Dominant and recessive central core disease associated with RYR1 mutations and fetal akinesia

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Summary
We studied seven patients (fetuses/infants) from six unrelated families affected by central core disease (CCD) and presenting with a fetal akinesia syndrome. Two fetuses died before birth (at 31 and 32 weeks) and five infants presented severe symptoms at birth (multiple arthrogryposis, congenital dislocation of the hips, severe hypotonia and hypotrophy, skeletal and feet deformities, kyphoscoliosis, etc.). Histochemical and ultrastructural studies of muscle biopsies confirmed the diagnosis of CCD showing unique large eccentric cores. Molecular genetic investigations led to the identification of mutations in the ryanodine receptor (RYRI) gene in three families, two with autosomal recessive (AR) and one with autosomal dominant (AD) inheritance. RYRI gene mutations were located in the C-terminal domain in two families (AR and AD) and in the N-terminal domain of the third one (AR). This is the first report of mutations in the RYRI gene involved in a severe form of CCD presenting as a fetal akinesia syndrome with AD and AR inheritances.

Keywords: central core disease; fetal akinesia; RYRI mutations

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; CCD = central core disease; dHPLC = denaturing high-performance liquid chromatography; MHS = malignant hyperthermia susceptibility; RYR = ryanodine receptor

Introduction
Central core disease (CCD) was the first congenital muscle disorder described involving structural changes of the muscle fibres (Shy and Magee, 1956; Greenfield et al., 1958). It is generally considered as one of the most frequent congenital myopathies (Fardeau and Tomé, 1994). The clinical phenotype is classically described as relatively benign and non-progressive with mild hypotonia during early childhood, delayed motor milestones, diffuse and moderate muscle weakness and gracility, frequent spinal deformities, hip dislocation, arched feet and pectus excavatus. A large phenotypic variability has been demonstrated from the early descriptions, but mainly to emphasize the frequency of mild, almost asymptomatic forms (Fardeau and Tomé, 1994).

The histological hallmark of this disorder is the presence of well-limited rounded areas of abnormal myofibrillar architecture, with a variable degree of sarcromeric disorganization and absence of mitochondria, allowing an easy detection of the cores on oxidative enzyme stainings in transverse cryostat sections. Several morphological aspects of the cores have been described in CCD patients: classical or typical ‘central cores’, ‘eccentric cores’ and variants associating single or multiple ‘peripheral or central cores’ in the same muscle fibre (Monnier et al., 2001; De Cauwer et al., 2002). In all cases, the cores had abrupt borders with the normal regions of the muscle fibres, and they extended almost along the full length of the fibre.

CCD has been considered to be generally transmitted according to an autosomal dominant (AD) inheritance. Linkage analysis (Haan et al., 1990) mapped the locus to chromosome 19q13, and association with malignant hyperthermia susceptibility (MHS) subsequently led to the identification of mutations in the ryanodine receptor gene (RYRI) for ~40% of CCD families (Lynch et al., 1999; Monnier et al., 2000, 2001). The majority of the CCD mutations have been found in the C-terminal part of the protein (Lynch et al., 1999; Monnier et al., 2000, 2001; Tilgen et al., 2001; Davis et al., 2003), and functional studies have confirmed the pathogenic role of the RYRI mutations in some of them (Lynch et al., 1999).
An autosomal recessive (AR) inheritance of RYR1 mutations was shown recently in two unrelated families (Ferreiro et al., 2002; Jungbluth et al., 2002). In the last 20 years, from a total series of 70 CCD families, we have studied seven cases of CCD, belonging to six families, presenting with a fetal akinesia syndrome. Histopathological analysis showed large eccentric unstuctured cores in the muscle fibres. Molecular genetic studies allowed the identification of mutations in the RYR1 gene in three out of the four families for which the samples were available for extensive molecular studies. Three patients from two unrelated AR families were compound heterozygous and one patient from an AD family was heterozygous, supporting an involvement of the RYR1 gene in fetal akinesia associated with CCD.

**Subjects and methods**

We analysed the clinical, histochemical, ultrastructural and genetic data of seven patients (fetuses/infants) from six unrelated families. Eight muscle biopsies (case 1 from family I had two muscle biopsies) were studied with histochemical and ultrastructural techniques. The molecular analysis of the RYR1 gene could only be performed in the four families whose DNA or RNA samples were available. This study was authorized by the ethical committee of Pitié-Salpêtrière Hospital (CCPRPB) and the DRC of the Assistance Publique, Hôpitaux de Paris.

