Variation in the Lamin A/C Gene
Associations With Metabolic Syndrome

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Objective—Metabolic syndrome is associated with increased risk for cardiovascular disease and type 2 diabetes mellitus (T2DM). The lamin A/C (*LMNA*) gene, mutations of which cause rare syndromes of severe insulin resistance and dyslipidemia, is located on chromosome 1q21-q24, a region linked to T2DM in several genome wide scans, including in the Old Order Amish. To determine whether polymorphisms in *LMNA* influence susceptibility to metabolic syndrome and its constituent components.

Methods and Results—We performed DNA sequence analysis of *LMNA.* Six single-nucleotide polymorphisms (SNPs) were identified: c.141889C>T (intron 3), c.141906G>T (intron 3), A287A (c.141253T>C; exon 5), c.140353G>A (intron 6), c.139418C>T (intron 8), and H566H (c. 138747C>T; exon 10). In 971 participants from the Amish Family Diabetes Study, the H566H polymorphism of *LMNA* was associated with metabolic syndrome diagnosed according to National Cholesterol Education Program ATP III criteria and also higher mean fasting triglyceride and lower mean high-density lipoprotein-cholesterol concentrations. However, no differences in allele frequencies were observed for any SNP among participants with T2DM or impaired glucose homeostasis (IGH) and normoglycemic controls. Haplotype analysis showed that >87% of individuals carried 1 of 2 common *LMNA* haplotypes. There were no significant differences in haplotype frequencies among subjects with metabolic syndrome T2DM, IGH, and controls.

Conclusion—Sequence variation in *LMNA* may confer modest susceptibility for development of metabolic syndrome and dyslipidemia in the Amish. **(*Arterioscler Thromb Vasc Biol.* 2004;24:1708-1713.)**

Key Words: lamin A/C ■ lipids ■ triglyceride ■ metabolic syndrome ■ insulin resistance ■ positional cloning ■ chromosome 1q21-q24

Metabolic syndrome is characterized by the presence of ≥3 of the following characteristics: abdominal obesity, elevated triglyceride concentrations, low high-density lipoprotein (HDL), high blood pressure, and elevated fasting glucose. On the basis of estimates from the third National Health and Nutrition Examination Survey, the prevalence of metabolic syndrome is 44% among adults in the United States >50 years of age.[1] Metabolic syndrome is associated with increased risk of type 2 diabetes mellitus (T2DM) and cardiovascular disease. Evidence from the West of Scotland Study indicates that men with metabolic syndrome had 1.7-fold increased risk of a cardiovascular event and 3.5-fold increased risk of developing diabetes.[2] The mechanisms that cause metabolic syndrome are poorly understood but likely involve insulin resistance, which is associated closely with abdominal obesity and is an independent risk factor for cardiovascular disease. Indeed, a large body of evidence supports the notion that adipose tissue factors may mediate several of the metabolic changes that occur with metabolic syndrome.[3] Metabolic syndrome, along with its individual components, is likely to be under the influence of both environmental and genetic factors.

To localize genes contributing to T2DM, cardiovascular disease, and related traits, including obesity and metabolic syndrome, we conducted a genome-wide linkage analysis in large Old Order Amish families from Lancaster County, Pa.[4,5] Evidence for linkage with T2DM, impaired glucose tolerance, or impaired fasting glucose was observed on chromosome 1q21-q24 (logarithm of odds=2.35; $P<0.0005$).[5] This region is a gene-rich region that includes ≥530 genes. Excellent positional candidate genes include *APOA* (apolipoprotein A2),[6] *PBX1* (pre–B-cell leukemia transcription factor 1),[7] *slc19a2* (solute carrier family 19 [thiamine transporter] member 2),[8] *INSRR* (insulin receptor–related receptor gene),[9] *KCNJ9* and *KCNJ10* (potassium inwardly-rectifying channel, subfamily J, members 9 and 10),[10] *PEA 15* (phosphoprotein enriched in astrocytes 15),[11] *RXRG* (the γ subunit

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of the retinoid X receptor),[12] *CRP* (C-reactive protein),[13] *HYPLIP1* (familial combined hyperlipidemia gene),[14] *CASQ1* (calsequestrin 1),[15,16] *VAMP 4* (vesicle-associated membrane protein 4;[17] M Sabra, S Ott, J Lee, JR O'Connell, BD Mitchell, AR Shuldiner, unpublished data, 2004), *USF1* (upstream stimulatory factor 1),[18,19] and LMNA (lamin A/C).[20] This region is approximately 160 to 180 cM from p-telomere and corresponds to a region of chromosome 1 that has been linked to T2DM in several other populations, including Pima Indians,[21] Utah Mormons,[22] British whites,[23] French whites,[24] and Chinese.[25]

