

## ARTICLE

# The myosin chaperone *UNC45B* is involved in lens development and autosomal dominant juvenile cataract

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Genome-wide linkage analysis, followed by targeted deep sequencing, in a Danish multigeneration family with juvenile cataract revealed a region of chromosome 17 co-segregating with the disease trait. Affected individuals were heterozygous for two potentially protein-disrupting alleles in this region, in *ACACA* and *UNC45B*. As alterations of the *UNC45B* protein have been shown to affect eye development in model organisms, effort was focused on the heterozygous *UNC45B* missense mutation. *UNC45B* encodes a myosin-specific chaperone that, together with the general heat shock protein HSP90, is involved in myosin assembly. The mutation changes p.Arg805 to Trp in the UCS domain, an amino acid that is highly conserved from yeast to human. *UNC45B* is strongly expressed in the heart and skeletal muscle tissue, but here we show expression in human embryo eye and zebrafish lens. The zebrafish mutant *steif*, carrying an *unc45b* nonsense mutation, has smaller eyes than wild-type embryos and shows accumulation of nuclei in the lens. Injection of RNA encoding the human wild-type *UNC45B* protein into the *steif* homozygous embryo reduced the nuclei accumulation and injection of human mutant *UNC45B* cDNA in wild-type embryos resulted in development of a phenotype similar to the *steif* mutant. The p.Arg805Trp alteration in the mammalian *UNC45B* gene suggests that developmental cataract may be caused by a defect in non-muscle myosin assembly during maturation of the lens fiber cells.

European Journal of Human Genetics (2014) 22, 1290–1297; doi:10.1038/ejhg.2014.21; published online 19 February 2014

**Keywords:** congenital cataract; *UNC45B*; non-muscle myosin; epithelial lens cell; zebrafish *steif* mutant

## INTRODUCTION

Congenital/infantile cataract (CC) is a developmental anomaly characterized by opacities in the crystal lens of the eye and is a common cause of restricted vision and blindness in children. Environmental and intrinsic factors are involved, including metabolic and genetic causes for CC. Mendelian forms of CC comprise a broad spectrum of syndromic and nonsyndromic phenotypes characterized by a set of associated ocular and/or systemic abnormalities. More than 35 loci, including at least 25 known genes, have been associated with nonsyndromic cataract, the majority showing autosomal dominant inheritance with high penetrance (ADCC).<sup>1,2</sup> Mutations in crystallins, particularly *CRYAA*, *CRYBB2*, and *CRYGD* and the connexin genes *GJA3* and *GJA8* comprise the largest group of loci causing ADCC, but mutations are also found in the membrane proteins *MIP*, *LIM2*, *TMEM114* and *CHMP4B*, in cytoskeleton proteins (*BSFP1* and *BSFP2*) and in transcription factors (*HSF4* and *MAF*).<sup>1,2</sup>

In a recent study,<sup>3</sup> we reported that these loci represented most of the causative mutations in 19 out of 28 unrelated Danish CC families. Among the unsolved families was one in which genome-wide linkage analysis revealed two candidate loci at 2q32.3–q33.3 and 17q11.2–q21.2, respectively.<sup>3</sup> The family comprised three generations with nine

affected members and the hereditary mode was consistent with autosomal dominant inheritance with complete penetrance (Figure 1a). Here we present the targeted resequencing of the linkage regions resulting in identification of a causative mutation in the myosin chaperone *UNC45B*. As zebrafish (*Danio rerio*) is a widely used model for human congenital disorders of the eye including cataracts,<sup>4</sup> we show the zebrafish homolog is necessary for normal early lens development.

