

Variants in the Ghrelin Gene Are Associated with Metabolic Syndrome in the Old Order Amish

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Context: Mature ghrelin has been shown to stimulate eating and to participate in the regulation of insulin signaling and glucose homeostasis. Its gene, *GHRL*, is located on chromosome 3 in a region where we have shown linkage to eating behavior, percentage body fat, and total and low-density lipoprotein cholesterol levels in subjects of the Amish Family Diabetes Study.

Objective: Our objective was to determine whether mutations in *GHRL* might influence eating behavior and risk for metabolic syndrome, obesity, diabetes, and related traits.

Design: We genotyped 856 Amish samples for three missense polymorphisms in *GHRL*, Arg51Gln, Leu72Met (rs696217), and Gln90Leu (rs4684677) and performed association analyses with eating behavior traits and metabolic syndrome as defined by the National Cholesterol Education Program Adult Treatment Panel III guidelines.

Subjects: Our subjects were adult participants in the Amish Family Diabetes Study.

Results: The allele frequencies of these variants were 0.03, 0.04, and 0.03, respectively. The prevalence of metabolic syndrome was lower among those carrying the 51Gln allele (3.8 vs. 15.8%; age- and sex-adjusted odds ratio = 0.22; $P = 0.031$). Hunger scores tended to be lower among 51Gln allele carriers but did not reach statistical significance ($P = 0.07$). The Leu72Met variant was also associated with increased prevalence of metabolic syndrome (23.2 vs. 13.4%; age- and sex-adjusted odds ratio = 2.57; $P = 0.02$) as well as higher fasting glucose, lower high-density lipoprotein, and higher triglyceride levels ($P = 0.02$, $P = 0.007$, and $P = 0.04$, respectively). The two variants were not in linkage disequilibrium with each other, suggesting independent effects. We conclude that mutations in *GHRL* may confer risk for the metabolic syndrome. (*J Clin Endocrinol Metab* 90: 6672–6677, 2005)

METABOLIC SYNDROME IS a heterogeneous disorder characterized by the presence of three or more of the following characteristics: abdominal obesity, elevated triglyceride concentrations, low high-density lipoprotein (HDL) cholesterol, high blood pressure, and elevated fasting glucose (1). It is associated with increased risk of type 2 diabetes (T2DM) and cardiovascular disease, global health problems, with increasing prevalence and growing impact on morbidity, mortality, and health care costs (2, 3). Nutrition habits, food intake, and physical inactivity impact risk of developing metabolic syndrome, T2DM, and cardiovascular disease (4). Yet not all people with poor nutritional habits and physical inactivity develop metabolic syndrome, suggesting important interactions between genetic, behavioral, and environmental factors. A deeper understanding of these complex interactions could provide opportunities for more effective treatment and preventive strategies.

Mature ghrelin is a 28-amino-acid peptide secreted from neuroendocrine cells, particularly of the gastrointestinal tract, and has been shown to stimulate eating and play a role in regulating energy balance, insulin signaling, and glucose levels (5–12). Plasma ghrelin levels rise before eating and are

positively associated with visual analog-quantified hunger scores (13). Ghrelin secretion appears to be modulated by food intake. Plasma ghrelin levels are reduced by consumption of glucose, lipid, and protein, but to varying degrees (14–16).

The ghrelin gene (*GHRL*) consists of four exons spanning 5199 base pairs and encodes a 115-amino-acid prepropeptide (17). Posttranslational processing, including proteolytic cleavages and *O*-*n*-octanoylation at serine-3 results in the active 28-amino-acid hormone. At least three common polymorphisms in *GHRL* have been reported: Arg51Gln, Leu72Met (rs696217) (18), and Gln90Leu (rs4684677) (19). The Arg51Gln polymorphism is at the junction of a cleavage site necessary to generate the mature active hormone, whereas the Leu72Met and Gln90Leu polymorphisms reside in regions of the precursor peptide that are not part of the mature hormone. Polymorphisms in *GHRL* have been shown to be associated with obesity (18–20) and obesity-related phenotypes (21, 22). The Arg51Gln variant was associated with T2DM, and elevated blood pressure in middle-aged Finnish subjects (23, 24); however, this variant was not associated with obesity phenotypes in three other studies (21). The Leu72Met polymorphism was associated with serum creatinine and lipoprotein (a) variation among Finnish subjects with T2DM (25) as well as with lower insulin levels among euglycemic tall and obese children (22), lower prevalence of hypertension among women participating in the Swedish Obese Subjects study (21), and lower fat mass among Black subjects participating in the HERITAGE Family Study and subjects enrolled in the Québec Family Study (21). Despite these modest associations with metabolic traits, results to date