**Subjects**

**Family I, cases 1 and 2**

This was a non-consanguineous family with one affected child and one affected fetus who died at 32 weeks of gestation age. There were no neuromuscular familial antecedents. The mother was completely asymptomatic and the father presented a discrete facial hypomimia without any skeletal muscle weakness. Open deltoid muscle biopsies were performed in both parents and did not show any structural abnormality (Fig. 1).

Case 1 was a boy born at 37 weeks. A hydramnion was noted during pregnancy; a normal chromosome chart was established by amniocentesis. At birth: weight, 2500 g; head circumference, 34.5 cm; Apgar score, 6/8; immediate respiratory distress requiring mechanically assisted ventilation. Complete generalized hypotonia, absence of spontaneous movements, thin muscular masses and facial hypomimia...
were the major clinical features. Multiple malformations were present: short femurs, facial dysmorphism with retrognathia and hypotelorism, and bilateral clinodactyly of the second and fifth fingers. During the first 6 months of life, he showed complete respiratory dependence and needed permanent ventilation through a tracheotomy. Awakening was normal, but severe global hypotonia persisted. During infancy, he developed a craniostenosis and an early kyphoscoliosis; spinal MRI showed a vertebral malformation in D4–D5 without medullar compression.

At 2 years, the tracheotomy-assisted ventilation was continued in hospital care. The infant improved his motor development but with persistence of severe muscular weakness and amyotrophy: he was able to sit without assistance at 22 months of age and to move in a sitting position from 28 months of age. Scoliosis was corrected by an orthopaedic corset and physiotherapy. In spite of the tracheotomy, he acquired language towards the age of 3 years although facial weakness was still present. He progressively developed a bilateral ptosis and strabismus (Fig. 2). Muscle biopsies were performed at 15 days and 1 year of life (Fig. 1).

From 2 to 9 years of age, partial respiratory autonomy was acquired allowing diurnal weaning. He had good thoracic growth, indicating that closure of the tracheotomy could be planned in the years to come. There was good control of the deformation of the rachis thanks to orthopaedic and physical care, but his muscles remained severely atrophic. There was a normal growth of the cranial perimeter and a normal echocardiographic follow-up. He started walking with assistance at 5 years of age, and without assistance 6 months later. Normal schooling took place successfully.

Case 2, the younger brother of case 1, was an affected male fetus who died at 32 weeks of gestation. Frequent echography surveillance was performed during the pregnancy, since the family had an antecedent of severe CCD congenital myopathy. During the second and third trimester of pregnancy, large hydramnion, absence of fetal movements and multiple malformations were noted. An interruption of the pregnancy was performed in accordance with ethical regulations, and a muscle biopsy was analysed.

**Family II, case 3**

This was a non-consanguineous family with one affected female child and one non-affected male child. There were no neuromuscular antecedents. The parents were asymptomatic.

During the pregnancy, at 36 weeks of gestation, poor fetal movements and hydramnion were noted. At 37 weeks, a Caesarean section was performed due to a breech presentation. At birth, the infant presented global hypotonia with frog position, no spontaneous antigravity movements and multiple arthrogryposis with left hip and knee blocked, bilateral valgus feet, adductus thumb and left femur fracture. A dysmorphic face with retrognathism was associated. Permanent ventilatory assistance was necessary during the first month of life. She presented serious swallowing difficulties and was kept at the hospital until 5 months of age. A muscle biopsy was performed at 2 months of age (Fig. 3). During the first year of life, there were persistent hypotonia, weakness and muscular hypotrophy; swallowing function improved. From an early age, she developed bilateral ptosis and strabismus. Intensive orthopaedic care and physical therapy were performed during infancy. At 5 years of age, she could crawl on the floor but not walk unsupported. A normal schooling was adapted successfully.
Family III, case 4
This was a non-consanguineous family with one affected girl presenting with a severe form of CCD. The mother, a 17 year old, presented with a classic form of CCD. After a high-risk pregnancy and premature delivery, the child was born at 34–35 weeks of gestation with a weight of 1860 g. She presented at birth with severe hypotonia, thin ribs, swallowing difficulty, respiratory distress and cyanosis needing mechanical assistance from the second day of life. A muscle biopsy was performed at 8 days of life. She needed permanent mechanically assisted ventilation, and died at 8 days of age.