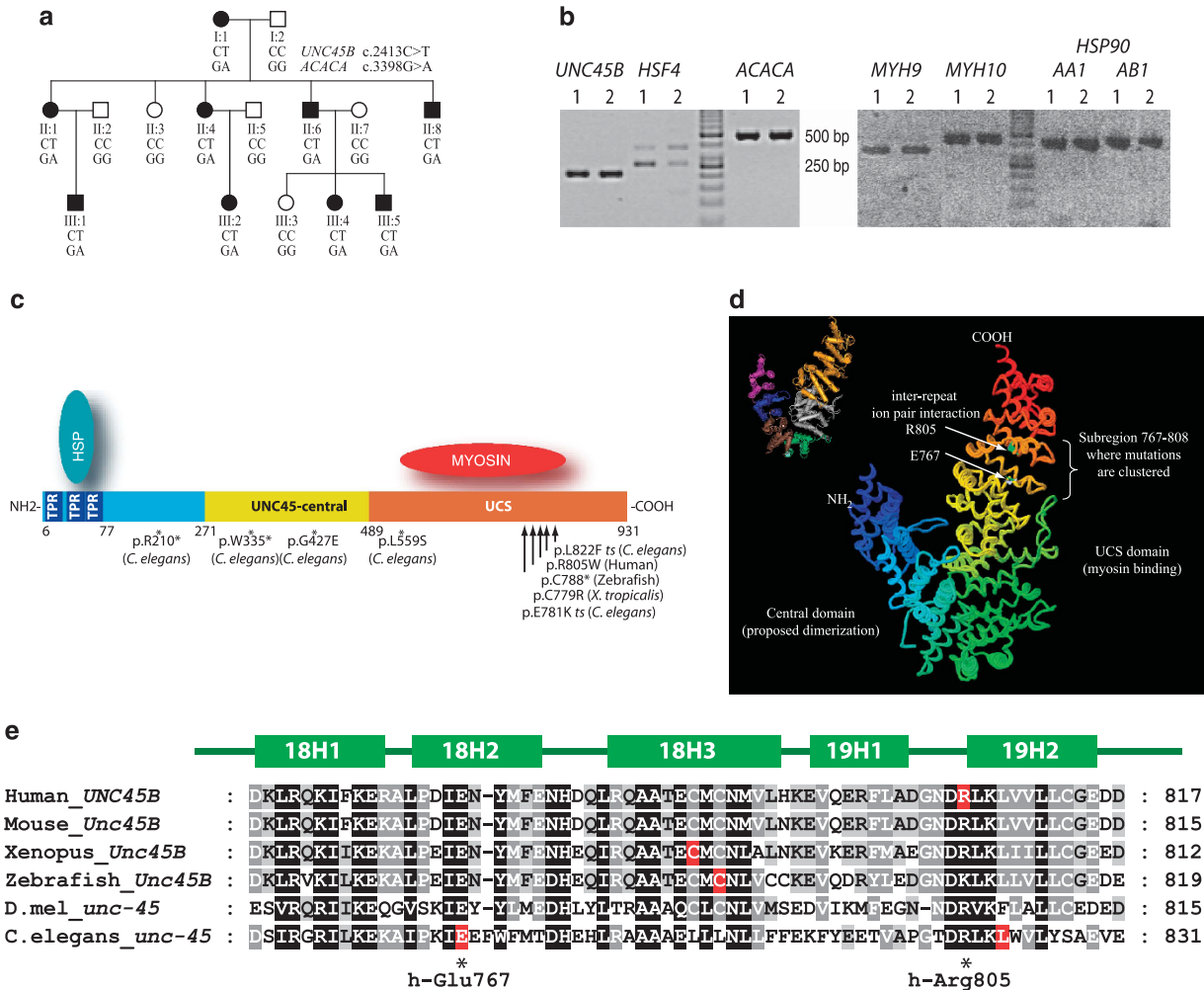
## MATERIALS AND METHODS

The family CC00116 was recruited from The National Danish Register of Hereditary Eye Diseases at the National Eye Clinic, Kennedy Center (<http://www.kennedy.dk/>). The study adhered to the tenets of the Declaration of Helsinki and was approved by the Copenhagen Scientific Ethics Committee and after being informed, all subjects gave written consent to participate in the study. Retrospective clinical information was obtained from ophthalmologists in private practice and local ophthalmic hospital departments. Genome-wide linkage analysis was made using Affymetrix 10K SNP arrays (Affymetrix, Santa Clara, CA, USA), multipoint genetic linkage analysis and haplotyping was done using standard methods.<sup>5,6</sup> PCR, Sanger sequencing and diagnostic restriction enzyme analyses (New England Biolabs, Ipswich, MA, USA) were carried out according to standard protocols. The linkage regions

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Received 16 August 2013; revised 17 January 2014; accepted 22 January 2014; published online 19 February 2014



**Figure 1** (a) The pedigree of family CC00116; filled symbols denote affected persons, circles denote females and squares denote males. (b) RT-PCR gene expression analyses of *UNC45B*, *HSF4*, *ACACA*, *MYH9*, *MYH10*, *HSP90AA1* and *HSP90AB1* in human embryo 43-day-old (1) and 54-day-old (2) eyes. The *HSF4* transcript was represented by two transcript variants (NM\_001040667.2 and NM\_001538.3). (c) Schematic domain structure of the UNC-45 family of proteins depicts the tetratricopeptide repeat (TPR) Hsp90 interaction domain, the central UNC-45 domain and the UCS myosin interaction domain. The clustered mutations in the UCS domain are shown by arrows, and the positions of the lethal (p.Arg210\* and p.Trp335\*) and the temperature-sensitive (ts) mutations in the *C. elegans*<sup>30</sup> homolog (p.Gly427Glu and p.Leu559Ser) are shown by an asterisk. The protein numbering refers to human UNC45B. (d) Structure of human UNC45B, central region and UCS domain based on the *D. melanogaster* 3D structure (PDB ID:3now).<sup>33</sup> The five ARMR structures are shown in different colors with the conserved groove in the brown. The h-Glu768 and the h-Arg805 residues are shown by arrows. The  $\alpha$ -helices structure is shown in upper left corner. Phyre2 and FirstGlance<sup>34</sup> were used for making the human model. (e) Alignment of the highly conserved subregion of the UCS domain with part of the  $\alpha$ -helix structure for 18H–19H (modified from<sup>33</sup>). Altered amino-acid positions are colored red and the conserved inter-repeat interacting ion pair h-Glu768 and h-Arg805 residues (Glu766 and Arg803 in the *D. melanogaster* sequence) are denoted.

(Supplementary Table S1) were captured in the affected individual I:1 (Figure 1a) using a NimbleGen custom-designed chip (Roche NimbleGen, Inc., Madison, WI, USA) and deep sequenced by paired-end tags using an Illumina Genome Analyzer IIx platform (Illumina, Inc., San Diego, CA, USA), and data were analyzed using standard protocols. The *UNC45B* cDNA clone NM\_001033576 purchased from Origene (SC306792, Origene, Rockville, MD, USA) was used for site-directed mutagenesis. Total RNA was isolated from human embryo 43- and 54-day-old eyes and analyzed for gene expression by RT-PCR (Figure 1; Supplementary Information).

### Zebrafish maintenance

Zebrafish were maintained and manipulated as described.<sup>7–9</sup> The *unc45b* mutant allele *steif* (*sb60*) was generously provided by the Max-Planck-Institut für Entwicklungsbiologie (Tübingen, Germany).<sup>10</sup> Zebrafish embryonic genotypes were determined phenotypically or by using dCAPS analysis.<sup>11,12</sup>

### Immunohistochemistry and cryosection of Zebrafish embryonic eyes

Polyclonal antibodies were raised in guinea pigs against bacterially expressed recombinant zebrafish *Unc45b* protein. Cryosections of embryonic eyes were prepared and immunostained as described.<sup>13,14</sup> non-muscle myosin (NMM) antibody (NMMII;M8064, Sigma-Aldrich, Oakville, ON, Canada) was used at 1:100 dilution, whereas ZL-1 (Zebrafish International Resource Center, University of Oregon) or *Unc45b* was used at 1:250 dilution.

