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CASE REPORTS

# Phenotypic variability in siblings with Calpainopathy (LGMD2A)

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**Calpainopathy is an autosomal-recessive limb girdle muscular dystrophy (LGMD2A) characterized by selective atrophy and weakness of proximal limb girdle muscles. The clinical phenotype of the disease is highly variable inter-familial, but little is known about intra-familial variability. This study reports the phenotypic variability in eight sibling pairs with genetically proven LGMD2A. Although siblings with identical mutations were often similarly affected, in some families the age of onset and the clinical course varied considerably.**

**Key words:** Calpainopathy, limb-girdle muscular dystrophy, genotype phenotype correlation, LGMD2A, sibling

## Introduction

Limb-girdle muscular dystrophies (LGMDs) are a clinically and genetically heterogeneous group of disorders characterized by progressive proximal weakness not caused by a primary dystrophin deficiency. Calpainopathy (LGMD2A) is the most common form of recessive LGMD (1). LGMD2A is caused by mutations in the *CAPN3* gene (15q15.1-15.3), encoding calpain 3, a calcium dependent protease (2). So far, 445 unique allelic variants are known to be responsible for LGMD2A (Leiden database: www.dmd.nl). The phenotypic features involve atrophy of the pelvic, scapular and trunk muscles, but not of cardiac or facial muscles. Mental status is normal. Age at onset is between 8 and 15 years for at least two-thirds of the patients, with a range of about 2 to 40 years. The slowly progressive course of the disease leads to loss of ambulation during adulthood and a near-normal life expectancy. Serum creatine kinase (CK) is markedly elevated and a muscular biopsy shows a dystrophic picture with evidence of necrosis and regeneration. Due to the variability of the phenotype the final diagnosis of LGMD2A relies on protein analysis of the muscle biopsy (Western blot) and mutation detection in the *CAPN3* gene. Although there is marked phenotypic variability between different families with LGMD2A, so far little is known about the intra-familial variability. Here we report on 8 siblings and their phenotypic variation which is particularly important for counselling of family members of patients with LGMD2A.

## Materials and methods

Families were included when two siblings showed a LGMD phenotype with a more than ten-fold elevation of serum creatine kinase, and the findings of muscle biopsy and mutation analysis of *CAPN3* confirmed the diagnosis of LGMD2A in at least one sibling. Information on age at onset and course of the disease were collected by chart review and each patient was examined clinically by one of the authors.

Muscle biopsies were analyzed by routine histology in 9/16 patients and Western blot analysis using a Calpain-3 antibody (Novocastra) was performed in 6/16 patients. Mutation analysis was performed by direct sequencing of the 24 exons and flanking intronic sequences of *CAPN3* after PCR from genomic DNA, as previously described (3).

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## Case study

We identified 8 families with 2 affected siblings (Table 1). Of the 16 patients, 10 were female and 6 male with an age range from 9.5 to 36.8 years (mean 21.8, SD 9.6). The mean age at onset of LGMD2A, in these patients, was 9.3 years (range 4-17 years). Within siblings, the difference of age at disease onset was between 1 and 11.5 years (mean 3.1 years). First symptoms were toe walking, weakness in the lower limbs, proximal weakness, and scapular winging. In 4 patients, the first recognized distinctive feature was an increased CK between 1300 and 8688 U/L (mean 4290 U/L). CK level at onset was markedly increased in all patients tested (mean 4046 U/L) and there was no difference between the sexes. Current age of the patients is between 9.5 and 36.8 years and 4 patients are no longer able to walk independently (patients 2A, 2B, 7B, 8A). Seven sib pairs have the same or similar clinical course and symptoms. However, the older sibling is usually more affected. In one sib pair (6A and 6B), the younger sibling has a proven mutation, but, at age 9, no clinical symptoms. There is no clear correlation between age of onset and clinical course. Although increased serum creatine kinase was the only manifestation at the time of diagnosis, all patients developed clinical symptoms during the course of the disease. A muscle biopsy was performed in 9 patients and showed a dystrophic picture with increase of connective tissue in all patients. Frozen muscle tissue for immunoblot analysis of Calpain-3 expression was available in 6 patients. In 5 patients, there was no detectable expression of Calpain-3 and in one it was markedly reduced. We have identified 8 different mutations, all of which previously described (Table 1). In 3 families, the patients carried homozygous mutations whereas 4 sib-pairs were compound heterozygotes and in one family only one mutation could be detected. The most frequent mutation was c.550delA in exon 4, present in 5 families; one Russian family (family 8) was homozygous for this mutation.

