Segregation of novel p.(Ser270Tyr) MAF mutation and p.(Tyr56∗) CRYGD variant in a family with dominantly inherited congenital cataracts

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ABSTRACT

**Background:** A bilaterally blind woman, with a three generation family history of autosomal dominant congenital cataracts, variably associated with iris colobomata and microcornea, sought preconception genetic consultation.

**Methods and Results:** Whole-exome sequencing was performed in three affected family members, one unaffected first degree relative, and one spouse. The sequence variant c.168C>G; p.(Tyr56*) in *CRYGD*, previously reported as pathogenic, and a novel mutation c.809C>A; p.(Ser270Tyr) in *MAF*, were identified in two affected family members; the grandmother, and half-brother of the proband. The proband inherited only the *MAF* mutation, whereas her clinically unaffected sister had the *CRYGD* change. *In silico* analysis supported a pathogenic role of p.(Ser270Tyr) in *MAF*, which was absent from publically available whole-exome dataset, and 1,161 Czech individuals. The frequency of *CRYGD* p.(Tyr56*) in the ExAC dataset was higher than the estimated incidence of congenital cataract in the general population.

**Conclusions:** Our study highlights that patients with genetically heterogeneous conditions may exhibit rare variants in more than one disease-associated gene, warranting caution with data interpretation, and supporting parallel screening of all genes known to harbour pathogenic mutations for a given phenotype. The pathogenicity of sequence variants previously reported as cataract-causing may require re-assessment in light of recently released public genome datasets.

**Keywords:** congenital cataract; *CRYGD*; *MAF*; coloboma; microcornea
INTRODUCTION

Non-syndromic congenital cataract, characterized by opacification of the lens at birth or during the first decade of life, has an estimated incidence of 1–6 per 10,000 live births [1-3]. Genetic factors are predicted to be involved in approximately half of the cases [2]. To date, mutations in at least 30 genes are known to be implicated in nonsyndromic childhood cataracts (http://cat-map.wustl.edu/) [4, 5].

Genes associated with congenital cataract encode for lens-expressed cytoskeletal and membrane proteins, crystallins, and transcription factors [6]. α-, β-, and γ-crystallins form 90% of water soluble proteins in the vertebrate lens, maintaining its transparency and refractive index [7]. The human γ-crystallins (CRYG) comprise four protein coding genes and two pseudogenes [8]. A number of mutations in CRYG genes, including CRYGD (MIM *123690), leading to diverse cataract phenotypes, have been reported [9-11].

V-Maf avian musculoaponeurotic fibrosarcoma oncogene homolog (MAF; MIM *177075) gene belongs to a family encoding basic leucine zipper (bZIP) transcription regulators [12, 13]. bZIP transcription factors act both as a homo- and heterodimer, and bind to Maf response elements (MARE) located in promotors of crystallin genes [14]. Mutations within MAF are reported to be associated with progressive forms of dominantly inherited congenital cataract, microcornea, coloboma and anterior segment dysgenesis [15, 16].

In this study we report on two rare heterozygous sequence variants in MAF and CRYGD identified in several affected members of one family, presenting with autosomal dominant congenital cataract, variably associated with iris colobomata and microcornea.
MATERIAL AND METHODS

Clinical examination

The study followed the tenets of the Declaration of Helsinki and was approved by the local Ethical review board. All investigated subjects, and/or their legal guardians, provided informed consent prior to inclusion into the study. Venous blood samples were collected, and DNA extracted from leukocytes using Gentra Puregene™ Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

Our investigation was initiated after a 24-year-old bilaterally totally blind proband (III:1; Fig. 1A) with a family history of an autosomal dominant congenital cataract sought preconception genetic counselling. Five family members, including the probands, underwent a comprehensive ophthalmological examination. Snellen visual acuity testing (converted to decimal values), intraocular pressure measurements, and slit-lamp biomicroscopy were performed. Past medical records were reviewed. The proband underwent neurological examination including magnetic resonance imaging (MRI) brain imaging.

Whole Exome Sequencing (WES)

WES was performed on samples from three affected individuals (I:1, III:1 and III:3), one clinically unaffected first degree relative (III:2), and one spouse (II:3) (Fig. 1A). DNA was captured according to manufacturer’s instructions using TruSeq exome enrichment kit (Illumina, Inc., San Diego, CA, USA), and sequenced on an Illumina HiSeq 2000 machine (fee for service, Axeq Technologies, Rockville, MD, United States). Raw FASTQ reads were aligned to the GRCh37/hg19 human reference sequence, using the Burrows-Wheeler Alignment tool (http://bio-bwa.sourceforge.net/) [17]. Variant calling was performed with SAMTOOLS (http://samtools.sourceforge.net/) [18].
Heterozygous changes present in at least two affected family members, with a frequency of less than 0.01 as mined from public databases (dbSNP; http://www.ncbi.nlm.nih.gov/SNP/), the 1000 Genomes dataset (http://www.1000genomes.org), the Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS), the Exome Aggregation Consortium (ExAC, Cambridge, MA; http://exac.broadinstitute.org), (accessed 5 May 2016) were further evaluated for possible pathogenicity. In house data, available through next-generation sequencing projects of The National Centre for Medical Genomics, were used to check for population specific variant frequencies.

