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A novel nonsense *PIEZO2* mutation in a family with scoliosis and proprioceptive defect

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Abstract

PIEZO2 mutations have been described in dominant arthrogryposis, but homozygous mutations of *PIEZO2* may also be responsible for more complex clinical patterns, associating distal arthrogryposis, neonatal respiratory insufficiency, scoliosis and proprioceptive impairment. We report here two sisters presenting with these clinical and genetic features. They had a similar phenotype, with severe hypotonia and respiratory distress at birth, delayed acquisition of motor milestones and need of scoliosis surgery. Hypotonia and alteration of proprioception were at the forefront of clinical examination for both, along with areflexia, hyperlaxity, *cutis laxa*, and discrete facial dysmorphy. Electrophysiological studies, including electroneuromyography and sensory evoked potentials, showed a mild sensory axonopathy without any myopathic features, but revealed a peripheral proximal lemniscal defect. Creatine kinase, muscular MRI and biopsy were normal, as well as cerebral MRI and neurometabolic biological explorations. They had a moderate restrictive syndrome on respiratory function tests and cardiac function was normal. Molecular studies performed on a panel of genes involved in distal arthrogryposis disclosed a nonsense homozygous c.3241C > T (p.Arg1051*) mutation in the *PIEZO2* gene, which was also present at the heterozygous state in their mother's DNA. This new *PIEZO2* mutation was in accordance with the phenotype combining arthrogryposis, scoliosis, hyperlaxity and proprioceptive impairment.

Keywords: PIEZO2; Arthrogryposis; Proprioceptive impairment.

1. Introduction

Arthrogryposis is defined by reduced mobility of multiple joints and highly heterogeneous, with more than 400 genes described. Arthrogryposis can be of both dominant and recessive inheritance, and associated with other clinical features, namely orthopedic malformations, facial deformities, central nervous system (CNS) abnormalities, peripheral neuropathy or myopathy [1]. Among these genes, heterozygous missense *PIEZO2* mutations have been associated with either distal Gordon Syndrome (Distal arthrogryposis (DA) type 3 or DA type 5 or Marden-Walker Syndrome (MWS) [5]. Later on, homozygous nonsense mutations in the *PIEZO2* gene have been held responsible for arthrogryposis, scoliosis and, strikingly, proprioception defects [6], neonatal respiratory insufficiency [7,8] and muscle weakness [9].

PIEZO polypeptides are mechanosensitive transmembrane cation channels [2,3]. They are expressed in several mechanosensitive tissues, such as neurons of the dorsal root ganglia, endothelium and visceral tissues, including lungs. Mouse models lacking PIEZO2 in mechanosensory neurons showed impaired limb coordination, and unstable gait [4].

We describe here the cases of two sisters prominently characterized by distal arthrogryposis and proprioception loss; a clinical phenotype which led us to find a novel nonsense homozygous mutation in the *PIEZO2* gene.

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2. Materials and methods

2.1. Patients

We report the cases of two twin sisters of Tunisian origin, followed from age 20 to 24 years (Patient II.3 and II.4), the first one (Patient II.3) at the Neuromuscular Reference Center of Pitié Salpêtrière Hospital (Paris) and the other (Patient II.4) at the Neuromuscular Reference Center in the University Hospital of Bordeaux.

2.2. Clinical, electrophysiological and muscle magnetic resonance imaging features

Both sisters have undergone clinical examination, extended neurophysiological evaluations, including electroneuromyography (ENMG) and sensory evoked potentials (SEP). Routine biological analyses, including lysosomal enzymes and vitamin dosages, have been performed, as well as respiratory function tests, cardiac explorations (Patient II.3 and II.4), muscle Magnetic Resonance Imaging (MRI) (Patient II.3) and biopsy (Patient II.3 and II.4).

2.3. Genetic studies

All samples were processed after informed consents for molecular diagnosis had been obtained from the patients. Mutations in the probands' DNA have been screened on a panel of eleven genes involved in distal arthrogryposis. The design of the gene panel was realized with the AmpliSeq Designer tool (Thermo Fischer Scientific) and libraries for Next Generation Sequencing (NGS) were prepared according to manufacturer recommendations and analyzed on IonTorrent PGM instrument (Thermo Fischer Scientific). The coverage for the *PIEZO2* gene (NM_022068) was 99.3% with a mean depth of 390X (ranging from 34 to 1486). Results of the sequencing were processed with the Torrent Suite 5.0 (Thermo Fischer Scientific) and annotation of the variants performed with a homemade script based on the Variant Effect Predictor tool [10]. The presence of the mutation was controlled by Sanger sequencing using specific primer designed to amplify exon 22 of the *PIEZO2* gene and an ABI PRISM Big Dye Terminator Cycle Sequencing (V3.0) reaction kit with an analysis on an ABI 3130 DNA Analyzer (Thermo Fischer Scientific).

