

# A novel dominant mutation in *CRYAB* gene leading to a severe phenotype with childhood onset

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## Abstract

**Background:**  $\alpha$ B-crystallin is a promiscuous protein involved in numerous cell functions. Mutations in *CRYAB* have been found in patients with different pathological phenotypes that are not properly understood. Patients can present different diseases like cataracts, muscle weakness, myopathy, cardiomyopathy, respiratory insufficiency or dysphagia, but also a variable combination of these pathologies has been found. These mutations can show either autosomal dominant or recessive mode of inheritance and variable penetrance and expressivity. This is the first report of congenital cataracts and myopathy described in childhood due to a *CRYAB* mutation with autosomal dominant mode of inheritance.

**Methods:** The whole exome sequence was subjected to phenotype-driven analysis and a novel variant in *CRYAB* was detected: c.514delG, p.(Ala172ProfsTer14). The mutation was located in the C-terminal domain of the protein, which is essential for chaperone activity. The deduced protein was analyzed searching for alterations of the relevant physico-chemical properties described for this domain. A muscle biopsy was also tested for *CRYAB* with immunohistochemical and histoenzymatic techniques.

**Results:** *CRYAB* displayed a mild immunoreactivity in the subsarcolemmal compartment with no pathological sarcoplasmic accumulation. It agrees with an alteration of the physico-chemical properties predicted for the C-terminal domain: hydrophobicity, stiffness, and isomerization.

**Conclusions:** The described mutation leads to elongation of the protein at the carboxi-terminal domain (CTD) with altered properties, which are essential for solubility and activity. It suggests that can be the cause of the severe conditions observed in this patient.

## KEYWORDS

cardiomyopathy, cataracts, *CRYAB*, crystallinopathy, HspB5, myopathy,  $\alpha$ B-crystallin

## 1 | INTRODUCTION

$\alpha$ B-crystallinopathy is a multisystem disorder characterized by variable combinations of cataracts, cardiomyopathy, myopathy, progressive muscle weakness affecting both the proximal and distal skeletal muscles, respiratory insufficiency, and dysphagia. Cataracts are the most common affection, but myopathies and cardiomyopathies have also been described alone in individuals with mutations in *CRYAB* (MIM: 123,590). About 20 mutations have been reported in *CRYAB*, but they seem to be nonrecurrent. The correlation between gene mutations and the clinical phenotype is not properly understood. The mutations found in *CRYAB* can show either dominant or recessive modes of inheritance and variable penetrance and expressivity.

*CRYAB* (NM\_001885.3) is located in chromosomal region 11q23.1 and it encodes for the  $\alpha$ B-crystallin protein (NP\_001876.1), also called HspB5.  $\alpha$ B-crystallin is a small heat shock protein (sHSP) with molecular ATP-independent chaperone activity. The activity assays in vivo and in vitro showed its role in protein folding and cytoprotection (Jakob, Gaestel, Engel, & Buchner, 1993). sHSPs have a large capacity for binding to nonnative proteins ranging from peptides to large proteins (Ehrnsperger, Hergersberg, Wienhues, Nichtl, & Buchner, 1998). Particularly under stress conditions, such as elevated temperature, sHSPs prevent the irreversible aggregation process, binding and maintaining nonnative proteins in a reversible state until they can be refolded to the native state by an ATP-dependent chaperones (Ehrnsperger, Gräber, Gaestel, & Buchner, 1997; Lee, Roseman, Saibil, & Vierling, 1997; Stromer, Ehrnsperger, Gaestel, & Buchner, 2003). sHSPs are highly promiscuous and bind hundreds of different cytosolic proteins (Haslbeck et al., 2004; Peschek et al., 2013). The monomers of sHSPs have in common a small molecular mass (about 22 kDa), but they can bind to each other and form large oligomers (usually composed of 12–32 subunits), with a mass of up to 650 kDa between either homogenous or heterogeneous HSP assemblies (Haley, Horwitz, & Stewart, 1998). In normal conditions, sHSPs are reserved in larger oligomeric species, but these proteins can sense the presence of unfolded proteins when changes occur in the environmental temperature, cold or heat shock, or oxidative stress (Haslbeck & Vierling, 2015; Hayes, Napoli, Mazurkie, Stafford, & Graceffa, 2009; Mchaourab, Godar, & Stewart, 2009). Under stress conditions, when the substrate proteins begin to unfold, the high-mer oligomeric species follow a disassembly process to increase the number of small species that can hold the substrate proteins, thus preventing the unfolding.

The  $\alpha$ B-crystallin has three domains. The hallmark of all the members of the sHSP family is the  $\alpha$ -crystallin domain (ACD), which is flanked by an N-terminal domain (NTD) and a C-terminal domain (CTD) (Bagn  ris et al., 2009). The isolated ACD domain does not have a chaperone activity (Haslbeck et al., 2004; Lindner et al., 2000). This means that

either NTD, CTD, or both, are required for activity. The NTD has mainly hydrophobic residues and provides sites for interactions leading to the formation of higher order sHSP oligomers. Deletion of the SRLFDQFFG or FLRAPSWF sequence from the NTD leads to a significant decrease in oligomeric size and increased chaperone activity (Pasta, Raman, Ramakrishna, & Rao, 2003; Santhoshkumar, Murugesan, & Sharma, 2009). The ERTIPITREEKPAVTAAPKK sequence of  $\alpha$ B-crystallin CTD weakly binds to the ACD dimers (Hilton et al., 2013). Specifically, a conserved tripeptide motif IPI in  $\alpha$ B-crystallin, is involved in stabilization of the oligomers. Said stabilization is achieved when an IPI motif binds into the hydrophobic ACD groove formed by the  $\beta$ 4/ $\beta$ 8 strands of ACD, which is the binding pocket where unfolding proteins are held (Delbecq, Jehle, & Klevit, 2012; Laganowsky et al., 2010; Treweek, Rekas, Walker, & Carver, 2010; van Montfort, Basha, Friedrich, Slingsby, & Vierling, 2001). In normal conditions the binding pocket is hidden because CTD (IPI motif) binds to it, however, when temperature is greater than 37°C, IPI-CTD leaves the ACD groove so that both are exposed for chaperone activity (Bagn  ris et al., 2009; Laganowsky et al., 2010). Once folding has been achieved and the normal conditions have been reestablished,  $\alpha$ B-crystallin dimers are again assembled into higher oligomers, serving as a depot of sHSPs (Horwitz, 1992; Jakob et al., 1993; Mchaourab, Dodson, & Koteiche, 2002).

