On Symptomatic Heterozygous Alpha-Sarcoglycan Gene Mutation Carriers

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Mutations in the human alpha-sarcoglycan gene on chromosome 17q21.2 have been shown to cause a severe childhood autosomal recessive muscular dystrophy, a less severe limb girdle muscular dystrophy, exercise intolerance, or asymptomatic hyperCKemia. Here, we describe the clinical findings in a German family harboring a 371 T > C (Ile124Thr) missense mutation in the alpha-sarcoglycan gene. Whereas our index patient, an 11-year-old girl homozygous for this mutation, presented with a severe Duchenne-like phenotype, 7 out of 12 heterozygous mutation carriers from three generations showed mild to moderate scapular winging. In analogy to symptomatic female dystrophinopathy carriers, our results suggest that heterozygous alpha-sarcoglycan gene mutation carriers can be symptomatic with selective muscle weakness. This finding may be attributed to an additional negative variation in a yet unknown modifier gene essential to the function of the sarcoglycan complex in shoulder girdle muscles.

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Autosomal recessive limb–girdle muscular dystrophies (LGMD) are a heterogeneous group of genetic diseases with a wide spectrum of clinical involvement and severity (see Bushby, 1999, and Bonnemann and Finkel, 2002, for reviews).[1,2] Alpha-sarcoglycan, initially called adhalin, is a 50kDa component of the dystrophin-associated sarcoglycan complex that functions as a 1:1:1:1 heterotetramer with beta, gamma, and delta-sarcoglycan at the sarcolemma.[3,4] Mutations affecting any of the sarcoglycan genes disrupt this complex and cause muscle fiber degeneration and autosomal recessive limb–girdle muscular dystrophies.[5–10] A broad spectrum of phenotypes have been associated with mutations in the human alpha-sarcoglycan gene on chromosome 17q21.2 that range from severe to mild limb–girdle muscular dystrophy (LGMD 2D), exercise intolerance, or asymptomatic hyperCKemia.[11–14] This report describes the clinical findings in a homozygous and 12 heterozygous carriers of a 371 T > C (Ile124Thr) missense mutation in the alpha-sarcoglycan gene.

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Materials and Methods

Patients

Twenty members of a large German kinship were seen for clinical and molecular genetic evaluation and underwent neurological examination using a standardized protocol to determine clinical involvement (MRC) as described.[15] Written and informed consent was obtained from all individuals.

Molecular Genetic Analysis

DNA extraction from blood samples was performed by standard procedures, and mutation scanning of all four sarcoglycan exons was performed by a 3500-HT denaturing high-performance liquid chromatography (HPLC) instrument (Transgenomic) using the melting conditions predicted by the Wavemaker software. Capillary sequencing of polymerase chain reaction products with abnormal profiles was performed using an ABI3100 (Applied Biosystems).

Muscle Biopsy, Antibodies, Immunohistochemistry, and Immunoblotting

An open diagnostic biopsy, taken from the left vastus lateralis muscle was performed from patients I.3 and III.5. Six-micrometer-thick cryostat sections of snap-frozen unfixed muscle were stained by standard procedures. Immunohistochemistry and immunoblotting were performed as described.[15] The following primary mouse monoclonal antibodies were used: dys-1, dys-2, dys-3, alpha-, beta-, gamma-, and delta-sarcoglycan, dysferlin, and calpain-3 (Novocastra, Newcastle, UK). All specimens were examined and pictures were digitally acquired using a Nikon E800 microscope (Nikon, Düsseldorf, Germany) equipped with a CCD camera.

Results

Family Pedigree and Mutation Analysis

The pedigree of the reported family is shown in Figure 1A. DNA mutation analysis of the index patient (see Fig 1A, patient III.4) showed a homozygous 371 T > C mutation in exon 4 of the alpha-sarcoglycan gene causing a 124 isoleucine → threonine amino acid change (see Fig 1B). This mutation had been reported previously in two compound heterozygous LGMD patients with a Duchenne's muscular dystrophy–like phenotype.[5,10] However, as the homozygosity indicated consanguinity, we performed further genetic analysis in the parents, and, subsequently in 17 additional family members by denaturing HPLC (dHPLC) and direct sequencing. Both parents and 10 other family members

