A Novel Mutation in a Large French-Canadian Family with LGMD1B

Nicolas Chrestian, Paul N. Valdmanis, Najmeddine Echahidi, Denis Brunet, Jean-Pierre Bouchard, Peter Gould, Guy A. Rouleau, Jean Champagne, Nicolas Dupré

ABSTRACT: Background: Limb girdle muscular dystrophy type 1B is an autosomal dominant disease characterized by late onset proximal muscle involvement associated with cardiac complications such as atrioventricular conduction blocks, dilated cardiomyopathy, and sudden death. Objective: Define the full phenotypic spectrum of a new mutation in the LMNA gene causing limb girdle muscular dystrophy type 1B. Methods: We identified a large French Canadian family with the LGMD 1B phenotype and a cardiac conduction disease phenotype that carried a new mutation in the LMNA gene and sought to define its full phenotypic spectrum by performing complete neurological and cardiac evaluations, muscle biopsy, RNA and DNA studies. Results: The proband and 12 living at risk relatives were tested. In total, we identified seven carriers of a new (IVS9-3C>G) LMNA gene mutation. Of the three symptomatic patients, all had cardiac involvement, but only two presented proximal limb weakness. The one available muscle biopsy demonstrated a normally expressed lamin A/C protein, localized at the nuclear envelope. RNA study revealed a loss of exon 10 transcription caused by the IVS9-3C to G splicing mutation. Conclusions: We have identified a new mutations in the LMNA gene in a French-Canadian family. This diagnosis has important implications for affected patients and their siblings since they may eventually require pacemaker implantation.

RÉSUMÉ: Une nouvelle mutation dans une grande famille canadienne-française atteinte de LGMD 1B. Contexte : La dystrophie musculaire des ceintures type 1 B est une maladie autosomique dominante caractérisée par un début tardif, une atteinte des muscles proximaux associée à des complications cardiaques comme des blocs de conduction auriculo-ventriculaires, une cardiomyopathie congestive et une mort subite. Objectif : Le but de cette étude était de définir l’expression phénotypique d’une nouvelle mutation du gène LMNA qui cause la dystrophie musculaire des ceintures de type 1B. Méthodes : Nous avons identifié une grande famille canadienne-française présentant le phénomène LGMD 1B accompagné d’un trouble de conduction cardiaque, qui était porteuse d’une nouvelle mutation du gène LMNA. Nous définissons le spectre de ses variations phénotypiques dans cette famille au moyen d’une évaluation neurologique et cardiaque complète, d’une biopsie musculaire et d’études de l’ARN et de l’ADN. Résultats : Le cas index et 12 apparentés à risque ont été évalués. En tout, nous avons identifié sept porteurs d’une nouvelle mutation du gène LMNA (IVS9-3C>G). Les trois patients qui présentaient des symptômes avaient tous une atteinte cardiaque, mais seulement deux présentaient une faiblesse proximale. La seule biopsie musculaire disponible a montré une expression normale de la lamine A/C localisée à l’enveloppe nucléaire. L’étude de l’ARN a montré une perte de transcription de l’exon 10 causée par une mutation d’épissage IVS9-3C à G. Conclusions : Ce diagnostic a des implications importantes pour les patients atteints et leur fratrie parce qu’ils peuvent éventuellement avoir besoin d’un stimulateur cardiaque.


From the Faculty of Medicine, Laval University (NC, DB, JPB, PG, ND), Department of Neurological Sciences, CHAUQ - Enfant-Jésus, Quebec City; Faculty of Medicine, Laval University (NE, JC), Institut Universitaire de cardiologie et pneumologie de l’Université Laval, Laval Hospital, Quebec City; Centre d’excellence en neuroimagerie de l’Université de Montréal (PNV, GAR, ND), CHUM Research Center - Notre-Dame Hospital, J.A. de Sève Pavilion, Montreal, QC, Canada.

Reprint requests to: Nicolas Dupré, CHAUQ - Enfant-Jésus, 1401, 18th street, Quebec City, QC, G1J 1Z4, Canada.
PATIENTS AND METHODS

