Short Report

Congenital posterior pole cataract and adult onset dilating cardiomyopathy: expanding the phenotype of αB-crystallinopathies


Mutations in the αB-crystallin gene (CRYAB) have been reported in desmin-related myopathies, with or without cardiac involvement. Mutations in this gene have also been documented in large multi-generation families with autosomal dominant congenital posterior pole cataract (CPPC). In these congenital cataract families no cardiac or muscular phenotype was reported. This report describes a family with an unusual read-through mutation in CRYAB, leading to the elongation of the normal αB-crystallin protein with 19 amino acid residues. Affected family members combine a CPPC with an adult onset dilated cardiomyopathy (DCM), thereby expanding the αB-crystallinopathy phenotype. Repolarisation abnormalities preceded the onset of cardiomyopathy and were already present in childhood. No skeletal myopathy was observed. This report illustrates that congenital cataract can be a prelude to more severe disease even outside the context of inborn errors of metabolism. The identification of a CRYAB mutation in this family supports the notion that mutations in this gene are a rare cause of genetically determined DCM. The combined congenital cataract/cardiomyopathy phenotype adds to our understanding of the complex phenotypic spectrum of αB-crystallinopathies.

α-, β-, and γ-crystallins are multi-meric protein complexes and make up approximately 90% of lens proteins. The crystallins are water soluble and are very stable in order to ensure life-long lens transparency following synthesis during lens development. The most abundant protein complex, α-crystallin, is constituted by 30 to 40 CRYAA and CRYAB subunits in a three to one ratio (1).

α-crystallins belong to the family of small heat-shock proteins and can function as molecular chaperones, thus contributing to the structural integrity of other proteins and cellular structures. While CRYAA is expressed almost exclusively in the lens, CRYAB is expressed in a wide variety of other tissues, most notably in skeletal and cardiac muscle. CRYAB is the most abundant small heat shock protein in cardiomyocytes (2). Mutations in CRYAB are an infrequent cause of desmin-related myopathies (DRM) (3, 4). DRM constitute a highly heterogeneous group of disorders characterised by accumulation of electron-dense granulofilamentous aggregates containing desmin, cryab and other proteins. DRM may or may not be associated...
with hypertrophic, dilated or restrictive cardiomyopathy. Mutations in DES, the gene coding for desmin on rare occasions also have been associated with arrhythmogenic right ventricular dysplasia/cardio-myopathy in DRM patients (5).

In DRM, cardiac disease can sometimes precede skeletal myopathy. An Arg120Gly mutation in CRYAB has been found to co-segregate with a phenotype of hypertrophic cardiomyopathy, velocar-yngael weakness and skeletal myopathy of varying severity in a large French DRM pedigree (3, 6). A number of patients developed cataract in this family. However, cataract was not congenital, did not require surgery at a young age and did not cause significant visual impairment in a large proportion of the patients. In addition to the Arg120Gly mutation, two truncating mutations in the C-terminal part of the CRYAB gene have been reported, leading to a different DRM phenotype, without cataract or cardiac involvement (4). In addition to the DRM phenotypes CRYAB mutations have been found in large multi-generation families with autosomal dominant congenital posterior pole cataract (CPPC) (7, 8), as well as other types of congenital cataract. In these very large CPPC families there were no signs of either cardiomyopathy or skeletal myopathy. Furthermore, CRYAB missense mutations have been described in unrelated single patients with isolated late onset familial dilated cardiomyopathy (DCM) (9, 10), and isolated distal myopathy (11). Finally a 5’ truncating c.60delC p.Ser21fs mutation leads to autosomal recessive fatal infantile hypertonic muscular dystrophy in Canadian Cree natives (OMIM 613869). Affected individuals invariably die from respiratory insufficiency before the age of 3, have no cardiomyopathy or cataract and antibodies against full length CRYAB protein show complete absence of the protein from muscle, while heterozygotes are healthy (12). Consistent with this loss of function phenotype in man, CRYAB knock-out mice are viable, do not develop cataracts and do not develop significant cardiomyopathy under standard laboratory conditions (13). However, these animals do develop a skeletal myopathy most notably of the tongue and axial muscles.

How mutations in the same gene lead to such widely divergent phenotypes largely remains to be clarified, although it appears that a gain-of-function or dominant negative disease mechanism is more probable than haploinsufficiency for the CRYAB gene product in most cases.

The current family study further expands the phenotypic spectrum of αB-crystallinopathies. The index patient in this family (Fig. 1 III-2) presented with a combination of autosomal dominant CPPC and adult onset cardiomyopathy. This unusual combination of symptoms was present in all affected adults, thereby apparently bridging the gap between the congenital cataract and DRM phenotypes. However, no skeletal myopathy was observed. We hypothesised that this particular phenotype could be the result of a mutation in CRYAB.

