.77)	0.0057	0.0083	1.61	0.0085	0.0124
Total study	76	90	23	0.36	454	396	81	0.30	1.31 (1.04–1.66)	0.0212	0.0310	1.70 (1.01–2.86)	0.0458	0.0669	1.31	0.0203	0.0296
rs519384																	
Matched	48	49	12	0.33	178	148	22	0.28	1.32 (0.95–1.83)	0.0939	...	2.02 (0.93–4.38)	0.070	...	1.37	0.0861	...
Total study	94	79	18	0.30	502	378	65	0.27	1.17 (0.92–1.49)	0.1973	...	1.48 (0.84–2.61)	0.1737	...	1.19	0.1949	...
rs512692																	
Matched	44	47	12	0.34	174	142	20	0.27	1.42 (1.01–1.98)	0.0407	0.0594	2.37 (1.08–5.22)	0.0282	0.0412	1.48	0.0358	0.0511
Total study	89	79	18	0.31	491	367	62	0.27	1.23 (0.96–1.57)	0.0953	...	1.60 (0.91–2.84)	0.1036	...	1.25	0.0930	...
rs863441																	
Matched	47	45	11	0.33	173	142	23	0.28	1.25 (0.89–1.75)	0.1914	...	1.76 (0.80–3.87)	0.1554	...	1.29	0.1843	...
Total study	93	78	17	0.30	493	368	64	0.27	1.16 (0.91–1.48)	0.2377	...	1.41 (0.79–2.51)	0.2447	...	1.17	0.2355	...

MAF indicates minor allele frequency; OR, odds ratio; CI confidence interval.

*Defined as LVM_h^{2,7} >50 g/m² in men and >47 g/m² in women.

†SNPs shown as rs numbers from the dbSNP database.

‡OR reported with their 95% CI are based on the Armitage test for trend.

§P value corrected for multiple testing.⁴¹

environmental, heritable, metabolic, hemodynamic, and neuroendocrine factors play a considerable role. Among those, the GHSR/ghrelin system may be a new link among GH activity, the neuroendocrine regulation of body weight, and energy balance and, thus, the development of LVH.³⁰ As the target of the endogenous ligand ghrelin, GHSR, integrates 2 anabolic actions: first, it mediates the stimulatory effect of ghrelin on GH release, and, second, it communicates the orexigenic and adipogenic activity of ghrelin, contributing to energy balance.³⁰ A positive energy balance, in turn, is necessary to maximize the anabolic actions of GH. GH could influence the growth of peripheral organs, for example, the heart, and favor the development of LVH. Indeed, it has been shown that GH and its local effector, insulin-like growth factor 1, increase the size of cultured cardiomyocytes and simultaneously induce muscle-specific gene expression,³¹ thereby promoting cardiac hypertrophy.

We have demonstrated previously that *GHSR* influences susceptibility to obesity and may be involved in the pathogenesis of this complex disease. We specifically identified linkage and association of common genetic variants within the *GHSR* gene region and body mass related phenotypes.¹⁷ Thus, one might speculate that heart size is driven by a resembling mechanism, because it is well known that an increased LVM and LVH are associated with obesity.^{32,33}

On the other hand, there is evidence, including the present data, that the actions of ghrelin mediated by GHSR, namely the regulation of GH secretion and the control of energy homeostasis, are performed through 2 independent mechanisms.^{34,35} Evidence that ghrelin-induced adiposity is inde-

pendent from ghrelin-mediated GH release is obtained from studies with GH-deficient rats, because ghrelin also increases body weight in those rats.³⁴ Furthermore, the orexigenic effects may not be mediated by GH, which is known to be lipolytic rather than lipogenic.³⁶

We were led to pursue *GHSR* to analyze whether common genetic *GHSR* variants affect LVM and size, independent of body mass, thereby influencing the development of LVH. The fact that we show very consistent associations between the 2 most frequent 5-marker haplotypes and 2 of the 5 SNPs with multiple parameters of LVM and heart size, independent of indexation, in the entire MONICA LVH substudy and in a subset of cases and controls well matched for hypertension and body mass argues in favor of 2 independent mechanisms.

We initially determined the extent of the region of high LD by covering the entire gene region, including the surrounding genomic regions close to the neighboring genes, with SNPs. The identified region of high LD encompasses part of the intron, exon 1, and the 5' adjacent region extending 8.8 kb past the 5' end of the gene but not encompassing the flanking genes. Therefore, it is unlikely that the association between variants of the *GHSR* gene and LVM and heart size parameters, as well as LVH, is seen because of LD with the proper causal mutation in 1 of the neighboring genes. However, we are not able to present a causal mutation at this point.

In our study, we evaluated exonic, intronic, and intergenic SNPs and found that the SNP residing in the intron (rs509035) showed the strongest association with our phenotypes, followed by the SNP in the exon (rs572169). Moreover, we detected a marginal association of 1 SNP located in the 5' region

(rs512692). These results support the hypothesis that regulatory elements, transcriptional initiation, or the promotor might be involved in the manifestation of variations underlying complex diseases, that is, that such variations might not be limited to the structure of the encoded protein.^{37–39}

The association between haplotypes and individual SNPs and LVM phenotypes could be observed in analyses of both quantitative and dichotomized phenotypes, revealing remarkably low *P* values, despite correction for multiple testing and permutation procedures. Thus, it seems unlikely that these associations are because of chance or confounding factors. Because the present LVH substudy is a random subset of the general population, the likelihood for a selection bias and population stratification might be low.^{40,41} However, further studies are needed to confirm the association of genetic *GHSR* variants and LVM and geometry phenotypes. Most importantly, functional studies might clarify whether individuals carrying the “risk” haplotype present with altered receptor activity and less favorable receptor properties, resulting in a higher susceptibility to LVH.

Perspectives

Common and complex phenotypes, such as LVH, are caused by the interaction of environmental and genetic factors. Multiple, and, in part, interacting, genes or genetic variants are likely to be involved in the expression of the disease phenotype. The current knowledge of the biological functions of genes, genetic variants, and encoded proteins in the pathogenesis of LVH is still very limited, and from this perspective, the susceptibility genes so far identified are likely to represent only a small contribution to the understanding of the molecular genetics of this complex trait. The combination of precise phenotypes and the definition of genetic variants, as performed in the present study, together with high-throughput genotyping methods, as well as the development of novel bioinformatic tools that integrate the complex interplay of multiple genes and environmental risk factors, will facilitate the elucidation of novel pathways in the pathogenesis of LVH in the future.

With this information at hand, early identification of high-risk individuals and preventive treatment will become feasible, for example, by using lower target BP levels or by providing background for the development of efficient drugs.

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Association of the Ghrelin Receptor Gene Region With Left Ventricular Hypertrophy in the General Population: Results of the MONICA/KORA Augsburg Echocardiographic Substudy

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