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ORIGINAL ARTICLE

Characterization of an acromesomelic dysplasia, Grebe type case: novel mutation affecting the recognition motif at the processing site of GDF5

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Abstract Acromesomelic dysplasia, Grebe type is a very rare skeletal dysplasia characterized by severe dwarfism with marked micromelia and deformation of the upper and lower limbs, with a proximodistal gradient of severity. *CDMP1* gene mutations have been associated with Grebe syndrome, Hunter–Thompson syndrome, Du Pan syndrome and brachydactyly type C. The proband is a 4-year-old boy, born of consanguineous Pakistani parents. Radiographic imaging revealed features typical of Grebe syndrome: severe shortening of the forearms with an acromesomelic pattern following a proximodistal gradient, with distal parts more severely affected than medial parts; hypoplastic hands, with the phalangeal zone more affected than the metacarpal zone; and severe hypoplastic tibial/femoral zones in both limbs. After molecular analyses, the p.Arg377Trp variant in a homozygous pattern was identified in the *CDMP1* gene in the affected child. In silico and structural analyses predicted the p.Arg377Trp amino acid change to be pathogenic. Of the 34 mutations described in the *CDMP1* gene, four different missense mutations have been associated with Grebe syndrome. The *CDMP1* gene encodes growth differentiation factor 5 (GDF5), which plays a role in regulation of limb patterning, joint formation and distal bone growth. Homozygous mutations in the mature domain of GDF5 result in severe limb malformations such as the Grebe type or the Hunter–Thompson type of acromesomelic chondrodysplasia. The p.Arg377Trp mutation is located within the recognition motif at the processing site of GDF5 where the sequence RRKRR changes to WRKRR. The genotype–phenotype correlation allowed not only confirmation of the clinical diagnosis but also appropriate genetic counselling to be offered to this family.

Keywords Skeletal · *CDMP1* · Grebe · Growth differentiation factor 5 · Pakistan

Introduction

Acromesomelic dysplasia, Grebe type (AMDG; OMIM 200700) is a rare autosomal recessive disorder characterized by severe dwarfism with marked micromelia and deformation of the upper and lower limbs, with a proximodistal gradient of severity, and by short and deformed middle long bones, fusion of carpal and tarsal bones, absence of proximal and middle phalanges and several metacarpal and metatarsal bones. In spite of this, the facial features and intelligence of the affected individuals are similar to those of the general population. In some cases, carriers also manifest milder signs such as brachydactyly and postaxial polydactyly [1]. Mutations in the *CDMP1* gene have been associated with AMDG, autosomal recessive acromesomelic dysplasia of Hunter–Thompson type, autosomal recessive

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Du Pan syndrome and autosomal dominant brachydactyly type C. This gene is located in 20q11.22, and is predominantly expressed in cartilaginous tissues of the developing long bones and the more distal elements of the appendicular skeleton [2]. *CDMP1* codifies a member of the transforming growth factor beta superfamily of secreted signaling molecules, growth differentiation factor 5 (GDF5), that plays a role in regulation of limb patterning, joint formation and distal bone growth [3] by the stimulation of the early steps of chondrogenesis increasing cellular adhesion and then the chondrocytic proliferation [4]. GDF5 is synthesized as a large precursor molecule consisting of a signal peptide, a prodomain and a mature domain. At the cleavage site between the prodomain and the mature domain, GDF5 is processed into an active protein that forms homodimers or heterodimers with other bone morphogenetic proteins. We report a Pakistani patient with clinical diagnosis of AMDG, the molecular methods which led to the identification of a previously unreported variant in the *CDMP1* gene and the respective genetic counselling.

Materials and methods

Patient ascertainment and sample collection

This molecular study was reviewed and approved by the Ethics Committee of the Fundacion Jimenez Diaz, and was performed according to the tenets of the Declaration of Helsinki and subsequent revisions (Tokyo, 2004). After informed consent had been obtained, blood samples were collected from the proband and the parents.

Clinical evaluation

A 4-year-old boy with severe skeletal deformities was referred to the Dysmorphology Clinic, and AMDG was suspected at the clinical examination. Radiographic imaging findings were consistent with features typical of Grebe syndrome. In a second gestation of this couple, severe shortening of the upper and lower limbs was diagnosed on ultrasound examination. The father of the proband had mild shortening of the digits in both hands.

