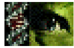 *Molecular Vision* 2007; 13:1475-82 <http://www.molvis.org/molvis/v13/a164/>
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HCCS loss-of-function missense mutation in a female with bilateral microphthalmia and sclerocornea: a novel gene for severe ocular malformations?

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Purpose: To analyze if mutations in *HCCS*, encoding the mitochondrial holocytochrome *c*-type synthase, are associated with phenotypes other than the microphthalmia with linear skin defects (MLS) syndrome, including severe eye malformations such as microphthalmia and/or anophthalmia. In addition, we investigated the impact of the p.E159K missense mutation on sorting of HCCS to mitochondria and its functional integrity.

Methods: In a cohort of 27 females obtained from a population-based study on infants and fetuses with congenital eye malformations we performed mutation analysis of *HCCS* by PCR amplification of the coding exons and direct sequencing. The X-inactivation pattern was determined by analyzing the methylation pattern at the *AR* locus in one patient. For functional analysis of the identified missense mutation, we transfected CHO-K1 cells with wild-type HCCS or HCCS E159K mutant construct and analyzed subcellular localization of the expressed proteins by immunofluorescence analysis and confocal microscopy. Functional integrity of the mutated HCCS protein was investigated by complementation studies in yeast. Therefore, we ectopically expressed HCCS wild type and the E159K mutant in the *S. cerevisiae* strain B-8025, carrying a deletion of the *HCCS* ortholog *CYC3*, and analyzed the capacity of the yeast strain to grow on nonfermentable carbon sources.

Results: We detected the heterozygous c.475G>A mutation in exon 5 of *HCCS*, predicting an amino acid substitution of the highly conserved glutamate at position 159 by lysine, in a female presenting with bilateral microphthalmia and sclerocornea. This point mutation was not found on more than 460 X chromosomes. We identified a skewed X-inactivation in the patient's peripheral blood cells. Similar to HCCS wild type, ectopically expressed HCCS E159K was targeted to mitochondria in CHO-K1 cells. In contrast, expression of HCCS E159K did not complement respiratory growth of the *CYC3*-deficient yeast strain B-8025, while wild-type HCCS and the yeast heme lyase Cyc3p could rescue growth on nonfermentable carbon sources.

Conclusions: Identification of the novel missense mutation p.E159K of *HCCS*, which leads to loss-of-function of the encoded holocytochrome *c*-type synthase, in a sporadic female patient with microphthalmia of both eyes and bilateral sclerocornea may suggest *HCCS* as candidate for severe ocular manifestations.

Severe structural malformations of the eye, such as anophthalmia (absence of the eye) and microphthalmia (reduction in eye size), have a birth prevalence of about 1 per 5,000 [1-3]. These ocular abnormalities are often apparent at birth and caused by very early disturbances in the normal program of development. Although the cause of most human ocular malformations is unknown, various genes for the anophthalmia and/or micophthalmia phenotype have been identified. Originally, heterozygous mutations in *PAX6*, encoding a paired-box transcription factor, have been described to cause human aniridia [4]. Nonetheless, *PAX6* was also the first gene to be identified for anophthalmia [5], and alterations of *PAX6* have recently been discovered in patients with microphthalmia [6]. Heterozygous, loss-of-function mutations in *SOX2* are most commonly associated with bilateral anophthalmia, although microphthalmia, sclerocornea and persistence of the hyperplastic vitreous are also seen (OMIM 206900) [7,8]. Moreover, in patients with the anophthalmia-esophageal-genital (AEG) syndrome (OMIM 206900) heterozygous mutations of *SOX2* have been detected [9,10]. *SOX2* codes for a transcription factor known to bind to PAX6, cooperatively activating expression of lens-specific genes [11]. Additionally, several other genes encoding transcription factors or repressors have been implicated in severe ocular malformations, such as *CHX10* (OMIM 610092 and 610093) [12], *RAX* (OMIM 611038) [13], *BCOR* (OMIM 300166) [14], and *OTX2* (OMIM 610125) [8,15].

