# An *LMNA* Variant Is Associated With Dyslipidemia and Insulin Resistance in the Japanese

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**Nuclear lamins A and C are encoded by *LMNA* and are present in terminally differentiated cells. Rare mutations in *LMNA* were shown to cause familial partial lipodystrophy, a syndrome characterized by regional loss of adipose tissue, glucose intolerance, and dyslipidemia, making *LMNA* a candidate gene for insulin-resistant diabetes. The aim of this study was to investigate whether genetic variation in *LMNA* can influence the risk of type 2 diabetes in a Japanese cohort. First, we performed mutational screening of *LMNA* by polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) and sequence analysis in 8 insulin-resistant males with acanthosis nigricans who were not lipodystrophic. One known single nucleotide polymorphism, 1908C/T, was found in exon 10. We subsequently screened samples of 171 nondiabetic and 164 type 2 diabetic male subjects for the presence of the 1908C/T polymorphism by PCR-restriction fragment length polymorphism (RFLP). The frequency of subjects with the 1908T allele tended to be higher in the diabetic group than in the nondiabetic group; however, the difference was not significant (43.9% *v* 32.2%) ($P = .084$). Carriers of the 1908T allele, both among diabetics and nondiabetics, showed significantly higher fasting insulin, triglycerides (TG), total cholesterol (TC), and lower high-density lipoprotein-cholesterol (HDL-C) levels than those of the 1908C/C subjects. These results suggest the *LMNA* 1908C/T single nucleotide polymorphism (SNP) is not associated with the prevalence of type 2 diabetes, although it may be a factor predisposing to insulin resistance and dyslipidemia in some Japanese.**


INSULIN RESISTANCE IS commonly seen among obese individuals and is associated with multiple linked defects in lipid metabolism and glucose intolerance.[1-3] On the other hand, a rare genetic form of insulin resistance, autosomal dominant Dunnigan-type familial partial lipodystrophy (FPLD; OMIM 151660), exists and is characterized by loss of subcutaneous fat from the extremities, trunk, and gluteal region and often by insulin resistance, hypertension, dyslipidemia, type 2 diabetes, and early development of atherosclerosis.[4-7] Some patients with FPLD show altered *LMNA* on chromosome 1q21-q23, which is the gene encoding nuclear lamins A and C.[8-11] The *LMNA* gene products, lamins A and C, are important elements of the nuclear lamina.[12] Alternative splicing within exon 10 of *LMNA* generates lamins A and C sequence identity for the first 566 amino acids, but distinctive carboxy-terminal tail regions.[12] Lamins A and C form heterodimers or homodimers and are coexpressed widely in well-differentiated cells and tissues.[12] Other mutations in *LMNA* underlie 2 additional autosomal dominant syndromes: Emery-Dreifuss muscular dystrophy (EMD2; OMIM 181350)[13] and familial dilated cardiomyopathy with conduction-system disease (CMD1A; OMIM 115200).[14] Although the mechanisms by which FPLD causes insulin resistance are not completely understood, *LMNA* could clearly be one of the candidate genes for insulin-resistant diabetes.

To investigate whether *LMNA* sequence variants are associated with insulin resistance, we performed mutational screening of *LMNA* in 8 men with acanthosis nigricans who are characteristically insulin-resistant. We found 1 single nucleotide polymorphism (SNP), a silent C→T substitution at nucleotide 1908 (1908C/T) in exon 10 in 3 of them. Recently, the same variant of *LMNA* was associated with indices of obesity in nondiabetic Oji-Cree, a population of aboriginal Canadians.[15] We consider that subjects with this *LMNA* variation may be at greater risk of type 2 diabetes. To prove or disprove this contention, we examined the association of this SNP and the prevalence of diabetes and then compared various clinical variables related to insulin resistance according to the genotype in unrelated nondiabetic and diabetic subjects.