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Abbreviations: AFDS, Amish Family Diabetes Study; BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism; T2DM, type 2 diabetes mellitus.

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have been inconsistent, and none have examined their potential relationship to eating behavior traits.

GHRL is located on chromosome 3p25–26, an area where we have previously shown linkage to eating behavior, percentage body fat, total and low-density lipoprotein cholesterol levels in subjects who participated in the Amish Family Diabetes Study (AFDS) (Fig. 1) (26, 27). We thus hypothesized that mutations in *GHRL* might influence eating behavior and risk for metabolic syndrome and related traits in this population. To address this hypothesis, we genotyped 856 samples from the AFDS for the three missense polymorphisms in *GHRL* to determine whether variants in this gene influence eating behavior or susceptibility to metabolic syndrome; its constituent components, obesity, hyperlipidemia, and hypertension; and T2DM.

Subjects and Methods

Subjects

The Old Order Amish of Lancaster County, Pennsylvania, represent a genetically closed homogeneous Caucasian population of Central European ancestry that is ideal for recruitment of large multiplex pedigrees for genetic studies. Large Amish families from Lancaster County, Pennsylvania, and surrounding counties were recruited between 1995 and 2000 (28). Families were recruited around an index case (proband) with T2DM with age of onset between 35 and 65 yr of age. All first- and second-degree family members aged 18 or over were recruited around the diabetic probands. If another individual with diabetes was identified in the family (e.g. aunt or uncle), the family was expanded further to include the first- and second-degree relatives aged 18 or over of that individual. All of these families could be joined into a single pedigree by including ancestors going back 12–14 generations (29, 30). For computational efficiency, this large pedigree was divided into 48 families, 11 of which had more than 20 participating family members (range, 22–188). The study was conducted in accordance with the standards involving human subjects of the Institutional Review Board of the University of Maryland, and informed consent was obtained from all subjects.

Phenotypic characterization for the 856 AFDS members in this study included a medical and family history obtained by interview. None of

the subjects included in the analysis of eating behaviors reported taking any medication known to affect appetite. Height and weight were measured, and percentage body fat was estimated by bioimpedance as described previously (27). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared to estimate total adiposity. Waist circumference was measured after removal of bulky clothing using an inelastic tape. The measurement was recorded to the nearest 0.1 cm. Blood pressure measurements were obtained in duplicate, with the use of a standard sphygmomanometer with the subject sitting for more than 5 min and were recorded to the nearest 1 mm Hg. Fasting lipid profiles (total cholesterol, HDL cholesterol, and triglycerides) were assayed by Quest Diagnostics (Baltimore, MD) (interassay coefficient of variation = 1.6% for total cholesterol, 5.0% for HDL cholesterol, and 1.6% for triglycerides). A 3-h 75-g oral glucose tolerance test (OGTT) was performed on subjects with no previous history of diabetes. Insulin and leptin concentrations were determined by RIA (Linco Research, Inc., St. Louis, MO) (interassay coefficient of variation = 4.4 and 4.2%, respectively). Diabetes was defined based upon criteria of the American Diabetes Association (2-h glucose \geq 11.1 mmol/liter or fasting glucose \geq 7 mmol/liter) (31), and metabolic syndrome was defined based upon criteria of National Cholesterol Education Program Adult Treatment Panel III (1).