## Discussion

We present here a retrospective analysis of a series of siblings with a genetically confirmed diagnosis of LGMD2A (calpainopathy). Although intra-familial variability has been described in other LGMD subtypes in more detail, there are only a few reports on siblings with LGMD2A. Saenz et al. published a series of 238 LGMD2A patients belonging to 187 different families (1). For many patients, details of the clinical course were not available but for one sib-pair a difference in the age of onset of two years was mentioned. Fardeau et al. reported 12 families from a remote area of the Réunion Island with a high degree of consanguinity (4). There were 5 sib pairs and one group of 4 siblings included. Age at onset differed up to 4 years in 4 of the sib pairs and was at the same age in one sib pair and in 3 out of the 4 siblings and delayed by 2 years in the fourth sibling. Age at loss of ambulation was recorded for at least two of the siblings in four families and differed by 5 to 12 years (4). Also Guglieri et al. reported 77 patients with LGMD2A, including 6 siblings, but without more detailed intra-familial clinical details (5). Another 23 patients with LGMD2A, from 14 families, have been described by van der Kooi et al., showing intra-familial clinical phenotypes in siblings (6). The age at onset in that study differs mostly within the families. In two families, the onset of the disease was at the same age in siblings. In our study, age of onset differed by more than two years between siblings in 4 out of 8 families, confirming data shown by van der Kooi et al. In some families, this might be due to the fact that symptoms were noted earlier in the younger child after the diagnosis had been made in the older. However, in family 1, the diagnosis of LGMD2A was first made in the younger brother at the age of 5 years, thus the range of difference in age of onset is 11 years. At that time, the sister (three years older) did not show any symptoms of muscle weakness and, in fact, she did not develop evidence of muscle weakness until the age of 16 and, at age 20, she has only minor weakness of hip girdle muscles. Serum creatine kinase was markedly elevated in all patients and muscle biopsies, where available, invariably showed a dystrophic picture. Although the sensitivity of immunoblot analysis seems to be lower than previously assumed, in our series it was abnormal in all samples tested. Mutations in the *CAPN3* gene could be identified in all families. The most frequent mutation was c.550delA in exon 4. This mutation is considered to be the most frequent one in Europe probably due to a founder mutation originating in the Eastern Mediterranean area (7-9). Our data suggest that it is also widely represented in German patients with LGMD2A as also observed by Hanisch et al., at the same time (10).

In conclusion, even in siblings with identical mutations, the age at onset and the clinical course of LGMD2A can vary considerably suggesting other genetic or environmental factors influencing the disease course. This is relevant for counselling family members of patients with LGMD2A and also leads to the conclusion that LGMD2A should not be excluded in siblings on the basis of absence of symptoms alone. Instead, evaluation of creatine kinase level in serum seems an adequate screening method, if clinically indicated.

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**Table 1.** Eight families with siblings with calpainopathy.