The association of developmental eye abnormalities with transcription factors highlights the existence of a complex genetic network important to control each stage of eye development [16]. Remarkably, molecules other than transcription regulators have been shown to be mutated in patients with syndromic microphthalmia. Recently, sequence alterations in *STRA6*, encoding a membrane receptor for the retinol binding protein which mediates cellular uptake of vitamin A [17], have been reported in individuals with anophthalmia, mental retar-

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dation, and a broad spectrum of organ malformations (OMIM 601186) [18,19]. The X-linked dominant, male-lethal microphthalmia with linear skin defects syndrome (also known as MLS or MIDAS; OMIM 309801) has been found to be associated with segmental monosomy of the Xp22.2 region in the majority of cases [20]. However, heterozygous, loss-of-function mutations of *HCCS* have been detected in patients with MLS and normal karyotype [21]. *HCCS* is an atypical gene for microphthalmia as its product is neither involved in transcriptional regulation nor belongs to the group of retinoic acid-inducible genes, such as *STRA6*, that are possible targets of the retinoid receptors [22]. *HCCS* encodes a mitochondrial enzyme, the holocytochrome *c*-type synthase, which catalyzes the covalent attachment of heme to both apocytochrome *c* and c_1, thereby leading to the mature forms, holocytochrome *c* and c_1 [23,24]. The product of the HCCS-catalyzed reaction, cytochrome *c*, has two cellular functions: First, it acts as an electron carrier in the mitochondrial respiratory chain and thereby is implicated in oxidative phosphorylation (OXPHOS), and second, it is released from mitochondria upon proapoptotic stimuli and plays an important role in caspase-dependent cell death, i.e., apoptosis [25].

The majority of MLS-affected females display the classic phenotypic features, including microphthalmia, sclerocornea, and linear erythrodermia, however, a high intra- and interfamilial phenotypic variability has been observed [21,26-29]. For example, several patients with severe eye malformations but without erythematous skin lesions at birth have been reported. These individuals show either isolated eye anomalies or a syndromic form with other organ manifestations, e.g., cardiomyopathy [21,30-32]. The broad spectrum of phenotypic abnormalities seen in patients with MLS led us to hypothesize that mutations in *HCCS* could be associated with isolated and syndromic forms of anophthalmia and/or microphthalmia in individuals with no apparent skin defects. Here, we describe mutation analysis of *HCCS* in a cohort of 27 females obtained from a population-based study on infants and fetuses with congenital eye malformations, including uni- and bilateral microphthalmia, and report functional analysis of a novel missense mutation leading to loss-of-function of the encoded HCCS protein.

METHODS

Patients: Clinical data were initially derived from the California Birth Defects Monitoring Program, a population-based active surveillance system for collecting information on births with congenital malformations. Diagnostic information was collected as previously described [3]. In that study, only infants with clinical anophthalmia or bilateral microphthalmia served as the analytic basis. In the period 1989-97, ascertainment among more than 2.5 million California births (liveborn and stillborn) resulted in the identification of 100 infants/fetuses with these eye anomalies. Ophthalmic examinations included slit lamp biomicroscopy, measurement of intraocular pressure (IOP) by applanation tonometry, gonioscopic evaluation of the anterior chamber angle, and perimetry by automated field analyzer.

For this study, phenotypes of 27 female cases with bilateral clinical anophthalmia and/or microphthalmia were reviewed. The cases were categorized as either isolated (9) or syndromic (18). Isolated cases had only anophthalmia/microphthalmia or anophthalmia/microphthalmia with other eye defects, whereas syndromic cases showed anophthalmia/microphthalmia and heart and/or brain defects and cataracts, respectively.

Mutation analysis: Total genomic DNA was isolated from Guthrie cards by standard procedures and amplified by using the GenomiPhi DNA Amplification Kit (GE Healthcare, München, Germany). We amplified the coding region of *HCCS* (exons 2 to 7, GenBank NM_005333), including the flanking intronic sequences, from whole genome amplified DNA. Primer sequences and PCR conditions are available on request. PCR products were directly sequenced with the Big Dye Terminator ready reaction kit (PE Applied Biosystems, Darmstadt, Germany) on an ABI Prism 377 (PE Applied Biosystems).

X-inactivation assay: Examination of the methylation pattern at the *AR* locus was performed as previously described [21].