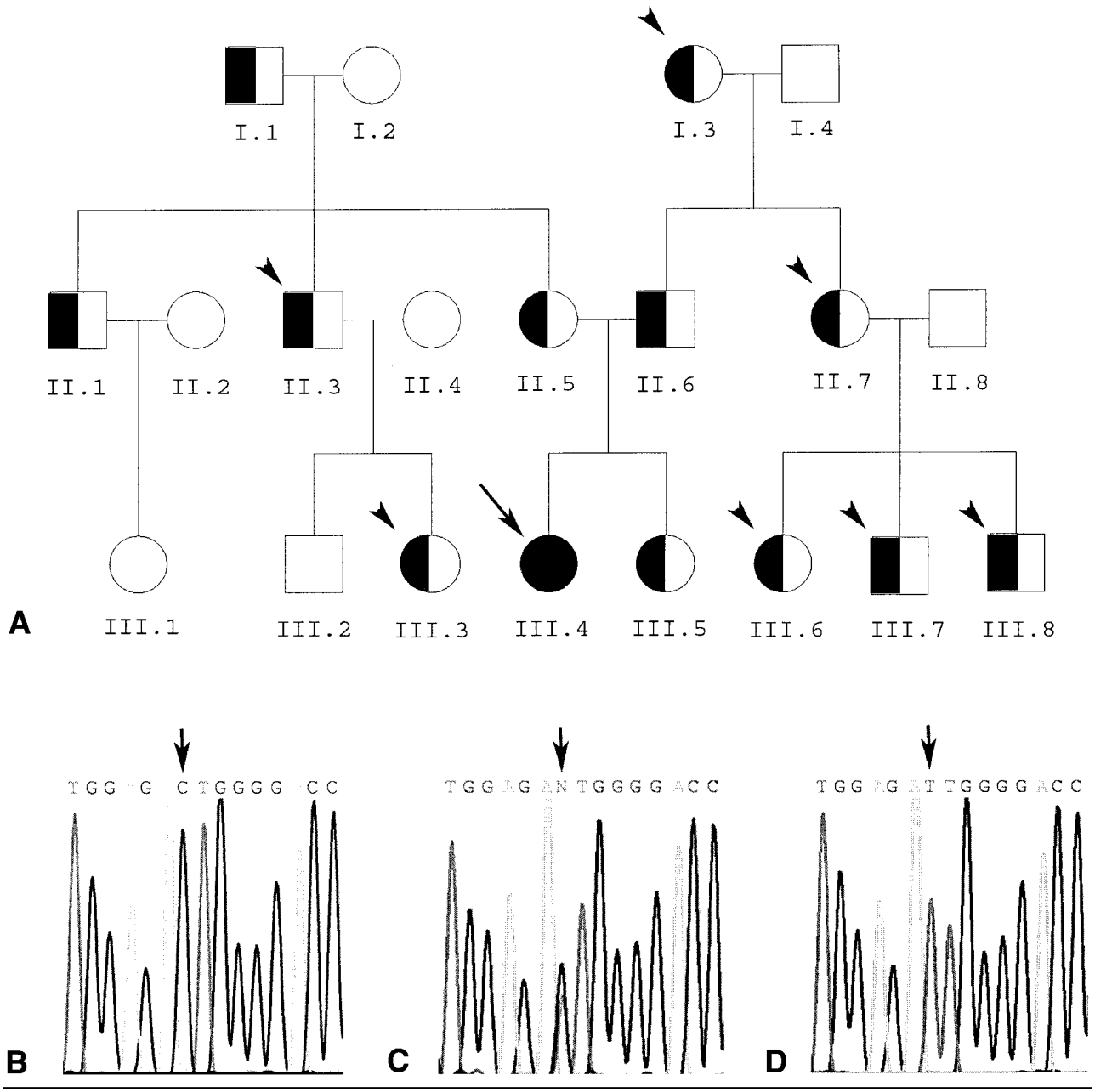

Fig 1. (A) Family pedigree and segregation of the α-sarcoglycan gene mutation. Symptomatic heterozygous mutation carriers that presented with scapular winging are indicated by arrowheads. Mutation detection and sequence analysis indicated in the index patient (III.4, arrow) a homozygous (B) and in the carriers a heterozygous (C) 371 T > C missense mutation in exon 4 of the α-sarcoglycan gene leading to an amino acid change from isoleucine → threonine. The sequence of normal controls is shown in D.

were identified as heterozygous mutation carriers (see Fig 1C). In addition, we excluded the presence of a reduced number of D4Z4 DNA (*Kpn*I) repeats in the FSH-locus on chromosome 4q35 in patient II.6 (not shown).

