A Recurrent Pathogenic Variant in *TPM2* Reveals Further Phenotypic and Genetic

Heterogeneity in Multiple Pterygium Syndrome-Related Disorders

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A Conflict of Interest Statement

The authors have no conflict of interest to declare.

A Data Availability Statement:

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Abstract

Multiple pterygium syndrome (MPS) disorders are a phenotypically and genetically heterogeneous conditions characterised by multiple joint contractures (arthrogryposis), pterygia (joint webbing) and other developmental defects. MPS is most frequently inherited in an autosomal recessive fashion but X-linked and autosomal dominant forms also occur. Advances in genomic technologies have identified many genetic causes of MPS-related disorders and genetic diagnosis requires large targeted next generation sequencing gene panels or genome-wide sequencing approaches. Using the Illumina TruSightOne clinical exome assay we identified a recurrent heterozygous missense substitution in *TPM2* (encoding beta tropomyosin) in three unrelated individuals. This was confirmed to have arisen as a *de novo* event in the two patients with parental samples. *TPM2* mutations have previously been described in association with a variety of dominantly inherited neuromuscular phenotypes including nemaline myopathy, congenital fibre-type disproportion, distal arthrogryposis and trismus pseudocamptodactyly, and in a patient with autosomal recessive Escobar syndrome and a nemaline myopathy. The three cases reported here had overlapping but variable features. Our findings expand the range of *TMP2*-related phenotypes and indicate that *de novo TMP2* mutations should be considered in isolated cases of MPS-related conditions.

Keywords: *TPM2*, beta tropomyosin, Multiple pterygium syndrome, arthrogryposis, distal contractures, camptodactyly, trismus

Introduction

Multiple pterygium syndrome (MPS) disorders are phenotypically and genetically heterogeneous. MPS is subdivided into Escobar variant MPS (EVMPS) and a prenatally lethal form (LMPS). Both are characterised by multiples joint contractures (arthrogryposis), pterygia or webbing across the joints, fetal akinesia and congenital abnormalities. Pterygia occur with immobility and are believed to result from early fetal oedema or subcutaneous fluid in the joint region[1] due to impaired connection between the venous and lymphatic systems.[2] Pterygia may become apparent with time, suggesting progression in the formation of the pterygia can occur.[3,4] LMPS and EVMPS have phenotypic overlap with fetal akinesia deformation sequence and other arthrogrypotic conditions respectively. MPS are most commonly autosomal recessively inherited although autosomal dominant and X-linked recessive families have been described.[5–9] MPS results from fetal akinesia and biallelic pathogenic mutations in the embryonal gamma subunit of the acetylcholine receptor gene (*CHRNG*) are a major cause of both LMPS and EVMPS.[10,11] Biallelic mutations in other fetal acetylcholine receptor function genes (*CHRNA1*, *CHRND*, *RAPSN*, *DOK7* and *MUSK)* can also result in MPS.[12–17] Other neuromuscular genes such as *SMA*, *GLE1* in the anterior horn cell of the central nervous system[18,19] and muscle such as ryanodine receptor-1, nebulin, actin, skeletal muscle alpha[20–23] have been identified in patients with MPS. Genetic heterogeneity makes molecular diagnosis challenging nevertheless it is important as there may be health implications for the patient, and to provide accurate recurrence risk information and options such as prenatal and pre-implantation genetic diagnosis for families. We utilized an exome sequencing approach to identify a recurrent monoallelic pathogenic *TPM2* variant in three unrelated children with an MPS spectrum disorder.

Materials and Methods

Patients with features of EVMPS were recruited to the study. All families gave informed consent. Clinical information was obtained from clinical questionnaires, medical records and clinical review. *CHRNG* was analysed and whole-exome sequencing (WES) undertaken in the *CHRNG* negative probands and segregation studies performed as described in Appendix S1, Supporting Information. The study was approved by the South Birmingham Research Ethics Committee.

Clinical Details

Cases 1, 2 and 3 had arthrogryposis predominantly affecting distal joints, congenital anomalies and facial features reminiscent of EVMPS (see Figures 1a-1c). Cases 2 and 3 also had neck pterygia. All three individuals had short stature and coeliac disease was identified in case 1 and isolated growth hormone deficiency in case 2. Clinical features of cases 1-3 and published cases with a sequence change affecting the amino acid at position 133 are summarised in Table 1. For detailed clinical information see Appendix S2, Supporting Information.