Generation of NH_2- and COOH-terminal tagged HCCS *constructs:* Generation of *HCCS* wild-type constructs has been described previously [21]. The *HCCS* (E159K) insert was established by PCR-mediated mutagenesis [33]. The purified PCR product was cloned into pENTR/D-TOPO (Invitrogen, Karlsruhe, Germany) according to the protocol provided. Constructs were sequenced for integrity and then used for cloning the *HCCS* (E159K) coding region into plasmids pcDNA-DEST53 (NH_2-terminal EGFP epitope; Invitrogen), pcDNA-DEST47 (COOH-terminal EGFP epitope; Invitrogen), pMT2SM-HA-DEST (NH_2-terminal HA epitope), and pcDNA3.2/V5-DEST (COOH-terminal V5 epitope; Invitrogen) via LR reaction following the manufacturer's instructions (Invitrogen).

Generation of yeast expression constructs: For generation of yeast expression constructs we used pYEX4Tps which drives expression of the GST-fusion proteins from the *Saccharomyces cerevisiae* CUP1 promoter and carries the selectable markers *leu2-d* and *URA3*. Generation of pYEX4Tps-HCCS-wild-type and pYEX4Tps-CYC3 has been described previously [21]. HCCS-E159K was established by PCR-mediated mutagenesis [33]. After purification of PCR products, the amplicon was restricted with *Bam*HI and *Not*I and cloned unidirectionally into pYEX4Tps. Primer sequences and PCR conditions are available on request. All constructs were sequenced for integrity, and large and pure amounts of plasmid DNA were prepared by using a plasmid maxi kit (QIAGEN, Hilden, Germany).

Immunofluorescence: CHO-K1 cells were cultured on fibronectin coated (10 µg/ml) coverslips in F12-Ham's Nutrient Mixture supplemented with 10% FCS, 1% L-glutamine, and penicillin-streptomycin at 37 °C in 5% CO_2. Immunofluorescence analysis was performed as previously described [21]. In brief, after transfection with pcDNA-DEST53-HCCS-wild type, pcDNA-DEST53-HCCS-E159K, pcDNA-DEST47-HCCS-wild type, pcDNA-DEST47-HCCS-E159K, pMT2SM-

HA-DEST-HCCS-wild type, pMT2SM-HA-DEST-HCCS-E159K, pcDNA3.2/V5-DEST-HCCS-wild type, or pcDNA3.2/V5-DEST-HCCS-E159K, CHO-K1 cells were incubated in medium containing MitoTracker Red CMXRos (Invitrogen) and fixed. HA- and V5-tagged HCCS proteins were detected with rat monoclonal anti-HA-Flourescein antibody (1.25 μg/ml; Roche, Mannheim, Germany) and mouse monoclonal anti-V5-FITC antibody (2.0 μg/ml; Invitrogen), respectively. Cells were analyzed on a Zeiss Axiovert 200 M confocal microscope equipped with a 63x Planapochromat/1.4 DIC lens.

Yeast complementation studies: Complementation studies were performed as previously described [21]. We used the *S. cerevisiae* strain B-8025 (MATα*can1-100 CYC1 cyc3-Δcyc7-Δ::CYH2 cyh2 his3-Δ1 leu2-3,112 trp1-289*), carrying a deletion of *CYC3* [34], as well as B-7553 (MATα*can1-100 CYC1 cyc7::CYH2 cyh2 his3-Δ1 leu2-3,112 trp1-289*), as a growth control [35].

Immunoblotting: Expression of GST-HCCS and GST-CYC3-fusion proteins was confirmed by immunoblotting of lysates from saturated yeast cultures (grown in minimal medium or YPG medium with copper) using horseradish peroxidase-conjugated goat polyclonal anti-GST antibody (0.3 μg/ml; Amersham Pharmacia Biotech, Vienna, Austria).

RESULTS

We performed mutation analysis of *HCCS* in a cohort of 27 newborn female patients with isolated or syndromic microphthalmia or anophthalmia [3]. In a single patient, we identified the heterozygous c.475G>A mutation in exon 5 which predicted an amino acid change from glutamate to lysine at position 159 of the holocytochrome *c*-type synthase (p.E159K; Figure 1A). The female patient has been diagnosed with bilateral microphthalmia and bilateral scleral cornea at the age of one week. Since parental DNA samples were not available, we analyzed 234 DNAs from healthy females for the c.475G>A change and could not detect it in any of these samples (data not shown).