Familial partial lipodystrophy is a condition associated with severe insulin resistance, diabetes, dyslipidemia, and atherosclerosis,[26] making *LMNA* an excellent positional candidate gene for metabolic syndrome, T2DM, and related traits. *LMNA*, located on chromosome 1q21.2, encodes a nuclear envelope protein that exists as a heterodimer of lamin A and lamin C and is expressed in several cell types including liver, fat, muscle, and pancreas.[27] The gene contains 12 coding exons. Alternative splicing within exon 10 of *LMNA* results in coding either the lamin A or lamin C protein. Mutations in *LMNA* have been shown to result in a number of disorders, including Emery–Dreifuss muscular dystrophy (Online Mendelian Inheritance in Man [OMIM] 181350),[28,29] dilated cardiomyopathy with conduction defects (OMIM 115200),[30] familial partial lipodystrophy (OMIM 151660),[31–34] mandibuloacral dysplasia (MAD; OMIM 248370),[35] Huchinson–Gilford progeria (OMIM 176670),[36–38] and autosomal dominant and recessive forms of Charcot Marie tooth disease (OMIM 605588).[39–40] The mechanism whereby mutations in *LMNA* lead to these diverse phenotypes is presently unknown.

The aim of this study was to search for novel sequence variation in *LMNA* and to determine whether variants in this gene influence susceptibility to metabolic syndrome and its constituent components. We sequenced *LMNA* in 18 Amish subjects diagnosed previously with T2DM from the Amish Family Diabetes Study (AFDS). These individuals were selected from families showing evidence for linkage to the region of interest on chromosome 1. We then performed association and haplotype analyses in the full AFDS cohort to determine whether the polymorphisms identified in *LMNA* are associated with diabetes, metabolic syndrome, and related traits in the Amish. We found that the polymorphism H565H in exon 10 was associated with metabolic syndrome as well as with higher fasting triglyceride and lower HDL-cholesterol concentrations, suggesting that *LMNA* variation may confer modest susceptibility for development of metabolic syndrome and dyslipidemia in the Amish.

Research Design and Methods

Subjects

Subject recruitment for the AFDS began in 1995. Details of the AFDS design, recruitment, phenotyping, and pedigree structures have been described previously.[4] Briefly, probands were defined as individuals with previously diagnosed diabetes with the age at diagnosis between 35 and 65 years. All first- and second-degree family members ≥18 years of age were recruited around the diabetic probands. If another diabetic individual was identified in the family (eg, aunt or uncle), the family was expanded further to include the first- and second-degree relatives of that individual ≥18 years of age. The AFDS participants were divided into 45 families, although all of these families can be joined into a single pedigree by including ancestors going back 12 to 14 generations.[41–42] None of the subjects had known muscular dystrophy, structural or cardiac conduction abnormalities, progeria, or familial partial lipodystrophy, conditions that have been associated previously with mutations in the *LMNA* gene.

Data regarding medical, social, and family history were obtained during an interview at our Amish Research Clinic or the subject's home. Height, weight, and waist circumference were measured. Body mass index (BMI) was calculated as weight (kilograms) divided by height squared (m^2) to estimate total adiposity. Waist circumference was measured by a single observer. For this procedure, subjects were asked to remove any unnecessary clothing. The subject stood erect with the abdomen relaxed, arms at sides, and feet together. The measurer faced the subject and placed an inelastic tape around him/her in a horizontal plane at the level of the natural waist, which is the narrowest part of the torso, as seen from the anterior aspect. In obese subjects, the smallest horizontal circumference was measured in the area between the ribs and iliac crest. The measurement was taken at the end of a normal expiration, without the tape compressing the skin, and recorded to the nearest 0.1 cm. Blood pressure was measured in the left arm with standard sphygmomanometer while the subject was seated after 10 minutes of rest. A single observer made duplicate measurements, and the average of these was recorded.

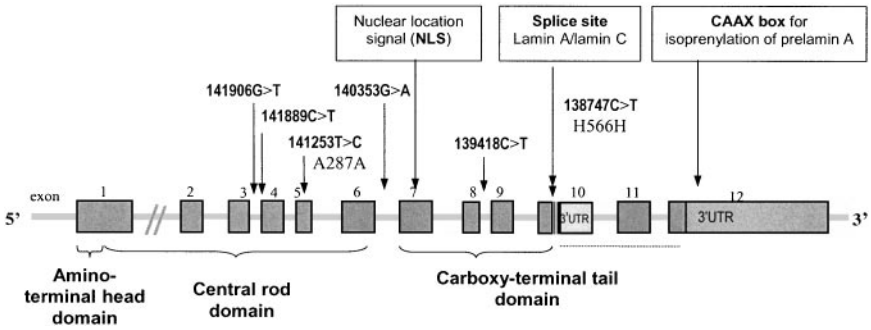
Fasting lipid profile (HDL-cholesterol and triglyceride concentrations) was assayed by Quest Diagnostics (Baltimore, Md; interassay coefficient of variation 5.0% for HDL-cholesterol; 1.6% for triglycerides). A 3-hour 75-g oral glucose tolerance test (OGTT) was performed on subjects with no previous history of diabetes. The T2DM diagnosis was defined on the basis of the OGTT using the plasma glucose criteria of the American Diabetes Association (2 hour glucose ≥11.1 mmol/L or fasting glucose ≥7 mmol/L). Subjects were also considered to have diabetes if they were currently taking medications for diabetes treatment. Subjects were excluded if there was a history of type 1 diabetes. Additionally, we defined impaired glucose homeostasis (IGH) as 2 hour glucose >7.7 mmol/L and <11.1 mmol/L or fasting glucose ≥6.1 mmol/L and <7 mmol/L. Metabolic syndrome was defined using the National Cholesterol Education Program (NCEP) ATP III guidelines.[43] It is characterized by the presence of ≥3 of the following characteristics: abdominal obesity (waist circumference >102 cm for men and >88 cm for women), elevated triglycerides (>1.68 mmol/L), low HDL (<1.03 mmol/L for men and <1.29 mmol/L for women), high blood pressure (≥130/85 mm Hg), and elevated fasting glucose (>6.0 mmol/L). Clinical characteristics of Amish subjects are shown in Table 1.