$\alpha$ B-crystallin was first described as an abundant protein in ocular lens interacting with  $\alpha$ A-crystallin, hence contributing to maintaining lens transparency (Horwitz, 2003). The dataBase for Gene Expression Evolution reports expression of the *CRYAB* human gene in 232 anatomical localizations, with the highest level of expression being found in the left ventricle of the heart. The  $\alpha$ B-crystallin location inside the cell is also diverse: it has been found as a soluble cytoplasmic protein, but also in the nucleus (van Rijk, Stege, Bennink, May, & Bloemendal, 2003), in perinuclear Golgi (Gangalum, Schibler, & Bhat, 2004), associated with centrosomes and midbodies during mitosis (Inaguma, Ito, Iwamoto, Saga, & Kato, 2001), close to cellular membranes (Maddala & Rao, 2005) and associated with mitochondria (Launay, Tarze, Vicart, & Lilienbaum, 2010). In this report, we present a novel mutation in *CRYAB* which alters drastically the physico-chemical properties of the CTD domain and leads to a severe pathogenic phenotype. This is the first mutation with autosomal dominant mode of inheritance described with childhood onset.

## 2 | METHODS

### 2.1 | Ethical compliance

The family came for genetic counseling to our Genetic Unit because of one child of a twin pregnancy born with

a syndrome and they were afraid of the healthy one. The study of this family was approved by the Institutional Ethics Review Boards of the Universidad Europea del Atlántico (Project 2 of the Agreement No. 28 celebrated on October 17, 2019), accompanied of the correspondent informed consents.

## 2.2 | Genetic analysis

Genetic analysis was performed. The genomic DNA was extracted from peripheral whole blood samples using Maxwell 16 System (Promega). Exomes of genes were enriched with the SureSelect<sup>QXT</sup> Clinical Research Exome V2 Library Preparation kit (Agilent Technologies) and sequenced on an Illumina Nextseq 500 sequencer with 150 cycles of paired-end reads. Variant annotation and interpretation: The primary data were filtered removing adapters and low-quality reads. Filtered reads were mapped to hg19 reference genome using bwa, duplicates were marked using PicardToolsMarkDuplicates, and variants were called using FreeBayes. The segregation study was done by Sanger sequencing on a 3130 Genetic Analyzer (Applied Biosystems) based on the mutations identified in the proband. Sequence variants were annotated using snpEff with population and literature databases including 1,000 Genomes, dbSNP, gnomAD, ClinVar, HGMD, and OMIM.

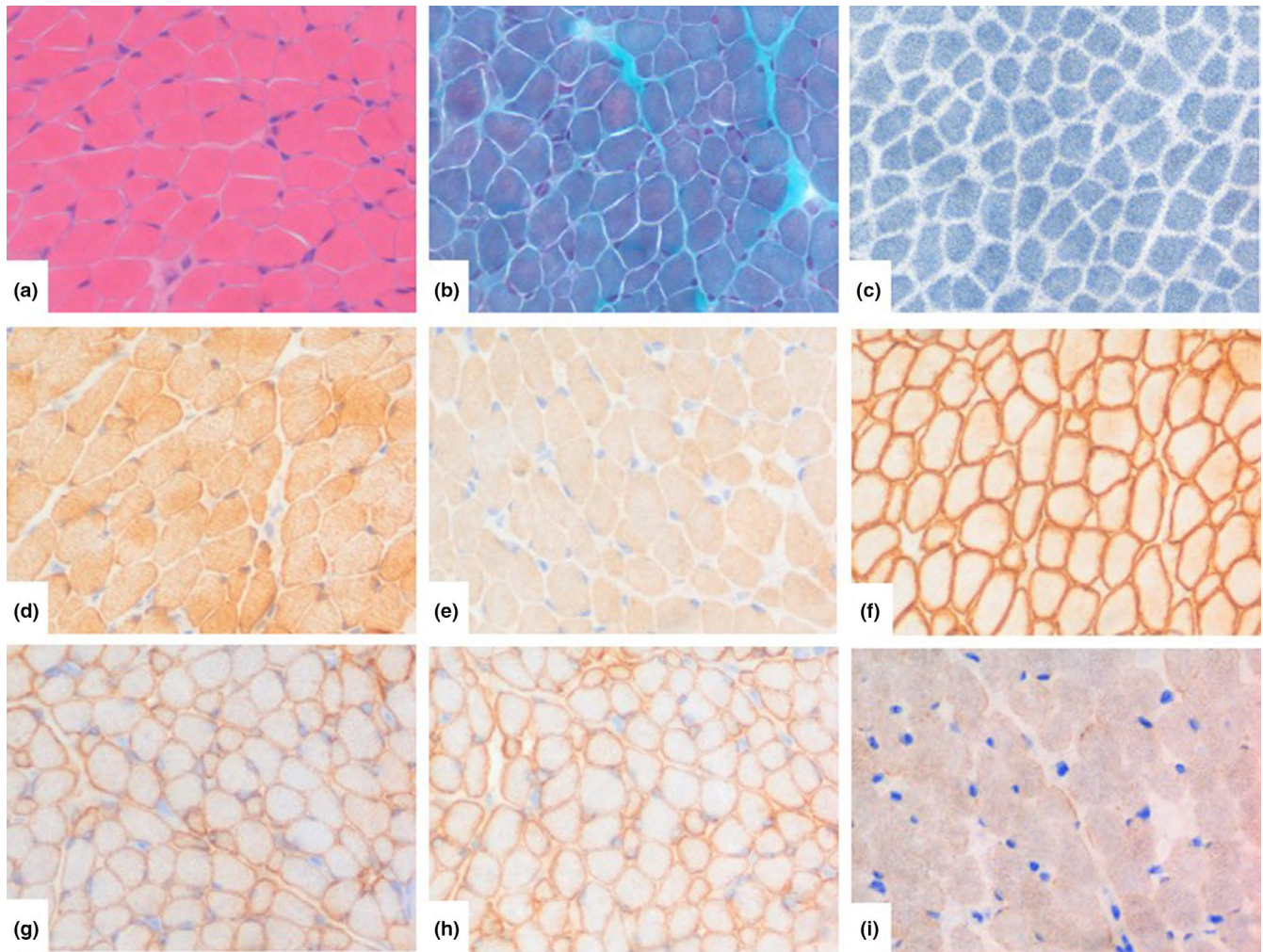
A muscle biopsy was performed. Muscle sample was obtained by open biopsy from cuadriceps and processed following the standard procedures. Histochemical and histochemical studies were performed on frozen sections and included: Hematoxylin and eosin (H&E), Modified Gomori trichrome (MGT), PAS, Oil red O, nicotinamide adenosine dinucleotide dehydrogenase (NADH), succinate dehydrogenase (SDH), cytochrome oxidase (COX), and ATPase after preincubation at pH 9.4, 4.5, and 4.3. Immunohistochemical analysis was performed using  $\alpha$ B-crystallin monoclonal antibody NCL-ABCrys-512 (Leica Biosystems Newcastle-Novocastra), C-terminus dystrophin monoclonal antibody NCL-DYS2 (Leica Biosystems Newcastle-Novocastra), myotilin monoclonal antibody NCL-myotilin (Leica Biosystems Newcastle-Novocastra), and desmin monoclonal antibody DE-R-11 (Ventana, Roche, Ventana Medical Systems, INC. Tucson). Leica Bond-III automated immunostainer was used with DAB detection.