Family IV, case 5
This was a non-consanguineous family with one affected boy born after Caesarean section. Permanent respiratory assistance was needed from birth until death at 10 months of life. A muscle biopsy was performed at 10 months of life.

Family V, case 6
This was a non-consanguineous family with one affected girl. Hydramnion and fetal akinesia were noted during gestation. At birth, she was diagnosed as having a Pena–Shokeir syndrome, with multiple arthrogryposis, facial dysmorphism, microretrognathia, diffuse amyotrophy and lung hypoplasia. She needed respiratory assistance from birth, and died at 1 month of age (Fig. 4). A muscle biopsy was performed at 1 month of age.

Family VI, case 7
This was a non-consanguineous family with one affected fetus presenting with multiple arthrogryposis detected by echography, facial malformations and diffuse amyotrophy. An interruption of the pregnancy was performed at 30–31 weeks of gestation, in accordance with ethical regulations, and a muscle biopsy was performed.

Histopathological studies
Skeletal muscle biopsies were analysed in seven patients (fetuses/infants) and in the two parents from family I. Parts of each muscle biopsy were frozen immediately in isopentane cooled in liquid nitrogen and stored at −80°C until processing, and another specimen was fixed for ultrastructural analysis. For three patients, a small specimen was frozen immediately in liquid nitrogen for RNA extraction. Histo-enzymological studies were carried out on 10 μm transverse cryostat sections according to protocols described previously (Romero et al., 1993). For the two dead fetuses (cases 2 and 7), immediate post-delivery muscle biopsies were taken.

Molecular genetic studies
Haplotyping analysis
A haplotyping study was performed in family I as described previously (Monnier et al., 2000) in chromosome 19q13.1 that includes the RYR1 gene. The following markers were used: D19S220, D19S909, RYR1, D19S422 and D19S417.

RYR1 mutation screening
Total RNA was extracted from frozen muscle specimens using a guanidium thiocyanate–phenol–chloroform method. First-strand cDNA was synthesized from total RNA using specific primer mixes and Long Expand Reverse Transcriptase (Roche, Switzerland) and then amplified in 31 overlapping fragments using Taq Plus Precision Polymerase (Stratagene, La Jolla, CA). The amplified products spanning the entire RYR1 cDNA subsequently were purified and directly sequenced.
When muscle samples were unavailable, RYR1 mutation screening was performed on genomic DNA extracted from blood samples using standard procedures. Exons 92–106 coding for the calcium channel domain and exons involved in the MH1 and MH2 domains were analysed by denaturing high-performance liquid chromatography (dHPLC). Exons showing a variation were then sequenced.

**Mutation analysis**

Mutation analysis was performed in the probands’ families and on 100 chromosomes from the general population. The G215E and G4899E mutations were screened, respectively, using dHPLC analysis of exon 8 and exon 102. BstUI and BstNI analysis of exon 95 and exon 97 was used to screen for the L4650P mutation and the K4724Q mutation, respectively. Both mutations created a restriction site. Fragment sizes obtained after enzymatic digestion were analysed by electrophoresis on acrylamide gels.

**Results**

**Clinical and histopathological data**

All patients (four males and three females) presented with a fetal akinesia syndrome and hydramnion during pregnancy. Two fetuses died after interruption of the pregnancy at 32 and 30–31 weeks of gestation, and five children were born with severe hypotonia and arthrogryposis. Among the children living at birth, three died at an early age (8 days, 30 days and 10 months of life) and two are still alive, at 5 and 9 years of age, after a long period of intensive care and respiratory assistance (cases 1 and 3). In spite of the severe hypotonia and hypotrophy, failure to thrive and severe malformations at birth (multiple arthrogryposis, congenital dislocation of the hips, severe hypotonia, skeletal and feet deformities, and kyphoscoliosis), the two surviving children improved their motor milestones and had a normal intellectual development. The muscular weakness appeared to be ‘non-progressive’; there was no cardiac involvement and the respiratory capacities were acceptable for their respective ages. It should be noted that, from an early age, bilateral ptosis and strabismus was observed in both surviving patients (Fig. 2). Clinical features are summarized in Table 1.