The mean age of diagnosis of diabetes in the AFDS cohort was 59.7±11.0 years (range 35 to 83 years), and the mean BMI was 28.8±5.2 kg/m^2 (range 16.4 to 40.7 kg/m^2). The 18 AFDS subjects selected for screening for mutations in *LMNA* in this study were individuals with diabetes who were members of different nuclear families that showed evidence for linkage to the area of interest on chromosome 1. The mean age of these 18 subjects was 61.3±12.3 years (range 49 to 90 years). The study was conducted in accordance with the human subject standards of the institutional review board of the University of Maryland, and informed consent was obtained from all subjects.

Laboratory Methods

Whole blood was collected from the subjects, and genomic DNA was isolated using Maxipreps according to the instructions of the manufacturer (Qiagen). All 12 exons as well as the exon–intron junctions of *LMNA* were amplified by polymerase chain reaction (PCR). Primers for DNA amplification and sequencing were derived using the published sequence for *LMNA* (GenBank AC007227; Table I, available online at http://atvb.ahajournals.org). DNA sequencing was performed using an Applied Biosystems 377 automated DNA sequencer (Perkin–Elmer). PCR products were sequenced in both directions, visually inspected, and compared with the GenBank sequence of human *LMNA*.

Genotyping was completed using an Orchid SNPstream UHT genotyping system (Orchid Bioscience). First, the target genomic sequences containing the single-nucleotide polymorphisms (SNPs) of interest were amplified in a 12-plex PCR reaction. After enzymatic clean-up, PCR products were subjected to an extension



Schematic of the genomic structure of *LMNA*. *LMNA* is composed of 12 exons. Alternate splicing within exon 10 results in 2 distinct mRNA transcripts corresponding to lamin A (664 amino acids) and lamin C (572 amino acids). The mature protein lamin A/C functions as a heterodimer. Also shown are SNPs detected in our mutation screen and their locations. Note that 138747C>T is a silent mutation in exon 10 at the alternate splice site. Promoter is not shown. NLS indicates nuclear location signal; UTR, untranslated region.

reaction using 5′-tagged extension primers and fluorescent dye–labeled terminators. In a thermal cycled extension step, the primers hybridized to the specific amplicons 1 base adjacent to the SNP site and were extended by 1 base at the 3′ end with a fluorescently labeled nucleotide. These reaction products were transferred to an array plate, where each of the 12 extension products in the multiplex reaction was sorted by hybridization of the unique 5′-tag sequence to its complementary probe immobilized in a miniarray within each well. The Orchid SNPscope reader imaged the microarray plates. The 2-color detection allowed SNP detection by comparing signals from the 2 fluorescent dyes. The image signals were then transferred to genotyping software that translated the images of the arrays into genotype calls. The error rate on the basis of blind replicates was 0.1% to 2% for the SNPs examined in the present study.

Analysis

Before analysis, genotypes were checked for Mendelian consistency, and inconsistencies, which were detected in <0.5% of genotypes, were removed from analysis. Genotype frequencies of all SNPs were tested for consistency with Hardy–Weinberg expectations by the χ^2 test. We then followed a 2-tiered analytic approach, first performing association analyses with each SNP individually and then considering SNPs jointly as haplotypes.

Because mutations in *LMNA* have been associated previously with diabetes[20] and dyslipidemia,[44] we examined the relationship of *LMNA* polymorphisms identified in the Amish with the metabolic syndrome and its component traits. For the quantitative traits associated with metabolic syndrome (ie, glucose, obesity, and lipid levels), we estimated mean trait levels according to *LMNA* genotypes, whereas for the qualitative traits (metabolic syndrome, T2DM, and abnormal glucose tolerance), we compared disease prevalence across genotypes. Subjects currently taking insulin (n=27), β-hydroxy-β-methylglutaryl coenzyme A reductase inhibitors or other lipid-lowering medications (n=7), aspirin (n=24), angiotensin-converting enzyme inhibitors, or other blood pressure–lowering agents (n=6), or estrogens (n=3) were excluded from analysis (n=63 subjects taking ≥1 of the above).

