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SHORT COMMUNICATION

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Single-nucleotide polymorphisms of the nuclear lamina proteome

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Abstract Familial partial lipodystrophy (FPLD) has been shown to be due to mutations in the *LMNA* gene encoding nuclear lamins A and C, indicating that defective structure of the nuclear envelope can produce this unique phenotype. Some patients with inherited partial lipodystrophy have normal *LMNA* coding, promoter, and 3′-untranslated region sequences. This suggests that the FPLD phenotype is genetically heterogeneous. Among the candidate genes to consider for the non-*LMNA*-associated forms of FPLD are other components of the inner nuclear membrane, such as lamin B1 and B2 and the lamin B receptor. We developed amplification primers for the coding regions of *LMNB1*, *LMNB2*, and *LBR*, which encode lamin B1, lamin B2, and the lamin B receptor, respectively. We found no putative disease mutations in any of these proteins in subjects with non-*LMNA* FPLD, but, through the screening of diseased and normal subjects, we identified several single-nucleotide polymorphisms (SNPs); specifically, five SNPs in *LMNB1* and four SNPs in *LBR*. The *LMNB2* gene was monomorphic in screening experiments. We conclude that mutations in other constituent proteins of the nuclear envelope are not present in subjects with non-*LMNA*-associated FPLD. However, the identification of amplification primers and SNPs provides tools to investigate these proteins for their association with other phenotypes.

Key words Nuclear envelope · Genomic DNA · Sequencing · Complex traits · Lipodistrophy · Diabetes · Muscular dystrophy

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Introduction

The nuclear envelope is composed of nuclear membranes, pore complexes, and the nuclear lamina (Morris and Manlial 1999). In dividing cells, the orderly dissolution and reconstitution of the nuclear envelope are essential for the perpetuation of genomic information (Morris and Manlial 1999). In non-dividing cells, components of the nuclear envelope mediate bidirectional molecular flow between cytoplasm and nucleus. However, appreciation of the complexity of nuclear envelope function has intensified after recent demonstrations that 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 diseases have so far been shown to result from mutations in *LMNB1*, *LMNB2*, or *LBR*, which encode the nuclear envelope proteins lamin B1, lamin B2, and the lamin B receptor, respectively. Furthermore, there is no information regarding common variants in these genes. *LMNB1* has been mapped to human chromosome 5q (Wydner et al. 1996), spans around 43 kb, consists of 11 exons (Lin and Worman 1995), and has conserved organization compared with other intermediate filament protein genes (Justice et al. 1992; Maeno et al. 1995). *LMNB2* has been mapped to 19p13.3, spans around 13 kb; consists of 3 exons (Giacca et al. 1994), and its product is expressed ubiquitously (Triboli et al. 1987). *LBR* has been mapped to 1q42.1, spans around 35 kb, has 13 exons, and its product is expressed widely in differentiated cells (Schuler et al. 1994). We developed a series of genomic DNA amplification primers for the coding sequences of *LMNB1*, *LMNB2* and *LBR*, in order to screen for nucleotide variants in these genes. In screening experiments, we identified five

single-nucleotide polymorphisms (SNPs) in *LMNB1* and four in *LBR*, but none in *LMNB2*. These SNPs can serve as markers for genetic linkage or association studies, and the amplification primers can facilitate mutation screening for other diseases.

Methods

Study subjects

Samples from five subjects with typical FPLD, determined by clinical and biochemical criteria — specifically, absence of fat on arms and legs, increased central fat stores with fat neck and face, and hyperinsulinemia — were evaluated. In addition, samples from a DNA archive of 22 clinically normal Caucasian subjects were screened to determine SNP allele frequencies. For SNPs that altered restriction sites, an expanded set of 140 clinically normal Caucasian subjects was studied to determine allele frequencies. The study subjects gave informed consent, and the study was approved by the Ethics Review Panel, University of Western Ontario.

Screening *LMNB1*, *LMNB2*, and *LBR* genes for DNA variants

In order to amplify coding regions and intron-exon boundaries from genomic DNA, a primer set was developed, using GeneBank sequences (see Table 1). Primer sequences were derived using GeneBank accession numbers L37737 through L37747 (for *LMNB1*), M94363 and M94362 (for *LMNB2*), and L25932 through L25941 and NM002296 (for *LBR*). Primer sequences are shown in Table 1. For each gene, primers were designed to anneal at a single temperature, which allowed for the use of a single amplification apparatus. Amplification conditions were: 94°C for 5 min, followed by 30 cycles comprised of 30 s each at 94°C, the specific annealing temperature, and 72°C, ending with a single 10-min extension step at 72°C. The specific annealing temperatures were 57°C, 60°C, and 58°C for all amplification reactions for *LMNB1*, *LMNB2*, and *LBR*, respectively.