Skeletal muscle biopsies were taken from all cases. The age at muscle biopsy ranged from 30–31 weeks of gestation to 1 year of age (Table 1). The diagnosis of CCD was shown on transverse muscle cryostat sections, which showed large, eccentric, well-limited areas devoid of any oxidative activity. The most frequent pattern was ‘unique large eccentric cores’ in most muscle fibres (Figs 1, 3 and 4). In all cases, a
<table>
<thead>
<tr>
<th>Family and heredity</th>
<th>Patient number</th>
<th>Pregnancy symptoms</th>
<th>Sex and clinical symptoms at birth</th>
<th>Evolution</th>
<th>Muscle biopsy (age)</th>
<th>RYR1 mutations (exons)</th>
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</thead>
<tbody>
<tr>
<td>I (AR)</td>
<td>Case 1</td>
<td>Hydramnion, fetal akinesia, born at 37 weeks of gestation</td>
<td>Male. Multiple arthrogryposis, severe hypotonia and amyotrophy, respiratory mechanical assistance, multiple malformations (vertebra, face)</td>
<td>Child alive at 9 years, delayed motor development, ptosis, strabismus, scoliosis, amyotrophy</td>
<td>(15 days and 1 year)</td>
<td>Type 1 fibre predominance, unique large eccentric cores, connective tissue increase</td>
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<td></td>
<td>Case 2</td>
<td>Hydramnion, fetal akinesia, multiple malformations</td>
<td>Male. Multiple arthrogryposis, amyotrophy</td>
<td>Fetus died at 32 weeks of gestation</td>
<td>(32 weeks gestation)</td>
<td>Large cores</td>
</tr>
<tr>
<td>II (AR)</td>
<td>Case 3</td>
<td>Hydramnion, breech presentation, fetal akinesia, born at 37 weeks of gestation</td>
<td>Female. Multiple arthrogryposis, severe hypotonia and amyotrophy, respiratory mechanical assistance, hypomimia</td>
<td>Child alive at 5 years, delayed motor development, ptosis, strabismus</td>
<td>(2 months)</td>
<td>Type I fibre predominance, unique large eccentric cores, few necrotic/regenerative fibres, connective tissue increase</td>
</tr>
<tr>
<td>III (AD)</td>
<td>Case 4</td>
<td>Hydramnion, born at 34 weeks of gestation</td>
<td>Female. Severe hypotonia, respiratory mechanical assistance, thin ribs</td>
<td>Death at 8 days</td>
<td>(8 days)</td>
<td>Type 1 fibre predominance, unique large eccentric cores</td>
</tr>
<tr>
<td>IV (sporadic)</td>
<td>Case 5</td>
<td>Hydramnion, severe fetal distress</td>
<td>Male. Multiple arthrogryposis, amyotrophy, severe hypotonia, multiple malformations</td>
<td>Death at 10 months</td>
<td>(10 months)</td>
<td>Type 1 fibre predominance, unique large eccentric cores</td>
</tr>
<tr>
<td>V (sporadic)</td>
<td>Case 6</td>
<td>Hydramnion, fetal akinesia</td>
<td>Female. Multiple arthrogryposis, amyotrophy, severe hypotonia, respiratory mechanical assistance, lung hypoplasia</td>
<td>Death at 1 month</td>
<td>(1 month)</td>
<td>Type I fibre uniformity, unique large eccentric cores, connective tissue increase</td>
</tr>
<tr>
<td>VI (sporadic)</td>
<td>Case 7</td>
<td>Hydramnion, fetal akinesia</td>
<td>Male. Multiple arthrogryposis, amyotrophy, face malformations</td>
<td>Fetus died at 30–31 weeks of gestation</td>
<td>(30–31 weeks gestation)</td>
<td>Large cores</td>
</tr>
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</table>

*Table 1 Summary of clinical, muscle biopsy and molecular data*
predominance of type I fibres was observed. An increase of endomysial connective tissue was also observed in cases 1, 3 and 6 (Figs 1, 3 and 4).

In all cases, ultrastructural analysis demonstrated large well-delimited areas with sarcomeric disorganization, myofibrillar compaction and absence of mitochondria, characteristic of non-structured cores (Fig. 4). Details of the histological findings are summarized in Table 1.

**Molecular genetics studies**

Molecular genetic studies allowed the identification of mutations in the *RYR1* gene in families I, II and III. Two compound heterozygous mutations were identified in two unrelated AR affected children and a heterozygous mutation was identified in an AD affected child.