## SUBJECTS AND METHODS

### *Subjects*

All individuals recruited for the present study were consecutive males over 18 years of age who consulted the Second Department of Internal Medicine, Kanazawa University, for any reason during the period extending from May 1995 to March 2000. A total of 8 insulin-resistant men with acanthosis nigricans whose fasting insulin levels were over 250 pmol/L, 171 nondiabetics and 164 type 2 diabetic subjects were enrolled in this study. The subjects in the nondiabetic group were confirmed to be nondiabetic by a 75-g oral glucose tolerance test according to the 1985 World Health Organization criteria.[16] Of the diabetics, 62 were controlled with sulfonylureas, 42 with insulin, and the rest with diet alone. None of the subjects had significant renal, hepatic, or cardiovascular disease, and none were receiving lipid-lowering or thyroid medication during the month preceding the time of blood sampling. The study was performed under informed written consent from all subjects and was approved by the Ethics Committee of Kanazawa University.

### *Biological Measurements*

Type 2 diabetes was defined according to the World Health Organization criteria.[16] Fasting plasma glucose (FPG) was measured by the hexokinase method (GA-1160; ARKRAY, Kyoto, Japan). Fasting plasma insulin (FIRI) was measured using a 2-site immunoenzymometric assay (mean intra- and interassay coefficients of variation [CVs] 1.7% and 3.3%, respectively) (ST AIA-PACK IRI, Tosoh, South San Francisco, CA). Insulin resistance and $\beta$-cell function were assessed by homeostasis model assessment (HOMA) (fasting insulin ($\mu$U/mL) × glucose (mmol/L)/22.5).[17] Blood samples were obtained in the morning

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after a 12-hour fast. Serum was obtained by low speed centrifugation for 0.5 to 1 hour after venipuncture, stored at −80°C, and analyzed enzymatically for total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG).[18] The CVs of these various lipid measurements were 0.5% for TC levels, 1.2% for HDL-C, and 0.7% for TG. Low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald formula when TG levels were ≤ 5 mmol/L.[19] Concentrations of fasting plasma leptin were determined by radioimmunoassay (Otsuka, Tokushima, Japan). Body mass index (BMI) ($kg/m^2$) was calculated as weight (kg) divided by height ($m^2$). Blood pressure was measured by auscultatory methods using a mercury sphygmomanometer.

### *Polymerase Chain Reaction Amplification of LMNA Exons and Sequencing*

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures.[20] All 12 exons and exon-intron boundaries were amplified from genomic DNA using specific primers derived from the 5′ and 3′ ends of intronic sequence. The annealing temperature for primers was 54°C to 59°C. Polymerase chain reaction (PCR) conditions were as follows: reaction volume was 50 μL, 50 to 500 ng genomic DNA, 0.2 μmol/L of the primers, 2.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxynucleotide triphosphate (dNTP), primers at a final concentration of 0.5 μmol/L and 1.0 U Taq DNA polymerase (Biotech International, Sugarland, TX). Single-strand conformation polymorphism (SSCP) analysis of amplified DNA was then performed by a previously described method,[21] with a slight modification. Sequencing reactions were performed using a Thermo sequence II dye terminator cycle sequencing kit (Amersham Pharmacia Biotech, Piscataway, NJ), and the products were analyzed on ABI 377 automated DNA sequencers (PE Applied BioSystems, Foster City, CA). The gel files were processed using the Sequence Analysis Software (PE Applied BioSystems) and then assembled and analyzed using Autoassembler 2.0 (PE Applied BioSystems).

