Mutations in the Caveolin-3 Gene: When Are They Pathogenic?

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Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of genetic disorders usually with autosomal recessive (AR) inheritance and, less often, displaying autosomal dominant (AD) inheritance. Mutations in the caveolin-3 gene (CAV-3) associated with a reduction of protein expression cause AD-LGMD1C muscular dystrophy. Based on a previous study in the American and Brazilian population, it has been suggested that CAV-3 mutations might also cause AR-LGMD. Here we report the analysis of the CAV-3 gene in 61 additional Brazilian LGMD patients and 100 additional Brazilian normal controls. Two rare G55S and C71W missense changes previously detected only in LGMD patients (and not detected in 100 normal controls from the American population) were now found in normal Brazilian controls. In addition, we have identified a novel R125H missense change in one LGMD female patient that was also found in two of her unaffected siblings. These observations, together with the normal immunofluorescence caveolin pattern in the muscle biopsy from two patients with the G55W and R125H changes in the CAV-3 gene suggest that the G55S, C71W, and R125H polymorphisms, on their own, are not sufficient to produce the pathology.

KEY WORDS: caveolin-3 mutations; limb-girdle muscular dystrophy

INTRODUCTION

Limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of genetic disorders in which there is a progressive weakness of the pelvic and shoulder girdle musculature. The clinical course is characterized by a great variability [Passos-Bueno et al., 1999], ranging from severe forms with onset in the first decade and rapid progression, resembling clinically Xp21 Duchenne dystrophy (DMD), to milder forms with later onset and a slower course.

Nine autosomal recessive (AR) and six autosomal dominant (AD) genes for LGMD have already been mapped. The AR-LGMDs are LGMD2A at 15q or calpainopathy [Beckmann et al., 1991], LGMD2B at 2p or dysferlinopathy [Bashir et al., 1994], LGMD2C at 13q or γ-sarcoglycanopathy [Nogush et al., 1995], LGMD2D at 17q or α-sarcoglycanopathy [Roberds et al., 1994], LGMD2E at 4q or β-sarcoglycanopathy [Lim et al., 1995; Bonnemam et al., 1995], LGMD2F at 5q or δ-sarcoglycanopathy [Passos-Bueno et al., 1996], LGMD2G at 17q or telothelinopathy [Moreira et al., 2000], LGMD2H at 9q [Weiler et al., 1998], and LGMD2I at 19q [Driss et al., 2000]. The LGMD 2H and LGMD 2I products are still unknown.

The AD-LGMD genes are LGMD1A at 5q22 [Speer et al., 1992], LGMD1B at 1q11-21 [van der Kooi et al., 1997], LGMD1C at 3p25 [McNally et al., 1998; Minetti et al., 1998], LGMD1D at 6q23 [Messina et al., 1997], LGMD1E at 5q31 [Feit et al., 1998], and LGMD1F at 7q [Speer et al., 1999].

Mutations in the gene coding for caveolin-3 (CAV-3), a muscle-specific form of the caveolin family causing a reduction of more than 95% in protein expression, were first reported in two Italian families with AD-LGMD. One of them has a missense mutation (a C→T transition resulting in a Pro→Leu substitution at

Grant sponsor: Fundação de Amparo a Pesquisa do Estado de São Paulo, Centro de Pesquisa, Inovaçõe Divulgação (FAPESP-CEPID); Grant sponsor: Programa de Apoio a Núcleos de Excelência (PRONEX); Grant sponsor: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Grant sponsor: International Atomic Energy Agency (IAEA); Grant sponsor: NIH; Grant sponsor: MDA.

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Received 25 May 2000; Accepted 18 December 2000
Published online 15 February 2001

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amino acid position 104), while the other one had a 9-pb deletion, resulting in the loss of three amino acid residues at positions 63–65, without changing the open reading frame. This form of muscular dystrophy was classified as LGMD1C [Minetti et al., 1998].

More recently, two other pathogenic heterozygous missense mutations (A46T and R26Q) were reported in three unrelated patients in two independent studies [Carbone et al., 2000; Herrmann et al., 2000, respectively].

Caveolae are small membrane invaginations on the surface of cells that participate in membrane trafficking, sorting, transport, and signal transduction [Anderson, 1993; Lisanti et al., 1995]. Cav-3, or M-caveolin, a small molecular-weight protein localized to the sarcolemma, mapped at 3p25 [McNally et al., 1998], forms a complex with dystrophin and associated glycoproteins [Song et al., 1996; Tang et al., 1996].