We identified a proband (III:2) with the LGMD 1B phenotype that carried a new mutation in the \textit{LMNA} gene (Figure 1). All 12 living at risk relatives of the proband were evaluated clinically by a neurologist (ND, DB, JPB) and a cardiologist (JC, NE), and underwent basic work-up (echocardiogram, electrocardiogram, Holter monitoring). Neuromuscular workup was limited since subjects presented mostly with cardiac symptoms, and did not show signs of peripheral neuropathy. Muscle biopsy of a deceased family member (III:5) was studied using immunohistochemistry, and was not repeated on other subjects in view of its lack of diagnostic value. Genetic testing was performed on the proband and all 12 living at risk relatives to evaluate their carrier status. All 12 coding exons of the \textit{LMNA} gene were polymerase chain reaction (PCR) amplified and cycle sequenced on both forward and reverse directions from genomic deoxyribonucleic acid (DNA) isolated from blood by standard methods. A blood sample from the proband was also obtained for ribose nucleic acid (RNA) study. Ribose nucleic acid was extracted from immortalized patient lymphoblast cell lines using TRIZOL total RNA isolation agent (Invitrogen). A reverse-transcription reaction was performed to obtain cDNA. The \textit{LMNA} transcript was amplified by PCR using a forward primer from exon 9 (5’- TGTCCGGCTGCAGCAAAAGTC) and a reverse primer in exon 12 (5’- CCGACCGGTCCCGCACAG). Polymerase chain reaction conditions were as follows: an initial denaturation step at 94°C for 5 minutes followed by 30 cycles of denaturation (94°C for 30 seconds), annealing (55°C for 30 seconds) and extension (72°C for 45 seconds), concluding with a final extension step for 7 minutes at 72°C. Polymerase chain reaction products were run on a 1.5% agarose gel and their size was identified by comparison to a 1 Kb DNA ladder.

RESULTS

A total of seven carriers of the (IVS9-3C>G) \textit{LMNA} gene mutation were identified (Table). This mutation was not found in 100 ethnically matched normal controls. The index case (III:2) presented hip flexor weakness (MRC 4.5/5) at age 34. When she was assessed at 65 years of age, she had significant atrophy of hip-girdle muscles with increased hip flexor weakness (MRC 3.5/5). Her serum creatine kinase was normal when assessed then. She was able to walk for short distances with a cane. She was incidentally diagnosed with a type 1 AVB at age 26 during her first pregnancy. Then, at age 40, she developed a complete symptomatic AVB leading to pacemaker implantation. Her brother (III:3) presented with symptomatic dysrhythmias and dilated cardiomyopathy. He had a syncope caused by paroxysmal bradyarrhythmia at age 30. He had a pacemaker and defibrillator implanted for paroxysmal AVB at age 42 and showed hip flexor weakness (MRC 4.5/5) that had begun at age 37. Another

The one available muscle biopsy from a deceased subject (III-5) demonstrated a normally expressed lamin A/C protein localized at the nuclear envelope (Figure 2). The cDNA sequencing on the proband (III:2) revealed a 319 bp product lacking LMNA exon 10 along with a normal product of 409 bp (Figure 3). This loss of 90 base pairs of sequence results in an in-frame deletion of 30 amino acids. The RNA study therefore revealed a loss of exon 10 transcription caused by the IVS9-3C to G splicing mutation (Figure 4). The consensus sequence before an exon is critical for determining whether an exon is included in the mRNA product. In 70% of cases, a cytosine residue is at position -3, while a guanidine is present only 0.3% of the time. Thus, splice assembly factors likely are unable to recognize the start of LMNA exon 10, and skips directly to exon 11, as we observe in heterozygous form.

**DISCUSSION**

Lamins are nuclear intermediate filament proteins consisting of a central alpha-helical rod domain flanked by a short N-terminal and by C-terminal Ig-like globular domains, which form dimers assembling in a head to tail fashion. Lamins are encoded by three genes: LMNA, which encodes lamin A and C by alternative splicing; LMNB1 and LMNB2, which encode lamin B. Alterations in the LMNA gene have been associated with a heterogeneous series of human disorders denominated laminopathies. These disorders display striking clinical variability: autosomal Emery-Dreifuss muscular dystrophy, LGMD1B, dilated cardiomyopathy, isolated cardiac conduction defects, Charcot-Marie-Tooth neuropathy type 2B, or partial lipodystrophy syndromes with or without developmental abnormalities / premature aging.

Thus far, only four splicing mutations of the LMNA gene have been reported to give a LGMD1B phenotype and only two mutations are localized on intron 9 (http://www.hgmd.cf.ac.uk). The IVS9-3C>G LMNA splicing mutation reported herein is thus

![Figure 4: Schematic of the position of the mutation and the subsequent effect on LMNA splicing.](image-url)
the fifth splicing mutation to be identified. By studying the cDNA of an affected patient, we were able to show that exon 10 was not transcribed. This did not, however, alter protein expression since the resulting protein product was shown to be expressed at the nuclear envelope on a muscle biopsy specimen, suggesting that the normal allele can localize to the nuclear envelope. Since this mutation was identified in a large French-Canadian family, we were able to show its full phenotypic spectrum that indicated clear cardiac predilection that is eventually associated with mild to moderate hip-girdle weakness. In the family described above, early cardiac symptoms generally lead to pacemaker implantation in middle age. Cardiac symptoms seemed to aggravate beginning with AVB type 1, then progressing to AVB type 2 or 3. Pacemaker-defibrillator was eventually implanted for primary prevention in patients presenting significant arrhythmias, following current recommendations in the literature. Although a founder effect was not demonstrated in the present study, we have recently identified a second family (personal communication, ND) from the same region (Chaudière-Appalaches, PQ, Canada) that bears the same mutation, prompting us to advise clinicians to perform cardiac evaluation and careful family study of patients from that area presenting with a LGMD phenotype.

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