### *Detection of the LMNA 1908C/T Polymorphism by PCR-Restriction Fragment Length Polymorphism*

The *LMNA* 1908C/T polymorphism was caused by a C→T substitution at nucleotide 1908 in exon 10. This SNP was determined by the PCR-restriction fragment length polymorphism (RFLP) method. The sequences of the primers were 5′-AAAGGGCAGGCCACAAGAAAAG-3′ (forward primer) and 5′-GGCTCGGCCTCAGCGGCGGCTACCACTCCC-3′ (reverse primer). The reverse primer contained 1 nucleotide mismatch (underlined), which made it possible to use restriction enzyme *Nco*I (Toyobo, Osaka, Japan) for the detection of the 1908C/T SNP. PCR products were digested with *Nco*I at 37°C for 180 minutes, electrophoresed through 10% to 20% gradient acrylamide gels (Pagel, Atto, Tokyo, Japan), and stained with ethidium bromide. The 1908C allele gives one 223-bp fragment, whereas the 1908T allele gives 190-bp and 33-bp fragments.

### *RNA Isolation, cDNA Synthesis, and LMNA cDNA Amplifications*

Total cellular RNA was isolated from leukocytes of a subject with *LMNA* 1908C/C and a subject with 1908T/T, respectively, using RNAzol (Tel-Test, Friendswood, TX), and the cDNA synthesis was performed using a cDNA synthesis kit (First-Strand cDNA Synthesis Kit, Amersham Pharmacia Biotech). The cDNA products were amplified in a 50-μL PCR using the exonic primers for lamin A (forward primer in exon 9: 5′-AGCCTGCGTACGGCTCTCAT-3′ and reverse primer in exon 11: 5′-GCTCCTGAGCCGCTGGCAGA-3′) and lamin C (forward primer in exon 9: 5′-AGCCTGCGTACGGCTCTCAT-3′ and reverse primer in exon 10: 5′-TCAGCGGCGGCTACCACTCAC-3′), respectively. The annealing temperature for primers was 58°C. The sizes of normal (C/C) and mutated (T/T) cDNA-PCR fragments were assessed on agarose gels. After extraction and purification of the normal and putative mutated cDNA, they were sequenced and analyzed on ABI 377 automated DNA sequencers (PE Applied BioSystems).

### *Statistical Analysis*

Clinical values were expressed as means ± SD. The proportions of genotypes or alleles were compared by $\chi^2$ analysis or Fisher's exact test. Odds ratio and 95% confidence interval (CI) nonadjusted or adjusted for age and BMI were calculated by logistic regression analysis. Because the sample size for analysis of the homozygotes for the *LMNA* 1908C/T polymorphism was quite small, the Kruskal-Wallis test was performed to compare mean variables among 3 genotypes. For pairwise comparison among 3 genotypes, Mann-Whitney *U* test was performed. For the comparison of multiple variables, $P < .01$ was considered statistically significant. All statistical analyses were performed using Stat View 5.0 software (Abacus Concepts, Berkeley, CA).

## RESULTS

### *Identification of a Polymorphism in the LMNA Gene*

All exons and the exon-intron splicing boundaries of the *LMNA* were screened by PCR-SSCP and sequenced in the 8 insulin-resistant men with acanthosis nigricans. All amplified fragments of the *LMNA* exhibited the expected lengths, consistent with the absence of deletions, duplications, or rearrangements within these fragments. One polymorphism was discovered in the exons in 3 of 8 subjects. Based on the predicted amino acid sequence for this gene, this polymorphism in exon 10 is silent with a C to T substitution, which does not alter the predicted histidine at codon 566. Because this affected residue is located at nucleotide +1908, position −1 of the exon 10 alternative splice donor site, we performed reverse transcriptase PCR (RT-PCR) on leukocyte transcripts using exonic primers localized in exon 9, 11, and 10 to check the consequences of this substitution at the RNA level of lamin A and lamin C, respectively. The results showed the presence of normally spliced *LMNA* mRNA in such affected (1908T homozygotes) subjects, as well as in normal 1908C homozygotes (data not shown).

