Novel Heterozygous Mutation in *YAP1* in A Family with Isolated Ocular Colobomas

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Running Title: Novel *YAP1* Mutation in Ocular Coloboma

Congenital ocular colobomas are tissue defects which occur as a result of failure of the ectodermal optic vesicle fissure to close at around 5-7 weeks of fetal life.[1] These defects can affect one or more ocular structures including the iris, ciliary body, lens, retina, choroid, and optic nerve. The incidence of WHO defined blindness (<3/60 best corrected vision in the better eye) has been reported as low as 7% in simple ocular coloboma to 66% in eyes with ocular coloboma and microphthalmos.[2] Most cases are sporadic, but may be familial, in which case autosomal dominant inheritance is most common. Ocular colobomas occur in a variety of chromosomal abnormalities and syndromic disorders. Recently, Williamson et al. identified a heterozygous nonsense mutation in *YAP1* in a family with isolated ocular coloboma.[3] We describe a further family with isolated ocular coloboma associated with a *YAP1* mutation where there is evidence of incomplete penetrance.

A 20-year-old male (patient 1) presented with a history of visual impairment since birth. He had been diagnosed with iris and retinal colobomas in each eye at a young age. His medical history was otherwise unremarkable and there were no systemic abnormalities. On examination, his best corrected visual acuities (BCVA) were 20/200 with no pinhole improvement in the right eye and 20/200 with pinhole improvement to 20/125 in the left eye. After 3 years of follow-up, BCVA had deteriorated to hand movements in the right eye and 20/400 in the left. He had bilateral inferonasal iris colobomas (Figure 1A). On dilated examination, he had bilateral large inferior chorioretinal colobomas encompassing the discs (Figure 1A). There was a shallow area of subretinal fluid in the left eye in the region of the coloboma and immediate adjacent temporal retina. Optical coherence tomography revealed fluid beneath residual glial tissue in the area of the left chorioretinal coloboma (Figure 1A). There was a marginal increase in fluid after 3 years

in the left eye only. Patient 2, the maternal half-brother of patient 1, is a 12-year-old boy who

had no visual symptoms with refractive correction, but did notice his right eye was smaller than

his left. His medical history was otherwise unremarkable and there were no other systemic

abnormalities. On examination, his BCVA was 20/20 in each eye. He had bilateral inferior

retinal colobomas and bilateral optic disc colobomas (Figure 1B). The mother of the two boys

was one of four children born to unaffected, unrelated parents. She had normal visual acuity and

a normal fundus examination. Patient 2's sister (patient 1's maternal half sister) also had a

normal examination.

DNA samples of both patients underwent whole exome sequencing (WES, AROS Applied

Biotechnology, Aarhus, Denmark) using the Illumina TruSeq exome capture kit and the Illumina

HiSeq 2000 sequencer (Illumina Inc.). There were no shared homozygous, X-linked or

compound heterozygous rare (MAF<0.001) variants. There were 54 shared, rare heterozygous

variants of which only 1 arose in a gene implicated in coloboma, *YAP1* (Yes-associated-protein

1, MIM#606608). Sanger sequencing confirmed the heterozygous mutation c.284T>C (p.F95S)

in both affected half brothers as well as in their unaffected mother (Figure 2). This mutation is

absent from all variant databases (dbSNP, 1kgenome, EVS and ExAC), affects an amino acid

highly conserved across a diverse range of species (Figure 2), and had equivocal pathogenic

predictions *in silico* (Sift 0.04 damaging, Polyphen2 0.085 tolerated). Mutation nomenclature

was assigned in accordance with GenBank Accession number NM_001130145.2, with

nucleotide position 1 corresponding to the A of the ATG translation initiation codon. There was

no evidence of mosaicism for this mutation in the peripheral-blood-derived DNA from the

mother (Figure 2). No other tissues were available from the mother nor DNA from other family members for further study.

This report describes a novel mutation in *YAP1* associated with isolated ocular coloboma in a family with an autosomal dominant pattern of inheritance with apparent incomplete penetrance. Unaffected mutation carriers have been described previously in families with autosomal dominant coloboma including one family with a *YAP1* mutation reported by Williamson et al.[3] It is unclear whether this represents true incomplete penetrance or germ line mosaicism. Similar mutation carriers with a normal ocular phenotype have been reported in families with anophthalmos/microphthalmos associated with mutations in OTX2 and SOX 2.[4,5]