### Ectopic Unc45b in zebrafish

Plasmids containing human wild-type and mutant *UNC45B* clones (described above) were linearized and *UNC45B* capped mRNA was synthesized using T7 polymerase (Invitrogen, Carlsbad, CA, USA) and the mMessage mMachine kit (Life Technologies Inc., Burlington, ON, Canada). Two hundred and fifty pg of

mRNA was injected into embryos at the one-cell stage. Embryos were genotyped by dCAPS as described above. More than 20 embryos were injected in each trial.

## RESULTS

### Clinical data

The pedigree of the family with autosomal dominant CC is shown in Figure 1a. Clinical data (obtained retrospectively) were limited for this family, as most of the affected individuals had already been operated upon and were aphakic at the time of investigation. The age at first operation varied between 6 and 45 years of age (mean = 19, median = 14 ( $N = 7$ )). In two individuals, retinal detachments occurred after operation for secondary cataract and one individual developed a severe secondary glaucoma. Individual III:4 (born in 1950) was diagnosed with cataract at the age of 18 and not operated before the age of 45. The phenotype was described as posterior subcapsular and central. During intraocular lens implantation, a central fibrous opacity in the lens capsule had to be left owing to a risk of rupture of the capsule. In the youngest generation, the two affected individuals were followed for years before lens opacities developed at ages 9 and 16, respectively.

### NGS and characterization of the mutations

Genome-wide linkage analyses resulting in two candidate loci on chromosomes 2 and 17 has previously been published (Supplementary Figures S1A and S2).<sup>3</sup> The linkage regions covered 24 Mbp and 254 annotated reference genes (Supplementary Table S1). Ten genes selected as candidates based on EST and cDNA expression data were all excluded by Sanger sequencing (Supplementary Figure S1B). Subsequently targeted deep sequencing of the two linkage regions in the index patient I:1 resulted in a total of 203 public (dbSNP138) and 5 private SNVs (Supplementary Table S1). One hundred and five public and four private SNVs were nonsynonymous variants. Sanger sequencing and segregation analyses excluded three of the four private nonsynonymous SNVs, leaving an *ACACA* mutation as a disease candidate. One hundred four of the 105 public nonsynonymous SNVs were excluded either by a MAF value  $> 0.01$  or by Sanger sequencing and segregations analysis (data not shown), leaving an *UNC45B* mutation as a putative disease-causing lesion. Five mutations in splice regions were excluded owing to high MAF values; gain or loss stop variants were not found in the exons (Supplementary Figure S1B and Supplementary Table S1). *UNC45B* and *ACACA* mutations segregated heterozygously with the disease trait and homozygous for the canonical sequence in unaffected members confirming autosomal dominant inheritance (Figure 1a).

The *ACACA* (MIM 200350) mutation was a G>A transversion (c.3398G>A) in exon 27 of transcript variant 1 causing a substitution of Arg1133 with His. The mutation affects the central region of the *ACACA* protein and was predicted to be damaging by the prediction servers PolyPhen2 (0.986), MutationTaster (0.79) and SIFT.

The *UNC45B* (MIM 611220) mutation was a C>T transition (c.2413C>T) in exon 19 of transcript variant 1 and replaces Arg805 with a tryptophan residue. The mutation is located in the C-terminal UCS domain of the protein (Figure 1) and predicted damaging by PolyPhen2 (1.000), MutationTaster (1.000) and SIFT. The human *UNC45B* Arg805 residue is highly conserved in vertebrate as well as in invertebrate and fungal homologs (Supplementary Figures S3C and S3D).

Neither of the mutations was found by Sanger sequencing or restriction enzyme digest in 230 unrelated healthy Danes of both sexes. The *ACACA* substitution has been reported by the COSMIC

project (Id: COSM175625) as a large intestine carcinoma somatic mutation, and the *UNC45B* mutation (rs370424081) by the NHLBI Exome Sequencing Project (ESP6500, November 2013) in the African American population (genotype frequencies TT = 0/TC = 1/CC = 2202).

### Gene expression data

To test whether the expression of either of the two candidate genes was consistent with a role in the lens, RNA from 43- and 54-day human embryonic eye tissues was used for qualitative PCR analyses of *UNC45B* and *ACACA*, the human HSP90 genes *HSP90AA1* (MIN 140571) and *HSP90AB1* (MIN 140572), the NMM heavy chain genes *MYH9* (MIM 160775) and *MYH10* (MIM 160776), and the cataract disease gene *HSF4* (MIM 602438). Expression of *UNC45B*, *ACACA*, *MYH9*, *MYH10*, the two HSP90 genes and the *HSF4* gene was detected at both developmental stages, which did not allow exclusion of either gene from consideration (Figure 1b).

Given that the *ACACA* gene is proposed to encode an enzyme with a housekeeping function, and is most likely to have an effect in highly lipogenic tissues, while the *UNC45B* locus has been shown in model systems to have both an eye phenotype when mutant as well as regulating the function of NMM in non-muscle tissues,<sup>10,15</sup> and heterozygous mutations in NMMs have been associated with premature cataract formation in mouse models,<sup>16</sup> our attention focused on *UNC45B* as the more likely candidate gene. We cannot rule out the formal possibility that the CC phenotype in this family may result from contributions from multiple heterozygous loci.