### *Associations Between LMNA 1908 C/T Genotype and Type 2 Diabetes*

The frequency of the 1908T allele in a total of 335 subjects was 20%. The genotype distribution of the 1908 C/T SNP was: C/C, 62.1%; C/T, 35.5%; and T/T, 2.4%. Genotype frequencies were in a Hardy-Weinberg equilibrium. The frequency of subjects bearing the 1908T allele tended to be higher in the diabetic group (43.9%) than in the nondiabetic group (32.2%), although we found no significant differences among the 3 genotypes ($P = .084$) (Table 1). The allelic frequency of 1908T also did not significantly differ between the groups ($P = .068$) (Table 1). Subjects with the 1908T allele had an increased risk of type 2 diabetes (odds ratio [OR] = 1.651; 95% CI, 1.058 to 2.576). After adjustment for age and BMI, this increased risk was still observed (OR = 1.208; 95% CI, 1.003 to 1.502). Among 1908C/C homozygotes, 44.2% had diabetes: C/T, 57.1%; T/T, 50.0%.

**Table 1. Genotype and Allelic Distribution of the *LMNA* 1908C/T Polymorphism in Type 2 Diabetic and Nondiabetic Subjects**

| Subjects | C/C (%) | C/T (%) | T/T (%) | Allelic Frequency of 1908T |
|---|---|---|---|---|
| Type 2 diabetic patients (n = 164) | 92 (56.1) | 68 (41.5) | 4 (2.4)* | 0.232* |
| Nondiabetic subjects (n = 171) | 116 (67.8) | 51 (29.9) | 4 (2.3)* | 0.173* |

*Not significant.

*Effect of LMNA 1908 C/T Genotype on Clinical Variables*

We also tested whether there were differences in clinical parameters between subjects of each *LMNA* genotype. Nondiabetic subjects with the 1908T allele had significantly higher levels of FIRI and HOMA compared with those without (Table 2). Carriers of the 1908T allele also had significantly higher TG, TC, LDL-C, and they had lower HDL-C levels than the 1908C homozygotes, both in the nondiabetic and diabetic groups (Table 2). Notably, these differences remained significant after adjustment for age and BMI. There were no differences between the genotypes with respect to BMI, fasting glucose, or blood pressure. Among some nondiabetic subjects (n = 19, C/T + T/T *v* C/C, 7 *v* 12), we observed an increase in plasma leptin levels in carriers of *LMNA* 1908T compared with noncarriers (3.8 ± 1.3 *v* 2.5 ± 1.5 ng/mL; $P = .096$), although this difference was not significant, probably due to the small sample size.

We subsequently assessed the relationship between 1908C/T SNP frequencies and obesity. The *LMNA* genotype in obese subjects was not significantly different from those in nonobese subjects (Table 3).

## DISCUSSION

The widely expressed *LMNA* gene products, lamins A and C, are important elements of the nuclear lamina.[12] Some rare mutations in *LMNA* underlie FPLD, a syndrome characterized by a regional loss of adipose tissue that is associated with insulin resistance and dyslipidemia.[4-7]

We report here that a common genetic variant in *LMNA*, a silent C→T substitution at position 1908 in exon 10, which was previously reported,[22] is associated with hyperinsulinemia and dyslipidemia. However, this variant was not significantly associated with the prevalence of type 2 diabetes in the Japanese. We confirmed that this SNP led to normal lamin A/C transcripts in this study; thus, we suggest that it may affect the ratio of lamin A to lamin C in peripheral tissues. However, it must be kept in mind that our association results could be due to linkage disequilibrium of the 1908C/T polymorphism in the *LMNA* gene with variations in other genes that affect glucose and lipoprotein metabolism. The allelic frequency of the 1908C/T SNP was 0.2 in our subjects. This frequency is lower than that reported in Pima Indians (0.43),[22,23] Canadian Oji-Cree (0.77),[15] and Canadian Inuit (0.48).[24]