The finding of mutations in the CAV-3 gene in two isolated female patients led to the suggestion that they might also cause AR-LGMD. One female patient was found to have a homozygous missense G55S change. The other C71W missense substitution was identified in only one allele in an 11-year-old Brazilian patient. The immunofluorescence staining for Cav-3 in the muscle from the first patient was apparently normal. However, these two missense mutations were not seen in a control population of 200 chromosomes from normal American individuals and 100 chromosomes from muscular dystrophy patients with known mutations, suggesting, therefore, that they might represent pathogenic mutations [McNally et al., 1998].

We have analyzed 61 additional Brazilian LGMD patients and 100 normal controls for the CAV-3 gene in an attempt to: (1) estimate the frequency of mutations in the CAV-3 gene among still unclassified Brazilian LGMD patients, as compared to the normal control population; and (2) better characterize the effect of CAV-3 changes in the pathogenesis of LGMD.

PATIENTS AND METHODS

A total of 61 unrelated affected patients (38 males and 23 females) were included in the present investigation. All studies were performed following patients’ informed consent. The diagnosis of LGMD was based on clinical and neurological examination, family history, muscle biopsy studies, elevated serum creatine-kinase, electromyography, and DNA analysis.

Patients were classified as LGMD according to the criteria reported in Bushby and Beckmann [1995] and included 45 isolated cases (15 females and 30 males), 3 belonging to families with AR inheritance (2 males and 1 female), and 12 patients (6 males and 6 females) with other affected relatives (6 with AD inheritance and the remaining with an atypical pattern of inheritance). In familial cases, only one patient from each genealogy was considered.

DNA was analyzed through single-strand conformation polymorphism (SSCP) and sequencing of abnormal migrating fragments (in a 377 ABI sequencer), as previously reported [McNally et al., 1998].

In patients from which it was possible to have muscle biopsies, protein analysis allowed the exclusion of dystrophinopathies in 36 patients and sarcoglycanopathies, calpainopathies, and dysferlinopathies in 24 of the 36 patients.

The caveolin-3 immunofluorescence analysis was done using the double labeling methodology [Vainzof et al., 1991], utilizing a rabbit polyclonal antibody for the N-terminal domain of dystrophin and the monoclonal anti-CAV3 antibody, raised against a synthetic peptide containing amino acids 3–24 (Transduction Laboratories).

Additional antibodies for sarcoglycans [Vainzof et al., 1996], calpain [Anderson et al., 1998], dysferlin [Anderson et al., 1999], and telethonin [Moreira et al., 2000] were also used. Western blot methodology was done as described in Ho-Kim et al. [1991].

RESULTS

DNA analysis revealed in three isolated unrelated affected patients the following heterozygous missense changes: G55S in two affected males and a novel R125H change in a female patient, all with a mild course, described below.

Patient 1, with the G55S change, is currently 49 years old. Pedigree analysis revealed that his parents (both deceased) are not consanguineous. He has nine normal siblings (six sisters and three brothers). This patient has a proximal weakness, which started at age 22, affecting both upper and lower limbs. Presently, he can walk only with support. He has very enlarged calves. His serum CK is increased 16-fold above normal.

Patient 2, with the same G55S change, is also currently 49 years old. Pedigree analysis revealed that his parents are distant cousins. He has four normal siblings (three brothers and one sister). This patient is much less affected than patient 1. He has calf hypertrophy but weakness mainly in the lower limbs. Since age 39, he needs help with standing up, but he can walk unassisted both on his toes and heels. His serum CK is about ninefold above normal.

Patient 3, with the novel R125H change, is currently 35 years old. Her parents are first-degree cousins and she has five normal siblings. She refers onset when she was a teenager, with a more rapid progression after she was 18 years old. She has proximal weakness and very enlarged calves. Her serum CK is increased 11-fold above normal. Since her father is already deceased, DNA analysis was performed in her mother and all of her unaffected sibs. The same R125H change was also observed in two of her unaffected sibs (one female aged 26 and one male aged 38). Both have normal serum CK and both are very strong and athletic. The proband’s mother does not carry this mutation, suggesting that it was inherited from her deceased father (who died at age 54 of heart failure but referred to have no muscular weakness).

Screening of 200 normal chromosomes (from 100 normal controls belonging to the same ethnic group as the patients) revealed in five unrelated subjects the following missense polymorphisms, all of them in only
one of the alleles: a C71W change in one subject and a G55S change in four other controls. The R125H change was not detected in this control group.

In addition, a silent (GTG→GTA) polymorphism (V56V) was observed in one patient and in one normal control.

Caveolin-3 immunostaining in muscles from the patients with the G55S and R125H substitutions showed a strong positive sarcolemmal labeling pattern that did not differ from the normal control, suggesting normal localization of the protein (Fig. 1). Additional analysis of muscle protein in these two patients revealed a normal staining for dystrophin, α,β,γ- and δ-sarcoglycan, and telethonin and normal molecular-weight bands on Western blot for calpain and dysferlin (not shown).