Results

Molecular Findings

An identical heterozygous missense substitution in exon 4 of *TPM2*, c.397C>T (p.Arg133Trp), was detected in all three patients. Parental samples were available for cases 2 and 3 and none of the parents carried the variant. The variant was not present in the Gnomad database (accessed 27/2/19) and was classified as pathogenic by ACMG criteria (using criteria PS2, PS4, PM2, PP5).

Discussion

Tropomyosins are a family of tissue specific protein isoforms. Tropomyosin 2 (*TPM2*) encoding beta tropomyosin is mainly expressed in slow muscle fibres and to a lesser extent in

fast muscle and heart muscle fibres.[24] It is an important component of the skeletal muscle machinery and is intimately involved with other components such as actin and troponin in calcium regulated muscle contraction.[25]

Mutations in *TPM2* have been associated with a variety of phenotypes including nemaline myopathy, congenital fibre-type disproportion, cap myopathy, distal arthrogryposis and trismus pseudocamptodactly.[24,26–30] The c.397C>T(p.Arg133Trp) variant predicted to be possibly damaging or damaging by PolyPhen-2 and FATHMM respectively was described in an adult mother and daughter with distal arthrogryposis type 2B and muscle weakness and in a patient with congenital fibre-type disproportion and progressive weakness.[30,31] A further patient with a different missense substitution c.398G>C (pArg133Pro) affecting the same amino acid had a congenital fibre-type disproportion, hypotonia and respiratory failure at birth.[30]

TPM2 loss of function mutations result in decreased calcium sensitivity and congenital muscle weakness whereas gain of function mutations cause a higher calcium sensitivity, hypercontractile state and arthrogryposis.[25] Distal arthrogryposis with the p.R133W gain of function mutation would fit with a hypercontractile state secondary to an heightened sensitivity to calcium however this has not been demonstrated and instead there may be a reduction of myosin head molecules in the strong actin-binding state and altered myosin–actin cross bridge kinetics resulting in muscle weakness without muscle wasting.[30,32] Beta tropomyosin is the main isoform expressed in-utero in skeletal muscle.[33] As arthrogryposis arises from fetal akinesia, the altered muscle contraction forces and decreased fetal movement provide a mechanism for congenital joint contractures.

In the three cases harbouring the p.R133W mutation in our series, the joint contractures were also most marked distally and mild trismus was present in two of our three patients. Our

patients had striking facial features and neck webbing reminiscent of Escobar variant MPS (EVMPS) and congenital anomalies . Neither patient described by Marttila et al[30] was reported to have an EVMPS phenotype. This may have been because of a predominantly neuromuscular phenotype or because the pattern of features was not present or sufficiently distinctive. Interestingly, the adult mother and daughter reported by Tajsharghi et al[31] presented with distal arthrogryposis and other features including hearing impairment, short stature, a scoliosis, proximal joint contractures, a high-arched palate, micrognathia and a short neck, in the EVMPS spectrum. There is a single report of EVMPS and a nemaline myopathy with complete absence of the skeletal muscle isoform due to a homozygous null mutation in *TPM2*.[34] Patient 1 was diagnosed with coeliac disease and patient 2 had isolated growth hormone deficiency. These may be coincidental findings however is possible that these complications are under-recognised in EVMPS as small stature may be considered part of the condition. Importantly, the arthrogryposis in patients 1 and 2 improved with physiotherapy however improving joint contractures in patients with the p.R133W mutation has previously been noted.[31]

The detection of identical *TPM2* mutations in patients with distal arthrogryposis and those with additional features more characteristic of EVMPS suggest the phenotype is modified by other genetic and environmental factors. Furthermore, the coexistence of arthrogryposis and weakness suggests differential tissue specific factors. A possible explanation may be the extent to which other tropomyosin isoforms form heterodimers with beta tropomyosin under certain circumstances, altering its physical properties and affecting its role in muscle contraction. Further study of *TPM2* mutations and variable phenotypes may identify of other important genetic modifiers and environmental factors and targets for future treatments.

De novo TPM2 mutations are likely to be under-recognised as MPS may be presumed to result from biallelic recessively inherited mutations and testing for autosomal dominant causes may not be considered . This report adds to our knowledge of the genetic heterogeneity underlying the EVMPS and broadens the phenotypic variability associated with the R133W mutation. Importantly, as *TPM2* is expressed in fetal and postnatal life, mutations may be associated with an evolving phenotype with improving joint contractures and possibly progressive weakness due to altered muscle kinetics over time. Therefore these individuals require follow up by a neuromuscular clinician as well as cardiorespiratory surveillance. Identification of an autosomal dominant *TPM2* mutation will also inform the recurrence risk for the parents and the proband.