Recently, several studies have suggested that *LMNA* might play a role in adipose tissue metabolism and might even be a candidate gene for obesity and type 2 diabetes. First, mice with a targeted disruption of the *LMNA* gene have an almost complete absence of white adipose tissue.[25] Second, a new nuclear protein named lipin, which was found to be responsible for lipodystrophy in *fld* mice when mutated, induced differentiation of adipocytes.[26] The identification of lipin indicates that some nuclear structural proteins, such as lamins, may also be required for normal adipose tissue development. Third, lamin A/C mRNA content increases during differentiation in preadipocytes, with a higher lamin A/lamin C ratio in subcutaneous than omental mature adipocytes found recently.[27] Finally, it was reported that 1908C/T was associated with overall adiposity and plasma leptin levels in an aboriginal Canadian population,[15] with physical indexes of obesity in Canadian Inuit[24] and with subcutaneous abdominal adipocyte size in Pima Indians.[22] Collectively, these findings suggest that genetic variation in *LMNA* may cause not only the rare and severe abnormalities in

**Table 2. Characteristics of Nondiabetic and Type 2 Diabetic Subjects According to the *LMNA* 1908C/T Genotype**

| Variable | Nondiabetic Subjects (n = 171) | | | | Diabetic Subjects (n = 164) | | | |
|---|---|---|---|---|---|---|---|---|
| | C/C | C/T | T/T | *P* Value | C/C | C/T | T/T | *P* Value |
| No. | 116 | 51 | 4 | | 92 | 68 | 4 | |
| Age (yr) | 48.9 ± 9.0 | 47.0 ± 10.1 | 48.4 ± 15.3 | .486 | 54.7 ± 14.2 | 52.9 ± 11.1 | 54.3 ± 13.6 | .357 |
| Body mass index (kg/m$^2$) | 23.4 ± 2.8 | 23.7 ± 2.4 | 22.3 ± 1.1 | .340 | 23.2 ± 3.2 | 24.1 ± 3.5 | 22.7 ± 4.3 | .637 |
| Systolic blood pressure (mm Hg) | 123.4 ± 14.2 | 125.2 ± 10.1 | 128.0 ± 14.2 | .122 | 123.2 ± 15.6 | 120.0 ± 16.1 | 119.4 ± 25.7 | .161 |
| Diastolic blood pressure (mm Hg) | 70.7 ± 8.9 | 66.9 ± 6.9 | 80.5 ± 7.8 | .667 | 71.1 ± 10.3 | 74.5 ± 10.8 | 75.9 ± 15.1 | .099 |
| Total cholesterol (mmol/L) | 4.81 ± 0.71 | 5.19 ± 0.75* | 5.60 ± 0.28† | .003 | 4.74 ± 0.81 | 5.02 ± 0.71* | 5.51 ± 1.30† | .001 |
| HDL-cholesterol (mmol/L) | 1.31 ± 0.34 | 1.19 ± 0.28* | 1.08 ± 0.40† | .008 | 1.26 ± 0.33 | 1.15 ± 0.31* | 1.01 ± 0.42† | .008 |
| LDL-cholesterol (mmol/L) | 2.88 ± 0.76 | 3.21 ± 0.70* | 3.89 ± 0.46† | .006 | 2.85 ± 0.85 | 3.19 ± 0.71* | 3.88 ± 0.93† | .009 |
| Triglycerides (mmol/L) | 1.42 ± 0.88 | 1.71 ± 1.15* | 1.99 ± 0.15† | .009 | 1.44 ± 0.82 | 1.67 ± 1.45* | 2.09 ± 1.68‡ | .009 |
| Fasting glucose (mmol/L) | 5.22 ± 0.49 | 5.37 ± 0.51 | 5.65 ± 0.52 | .051 | 9.11 ± 2.99 | 8.41 ± 2.76 | 8.82 ± 3.07 | .194 |
| Fasting insulin (pmol/L) | 52.4 ± 12.9 | 59.6 ± 14.1* | 80.2 ± 10.5† | .001 | 57.4 ± 19.1 | 75.8 ± 39.2 | 77.2 ± 43.3 | .083 |
| HOMA | 1.70 ± 0.48 | 1.99 ± 0.40* | 2.81 ± 0.69† | .001 | 3.01 ± 1.78 | 4.10 ± 3.29 | 4.24 ± 3.53 | .128 |

NOTE. Data are means ± SD.

