SHORT COMMUNICATION

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Identification of single-nucleotide polymorphisms in the human *LPIN1* gene

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Abstract Because mutations in the murine analog of human *LPIN1* cause lipodystrophy in mice, *LPIN1* is a candidate gene for human lipodystrophy syndromes. To identify possible disease mutations and/or common single-nucleotide polymorphisms (SNPs), we developed primer pairs to amplify the 21 exons of *LPIN1*. We used these primer pairs to sequence *LPIN1* in lipodystrophy patients who had no mutations in known lipodystrophy genes, and also in normal control subjects. We found no rare *LPIN1* coding sequence variants that were exclusive to patients with lipodystrophy. However, we found four silent SNPs, namely, +17C>T in exon 3, 935C>T in exon 5, and 1040G>A and 1079G>C in exon 6, and one nonsynonymous SNP, namely, 2211C>T (P616S) in exon 15. The findings suggest that *LPIN1* mutations are not commonly seen in patients with lipodystrophy who had no mutations in known disease genes. However, the identification of amplification primers and SNPs provides tools to further investigate *LPIN1* for association with other phenotypes.

Key words Adipose tissue · Lipodystrophy · Diabetes · Genomic DNA · Sequencing · Complex traits

Introduction

Lipodystrophy refers to a heterogeneous group of disorders characterized by loss of body fat, insulin resistance, dyslipidemia, hypertension, diabetes, and atherosclerosis (Hegele 2001). Two forms of lipodystrophy have been

characterized at the molecular genetic level. The first is Dunnigan-type familial partial lipodystrophy (FPLD; MIM 151660), which is due to mutations in the *LMNA* gene (Cao and Hegele 2000; MIM 150330). The second is Berardinelli-Seip complete lipodystrophy (BSCL; MIM 260700), which results from mutations in the *BSCL2* gene (Magre et al. 2001; MIM 606158) in some families. However, there is another locus for BSCL called *BSCL1* (Garg et al. 1999; MIM 606166) and, similarly there might be other loci for FPLD. Thus, it remains important to evaluate new candidate genes for lipodystrophy syndromes based on function, expression, genetic map position, and/or analogy with other human or animal phenotypes.

Mice carrying mutations in the fatty liver dystrophy (*fld*) gene have features of human lipodystrophy (Reue et al. 2000). Last year, the murine *fld* gene was isolated and two mutant alleles were characterized (Peterfy et al. 2001). The murine gene was designated *Lpin1* and the novel nuclear protein gene product was designated lipin (Peterfy et al. 2001). Database analysis identified several mouse and human sequences with similarities to *Lpin1*, including two *Lpin1*-related mouse genes called *Lpin2* and *Lpin3*, and three human homologs called *LPIN1* (MIM 605518), *LPIN2* (MIM 605519), and *LPIN3* (MIM 605520). *LPIN1* is identical to the *KIAA0188* gene (Nagase et al. 1996), and maps to chromosome 2p21 (Peterfy et al. 2001). Because of the findings in the mouse, the product of *LPIN1* appears to be required for normal adipose tissue development, and thus represents a candidate gene for human lipodystrophy. To identify possible disease mutations and/or common single-nucleotide polymorphisms (SNPs), we sequenced *LPIN1* in (1) lipodystrophic patients with no sequence abnormality in known lipodystrophy genes and (2) normal controls.

Subjects and methods**Study subjects**

We studied 11 subjects with typical FPLD and 4 subjects with apparently typical BSCL. All subjects had normal se-

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quences of both the *LMNA* and *BSCL2* genes. All affected subjects were of European descent. Subjects with acquired or drug-induced lipodystrophy were excluded. All subjects were isolated affected cases, and none came from a family that was sufficiently large to perform linkage analysis. In addition, samples from 100 clinically normal Caucasian subjects were screened to determine SNP allele frequencies. The study was approved by the Ethics Review Panel, University of Western Ontario.

Screening the *LPIN1* gene for DNA variants

To amplify coding regions and intron–exon boundaries from genomic DNA, we developed a primer set using GenBank accession numbers XM_041136 and 17444450. Primer sequences are shown in Table 1. Amplification conditions for all exons were as follows: 94°C for 5 min, followed by 30 cycles of 30s each at 94°C, 60°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Genotyping of *LPIN1* gene SNPs

The *LPIN1* exon 3 +17C>T SNP was genotyped by amplifying genomic DNA using the primers in Table 1, and the preceding amplification program. This was followed by digestion of the 350-bp product with endonuclease *NlaIII*. The +17C allele yielded two fragments with sizes 341 and 9-bp after *NlaIII* digestion. In contrast, the +17T allele yielded three fragments with sizes 207, 134, and 9bp after *NlaIII* digestion. The polymorphic fragments were resolved on 2% agarose gels.

The *LPIN1* exon 5 935C>T SNP was genotyped by amplifying genomic DNA using the primers in Table 1, and the preceding amplification program. This was followed by digestion of the 462-bp product with endonuclease *TaqI*. The 935C allele yielded three fragments with sizes 211, 135, and 116bp after *TaqI* digestion. In contrast, the 935T allele yielded two fragments with sizes 346 and 116bp after *TaqI* digestion. The polymorphic fragments were resolved on 2% agarose gels.