Sequence variants previously reported as disease-causing, and novel sequence variants within genes listed in CatMap (http://cat-map.wustl.edu/); an online reference database for inherited cataracts [19] were given a priority for further evaluation of pathogenicity. SIFT [20], PolyPhen2 [21], MutPred [22], PROVEAN [23], MutationTaster [24] and SNPs&GO [25] algorithms were used to predict the pathogenicity of missense variants. Conservation analysis of affected amino acid residues was performed using T-coffee [26].

**Karyotyping and SNP array analysis**

Chromosomal aberrations and DNA copy number variations (CNVs) were analysed by conventional karyotyping and SNP array HumanCytoSNP-12 v2.1 BeadChip (Illumina) in the proband. Labelling and hybridization were done according to the recommended Illumina protocol. Scanning was performed with an Illumina iScan System, and genotype calling and quality control using GenomeStudio Software v2011.1 (Illumina). Results were analysed with the CNV Partition 2.4.4 algorithm plug-in within the GenomeStudio software.
**Sanger sequencing**

Verification of the prioritized possibly pathogenic sequence variants detected by WES was performed by conventional Sanger sequencing using Big Dye terminator chemistry on an ABI PRISM 3100 genetic analyser (Applied Biosystems, Foster City, CA). Reaction conditions and primers pair sequences are provided in online resource (Table 1). Prior to this step DNA samples from the proband and her sister were recollected for verification of the detected findings. NM_001031804.2 and NM_006891.3 were used as the reference sequences for *MAF* and *CRYGD* genes, respectively. Mutation description followed Human Genome Variation Society guidelines [27].

**RESULTS**

The pedigree of the investigated family is shown in Fig. 1A. Ophthalmological findings in all examined family members are summarized in Table 1.

Clinical records of grandmother of the proband (I:1) indicated the presence of bilateral congenital cataracts and congenital vertical iris coloboma in the left eye. She underwent cataract surgery at 15 years of age in the left eye and at 16 years of age in the right eye. Subsequently she developed bilateral secondary glaucoma. Despite a series of surgeries to achieve control of her eye pressure, her left eye had to be enucleated when she was aged 55 years. The father of the proband (individual II:4) was not available for examination. He was known to have congenital cataracts and had his cataracts removed around 20 years of age. The proband (III:1) presented with nystagmus at the age of 2 months. She was diagnosed with nuclear cataracts and microcorneas (corneal diameters 9-10 mm), and underwent bilateral lens extraction at the age of 9 months, followed by multiple surgeries: secondary cataracts, removal and elongation of inner rectus muscles at 2 years of age, synechiolysis in the right
eye at 4 years of age, removal of secondary cataract in the left eye combined with anterior
vitrectomy and basal iridotomy and sphincterotomy at 6 years of age. BCVA (best corrected
visual acuity) in both eyes when she was aged 14 years was 0.08 with an aphakic correction.
At 16 years of age, she experienced a sudden bilateral decrease of visual acuity, for which no
particular reason, such as elevated intraocular pressure increase, nor marked retinal pathology
was found. By 19 years of age, she became completely blind. Neurological examination was
normal and MRI brain scan performed when she was 22 years old did not reveal any
abnormalities.
The sister of the proband (III:2) had clear crystalline lenses when examined at 22 years of age.
(Fig. 1A-C). No other ocular pathology was found. The half-brother of the proband (III:3; Fig.
1A), age 17 years, was documented to suffer from congenital cataracts, in addition to high
myopia and astigmatism, and underwent cataract surgery at 4 years of age in the right eye and
at 14 years in the left eye. No microcornea or iris abnormalities were noted.
WES data and confirmatory Sanger sequencing identified rare heterozygous variants within
two different genes known to be implicated in childhood cataract development; c.809C>A in
MAF (Fig. 1D) and c.168C>G in CRYGD (Fig. 1E). No other variants in known cataract
genes were identified in affected individuals. Individuals I:1 and III:3 harboured both of these
sequence changes, whereas the MAF mutation was found in isolation in the proband (III:1)
while her unaffected sister (III:2) inherited only the CRYGD variant (Fig. 1A). In addition,
segregation of the sequence changes within the family indicated that proband’s father (II:4),
who was unavailable for testing, must be an obligate carrier for both sequence changes.
The c.168C>G in CRYGD is predicted to truncate the protein; p.(Tyr56*), and the c.809C>A
in MAF to cause a Ser to Tyr substitution at codon 270; p.(Ser270Tyr). The CRYGD sequence
variant has been previously observed in association with congenital cataract [28], but the
MAF mutation has not been reported. All six pathogenicity prediction algorithms (Online
Resource; Table 2) indicated that the \textit{MAF} mutation leading to missense change in a highly conserved region, known to be critical for DNA binding, is disease-causing. While \textit{c.809C>A} in \textit{MAF} was absent from the ExAC and EVS datasets, \textit{c.168C>G} in \textit{CRYGD} had an ExAC frequency of 0.005284, and it was also present in 1 allele of 1,161 population specific control individuals (online resource; Table 3). Any chromosomal abnormalities or CNVs encompassing cataract genes were detected in the proband.