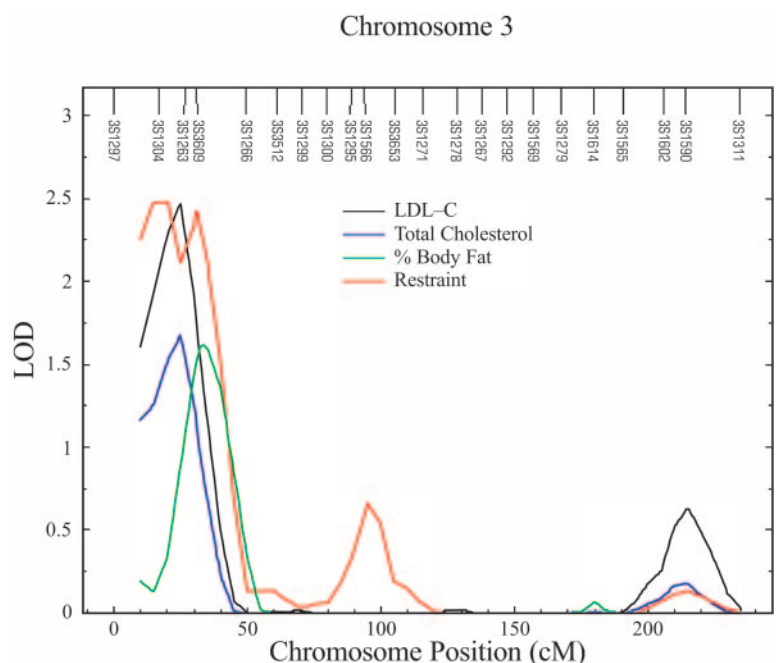
Eating behavior scores were obtained from the Three Factor Eating Questionnaire as previously described (32). The three dimensions of eating behavior measured were restraint, disinhibition, and hunger (32, 33).

Laboratory methods

Genotyping was completed using template-directed dye terminator incorporation with fluorescence polarization detection method for the Arg51Gln and Leu72Met polymorphisms. The AcycloPrime FP single nucleotide polymorphism (SNP) detection kit was used (PerkinElmer Life Sciences, Inc., Boston, MA). This process involved subjecting genomic DNA to PCR, followed by use of a thermostable polymerase that extends by one base an oligonucleotide primer that ends immediately upstream of the SNP position, using one of two fluorescent dye-labeled terminators. Reaction plates were then transferred to a fluorescence polarization reader. The identity of the base added was determined by the increased fluorescence polarization of its linked dye.

For the Gln90Leu polymorphism, the Beckman SNPstream UHT Genotyping System (Beckman Coulter, Inc., Fullerton, CA) was used. First, the target genomic sequences containing the SNPs of interest were amplified in a 12-plex PCR. After enzymatic cleanup, the PCR products

FIG. 1. Chromosome 3 linkage to lipids, percentage body fat, and eating behavior in the Old Order Amish.



were subjected to an extension reaction using 5'-tagged extension primers and fluorescent dye-labeled terminators. In a thermal cycled extension step, the primers were hybridized to the specific amplicons one base adjacent to the SNP site and extended by one base at the 3' end with a fluorescently labeled nucleotide. Beckman's SNPscope reader imaged the microarray plates.

For both methods, the image signals were transferred to genotyping software that translated the images into genotype calls, and allele calls were transferred to data files. The error rates based on blind replicates were 2, 0.1, and 0% for the Arg51Gln, Leu72Met, and Gln90Leu polymorphisms, respectively.

Statistical analyses

The analysis set included a total of 856 individuals who were genotyped for one or more of the three GHRL polymorphisms and phenotyped for one or more metabolic syndrome-related traits. These subjects included probands from ascertained families and their family members, although not all individuals were included in each analysis, either because of missing data and/or outcome-specific exclusions. The analysis of diabetes was based on 90 subjects with T2DM and 286 normoglycemic subjects aged 38 yr and over. The analysis of metabolic syndrome was based upon 106 subjects with metabolic syndrome and 621 subjects who did not meet the criteria for metabolic syndrome. The number of subjects included for analysis of quantitative traits was 785. This number did not include subjects currently taking insulin ($n = 13$), β -hydroxy β -methylglutaryl coenzyme A reductase inhibitors or other lipid-lowering medications ($n = 11$), aspirin ($n = 21$), angiotensin converting enzyme inhibitors or other blood pressure-lowering agents ($n = 62$), and/or estrogens ($n = 3$). In total, 86 subjects taking one or more of the above medications were excluded from quantitative trait analysis. In addition, for analysis of eating behavior traits, subjects with prevalent diabetes ($n = 18$) were excluded to avoid influence of previous exposure to dietary advice and exposure to diabetes medications, which may indirectly affect appetite.