To account for the relatedness among family members, we used the measured genotype approach,[45] 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. Triglyceride values were transformed by their natural logarithms (lns) to reduce skewness. All analyses were conducted using the Sequential Oligogenic Linkage Analysis Routines program.[46]

The strength of linkage disequilibrium between all pair-wise combinations of SNPs and haplotypes were inferred for each individual using an expectation maximization algorithm implemented into the ZAPLO software program.[47] Additional association analyses were then performed treating each haplotype as a super-allele as described above.

Results

In sequence analysis of 18 relatively unrelated Amish T2DM subjects (36 alleles), we identified 6 SNPs, including c.141906G>T and c.141889C>T (intron 3); A287A (c.141253T>C; exon 5); c.140353G>A (intron 6); c.139418C>T (intron 8); and H566H (c. 138747C>T; exon 10). This exon 10 SNP is also designated as rs4641 and has been identified as GenBank No. X03444 C1908T previously.[40] Although this SNP is silent, it is of particular interest because it occurs at the last nucleotide in exon 10, where alternate splicing determines the relative amounts of lamin A and lamin C transcripts (Figure). The exon 5 A287A (c.141253T>C) SNP is also a silent mutation. The intronic SNPs do not predict any obvious functional sequelae.

The frequencies of the minor alleles at each of the 6 SNPs ranged from 0.05 to 0.35 (Table 3). The distribution of genotypes for all SNPs was consistent with Hardy–Weinberg equilibrium. The degree of linkage disequilibrium was strong between the 2 intron 3 SNPs (r^2=0.93; D′=1), moderate between the intron 3 SNPs and SNPs at exon 5, intron 6, and intron 8, and among the latter SNPs (r^2=0.45 to 0.84; D′=0.94 to 1) and weaker between the exon 10 SNP and all others (r^2=0.02 to 0.12; D′=0.35 to 1; see Table 3).

The prevalence rates for T2DM and IGH were 9.8% and 25.9%, respectively, in this sample. Allele and genotype frequencies did not differ between T2DM cases and controls for any of the 6 SNPs, nor was there evidence for association of any of the SNPs with the composite trait T2DM/IGH (Table 2). We examined the relationships between BMI and metabolic syndrome–related traits and *LMNA* sequence variants. Mean levels of obesity and lipid measures are also shown in Table 2 according to the exon 10 H566H (c.138747C>T) genotype. Triglyceride concentrations were higher and HDL concentrations lower in subjects with the T allele (P=0.004 and 0.03, respectively). There was also a significant association with metabolic syndrome as defined by NCEP ATP III guidelines (P=0.017, odds ratio 1.43; CI 1.05 to 1.80).

Corresponding analyses of the exon 5 A287A (c.141253T>C) SNP showed higher fasting glucose among those with the C allele than those with the TT genotype (P=0.001). No significant association with obesity measures, lipid traits, or blood pressure was seen for this SNP. Analysis of the intronic SNPs showed no significant association with obesity, lipid traits, or metabolic syndrome (data not shown).

As a secondary analysis of the exon 10 variant H566H (c.138747C>T), we evaluated whether the effects of the exon

TABLE 1. Clinical Characteristics of Amish Subjects

Trait	Means	
	Women (n=486)	Men (n=422)
Age (years)	45.2±16.2	46.7±16.0
BMI (kg/m^2)	27.6±5.6	26.4±3.9
Waist circumference (cm)	89.1±11.6	95.5±11.3
HDL (mmol/L)	1.41±0.34	1.22±0.33
ln (TGs; mmol/L×100)	4.85±0.62	4.85±0.61
Systolic blood pressure (mm Hg)	121.6±16.6	122.0±15.1
Diastolic blood pressure (mm Hg)	77.2±9.0	78.5±10.2
Metabolic syndrome (%)	15.5	9.2
T2DM (%)	10.1	9.4
T2DM/IGH (%)	32.6	18.3

TGs indicates triglycerides.

10 CT/TT genotype on metabolic syndrome and related traits differed between men and women or between younger or older individuals. There was little evidence that the genotype effect differed between men and women for any trait. For triglycerides, age-stratified analyses indicated that the genotype effect differed little between younger and older men, with the CT/TT genotype associated with a 3.3% and 3.6% increase in ln triglyceride levels in men younger and older than age 50, respectively. However, in women, the effect of the CT/TT genotype was restricted almost entirely to those ≥50 years of age; among these older women, being exposed to the T allele was associated with a 3.8% increase in ln triglyceride levels compared with only a 0.3% increase for women <50 years of age. This interaction effect did not achieve statistical significance ($P=0.17$).

