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SHORT COMMUNICATION

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Single nucleotide polymorphisms of the *fukutin* gene

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Abstract Mutations in the *LMNA* gene, which encodes nuclear lamins A and C, underlie both Emery-Dreifuss muscular dystrophy (EMD2) and Dunnigan-type familial partial lipodystrophy (FPLD). This indicates that one gene can cause different phenotypes characterized by tissue degeneration. The gene for one form of Berardinelli-Seip-type congenital total lipodystrophy (BSCL) has been mapped to chromosome 9q34. Based on the observation that one gene caused both FPLD and EMD2, we considered that a known gene for muscular dystrophy at or near the BSCL locus on chromosome 9q would be an appropriate candidate for BSCL. The gene encoding fukutin, which is mutated in Fukuyama congenital muscular dystrophy has been mapped to 9q31. We thus developed amplification primers for the coding regions of the *fukutin* gene. We found no putative disease mutations, but through screening of diseased and normal subjects, we identified three novel single nucleotide polymorphisms (SNPs). We conclude that mutations in *fukutin* are not present in subjects with BSCL. However, the identification of SNPs provides tools to investigate this protein for association with other phenotypes.

Key words Lipodystrophy · Muscular dystrophy · Diabetes · Genomic DNA · Sequencing · Complex traits · Genetic pleiotropy

Introduction

Naturally occurring mutations within *LMNA*, which encodes nuclear lamins A and C, underlie several autosomal dominant diseases: a form of Emery-Dreifuss muscular dystrophy (EMD2; OMIM 181350), a form of dilated cardiomyopathy (CMD1A; OMIM 115200), and a form of limb girdle muscular dystrophy (LGMD1B; OMIM 159001). In addition, Dunnigan-type familial partial lipodystrophy (FPLD; OMIM 151660) results from mutations in *LMNA* (Cao and Hegele 2000). In contrast, no disease mutations have been found for Berardinelli-Seip congenital total lipodystrophy (BSCL; OMIM 269700), one form of which was mapped to chromosome 9q34 (Garg et al. 1999). Because mutations in one gene, namely, *LMNA*, caused both lipodystrophy and muscular dystrophy, we analogously prioritized positional candidate genes for BSCL on the basis of whether they were known to cause muscular dystrophy. The gene encoding fukutin (Kobayashi et al. 1998), which is mutated in Fukuyama congenital muscular dystrophy (FCMD; OMIM 253800), has been mapped to 9q31 (Toda et al. 1996). We thus chose to examine *fukutin* as a candidate gene for BSCL on the basis of analogy with *LMNA* as the cause of both EMD2 and FPLD.

Subjects and methods

Study subjects

Samples from 3 subjects with BSCL were evaluated. In addition, samples from a DNA archive of 20 clinically normal Caucasian subjects were screened to determine single nucleotide polymorphism (SNP) allele frequencies. The study was approved by the Ethics Review Panel, University of Western Ontario.

Screening the *fukutin* gene for DNA variants

To amplify coding regions and intron–exon boundaries from genomic DNA, we developed a primer set by using GenBank sequences (see Table 1). Primer sequences were derived by using GenBank accession numbers NM__006731 and AB038490. Primer sequences are shown in Table 1. These differed from previously reported primers

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Table 1. Amplification primers for *fukutin*

