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Analysis of the *Lamin A/C* gene as a candidate for Type II diabetes susceptibility in Pima Indians

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Abstract

Aims/hypothesis. *Lamin A/C* (*LMNA)* is located within a region on chromosome 1q that has been linked with Type II (non-insulin-dependent) diabetes mellitus in Pima Indians. Rare mutations in exon 8 of *LMNA* underlie Dunnigan-Type familial partial lipodystrophy, a disease characterized by regional adipocyte degeneration and frequently accompanied by insulin resistance, glucose intolerance, and diabetes. A more common variant in exon 10 (*3408C/T*) has recently been associated with obesity in non-diabetic aboriginal Canadian subjects. Because obesity is a strongly predisposing factor for Type II diabetes, we hypothesized that the *LMNA 3408C/T* variant could be associated with diabetes and body mass index in Pima Indians.
Methods. To determine whether the *LMNA 3408C/T* variant contributes to Type II diabetes susceptibility, we genotyped the polymorphism in 1338 Pimas using allelic discrimination technology. The locus was screened for additional variants in 20 diabetic Pima Indians and non-diabetic Pima Indians using denaturing high performance liquid chromatography and dideoxy sequencing.
Results. We found no evidence for association of *3408C/T* with diabetes, body mass index, total cholesterol, HDL cholesterol, triglycerides, leptin concentrations, or indices of insulin sensitivity and secretion. Subsequent screening of the remaining *LMNA* exons and flanking sequences revealed only rare variants in intron 4 and the 3'UTR, showing no frequency differences between diabetic and non-diabetic Pima Indians. We reassessed the linkage with diabetes following adjustment for the *LMNA 3408C/T* variant; adjustment for the effects of *LMNA* did not substantially modify the evidence for linkage.
Conclusion/interpretation. We conclude that the *LMNA 3408C/T* variant probably does not play a role in susceptibility to diabetes or obesity in Pima Indians. [Diabetologia (2001) 44: 779–782]

Keywords *LMNA*, Type II diabetes, Pima Indians, association analysis, single nucleotide polymorphism, 1q21

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Abbreviations: LMNA, lamin A/C gene; SNP, single nucleotide polymorphism; FPLD, familial partial lipodystrophy; DHPLC, denaturing high performance liquid chromotography; 3'UTR, 3' untranslated region.

Type II (non-insulin-dependent) diabetes mellitus is a complex heterogeneous disorder and although the underlying causes are not well understood, it is widely accepted that disease susceptibility has both genetic and environmental components [1].

To date, the most consistent evidence for linkage to Type II diabetes is for a diabetes locus on chromosome 1q21-q23 reported in Pima Indians [2], Utah Caucasians [3], the Old Order Amish [4] and European Caucasians [5, 6]. Our strategy to identify the diabetes locus on 1q21-q23 in the Pima Indians includes analysis of candidate genes positioned within this in-

terval. One such candidate is *LMNA*, encoding lamins A and C, which constitute the nuclear lamina envelope. Both proteins are widely expressed and originate from *LMNA* as a result of alternative splicing. The isoforms share sequence homology for the first 566 residues, differing only in their COOH termini. Both lamins A and C have globular head and tail domains with an intervening central rod domain; the proteins heterodimerize to form the dense laminar latticework of the nuclear membrane.

Rare mutations in *LMNA* exon 8 underlie Dunnigan-Type familial partial lipodystrophy (FPLD: OMIM 151660), an autosomal dominant disease characterized by progressive regional loss of subcutaneous adipose tissue following puberty [7]. This disease is commonly associated with severe insulin resistance, glucose intolerance, and diabetes [8]. Other mutations in *LMNA* underlie two additional autosomal dominant syndromes: Emery-Dreifuss muscular dystrophy (EMD2; OMIM 181350) and dilated cardiomyopathy with conduction-system disease (CMD1A: OMIM 115200). Recently, a common variant in exon 10 of *LMNA* (*3408C/T*: Genbank L12401) was associated with indices of obesity and plasma leptin concentrations in non-diabetic Oji-Cree, a population of aboriginal Canadians [9]. Because the C→T substitution does not produce an amino acid change and no other coding sequence variants were found, the authors postulated that common genetic variation in *LMNA* might be an important determinant affecting plasma leptin concentrations and obesity-related phenotypes.

