Unveiling hidden genetic complexity: coexistence of *HGSNAT* and *EYS* variants in a patient with retinal dystrophy

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Dear Editor,

In a previous publication in this journal (AJMG Part C), Schiff *et al* reported on a series of 17 patients with mild or late-onset non-syndromic retinitis pigmentosa attributed to biallelic variants in *HGSNAT* [Schiff et al., 2020]. This report presents additional molecular findings in a patient previously featured in the series (MEH2; GC16348) and discusses the patient's clinical findings in the context of her complete molecular diagnosis.

The patient, an 85-year-old female patient of Asian Pakistani descent, initially reported night vision difficulties at age 55, followed by central vision problems at age 65. Significant visual field constriction noted at age 66. At age 72, she developed late onset bilateral hearing loss requiring hearing aids, and had a medical history of treated breast carcinoma for which she received treatment. Her parents were first cousins, and she had two unaffected siblings. No other individuals in the family were known to be affected (Figure 1a).

At her most recent ocular examination aged 84 years, her vision had deteriorated to hand movement perception in both eyes. Fundus examination revealed optic disc pallor, attenuated retinal vasculature, mid peripheral pigment spicules and pigmentary mottling, and extensive central and peripheral retinal pigment epithelium atrophy. Optical coherence tomography (OCT) scans demonstrated marked outer retinal degeneration with no macular oedema (Figure 1b).

Genome sequencing was performed via the Genomics England 100,000 Genomes Project (100kGP) [Genomics England, 2020], identifying a novel homozygous missense variant in

HGSNAT; NM_152419.3: c.1283T>G; p.(Phe428Cys), which was initially classified as a variant of uncertain significance. Subsequent HGSNAT enzymology testing identified a mild but significant reduction of HGSNAT activity in leukocytes, measured at 0.3 nmol/hr/mg protein, below the healthy control range of 0.64-4.2. This finding supported the molecular diagnosis of pathogenic *HGSNAT* variants causing non-syndromic retinitis pigmentosa (RP).

We subsequently conducted a systematic analysis of genome sequencing data from the 100kGP inherited retinal disease (IRD) patient cohort for structural variants (SVs) (genomic aberrations of >1 kb in size), using two SV calling algorithms, Manta and Canvas[Roller et al., 2016; Chen et al., 2016]. This analysis identified a homozygous 3.6 kb intragenic deletion in *EYS* [(GRCh37) Chr6:64708420_64712049del] in the same patient. The deletion encompasses exon 34 of *EYS* (NM_001142800.2: c.6726-2973_6834+548del) and is predicted, if transcribed, to result in a frameshift variant leading to premature truncation [p.(Tyr2243HisfsTer32)].

Biallelic variants in *EYS* are a well-established cause of autosomal recessive retinitis pigmentosa (MIM #602772) and represent a common cause of disease within our molecularly characterised IRD patient cohort [Lin et al., 2024; Pontikos et al., 2020]. This specific SV has been reported in homozygous form, causing RP, in an individual of Asian Indian descent [Carss et al., 2017] and has also been identified in compound heterozygous form with a c.892del; p.(Cys298ValfsTer41) *EYS* variant in another affected individual of mixed Indian, Portuguese and Guyanese ancestry within our IRD cohort. These findings support the pathogenic nature of this SV and suggest a potential enrichment within the Asian subpopulation.

These data strongly suggest that this patient has two independent pathogenic genotypes, one in *EYS* and one in *HGSNAT*, both contributing to her retinal disease. Notably, while this patient exhibited more extensive peripheral retinal involvement than typically seen in *HGSNAT*-associated retinal dystrophy, her clinical presentation was not considered inconsistent with *HGSNAT*-associated retinopathy, given her advanced age and the variable disease severity demonstrated in our previous case series [Schiff et al., 2020]. It is important to highlight this new finding, so that her clinical phenotype can be assessed in the context of her dual molecular pathology, and ascribing her clinical findings solely to the effect of *HGSNAT* dysfunction alone would be misleading.

SVs are increasingly recognised as important contributors to IRD, with an estimated prevalence in IRD patient cohorts ranging from 0.25% to 9% [Zampaglione et al., 2020; Wen et al., 2023]. Genome sequencing, with its superior ability to detect SVs compared to earlier exome sequencing methods, has been increasingly incorporated into our research and diagnostic pipelines. Despite this, SVs still only account for a relatively modest 2.5% to 4.3% of IRD cases within our patient cohort [Carss et al., 2017; Pontikos et al., 2020; Lin et al., 2024], likely underestimating their true impact. Improved bioinformatic tools for SV annotation [Yu et al., 2021], and emerging single-molecule long-read sequencing approaches [Ascari et al., 2021] will enhance the detection of SVs and hopefully go some way towards addressing missing heritability in IRD [Stone et al., 2017; Carss et al., 2017].

This case serves to remind us of the possibility of two or more independent disorders co-occurring in the same patient and family, a situation likely to be more common in the presence of consanguinity [Lal et al., 2016; Mahroo et al., 2018]. Such a finding would have important implications for screening of other family members, and underscores the importance of continuing genomic analysis to consider all possibly pathogenic genotypes, even when a likely molecular diagnosis has been identified.