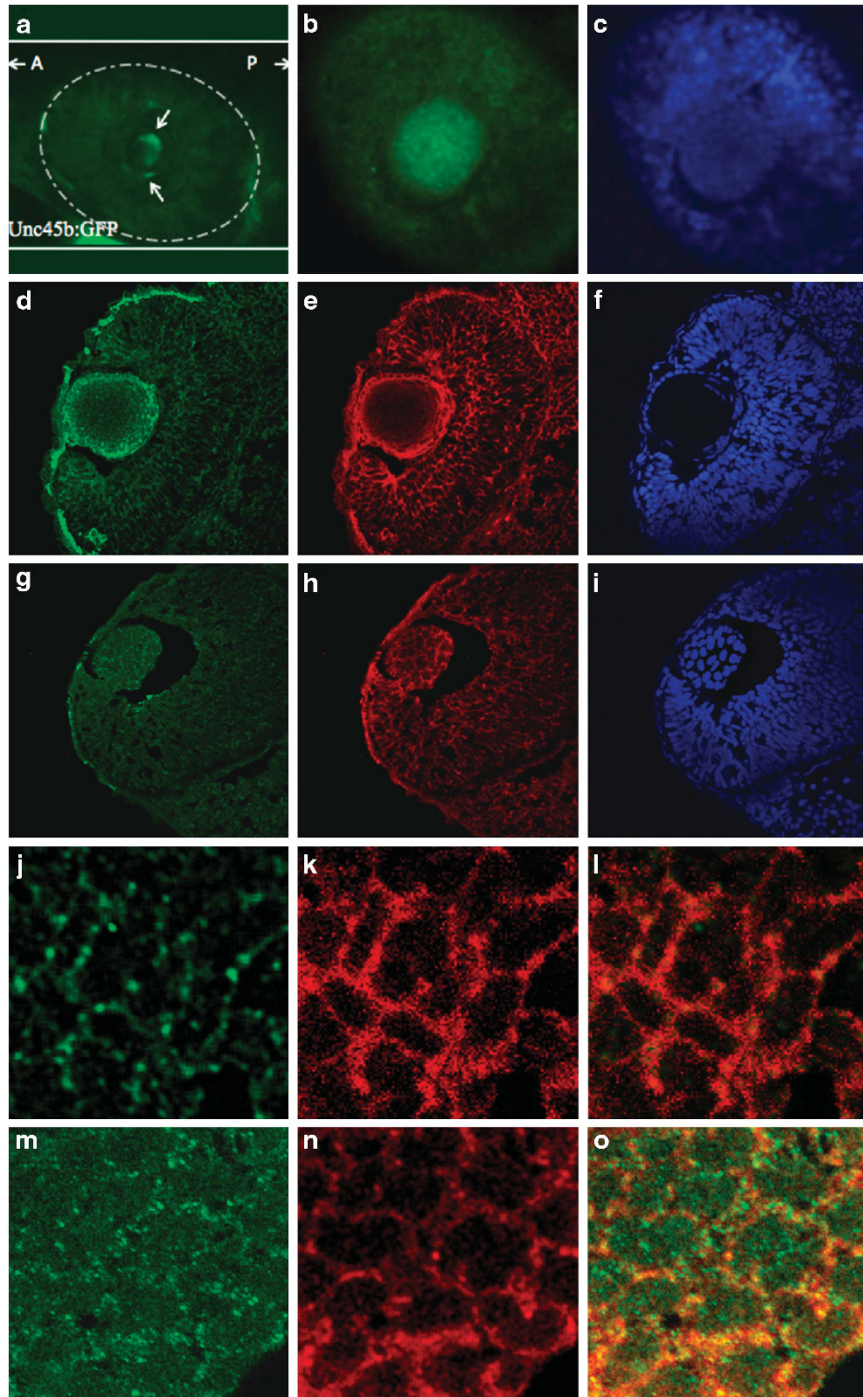
### Zebrafish studies

The *Unc45b* ortholog in zebrafish shows expression in the embryonic eye, both by antibody staining and by reporter gene (Figures 2a–c). Differentiation of lens fiber cells begins in the zebrafish at ~24 h post fertilization (hpf) and involves processes including cellular reorganization and organelle degeneration that result in a transparent lens and functional visual system by 72 hpf.<sup>17, 18</sup> Disassembly of actin stress fibers is believed to induce lens cell differentiation and lead to the formation of cortical fibers through the reorganization of actin filaments.<sup>18,19</sup> Zebrafish embryonic eyes were examined for lens fiber cell boundaries, differentiation, and nuclear retention. Expression of zebrafish *Unc45b* is seen both in the epithelial layer of the developing lens and in the surrounding tissues, including the ganglion cell layer (Figure 2d). NMM is also robust in these tissues (Figure 2e), and *Unc45b* and NMM colocalize at the cortex of these cells (Figure 2l), consistent with both a role for NMM in lens development as well as association of *Unc45b* with NMM. At this stage, the lens is almost clear of cell nuclei (Figure 2f), but NMM and actin are still present in the retracting cells. At 3–5 dpf, the eye continues to grow and differentiate, and the lens is clear of nuclei as well as actin-myosin staining (Supplementary Figure S4).

The *unc45b* mutant, *steif*, carries an embryonic lethal nonsense mutation in the UCS domain (Table 1 and Figure 1c).<sup>10,20</sup> In addition to the paralyzed phenotype, *steif*<sup>-/-</sup> mutants have smaller eyes compared with wild-type siblings (Figure 3). Both muscle and eye phenotypes are also seen in *Unc45b dicky ticker* mutants in *Xenopus*.<sup>21</sup> In CC patients, the eye size is normal and none reported muscular weakness or cardiac disease.