Due to its chromosomal localization within an area linked to Type II diabetes in several groups, the frequent occurrence of insulin resistance and diabetes in FPLD patients and the reported association of the *LMNA 3408C/T* SNP with obesity in the Oji-Cree [9], we investigated the association of *LMNA* with Type II diabetes, obesity, and serum lipid concentrations in Pima Indians.

Subjects and methods

Subjects. Subjects selected for genotyping comprised 1338 individuals selected for linkage studies from participants in ongoing longitudinal studies of diabetes conducted in the Gila River Indian Community [2]. Diabetes was diagnosed according to the World Health Organization criteria as described [2]. This study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and the Tribal Council of the Gila River Indian Community. All subjects provided written informed consent before participation.

LMNA 3408C/T Genotyping. The *3408C/T* SNP was genotyped in 1338 Pima Indians by allelic discrimination using the TaqMan Reagent Kit and the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, Calif., USA). The PCR was performed using primers 5'-GTTGAGGACGACGAGCATCAG and 5'-GCCAGCGAGTAAAGTTCCAAA. The wild type allele was detected using the Tet-labelled probe 5'-ATCACCACCACGTGAGTGGTAGCC and the mutant allele was detected using the Fam-labelled probe 5'-CATCACCACCATGTGAGTGGTAGCC (the variant nucleotide is underlined in each sequence). Reaction components and amplification parameters were based on the manufacturer's instructions using an annealing temperature of 64 °C and optimized concentrations for primers and probes of 900 nmol/l and 100 nmol/l, respectively. Genotyping reproducibility was evaluated in 75 duplicate samples.

LMNA genomic screening. The gene was screened in 10 diabetic and 10 non-diabetic Pima Indians by denaturing HPLC using the Transgenomic WAVE system (Transgenomic, Omaha, Neb., USA) as described [10]. Melt temperatures were calculated for each amplicon using the WAVEmaker software, Version 3.3.3 (Transgenomic) and are listed in Table 2.

Statistical analysis. Diabetes prevalence among *LMNA* genotypes was compared by logistic regression with adjustment for age, sex, ethnicity and birth year. Logistic regression analyses were conducted by binomial generalized estimating equations to account for non-independence of siblings (PROC GENMOD of SAS). To maximize power, we used two different models, one assuming the *T* allele is dominant and the other assuming it is recessive to the *C* allele.

Clinical measurements also included serum cholesterol and insulin concentrations, height and weight, and – since 1993 – HDL cholesterol and serum triglycerides. Plasma leptin concentrations have also been measured among non-diabetic genomic scan participants. Propensity to obesity was taken as the maximum BMI observed in the longitudinal study. Among non-diabetic individuals, indices of insulin sensitivity and secretion, based on the fasting and 2-h glucose (G_0, G_2) and insulin (I_0, I_2) were also analyzed. The fasting insulin sensitivity index $[1/(G_0{*}I_0)]$ and 2-h corrected insulin response $[I_2/(G_2{*}(G_2{-}70\ \text{mg/dl})]$ were chosen to provide the best estimates of insulin sensitivity and insulin secretion, respectively. Linear generalized estimating equations were used to examine the association of the *3408C/T* variant with these quantitative traits. As in the analyses of diabetes, both dominant and recessive models were employed with adjustment for age, sex, birth year, ethnicity and sibship. Analyses of serum lipid concentrations were additionally adjusted for diabetes and, among diabetic subjects, for duration of diabetes. The association was partitioned into a component representing differences among sibships (which is potentially confounded by population stratification) and a component representing differences within sibships (which reflects linkage disequilibrium between marker alleles and a potential trait locus). We obtained statistically significant *p*-values only in those analyses partitioned among sibships.