Morphological Analysis

Morphological analysis of the muscle biopsy from the homozygous index patient (III.4) showed severe dystrophic changes consisting of an increase of endomysial connective tissue, pathological variation of fiber size, increased number of internal nuclei, regenerating and degenerating fibers (Fig 2A). Immunohistochemistry (see Fig 2C) and immunoblotting (not shown) demonstrated the complete absence of alpha-sarcoglycan and a partial reduction of beta-, gamma-, and delta-sarcoglycan (not shown). A biopsy specimen taken from a carrier (patient I.3), which had been performed 10 years ago because of exercise-related myalgias and CPK elevation, showed slight nonspecific changes such

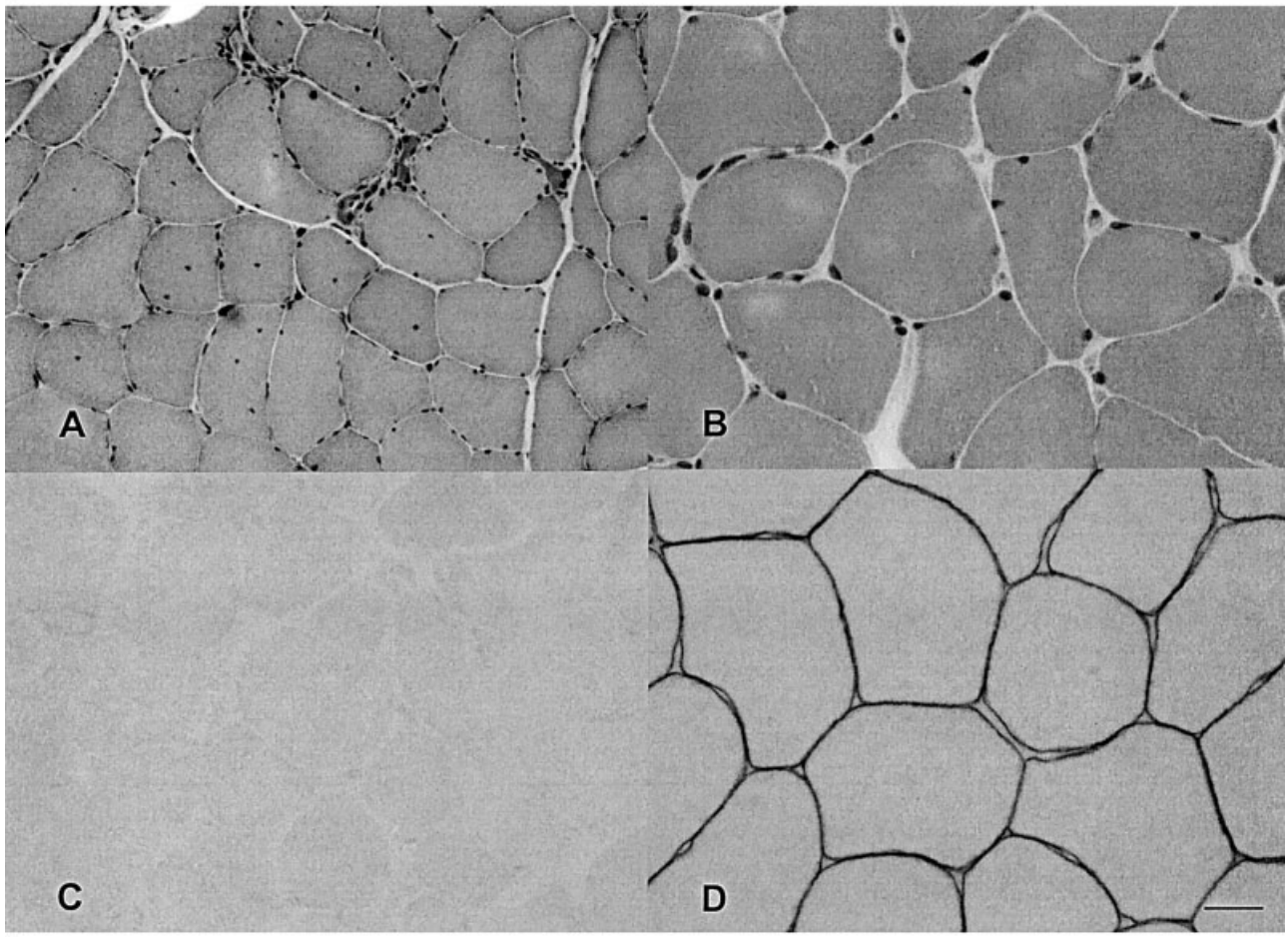

Fig 2. Morphological analysis of skeletal muscle tissue of the homozygous patient (A) showed dystrophic changes consisting of pathological fiber-size variability, increased internalization of nuclei and fiber de- and regeneration. In contrast, skeletal muscle tissue of a heterozygous patient (B) with nonspecific fiber size variability and few atrophic fibers. Absence of α-sarcoglycan immunostaining in the homozygous patient (C) when compared with controls (D). Scale bar = 25μm.

as increased fiber-size variability and abnormally rounded and few atrophic fibers (see Fig 2B). In contrast to the homozygous patient, sarcoglycan immunostaining and alpha-sarcoglycan Western blotting did not differ from controls (not shown). Furthermore, calpain-3 Western blotting of muscle tissue from both homozygous and heterozygous patients gave normal results (not shown).