Materials, methods and results

The family was ascertained at the department of clinical genetics of the Utrecht University Medical Centre 20 years ago, when the female index patient (III-2), known to have had congenital cataract came in for reproductive counselling. Congenital cataract ran in her family, as well as severe cardiac disease. At that time all affected family members were deceased. Contact with the family of her affected biological father had been lost. III-2 was referred again at age 40 when she started to have cardiac problems.

Molecular analysis

Genomic DNA from the index patient was isolated from peripheral blood using standard procedures. The coding regions and intron–exon boundaries of the CRYAB gene were analysed using direct Sanger sequence analysis (reference sequence NM_001885.1).

Immunohistochemistry on explanted heart III-2

For immunohistochemical desmin analysis, a monoclonal mouse anti-human desmin antibody (dilution 1:100 Biogenex, Fremont, CA) was used, followed by incubation with poly-HRP anti-mouse IgG (Immunologic, Duiven, The Netherlands). Sections were developed in Diamino benzidine. For αB-crystallin staining, a monoclonal mouse anti-human αB-crystalline antibody (dilution 1:150 Novacastra, Newcastle, UK) was used, followed by incubation with poly-AP anti-mouse IgG (Immunologic). Sections were pretreated by boiling in citrate buffer (pH 6.0). All sections were counterstained with haematoxylin. For double staining, sections were first stained for αB-crystallin followed by boiling in citrate buffer. Subsequently, sections were stained for desmin and developed with fast blue.

Results

Family study

For a pedigree of the family see Fig. 1. For clinical information see table 1.

Additional clinical information on affected family members is available in the supplementary data online.

Sequencing of CRYAB

Sequencing of the CRYAB gene revealed a single nucleotide substitution at position 527 (c.527A>G p.*176Trp) in the index patient III-2, leading to replacement of the stop-codon by a tryptophan residue (TAG→TGG). As a result, the CRYAB protein is elongated by 19 amino acid residues. Presence of the mutation could be confirmed in individuals IV-2 and IV-3, but was absent in IV-1, the mutation was
Congenital posterior pole cataract and adult onset dilating cardiomyopathy

**Fig. 1.** Clear symbols represent healthy individuals, dashed symbols represent deceased individuals. The index patient is indicated with an arrow. Black symbols represent individuals with both a congenital cataract and a cardiomyopathy, individuals that are only half blackened had a congenital cataract, but did not yet have a cardiomyopathy. + and − represent the presence and absence of the CRYAB c.527A>G, p.X176Trp mutation respectively, individuals without a + or − were not available for study.

<table>
<thead>
<tr>
<th>Individuals</th>
<th>Eye</th>
<th>abRP</th>
<th>Cardiac pathology</th>
<th>ESHF or CP</th>
<th>Skeletal muscle myopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>II-4</td>
<td>Ccr</td>
<td>n.a.</td>
<td>n.a</td>
<td>DESHF at (32)</td>
<td>n.a.</td>
</tr>
<tr>
<td>II-6</td>
<td>Ccr</td>
<td>n.a.</td>
<td>n.a</td>
<td>DESHF at (29)</td>
<td>Not clinically</td>
</tr>
<tr>
<td>III-1</td>
<td>Ccr</td>
<td>at (9)</td>
<td>DCM with inclusions in cardiomyocytes</td>
<td>Death from embolic cva at (23)</td>
<td>Not clinically, not at PM</td>
</tr>
<tr>
<td>III-2</td>
<td>Ccr Ra (2×)</td>
<td>at (28)</td>
<td>DCM at (40) with myocardial inclusions</td>
<td>Normal EC at (28)</td>
<td>Not clinically, normal EMG at (41) CK: normal</td>
</tr>
<tr>
<td>IV-2</td>
<td>Ccr</td>
<td>(8)</td>
<td>Mwma at (13)</td>
<td>Not clinically</td>
<td></td>
</tr>
<tr>
<td>IV-3</td>
<td>Ccr</td>
<td>(9)</td>
<td>Mwma at (13)</td>
<td>Not clinically</td>
<td></td>
</tr>
</tbody>
</table>

abRP, abnormalities of repolarisation; CP, cardiac pathology/cardiac post mortem; ESHF, end stage heart failure; Ccr, congenital cataract; n.a., no data available; DESHF, death from end stage heart failure; numbers between (), age in years; DCM, dilated cardiomyopathy; cva, cerebro vascular accident; PM, post-mortem investigation; Ra, retinal ablation; EC, echocardiography; HTX, heart transplantation; EMG, electro-myography of proximal and distal muscles; Mwma, minor ventricular wall motion abnormalities, CK, creatine kinase; n, normal.

Also absent in the mother of the III-2. The same mutation was not found in over 200 ethnically matched control chromosomes and was not observed in over 6500 exomes (http://evs.gs.washington.edu/, accessed 29 October 2012).