A total of 1166 subjects were included in analyses of diabetes. Because of restrictions related to age, availability of samples and presence of diabetes, a subset of these individuals was included in analyses of quantitiative traits (Table 1).

Linkage analyses were conducted using the Haseman-Elston test [2]. The extent to which the *3408C/T* SNP contributed to the observed linkage results was assessed by determining the association of genotypes with young-onset diabetes and 'adjusting' the diabetes score for the effect of the single nucleotide polymorphism by subtracting the prevalence of young-onset diabetes in the individual's genotypic category from an indicator variable for affection status. The Haseman-Elston regression with this 'adjusted' diabetes score in the chromosome 1q21 region was then compared with the result for linkage obtained without adjustment for the effect of the SNP.

Table 1. Analyses are adjusted for age, sex, birth year, ethnicity and nuclear family membership for all variables. Analyses of serum lipids are also adjusted for diabetes and, among diabetic individuals, for duration of diabetes. The association parameter (β) represents the difference between genotypes, in standard deviation units on the logarithmic scale

	Dominant Model				Recessive Model			
	Among Sibships		Within Sibships		Among Sibships		Within Sibships	
	β	p value	β	p value	β	p value	β	p value
Body Mass Index (kg/m^2)	–0.05	0.62	–0.01	0.91	–0.11	0.45	0.13	0.05
Total Cholesterol (mg/dl)	0.17	0.08	0.12	0.27	0.25	0.02	–0.04	0.63
HDL Cholesterol (mg/dl)	–0.28	0.02	0.15	0.16	–0.13	0.37	–0.05	0.73
Triglycerides (mg/dl)	0.45	< 0.01	0.05	0.73	0.45	< 0.01	–0.10	0.54
Leptin (ng/dl)	–0.16	0.13	0.15	0.19	–0.16	0.31	0.15	0.25
Insulin Sensitivity Index (SD units)	0.08	0.53	0.01	0.90	–0.01	0.93	0.06	0.62
Corrected Insulin Response (SD units)	–0.04	0.77	–0.02	0.89	–0.01	0.96	–0.25	0.10

Table 2. Primers and assay conditions for *LMNA* screening by DHPLC

Exon	Size (bp)	Forward primer (5'→ 3')	Reverse primer (5'→ 3')	Tm (°C)*
1	406	GCAGCGCTGCCAACCTG	AGGCACGCAGCCACCTG	66, 67
2	257	CTCCTTCTCTTAAATCTACTCTC	TAGAAGAGTGAGTGTACATGTGTTA	65–68
3	171	TCTCTGCCTGCTTCCTCAC	CTGGCTTGCAAAGCACAGTC	62, 63
4	245	CTAATTCTGATTTTGGTTTCCTGTG	GCAGTGAGGGAACCAATCG	61, 62, 65, 66
5	309	GCAGTGATGCCCAACTCAG	CATCCCTCTCCTCCTCAG	65, 67
6	372	CAGCTGTCTCCTACACCG	CTAGTCAAGGCCAGTTGCCG	66, 67
7	371	CGGCAACTGGCCTTGACTAG	CTCTGAGGGCAAGGATGTTC	63, 64
8 & 9	371	TCTTCCCTATCTTCCCGGC	GCCTCGTCCAGCAAGCAGC	63, 64, 66
10	364	GTAGAGATCTTGTACAACCCTT	TCCCTTCCTTACCAGAGTAG	62, 63
11	335	TCAGTCCCAGACTCGCCG	AAAGCAGAAGACAACTCACCTG	66, 67, 68
12a$^+$	497	TTCTCTCTTAGAGCCCCCAG	GTGAGCCTGCCGTCCAGAG	58, 62, 65
12b$^+$	584	CTCTGGACGGCAGGCTCAC	CCTCAGTACACACAGCGAC	64, 65, 66

* Tm refers to the optimal melt temperatures used for heteroduplex analysis.
$^+$12a and 12b represent overlapping segments spanning the last exon and including the 3' untranslated region.