The *YAP* (Yes-associated-protein) gene was originally characterized by Sudol et al. who identified a regulatory protein responsible for protein-protein interactions with ubiquitous expression at the RNA level,[6] later shown to be associated with the Hippo signaling pathway responsible for cell contact inhibition integral in the control of organ size during development.[7] Using in situ hybridization and immunohistochemistry, Williamson et al. observed expression of *YAP1* in the otic vesicle and future brainstem at 9.5 days postconception which continued as strong expression on the distal optic cup, suggesting that the protein is present in the developing lens and optic stalk.[5] This same group also identified different heterozygous nonsense mutations in *YAP1* in a family with isolated ocular coloboma, and in another family with ocular coloboma and variable multisystem involvement (hearing loss, intellectual disability, hematuria, and orofacial clefting). In the first family, apparent non-penetrance was identified in an unaffected carrier similar to the mother in this report. They additionally identified 4 families with

heterozygous missense variants in *YAP1* all thought to be non-pathogenic. In one family, this was based on non-segregation with disease. In the other 3 families, the identified mutations arose in residues that were not evolutionarily conserved when compared with mouse, chick and zebrafish. Phenylalanine-95 in this study is highly conserved across all 9 species analyzed including mouse, chick and zebrafish (Figure 2). This study supports the idea that mutations affecting only the long transcript (those occurring before Met179 of NM_001130145.2) might give rise to the localized ocular phenotype, while those that affect both long and short transcripts (after Met179) might cause a multi-system disorder.

To date, only two families with coloboma have been reported with mutations in *YAP1* gene, a gene with known strong expression in developing ocular structures and known to have a role in cell proliferation during organogenesis. Here, we report a third family with autosomal dominant coloboma with a novel heterozygous mutation in *YAP1* and confirm that mutations in this gene may be associated with incomplete penetrance, or germ line mosaicism a well recognized feature of autosomal dominant coloboma.

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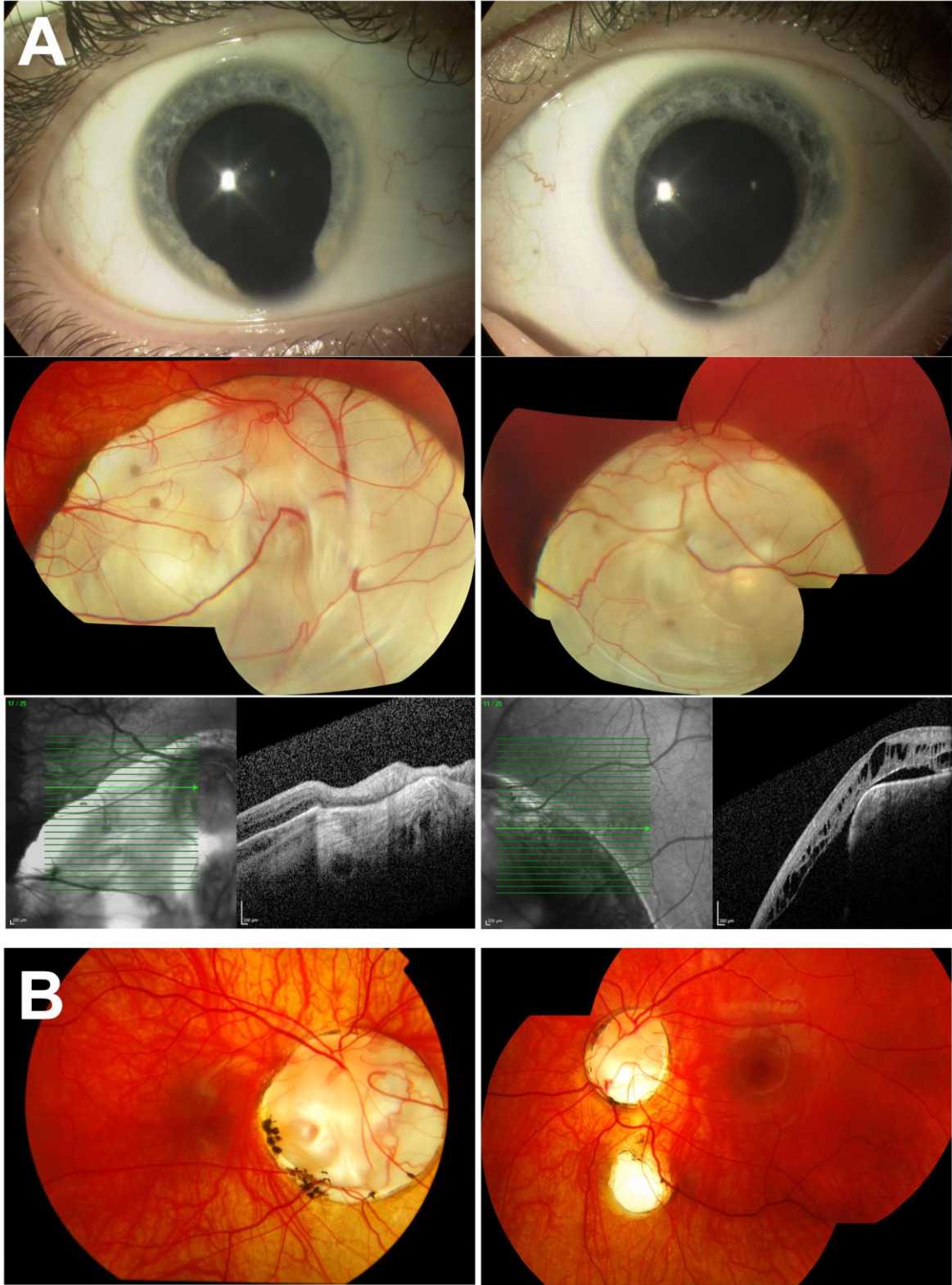
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FIGURE 1. Color fundus image montages, fundus autofluorescence imaging (FAF) and optical