Before analysis, genotypes were checked for Mendelian consistency, and inconsistencies, comprising less than 0.5% of genotypes, were removed from analysis. χ^2 tests were used to evaluate whether the distribution of SNP genotypes were consistent with expected genotype distribution under Hardy-Weinberg equilibrium.

We estimated mean levels of glucose, insulin, obesity, blood pressure, and lipid traits according to GHRL genotypes in the AFDS sample. To account for the relatedness among family members, we employed the measured genotype approach (34), in which we estimated the likelihood of specific genetic models given the pedigree structure. For example, we compared the likelihood of a full model, which allowed for genotypic-specific means, to that of a nested model in which genotypic means were restricted to be equal to each other. Parameter estimates were obtained by maximum likelihood methods, and the significance of association was tested by likelihood ratio tests. Within each model, we simultaneously estimated the effects of age and sex. Insulin, leptin, and triglyceride values and eating behavior scores were transformed by their

natural logarithms (ln) to reduce skewness. Analyses of insulin levels were performed excluding data from subjects meeting criteria for diabetes. Insulin secretion was calculated as $\ln(\text{insulin } 30 - \text{fasting insulin})$. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as $[(\text{fasting glucose (mmol/liter)} \times (\text{fasting insulin } (\mu\text{U/ml})/22.5)]$.

Analysis of the dichotomized traits, diabetes and metabolic syndrome, was conducted using a variance component threshold model. This analysis is analogous to logistic regression with disease status as the dependent variable and genotype (with covariates age and sex) modeled as the independent variables. As before, all correlations were estimated conditional on the familial correlations implied by the pedigree structure. All analyses were conducted using the SOLAR program (35).

In addition to computing P values by likelihood ratio test, we also computed P values empirically for low-frequency variants to guard against the possibility of elevated type 1 error rates. Briefly, we generated empirical P values for SNP-trait associations by dropping 10,000 randomly generated SNPs with a given allele frequency through the pedigrees and testing each one for association. The empirical P value for the association between the SNP and the trait was defined as the proportion of the 10,000 simulated replicates having a test statistic (*i.e.* likelihood value) greater than or equal to that of the model containing the real SNP.

The strength of linkage disequilibrium between all pairwise combinations of SNPs was inferred for each individual by maximum likelihood methods using the ZAPLO software program (36).

Results

The clinical characteristics for the population studied are shown in Table 1. The prevalence of metabolic syndrome was 9.8% among men and 15.2% among women ($P < 0.001$). The prevalence of T2DM was 17.2% among men and 30.4% among women ($P = 0.004$). BMI and serum levels of leptin and HDL were higher among women compared with men ($P < 0.001$ for all).

The frequencies of the variant alleles, Gln51 (G→A), Met72 (C→A), and Leu90 (A→T) were 0.03, 0.04, and 0.03, respectively. No subject was homozygous for the Gln51 or the Met72 variant; one was homozygous for the Leu90 variant. There was no association with BMI, waist circumference, or T2DM for any variant.

The Gln51 variant was associated with lower ln leptin ($P = 0.02$), despite similar BMI compared with those homozygous for the Arg51 allele (Table 2). Hunger scores among subjects heterozygous for the Arg51Gln variant trended toward being lower, but did not reach statistical significance ($P = 0.07$). Glucose levels during the OGTT, insulin secretion, and insulin

TABLE 1. Mean (\pm SE) values of selected clinical characteristics of Amish men and women