Table 1. Amplification primers for *LPIN1*

Exon	Primer sequence (5' to 3')	Product size (bp)
1	F-AAA GCA AGC TGG AGA GGG AG R-CTG TGT CAC CGG GAA GCT CT	354
2	F-ATG TCG TGT GTC CAC AGG TAA C R-AAT GTT TGG AAC CAA TTT ACG C	210
3	F-CTT GGA TTA ATT GTG TGT CTG TGT G R-TTT CAA TTA GTC ACT GTC AGT TCC G	400
4	F-GGT GAA GGC AGT TCA TTC TTT T R-ACT TCT GCC GTG TTT CCA GAT A	218
5	F-CAA GGC CCT GCT TCT TAT ACC T R-AGG AAT TAT GGG GGA AAA GTT C	462
6	F-CAA AGC ACT TAG AGC TAA TCA AGA AA R-TGA CTT CTA AGC CTC TGC ACT G	269
7	F-TCT TTT CCC ACC TTA ATT TTC G R-GCT CCT GGT AGC TGT GTT TCT T	271
8	F-TCT CCT TGG GTC ACT CAG TTT T R-AAC TAT GAA CAG AAA AAG AGC CG	432
9	F-GAG TGG TTT TCT GAC TTG AGC C R-ATA AGG GGA AAC TGG TCT CAC A	206
10	F-ATG GTG ATG TAG GAC CCT GAA C R-GAG AAG CCT AAG CCT CTG CG	326
11	F-AGT GAG CAA ATG AAA GGT AGG C R-ATC ATG CTA CTG CCA AAG TGA C	215
12	F-TCA GTC TGA GCC TCG GTT TT R-TCC AAC CAC CCC AGA TTA AG	188
13	F-TTT CTT TTG GTT TGA ATG TGC T R-CAC TAC ACA AAA CCA ACA CTT GC	223
14	F-CCC CTT CTC TGT GGT ACT TCT G R-GGA ACT GTG TGG TGA ACC CTA T	227
15	F-ATT TTT CAT GGC TAC CCA GAT G R-AAA CCT CAT GTG CTC ACA ACA C	235
16	F-CTT TCC CTC AAG CAG ACA AAT A R-CGG TGA AGC AAA GAA ATC TAC C	240
17	F-GAA TGG TGC AAT GAA TCT GAA A R-CGG GAA ACA GGA ACT CTG TAC T	174
18	F-GAA AGG TTC CAT TTT CTG ATG C R-ACT TGC AGT TAG GAA GAG AGC G	274
19	F-TGA TGC TAA AGG AAG CTT AGC C R-CAG ATG ACA GCA ACC AGA AAA G	236
20	F-TGA ACA CTT TAA ATC ACC TTT ACC A R-GAA AAG GTA CAC CGC AAA CTG	221
21	F-GCA TAG CTG GTA TCG CTC AGT R-CCG GGG AGA CCT ATC CTT TAA T	280

F, Forward primer; R, reverse primer

LPINI exon 6 SNPs were genotyped by amplification with the primers in Table 1, using the preceding program, followed by direct sequencing of genomic DNA using these primers. *LPINI* exon 15 SNP was genotyped by amplifying genomic DNA with the primers in Table 1, using the preceding program, followed by direct sequencing of genomic DNA using these primers.

Statistical analysis

SAS version 6.12 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Allele frequencies were determined from electropherogram tracings of genomic DNA sequence, except for two SNPs assayed using restriction digestion. Chi-square analysis tested the deviation of genotype frequencies from Hardy-Weinberg predictions, with the nominal $P < 0.05$.

Results

Identification of SNPs

Genomic DNA sequencing experiments in 11 FPLD subjects with normal *LMNA*, 4 BSCL subjects with normal *BSCL2* and 10 normal controls uncovered five SNPs. These were a silent SNP +17C>T in exon 3, a silent SNP 935C>T in exon 5, silent SNPs 1040G>A and 1079G>C in exon 6, and a nonsynonymous SNP 2211C>T (P616S at the amino acid level) in exon 15. These SNPs are shown in Table 2. All SNPs were seen in both affected subjects and normal controls. There were no other DNA variants detected in the samples taken from the diseased subjects.

SNP frequencies in normal samples

The observed genotype frequencies of all SNPs did not deviate from the predictions of the Hardy-Weinberg equation. The allele frequency in 100 Caucasians for each SNP is shown in Table 2.

Discussion

We report (1) primer sets to amplify coding sequences of the *LPINI* gene and (2) use of these primers to sequence

genomic DNA. This has resulted in (1) identification of five SNPs in *LPINI* and (2) demonstration that there are no additional coding sequence variants in *LPINI* in lipodystrophic subjects. In the present report, we did not examine the *LPINI* promoter region; therefore, we cannot absolutely rule out the possibility of such SNPs and/or mutations in the affected subjects. However, more than two dozen mutations causing FPLD and BSCL alter the coding sequence and none has been found in promoter or intron sequences (Hegele 2001; Magre et al. 2001), possibly implying that promoter or intron sequence variants are uncommon in lipodystrophy. Genomic DNA from these subjects will continue to be sequenced as new candidate genes are identified and prioritized for possible involvement in this phenotype. Thus, *LPINI* mutations do not appear to be a common cause of lipodystrophy, although we cannot rule this possibility out because of the limited number of subjects studied herein. It is possible that others might find *LPINI* mutations in lipodystrophic subjects. Despite the absence of disease association in our sample, the reagents described in this report could be helpful to screen the *LPINI* gene for other studies.

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Table 2. *LPINI* gene SNP allele frequencies in normal Caucasians

SNP name	Allele frequency	Detection method
Exon 3 +17C>T	+17T: 0.35	<i>Nla</i> III digestion
Exon 5 935C>T	935T: 0.20	<i>Taq</i> I digestion
Exon 6 1040G>A	1040A: 0.01	Direct sequencing
Exon 6 1079G>C	1079C: 0.02	Direct sequencing
Exon 15 2211C>T	2211T: 0.06	Direct sequencing

SNP, Single-nucleotide polymorphism