**Family I (AR)**

A preliminary linkage study performed with microsatellite markers flanking the *RYR1* locus on chromosome 19q13.1 showed that the two affected children carried the same haplotypes inherited from their unaffected parents. Sequencing of the entire *RYR1* cDNA from one affected boy led to the identification of two mutations in the *RYR1* gene. A R614C mutation at the amino acid level was identified in exon 17 of the maternal allele. A G215E mutation was identified in exon 8 of the paternal allele. These two mutations were present in both affected children. The R614C mutation is the most frequent mutation identified in the French population affected by MHS. The G215E mutation affected a very well conserved glycyl residue localized in the N-terminal domain of the protein; it introduced a negative charge and was absent in 100 chromosomes from the general population. This change was inherited from the father, who was clinically and histologically unaffected (Fig. 1).

**Family II (AR)**

Sequencing of the entire *RYR1* cDNA from the affected girl led to the identification of two mutations in the *RYR1* gene, a L4650P mutation in exon 95 and a K4724Q mutation in exon 97. The L4650P mutation affected a well-conserved leucine localized in the M6 transmembrane region of the protein, while the K4724Q mutation affected a well-conserved lysyl residue that mapped to the cytoplasmic loop M6–M7 according to the recently described calcium channel topology (Du *et al*., 2002). Both mutations were absent from 100 chromosomes analysed from the general population. The K4724Q mutation was inherited from the proband’s father and the L4650P mutation from her mother. Both parents were clinically unaffected. Unfortunately, no muscle samples were available for histological studies.

**Family III (AD)**

Since a muscle sample was unavailable for mutation screening, genomic DNA was investigated using dHPLC screening of the C-terminal domain containing the calcium channel (exons 92–106). Furthermore, exons involved in known MHS domains were screened using dHPLC. A G4899E mutation in exon 102 of the *RYR1* gene was identified in the severely affected newborn. Her less severely affected mother transmitted this mutation. The mutation affected the last glycyl residue of a very well conserved GVRAGGGIGD luminal motif (amino acids 4891–4900) that was proposed as a pore-forming fragment (Zhao *et al*., 1999).

**Family VI (sporadic)**

Sequencing of the entire *RYR1* cDNA from the affected fetus did not allow the identification of mutations in the *RYR1* gene.

**Families IV and V**

For the two remaining sporadic cases, neither probands’ biological samples were available for molecular investigation.

**Discussion**

Fetal akinesia syndrome is an aetiologically heterogeneous group of development abnormalities resulting from a lack of intra-uterine fetal movements (Hammond and Donnenfeld, 1995; Fallet-Bianco, 1997). This group includes cases previously known as Pena–Shokeir syndrome (Pena and Shokeir, 1976; Brueton *et al*., 2000; Ho, 2000). The fetal akinesia syndrome is well known in several congenital myopathies such as nemaline and myotubular myopathies, but as yet has not been associated with CCD (Lammens *et al*., 1997; Mulder *et al*., 2001; Biancalana *et al*., 2003). The classic description of CCD patients includes delayed motor milestones, hypotonia during infancy and diffuse muscle weakness with reduced muscle bulk. Here, we describe a ‘severe neonatal form’ of CCD with antenatal onset of symptoms; all cases (four males and three females) presented with fetal akinesia, multiple arthrogryposis and hydramnion during pregnancy. At birth, the clinical picture was completed by severe hypotonia and respiratory distress, frequently often associated with multiple malformations. The prognosis was most often severe, but two patients (cases 1 and 3) survived after long-term ventilatory assistance and nursing care. In these two children, the muscle weakness remained apparently stable and ‘non-progressive’, and they were able to follow normal schooling. It is also important to note that both surviving children developed bilateral ptosis and strabismus during infancy; it is interesting that these particular ocular manifestations occurred in patients presenting compound heterozygous *RYR1* gene mutations (Table 1 and Fig. 2).
Ocular symptoms are quite uncommon and have rarely been described in CCD patients (Shaib et al., 1987).