Trait	Men (n = 402)	Women (n = 454)
Age (yr)	46.2 \pm 0.9	45.5 \pm .09
BMI (kg/m ²)	26.5 \pm 0.9	28.0 \pm 0.3 ^b
Waist circumference (cm)	95.1 \pm 0.6	89.4 \pm 0.7 ^b
Systolic blood pressure (mm Hg)	122.6 \pm 0.8	124.1 \pm 1.0
Diastolic blood pressure (mm Hg)	78.7 \pm 0.5	77.9 \pm 0.6
Fasting glucose (mmol/liter)	5.05 (4.78, 5.36) ^c	5.00 (4.66, 5.35) ^c
Fasting insulin (pmol/liter)	358.9 (286.6, 456.5) ^c	350.3 (281.5, 452.5) ^c
Leptin (ng/dl)	2.68 (1.51, 4.97) ^c	13.8 (7.2, 24.1) ^{b,c}
Total cholesterol (mmol/liter)	5.52 \pm 0.07	5.58 \pm 0.08
HDL cholesterol (mmol/liter)	1.21 \pm 0.02	1.39 \pm 0.02 ^{b,c}
Triglycerides (mmol/liter)	0.76 (0.55, 1.14) ^c	0.78 (0.54, 1.21) ^c
% T2DM (no. of cases/no. of controls)	17.2 (32/153)	30.4 (58/133) ^a
% Metabolic syndrome (no. of cases/no. of controls)	9.8 (38/348)	15.2 (68/379) ^b

^a $P < 0.01$, men vs. women, adjusted for age.

^b $P < 0.001$, men vs. women, adjusted for age.

^c Median (lower 25% and upper 75% values of the distribution).

TABLE 2. Mean levels (\pm SE) of clinical characteristics among the Amish according to ghrelin genotype, adjusted for age and sex

Trait	Arg51Gln (R51Q)		Leu72Met (L72M)	
	RR (n = 756)	RQ (n = 67)	LL (n = 746)	LM (n = 54)
BMI (kg/m ²)	27.1 \pm 0.3	26.0 \pm 0.7	27.1 \pm 0.3	27.4 \pm 0.7
Waist circumference (cm)	97.1 \pm 0.8	95.1 \pm 1.7	97.0 \pm 0.8	99.2 \pm 1.5
Fasting glucose (mmol/liter)	5.21 \pm 0.11	5.27 \pm 0.22	5.19 \pm 0.11	5.66 \pm 0.21 ^a
Systolic blood pressure (mm Hg)	121.3 \pm 1.0	119 \pm 2.2	121.2 \pm 1.1	121.8 \pm 2.1
Diastolic blood pressure (mm Hg)	80.6 \pm 0.7	80.6 \pm 1.5	80.3 \pm 0.7	81.0 \pm 1.4
Ln leptin (ng/dl)	1.10 \pm 0.06	0.86 \pm 0.12 ^a	1.09 \pm 0.06	1.23 \pm 0.11
Total cholesterol (mmol/liter)	5.68 \pm 0.09	5.59 \pm 0.18	5.70 \pm 0.09	5.58 \pm 0.17
HDL cholesterol (mmol/liter)	1.24 \pm 0.03	1.23 \pm 0.05	1.25 \pm 0.03	1.11 \pm 0.05 ^b
Ln triglycerides (mmol/liter) \times 100	4.90 \pm 0.05	4.81 \pm 0.10	4.88 \pm 0.05	5.06 \pm 0.09 ^a
Ln hunger score	1.48 \pm 0.06	1.22 \pm 0.12	1.50 \pm 0.06	1.59 \pm 0.11
Ln restraint score	1.37 \pm 0.06	1.29 \pm 0.12	1.38 \pm 0.06	1.53 \pm 0.11
Ln disinhibition score	1.50 \pm 0.04	1.41 \pm 0.09	1.49 \pm 0.04	1.57 \pm 0.09
% T2DM (no. of cases/no. of controls)	23 (77/259)	39 (11/17)	24 (81/254)	22 (4/14)
% Metabolic syndrome (no. of cases/no. of controls)	15.8 (100/634)	3.8 (2/53) ^a	13.4 (85/634)	23.3 (14/60) ^a

Means are scaled for men of the mean age of the population. Analysis of diabetes was restricted to subjects aged 36 yr and older. For Gln90Leu, there was no significant association with above phenotypes.

^a $P < 0.05$ (see text for precise P values).

