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SHORT COMMUNICATION

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Identification of polymorphisms in the human *SHP1* gene

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Abstract Because mutations in human *SHP1* underlie obesity and diabetes, *SHP1* 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 promoter and coding region of *SHP1*. We used these pairs to sequence *SHP1* in lipodystrophy patients who had no mutations in known lipodystrophy genes, and also in normal control subjects. We found no rare *SHP1* coding sequence variants that were exclusive to patients with lipodystrophy. However, we found four polymorphisms, namely, an SNP [−394]C>T in the promoter, a micro-deletion polymorphism [−195]delCTGA in the promoter, a missense SNP 541G>C in exon 1 (which changed the amino acid sequence G171A), and an SNP 903C>T in exon 2. The findings suggest that *SHP1* mutations are not commonly seen in patients with lipodystrophy who had no mutations in known disease genes. However, the identification of amplification primers and polymorphisms provides tools to further investigate *SHP1* for association with other phenotypes.

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

Introduction

Lipodystrophy is characterized by repartitioning of body fat, insulin resistance, dyslipidemia, hypertension, diabetes, and atherosclerosis (Hegele 2001). Two forms of lipodystrophy have been characterized at the molecular level. The first is Dunnigan-type familial partial lipodystrophy (FPLD; MIM 151660) caused by mutant *LMNA* (Cao and Hegele 2000; MIM 150330). The second is Berardinelli-Seip complete lipodystrophy (BSCL; MIM 260700) caused by mutant *BSCL2* (Magre et al. 2001; MIM 606158). However, there is at least one other locus for BSCL called *BSCL1* (Garg et al. 1999; MIM 606166) and there might also be other loci for FPLD. Thus, it remains important to evaluate new candidate genes for lipodystrophies based on function, expression, genetic map position, and/or analogy with other phenotypes.

Nuclear receptors are transcription factors regulated by small hydrophobic hormones such as retinoic acid, thyroid hormone, and steroids (Lee et al. 1998). This superfamily also includes orphan nuclear receptors, which are related proteins with unspecified ligands. Small heterodimer partner 1 (SHP1), also called nuclear receptor subfamily 0, group B, member 2 (NR0B2), is a 257-amino acid protein that contains ligand-binding and dimerization domains, but lacks a DNA-binding domain (Seol et al. 1996). Loss-of-function mutations in the *SHP1* gene were reported in Japanese subjects with obesity and diabetes (Nishigori et al. 2001). Because these traits are related to lipodystrophy, *SHP1* is a potential candidate gene for lipodystrophy that is not due to mutant *LMNA* or *BCSL2*. To find disease mutations and/or single-nucleotide polymorphisms (SNPs), we sequenced *SHP1* 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 typical BSCL. All subjects had normal sequences 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

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Table 1. Amplification primers for *SHP1*

Exon	Primer sequence (5′ to 3′)	Product size (bp)
Promoter	F- TAG ACT GGA CAG TGG GCA AAG T R- CAG CTC TCT GGC TCT GTG TTC T	575
1	F- GGA AGT GAG AGC AGA TCC CTA A R- TTA GAA GCT ACC TTC CCT GGC T	627
2	F- AGA TCT TGG GCC AGT CTT GTC R- AGC TGT TCC TAA GGA GCC AAG T	455

F, Forward primer; R, reverse primer

sufficiently large to perform linkage analysis. In addition, samples from 74 clinically normal Caucasian subjects were screened to determine polymorphism allele frequencies. The study was approved by the Ethics Review Panel, University of Western Ontario.

Screening the *SHP1* gene for DNA variants

To amplify coding regions and intron–exon boundaries from genomic DNA, we developed a primer set using GenBank accession numbers 17438593 and NM_021969. Primer sequences are shown in Table 1. Amplification conditions for all exons were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 60°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Genotyping of *SHP1* gene polymorphisms

The promoter [−394]C>T SNP and the [−195]delCTGA polymorphism were genotyped by amplifying genomic DNA using the primers in Table 1, and the amplification program described earlier. For the [−394]C>T SNP, the 575-bp product was digested with endonuclease *Bst*N1. The [−394]C allele yielded eight fragments with sizes 177, 131, 87, 74, 48, 35, 16, and 7 bp after *Bst*NI digestion. The [−394]T allele yielded seven fragments with sizes 177, 131, 122, 87, 35, 16, and 7 bp after *Bst*NI digestion. The fragments were resolved on 10% polyacrylamide gels. For the [−195]delCTGA polymorphism, the 575-bp product was digested with endonuclease *Dde*I. The allele without the deletion yielded five fragments with sizes 200, 181, 96, 49, and 49 bp after *Dde*I digestion. The allele with the deletion yielded four fragments with sizes 249, 181, 96, and 49 bp after *Dde*I digestion. The fragments were resolved on 2% agarose gels.

The *SHP1* exon 1 541G>C SNP (which changed the amino acid sequence G171A) was genotyped by amplifying genomic DNA using the primers in Table 1, and the amplification program described earlier. This step was followed by digestion of the 627-bp product with endonuclease *Ava*II. The 541G allele yielded three fragments with sizes 270, 266, and 91 bp after *Ava*II digestion. The 541C allele yielded two fragments with sizes 357 and 270 bp after *Ava*II digestion. The fragments were resolved on 2% agarose gels. The *SHP1* exon 2 SNP was genotyped by amplification with the primers in Table 1, followed by direct genomic DNA sequencing.

Table 2. *SHP1* gene allele frequencies in normal Caucasians

Polymorphism name	Allele frequency	Detection method
Promoter [−394]C>T	−394T: 0.03	*Bst*NI digestion
Promoter [−195]delCTGA	delCTGA: 0.03	*Dde*I digestion
Exon 1 541G>C	541C: 0.09	*Ava*II digestion
Exon 2 903C>G	903C: 0.04	Direct sequencing

Statistical analysis

SAS version 6.12 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Allele frequencies were determined from electropheretogram 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 polymorphisms

Genomic DNA sequencing experiments in 11 FPLD subjects with normal *LMNA*, 4 BSCL subjects with normal *BSCL2*, and 10 normal controls uncovered four polymorphisms. These were an SNP [−394]C>T in the promoter, a microdeletion polymorphism [−195]delCTGA in the promoter, a missense SNP 541G>C in exon 1 (which changed the amino acid sequence G171A), and an SNP 903C>T in exon 2. These SNPs are shown in Table 2. All SNPs were seen in affected subjects and normal controls. There were no other DNA variants detected in the samples taken from the diseased subjects.

Polymorphism frequencies in normal samples

The observed genotype frequencies of all polymorphisms did not deviate from the predictions of the Hardy-Weinberg equation. The allele frequency in 74 Caucasians for each polymorphism is shown in Table 2.

Discussion

We report (1) primer sets to amplify coding sequences of the *SHP1* gene and (2) use of these primers to sequence

genomic DNA. This has resulted in (1) identification of four polymorphisms in *SHP1* and (2) demonstration of no additional coding sequence variants in *SHP1* in lipodystrophic subjects. 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, *SHP1* 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 *SHP1* 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 *SHP1* gene for other studies.

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