The prediction properties of the deduced mutated protein and comparison with the nonmutated were performed with ProtScale Analysis Tools on the ExPASy Server (Gasteiger et al., 2005). Hydrophobicity was performed with Kyte-Doolittle Scale, Polarity with Grantham Scale and Flexibility with Bhaskaran and Ponnuswamy Scale.

## 3 | RESULTS

Here we report on the clinical case of a dichorionic diamniotic twin pregnancy in which two boys were delivered in 2015 and where one of them presented a multisystem syndrome of unknown etiology. At birth, he presented congenital bilateral cataracts, palpebral bilateral ptosis, congenital hypotonia and a slight delay in motor skills acquisition. At the age of 3 months, the patient underwent ocular surgery to correct the cataracts. At the age of 13 months, as the consequence of a pulmonary infection, he was admitted to the Intensive Care Unit for 2 months with acute respiratory failure. Since then, he has a permanent tracheostomy for intermittent mechanical respiratory assistance and frequently requires a nebulizer. Today, he finds himself in need of mechanical ventilation while sleeping and in contexts of respiratory exacerbation. The patient also presented dysphagia, since then he is basically fed by gastrostomy, although some liquids and soft foods are tolerated orally. He does not present cognitive delay and he is acquiring and improving speech with therapy. He presents generalized hypotonia and weakness, with facial involvement and palpebral ptosis, but he remains ambulatory, although walks unstably. Basic biochemical and metabolic studies showed normal values, except for a slight elevation of creatine kinase (maximum value 391 UI/l). Cranial magnetic resonance imaging evidenced no pathological findings, but only prominence of the subarachnoid spaces at the frontal level. Echocardiographies performed from the onset of the symptoms until the age of 4 years have been normal. Muscle biopsy from quadriceps showed moderate variability in fiber size, frequent atrophic fibers of less than 10–12 microns, and focal signs of muscle degeneration. No rimmed vacuoles or sarcoplasmic inclusions were identified with Modified Gomori trichrome (MGT, Figure 1b). Mild increase in lipids was observed with oil red O staining. The histochemical study with NADH, SDH (Figure 1c), and COX showed a regular intermyofibrillar pattern without cores, minicores, or other relevant structural alterations. Immunohistochemical study showed normal staining with desmin, myotilin, and dystrophin (Figure 1d–e).  $\alpha$ B-crystallin displayed a mild immunoreactivity in the subsarcolemmal compartment with no pathological sarcoplasmic accumulation (Figure 1g and h). The muscle biopsy of a healthy control immunostained with CRYAB antibodies shows that the  $\alpha$ B-crystallin is located homogeneously in the muscle cell (Figure 1i). If we compare with the proband biopsy CRYAB immunostained, the sarcoplasm is significantly depleted of  $\alpha$ B-crystallin, which is mainly relegated at the submembrane compartment of the muscle fibers (Figure 1g and h). The important reduction of  $\alpha$ B-crystallin observed in muscle fibers suggests that the function of the protein can be severely impaired, supporting the hypothesis of the pathogenicity of this mutation.