3. Results

3.1. Clinical, electrophysiological and muscle MRI features

Our 2 patients were born from healthy parents, who were not known to be consanguineous. They had 5 siblings, including one sister (II.2) who died at six years of age, with similar symptoms. Their father died in a car accident at age 32 (Fig. 1).

Pregnancy was uneventful, but the 2 sisters were severely hypotonic at birth and experienced transient neonatal respiratory failure. Later on, they displayed delayed acquisitions of motor skills and started walking independently at 5 (Patient II.4) and 7 (Patient II.3) years only. From the onset of the disease, gait was very unstable, and a wheelchair was needed early on during childhood. They also had severe scoliosis, requiring arthrodesis in both cases.

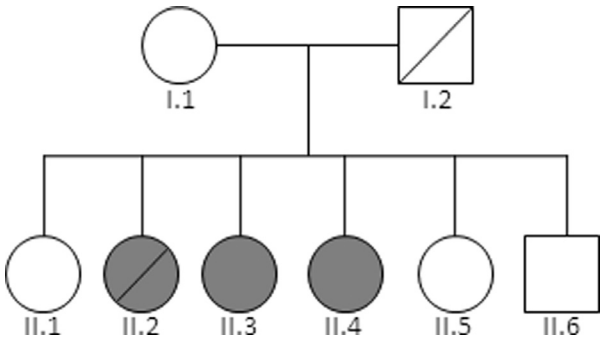

Fig. 1. Family pedigree.

There was no cognitive impairment, and both sisters were able to pursue higher studies.

On examination, at age 20, hypotonia was still at the fore. Both patients displayed diffuse distal wasting along with muscular deficit and discrete fingers and wrist retractions, compatible with arthrogryposis. Hyperlaxity was noticed in multiple joints. Proprioceptive abnormalities were major, with diffuse apallesthesia, positive Romberg sign, and unsteady wide-based gait, as well as dysmetria appearing with eyes closed. Tendon reflexes were abolished in the four limbs. There was no facial involvement, no cerebellar ataxia, no pyramidal or bulbar syndrome, and no ocular motor impairment. Patient II.3 also displayed *cutis laxa* (Fig. 2)

ENMG showed a mild sensory axonal neuropathy in patient II.3 and was normal in patient II.4. There was no myopathic feature in both sisters. SEP revealed bilateral impairment of lemniscal sensory pathways for both (Fig. 3). Cerebral and medullar MRI were normal, apart from the scoliosis. Muscular MRI showed no abnormality in patient II.3. Muscular biopsy was normal for patient II.3 and showed mild muscular abnormalities for patient II.4: type 2 fiber predominance and rare isolated concentric lamellar bodies.

Respiratory function tests revealed a mild restrictive syndrome. The forced vital capacity was 46% (Patient II.3) and 69% (Patient II.4) of predicted value. No other functional studies were realized to assess diaphragmatic function.

Echocardiography and electrocardiogram were normal.

3.2. Molecular findings

Patient 1 was first screened for the *FKBP14* gene, known to be responsible for Ehlers-Danlos Syndrome with progressive kyphoscoliosis and myopathy [11]. No mutation was found, and DNA of the patient was then screened for genes involved in distal arthrogryposis on a gene panel with an IonTorrent PGM instrument. The mean coverage for a minimal depth of 30X for the 11 genes explored was 92.45%. The analysis of SNP with an allelic frequency under a threshold of 0.5% in the EXAC database identified only