We next considered the possibility that haplotypes defined by allelic combinations at these 6 SNPs might show associations with either disease status or with quantitative trait levels. Because of marked linkage disequilibrium between markers (Table 3), 2 most frequent haplotypes (C138747-T141253-C139418-G140353-C141889-T141906 and T138747-T141253-C139418-G140353-C141889-T141906) together accounted for >87% of the haplotypes in the Amish. The estimated frequencies of the first 2 most common haplotypes are 0.548292 and 0.327621, respectively. There were no significant differences in haplotype frequencies among subjects with T2DM or metabolic syndrome and controls, nor were there any differences in glucose, insulin, obesity, or lipid traits between haplotypes.

Discussion

Mutations in *LMNA* have been found in diseases involving muscle and fat cells and include Emery–Dreifuss muscular dystrophy,[28,29] dilated cardiomyopathy with conduction defects,[30] familial partial lipodystrophy,[31–34] MAD,[35] and Charcot Marie tooth disease,[39–40] as well as premature aging.[36–38] Familial partial lipodystrophy is associated with severe insulin resistance, diabetes, dyslipidemia, and atherosclerosis.[26] Our genome-wide scan in the Amish[5] and those of several others[21–25] provide strong evidence for the existence of ≥1 T2DM susceptibility genes on chromosome 1q21-q24, the region in which *LMNA* resides. These observations led us to sequence all of the exons and exon–intron boundaries of *LMNA* in subjects with diabetes from Amish families, providing evidence for linkage to chromosome 1q21-q24. Although our mutation screen detected 6 SNPs, none resulted in obvious alterations in protein structure, and none were associated with T2DM or IGH. We did not screen for mutations in regulatory regions or the entirety of all introns, and thus cannot rule out the possibility that significant mutations may be present in these regions. However, lack of association of any haplotype with T2DM or related traits make this possibility less likely. Thus, *LMNA* is unlikely to be the gene on chromosome 1q21-q24 detected through linkage analysis contributing to risk of T2DM.

Modest associations with a common variant in exon 10 of *LMNA* (H566H) have been shown to be associated with adipose tissue metabolism and obesity phenotypes.[48–50] In Canadian Oji-Cree and Inuit, this variant was associated with

TABLE 2. Means (SEs in Parentheses) for Metabolic Syndrome–Related Traits in AFDS Subjects With and Without the Exon 10 H566H Polymorphism

Trait	Means Exon 10 (H566H) Polymorphism		Age and Sex Adjusted x^2 and P Value	
	Non-T Allele Carriers n=372	T Allele Carriers n=536	x^2	P
BMI (kg/m^2)	26.4 (0.3)	26.5 (0.3)	0.10	0.75
Waist circumference (cm)	95.2 (0.8)	95.8 (0.8)	0.63	0.43
HDL (mmol/L)	1.24 (0.02)	1.19 (0.02)	4.49	0.03
ln (TGs; mmol/L×100)	4.78 (0.04)	4.91 (0.04)	8.44	0.004
Systolic blood pressure (mm Hg)	121.7 (1.0)	122.2 (1.0)	0.46	0.50
Diastolic blood pressure (mm Hg)	78.1 (0.7)	78.9 (0.7)	1.14	0.28
Fasting glucose	5.20 (0.09)	5.25 (0.10)	0.28	0.60
Metabolic syndrome (%)	8.6	15.3	5.68	0.017
T2DM (%)	7.6	11.4	1.92	0.16
T2DM/IGT (%)	23.1	28.0	0.76	0.38

TGs indicates triglycerides.
Metabolic syndrome defined according to NCEP ATP III guidelines (see text).

TABLE 3. Pairwise Linkage Disequilibrium Coefficients (D′ and r^2) for *LMNA* SNPs in the Old Order Amish

SNP	Intron 3 141889C>T (0.05)	Intron 3 141906G>T (0.06)	Exon 5 A287A 141253T>C (0.08)	Intron 6 140353G>A (0.09)	Intron 8 139418C>T (0.11)	Exon 10 H566H 183747C>T (0.35)
Intron 3 141889C>T (0.05)		0.93	0.64	0.63	0.49	0.02
Intron 3 141906G>T (0.06)	1.00		0.63	0.59	0.45	0.03
Exon 5 A287A 141253T>C (0.08)	0.97	1.00		0.84	0.64	0.12
Intron 6 140353G>A (0.09)	1.00	1.00	0.95		0.77	0.01
Intron 8 139418C>T (0.11)	1.00	1.00	0.94	1.00		0.01
Exon 10 H566H 138747C>T (0.35)	0.83	1.00	0.48	0.35	0.41	

Allele frequency is in parentheses. Upper triangle, r^2; lower triangle, D′.