At the earliest time point examined (48 hpf), the lens in *steif* mutants is noticeably smaller than in wild-type, and has not yet begun to clear of nuclei (Figure 2). At 3 dpf, the central lens of *steif* mutants contained ectopic actin fibers and nuclei that were not present in the wild-type embryos. In the wild-type lens, central lens





**Figure 2** Expression of zebrafish *unc45b* is detected in the lens of animals carrying a *punc45b::GFP* transcriptional reporter (a), as well as in fixed wild-type animals with a polyclonal zebrafish Unc45b antibody (b). Counterstain with DAPI shown in c. (d–o) Cryosections of zebrafish eye from 2 dpf embryos. Wild-type (d–f) and homozygous *steif* embryos (g–i) immunostained with polyclonal antibody against zebrafish Unc45b (d, g), polyclonal antibody against human Non-muscle Myosin 2A (e, h) or DAPI (f, i). Panels j and k are magnified images from panels c and d, and l is a merge of j and k, showing colocalization of Unc45b and NMM. Panels m and n are magnified images from panels g and h, and o is a merge of g and h, showing loss of cortical Unc45b staining in the mutant embryos. The *steif* mutation may still result in expression of a truncated, but nonfunctional protein, so polyclonal antisera still produce a signal. The transgenic line, *Tg(unc45b:EGFP)ua1*, in which eGFP is driven by a 1128-bp fragment of the zebrafish *unc45b* promoter, was constructed at the University of Alberta by Eva Guznowski.

fibers lack nuclei, and at the proximal lens pole in the lens transition zone, nuclei of the differentiating secondary fibers have a flat and elongated shape. The nuclei of *steif* mutants are swollen and located throughout the anterior region of the lens and the nuclei of the distal

epithelium cells stain just as intensely as in wild-type embryos. In contrast to wild-type, colocalization of Unc45b and NMM is lost in *steif* embryos, where the presumably nonfunctional truncated protein is now seen throughout the cytoplasm (Figure 2o). Smaller eyes are

**Table 1** Clustered mutations in the UCS domain of the *UNC5B* proteins

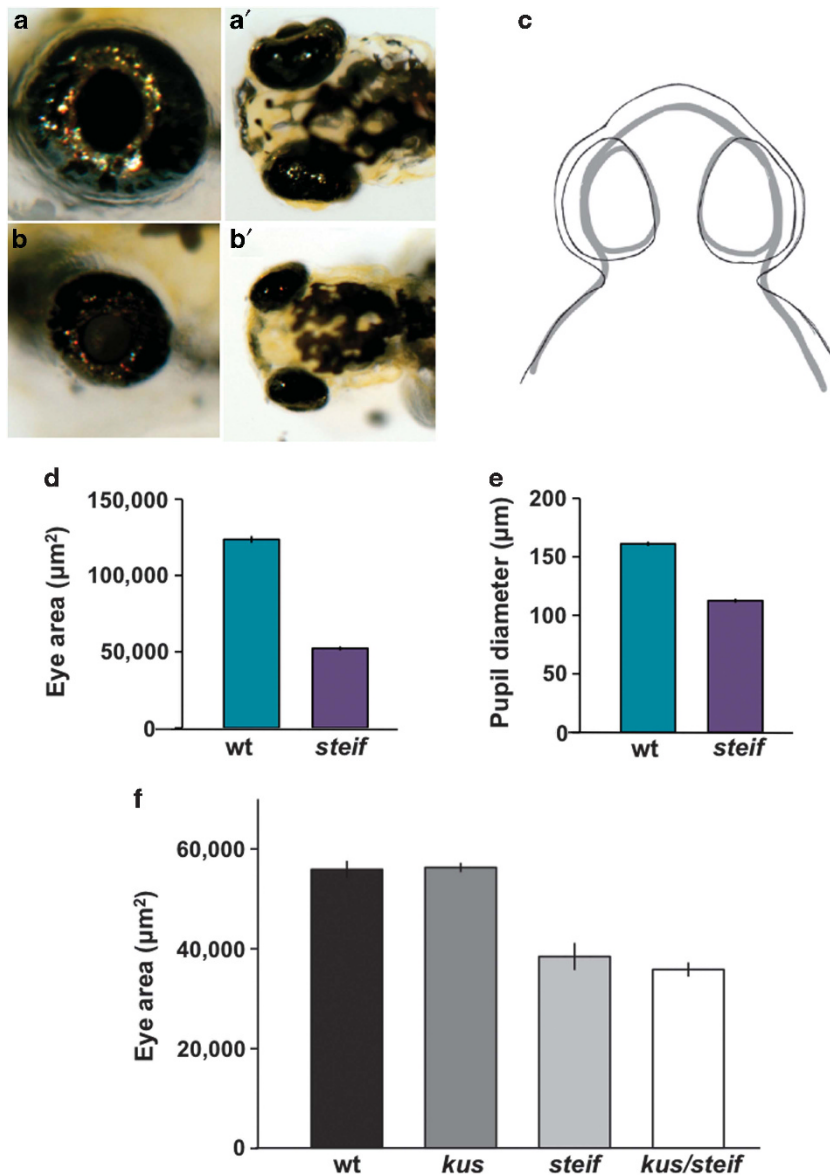
Species	Gene <sup>a</sup>	Nucleotide change	Amino-acid change	Type of mutation	Inheritance	Consequence <sup>b</sup>	Phenotype	Reference
<i>X. tropicalis</i>	<i>Unc45b</i>	c.2335T>C	p.Cys779Arg <sup>c</sup>	Missense	Recessive	Damaging/lethal	Muscle	2
Zebrafish	<i>unc45b</i>	c.2364C>A	p.Cys788 <sup>*,d</sup>	Nonsense	Recessive	Damaging/lethal	Muscle	10
<i>C. elegans</i>	<i>unc-45</i>	c.2341G>A	p.Glu781Lys	Missense	Recessive	Tolerant/paralyses	Muscle (ts)	30
<i>C. elegans</i>	<i>unc-45</i>	c.2464C>T	p.Leu822Phe	Missense	Recessive	Tolerant/paralyses	Muscle (ts)	30
Human	<i>UNC5B</i>	c.2413C>T	p.Arg805Trp	Missense	Dominant	Damaging	Cataract/lens	Present study

<sup>a</sup>Referenced sequence accession numbers are given in Supplementary Information.