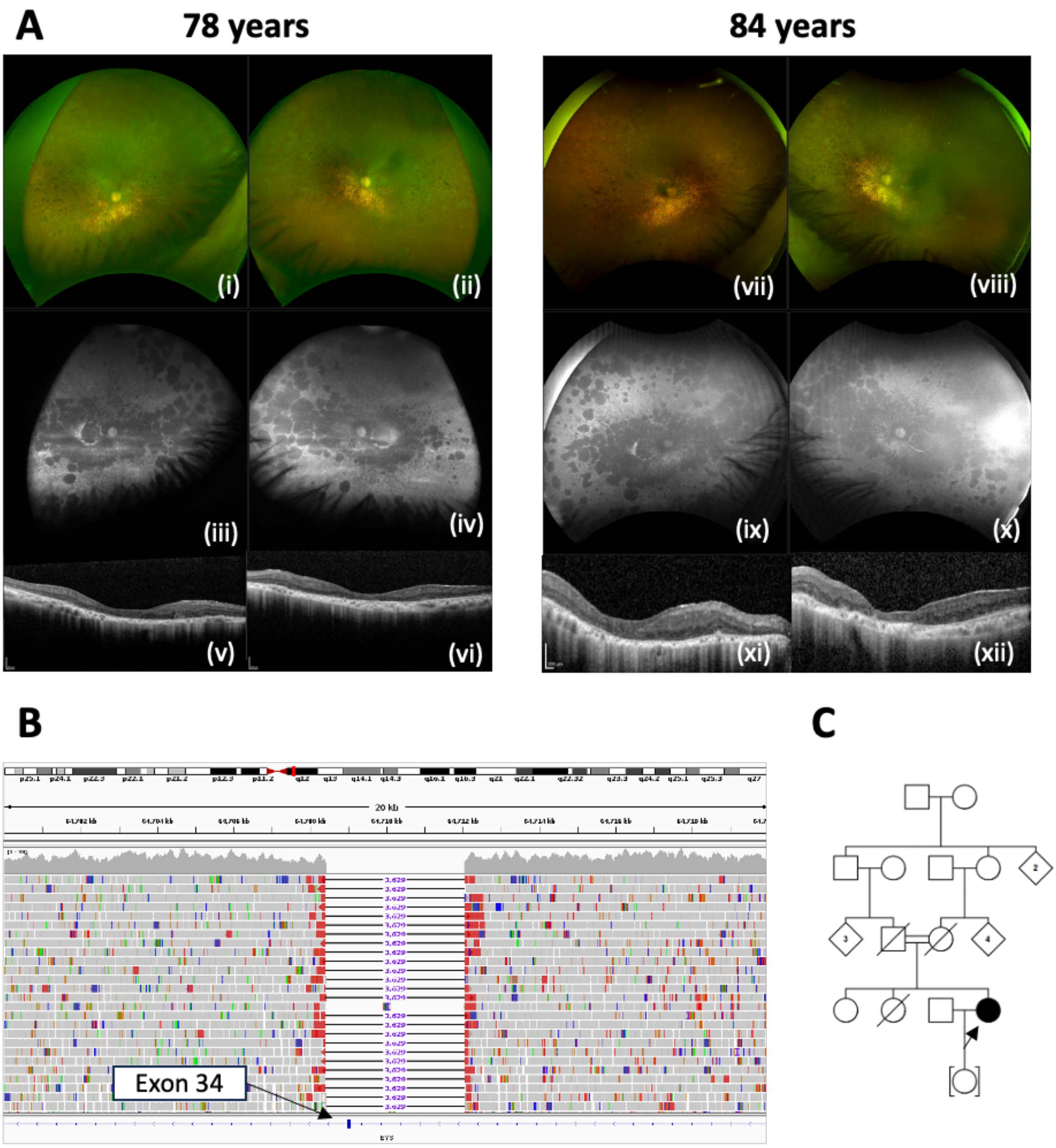

Figure 1: Clinical information, pedigree and sequence analysis in Individual MEH2.

A: Serial multimodal imaging including widefield fundus pseudo-colour photographs (i, ii, vii, viii), autofluorescence (iii, iv, ix, x)(Optos PLC, Dunfermline, UK), and OCT imaging (v, vi, xi, xii)(SD-OCT, Heidelberg Spectralis) for right (i, iii, v, vii, ix, xi) and left (ii, iv, vi, vim x, xii) eyes, depicting the slow progression of the disease over a 6-year period.
B: Integrative Genomics Viewer image displaying genomic sequence data across *EYS* exon 34, showing a homozygous 3.6 kb intergenic deletion (GRCh37 Chr6:64,708,420-64,712,049). This deletion can be visualised through a decrease in read depth and an increased distance between mate-paired reads spanning the breakpoint.
C: Family pedigree demonstrating consanguinity, with MEH2 being the sole known affected individual in the family.

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