BMI, waist-to-hip ratio, leptin, and body fat percentage.[48,49] Additionally, this variant has been studied in a Japanese cohort[44] and in Pima Indians.[50,51] In Pimas, no evidence for association of H566H with T2DM, BMI, total cholesterol, HDL-cholesterol triglycerides, or indices of insulin sensitivity and secretion was found.[51] However, Pimas with the T allele had significantly greater subcutaneous fat cell size.[50] In the Japanese cohort, this variant was associated with higher fasting insulin, triglyceride, total cholesterol concentrations, and lower HDL-cholesterol concentrations.[44]

Amish individuals who were exposed to the T allele of the c.138747C>T variant had higher triglyceride concentrations and lower HDL-cholesterol and were more likely to have the presence of metabolic syndrome. Thus, it is possible that this variant plays a modest role in modulating the metabolic syndrome, particularly lipid components.

Although this SNP encodes a silent mutation H566H, it is the terminal exonic nucleotide of exon 10, the site of alternate splicing for lamin A and C mRNA. This polymorphism could alter mRNA splicing and thus the relative amounts of lamin A and C mRNA and protein. Indeed, the silent *LMNA* G608G SNP has been shown to cause aberrant mRNA splicing and to cause Hutchinson–Gilford progeria.[36]

In summary, screening of *LMNA* revealed 6 SNPs. One SNP, H566H (c.138747C>T), showed significant association with metabolic syndrome and with lower HDL-cholesterol and higher triglyceride concentrations. These associations are consistent with other studies reporting association of this same variant with metabolic syndrome traits. None of the SNPs or haplotypes examined showed evidence of association with T2DM or IGH. We conclude that genetic variation in *LMNA* may play a modest role in susceptibility to metabolic syndrome and dyslipidemia in our population but is unlikely to be the gene on chromosome 1q21-q24 detected through linkage analysis contributing to risk of T2DM.

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References

1. Alexander CM, Landsman PB, Teutsch SM, Haffner SM. Third National Health and Nutrition Examination Survey (NHANES III); National Cholesterol Education Program (NCEP). NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* 2003;52:1210–1214.
2. Sattar N, Gaw A, Scherbakova O, Ford I, O'Reilly DS, Haffner SM, Isles C, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J. Metabolic syndrome with and without C-reactive protein as a predictor of coronary heart disease and diabetes in the West of Scotland Coronary Prevention Study. *Circulation*. 2003;108:414–419.
3. Reilly MP, Rader DJ. The metabolic syndrome: more than the sum of its parts? *Circulation*. 2003;108:1546–1551.
4. Hsueh W-C, Mitchell BD, Aburomia R, Pollin T, Sakul H, Gelder Ehm M, Michelsen BK, Wagner MJ, St. Jean PL, Knowler WC, Burns DK, Bell CJ, Shuldiner AR. Diabetes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. *Diabetes Care*. 2000;23:595–601.
5. Hsueh W-C, St. Jean PL, Mitchell BD, Pollin TI, Knowler WC, Ehm MG, Bell CJ, Sakul H, Wagner MJ, Burns DK, Shuldiner AR. Genome-wide and fine-mapping linkage studies of type 2 diabetes and glucose traits in the Old Order Amish: evidence for a new diabetes locus on chromosome 14q11 and confirmation of a locus on chromosome 1q21-q24. *Diabetes*. 2003;52:550–557.
6. Deeb SS, Takata K, Peng R, Kajiyama G, Albers JJ. A splice-junction mutation responsible for familial apolipoprotein A-II deficiency. *Am J Hum Genet*. 1990;46:822–827.
7. Kim SK, Sellleri L, Lee JS, Zhang AY, Gu X, Jacobs Y, Cleary ML. Pbx1 inactivation disrupts pancreas development and in Ipf1-deficient mice promotes diabetes mellitus. *Nat Genet*. 2002;30:430–435.
8. Thameem F, Wolford JK, Bogardus C, Prochazka M. Analysis of slc19a2, on 1q23.3 encoding a thiamine transporter as a candidate gene for type 2 diabetes in Pima Indians. *Mol Genet Metab*. 2001;72:360–363.
9. Wolford JK, Thameem F, Bogardus C, Prochazka M. Polymorphism screening of the insulin receptor-related receptor gene (INSRR) on 1q in Pima Indians. *Mol Cell Probes*. 2001;15:223–227.
10. Wolford JK, Hanson RL, Kobes S, Bogardus C, Prochazka M. Analysis of linkage disequilibrium between polymorphisms in the KCNJ9 gene with type 2 diabetes mellitus in Pima Indians. *Mol Genet Metab*. 2001;73:97–103.
11. Wolford JK, Bogardus C, Ossowski V, Prochazka M. Molecular characterization of the human PEA 15 gene on 1q21–q22 and association with type 2 diabetes mellitus in Pima Indians. *Gene*. 2000;241:143–148.
12. Lenhard JM. PPAR γ/RXR as a molecular target for diabetes. *Receptors Channels*. 2001;7:249–258.
13. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. *J Am Med Assoc*. 2001;285:2481–2485.
14. Pajukanta P, Bodnar JS, Sallinen R Chu M, Airaksinen T, Xiao Q, Castellani LW, Sheth SS, Wessman M, Palotie A, Sinsheimer JS, Demant P, Lusis AJ, Peltonen L. Fine mapping of Hyplip1 and the human homolog, a potential locus for FCHL. *Mamm Genome*. 2001;12:238–245.
15. Howarth FC, Glover L, Culligan K, Qureshi MA, Ohlendieck K. Calsequestrin expression and calcium binding is increased in streptozotocin-