| Family | Patient No. | Sex | Age onset (y) | First symptoms | Current age and clinical status |
|---|---|---|---|---|---|
| **1** | 1A | Female | 16 | Proximal weakness in lower limbs, toe walking | 20 y, weakness of hip girdle muscle (MRC 4/5), normal strength of shoulder girdle, no scapular winging, mild ankle contractures, independent walking |
| | 1B | Male | 5 | Toe walking, walking on heels not possible | 16 y, weakness of limb girdle muscles (MRC 3-4/5) sparing shoulder and hip abductors, mild hyperlordosis, mild ankle contractures, independent walking |
| **2** | 2A | Female | 11 | Proximal weakness in lower limbs | 37 y, marked weakness of proximal muscles (MRC 2-3/5), extreme hyperlordosis, severe elbow, hip, knee, and ankle contractures, wheelchair-bound since age 30 y |
| | 2B | Male | 7 | Toe walking | 33 y, marked weakness of proximal muscles (MRC 2-3/5), hyperlordosis, severe elbow and mild ankle contractures, wheelchair- bound since age 33 y |
| **3** | 3A | Female | 6 | Pes equinus | 17 y, weakness of limb girdles muscles (MRC 3-4/5), sparing hip abductors, scapular winging, hyperlordosis, elbow and ankle contractures, walking with difficulties |
| | 3B | Male | 6 | Muscle weakness, muscle cramps | 14 y, weakness of limb girdle muscles (MRC 3+-4/5), sparing hip abductors, scapular winging, mild hyperlordosis, mild ankle contractures, independent walking |
| **4** | 4A | Male | 10 | Mild weakness in getting up from squatting position | 21 y, weakness of proximal muscles (MRC 2-3/5), moderate Achilles tendon contractures, hyperlordosis, arm abduction up to 40°, getting up from chair or floor not without help, walking unsupported only for short distances |
| | 4B | Female | 9 | Foot extensor weakness | 18 y, weakness proximal muscles (MRC 3-4/5), mild Achilles tendon contractures, mild scoliosis, no scapula alata |
| **5** | 5A | Female | 7 | CK increased | 12 y, only muscle-cramps after sports |
| | 5B | Female | 7 | CK increased | 12 y, only muscle-cramps after sports |
| **6** | 6A | Female | 4 | CK increased | 11 y, mild proximal weakness (MRC 4-/5), muscle-cramps after sports, toe walking, scapular winging, |
| | 6B | Male | 4 | CK increased | 9 y, no symptoms |
| **7** | 7A | Female | 12 | Problems with sports in school, difficulties in running, getting up from floor, and climbing stairs | 25 y, muscle weakness: shoulder: (MRC 3/5), arm flexion/ext (MRC 4/5), cannot walk on heels, minimal tiptoe stand is possible, scoliosis, walking with a frame at home, for longer distance needs a wheelchair since age 24 y |
| | 7B | Female | 17 | Scapular winging | 22 y, muscle weakness: shoulder (MRC 3/5), arm flexion/ext (MRC 4/5), cannot walk on heels, mild tiptoe walking, scoliosis, scapular winging, waddling gait, able to walk without aid for 500 m |
| **8** | 8A | Male | 10 | Toe walking, difficulties in running | 33 y, proximal weakness arm and leg muscles (MRC 2-3/5), foot extensors (MRC 3/5), no distal pareses in arm muscles, marked hyperlordosis, walking with aid of crutches since age 19, wheelchair bound since age 29 y |
| | 8B | Female | 6 | Toe walking | 23 y, similar severity as affected brother, climbing stairs with aid of railing, difficulties in walking independently, not examined personally |

n.d. = not done; CK = creatine kinase; y = years

| Calpain 3 mutation | Muscle biopsy | Westernblot for Calpain-3 | CK onset (U/L) |
|---|---|---|---|
| *Homozygous* Exon 21: c.2243G > A, p.Arg748Gln | no | n.d. | 6.949 |
| | yes | No expression | 4.570 |
| *Compound heterozygous*<br>Exon 4: c.598_612del, p.Phe200_Leu204del<br>Exon11: c.1468C > T, p.Arg490Trp | yes | n.d. | 4.200 |
| | yes | n.d. | 5.160 |
| *Compound heterozygous* Exon 1: c.146G > A, p.Arg49His<br>Exon 4: c.550delA, p.Thr184ArgfsX36 | yes | No expression | 2.078 |
| | no | n.d. | 8.688 |
| *Heterozygote*<br>Exon 4: c.550delA, p.Thr184ArgfsX36<br>no second mutation identified. | Yes | No expression | 3.000 |
| | No | n.d. | 2.100 |
| *Compound heterozygous* Exon 4: c.550delA, p.Thr184ArgfsX36 Exon 18: c.2036-2037del, p.Thr679SerfsX20 | No | No | 4.790 |
| | Yes | n.d. | 5. 946 |
| *Homozygous*<br>Exon 11: c.1468C > T, p.Arg490Trp | Yes | No expression | 4.552 |
| | No | No | 2.660 |
| *Compound heterozygous*<br>Exon 4: c.550delA, p.Thr184ArgfsX36<br>Exon 8: c.1063C>T, p.Arg355Trp | No | n.d. | 2.200 |
| | Yes | No expression | 1.300 |
| *Homozygous*<br>Exon 4: c.550delA, p.Thr184ArgfsX36<br>Not analyzed in patient 8B | Yes | Bands for calpain markedly reduced | 250 |
| | No | n.d. | n.d. |

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