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Figure legends

Fig.1a Case 1 age 6 years. Face i) periorbital fullness, broad nasal root and hypertelorism ii) bilateral finger contractures; age 10 years. Face iii) frontal view, iv) brachycephaly, low set posteriorly rotated ears and hearing aid v) mild trismus

Fig. 1b Case 2 age 3 months. Face i) hypertelorism, short downslanting palpebral fissures, small mouth ii) brachycephaly, low set posteriorly rotated ears and short neck; age 5 years. Face iii) downslanting palebral fissures, small mouth and neck webbing iv) flat facial profile and low set ears and arthogryposis of the v)lower limbs and vi) hands

Fig 1c Case 3. i), Posterior view of low hairline, neck webbing and kyphoscoliosis, arthrogryposis ii) Lower limb athrogryposis and decreased muscle bulk iii) Feet with full heels and iv) Hand and finger contractures.

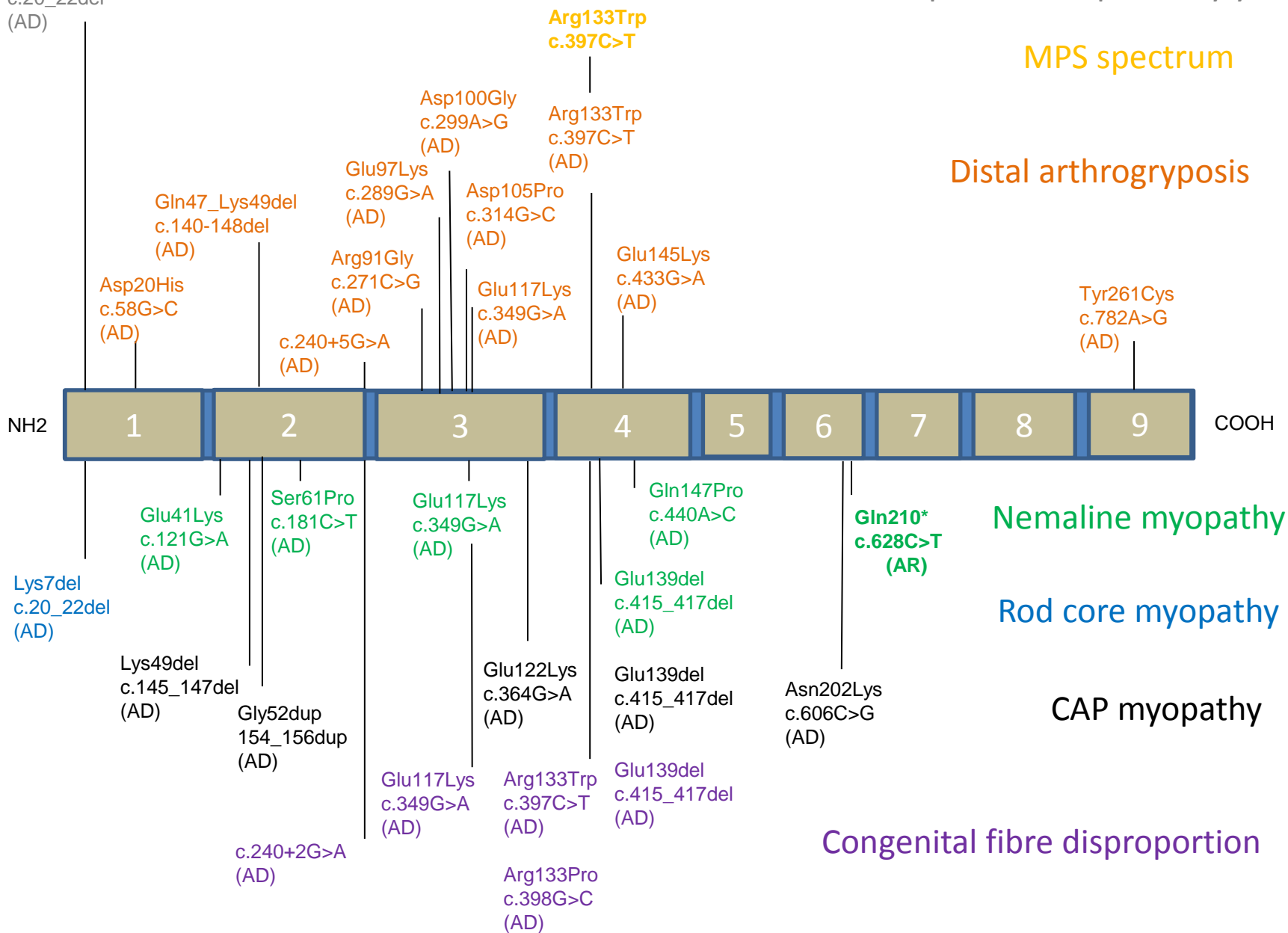Figure 2 Schematic of reported *TPM2* mutations