Phenotype Analysis

Neurological examination of the 11-year-old homozygous female index patient showed severe weakness of proximal limb muscles with predominant involvement of the scapular fixator muscles (serratus anterior, rhomboid, trapezius, and infraspinatus), pelvic girdle muscles (glutei, adductors, and quadriceps) and trunk extensors (Fig 3A–C). The CPK was markedly elevated (7449 U/L, normal < 80). Phenotypic analysis of the 12 carriers showed mild to moderate symmetric scapular winging in 7 carriers (see Fig 3E–H). Furthermore, prominent calf hypertrophy was found in two patients (see Fig 3D). None of the carriers had weakness of the pelvic girdle muscles or showed an elevated creatine kinase (CK) level at rest.

Discussion

In this report, we present a large German family harboring a 371 T → C missense mutation in exon 4 of the alpha-sarcoglycan gene causing an 124 isoleucine → threonine amino acid change. This missense mutation led to a severe Duchenne-like phenotype in an 11-year-old homozygous girl. Because LGMD 2D is, by definition, an autosomal-recessive disorder, it is remarkable to note that phenotypic analysis in 7 out of 12 heterozygous mutation carriers showed mild to moderate symmetric winging of the scapula. Furthermore, two patients with this heterozygous mutation had signs of calf hypertrophy. However, CK testing was normal in all individuals analyzed. Because other muscular dystrophies have marked involvement of