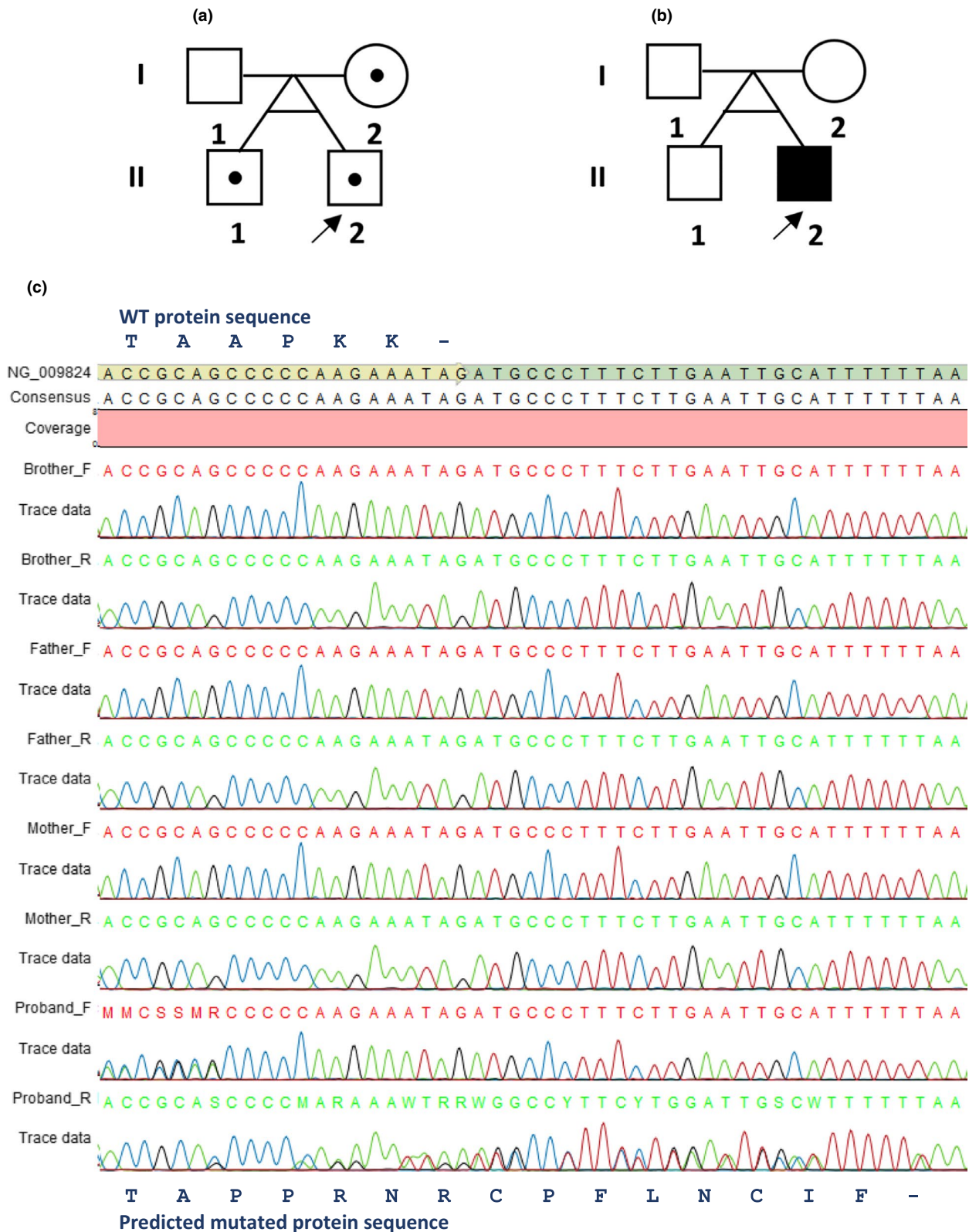


**FIGURE 1** Muscle biopsy from quadriceps: Hematoxylin and eosin staining (a) and Modified Gomori trichrome (b) shows a myopathic pattern with moderate variability in fiber size and scattered atrophic fibers. No vacuoles, hyaline bodies, or sarcoplasmic inclusions were observed. Succinate dehydrogenase (SDH) (c) reveal uniform sarcoplasmic staining with no cores, minicores, moth-eaten fibers, or other structural alterations. Immunohistochemistry with desmin (d), myotilin (e), and dystrophin-CT (f) displayed a normal staining.  $\alpha$ B-crystallin (g,h) revealed a subtle immunostaining in the subsarcolemmal compartment when compared to the control (i). Magnification  $\times 400$

A study of the enzymatic activity of the mitochondrial respiratory chain showed a combined deficit of complex III (43.38, Reference values: 51–79), complex I–III (3.2, Reference values: 8–24), and complex II–III (12.78, Reference values: 21–48). Hence, the first genetic analysis was mitochondrial DNA sequencing, with negative results, therefore depletion and significant deletions of mitochondrial DNA were discarded. The whole exome sequence testing for mitochondrial-related genes revealed two variants: the first, was a variant of uncertain significance in *OPA1* (Optic Atrophy) gene (MIM:605,290, NM\_015560.2, NP\_570850.2):c.113\_130delGAAGCATTTATCATTAC p.(Arg38\_Ser43del); and the second, is a probably pathogenic variant inherited in an autosomal recessive disorder produced by the mutations in gene *HSD17B4* (MIM: 601,860, NM\_000041.4, NP\_000405.1): c.46G > A p.(Gly16Ser) detected in heterozygosis, hence the latter appears to bear no

relevance in this case. The *OPA1* gene encodes for a dynamin GTPase involved in mitochondrial fusion and is mainly related with optic neuropathy. The segregation analysis showed that the *OPA1* variant was also present in the mother (I2) and in the twin brother (II1), both of whom are unaffected (Figure 2a). Moreover, no optic neuropathy was observed in the index case, nor in the mother's family.

A new phenotype-driven analysis of exome data was filtered by eliminating low-quality and low coverage variants first, as well as deep intronic variants, high-frequency variants in population databases, then synonymous variants without splicing effects and, finally, variants with benign or/and likely benign ClinVar classification. The remaining sequences were analyzed focusing on genes related to cataracts and myopathy according to the Human Phenotype Ontology Browser (HPO, <https://hpo.jax.org/>) and the resulted variants after this filtering step are shown in Table 1.



**FIGURE 2** Genetic studies. (a) Pedigree for *OPA1* variant. Carriers are indicated by dots. The proband is indicated by an arrow. (b) Pedigree for *CRYAB* mutation (c.514delG, p.(Ala172ProfsTer14)). The proband is indicated by an arrow and by filled symbol. (c) Sanger sequence of the four family member's brother (up), father, mother, proband (down). The nonmutated protein sequence is indicated at the top and the mutated protein sequence is indicated at the bottom