^b $P < 0.01$.

sensitivity estimated by HOMA-IR were not significantly different among subjects categorized according to Arg51Gln genotype. Metabolic syndrome was less prevalent among those with the Gln51 allele compared with Arg51 homozygotes (3.8 vs. 15.8%; age- and sex-adjusted odds ratio = 0.22; nominal P value = 0.03; empirical P value based on 10,000 simulations = 0.04).

The Leu72Met variant was associated with higher fasting glucose, lower HDL cholesterol, and higher triglyceride levels ($P = 0.02$, $P = 0.007$, and $P = 0.04$, respectively) (Table 2). There was no association of this variant with HOMA-IR or insulin secretion, although metabolic syndrome was more prevalent among carriers of the Met 72 variant (23.2 vs. 13.4%; age- and sex-adjusted odds ratio = 2.57; nominal P value = 0.02; empirical P value = 0.03). There was no evidence for association of the Met72 allele with eating behavior traits. There were no significant associations between the Gln90Leu variant and measures of obesity, eating behavior, blood pressure, lipids, glucose, or insulin levels (data not shown).

None of the three SNPs were in significant linkage disequilibrium with each other (pairwise r^2 values were all < 0.01). For this reason, and because the minor allele at each SNP was relatively infrequent, haplotype analyses were not carried out.

Discussion

The prevalence of metabolic syndrome among U.S. adults is reported to be approximately 22% according to data from the Third National Health and Nutrition Examination Survey (NHANES III) data (37). The prevalence of metabolic syndrome increases with age and is impacted by race and ethnicity. Data from NHANES III also indicate that metabolic syndrome is more prevalent among African-American women compared with African-American men, but the prevalence is similar according to gender among whites and other races and ethnic origins (37). Interestingly, among the Amish, a Caucasian cohort of European descent, metabolic syndrome as well as T2DM is more prevalent among women compared with men. The women in the current study had higher BMI than men, although they were not older and did

not have significantly different prevalence of individual abnormalities of the metabolic syndrome. It is likely that Amish men are physically more active than women, which might also contribute to the observed sexual dimorphism in metabolic syndrome and T2DM prevalence in this population.

Ghrelin is a ligand of a G coupled-protein receptor belonging to the GH secretagogue receptor family and has been shown to have neuroendocrine activities including modulation of insulin secretion, glucose and leptin levels, food intake and energy balance, gastric motility, and acid secretion, among others (6, 38, 39). Thus, *GHRL* is a plausible candidate gene for obesity, T2DM, and the metabolic syndrome. Missense mutations at codons 51, 72, and 90 have been reported to be related to obesity and obesity-related phenotypes (18–22). However, these associations have not been shown consistently. Inconsistency between findings could be real and caused by genetic heterogeneity or differences between populations in environmental factors that influence phenotypic expression of the gene variant. Alternatively, positive results could represent false positives because of population stratification, type 1 error, or multiple comparisons, or false negatives may be a result of inadequate power or population stratification.

A rare 2-bp deletion in *GHRL* at codon 34 leading to the insertion of 36 aberrant amino acids was reported in a normal-weight individual (19). This suggests that haploinsufficiency of *GHRL* is not accompanied by a significant phenotype, at least with respect to obesity. However, because there are many genetic and environmental influences on obesity, modest or even moderate functional consequences of genetic variants in *GHRL* might not be discernible. This provided the rationale for us to examine association of variation in *GHRL* with eating behavior, traits more directly related to ghrelin action.

In the Old Order Amish, the frequency of the Arg51Gln mutation is similar, whereas those of the Leu72Met and Gln 90Leu mutations are lower, compared with frequencies reported in Swedish and German populations (18, 19). The Arg51Gln mutation occurs at the carboxyl-terminal cleavage site of proghrelin to produce mature ghrelin. Mutations at this site have been shown to be associated with lower mature

ghrelin levels (21, 24). Unfortunately, plasma ghrelin levels were not measured in the current study. Individuals with this variant had hunger scores that trended toward being lower, although they did not reach statistical significance. This finding is of particular interest given the known role of ghrelin on feeding behavior (12–14). These findings suggest greater leptin sensitivity in those with the Arg51 allele or alternatively leptin-independent effects of the Gln51 allele on hunger. Subjects with the Gln51 variant also had lower age- and gender-adjusted leptin levels, despite similar BMI, which would not have been expected in subjects who also had lower hunger scores. Ghrelin is reported to antagonize leptin action via neuropeptide Y/Y1 neural pathways (10, 40, 41); however, disparate findings have been reported regarding the correlation between fasting ghrelin and leptin levels (42, 43).