Molecular methods

DNA extraction

Genomic DNA obtained from proband and parental peripheral blood was extracted using an automatic DNA extractor (BioRobot EZ1, Qiagen, Hilden, Germany).

Mutation detection

The *CDMP1* gene was PCR-amplified with use of previously described primers [5]. PCR products were sequenced using a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA), and were analysed with an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems) with sequence analysis software in accordance with the manufacturer's protocols.

Results

The proband is a 4-year-old boy, the first child of a consanguineous couple originally from Pakistan. He was referred to the Dysmorphology Clinic because of severe skeletal deformities with extremely short limbs. At the clinical examination, his height was 78 cm and his cranial circumference was 47.5 cm, both below two standard deviations for his age. He had severe shortening of the forearms with an acromesomelic pattern following a proximodistal gradient, with distal parts more severely affected than medial parts. Hypoplastic hands also followed this gradient, with the phalangeal zone more affected than the metacarpal zone. The lower limbs showed a severer defect, with extremely hypoplastic tibial/femoral zones in both limbs; there were severely hypoplastic club feet, with rudimentary toes (Fig. 1). He was unable to walk alone, but stayed in a semisitting position, and he had a remarkable limitation on extension movements of the knees. He had an acceptable level of comprehension, but low oral expression. AMDG was suspected at the clinical examination. Radiographic imaging findings were consistent with features typical of Grebe syndrome. In a second gestation of this couple, severe shortening of the upper and lower limbs was diagnosed on ultrasound examination, and termination of the pregnancy was requested by the parents. The father of the proband had mild shortening of the digits in both hands, with no other limbs anomalies (Fig. 1), and he (III:6 in Fig. 2) informed us that a nephew (IV:6 in Fig. 2), a first cousin of proband, had the same pattern of shortening of the limbs as his son (IV:1 in Fig. 2).

The clinical diagnosis of Grebe syndrome was confirmed by molecular analysis of the proband sample. The p.Arg377Trp (c.1129C>T) variant was identified in the proband in a homozygous pattern (Fig. 3a). The parents exhibited the variant heterozygously, confirming the carrier status for both of them (Fig. 3b, c). This p.Arg377Trp variant had not been previously described, so in silico prediction was performed in order to determine the pathogenicity of this variant. It was predicted to be probably pathogenic by SIFT, with a score of 2.65, and by PolyPhen, with a score of 1.00.