A skewed X-inactivation pattern has been commonly identified in MLS-affected individuals with segmental monosomy

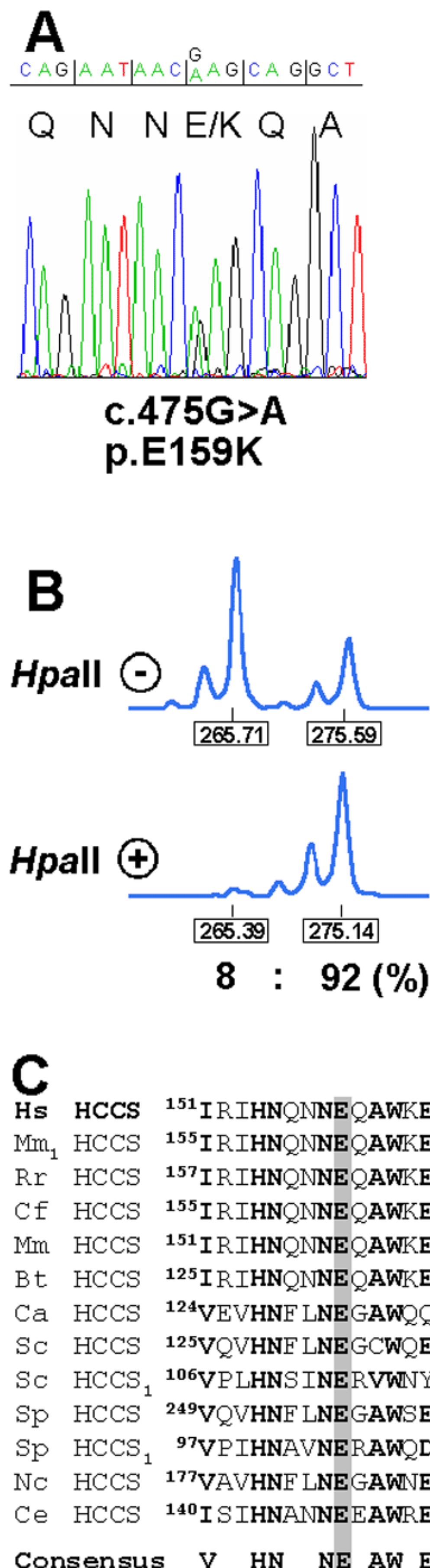

Figure 1. An *HCCS* missense mutation in a female with nonsyndromic microphthalmia. **A**: Sequence electropherogram of part of *HCCS* exon 5 from genomic DNA of the proband. Triplets and encoded amino acids are indicated. The patient is heterozygous for the c.475G>A mutation (p.E159K) in exon 5. **B**: X chromosome inactivation determined by amplification of an *AR* sequence polymorphism and digestion of genomic DNA isolated from lymphocytes with *Hpa*II (indicated with + or -). The allele sizes and ratio of the X-inactivation pattern are given below the peaks. **C**: Partial amino acid sequence alignment of the first heme lyase targeting motif from various species. The position of amino acids is given. Evolutionary conserved residues are presented in bold. The invariant glutamate at position 159 is shaded in gray. The consensus sequence is indicated below the amino acid alignment. $HCCS_1$ indicates specificity of this heme lyase for cytochrome c_1. Hs, *Homo sapiens*; Mm_1, *Mus musculus*; Rr, *Rattus norvegicus*; Cf, *Canis familiaris*; Mm, *Macaca mulatta*; Bt, *Bos taurus*; Ca, *Candida albicans*; Sc, *S. cerevisiae*; Sp, *Schizosaccharomyces pombe*; Nc, *Neurospora crassa*; Ce, *Caenorhabditis elegans*.

of Xpter-p22.2 or an *HCCS* mutation [21,29,36]. Indeed, the holocytochrome *c*-type synthase acts in a cell-autonomous manner and loss-of-function mutations are supposed to cause selective cell loss resulting in a skewed X-inactivation pattern [21]. We used the methylation-specific PCR at the polymorphic human androgen receptor (*AR*) locus to study the X-inactivation pattern in the patient carrying the *HCCS* missense mutation. As expected, we detected preferential inactivation of one X chromosome with a ratio of 92:8 in the patient's peripheral blood cells (Figure 1B).