Genotyping of coding SNPs that affected naturally occurring restriction sites

The *LMNB1* A501V SNP was genotyped using the primers F5′-ATCAAGTGTTTGAGTCTCATCGC-3′ and R5′-TCTTGATTATACTTGCACAGTGTTCTT-3′. The amplification conditions were: 94°C for 5 min, followed by 30 cycles comprised of 30 s each at 94°C, 57°C, and 72°C, ending with a single 10-min extension step at 72°C. The resulting fragment was 280 base pairs in length. Digestion of the V501 allele with *Bbv*I produced a single fragment of 280 base pairs, whereas digestion of the A501 allele produced two fragments, with lengths of 193 and 87 base pairs. These fragments were resolved after electrophoresis in 2% agarose gels.

The *LBR* 336C>T SNP was genotyped using the primers F5′-TAAAGCTCTTTGCCTTCTTCCC-3′ and R5′-ACATAAAGCGGAAGACAAAAGG-3′. The amplification conditions were: 94°C for 5 min, followed by 30 cycles comprised of 30 s each at 94°C, 57°C, and 72°C, ending with a single 10-min extension step at 72°C. The resulting fragment was 278 base pairs in length. Digestion of the 336T allele with *Msp*I produced a single fragment of 278 base pairs, whereas digestion of the 336C allele produced two fragments, with lengths of 152 and 126 base pairs. These fragments were resolved after electrophoresis in 2% agarose gels.

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 sequences, except for two SNPs assayed using restriction digestion. We used χ^2 analysis to test 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 five non-*LMNA*-associated FPLD subjects and 22 normal controls uncovered five relatively common SNPs in *LMNB1*, designated 414T>C, 852T>C, A501V, 1779C>T, and 1804C>T, of which only *LMNB1* A501V affected the coding sequence. No genomic sequence variants were detected in *LMNB2*. Four relatively common SNPs were detected in *LBR*, designated 165C>T, 192A>G, 336C>T, and N154S, of which only *LBR* N154S affected the coding sequence. 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: (1) the definition of primer sets to amplify the coding sequences of *LMNB1*, *LMNB2*, and *LBR* and (2) the use of these amplification primers for genomic DNA

Table 1. Amplification of nuclear envelope proteins

Gene	Exon(s)	Primer sequence	Product size
LMNB1	Exon 1	F 5′-GTTTGTGCCTTCGGTCCC-3′ R 5′-CCCCTCCTTGGCTAACGC-3′	491 bp
	Exon 2	F 5′-GAAGACCTCAAGTCATCAGTATGG-3′ R 5′-CTATGGCTTAAGGCAGATGAGG-3′	279 bp
	Exon 3	F 5′-TTCATTATTAAATGATTGCCATGT-3′ R 5′-CATGTTTAGCTATGACAAAGTCATTC-3′	254 bp
	Exon 4	F 5′-CCATGGTAAGTAGCTTGGCTTT-3′ R 5′-ATGTTCATCATGGGAATTGTGA-3′	283 bp
	Exon 5	F 5′-AGCTTGAGAATGCCAGACTGT-3′ R 5′-GAGGAGAGTGACATCAGCCC-3′	220 bp
	Exon 6	F 5′-GAGATCATAGGTGTACTGTTTCCA-3′ R 5′-TAAAAAGGGTGAGAGGCAAAAA-3′	381 bp
	Exon 7	F 5′-CGAGAAGGGCATATGTGTTTTA-3′ R 5′-GGTCATTTATACCTGTTCAGAAGTG-3′	290 bp
	Exon 8	F 5′-ATCTTTGAAGTAACACCCCTC-3′ R 5′-AACGGCCATTTCTAAAATGCTT-3′	250 bp
	Exon 9	F 5′-ATCAAGTGTTTGAGTCTCATCGC-3′ R 5′-TCTTGATTATACTTGCACAGTGTTCTT-3′	280 bp
	Exon 10	F 5′-TGGCAAGAAATGCTGTCTCTTA-3′ R 5′-CAACCACACTCTTGGTCTTCAT-3′	282 bp
	Exon 11	F 5′-GGTATCCCTGTAGTTAAAAGTTTTTGT-3′ R 5′-CATCTTGCAACAAAGTGTATGGA-3′	334 bp
LMNB2	Exon 2	F 5′-CAGTGCCGCTGAAGATCG-3′ R 3′-GCTCTCCTCCTCGCCCTC-3′	220 bp
	Exon 3	F 5′-CTGAAGCTGTCCCCCAGC-3′ R 5′-GGATGTACTTGGGCGTGAAC-3′	361 bp
	Exons 4 and 5	F 5′-GTCCCTGGAACCAGAGGTGA-3′ R 5′-CATGTGTATGTGTGTGCACGAG-3′	483 bp
	Exon 6	F 5′ TAGACACTGTTGTTGCTCAGCC-3′ R 5′-TTTGACCAAATGGTGAGATGAG-3′	406 bp
LBR	Exon 1	F 5′-GATTCAAAAGGTCGAGGAGC-3′ R 5′-GGGTGGAAGCACTCACCTG-3′	250 bp
	Exon 2	F 5′-TAGTGATCAGCCTGTGGAAAAA-3′ R 5′-AACCGAGCTGAACTGACTATCC-3′	355 bp
	Exon 3	F 5′-TAAAGCTCTTTGCCTTCTTCCC-3′ R 5′-ACATAAAGCGGAAGACAAAAGG-3′	278 bp
	Exon 4 and 5	F 5′-TTGCTTGATTCCAAAACAGTAA-3′ R 5′-CAGTTCACCTTGTGGCAACC-3′	698 bp
	Exon 6	F 5′-AAATAAACCAGATTGAAATTTGACC-3′ R 5′-CAAAACAGAATTACCATAAACGTACC-3′	244 bp
	Exon 7	F 5′-TGTATATATGATGGTGATGTGCTTAG-3′ R 5′-ACTCTGGTCTAATCAGCTTTAACAAA-3′	202 bp
	Exon 8	F 5′-TCATGTGAGCCTTGGGTTAATA-3′ R 5′-ATTCAAATCTGGAAATGGCTGT-3′	322 bp
	Exon 9	F 5′-GTAAAGCCTCCTGACTGGGTTT-3′ R 5′-CCCACTGCTTATTTTTGAATGG-3′	254 bp
	Exon 10	F 5′-TAGTGCTAAGGTCTCTGGGGAG-3′ R 5′-CCCTCACTTTGTGTGCAAGA-3′	280 bp
	Exon 11	F 5′-ACAGAAGCTCCTTCTCCCTTTC-3′ R 5′-TCAAAATGGCATGTTTCAAGTA-3′	306 bp
	Exon 12 and 13	F 5′-TCATTTTTAAGAGAAATTACAGATGC-3′ R 5′-CACACAAAGGACACACACACAC-3′	460 bp
	Exon 14	F 5′-GAAAATGAAGTACCTTGTGGTTCC-3′ R 5′-TTTCCTTGTTTTTGCAAATGG-3′	338 bp