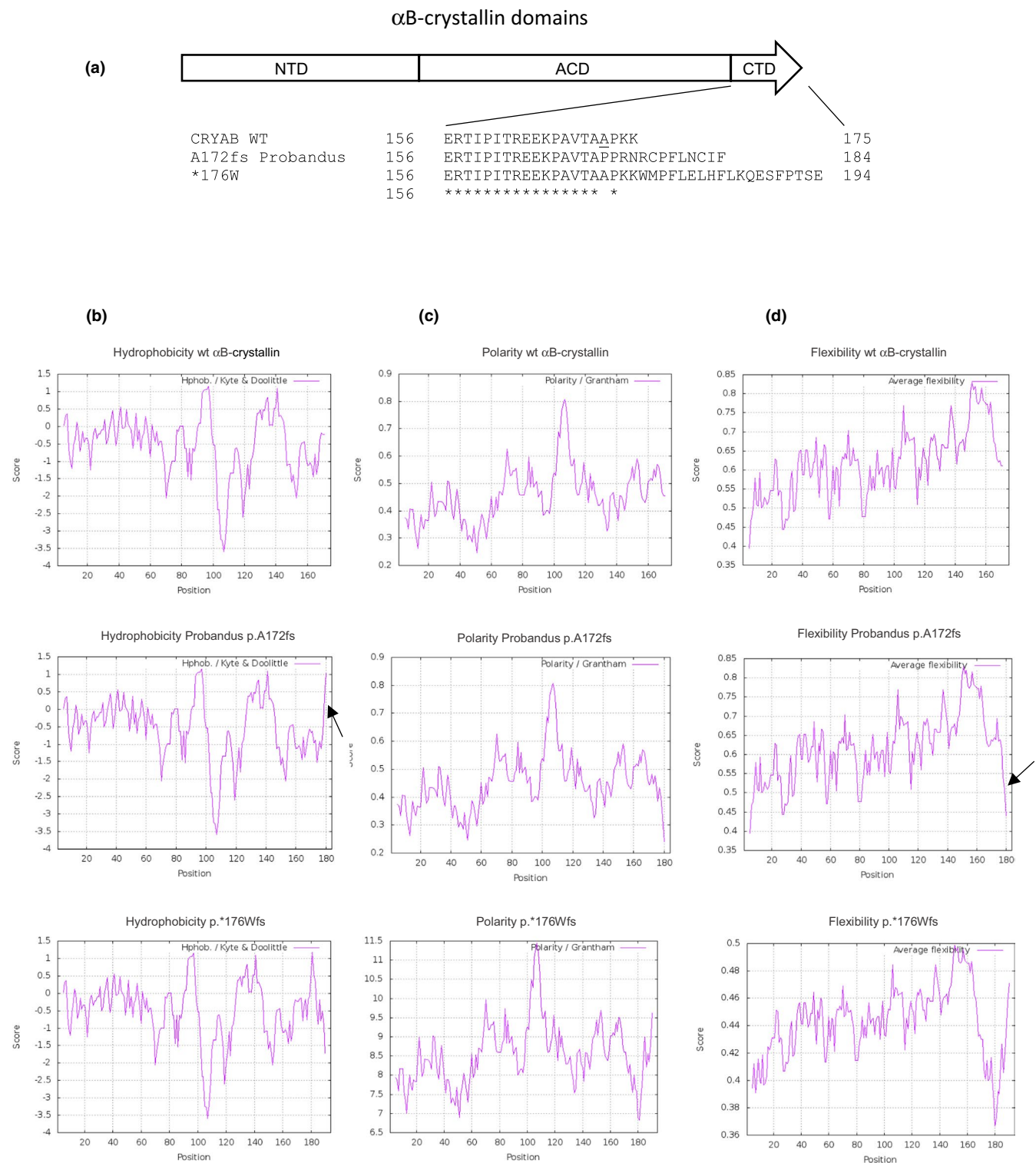


The selected variants underwent a prioritization based on protein impact, phenotypic overlap, and inheritance mode. The *CRYAB*: c.514delG, p.(Ala172ProfsTer14) variant is the one that best explains the phenotype observed in the affected patient (II2 in Figure 2b). According to ACMG for variant classification, p.(Ala172ProfsTer14) fits the criteria PVS1, PS2, PM2, and PP3 and all of them coincide in classifying it as a pathogenic variant. This variant was not

present in the 1,000 Genomes Project Database, gnomAD or ClinVar. To our knowledge, this mutation has never been described previously in scientific literature, meaning that it represents a novel variant for this gene which produces a severe pathological phenotype and is the first report of myopathy described in childhood due to a *CRYAB* mutation with autosomal dominant mode of inheritance. Other lethal myopathies in childhood were described in

**TABLE 1** List of variants filtered by phenotype: myopathy and cataracts

Genomic Position (hg19/GRCh37)	Zygosity	Gene	Gene level annotation
chr1:5937203G > A	Het	NPHP4	NM_015102.4:c.2767C > T (p.Arg923Cys)
chr1:26131638A > G	Het	SEPN1	NM_020451.2:c.409A > G (p.Thr137Ala)
chr1:100382037A > G	Het	AGL	NM_000028.2:c.4331A > G (p.Asn1444Ser)
chr2:71738977G > A	Het	DYSF	NM_001130987.1:c.386G > A (p.Gly129Glu)
chr2:152359922G > A	Het	NEB	NM_001271208.1:c.23881C > T (p.Pro7961Ser)
chr2:179451454G > A	Het	TTN	NM_001267550.2:c.64174C > T (p.Arg21392Cys)
chr2:215865549G > C	Het	ABCA12	NM_173076.2:c.3059C > G (p.Ala1020Gly)
chr2:219857880G > A	Het	CRYBA2	NM_057093.1:c.19C > T (p.Pro7Ser)
chr3:193332586TTTCACGAAGCATTTATCA > T	Het	OPA1	NM_130837.2:c.113_130delGAAGCATTTATCATTAC (p.Arg38_Ser43del)
chr4:123171659T > A	Het	KIAA1109	NM_015312.3:c.5853T > A (p.Asp1951Glu)
chr5:89923101G > A	Het	ADGRV1	NM_032119.3:c.746G > A (p.Arg249Lys)
chr6:7248990G > A	Het	RREB1	NM_001003699.3:c.5018G > A (p.Arg1673Gln)
chr6:112461987A > G	Het	LAMA4	NM_001105206.2:c.2951T > C (p.Val984Ala)
chr6:152751829T > C	Het	SYNE1	NM_182961.3:c.4477A > G (p.Ile1493Val)
chr8:87755776T > C	Het	CNGB3	NM_019098.4:c.80A > G (p.Asn27Ser)
chr8:144995938C > T	Het	PLEC	NM_201380.3:c.8462G > A (p.Arg2821Gln)
chr10:50827939C > T	Het	CHAT	NM_020549.4:c.556C > T (p.Arg186Trp)
chr10:73464812G > A	Het	CDH23	NM_022124.5:c.2878G > A (p.Glu960Lys)
chr10:85956268C > A	Het	CDHR1	NM_033100.3:c.159C > A (p.His53Gln)
chr11:8060566G > A	Het	TUB	NM_003320.4:c.146G > A (p.Arg49Gln)
chr11:64519958T > C	Het	PYGM	NM_005609.3:c.1537A > G (p.Ile513Val)
chr11:77823791C > T	Het	ALG8	NM_024079.4:c.803G > A (p.Arg268Gln)
chr11:111779501GC > G	Het	CRYAB	NM_001289807.1:c.514delG (p.Ala172fs)
chr12:88512301C > T	Het	CEP290	NM_025114.3:c.1670G > A (p.Arg557His)
chr14:64634063G > A	Het	SYNE2	NM_182914.2:c.16718G > A (p.Arg5573Gln)
chr16:1569961TCTTGGC > T	Het	IFT140	NM_014714.3:c.3955_3960delGCCAAG (p.Ala1319_Lys1320del)
chr16:58051264CA > C	Het	USB1	NM_024598.3:c.531delA (p.His179fs)
chr16:77328872G > C	Het	ADAMTS18	NM_199355.3:c.2954C > G (p.Ala985Gly)
chr17:4802308C > G	Het	CHRNE	NM_000080.3:c.1314G > C (p.Glu438Asp)
chr17:38907448C > T	Het	KRT25	NM_181534.3:c.800G > A (p.Arg267His)
chr20:50407735A > C	Het	SALL4	NM_020436.4:c.1287T > G (p.Phe429Leu)
chr20:57429447C > T	Het	GNAS	NM_080425.3:c.1127C > T (p.Pro376Leu)
chr22:50665165G > C	Het	TUBGCP6	NM_020461.3:c.1598C > G (p.Thr533Ser)



