Case report

Mutation analysis in the FKRP gene provides an explanation for a rare cause of intrafamilial clinical variability in LGMD2I

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Abstract

We report a limb-girdle muscular dystrophy 2I family with three affected sisters and a highly variable clinical course. FKRP gene sequencing showed that all three sisters carried a nonsense paternal mutation (W225X). The two oldest sisters with a severe phenotype carried two maternal mutations V79M and P89A. However, the youngest sister with a milder course carried the paternal and only the V79M maternal mutation, due to an intragenic recombination.

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1. Introduction

Limb-girdle muscular dystrophies (LGMD) are a heterogeneous group of progressive muscle disorders with a primary or predominant involvement of the shoulder-girdle or pelvic muscles. There are at least 17 different genetically defined subtypes of LGMD, most autosomal recessive (AR), characterized by normal intelligence and great clinical variability [1].

The fukutin-related protein (FKRP) gene is associated to LGMD2I and it is composed of four exons containing a 1488-bp open reading frame that encodes a 495-amino-acid protein [2]. The FKRP is a type II transmembrane protein Golgi-resident thought to be a glycosyltransferase or phosphoryl-ligand transferase [3].

Mutations in this gene cause also congenital muscular dystrophy type 1C (MDC1C), characterized by severe weakness presenting at birth or in the first few weeks of life [2]. LGMD2I has a variable course, ranging from severe Duchenne-like forms with early onset and rapid progression, to mild forms with late onset and mild phenotype or no visible weakness [2,4]. In both disorders, weakness and wasting of shoulder-girdle muscles, primary restrictive respiratory and cardiac involvement has been reported [2]. Understanding the spectrum of severity associated with FKRP mutations remains a great challenge.

Here, we report a family with three affected sisters with a variable course, who were followed in our center for more than 20 years. The two oldest ones had a typical Duchenne-like course while the youngest shows a milder phenotype. Screening of mutations in the FKRP gene as well as linkage analysis revealed that the two more affected sisters carried an additional pathogenic mutation, which was not present in the third one.

2. Subjects and methods

2.1. Subjects

The three sisters were first seen when they were 2, 4 and 6 years old. The parents were not consanguineous
and there were no other cases in the family (Fig. 1A). When first examined, the youngest sister was asymptomatic, but the two older sisters had hypertrophic calves and performed Gower’s maneuver to raise up from the floor. By ages 11 and 12 they were confined to a wheelchair and died of cardiopulmonary failure at ages 14 and 15, respectively. The youngest sister, who is currently 27 years old, has a milder course and was able to walk short distances with support until the age of 24. All three had very high serum CK levels (on average 50-fold above normal) assessed on different occasions.

2.2. Methods

DNA was extracted from whole blood using standard procedures following informed consent. Mutation analysis was performed in the three sisters and both parents, as previously reported, amplifying the entire FKRP coding region of genomic DNA and subsequent sequencing [4]. Linkage analysis was performed using microsatellite markers D19S420, D19S571, D19S201, and D19S902 (Fig. 1B).

3. Results

Sequencing analysis, performed first in the youngest sister (the only one who is still alive), revealed that she is a compound heterozygote for one stop codon (W225X), inherited from the father, and one missense change (V79M), from the mother as previously reported [4]. Unexpectedly a third missense mutation (P89A) was identified in the two oldest sisters (Fig. 2). This additional mutation, which is 30 bp downstream of V79M, was also present in the mother. The P89A is a novel mutation which was not found in 300 normal control chromosomes.

In an attempt to explain the absence of the P89A mutation in the third sister we performed haplotype analysis using four flanking markers. It revealed that the three sisters inherited the same paternal allele with a null mutation and the same maternal allele. However,
the youngest sister received a maternal allele with only one missense mutation due to a recombination event that occurred between the two maternal missense mutations. The markers \textit{D19S571} and \textit{D19S902} showed that the telomeric region of the \textit{FKRP} gene is not shared among the three sisters (Fig. 1B).

4. Discussion

LGMD2I, one of the most prevalent form of AR-LGMD in several European countries, has been associated with a broad clinical variability ranging from severe MDC to milder or even asymptomatic cases [5]. Patients with two missense mutations usually show a milder progression course while compound heterozygotes who carry a null mutation in one allele and a missense in the other one usually have a more severe DMD-like course [6]. We found another Brazilian family with two affected sisters who are compound heterozygotes for the common mutation (\textit{L276I}) and another mutation (\textit{P89L}), in the same codon as the \textit{P89A} mutation. The youngest has an apparently less severe phenotype than the oldest but none have a typical Duchenne-like course. No additional mutation in the \textit{FKRP} gene was found but the \textit{L276I} mutation has been reported to cause clinical variability [6]. Matsumoto et al. [7] reported a Japanese MDC1C patient, who also carries a mutation in codon 89 (\textit{P89R}) in trans with a 2-bp deletion, which causes a premature stop codon, which has been associated to a severe phenotype [3].

The occurrence of two pathogenic mutations in the same allele has been reported previously for other conditions [8], but apparently not for LGMD2I. It might provide an explanation for the more severe course in the two older sisters, possibly due to the presence of a less functional maternal allele than in the youngest one. Although mutations in the \textit{FKRP} gene have been associated with a variable phenotype it is noteworthy that the two sisters who carried two mutations in cis had a very similar severe course. In addition, the observation of an intragenic recombination which was identified in the present family due to the presence of two mutations, which are only 30 bp apart, was unexpected.

Understanding intrafamilial clinical variability in Mendelian disorders has been a great challenge. Different mechanisms such as genetic modifiers [9], different genetic background [10], or epigenetic factors have been proposed. For LGMD2I the pathogenesis appears to be linked to a downregulation of \(\alpha\)-dystroglycan and clinical severity with depletion of \(\alpha\)-dystroglycan and secondary reduction in laminin-\(\alpha_2\) [3]. The occurrence of an intragenic recombination may be an additional...
mechanism to explain such variability in a small subgroup. Although the existence of two pathogenic mutations in the same allele is rare, clinical variability caused by the association or not of a pathogenic mutation and a relatively common polymorphism have been reported for other conditions [9]. These results emphasize the importance of sequencing the entire gene even after the identification of a pathogenic mutation in sibs with discordant phenotypes.

References