Table 1 Clinical features with a sequence change affecting the amino acid at position 133 summarised with published cases

Lys7del
c.20_22del
(AD)

Trismus pseudocamptodactyly

Arg133Trp
c.397C>T

MPS spectrum

Asp100Gly
c.299A>G
(AD)

Arg133Trp
c.397C>T
(AD)

Glu97Lys
c.289G>A
(AD)

Asp105Pro
c.314G>C
(AD)

Distal arthrogryposis

Gln47_Lys49del
c.140-148del
(AD)

Arg91Gly
c.271C>G
(AD)

Glu117Lys
c.349G>A
(AD)

Glu145Lys
c.433G>A
(AD)

Tyr261Cys
c.782A>G
(AD)

Asp20His
c.58G>C
(AD)

c.240+5G>A
(AD)

NH2 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | COOH

Glu41Lys
c.121G>A
(AD)

Ser61Pro
c.181C>T
(AD)

Glu117Lys
c.349G>A
(AD)

Gln147Pro
c.440A>C
(AD)

Gln210*
c.628C>T
(AR)

Nemaline myopathy

Lys7del
c.20_22del
(AD)

Glu139del
c.415_417del
(AD)

Rod core myopathy

Lys49del
c.145_147del
(AD)

Glu122Lys
c.364G>A
(AD)

Glu139del
c.415_417del
(AD)

Asn202Lys
c.606C>G
(AD)

CAP myopathy

Gly52dup
154_156dup
(AD)

Glu117Lys
c.349G>A
(AD)

Arg133Trp
c.397C>T
(AD)

Glu139del
c.415_417del
(AD)

c.240+2G>A
(AD)

Arg133Pro
c.398G>C
(AD)

Congenital fibre disproportion

Appendix S1, Supporting Information

Molecular methods

DNA was collected from blood samples using a standard extraction methodology. Targeted resequencing was performed using Illumina TruSightTM Rapid Capture kit to cover coding sequences from 4813 clinically relevant genes from the TruSightOne panel. Using Nextera library preparation technology, genomic DNA was fragmented and tagged prior to multiplex pre-enrichment sample pooling, an average 400bp fragment library size was achieved and a final pooled library concentration of 8-12Pm was assembled. For the assessment of the genomic library quality, 2100 Bioanalyzer tools (Agilent Technology) were used. Sequencing was performed on NGS Illumina's HiSeq2500 analyser employing a pair-end 150-cycle sequencing run. Bioinformatics analysis and annotation of rare genetic variants was undertaken. Any variant reported >2% heterozygote frequency in 1,000 genomes project and variant exome server data (www.1000genomes.org/ and http://evs.gs.washington.edu/EVS/ respectively) was removed. Sequence data was inspected in a total of 86 candidate genes of potential relevance to MPS-related phenotypes. Variants identified as candidate pathogenic variants were confirmed by Sanger sequencing in the proband sample and segregation was checked in DNA from other available family members. PCR products were sequenced in forward and reverse orientations using a standard sequencing method (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems®).

Clinical details

Case 1

This female patient was the second child of unrelated white parents. From 34 weeks of pregnancy there was poor growth and reduced fetal movements. She was born at 41 weeks of pregnancy, birth weight 2.944 kg (13 percentile), length 43 cm (< 0.4 percentile) and head circumference 33.5 cm (25 percentile). Poor feeding and almost 10% weight loss of after birth weight required admission. Multiple joint contractures were present with ulna deviation and mild restriction of wrist movement, flexion contractures of fingers 2-5 and paucity of palmar creases. She had Bilateral congenital talipes equinovarus with full heels, full knee flexion but lacked 30 degrees of extension in her lower limbs. Her muscle bulk appeared normal with normal power in both quadriceps. Facially she had brachycephaly, periorbital fullness, a slightly broad nasal root and a smooth philtrum. She had a small mouth with slightly reduced mouth opening. She had a low posterior hairline. She required removal of a left brachial cyst remnant and grommets (see Figure 1).