**FIGURE 3** Mutations at CTD of  $\alpha$ B-crystallin (a) Schematic representation of the  $\alpha$ B-crystallin protein formed by NTD (amino-terminal domain), ACD ( $\alpha$ -crystallin domain), and CTD (carboxi-terminal domain). Alignment of CTD sequences of human  $\alpha$ B-crystallin, proband (p.A172fs) and p.X176Wfs, following Bagn  ris description of the protein domains<sup>16</sup>. Conserved residues are indicated by asterisks. (b) Comparison of  $\alpha$ B-crystallin profile of nonmutated (up), Proband (p.A172fs) and p.X176Wfs mutated proteins, related to hydrophobicity. (c) Same comparison but related to polarity and (d) related to flexibility. The arrows indicate the increased hydrophobicity and reduced flexibility of the CTD extend in the mutated protein of the proband

autosomal recessive mode of inheritance, but previous autosomal dominant mutations reports belong to adult onset of one or some crystallinopathies conditions. The mutation c.514delG generates a frameshift at position Ala172, which presumably moves the stop signal to the next frame, while the addition of new amino acids elongates the protein to 184 residues (instead of 175 residues) (Figure 3a). The segregation analysis was done by Sanger sequencing and the results showed that this mutation was de novo, as neither of the unaffected parents carried the mutation (Figure 2b and c). This segregation pattern suggests an autosomal dominant mode of inheritance. No other variant of clinical significance was found in this whole exome sequencing study. The detected variant was deposited in ClinVar with the Accession Number: SCV001132042.

The p.Ala172fs mutation predicts that the last APKK residues (172–175) from the  $\alpha$ B-crystallin change to the PPRN sequence in the mutated protein, which also has nine new RCPFLNCIF residues added at the C-terminus of the protein (Figure 3a). It means that these new amino acids, added to this important domain, may alter the CTD tail and can lead to pathological condition. The physico-chemical properties of the new deduced protein (Figure 3a) were predicted with the ProtScale Tool (Gasteiger et al., 2005). The Figure 3b–d compares the physico-chemical properties (hydrophobicity, polarity and flexibility) of the nonmutated  $\alpha$ B-crystallin (up), the proband p.Ala172fs (middle), and the p.X176Trp mutation (down). The p.X176Trp is the only mutation previously described with modification of the *CRYAB* CTD tail. The new CTD tail in the p.Ala172fs mutated protein, here described, presents increased hydrophobicity instead of charged predominant amino acids observed in the nonmutated protein and reduced polarity, but also presents a reduced flexibility, which are important properties described as essential for the activity of CTD domain.

## 4 | DISCUSSION

The widespread expression of *CRYAB* makes possible a variety of phenotypes in mutation carriers. The phenotypes associated with different mutations have been summarized in Table 2 showing the variability of the phenotypes described, regardless of the domain of the protein in which the mutation is produced. In lens, the  $\alpha$ B-crystallin acts maintaining the solubility of other lenticular proteins, but the formation of aggregates due to the inactivity of the  $\alpha$ B-crystallin protein causes cataracts (Berry et al., 2001), a fact that is coincident with the congenital cataract observed in the present case, suggesting that the mutation here described is pathogenic.

The first pathogenic mutation described in  $\alpha$ B-crystallin was the change Arg120Gly, an autosomal dominant desmin-related myopathy observed in several generations of a

French family. The patients were affected by weakness of the proximal and distal limb muscle (including the neck, velopharynx, and trunk muscles), cardiomyopathy and cataracts. The skeletal and cardiac biopsies of these patients showed that aggregates, amyloid positive composed of desmin and  $\alpha$ B-crystallin, were responsible for triggering degenerative changes in the muscle myofibrils (Vicart et al., 1998). The Arg120Gly mutation and a mutation in the muscle-specific desmin protein were found to phenocopy one another (Goldfarb et al., 1998). The Arg120Gly mutation decreases the dissociation constant by half for desmin, causing an aggregation of desmin filaments both in transfected cells and in in vitro assembly assays (Perng, Wen, Ijssel, Prescott, & Quinlan, 2004). The aggregation of diverse proteins and mitochondrial abnormalities is typical in myopathies caused by human desmin or  $\alpha$ B-crystallin mutations. This mutation has important differences with the case here described because the cataract was not congenital and did not cause significant visual impairment in the French family. Furthermore, the residue Arg120 is intact in the proband protein and it does not form aggregates with desmin, as revealed the immunohistochemical analysis in the muscle biopsy, where no protein aggregations of desmin, myotilin, or dystrophin were found (Figure 1d–f). Other mutations as Gly154Ser (myopathy and cardiomyopathy), Arg157His (cardiomyopathy), and Ala171Thr (cataracts only) are closest to the one described here, but each of them produces a different phenotype. The physico-chemical properties of these and other point mutations do not exhibit a dramatic change, suggesting that these mutations may affect the interaction site with sHSPs (homolog or heterologous) or with their target proteins (Gerasimovich, Strelkov, & Gusev, 2017). In the proband all these interactions seem to be preserved.

Other two mutations have been described related to the CTD of  $\alpha$ B-crystallin in patients without cataracts or cardiac affection: (a) The  $\alpha$ B-crystallin mutation Gln151X, due to a premature stop codon, lacking the complete CTD was observed in a patient of 53 years old with slowly progressive leg weakness and occasional cramping and stiffness of the leg muscles after exercise. Our proband has coincident muscle condition with extreme weakness in his legs after exercising probably due to the myofibrils requirement of  $\alpha$ B-crystallin chaperone activity after heat stress. But proband, in the absence of exercise, also presents weakness, hypotonia and hyporeflexia, meaning that he presents a more severe phenotype and significantly earlier onset (Selcen & Engel, 2003). (b) The  $\alpha$ B-crystallin mutation Pro155fs, lacks the complete CTD, but eight residues were added to the ACD domain. The mutation Pro155fs was observed in a patient with ventilatory insufficiency and difficulty swallowing liquids at the age of 52 years old (Selcen & Engel, 2003), coincident symptoms were observed in the proband but they appeared when he was 13 months old.