Our studies did not show an association between the Gln51 allele and risk for hypertension or diabetes, in contrast to the findings of the OPERA study (23). However, we did find significant association of this variant with the metabolic syndrome, those with the Gln51 allele being protected relative to Arg51 homozygotes. To date, few studies of variants in *GHRL* have been performed to determine the relationship between the Gln51 allele and risk for conditions related to metabolic syndrome in humans, but evidence regarding the association of the Gln51 variant with obesity is discordant (21). Other evidence linking variants in *GHRL* to metabolic parameters comes from the Finnish OPERA study (23, 24). These studies included over 1000 randomly selected subjects with hypertension and age-matched controls. Among these subjects, the Gln51 variant was associated with T2DM and hypertension. Subjects with this variant also had lower ghrelin levels, which were also associated with high insulin concentration, insulin resistance, and elevated blood pressure.

A potential explanation for the different findings in OPERA compared with AFDS may be a difference in ascertainment strategy or underlying *GHRL* variant-environment interaction. Subjects in the OPERA study were ascertained based on use of blood pressure-lowering medication. Amish subjects were ascertained based on family history of T2DM. One potential environmental characteristic unique to the Old Order Amish is a high level of physical activity. The Old Order Amish rely little on motorized mechanical devices and modern technology. A study of an Old Order Amish community living in Ontario, Canada, reports that men typically engage in 10 h of vigorous and over 40 h of moderate activity weekly, whereas women report engaging in over 3 h of vigorous and nearly 40 h of moderate activity weekly (44). A hypothesis might be that Amish with the Gln51 allele are less hungry and more active and have greater insulin and leptin sensitivity and lower prevalence of metabolic syndrome.

The Leu72Met mutation results in an amino acid exchange in the proghrelin coding sequence of exon 2. The functional consequences of this variant are not known. Although not at a site of proteolytic cleavage, this variant could impair proteolytic processing similar to the proinsulin Providence gene variant in subjects with familial hyperproinsulinemia (45). In our study, individuals with this variant had higher fasting glucose, lower HDL, and higher triglyceride levels than individuals without the variant. Elevated glucose and trigly-

cerides and low HDL are associated with risk for metabolic syndrome, and indeed we found that this variant was significantly associated with increased risk of the metabolic syndrome. It has been suggested that this variant might modulate insulin secretion. In a study of tall obese children, insulin secretion was lower in a setting of euglycemia among subjects with this variant (22). In the present study, however, there was no difference in insulin levels during the 3-h OGTT or insulin secretion between Met72 carriers and Leu72 homozygotes.

It is of interest that the Leu72Met and Arg51Gln variants are not in significant linkage disequilibrium with each other, suggesting that the effect of both of these variants on risk for metabolic syndrome are independent of each other. Although the functional consequences of these variants are not known, the independent associations provide supportive evidence for a role of *GHRL* polymorphisms in influencing metabolic syndrome risk. In a *post hoc* analysis in which risk of metabolic syndrome in subjects with either at-risk variant (Arg51 or Met72) was compared with subjects with neither at-risk variant, we found that those with one of the risk variants had a significantly higher prevalence of metabolic syndrome compared with those without an at-risk variant (15.6 vs. 2.2%; age- and sex-adjusted odds ratio = 13.0; *P* value = 0.01).

In summary, we found that two missense mutations in *GHRL* influence risk of metabolic syndrome. Furthermore, the Arg51Gln variant was associated with a trend toward lower hunger scores, suggesting that this variant may decrease risk for metabolic syndrome through its effects on eating behavior; however, the interaction of *GHRL* variants and metabolic processes remains controversial. More detailed phenotypic characterization as well as functional studies of these variants will be necessary to further define the role of *GHRL* polymorphisms in defining risk for metabolic syndrome and to delineate the molecular and physiological mechanisms of their effects.

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