Abbreviation: HOMA, homeostasis model assessment of insulin.

*$P < .01$ *v* C/C genotype, †$P < .01$ *v* C/T genotype, ‡$P = .043$ *v* C/T genotype.

**Table 3. Genotype and Allelic Distribution of the *LMNA* 1908C/T Polymorphism in Obese and Nonobese Subjects**

| Subjects | C/C (%) | C/T (%) | T/T (%) | Allelic Frequency of 1908T |
|---|---|---|---|---|
| BMI $< 25$ kg/m$^2$ (n = 249) | 153 (61.4%) | 91 (36.6) | 5 (2.0)* | 0.203* |
| BMI $\geq 25$ kg/m$^2$ (n = 86) | 53 (61.6%) | 30 (34.9) | 3 (3.5)* | 0.209* |

*Not significant.

adipose tissue and glucose homeostasis seen in FPLD, but may also contribute to variation in adipose tissue cellularity in the general population.

In the present study, the *LMNA* 1908T genotype (C/T and T/T) was significantly associated with hyperinsulinemia, hypertriglyceridemia, high levels of LDL-C and low levels of HDL-C, all characteristic of the insulin-resistant syndrome. Previous studies in the Canadian Oji-Cree[15] and Inuit[24] have shown that carriers of the 1908T allele have more fat including higher BMI, plasma leptin concentrations, waist-to-hip circumference ratio, and skinfold thickness than those without it. However, a very recent study performed in Pima Indians did not show an association of this gene with BMI, leptin concentrations, type 2 diabetes, or serum lipids.[23] The discrepancy between the current observation and those in Pima Indians and Canadians may be due to linkage disequilibrium of the 1908T SNP with other ethnic-specific polymorphisms or the nature of the SNP studied. It would be interesting to elucidate whether these associations with insulin resistance and dyslipidemia are further enhanced or masked by the influence of environmental factors. One limitation of our study is that we did not assess anthropometric measurements characterizing the fat amount or distribution of the subjects. Although we found no association between this *LMNA* genotype and BMI, plasma leptin levels tended to be high in the subjects with the 1908T allele. Thus, we postulate that some variation in adipocyte tissue may be influenced by lamin A/C independent of body weight. Interestingly, high concentrations of TC and LDL-C were also dramatically associated with this 1908C/T SNP. This suggests that the *LMNA* may affect the synthesis and secretion of apolipoproteins, lipolytic enzyme activities, or the expression of lipoprotein receptors. Recently, it was found that the pattern of dyslipidemia shown in a family with FPLD carrying an R482W mutation within *LMNA* on chromosome 1q21-q23 was similar to familial combined hyperlipidemia (FCHL),[28] which has been shown to be linked also with 1q21-q23. Therefore, Schmidt et al[28] suggested that *LMNA* might play a role in the genesis of familial combined hyperlipidemia. Recent studies have shown that subjects with FCHL or combined hyperlipidemia are also insulin resistant. Although we did not measure in detail lipids and apolipoproteins, including very–low-density lipoprotein (VLDL) cholesterol, small dense LDL particles, or apoB levels, the *LMNA* 1908T carriers in our subjects showed a pattern of combined hyperlipidemia, with elevated levels of both serum TC and TG.

In conclusion, we demonstrated hyperinsulinemia and dyslipidemia in Japanese subjects, but not an increased prevalence of type 2 diabetes according to the *LMNA* 1908C/T SNP. This result suggests that *LMNA* 1908C/T SNP may play a role in the development of the insulin-resistant syndrome in some subjects. Additional studies on the regulation and consequences of *LMNA* gene expression, as well as the roles of lamins A and C in adipocyte metabolism, are needed. The various functions of *LMNA*, in addition to its role as a structural protein, also require further clarification.

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