**TABLE 2** Mutations described in  $\alpha$ -crystallin related to condition, inheritance, and clinical significance

	Mutations	Conditions	Inheritance	Reference	Clinical significance
NTD	Met1Leu	Cardiomyopathy	AR	Ma et al., 2019	Conflicting interpretations of pathogenicity
	Pro8Ser	Cardiomyopathy		ClinVar VCV000222530	Uncertain significance
	Arg11His	Cataract	AD	Chen et al., 2009	Pathogenic
	Arg11Cys	Cardiomyopathy		ClinVar VCV000222531	Uncertain significance
	Phe14Val	Cardiomyopathy		ClinVar VCV000544022	Uncertain significance
	Pro16Ler	Cardiomyopathy		ClinVar VCV000578197	Uncertain significance
	Pro20Ser	Cataract	AD	Li et al., 2008	Pathogenic
	Pro20Arg	Cataract	AD	Xia et al., 2014; Zhu et al., 2017	Pathogenic
	Arg22His	Cardiomyopathy		ClinVar VCV000569482	Uncertain significance
	Ser21AlafsTer24	Fatal infantile hypertonic muscular dystrophy	AR	Del Bigio et al., 2011	
	Leu23Pro	Cardiomyopathy		ClinVar VCV000518521	Uncertain significance
	Lys25Arg	Cardiomyopathy		ClinVar VCV000643345	Uncertain significance
	Glu34Asp	$\alpha$ B crystallinopathy		ClinVar VCV000302432	Uncertain significance
	Pro39Ala	$\alpha$ B crystallinopathy		ClinVar VCV000571646	Uncertain significance
	Pro39Ser	Cardiomyopathy		ClinVar VCV000657190	Uncertain significance
	Pro39Leu	$\alpha$ B crystallinopathy		ClinVar VCV000178013	Conflicting interpretations of pathogenicity
	Pro39Gln	Cardiomyopathy		ClinVar VCV000566247	Uncertain significance
	Thr40Met	Cardiomyopathy		ClinVar VCV000657757	Uncertain significance
	Ser45Asn	Cardiomyopathy		ClinVar VCV000477731	Uncertain significance
	Arg50Gln	Cardiomyopathy		ClinVar VCV000281506	Uncertain significance
	Pro51Leu	$\alpha$ B crystallinopathy		ClinVar VCV000044232	Conflicting interpretations of pathogenicity
	Arg56Trp	Cataract	AR	Khan, Abu Safieh, & Alkuraya, 2010	Pathogenic
ACD	Arg69Cys	Cardiomyopathy		ClinVar VCV000570808	Uncertain significance
	Leu89Phe	Cardiomyopathy		ClinVar VCV000477732	Uncertain significance
	Asp96Gly	Cardiomyopathy		ClinVar VCV000662761	Uncertain significance
	His101Asn	Cardiomyopathy		ClinVar VCV000579144	Uncertain significance
	Arg107Leu	Congenital Cataract		ClinVar VCV000264247	Likely pathogenic
	Gln108His	Cardiomyopathy		ClinVar VCV000264247	Uncertain significance
	Asp109Gly	Cardiomyopathy	AD	Brodehl et al., 2017	Pathogenic
	Asp109His	$\alpha$ B crystallinopathy	AD	Sacconi et al., 2012	Pathogenic
	Asp109Ala	$\alpha$ B crystallinopathy	AD	Fichna et al., 2016	Pathogenic
	Ser115ProfsTer129	Myopathy	AR	Forrest et al., 2010	Pathogenic
	Phe118Ser	Not specified		ClinVar VCV000228540	Uncertain significance
	Arg120Gly	$\alpha$ B crystallinopathy	AD	Vicart et al., 1998	Pathogenic
	Arg123Gln	Not specified		ClinVar VCV000228541	Uncertain significance
	Arg123Trp	Cardiomyopathy		ClinVar VCV000393088	Uncertain significance
	Ile124Val	Not specified		ClinVar VCV000227275	Likely benign
	Ser136Thr	Cardiomyopathy		ClinVar VCV000477734	Uncertain significance
	Asp140Asn	Cataract	AD	Liu et al., 2006	Pathogenic
	Lys150AsnfsTer184	Cataract	AD	Berry et al., 2001	Pathogenic

TABLE 2 (Continued)

	Mutations	Conditions	Inheritance	Reference	Clinical significance
	Gln151X	$\alpha$ B crystallinopathy	AD	Selcen & Engel, 2003	Pathogenic
	Ser153Phe	Cardiomyopathy		ClinVar VCV000619269	Uncertain significance
	Gly154Ser	$\alpha$ B crystallinopathy	AD	Reilich et al., 2010	Conflicting interpretations of pathogenicity
	Gly154Asp	Cardiomyopathy		ClinVar VCV000477735	Uncertain significance
	Pro155ArgfsTer163	$\alpha$ B crystallinopathy	AD	Selcen & Engel, 2003	Pathogenic
CTD	Arg157His	Cardiomyopathy	AD	Inagaki et al., 2006	Uncertain significance
	Arg157Cys	Cardiomyopathy		ClinVar VCV000641068	Uncertain significance
	Ala171Thr	Cataract	AD	Devi et al., 2008	Pathogenic
	Ala172ProfsTer14	Crystallinopathy	AD	This report	Pathogenic
	X176Trp	Cataract and cardiomyopathy	AD	van der Smagt et al., 2014	Pathogenic

These comparisons suggest that the absence of the CTD domain is not enough to produce cataracts or cardiac affection, furthermore, the described mutation in the proband CTD causes more dysfunction than its absence. In fact, most of mutations produce aggregates, but in this case we observed depletion of  $\alpha$ B-crystallin in the muscle biopsy cells and the comparison with the control suggests that the reduced amount of this protein in the cytoplasm contribute to the severe pathogenicity of this mutation. The stress conditions originated by the pulmonary infection that affected the proband at the age of 13 months, with the observed depletion of the  $\alpha$ B-crystallin chaperone in the sarcoplasm, may explain the critical state and the large period of time required to overcome the acute respiratory failure and the other symptoms described in this case.