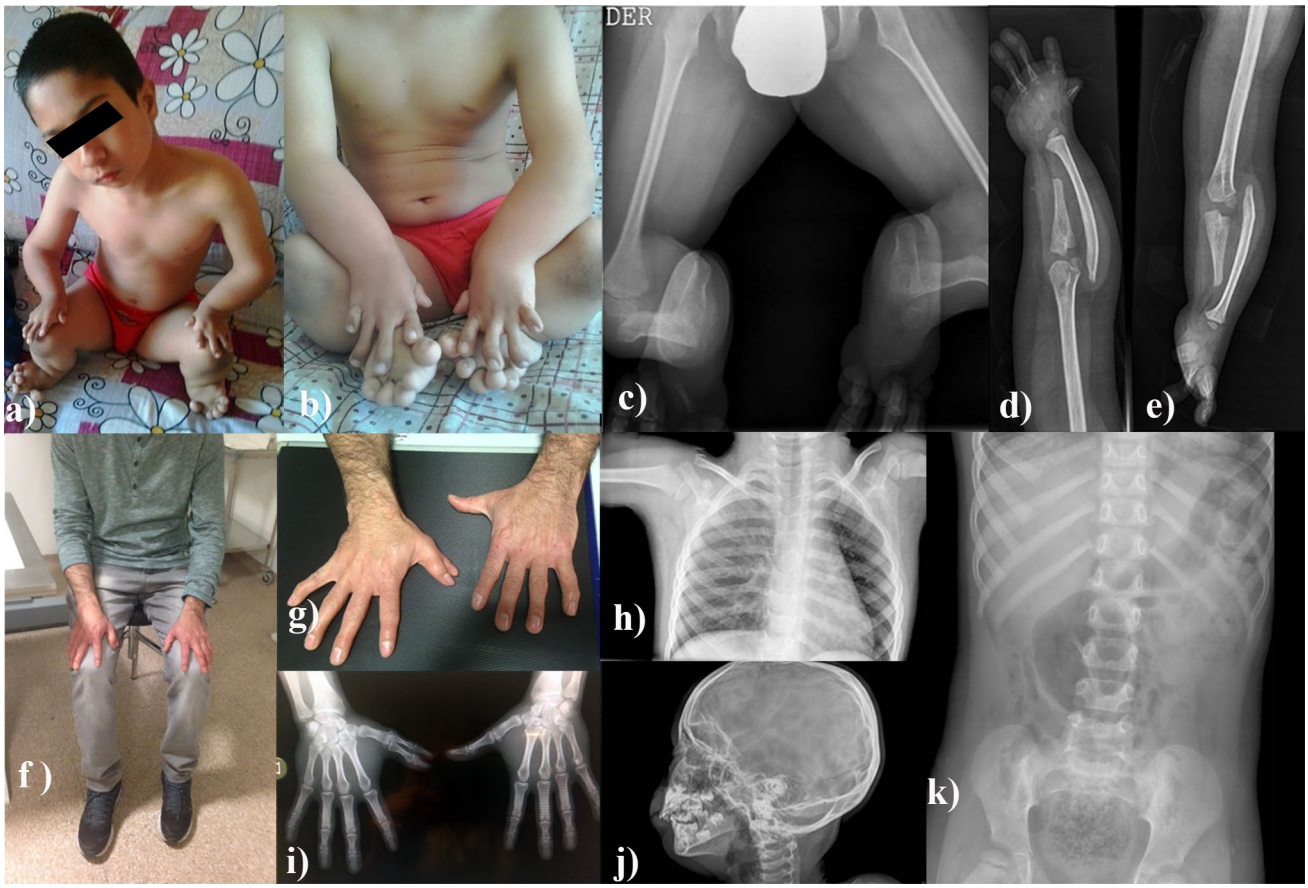

Fig. 1 **a**, **b** Photographs of the affected child. **c,–e**, **h**, **j**, **k** Radiographs of the upper and lower limbs of the affected child. **f**, **g** Photographs of the father. **i** Radiograph of the father's hands