On review at aged 10 years her weight was 23.2 kg (2 percentile), her height was 129 cm (9 percentile) and her head circumference was 50.8 cm (0.4 - 2 percentile). Fixed flexion contracture of her right 3rd finger proximal interphalangeal joint, her speech was slightly indistinct and nasal, and she had bone anchored hearing aids for bilateral conductive asymmetric hearing loss. Educational classroom support was in place in school because of difficulty retaining information. She was diagnosed with coeliac disease and commenced on a gluten free diet Prior *CHRNG* analysis was normal.

Case 2

The male proband is the second child of unrelated white parents. Antenatally there was polyhydramnios, decreased fetal movements, a right sided pleural effusion, bilateral talipes equinovarus and clenched hands. He was born by Caesarean section at 37 weeks, birth weight 3.34 kg (32 percentile) and head circumference 36 cm (83 percentile). Nasogastric tube feeds were required for the first 4 weeks of life. On examination he had brachycephaly and dysmorphic features with mild hypertelorism and short downslanting palpebral fissures, a small mouth, a high palate, micrognathia, small low set posteriorly rotated ears, a short neck with mild neck webbing and widely spaced nipples (see Figure 2). Multiple joint contractures with ulna deviation, camptodactyly of fingers 2-5 and bilateral talipes equinovarus were present. There was good muscle bulk in the thighs but small calves. He had a small secundum atrial septal defect, a left inguinal herniaand bilateral undescended testicles.

On review at the age 5 years his height and weight were around the 2 percentile and his head circumference was between the 2-9 percentiles. His diet was supplemented with high calorie milk and he a laxative for constipation. He had recurrent chest infections. Psychomotor development was delayed with walking achieved at 19 months however he could only manage short distances before tiring. He was toe walker with tightness of the ankles joints. He had mild fixed flexion deformities of elbows and knees and limited shoulder abduction on right. In the upper limbs windswept fingers with residual flexion contractures of fingers 2-5 were present however there was good hand function. Speech improved with speech and language therapy and he was making progress with classroom support at school. He wore glasses because of astigmatism and a right sided squint.

By aged 9 years his height velocity had tailed off to 4 cm/year and investigations showed a delayed bone age, an IGF1 of 14nmol/l, at the lower end of normal and a suboptimal growth

hormone peak of 3.9 mcg/L on an arginine stimulation test. Isolated growth hormone deficiency was confirmed on a glucagon stimulation test and growth hormone treatment commenced. On examination at 10 years of age his weight was 20.2 kg (< 0.4 percentile), height was123.4 cm (1 percentile) and head circumference was 51.4 cm (3 percentile. He had limited mouth opening and teeth erupting on the hard palate. There was chest wall asymmetry and a mild pectus excavatum., restriction of neck movement and the thoracic spine was slightly stiff. In the upper limb there was asymmetry of the scapulae but no winging and a good range of shoulder movement. There were dimples and fixed flexion of both elbows with reduced pronation and supination and fixed flexion of the wrists. His fingers were flexed with pseudocamptodactyly and he had fixed flexion of the left index fingers with webbing across joint. In the lower limb his hips and knees were very mobile and he had a fixed hindfoot equinovarus bilaterally and a forefoot adductus. He walked on his tip toes with an equinus posture loading the lateral border of the feet and a midfoot break

An MRI showed a small arachnoid cyst in the left middle cranial fossa, right occipital plagiocephaly and skull base dysplastic with a vertically orientated clivus. Postnatal CK and baseline metabolic screen were normal. Karyotyping, *MYH3* genetic testing exons 5, 8-11, 14-21 and 33, *CHRNG*, *RAPSN* and *DOK7* genetic testing was normal.

Case 3

This male patient was born to non-consanguineous South American parents. Reduced fetal movements were noted towards the end of pregnancy. His birth weight was 2.83 kg (5 percentile) and there were no neonatal problems. He required repair of bilateral inguinal herniae and bilateral tendon releases between his 1st and 2nd fingers of both hands and feet.

He also had release of a tongue tie. At age 6 years he was short with poor muscle bulk. His height was on the 0.4 percentile, his weight below the 0.4 percentile and his head circumference between the 0.4 -2 percentile. There were no other concerns about his health or development. Facially he was hyperteloric with down-slanting palpebral fissures and low set ears. He had a low posterior hairline and neck webbing (see Figure 3). He had sloping shoulders and flexion contractures of his, elbows, wrists and fingers, with ulna deviation at the metacarpalphalangeal joints and soft tissue syndactyly of fingers 2-5 and flexion contractures affecting his hips, knees and ankles. He had a mild thoracic kyphoscoliosis and eleven pairs of downward sloping ribs

On x-ray there was bilateral forefoot valgus deformities and hindfoot valgus. There was bilateral coxa vara. *CHRNG* genetic analysis was normal.