<sup>b</sup>The consequences of amino-acid changes are predicted by PolyPhen2.

<sup>c</sup>The *Xenopus tropicalis* *Unc45b* *dicky ticker* mutant.

<sup>d</sup>The zebrafish *steif* *unc45b* mutation.



**Figure 3** Analysis of zebrafish eye phenotypes in *unc45* mutants. Lateral and dorsal views of representative 4 dpf wild type (**a**, **a'**) and *steif* embryos (**b**, **b'**). *Steif* mutants have visibly reduced ocular size and the lens does not protrude from the optic cup. Camera lucida drawing depicting the difference in head size between wild type (thin line) and *steif* (thick line) embryos at 48 hpf (**c**). Graphs showing the average eye area (**d**), and average pupil diameter (**e**) at 4 dpf and the average eye area (**f**) at 48 hpf. For comparison, data from a strain (*kurzschluss* (*kus*)) mutant in the second *unc-45* paralog (*Unc45a*) as well as doubly mutant in *unc45a* and *unc45b* (*kus;steif*) is included.<sup>8,26</sup> *Steif* and *kus;steif* mutants show a significant reduction in eye area ( $P=0.000$ ). Error bars indicate SEM.

often seen in zebrafish mutants lacking normal circulation, but the lens fiber disorganization at 3 dpf is not detected in the smaller eyes from *pickwick* mutants, defective in the heart-specific isoform of titin (Supplementary Figure S4).<sup>22</sup> A defect in lens fiber development in *steif*, but not wild-type or *pickwick*, is also seen when eyes are stained with the ZL-1 antibody, which is fiber-cell-specific, although the nature of the antigen is unknown (Supplementary Figure S4).

RNA encoding human *UNC45B* was synthesized and injected into either wild-type one-cell embryos or those derived from an incross of *steif* heterozygous parents. Homozygous mutant embryos were determined by genotyping. Although some nuclei remain in the anterior region of the lens, their numbers appear to be reduced compared with uninjected *steif* embryos (Figure 4). Nuclei along the posterior edge of the lens have a flat appearance similar to those in wild-type embryos. Injection of wild-type human *UNC45B* mRNA was not able to rescue the ectopic F-actin localization pattern seen in *steif* mutants (Figure 4). RNA injections of the p.Trp805 altered form of human *UNC45B* into wild-type embryos did, however, recapitulate both the ectopic nuclei and F-actin staining phenotypes (Figure 4).

## DISCUSSION

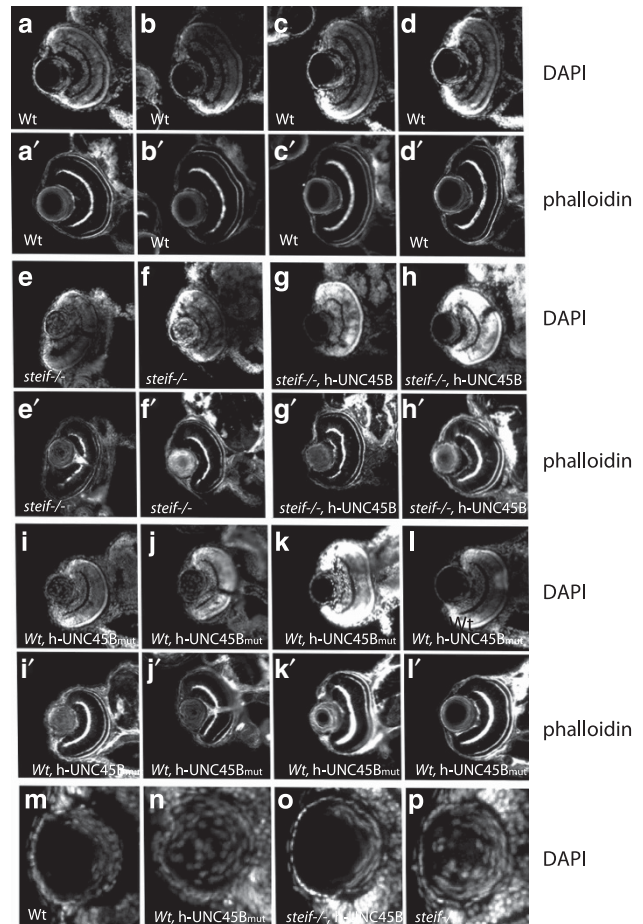
The genome-wide linkage analysis of family CC00116 resulted in two novel CC loci on chromosomes 2 and 17, respectively.<sup>3</sup> Additional family members were not available for exclusion of either linkage region, and targeted deep genome resequencing of the linkage regions resulted in two candidate mutations co-segregating with the disease trait. The mutations affected proteins of very different function; the *ACACA* gene encodes multifunctional enzyme involved in *de novo* biosynthesis of fatty acids and the *UNC45B* gene encodes a chaperone involved in correct assembly of type II muscle and NMM.

### The *ACACA* mutation

*ACACA* encodes the type I fatty-acid synthase, FAS, which is expressed in the soluble cytoplasm of lipogenic tissues such as adipose, liver and mammary gland. The domain structure of FAS is conserved in eukaryotes and has evolved into the large 264 kDa synthase with seven discrete distinct functional domains.<sup>23</sup> The p.Arg1133His mutation is in the less well-conserved central part of FAS, which has no assigned catalytic function.<sup>23</sup> The expression of *ACACA* in embryonic eye tissues (Figure 1b) is consistent with a housekeeping function for *de novo* fatty-acid biosynthesis. Homozygous *Acaca*<sup>-/-</sup> knockout (KO) mouse embryos were undeveloped and died, while heterozygotes were fertile with normal life spans and normal body weight.<sup>24</sup> Considering the function and housekeeping role of FAS and the KO mouse phenotypes, the *ACACA* mutation is unlikely to be the genetic cause of the CC phenotype in family CC00116. The report of the p.Arg1133His mutation as a somatic mutation in large intestine carcinoma must be considered as a passenger mutation in cancer progression.