Holocytochrome *c*-type synthases, also known as "heme lyases", contain a sequence element which is essential for the import of these proteins into the intermembrane space of mitochondria. The targeting signal is located in the third quarter of HCCS and comprises about 60 amino acid residues which are spread over two highly conserved motifs, from residues 151-169 and 190-216 of human HCCS [37]. Glutamate at position 159 of HCCS is evolutionary conserved and located within the first targeting motif (Figure 1C). Thus, by changing glutamate for lysine at position 159, import of HCCS into mitochondria could be impaired. We compared subcellular localization of ectopically expressed wild-type with the mutated HCCS protein. NH_2-terminally HA-tagged wild-type HCCS was found to be targeted to mitochondria (Figure 2C). Similarly, the mutated HCCS protein with lysine-159 (HCCS E159K) also localized to mitochondria (Figure 2F). We performed the same experiments with wild-type HCCS and HCCS E159K carrying an NH_2- or COOH-terminal EGFP tag or a COOH-terminal V5 tag and obtained similar results as described above (data not shown).

Mammalian cytochrome *c* heme lyases catalyze the covalent attachment of heme to the apoforms of both cytochrome *c* and c_1 [24,38]. Cytochrome *c* plays an important role in OXPHOS and is therefore required for respiratory growth. The *S. cerevisiae* yeast strain B-8025 carries a deletion of *CYC3* which encodes Cyc3p, the yeast ortholog of human HCCS, and is unable to grow on nonfermentable carbon sources indicating a severely depleted respiratory function [39]. While ectopic expression of human HCCS wild type is able to complement this deficiency [21,40], expression of HCCS mutants associated with MLS did not restore respiratory function of this yeast mutant [21]. We ectopically expressed HCCS E159K, HCCS wild type and yeast Cyc3p as glutathione-S-transferase (GST) fusion proteins in yeast strain B-8025 and analyzed their capacity to compensate for respiratory deficiency. Whereas human wild-type HCCS and yeast Cyc3p proteins restored growth on glycerol-containing medium, yeast cells expressing HCCS with p.E159K could not grow on nonfermentable carbon sources (Figure 3A). By western blot analysis of protein extracts from the transformed B-8025 strains grown in minimal medium we confirmed expression of the three different GST fusion proteins (Figure 3B).

DISCUSSION

Mutation analysis of *HCCS* in a cohort of 27 newborn females with nonsyndromic or syndromic microphthalmia and/