**Western blot on explanted heart of III-2**

Western blot analysis confirmed the presence of both wild type CRYAB protein around 20 kDa and elongated CRYAB around 24 kDa (see Supporting Information).

**Histopathology of explanted heart of III-2**

The explanted heart (weight, 370 g) had a macroscopic appearance of a DCM. Microscopy revealed hypertrophy of cardiomyocytes with interstitial fibrosis (Fig. 2a,b). Part of the cardiomyocytes contained intracellular accumulations (Fig. 2b), similar to the aggregates that have been described in patients with a desmin-related cardiomyopathy caused by the Arg120Gly missense mutation in αB-crystallin (3). Both desmin and CRYAB protein were present in the accumulations (Fig. 2f). Similar deposits were not observed in control heart muscle.

**Electron microscopy**

Using electron microscopy, it was shown that there is an abnormal architecture of the myofibrils in relation to the mitochondria (see Supporting Information). In addition, it was confirmed that remnants of the myofibrils were present in the accumulations.
Discussion

This report suggests that a read-through mutation of *CRYAB* can result in a not previously described alpha-B crystallinopathy characterised by CPPC and adult onset DCM in absence of significant skeletal myopathy. How mutations in *CRYAB* exactly cause disease and why so many different phenotypes remains to be explained. Zhang et al. (14) in an attempt to clarify the genotype–phenotype correlation, recently studied the effect of the DRM causing Arg120Gly mutation (3), both reported skeletal myopathy causing C-terminal truncating mutations (4), and the isolated CPPC causing protein elongating C-terminal frameshift (7) in cultured H9c2 rat heart cells in relation to the expression of a related heat shock protein *HSPB1*, showing that all four cryab mutants tend to form aggregations and cause a shift from the soluble to the insoluble cell fraction to a different extend, whereas WT cryab protein was present almost entirely in the soluble fraction. The presented family gives away some additional clues. First, it suggests that just elongation of wild type cryab protein is sufficient to cause both congenital cataract and DCM, probably by interfering with post translational modification of the flexible C-terminal part of the protein which is thought to be important for water solubility, chaperone function, as well as oligomerisation (1, 4). Second, the absence of clinical skeletal myopathy in an adult well over 40 years of age seems to indicate that cryab chaperone function in the long run is more vital (less redundant) in cardiomyocytes than other types of striated muscle. The cryab protein is a chaperone in the heart, that under ischaemic stress translocates from the cytosol to the myofibrils and binds for example desmin and actin filaments (15, 16). It also has been shown to bind to specific parts of the titin protein, coded by TTN in cardiomyocytes and is probably important for structural integrity of this very large protein during
mechanical stress (15). Recently, truncating mutations in TTN have been identified as the single most important cause of idiopathic DCM (17). Therefore, it is logical to hypothesise that mutations in CRYAB may cause cardiomyopathy and myopathy by defective chaperoning of the titin protein.

The combination of autosomal dominant CPPC with adult onset DCM in absence of skeletal myopathy is a recognisable new type of alpha-B crystallinopathy that may be caused by a read-through mutation in the CRYAB gene.

Supporting Information

The following Supporting information is available for this article:

Appendix S2. Methods

Fig. S1. Western blot of protein isolated from the myocardium. The left lane with protein isolated from the heart of the patient shows 2 bands for αB-crystallin (CRYAB), one at 20 kDa and a second band at ~24 kDa. In the right lane with protein from a control patient only one band at 20 kDa is present. An antibody directed against α-tubulin was used as loading control.

Fig. S2. Transmission electron microscopy of the myocardium. (a) Heart of a healthy control showing regular myofibrils with mitochondria in between the myofibrils, bar = 2 μm. (b) Heart of the patients showing abnormal architecture of the myofibrils in relation to the mitochondria. Same magnification as (a). (c) Transverse cross-section of a cardiomyocyte with an intracellular aggregate showing electron-dense and electron-poor areas, bar = 2 μm. (d) Higher magnification of the area indicated by a box in C showing remnants of myofibrils in the intracellular accumulations indicated by an arrow, bar = 1 μm.

Fig. S3. (a) Last pre-transplant ECG of index III:2: sinus rhythm 85 bpm, intermediate heart axis, PQ 180 ms, QRS 105 ms, (no evidence for conduction disease), low voltage in standard leads, negative T waves in V1–V3, negative T waves in V4–V6. (b) Individual IV:2 13 years: Regular sinus rhythm, repolarisation abnormalities: positive T waves in V1–V3, negative T waves in V4–V6. (c) Individual IV:3 13 years: Regular sinus rhythm, repolarisation abnormalities: positive T waves in V1–V2, negative T waves in V4–V6.

Additional Supporting information may be found in the online version of this article.

Acknowledgement

We acknowledge the participating family for their cooperation.

Conflict of interest

No conflicts to report.