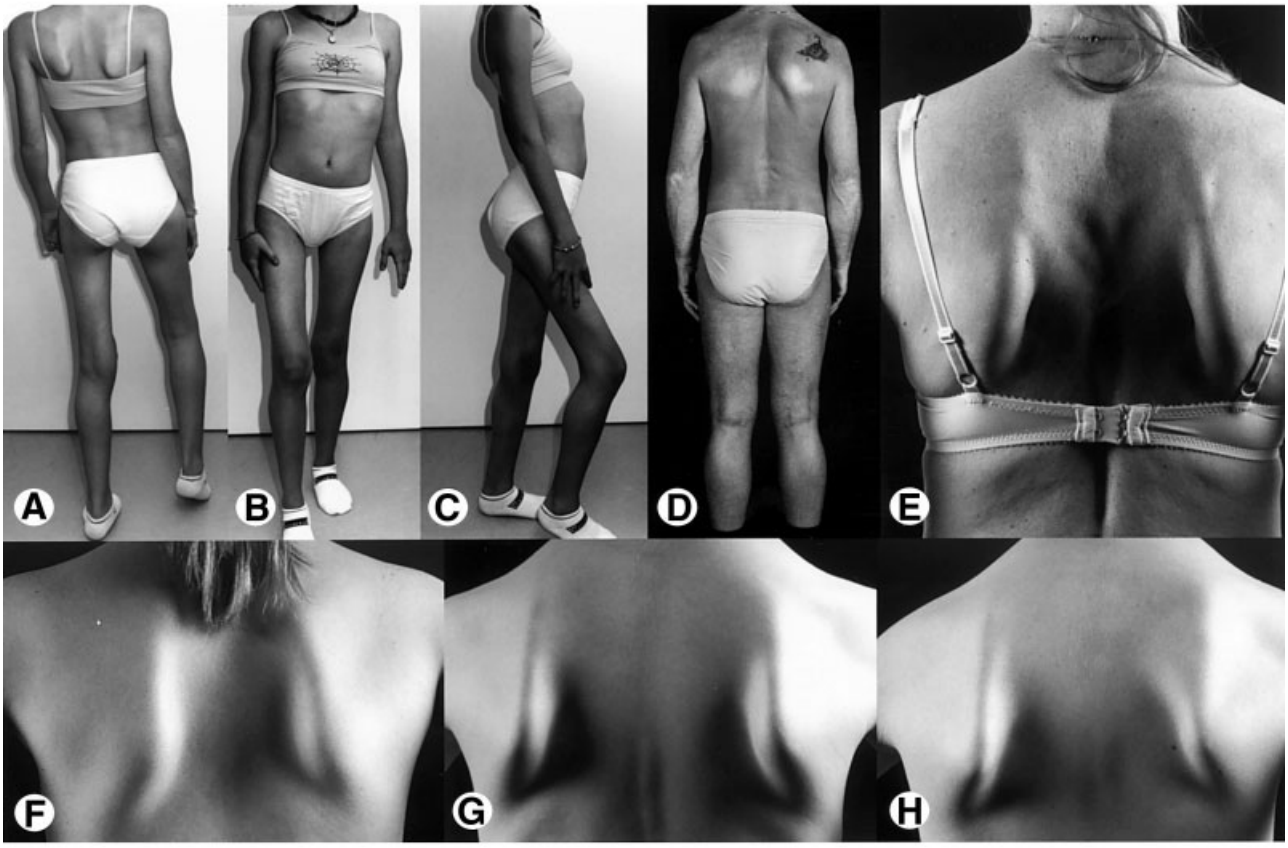

Fig 3. Clinical features of the 11-year-old homozygous girl (III.4) with a severe muscular dystrophy phenotype and marked proximal weakness with scapula alata and wasting of thigh muscles (A–C). Phenotypes of carriers with calf hypertrophy in Patient II.6 (D) and scapular winging in Patients II.7 (E), III.6 (F), III.7 (G), III.8 (H) with marked weakness of the serratus anterior and rhomboid muscles.

shoulder girdle muscles, facioscapulohumeral dystrophy[16] and LGMD 2A[17,18] were ruled out by genetic and calpain-3 Western blotting analysis. These findings indicate that heterozygous alpha-sarcoglycan gene mutation carriers can present with a mild clinical phenotype. This notion is further corroborated by earlier reports showing that approximately 40% of parents of affected children with alpha-sarcoglycanopathy had moderately elevated CK levels.[19] However, muscular weakness in carriers has not been reported so far. In this context, it is noteworthy, that approximately 17% of female carriers of Duchenne's or Becker's muscular dystrophy show signs of clinical involvement consisting of pelvic rather than the shoulder girdle muscle weakness and sometimes cardiomyopathy.[20,21] Because dystrophinopathy is an X-linked disorder, female carriers have a mosaic of fibers with wild-type or absent dystrophin expression. In contrast, the situation is more complex in autosomal recessive alpha-sarcoglycanopathy.

Morphological analysis of skeletal muscle tissue of the 11-year-old homozygous female patient showed severe dystrophic changes as well as a complete absence of the alpha-sarcoglycan protein expression both on immunohistochemistry and Western blot analysis. The lack of the alpha-sarcoglycan protein appears due to a premature decay of alpha-sarcoglycan mRNA or protein. In contrast, myopathological evaluation of a muscle biopsy that had been taken from the (clinically unaffected) left vastus lateralis muscle of a 70-year-old female carrier showed only unspecific changes. Alpha-sarcoglycan immunohistochemistry and Western blotting indicated normal protein expression. Furthermore, beta-, gamma-, and delta-sarcoglycan immunohistochemistry did not differ from normal controls. These findings suggest that one allele is sufficient to produce a normal amount of alpha-sarcoglycan protein in clinically unaffected muscle tissue. However, the presence of shoulder girdle muscle weakness indicate that the function of the sarcoglycan complex is selectively disturbed in these muscles. Sequence comparison data show that the amino acid Ile 124 is highly conserved in humans, mouse, rat, rabbit, and hamster, suggesting that Ile 124 is critical for the alpha-sarcoglycan function. Although we could not address this issue experimentally, one has to consider the possibility that the Ile124Thr mutation exerts a dominant negative effect

on the function of the sarcoglycan complex in affected shoulder girdle muscles. With regard to the pedigree, however, it appears possible that symptomatic carriers harbor an additional negative variation in a yet unknown modifier gene essential to shoulder girdle muscle groups, which influences the function of the sarcoglycan complex.

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Dually Infected (HSV-1/ VZV) Single Neurons in Human Trigeminal Ganglia

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Human trigeminal ganglia were tested by double fluorescence in situ hybridization for the presence and distribution of herpes simplex virus type 1 (HSV-1) and varicella-zoster virus (VZV) latency. Latency transcripts of both viruses were detected in common areas within the ganglia. Also, a few single neurons were shown to harbor HSV-1 and VZV together.

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Herpes simplex virus type 1(HSV-1) and varicella-zoster virus (VZV) are alphaherpesviruses, a viral fam-

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