induced diabetic rat skeletal muscle though not in cardiac muscle. *Pflügers Arch.* 2002;444:52–58.
16. Fu M, Damcott C, Sabra M, Ott J, Lee J, Pollin TI, Garant M, O'Connell J, Mitchell BD, Shuldiner AR. Polymorphism in the Calsequestrin 1 gene on chromosome 1q21 is associated with type 2 diabetes in Old Order Amish. *Diabetes*. In press.
17. Steegmaier M, Klumperman J, Foletti DL, Yoo J-S, Scheller RH. Vesicle-associated membrane protein 4 is implicated in trans-Golgi network vesicle trafficking. *Mol Biol Cell*. 1999;10:1957–1972.
18. Iynedjian PB. Identification of upstream stimulatory factor as transcriptional activator of the liver promoter of the glucokinase gene. *Biochem J*. 1998; 333:705–712.
19. Pajukanta P, Lilja HE, Sinsheimer JS, Cantor RM, Lusis AJ, Gentile M, Duan XJ, Soro-Paavonen A, Naukkarinen J, Saarela J, Laakso M, Ehnholm C, Taskinen MR, Peltonen L. Familial combined hyperlipidemia is associated with upstream transcription factor 1 (USF1). *Nat Genet.* 2004;36:371–376.
20. Hegele RA, Anderson CM, Wang J, Jones DC, Cao H. Association between nuclear lamin A/C R482Q mutation and partial lipodystrophy with hyperinsulinemia, dyslipidemia, hypertension, and diabetes. *Genome Res.* 2000;10:652–658.
21. Hanson, RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almsay L, Blangero J, Garvey WT, Bennett PH, Knowler WC. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet.* 1998;63:1124–1132.
22. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ. A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes.* 1999;48:1175–1182.
23. Wiltshire S, Hattersley AT, Hitman GA, Walker M, Levy JC, Sampson M, O'Rahilly S, Frayling TM, Bell JL, Lathrop GM, Bennett A, Dhillon R, Fletcher C, Groves CJ, Jones E, Prestwich P, Simecek N, Rao PV, Wishart M, Foxon R, Howell S, Smedley D, Cardon LR, Menzel S, McCarthy MI. A genomewide scan for loci predisposing to type 2 diabetes in a UK population (the diabetes UK Warren 2 repository): analysis of 573 pedigrees provides independent replication of a susceptibility locus on chromosome 1q. *Am J Hum Genet.* 2001;69:553–569.
24. Vionnet H, Hani El-H, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–24. *Am J Hum Genet.* 2000;67:1470–1480.
25. Xiang K, Wang Y, Zheng T, Jia W, Li J, Chen L, Shen K, Wu S, Lin X, Zhang G, Wang C, Wang S, Lu H, Fang Q, Shi Y, Zhang R, Xu J, and Weng Q. Genome-wide search for type 2 diabetes/impaired glucose homeostasis susceptibility genes in the Chinese: significant linkage to chromosome 6q21–q23 and chromosome 1q21–q24). *Diabetes.* 2004;53:228–234.
26. Hegele RA. Premature atherosclerosis associated with monogenic insulin resistance. *Circulation.* 2001;103:2225–2229.
27. Broers JL, Machiels BM, Kuijpers HJ, Smedts F, van den Kieboom R, Raymond Y, Ramaekers FC. A- and B-type lamins are differentially expressed in normal human tissues. *Histochem Cell Biol.* 1997;107:505–517.
28. Bonne G, Di Barletta MR, Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D, Schwartz K. Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat Genet.* 1999;21:285–288.
29. di Baretta MR, Ricci E, Galluzzi G, Tonali P, Mora M, Morandi L, Romorini A, Voit T, Orstavik HK, Merlini L, Trevisan C, Biangalana V, Housmanowa-Petrusewicz I, Bione S, Ricotti R, Schwartz K, Bonne G, Toniolo D. Different mutations in the LMNA gene cause autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy. *Am J Hum Genet.* 2000;66:1407–1412.
30. Fatkin D, MacRae C, Sasaki T, Wolff MR, Maurizio P, Frennequx M, Atherton J, Humberto J, Vidaillet HJ Jr, Spudich S, De Girolami U, Seidman JG, Seidman CE, Muntoni F, Muehle G, Johnson W, McDonough B. Missense Mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction system disease. *N Engl J Med.* 1999;341:1715–1724.
31. Cao H, Hegele RA. Nuclear lamin A/C R482Q mutation in Canadian kindreds with Dunnigan-type familial partial lipodystrophy. *Hum Mol Genet.* 2000;9:109–112.
32. Shackleton S, Lloyd DJ, Jackson SN, Evans R, Niermeijer MF, Singh BM, Schmidt H, Brabant G, Kumar S, Durrington PN, Gregory S, O'Rahilly S, Trembath RC. LMNA, encoding lamin A/C, is mutated in partial lipodystrophy. *Nat Genet.* 2000;24:153–156.