Table 2. Nuclear envelope protein SNP allele frequencies

Gene	SNP name	Sample size	Allele frequency	Detection method
LMNB1	414T>C	22	414C: 0.091	Direct sequencing
	852T>C	22	852C: 0.045	Direct sequencing
	A501V	140	V501: 0.014	*Bbv*I digestion
	1779C>T	22	1779T: 0.091	Direct sequencing
	1804C>T	22	1804T: 0.409	Direct sequencing
LBR	165C>T	22	165T: 0.045	Direct sequencing
	192A>G	22	192G: 0.182	Direct sequencing
	336C>T	140	336T: 0.240	*Msp*I digestion
	N154S	22	S154: 0.227	Direct sequencing

SNP, Single-nucleotide polymorphism

sequencing, which has resulted in: (3) the identification of five novel SNPs in *LMNB1* and four novel SNPs in *LBR*, but none in *LMNB2*. In addition, we have ruled out the presence of coding sequence mutations in these genes in five subjects with FPLD whose *LMNA* sequence was normal. This latter finding emphasizes the extremely high specificity of the relationship between particular nuclear envelope mutations in *LMNA* and the disease phenotype (Hegele 2000).

The findings indicate that, while the nuclear lamin proteins might be good candidate genes for non-*LMNA*-associated FPLD, they are not mutated in these patients. The five subjects were each singleton cases from small families, and, thus, linkage analysis could not be performed to determine whether there was linkage to the region on chromosome 1q21 that harbors *LMNA*, or to any other genomic region. Genomic DNA from these subjects will continue to be sequenced as new candidate genes are identified and prioritized for possible involvement in this phenotype.

We did not examine the promoter regions of these candidate genes, and so we cannot absolutely rule out such mutations in the non-*LMNA*-associated FPLD subjects. However, *LMNA* mutations in FPLD are predominantly missense mutations that affect amino acids near the carboxy terminus. This suggests that some exposed structure of the mature laminar protein is defective in FPLD, possibly affecting several intracellular functions. Thus, at least for the first set of screening experiments, it could be argued that restricting the search for nuclear envelope mutations to those that could affect protein structure was a reasonable strategy, and that such mutations were absent in *LMNB1*, *LMNB2*, and *LBR* in the five FPLD subjects without a *LMNA* mutation.

At present, no mechanistic data can explain the role of mutant nuclear laminar proteins in disease. Nuclear lamins belong to the intermediate filament multigene family, and participate in DNA replication, organization, nuclear pore arrangement, nuclear growth, and membrane anchorage (Stuurman et al. 1998). In terminally differentiated cells, the *LMNA* gene products, lamins A and C, polymerize to form part of the nuclear lamina, a lattice of 10-nm filaments lining the inner nuclear envelope (Fisher et al. 1986). Structural mutations in nuclear lamin proteins could: (a) destabilize the nuclear lamina or (b) impair interactions with chromatin, nuclear proteins, transcription factors, or other cellular proteins, affecting differentiation and/or apoptosis. Differential tissue expression might alter the impact of mutant nuclear lamins (Hegele 2000).

The reagents described in this report could be helpful for screening the *LMNB1*, *LMNB2*, and *LBR* genes when these become candidates for other phenotypes, through positional cloning, analogy with other phenotypes, or expression data showing changes in response to particular experimental interventions. The SNPs in *LMNB1* and *LBR* could also be helpful in linkage mapping, and for association studies.

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