The CTD was defined as a flexible 12–20 residue tail with a preponderance of charged residues (Jehle et al., 2010). The mutated protein here described has a CTD tail with increased hydrophobicity (Figure 3b), that may reduce the sarcoplasmic solubility of the protein contributing to the more hydrophobic new membrane binding location observed in the CRYAB immunohistochemically stained muscular biopsy (Figure 1g and h). In concordance, the polarity properties are lost in the proband mutated CTD compared to the nonmutated (Figure 3c). A hydrophobicity peak is also found in the p.X176Trp mutation (van der Smagt et al., 2014), but at the end of the protein, it recovers the normal hydrophobicity conditions (Figure 3b) and the heart muscle biopsy of this patient shows aggregates that stain positive for desmin and  $\alpha$ B-crystallin, not observed in our proband.  $\alpha$ B-crystallin p.X176Trp mutation produces congenital posterior pole cataract and adult onset dilating cardiomyopathy, but neither myopathy, respiratory failure, nor dysphagia were observed, suggesting that different alterations of physico-chemical properties due to the sequence at the end of the protein can introduce variability in the phenotype.

In addition to the charged character of the CTD domain, NMR relaxation experiments have revealed that the CTD of  $\alpha$ B-crystallin is highly dynamic between the IPI motif bound to the ACD groove and the free state (Delbecq et al., 2012). This dynamic property has been related to the flexibility of the CTD domain, which enables it to change its position (Carver & Lindner, 1998; Delbecq & Klevit, 2013; Hochberg & Benesch, 2014; Treweek, Meehan, Ecroyd, & Carver, 2015). If we consider that the CTD domain is defined as a charged and flexible residue tail (Baldwin, Lioe, Robinson, Kay, & Benesch, 2011; Baldwin et al., 2012), the physico-chemical properties of the proband protein, contrary to the nonmutated one, make it hydrophobic and stiff by the addition of these new amino acids at the end of the protein, as is shown in Figure 3d. The prediction estimates that the proband protein is affected in the flexibility needed to trigger the association–dissociation oligomer dynamics observed in  $\alpha$ B-crystallin. CTD domain can be also involved in the interaction between the  $\alpha$ B-crystallin and the target protein during the chaperone function (Aquilina & Watt, 2007). The CTD flexibility is also involved in maintaining the solubility of the  $\alpha$ B-crystallin oligomer itself as well as the protein target complex (Carver, 1999). This means that changes in  $\alpha$ B-crystallin flexibility can affect the oligomerization, the protein solubility and the chaperone activity. Mutation of the CTD residue in  $\alpha$ B-crystallin (Lys175Leu) which enhances the flexibility, without altering the properties of the secondary and tertiary structure, shows changes in the quaternary structure where the oligomers formed are smaller than those formed by the nonmutated protein, suggesting that the increased flexibility disrupts packing of the  $\alpha$ B-crystallin subunit into the oligomer (Treweek et al., 2007). The enhanced chaperone ability of Lys175Leu  $\alpha$ B-crystallin may be due the greater flexibility and more efficient interaction with the highly amyloidogenic protein (Ecroyd et al., 2008). It suggests that a reduced flexibility of the CTD domain observed in the mutated protein

may impair the protein activity, leading to a particularly critical phenotype under stress conditions.

CTD also has been described as a disorder chain which is expected to form peptide bonds adopting a *trans* conformation in more than 99.5% of the cases. But peptides rich in proline residues have an increased population of *cis* conformation (5%–15%), showing a potential *cis-trans* proline isomerization somewhere around the Gly193-Pro194 of Hsp27 (Alderson, Benesch, & Baldwin, 2017). This was also demonstrated in bovine  $\alpha$ B-crystallin for Val169 to *cis* and *trans* conformers of the Lys166-Pro167 peptide bond (Carver & Lindner, 1998). The same is true for the second proline residue of Hsp27 situated in the highly conserved IPV motif (IPI motif in  $\alpha$ B-crystallin) and *cis-trans* isomerization between the Ile181-Pro182 peptide bond (Alderson et al., 2017). Resonances were also found for residues near Pro160 in the IPI motif of human  $\alpha$ B-crystallin (Mainz et al., 2015), suggesting a *cis-trans* proline isomerization in the IPI/V motif of the CTD domain. The isomerization changes in the IPI motif of  $\alpha$ B-crystallin have been related to the oligomer formation and monomer dissociation for substrate binding (Baldwin et al., 2011, 2012). The presence of other proline residues in the CTD could produce other conformations in combination with the proline of the IPI motif, altering the binding of CTD to the ACD groove and/or to other species for oligomer formation. Consistent with this, mutation of the central proline has been shown to modulate both the kinetics and the thermodynamics of the oligomerization of  $\alpha$ B-crystallin, resulting in slightly faster subunit exchange rates and an altered equilibrium of oligomeric distribution (Hilton et al., 2013). If we observe the residues incorporated in the predicted mutated CTD, another two prolines are predicted to be incorporated in this domain, which could affect its binding to the hydrophobic ACD groove and oligomer formation, causing pathogenicity.

In summary, here we describe a novel dominant frameshift mutation in *CRYAB*. This mutation changes the protein sequence at the end of the CTD domain of  $\alpha$ B-crystallin and produces a multisystem disorder characterized by congenital cataracts, hypotonia, myopathy, respiratory failure, and dysphagia. Critical muscle affection is mainly dependent on infections or exercise when stress and heat are produced and  $\alpha$ B-crystallin chaperone activity is highly required, but even in normal conditions weakness, hypotonia and hyporeflexia are present. The proband presented congenital cataracts revealing a very early onset of crystallinopathy. This new frameshift mutation closest to the stop codon predicts an alteration of the properties of the CTD domain, that suggest a low solubility that probably relegates its location to the membrane, hence reducing its presence and its chaperone activity in the cytoplasm. To understand the interference mechanism of this specific mutation, it is necessary to measure the consequences of the increment in

hydrophobicity, stiffness, and *cis* isomerization properties of the new CTD domain in the capability of oligomer assembly and disassembly, solubility and chaperone activity of the novel  $\alpha$ B-crystallin protein.

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## CONFLICT OF INTEREST

The authors have no competing interests to declare.

## AUTHOR CONTRIBUTIONS

DA, FG, and LA performed the genetic and sequencing study. BMC made a medical history and physical exams. ERI performed the anatomical pathology studies of the muscle biopsy. ATM and JMNP planned the work, coordinated clinical and molecular studies, and wrote the final version of the manuscript that was revised and approved by all authors.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request.

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