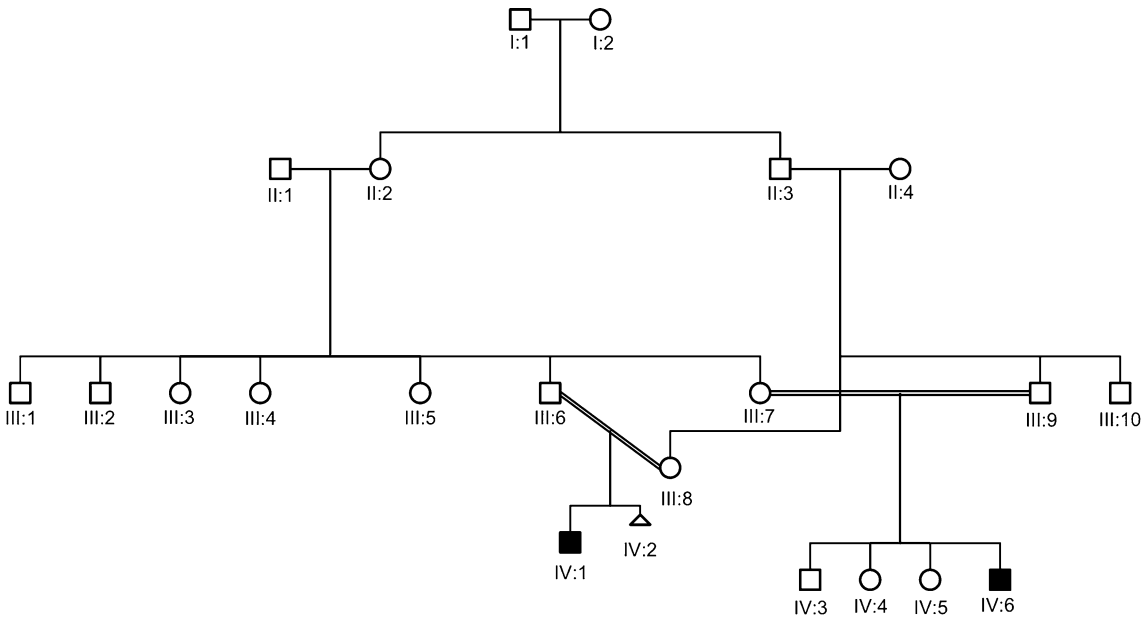

Fig. 2 Pedigree of the family. The proband (IV:1) was the first gestation of a consanguineous couple (III:6 and III:8). A first cousin (IV:1) of the proband is also affected, and is the fourth gestation of a consanguineous couple (III:7 and III:9) within the family