### *UNC45B* mutation

*UNC45B* is a molecular co-chaperone responsible for correct assembly of type II myosins and has a role in myoblast fusion and sarcomere organization. Within the family of UNC-45 proteins, one isoform (*unc-45*) is encoded in invertebrate genomes and two isoforms (*Unc45a* and *Unc45b*) in vertebrates. *Unc45a* is ubiquitously expressed and *Unc45b* is highly expressed in striated muscle<sup>25,26</sup> and the muscle myosin chaperone activity of UNC-45 proteins is dependent upon Hsp90 as co-chaperone.<sup>27</sup> UNC-45 has three functionally and structurally distinct regions (Figure 1c). The N terminus contains three tetratricopeptide repeat (TPR) motifs and is involved in



**Figure 4** Cryosections of zebrafish embryonic eyes following *Unc45b* mRNA injections. Production of the ectopic nuclear and actin accumulation phenotypes were compared either with WT (a–d, a'–d', m) or *unc45b* (*steif*)<sup>-/-</sup> homozygous embryos (e, f, e', f', p) as controls. Ectopic expression of human *UNC45B* in zebrafish eyes was achieved by injecting synthetic human *UNC45B* mRNA into one-celled embryos derived from WT or from an *unc45b*(*steif*)<sup>+/-</sup> incross. Genotype of embryos was determined retrospectively for the latter cross as described. Embryos were fixed at 3 dpf for all images. Stained with DAPI to indicate nuclei (a–p), or phalloidin to show actin localization (a'–l'). Multiple embryos are presented for each genotype. Injection of wild-type human *UNC45B* mRNA was able to rescue the ectopic nuclei phenotype, but not the ectopic F-actin localization pattern, seen in *steif* mutants (g, h, g', h', o). Although some nuclei remain in the anterior region of the lens, their numbers appear to be reduced compared with uninjected *steif* embryos. Injections of mRNA encoding the mutant form of human *UNC45B* into one-cell WT embryos did produce both the ectopic nuclei and F-actin staining phenotypes (i–l, i'–l', n) reminiscent of the *steif* phenotype. Panels m–p show DAPI staining at higher magnification to highlight nuclei in lens.

formation of a stable complex with the general Hsp90 protein. The central region is conserved in all metazoan UNC-45 proteins but its function still remains unclear. The C terminal consists of an UCS domain (Unc-45, Cro1 and She4,) homologous with fungal proteins involved in segregation of molecules during budding, endocytosis and cell division (Supplementary Figure S3C).<sup>25,27,28</sup>

Mutations are known in *Caenorhabditis elegans unc-45* and in zebrafish and *Xenopus tropicalis Unc45b*.<sup>10,21,29–31</sup> These are either lethal or temperature sensitive (*ts*) homozygous missense or nonsense mutations (Table 1 and Figure 1c–e). The phenotypes caused by



mutations in *C. elegans unc-45* or vertebrate *unc45b* include defective muscle development as well as effects in the vertebrate eye.<sup>10,32</sup> Heterozygote siblings have normal phenotypes in all systems examined. The majority of the mutations, including the human p.Arg805Trp mutation, are located in the UCS domain (Figure 1d). Lee *et al.*<sup>33</sup> proposed a 3D structure from X-ray analysis of *Drosophila melanogaster UNC-45*, which we used to make a 3D model of the human protein (Figure 1d).<sup>34</sup> The structure reveals a contiguous arrangement of 17 helical layers with five discrete armadillo (ARM) repeat subdomains, which is folded to an L shape with no obvious transition between the central region and the UCS domain.<sup>33</sup> Five of the six known mutations in the UCS domain are located in a small nonpolar subregion, which has the highest degree of sequence identity in the five most evolutionarily divergent UCS proteins. This ~40 amino-acid subregion forms the  $\alpha$ -helices 18H1-3 and 19H1-2 (Figure 1e) and with the exception of the *steif* nonsense allele, all the mutations in this subdomain are missense mutations. In addition, the Arg803 position in *Drosophila*, which corresponds to h-Arg805 and *C. elegans*-Arg819, forms an inter-repeat ion pair interaction with *Drosophila* Glu766, which corresponds to h-Glu767 and *C. elegans*-Glu781.<sup>33</sup> The *C. elegans*-Glu781 position is one of the *ts* paralyzing mutations, and the ion-interacting Arg residue is the C00116 Arg805Trp mutation. Both mutations may lead to ion pair instability of the  $\alpha$ -helix structures (18H2 and 19H2 in Figure 1e) and reduced myosin-binding ability of the UCS domain. The heterozygous genotype of the human p.Arg805Trp mutation suggests either a dominant-negative or neomorphic mutation. This is consistent with recent evidence that the *UNC45B* protein function is part of a multimeric complex.<sup>35,36</sup> Gazda *et al.*<sup>36</sup> have demonstrated that *C. elegans* UNC-45 forms linear protein chains that offer multiple binding sites for cooperating chaperones and client proteins. The backbone of the UNC-45 filament is composed of the tethered superhelix ARM1-8 and TPR1-3 unit, and the UCS domain extends away from the filament axis. This is consistent with the h-p.Arg805Trp alteration serving as a dominant-negative mutation that affects *UNC45B* multimeric complex, and suggests instability of the *UNC45B* polymer with structural hindrance or reduced binding stability during the assembly of nonmuscular myosin in the lens development. The occurrence of c.2413C>T heterozygous in the African American population may infer the person to have juvenile cataract. The NHLBI Exome Sequencing Project represents anonymized data, so a follow-up on the individual is not possible.