Results

The frequency of the *3408 T* and *C* alleles was 0.43 and 0.57, respectively, which is different from the Oji-Cree (0.77/0.23) [9]. The unadjusted prevalence of Type II diabetes was comparable between Pima patients grouped according to genotype (58% for the *TT* genotype, 59% for the *CT* genotype and 62% for the *CC* genotype). In analyses adjusting for age, sex, birth year, ethnicity, and family membership, no association between *LMNA* genotypes and diabetes was found. The odds ratio (OR) for the model comparing the *TT* and *CT* genotypes with the *CC* genotype (the dominant model) was 0.86 (95% confidence interval 0.46–1.16, $p = 0.31$). Similar results were found with a recessive model (OR = 0.92, 95% confidence interval 0.68–1.25, $p = 0.61$).

We also assessed the relationship of the *3408C/T* variant with BMI, serum lipid concentrations, plasma leptin, and indices of insulin sensitivity and secretion. Because these variables (with the exception of corrected insulin response), unlike young-onset diabetes, did not show strong evidence for linkage with this chromosomal region [2], the association was partitioned into a component representing differences among sibships and a component representing differences within sibships. The results are shown in Table 1. While subjects who carried the *T* allele generally had higher serum triglycerides than those who carried the *C* allele, these differences were largely attributable to differences among sibships. None of the within sibship tests was statistically significant; these data, therefore, do not support the hypothesis of linkage disequilibrium between alleles at the *3408C/T* SNP and alleles influencing BMI, serum lipid and plasma leptin concentrations, insulin sensitivity or insulin secretion in Pimas.

Discussion

Interestingly, the 1q21 interval shows a suggestive linkage to subcutaneous abdominal adipocyte size in the Pima Indians and the *3408 TT* genotype was modestly associated with a low subcutaneous abdominal adipocyte size in a subset of 255 non-diabetic Pima Indians ($p < 0.05$; Weyer: personal communication). It is therefore possible that this single base substitution is a marker for variation in adipocyte size, without a direct relationship to the degree of obesity or diabetes status. The *3408C/T* variant involves the last base of exon 10, representing the third position of

amino acid 566, the last codon shared between lamins A and C. The variant does not produce an amino acid change and both the *C* and *T* base are found at this position in different species. The SNP is therefore not likely to have a major functional consequence but could either be in linkage disequilibrium with a functional variant in the nearby vicinity of this locus or modulate splicing efficiency at the exon 10/intron 10 juncture.

To examine whether any other variants in *LMNA* contribute to diabetes susceptibility in Pima Indians, we screened through all 12 exons using DHPLC (Table 2). Two rare SNPs were detected: one in intron 4 (*T/A*; 13 bases upstream of the first base of exon 5) and another in the 3' UTR (*C/T*; 398 bases downstream from the stop codon). Because both variants were present at low frequency (< 5 %) and did not show frequency differences between diabetic and non-diabetic Pima Indians, they were not further investigated. Apart from the *3408C/T*, we did not detect any additional common variants within the *LMNA* exons, adjacent splice sites, approximately 2 kb upstream of the first ATG codon and in the 3'UTR. Furthermore, none of the reported rare FPLD mutations were identified in a subset of the 20 Pima Indians used in our screening experiments.

To determine whether *3408C/T* might contribute to the observed linkage results, we performed a Haseman-Elston test for linkage following adjustment for the effect of this SNP and compared this result (maximum LOD = 3.2) with the unadjusted linkage results (LOD = 3.0). The *3408C/T* SNP had no effect on the chromosome 1 LOD score and we therefore conclude that it does not explain the linkage to Type II diabetes in this sample. It is possible that mutations in more than one locus are responsible for the 1 q linkage in the Pima Indians and in such a case, a significant reduction in LOD score might not be apparent without adjusting for all mutations simultaneously. However, based on our findings, it is not likely that any other mutations within *LMNA* contribute to the linkage on 1 q in this group.

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