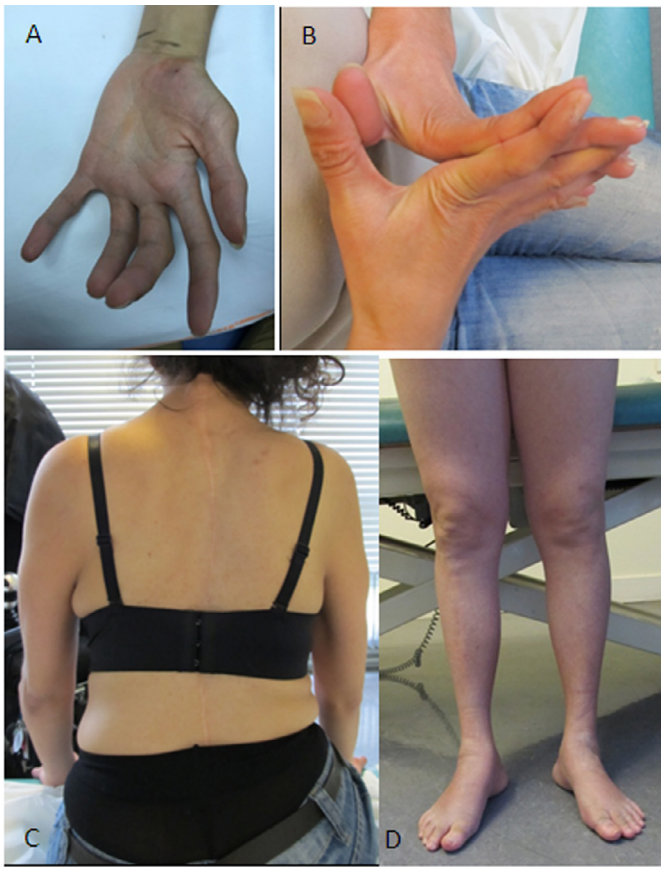

Fig. 2. Patients clinical features. Both patients displayed distal wasting and arthrogryposis (A and D), hyperlaxity (B) and severe scoliosis (C).

the c.3241C > T;p.Arg1051* variation in the exon 22 of the *PIEZO2* gene at the homozygous state. This variation resulted in a premature STOP codon in exon 22 and in a biallelic loss-of-function of the *PIEZO2* gene. The segregation of the variation performed on the DNA of the probands' mother and sister showed compatibility with a recessive mode on inheritance. The father's DNA was not available.

4. Discussion

A recessive condition associating arthrogryposis and a proprioception defect with transient respiratory failure, scoliosis, hypotonia, hyperlaxity is highly evocative of *PIEZO2* biallelic loss-of-function mutation, as established by previously published works [6–9] (Table 1). The clinical phenotype of our two patients, encompassing these features, led us to a specific DNA sequencing targeting the *PIEZO2* gene, and allowed us to identify a novel homozygous mutation, without using whole exome or mendeliome sequencing as previously described [6–9]. It is worth noticing that our two patients, although most of our symptoms were similar, displayed some phenotypical differences: age of motor skills achievement, ENMG and biopsy findings. The fact that they were followed in two different centers could account for those findings, this might suggest that there is some variability in the clinical expression of the disease.

4.1. Two types of PIEZO2 *mutations inducing two different phenotypes*

PIEZO2 gene mutations are particularly interesting and may cause two very distinguishable phenotypes according to the type of mutation and their mode of inheritance. Homozygous recessive mutations lead to a loss of function of the gene, whereas heterozygous dominant mutations cause a gain of function. Consequently, those two mechanisms induce very different conditions: although they share some clinical characteristics, namely arthrogryposis and scoliosis, there are distinguished by the presence of severe alterations of proprioception in the biallelic *PIEZO2* whereas the heterozygous mutations correspond to a purer form of distal arthrogryposis, such as Gordon Syndrome, Marden-Walker Syndrome, or Distal Arthrogryposis Type 5 [2,5].

4.2. PIEZO2 *and proprioception*

So far, mutations of PIEZO2 seem to be the only possible genetic cause of proprioception impairment with arthrogryposis. In humans, specialized mechanosensory neurons called proprioceptors convey information about the stretch and tension experienced by muscles, tendons, skin, and joints. PIEZO polypeptides are mechanosensitive cation channels and are activated in response to mechanical displacement of the cell surface membrane [3].

PIEZO2 is expressed, in particular, at the endings of somatosensory neurons, which convey information about tension and stretch experienced by joints, skin, muscles and tendons [4,8]. Mice models have evidenced that PIEZO2 is responsible for the transduction of rapidly-adapting mechanically-activated currents in the neurons of the dorsal root ganglia [2]. These findings explain why PIEZO2 loss-of-function mutations cause a very specific phenotype with a characteristic proprioceptive defect

The mild axonal neuropathy observed in Patient II.3 seems to be a frequent characteristic of patients with biallelic PIEZO2 mutations [7–9], although not explaining the proprioceptive defect in itself.