### Expression and zebrafish *steif* mutant

*UNC45B* expression was seen in 43- and 54-day-old human embryonic eyes together with the two potential client proteins MYH9 and MYH10 and two co-chaperones HSP90AA1 and HSP90AB1 (Figure 1b). Expression of both *Unc45b* and NMM in the zebrafish embryonic eye is consistent with this analysis. Colocalization of the two proteins is seen in several different cell layers, including lens epithelia and ganglion cells, and this colocalization is disrupted in homozygous *steif* embryos.

The *steif*<sup>-/-</sup> embryos have a smaller eye compared with wild-type embryos at the same developmental stage,<sup>10</sup> and quantitative analysis shows that the eyes were approximately half the size as seen for the wild-type or for embryos homozygous for the *kurzschluss* mutation, which truncates the *Unc45a* isoform and results in severe aortic arch defects (Figure 3).<sup>8,26</sup> The *steif*<sup>-/-</sup> embryos lens also showed persistence of nuclei compared with the wild-type embryo, suggesting the lens fiber cells maturation is affected by the *unc45b* mutation. The same lens phenotype showing high nuclei

accumulation was seen when wild-type embryos were injected with the altered human p.Trp805 *UNC45B* construct (Figure 4), consistent with an ability of the mutant human *UNC45B* protein causing the juvenile cataract phenotype seen in family CC00116. All other *unc-45/unc45b* mutations described are lethal in homozygous state, where the effect of the heterozygous human p.Arg805Trp alteration results in a milder phenotype of unclear lenses. Homozygous or compound heterozygote *UNC45B* mutation state may be lethal in humans as seen for the zebrafish and *Xenopus* mutations (Table 1).

The lens grows rapidly by embryonic cell division and differentiation in the epithelial cell layer region just above the lens equator. The immature lens cells migrate below the equatorial plane into the transitional zone, where they elongate and differentiate into fiber cells.<sup>37</sup> Data from *C. elegans* have shown that UNC-45 acts as chaperone in assembly of NMMs.<sup>15,38</sup> NMMs are known to be involved in differentiation and elongation of mouse embryo lens fiber cells where *MYH10* was localized in lens epithelium and was detected at the interface of lens epithelium and fiber cells.<sup>39</sup> The occurrence of NMM as well as its chaperone components *UNC45B* and *HSP90* in the human and zebrafish embryo lenses suggests *UNC45B* may act as a chaperone in differentiation, migration and maturation of the lens epithelia cells, and the accumulation of nuclei seen in the embryo *steif*<sup>-/-</sup> lens (Figure 2) may be a consequence of defective maturation and organelle apoptosis in the developing lens fiber cells.

Human mutations in *MYH9* (MIM 160775) are autosomal dominantly inherited<sup>40</sup> and display phenotypically distinct disorders including May-Hegglin anomaly (MIM 155100), Fechtner syndrome (MIM 153640), and Sebastian syndrome (MIM 605249). All three disorders involve megakaryocyte, platelet, and leukocyte defects and the Fechtner syndrome is further characterized by sensorineural deafness, cataract and nephritis implicating *MYH9* in the pathogenesis of cataract. Homozygous KO of *Myh9* in mice was embryonic lethal, whereas heterozygote *Myh9*<sup>+/-</sup> mice were viable and fertile and cataract was observed in two out of the three mouse lines heterozygous for human *MYH9* missense mutations.<sup>16</sup> In zebrafish, mutations that produce ocular disorders, including cataracts, are found in a similar spectrum of candidate genes as we have seen in humans, suggesting that eye development processes in the two systems are fundamentally similar at the cellular level despite differences in timing and morphology during development.<sup>4,17,41,42</sup>

In conclusion, we have characterized a human *UNC45B* mutation in a Danish family with autosomal dominant cataract developing in early childhood. Experimental data using a zebrafish *unc45b* mutant show an eye developmental phenotype. Human *UNC45B* has been shown to be present in human embryo eye supporting a function for *UNC45B* in the developing lens during fiber cells maturation and migration. Both the co-chaperone *HSP90* and nonmuscular myosin are expressed in the human embryo lens, which is suggestive of a role for the myosin chaperone *UNC45B* in lens maturation and a possible new mechanism for cataract formation.

### WEB RESOURCE

1000 Genomes Browser: <http://browser.1000genomes.org/index.html>  
AceView: <http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/index.html>  
CatMap:<sup>2</sup> <http://cat-map.wustl.edu/>  
COSMIC (Catalogue of Somatic Mutations in Cancer): <http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/>  
dbSNP, NCBI: <http://www.ncbi.nlm.nih.gov/snp/>  
FirstGlance in Jmol: <http://bioinformatics.org/firstglance/fgij/>  
GEPIS-tissue: <http://research-public.gene.com/Research/genentech/genetech-gepis/index.html>

NHLBI, Exome Sequencing Project (ESP): <http://evs.gs.washington.edu/EVS/>  
 Online Mendelian Inheritance in Man (OMIM): <http://www.omim.org>  
 Phyre2<sup>34</sup> (Protein Homology/analogy Recognition Engine V 2.0): <http://www.sbg.bio.ic.ac.uk/phyre2>  
 UCSC Genome Browser: [www.genome.ucsc.edu](http://www.genome.ucsc.edu)

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

We are grateful to the family for their participation. Lars Allan Larsen is thanked for donation of the human embryo RNA, Annemette Mikkelsen, Linda Boje Dalsgaard and Karen Friis Henriksen are thanked for library preparation, cDNA synthesis and expression analyses. The cataract study was supported by a grant from The Danish Association of the Blind, Wilhelm Johannsen Centre for Functional Genome Research hosted the targeted next generation sequencing, and RCLink hosted the RNA expression analyses. Research in the Pilgrim lab is supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC). We are grateful to Andrew Waskiewicz for the use of his zebrafish facility, which is supported by the NSERC MRS Program. SC was supported by a Graduate Studentship from NSERC.

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