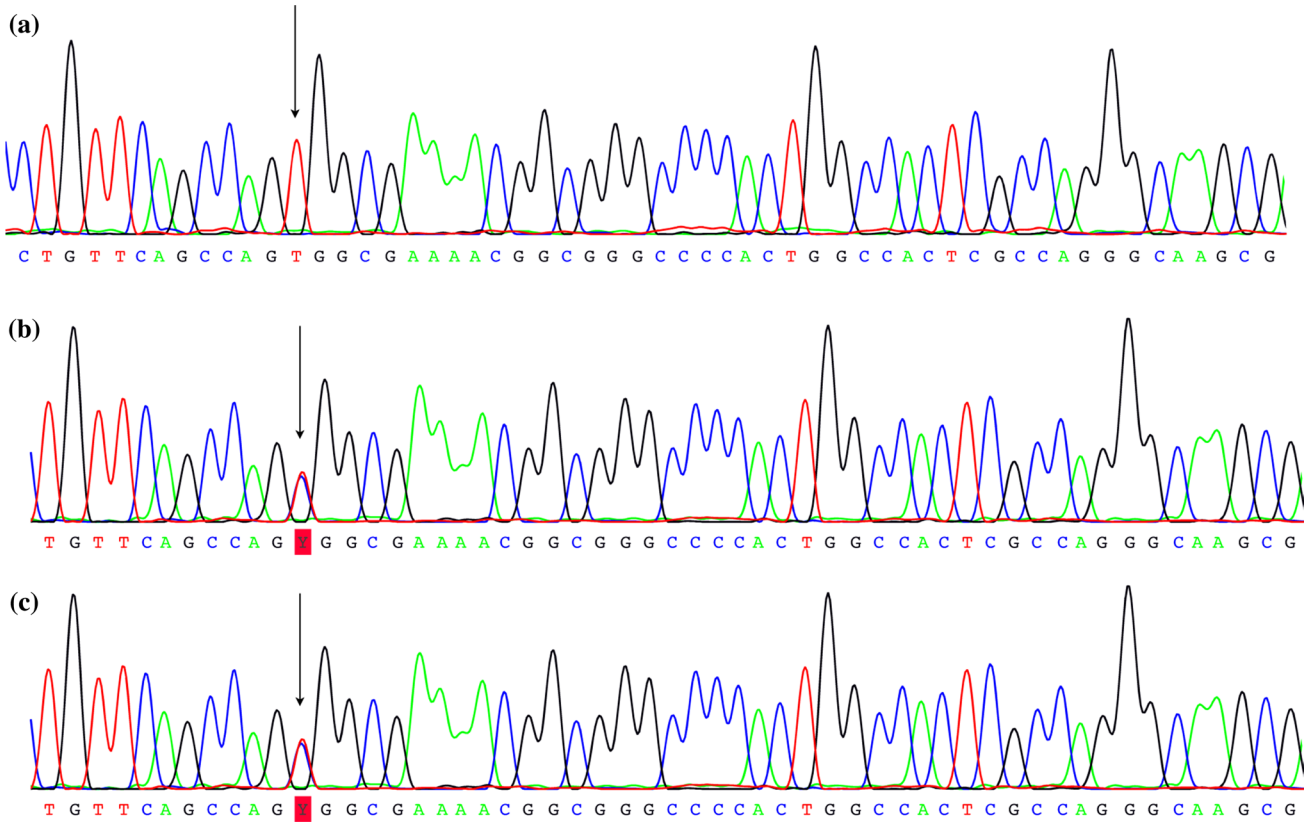

Fig. 3 **a** Electropherogram of the sequence showing the homozygous p.Arg377Trp (c.1129C>T) mutation present in the proband's sample. **b** The heterozygous mutation pattern present in the father's sample. **c** The heterozygous mutation pattern present in the mother's sample

According to HGMD, about 34 mutations have been described in the *CDMP1* gene to date, and these mutations had been associated with different phenotypes, specifically in the case of heterozygous missense mutations, ranging from mild brachydactyly with features overlapping to unaffected mutation carriers. The father of the proband manifested mild phenotypes related to the presence of a heterozygous mutation in the *CDMP1* gene [1]. Homozygous mutations in the mature domain of GDF5 result in severe limb malformations such as AMDG or acromesomelic chondrodysplasia of Hunter–Thompson type. Four different missense mutations have been associated with Grebe syndrome, and particularly the c.1111_1114dupGAGT mutation was identified in a Pakistani patient [5]. The p.Arg377Trp variant identified in a homozygous pattern in our affected individual and in a heterozygous pattern in his parents was not previously described. The Pakistani origin of the patient unable us to determine the allelic frequency in the control population, and they did not collaborate in the study of the other branch of the family that had similar clinical features.

ProGDF5 is thought to undergo proteolysis at a cluster of basic residues (RRKRR) by specific members of endoproteases [6]. The Arg377Trp mutation is located within the recognition motif at the processing site of GDF5 where the sequence RRKRR changes to WRKRR. Similar changes at neighbouring amino acids, Arg378Gln and Arg380Gln, were associated with Du Pan syndrome and brachydactyly type A2, respectively, and they were shown to interfere with the cleavage of proGDF5 at the recognition site [7, 8]. We postulate that p.Arg377Trp can be considered a mutant allele, taking into account the prediction tests, the conserved codon of the protein affected, the motif of the protein altered and the presence of another mutation in a Pakistani consanguineous family having a member with the same rare disease. Further functional studies are needed to fully understand the pathomechanism of these mutations.

The father of the patient informed us in subsequent consultations in the Genetic Clinic about a new marriage within the familial group, because of the awareness of the high risk of a new baby with Grebe dysplasia. Henetic counselling was offered because of the cultural context of this family. Communication of information regarding life-threatening situations or the risk of developing a severe disease involves important emotional, social and cultural considerations [9]. Several medical issues dealing with genetics single out specific ethnic groups, resulting in potentially negative psychological consequences and privacy/discrimination issues [9, 10].

Discussion

A multidisciplinary approach is crucial to ensure the final diagnosis for a patient with congenital malformations, especially in patients presenting with skeletal dysplasias. The approach described here led to the diagnosis of AMDG in an affected child. There are few cases of AMDG worldwide, so clinical expertise in rare skeletal dysplasias is needed in order to establish a precise clinical diagnosis. A new mutation, p.Arg377Trp, was identified in the proband. After an extensive review of the literature and in silico predictions, we proposed the mutation to be pathogenic. However, further analysis is required to confirm this finding. The genotype–phenotype correlation allowed not only confirmation of the clinical diagnosis but also appropriate genetic counselling to be offered to this family. Understanding the risk in subsequent pregnancies was essential in this case, because of the 25 % risk of this couple having another child with Grebe dysplasia and because of the high level of consanguineous marriages in this family. The couple were also informed about the risk of having another child with Grebe dysplasia with other consanguineous marriages and the possibility to test the heterozygous condition of the new wife.

A genetic counsellor must clarify the genetic cause of the phenotype, the risk of having subsequent children with the same signs and the possibilities to avoid or prevent them in order to minimize the feeling of guilt towards a pregnancy with congenital defects, a common behaviour for most patients of all backgrounds.

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Conflict of interest The authors declare that they have no conflict of interest.

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