\textbf{DISCUSSION}

In this study we report a family with autosomal dominant cataract, variably associated with iris colobomata and microcornea. Using WES we identified two heterozygous rare variants in genes that are involved in early cataract development; the novel \textit{c.809C>A; p.(Ser270Tyr)} in \textit{MAF} and the \textit{c.168C>G; p.(Tyr56*)} variant in \textit{CRYGD}, previously reported as pathogenic [28]. Both sequence variants were found in two affected family members, while the proband carried only the \textit{MAF} mutation and her unaffected sibling only the \textit{CRYGD} sequence variant. The pathogenicity of the novel \textit{MAF} mutation is supported by its location in a functional protein domain, absence from public datasets, segregation with the disease phenotype in the investigated family and by scoring of prediction algorithms. All six \textit{MAF} mutations reported to date, associated with dominantly inherited cataract [including \textit{p.(Ser270Tyr)} identified in the current study] are located either in the basic domain, or in an adjacent ancillary DNA-binding domain (Online Resource, Fig. 1); highly conserved regions known to play a role in MAF binding to MARE elements [16, 29]. In five of the seven families with \textit{MAF} disease-causing mutations (including this study), the phenotype included, in addition to congenital cataracts, iris colobomata and/or microcornea [16, 30].
The c.168C>G in CRYGD has been previously reported to be associated with congenital nuclear cataracts in a Brazilian family with three affected members. The fact that sister of the proband carrying this change had clear lenses at the age of 22 years, and that the frequency of c.168C>G in CRYGD in the ExAC database is 0.005284 (the variant was detected in 603 out of 114,118 alleles, including 4 homozygous individuals), i.e. about 10x more than the estimated prevalence of congenital cataracts, implies that c.168C>G is not disease-causing. Alternatively, the penetrance of the phenotype associated with this variant would have to be very low. In addition, we have found this change in a heterozygous state also in one Czech control subject. It should be however noted that individuals included to the ExAC project, as well as Czech in house controls, did not undergo a comprehensive ophthalmic examination.

One of the shortcomings of the current study is that, although we obtained past medical records from affected individuals III:1 and III:3 indicating the presence of nuclear cataracts, no confirmatory anterior segment photographs were available. Also the description of vertical iris coloboma in the left eye of individual I:1 was historical. Nevertheless, there is evidence of considerable intrafamilial phenotypical differences in cataract severity, as reflected by cataract surgery timing, and additional congenital ocular dysmorphology.

Interestingly, the proband harbouring only the MAF mutation seemed to have the most severe phenotype, with bilateral microcorneas and dense cataracts necessitating surgery early in life, compared to the other family members. The reason for the blindness in the proband remained unknown. Her records indicated that there was no optic cupping, nor elevation of intraocular pressures that would lead to a suspicion of glaucoma. In addition, there was no retinal detachment or other retinal pathology to explain the sudden visual loss.

In conclusion, next generation sequencing has not only revolutionized mutation detection in genetically heterogeneous conditions such as congenital cataracts, but also leads to the identification of many rare sequence variants of unknown significance. Mutations previously
reported as cataract-causing need to be revised in light of the recently available large exome data sets in relation to disease prevalences and information on penetrance. For many disease-causing mutations data on phenotype penetrance are currently insufficient.

**Conflict of Interest:** The authors declare that they have no conflict of interest.
REFERENCES


Fig. 1 Clinical and molecular genetic findings in the investigated family

A) Pedigree showing segregation of the identified sequence variants in MAF and CRYGD. B) Slitlamp examination of unaffected individual III:2 in dilation against the red reflex C) and in a narrow slit-beam view. D) Sequence chromatogram of c.809C>A in MAF. E) Sequence chromatogram of c.168C>G in CRYGD.