4.3. Possible mechanisms for other clinical features encountered in PIEZO2 *mutations*

It has been suggested that *PIEZO2* mutations may cause dysregulation of mechanotransduction affecting both cartilage constitution [12] and development of skeletal muscles [5] possibly leading to arthrogryposis and orthopedic abnormalities.

As a matter of fact, in patient 2, muscle biopsy revealed non-specific mild myopathic features, findings that have been described previously in recessive *PIEZO2* mutations [9].

Respiratory impairment has also been described and is at least partially explained in adulthood by severe scoliosis and diaphragmatic weakness, whereas in cases of neonatal respiratory failure, it is more likely the consequence of diaphragmatic weakness only. Alternatively, a role for PIEZO2 was

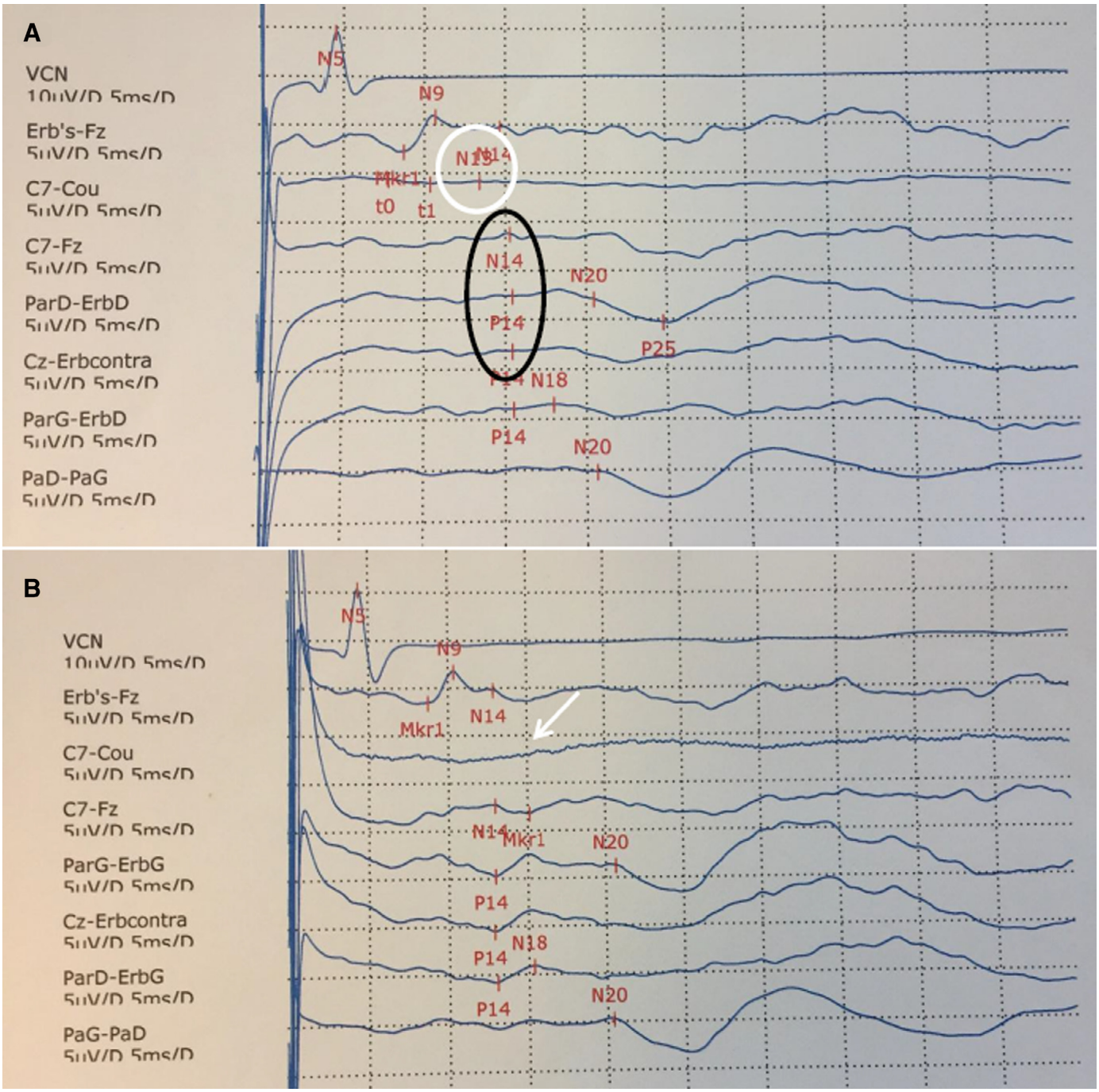

Fig. 3. Sensory evoked potentials in the upper limbs of Patient 1. Peripheric conduction was normal (N5). N13 amplitude was reduced on the left upper limb (A, white circle) and absent on the right (B, white arrow). Bulbar components (N/P14—black 294 circle) were reduced and latency of cortical components (N9-N20) were extended, indicating bilateral impairment of the lemniscal pathways.

suggested as a mechanosensor required for correct lung development [13].

4.4. A unique cause for proprioceptive defects and arthrogryposis

Hereditary proprioceptive defect is caused by numerous hereditary sensory neuropathies but usually with severe axonal neuropathy. It has much more rarely been described when caused by proximal lemniscal pathways impairment. Neurometabolic diseases such as Friedreich ataxia, Vitamin E deficiency or hypobetalipoproteinemia may show posterior cord involvement on SEPs, but patients also display central nervous system symptoms [14,15]. A case of hereditary sensory ataxic neuropathy has been reported in a Japanese family with a mild sensory axonal neuropathy but significantly prolonged central conduction time on SEPs. Patients were not screened for *PIEZO2* mutations, but, unlike ours, their sensory

Table 1
Genetic and clinical characteristics of previously published recessive mutations in the *PIEZO2* Gene.

	Clinical features	Mutation
Delle Vedove et al. [7]	10 individuals, 4 families Arthrogryposis, spontaneously resolving respiratory insufficiency at birth, muscular atrophy > distal lower limbs, scoliosis, mild sensory involvement	- c.5621delT (p.Leu1874Argfs*5) homozygous - c.3019_3029del(p.Pro1007Leufs*3) homozygous - c.1550_1552delGCTinsCGAA (p.Ser517Thrfs*48) homozygous - c.493-?_917 + ?del (p0?) homozygous