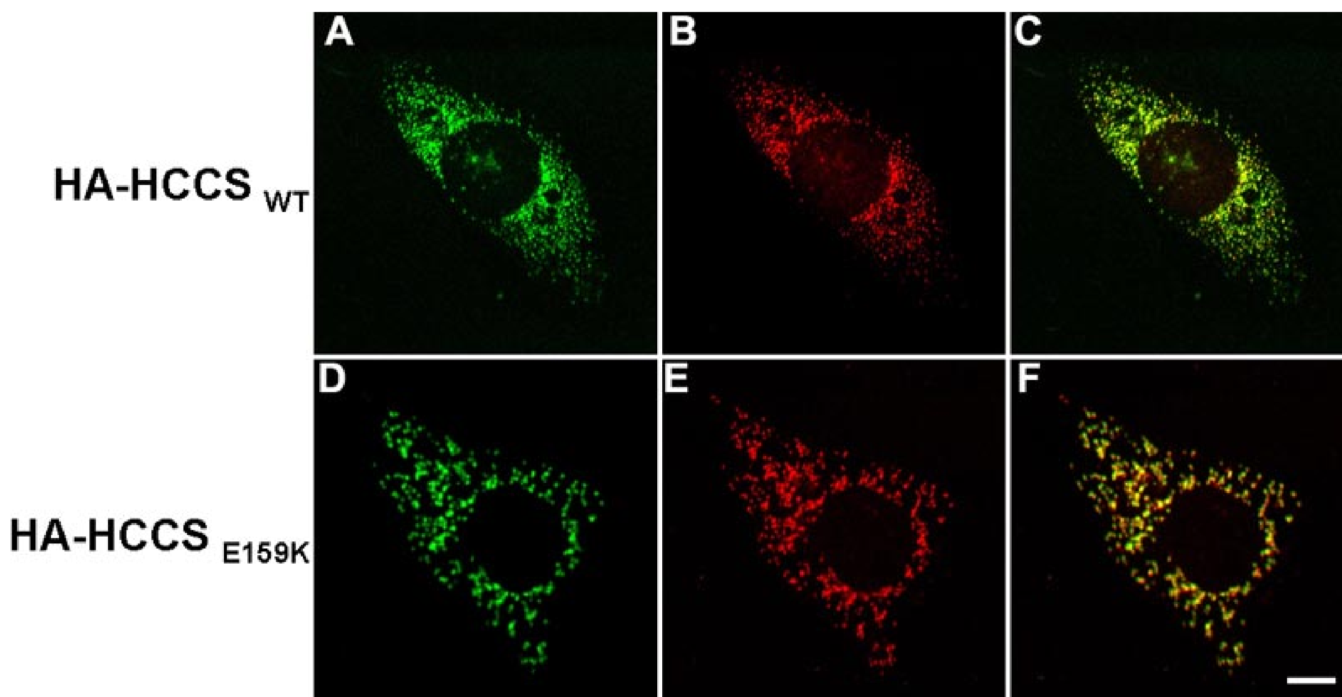

Figure 2. Targeting of ectopically expressed HCCS wild type and the E159K mutant to mitochondria. Subcellular localization of HA-tagged HCCS proteins ectopically expressed in CHO-K1 cells (**A** and **D**) and staining of endogenous mitochondria by MitoTracker are shown (**B** and **E**). HA-tagged HCCS wild type protein (**A**, green) is targeted to mitochondria (**B**, red) as shown by colocalization with the MitoTracker (yellow pseudocolor in **C**). Similarly, HA-tagged HCCS E159K mutant protein (**D**, green) shows a mitochondrial (**E**, red) distribution (yellow pseudocolor in **F**). The scale bar represents 10 μm.

or clinical anophthalmia identified the heterozygous missense mutation c.475G>A/p.E159K in a single female infant with bilateral microphthalmia and sclerocornea in both eyes. The findings that (1) mutated HCCS with E159K did not complement respiratory deficiency of the *CYC3⁻ S. cerevisiae* mutant strain, (2) this mutation was not detected on more than 460 X chromosomes, and (3) a skewed X-inactivation was found in the patient's lymphocytes strongly provide evidence for the pathogenic relevance of the c.475G>A mutation.

The mutated glutamate-159 is an invariant residue and located within the first motif of the sequence element responsible for sorting HCCS to the mitochondrial intermembrane space. Chemical properties of the heme lyase targeting element are distinct from those of other mitochondrial proteins as it is highly hydrophilic with 30% charged residues containing a similar number of positively and negatively charged amino acids. This topogenic signal has been suggested to determine the specificity of transport into mitochondria and mediate the import into the mitochondrial intermembrane space [37]. Thus, substitution of the negatively charged glutamate-159 by the positively charged lysine most likely causes a disequilibrium of positively and negatively charged residues within the targeting motif which might interrupt mitochondrial sorting of HCCS. Surprisingly, the missense mutation p.E159K did not have any impact on translocating the mutated heme lyase to mitochondria (Figure 2F), although the capacity of the altered targeting sequence to specifically guide HCCS to the intermembrane space has not been investigated. These data suggest that the total amount of charged residues within the targeting element rather than the physicochemical nature of a single amino acid is important for directing HCCS to mitochondria.

In yeast complementation studies human heme lyase with E159K was not able to compensate for respiratory deficiency of the *CYC3⁻S. cerevisiae* mutant strain indicating that glutamate-159 is important for functional integrity of the holocytochrome *c*-type synthase. In line with this finding, the two mitochondrial targeting motifs have also been suggested to be important for proper function of heme attachment [37]. Taken together, the p.E159K missense mutation of *HCCS* leads to loss-of-function of the encoded protein and is likely associated with the severe eye malformations in the female infant.