**DISCUSSION**

In our previous study, the C71W and the G55S changes identified in two LGMD isolated affected patients were not found either in 100 American normal controls or in 50 American patients with other forms of LGMD, suggesting that they might cause AR-LGMD [McNally et al., 1998]. However, the analysis of a new set of normal Brazilian controls, reported in the present study, revealed the presence of the C71W and the G55S changes in the normal population. This observation suggests that although they represent rare polymorphisms (with an estimated frequency of 0.5%, or 1 among 200 normal chromosomes for C71W, and 2%, or 4 in 200 for G55S in the Brazilian population), it is unlikely that they are causing the abnormal phenotype when present in just one allele.

The novel R125H substitution, identified in one patient and her two unaffected siblings, was apparently not reported before and was not found in 200 normal chromosomes from the control population. However, the fact that the two siblings of the propositus who carry this change are very strong and athletic suggests that this rare R125H polymorphism is apparently not causing the abnormal phenotype, by its own, when present in only one allele. This conclusion is reinforced by the normal IF CAV-3 pattern observed in muscle biopsy from patients with the G55S, as well as the R125H, polymorphism (Fig. 1), as opposed to the abnormal protein findings reported in the patients from Minetti et al. [1998], Carbone et al. [2000], and Herrmann et al. [2000].

On the other hand, a normal immunostaining was observed for patients 2 and 3 for all known proteins codified by AR-LGMD. This result suggests that the abnormal phenotype observed in these patients might be caused by some still unknown LGMD gene [Zatz et al., 2000]. However, it does not allow a definite exclusion of all forms of AR-LGMD, since almost normal calpain bands were reported in some patients with primary calpainopathy [Anderson et al., 1998] and very slight SG protein reduction was also described in sporadic LGMD2D patients [Vainzof et al., 2000].

It has been suggested that the LGMD1C mutations of caveolin-3 behave in a dominant-negative fashion [Galbiati et al., 1999]. According to previous studies [Garcia-Gardena et al., 1997; Venema et al., 1997], the R125H polymorphism may fall in a region that interacts with neuronal nitric-oxid synthase (nNOS). Therefore, it is interesting to observe that this change, on its own, is associated with a normal phenotype.

Although we have sequenced only patients with an abnormal migrating fragment through SSCP, one missense pathogenic mutation reported recently by Herrmann et al. [2000] was also identified through the same method.

The two first reported mutations [McNally et al., 1998; Minetti et al., 1998] were found in AD genealogies. However, the two additional mutations (A46T and R26Q) recently reported in three isolated patients were de novo mutations. These observations reinforce our suggestion that LGMD1C (screened in few AD families but in many isolated cases) is rare in the Brazilian population, moreover because AD-LGMD represents less than 5% of our familial LGMD cases [Zatz et al., 2000].

The possibility that mutations in the CAV-3 gene might cause muscular dystrophy in homozygosity or heterozygosity has been questioned. It has been already demonstrated for other conditions, such as nemaline myopathy [Nowak et al., 1999], oculopharyngeal muscular dystrophy [Brais et al., 1998], and, more recently, Emery-Dreifuss muscular dystrophy [Barletta et al., 2000] that mutations in the same gene might cause AD or AR or even act as a modifier of either a dominant or a recessive mutation.

Our results suggest that the polymorphisms C71W and G55S and the novel change R125H in the CAV-3 gene are unlikely to cause muscular dystrophy when present in one allele. However, the possibility that they can act as recessive mutations or interact with other genes involved in the dystrophic process cannot be ruled out. Caution regarding interpretation of such changes is ever more important in the increasingly complex array of LGMD genotypes and phenotypes.

In summary, the pathogenic effect of different mutations in the CAV-3 gene is still unclear. The present results emphasize the great importance of using appropriate ethnic and healthy sibling control in the identification of polymorphisms. Therefore, screening of patients from different populations, as well as the generation of a mouse model, will be important to better understand the role of caveolin-3 mutations in LGMD.
ACKNOWLEDGMENTS

The collaboration of the following persons is gratefully acknowledged: Dr. Maria Rita Passos-Bueno, Dr. Eloisa de Sá Moreira, Dr. Rita C. M. Pavanello, Dr. Ivo Pavanello, Dr. Suely K. Marne, Antonia Cerequeira, Marta Canovas, and Constancia Urbani. We would also like to thank the constructive comments of the anonymous reviewer. This work was partially supported with a grant from MDA (E.M.). L.M.K. is an investigator from Howard Hughes Medical Institute.

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