Exon(s)	Primer sequence	Product size
Exon 1	forward 5′-ATTATAAAAGCGAGGAATCGC-3′	392 bp
	reverse 5′-GACTGACTGAGGAGTACCCAGC-3′	
Exon 2	forward 5′-CAGGTACAAAATTAAAGAGGTTTTTG-3′	360 bp
	reverse 5′-GCAAGAATTTGAATACCCATGA-3′	
Exon 3	forward 5′-AATGTAATGTTGCATGCTGGAC-3′	259 bp
	reverse 5′-CCTTACCTGCCACTACCAACC-3′	
Exon 4	forward 5′-AATGATAAGCATGGTATCTATTGGG-3′	380 bp
	reverse 5′-TATTTTGTAAAAAGAGACACATTCCA-3′	
Exon 5	forward 5′-GTTGTTTCTTGATGTTTGATGCTT-3′	377 bp
	reverse 5′-CACTGCAAGAATTATGACTGAACA-3′	
Exon 6	forward 5′-CTCTGCACGCATTAGGTATTTG-3′	287 bp
	reverse 5′-CCCTCAAATATTAAAAATAATCCCAA-3′	
Exon 7	forward 5′-CAATTTAGAAATGGCTGTAATAGCTG-3′	238 bp
	reverse 5′-TAATCCCTAAAGCCAGAGGACA-3′	
Exon 8	forward 5′-ACAGCCTGGGCAATATAGTGAG-3′	300 bp
	reverse 5′-TATGAAATGAAGCCGACTTTTG-3′	
Exon 9	forward 5′-CCTTTGTTTCAGTGTGTGAAGG-3′	256 bp
	reverse 5′-TGGTTTTCTTTTCAGTTACTTCTTT-3′	
Exon 10	forward 5′-CACTGTTGAAGCCTAATCCCTC-3′	346 bp
	reverse 5′-TGGTTCCCACTTATGTTTGACA-3′	

(Kondo-Iida et al. 1999) in that they were designed both to amplify at least 50 bp flanking each exon and to anneal at a single temperature, which allowed for use of a single amplification apparatus. Amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Genotyping of *fukutin* gene SNPs

The *fukutin* R>Q203 SNP was genotyped by direct sequencing of genomic DNA using the primers F5′-GTTGTT TCTTGATGTTTGATGCTT-3′ and R5′-CACTGCAAG AATTATGACTGAACA-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-min extension step at 72°C.

The *fukutin* 1137C>A SNP was genotyped by direct sequencing of genomic DNA with the primers F5′-ACAGC CTGGGCAATATAGTGAG-3′ and R5′-TATGAAATG AAGCCGACTTTTG-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-min extension step at 72°C. The resulting fragment was 300 bp in length. Digestion of the 1137A allele with *Bsr*BI produced a single fragment of 300 bp, whereas digestion of the 1137C allele produced two fragments of 252 and 48 bp. These fragments were resolved after electrophoresis in 2% agarose gels.

The *fukutin* T>A433 SNP was genotyped by direct sequencing of genomic DNA with the primers F5′-CACTGT TGAAGCCTAATCCCTC-3′ and R5′-TGGTTCCCA CTTATGTTTGACA-3′. The amplification conditions were 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Statistical analysis

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

Results

Identification of SNPs

Genomic DNA sequencing experiments in three BSCL subjects and 20 normal controls uncovered three relatively common SNPs, designated R>Q203, 1137C>A, and T>A433. No other sequence variants were found in any study sample.

SNP frequencies

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

Discussion

We report the definition of primer sets to amplify the coding sequences of the *fukutin* gene and the use of these amplification primers for genomic DNA sequencing, which

Table 2. *Fukutin* gene SNP allele frequencies

SNP name	Location	Sample size	Allele frequency	Detection method
R>Q203	Exon 5	20	Q203: 0.45	Direct sequencing
1137C>A	Exon 8	140	1137A: 0.36	*Bsr*BI digestion
T>A433	Exon 10	32	A433: 0.031	Direct sequencing

SNP, Single nucleotide polymorphism

has resulted in the identification of three SNPs. The R>Q203 SNP has been recently reported by another group (Kobayashi et al. 2001). In addition, we have ruled out the presence of coding sequence mutations in the *fukutin* gene among three subjects with BSCL. *Fukutin* was a candidate gene for BSCL because of its position and because another muscular dystrophy gene also causes lipodystrophy. However, *fukutin* is not likely to be causative for BSCL. We did not examine the *fukutin* promoter, and so we cannot absolutely rule out such mutations in the BSCL 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. The reagents described in this report could be helpful to screen the *fukutin* gene when it becomes a candidate for other phenotypes, through positional cloning, analogy with other phenotypes, or expression data showing changes in response to particular experimental interventions. The SNPs could also be helpful in linkage mapping and for association studies.

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