Chelser et al. [8]	2 subjects, 2 families Scoliosis, hypotonia and shallow breathing during infancy, unsteady gait with proprioceptive defect	- c.4723 > T (p.R1575*)/ c.5053C > T (p.R1685*) - c.5054G > C (p.R1685P)/ c.5053C > T (p.R1685*)
Haligolu et al. [9]	Hypotonia, distal laxity, contractures, muscle weakness, scoliosis, proprioceptive defect	c.1384C > T, (p.R462*) homozygous
Mahmud et al. [6]	Scoliosis, contractures involving distal joints, proprioception and touch sensation defects	c.2708C > G; p.S903* homozygous

complaints went beyond sole ataxia, proximal muscle weakness was systematic, age at onset was much older (after 40 years), and there was neither scoliosis nor respiratory failure [16].

More recently, mutations in *RFN170* coding for the Ring Finger Protein 170 have been described, responsible for progressive sensory ataxia linked to degeneration of the posterior columns of the spinal cord. Patients with *RFN170* mutations had, however, a progressive, isolated, form of ataxia predominating in the legs; they were older, and inheritance was dominant [17]. Mutations in the *FLVCR1* gene also cause posterior column ataxia, sometimes associated with scoliosis and *retinis pigmentosa*, but ENMG reveals a ganglionopathy [18].

Finally, secondary arthrogtyposis may occur in several neuromuscular disorders, and thus could be associated with conditions causing proprioceptive defects and neuropathy. However, in those cases, it tends to appear later on in the course of the disease than in our patients.

PIEZO2 is a unique gene with two very distinct phenotypes corresponding to the inheritance mode. As heterozygous dominant mutations are responsible for arthrogryposis multiplex congenita, *PIEZO2* biallelic mutations have a typical clinical presentation, with scoliosis and proprioceptive deficit as main symptoms, together with arthrogryposis, hypotonia and transient neonatal respiratory failure. The role of *PIEZO2* as a mechanosensitive receptor in somatosensory neurons and its implication in bones and joint development as well as lung development probably intervene in the occurrence of those symptoms. The association of these clinical features is highly evocative and should prompt practitioners to screen *PIEZO2* gene, as few other disorders display the same characteristics.

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