The diagnosis of CCD was suggested following the examination of transverse muscle cryostat sections with oxidative enzyme staining that showed well-limited ‘unique large eccentric cores’ in the muscle biopsies. This pathological pattern was particularly evident in this group of CCD patients (Figs 1, 3 and 4). The diagnosis was confirmed by an ultrastructural study that showed non-structured core areas. As usual in congenital myopathies, all muscle biopsies showed a type I fibre predominance. Noticeably, three of the muscle biopsies (cases 1, 3 and 6) also showed a significant increase in the endomysial connective tissues that could lead to diagnostic errors in incompletely studied cases (Figs 1, 3 and 4).

Classically, CCD is an AD disorder associated with mutations mostly localized in the C-terminal domain of the RYR1 protein (Lynch et al., 1999; Monnier et al., 2000, 2001; Tilgen et al., 2001; Davis et al., 2003). However, in the present study, a recessive inheritance was strongly suggested in five unrelated families: I, II, IV, V and VI. Compound heterozygous mutations were identified in families I and II. No other change was found after the sequencing of the entire RYR1 cDNA extracted from probands’ muscles.

In family I, both affected children carried a R614C mutation on the maternal allele and a G215E mutation on the paternal allele. The mutations are localized in the N-terminal domain of the protein. The G215E mutation is a new mutation affecting a very well conserved amino acid among distant species and RYR1 isoforms. The R614C mutation is classically associated with the MHS phenotype, and its pathogenic effect on channel release activation by triggering agents has been well documented (Tong et al., 1999; McCarthy et al., 2000). Furthermore, we recently have identified this mutation in a homozygous state or associated with another MHS mutation in MHS patients with no myopathic phenotype (Monnier et al., 2002). To date, this mutation has never been associated with congenital myopathy phenotypes at either heterozygous, homozygous or compound heterozygous levels. To explain the severity of the disease observed in the two affected children, it will be necessary to evaluate the deleterious association of these two particular mutations in the holotetrameric RYR1 protein.

In family II, the affected child was a compound heterozygous for the L4650P and K4727Q mutations that mapped to the C-terminal calcium release channel. Both carrier parents were clinically unaffected. The L4650P mutation substitutes a well-conserved amino acid in the M6 transmembrane fragment according to the recently described calcium channel model (Du et al., 2002). This region was found to be a hot spot for AD CCD mutations (unpublished observations). Although the carrier mother was clinically asymptomatic, no biopsy was available for morphological investigation. The paternal K4727Q mutation substitutes a well-conserved amino acid in mammalian in the M6–M7 cytoplasmic loop of the channel release domain. The association of these two vicinal changes could account for the severity of the clinical picture observed in the affected child.

A recessive inheritance, or sporadic disease, could also be suggested for families IV, V and VI. In family VI, sequencing of the complete cDNA extracted from a proband muscle biopsy failed to identify mutations in the RYR1 gene. This clearly raises the question of a possible genetic heterogeneity. Unfortunately, no biological samples were available to study families IV and V.

Family III represents a case of AD CCD in which a G4899E mutation in the RYR1 gene was identified in the severely affected newborn and her less severely affected mother. The G4899E mutation substitutes a very well conserved amino acid located in the luminal GVRAGGGIGD pore-forming motif (Zhao et al., 1999). This motif can be considered to be a hot spot for CCD mutations (Lynch et al., 1999; Monnier et al., 2001; Davis et al., 2003). However, the severity of the disorder observed in the affected child compared with clinical pictures previously reported for patients mutated in this domain (Lynch et al., 1999; Monnier et al., 2001; Tilgen et al., 2001) suggests the implication of an additional factor. This factor could be a mutation in the paternal allele or a neomutation of the RYR1 gene not yet identified due to the partial screening of the 106 exons present in the RYR1 gene. Alternatively, one cannot exclude a mutation in another gene of the triad junction. Unfortunately, no muscle sample was available for extensive cDNA investigation. Interestingly, an aggravation of the clinical phenotype through generations had also been reported previously in one family presenting with CCD associated with the RYR1 gene (Monnier et al., 2000).

The diagnosis of CCD in these various families presenting with a fetal akinesia syndrome is supported by both histological studies and identification of mutations in the RYR1 gene, previously associated with classical forms of CCD. Implication of the RYR1 gene as a main component of the pathogenic process was clearly suggested in three out of the four families investigated at the molecular level. In the absence of functional data on the different RYR1 mutations identified in these children, functional studies are necessary to confirm whether the RYR1 gene alone is fully responsible for this severe phenotype or is part of a multigenic process involving an additional aggravating factor that might explain the variable severity of CCD.

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