The function of the protein encoded by *HCCS* is completely different from that of several genes responsible for severe eye malformation disorders, such as *PAX6*, *SOX2*, and *OTX2*. The latter three code for transcriptional regulators involved in a complex regulatory network. Cross-regulation for PAX6, SOX2, and OTX2 as well as regulatory mechanisms for their fine-tuned expression have been discovered indicating that these proteins cooperatively control each stage of eye development [16]. In marked contrast, HCCS is a mitochondrial housekeeping enzyme required for cytochrome *c* biosynthesis. On one hand, cytochrome *c* is necessary for proper functioning of the mitochondrial respiratory chain and on the other hand, release of cytochrome *c* from mitochondria induces apoptotic cell death [25]. Recently, we put forward the hypothesis that deficiency of *HCCS* may not only cause functional deficits in OXPHOS, but also leads to severe constraints in the process of apoptosis. Thus, functional nullisomy of *HCCS* may disturb the balance between apoptosis and necrosis and push cell death toward necrosis [21]. Importantly, necrosis bears the danger of inflammatory reactions leading to substantial damage of neighboring cells [41,42] that could be

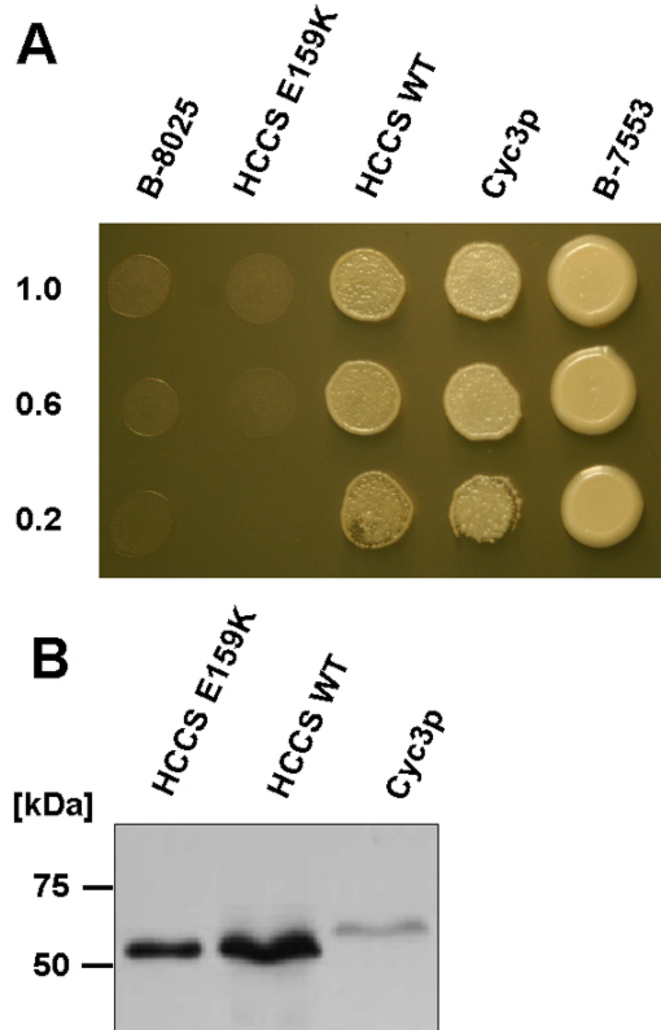

Figure 3. HCCS E159K is not able to complement *S. cerevisiae CYC3*-deficiency. **A**: Functional complementation of the *S. cerevisiae* strain B-8025. B-8025 was grown on minimal medium and transformed with human wild-type HCCS (HCCS WT), the HCCS mutant E159K (HCCS E159K), or yeast CYC3 (Cyc3p) expression constructs. Transformants were grown in liquid minimal medium, and aliquots of saturated (top row) and diluted cultures (middle and bottom rows) were spotted on glycerol medium containing copper (to induce expression of GST fusion proteins), and were incubated for 7 days at 30 °C. Dilution rates are indicated to the left of the figure. Note restoration of growth by ectopic expression of Cyc3p and HCCS WT, whereas no growth was observed for the untransformed strain and B-8025 expressing HCCS E159K. Strain B-7553 served as wild-type growth control. **B**: Expression of GST-HCCS fusion proteins in yeast strain B-8025. Expression of GST-HCCS E159K (left lane), GST-HCCS wild type (middle lane), and GST-Cyc3p (right lane) fusion proteins in the yeast mutant strain B-8025 grown in minimal medium was demonstrated by immunoblotting.