coherence tomography (OCT) of patient 1 (A) and color fundus image montages of patient 2 (B).

Images of the left eye of patient 1 show fluid under residual glial tissue confined to the left
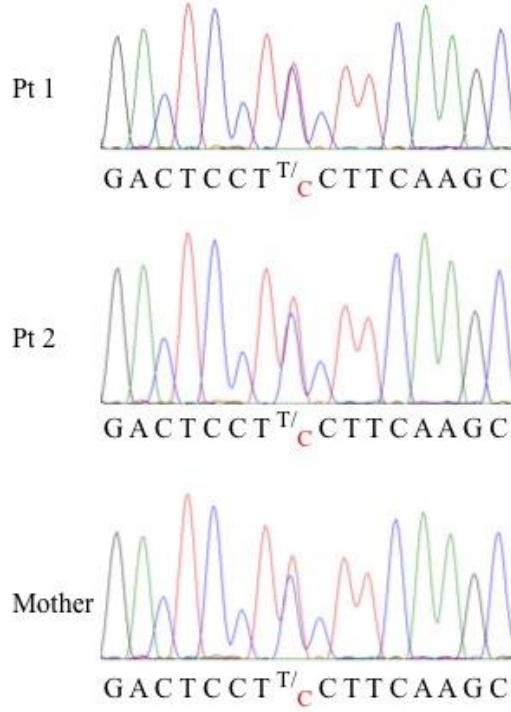
chorioretinal coloboma

FIGURE 2. A. Chromatograms from Sanger sequencing for patients 1, 2, and their mother. B.

Family pedigree (family GC20086) with *YAP1* segregation. C. Conservation across species,

generated by Clustal Omega. * (asterisk) indicates positions which have a single, fully conserved

residue, a : (colon) indicates conservation between groups of strongly similar properties, a .

(period) indicates conservation between groups of weakly similar properties.

A *YAP1* c.284T>C (p.F95S)

Pt 1

G A C T C C T ^{T/}_C C T T C A A G C

Pt 2

G A C T C C T ^{T/}_C C T T C A A G C

Mother

G A C T C C T ^{T/}_C C T T C A A G C

B

M/WT

Pt 1   Pt 2
M/WT   M/WT
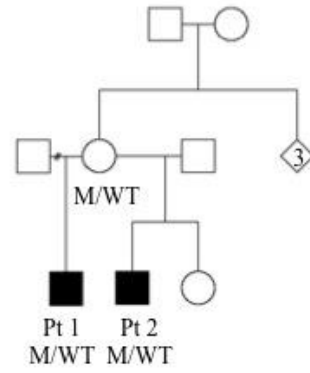
C

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rat        PQTVPMRLRKLPDSFFKPPEPKSHSRQ
mouse      PQTVPMRLRKLPDSFFKPPEPKSHSRQ
dolphin    PQTVPMRLRKLPDSFFKPPEPKSHSRQ
lizard     PHTLPMRLRKLPDSFFKPPEPKAHSRQ
chicken    PHTLPMRLRKLPDSFFKPPEPKAHSRQ
finch      PHTLPMRLRKLPDSFFKPPEPKAHSRQ
human      PQTVPMRLRKLPDSFFKPPEPKSHSRQ
monkey     PQTVPMRLRKLPDSFFKPPEPKSHSRQ
zebrafish  PPSVPMRLRKLPDSFFTPPEPKSHSRQ
cod        PPSVPMRMRKLPDSFFKPPEPKSHSRQ
           *  ::***:********.*****:****
                         F95S
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