33. Speckman RA, Garg A, Fenghe D, Bennett L, Veile R, Ariouglu E, Taylor SI, Lovett M, Bowcock AM. Mutational and haplotype analyses of families with familial partial lipodystrophy (Dunnigan variety) reveal recurrent missense mutations in the globular C-terminal domain of lamin A/C. *Am J Hum Genet.* 2000;66:1192–1198.
34. Vigouroux C, Magre J, Vantyghem MC, Bourut C, Lascols O, Shackleton S, Lloyd DJ, Guerci B, Padova G, Valensi P, Grimaldi A, Piquemal R, Touraine P, Trembath RC, Capeau J. Lamin A/C gene. Sex-determined expression of mutations in Dunnigan-type familial partial lipodystrophy and absence of coding mutations in congenital and acquired generalized lipoatrophy. *Diabetes.* 2000;49:1958–1962.
35. Novelli G, Muchir A, Sangiuolo F, Helbling-Leclerc A, D'Apice MR, Massart C, Capon F, Sbraccia P, Federici M, Lauro R, Tudisco C, Pallotta R, Scarano G, Dallapiccola B, Merlini L, Bonne G. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. *Am J Hum Genet.* 2002;71:426–431.
36. Cao H, Hegele RA. LMNA is mutated in Hutchinson-Gilford progeria (MIM 166670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). *J Hum Genet.* 2003;48:271–274.
37. Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW. Collins fractional shortening (FS). Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. *Nature* 2003;423:293–298.
38. De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, Lyonnet S, Stewart CL, Munnich A, Le Merrer M, Levy N. Lamin A truncation in Hutchinson-Gilford progeria. *Science.* 2003;300:2055.
39. De Sandre-Giovannoli A, Chaouch M, Kozlov S, Vallat JM, Tazir M, Kassouri N, Szepetowski P, Hammadouche T, Vandenberghe A, Stewart CL, Grid D, Levy N. Homozygous defects in LMNA, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot Marie Tooth disorder type 2) and mouse. *Am J Hum Genet.* 2002;70:726–736.
40. Goizet C, Yaou RB, Demay L, Richard P, Bouillot S, Rouanet M, Hermosilla E, Le Masson G, Lagueny A, Bonne G, Ferrer X. A new mutation of the lamin A/C gene leading to autosomal dominant axonal neuropathy, muscular dystrophy, cardiac disease, and leuconychia. *J Med Genet*. 2004;41:e29.
41. Agarwala R, Biesecker LG, Hopkins KA, Francomano CA, Schaffer AA. Software for constructing and verifying pedigrees within large genealogies and an application to the Old Order Amish of Lancaster County. *Genome Res.* 1998;8:211–221.
42. Agarwala R, Biesecker LG, Schaffer AA. Anabaptist genealogy database. *Am J Med Genet.* 2003;121C:32–37.
43. Adult Treatment Panel III. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. *J Am Med Assoc.* 2001;285:2486–2497.
44. Murase Y, Yagi K, Katsuda Y, Asano A, Koizumi J, Mabuchi H. An LMNA variant is associated with dyslipidemia and insulin resistance in the Japanese. *Metabolism*. 2002;51:1017–1021.
45. Boerwinkle E, Chakraborty R, Sing CF. The use of measured genotype information in the analysis of quantitative phenotypes in man. I. Models and analytical methods. *Ann Hum Genet.* 1986;50:181–194.
46. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet.* 1998;62:1198–1211.
47. O'Connell JR. Zero-recombinant haplotyping: applications to fine mapping using SNPs. *Genet Epidemiol.* 2000;19(suppl 1):S64–S70.
48. Hegele RA, Huff MW, Young TK. Common genomic variation in *LMNA* modulates indexes of obesity in Inuit. *J Clin Endocrinol Metab.* 2002;86: 2747–2751.
49. Hegele RA, Cao H, Harris SB, Zinman B, Hanley AJ, Anderson CM. Genetic variation in LMNA modulates plasma leptin and indices of obesity in aboriginal Canadians. *Physiol Genomics.* 2000;3:39–44.
50. Weyer C, Wolford JK, Hanson RL, Foley JE, Tataranni PA, Bogardus C, Pratley RE. Subcutaneous abdominal adipocyte size, a predictor of type 2 diabetes, is linked to chromosome 1q21–q23 and is associated with a common polymorphism in *LMNA* in Pima Indians. *Mol Genet Metab.* 2001;72:231–238.
51. Wolford JK, Hanson RL, Bogardus C, Prochazka M. Analysis of the lamin A/C gene as a candidate for type II diabetes susceptibility in Pima Indians. *Diabetologia*. 2001;44:779–782.