a key element in developing eye malformations as well as other MLS-specific symptoms in affected individuals. A more direct role of HCCS in apoptotic cell death has been proposed recently. In response to apoptotic stimuli, HCCS is released from mitochondria into the cytoplasm where it interacts with several members of the inhibitor of apoptosis protein (IAP) family [43]. IAPs suppress caspase-3 activity required for apoptotic cell death [44], and binding of HCCS to IAPs was shown to cause accelerated cell death by antagonizing IAP activity [43]. Thus, holocytochrome *c*-type synthase has a pro-apoptotic activity and *HCCS* deficiency may result in enhanced resistance to cell death by apoptosis. Taken together, direct and indirect implication of HCCS in apoptotic cell death and requirement of cytochrome *c*, the final product of HCCS activity, for OXPHOS may suggest that disturbance of these processes contribute to the variable clinical features observed in patients with *HCCS* mutations. In addition, the pattern of X-inactivation in the early embryo of a female carrying an *HCCS* alteration may determine in part the severity of the disease phenotype.

Up to date, one missense mutation, p.R217C, and one nonsense mutation, p.R197X, in *HCCS* have been described in patients with MLS. While the girl with p.R197X shows the classical MLS phenotype, the female patient with p.R217C presented with bilateral microphthalmia and sclerocornea, but no linear skin defects. Later in life, she developed idiopathic ventricular tachycardia and suffered an occlusion of her right arteria cerebri media. The female infant with the *HCCS* mutation p.E159K presented only with severe eye malformations at birth, including microphthalmia of both eyes and bilateral sclerocornea. However, other features occassionally seen in patients with MLS, such as chorioretinal abnormalities, infantile seizures, and tachycardia, might have developed later in life in this patient and would have been missed by the California Birth Defects Monitoring Program, which only collected data on structural anomalies diagnosed within 1 year of delivery [3]. Nonetheless, it is reasonable to speculate that heterozygous mutations of *HCCS* are associated with isolated eye malformations in affected female patients. Indeed, a female carrying a heterozygous deletion of 8.6 kb, encompassing part of *HCCS*, only presented with a left opaque cornea, congenital glaucoma with total anterior synechia, and a white anterior cataract [21]. Similarly, Cape and colleagues [32] reported a girl with bilateral sclerocornea and partial aniridia in the left eye and an X/Y translocation resulting in heterozygous deletion of the MLS critical region [32]. The wide variety of ocular findings in patients with MLS suggests *HCCS* as an attractive candidate for a broad spectrum of ocular malformations. Since mutations of *HCCS* seem to be rarely associated with microphthalmia and/or anophthalmia in female patients, X-inactivation studies prior molecular testing may provide a clue for finding an alteration in this gene. Although male lethality has been proposed to be associated with MLS in patients with deletions involving Xp22 and later on with *HCCS* mutations [20,21,45], males carrying a loss-of-function mutation of *HCCS* may survive due to somatic mosaicism or Klinefelter syndrome, as reported for e.g., X-linked dominant incontinentia pigmenti [46]. Alternatively, hypomorphic *HCCS* alleles might be present in male patients with various ocular malformations.

In conclusion, in this study we provide evidence for a loss-of-function mutation of *HCCS* in an individual with isolated eye abnormalities suggesting *HCCS* as candidate gene for a broad spectrum of human ocular phenotypes. In the future, mutation analysis in large cohorts of patients with severe ocular malformation disorders will shed light on the variable eye phenotypes associated with mutated *HCCS* alleles. Moreover, implication of a mitochondrial housekeeping enzyme in developmental ocular abnormalities is intriguing and opens the possibility of identifying other genes for these disorders whose products are involved in a wide spectrum of cellular functions.

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