

Interpretative intricacies of *ABCA4* variants in a patient with infantile nystagmus and a diagnosis of achromatopsia type 2

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Abstract:

Introduction: Infantile nystagmus is an eye movement disorder appearing before the age of 6 months. It can be of various aetiologies; however, in this age group, ophthalmic disorders predominate over neurological ones. The major cause is albinism, followed by inherited retinal dystrophies. Nonetheless, both are umbrella terms comprising numerous diseases caused by mutations in distinct genes, of different inheritance patterns and visual outcomes, necessitating a broad genetic screening.

Case report: The patient was a 4-month-old boy with infantile nystagmus and suspected ocular albinism or early-onset retinal dystrophy. Whole-exome sequencing identified four variants in retinal dystrophy genes, two pathogenic *CNGA3* mutations, corroborating the diagnosis of achromatopsia, and two concomitant *ABCA4* variants, a mild and a frequent hypomorphic allele. Based on the existing genotype-phenotype correlations and considering the visual limitations associated with a primary diagnosis, the detected *ABCA4* variants were assumed unlikely to be significant for the patient's visual functioning.

Conclusions: The present case highlights the need for judicious evaluation of multiple variants detected via high-throughput genetic testing methods and cautions against hasty diagnoses. The mere identification of a biallelic mutation in a recessive disease-associated gene is not always sufficient to make a diagnosis; in this instance, a diagnosis of Stargardt disease was not justified, given the mild character of the variants. Moreover, even a postulated association with age-related macular degeneration or late-onset macular dystrophic changes, considering the achromatopsia phenotype, appears to be of limited relevance for the patient, thereby underscoring the necessity of an individualised approach to each case.

Key words:

infantile nystagmus, achromatopsia, *CNGA3*, *ABCA4*.

Introduction

Infantile nystagmus (IN), previously referred to as congenital nystagmus, is a relatively infrequent eye movement disorder manifesting before the age of 6 months and reported to have an estimated prevalence of 6–14 per 10,000 live births [1]. Rather than representing a single disease entity, it is a unifying clinical sign of a broader range of disorders, the majority of which are associated with visual sensory defects, accounting for approximately 90% of cases [1, 2]. Frequent culprit conditions are albinism and inherited retinal dystrophies ([IRDs] together accounting for up to 80% of IN cases), and ocular development anomalies [1, 2].

The major diagnostic hurdle of IN stems from its considerable clinical and genetic heterogeneity. The underlying ophthalmic disorders are umbrella terms for numerous diseases that result from pathogenic variants in multiple distinct genes involved in phototransduction and visual cycle, retinal development, foveal morphogenesis, and photoreceptor maintenance [2, 3]. Furthermore, these conditions may follow all Mendelian inheritance patterns – autosomal dominant, autosomal recessive, or X-linked – and are associated with highly variable disease trajectories and visual outcomes, running a spectrum of relatively preserved visual acuity in *FRMD7*-associated nystagmus to progressive, debilitating visual impairment in severe retinal dystrophies, such as Leber congenital amaurosis [1, 2, 4].

Given the underlying genetic complexity, clinical examination alone is insufficient to achieve a precise aetiological diagnosis. Therefore, implementation of next-generation sequencing (NGS) – based high-throughput genetic testing methods, particularly targeted panel sequencing and whole-exome sequencing (WES), has become an essential component of an efficient modern diagnostic

approach [1]. However, the principal challenge lies in the proper interpretation of the detected variants' clinical significance, as broad sequencing may uncover additional variants beyond those sought as a primary diagnostic target.

We herein describe an individual with IN, harbouring an achromatopsia (ACHM) – causing *CNGA3* genotype and two concomitant *ABCA4* variants detected via WES. We also discuss interpretative issues regarding the incidental detection of multiple variants in IRD genes and their pathogenicity in the context of existing genotype-phenotype correlations.

Case report

The patient was an otherwise healthy 4-month-old boy with horizontal nystagmus since the age of 2 months. He was the third child born at term after an uneventful pregnancy through caesarean section at 39 weeks' gestation. His Apgar score was 10. The family anamnesis was unremarkable, and there was no history of parental consanguinity. Upon neurological evaluation, lack of visual fixation and poor eye tracking were noted. Transfontanellar ultrasound was unrevealing. Subsequent ophthalmologic evaluation was remarkable for high-rate hypermetropia of +10.0 Dsph, normal anterior segment of the eyes, and no iris transillumination. Fundus exam showed blunted foveolar reflexes, greyish optic discs, and tessellated peripheral retina. The boy was therefore referred to the Genetic Clinic due to suspicion of ocular albinism. The patient underwent WES with targeted analysis of the IRD and albinism gene panels, which yielded 4 variants in IRD – associated genes. Two heterozygous pathogenic missense variants in the *CNGA3* gene (NM_001298.3): c.572G>A, p.(Cys191Tyr) and c.1688G>A, p.(Arg563His), were consistent with the observed

phenotype and deemed diagnostic for ACHM. Two heterozygous *ABCA4* (NM_000350.3) variants: c.5603A>T, p.(Asn1868Ile) and c.5882G>A, p.(Gly1961Glu), were not related to the phenotype that prompted genetic evaluation, but they required consideration and interpretation in the clinical context. They are regarded as hypomorphic and mild pathogenic variants, respectively, and are therefore insufficient alone to cause a typical Stargardt disease (STGD) phenotype.

Discussion

The *CNGA3* gene is, next to *CNGB3*, one of the most common ACHM-causative genes, and it encodes the alpha subunit of a cyclic nucleotide-gated cation channel specific to cones; thus, biallelic pathogenic variants result in the phenotype reflecting dysfunction of these photoreceptors [5–7]. ACHM usually manifests in infancy with IN due to very low visual acuity of around 0.1–0.2 [2, 5]. Of note, rod-driven electrophysiological responses are typically preserved or slightly abnormal, and scotopic vision is usually similar to that of healthy individuals, owing to different ion channels in rods encoded by homologous *CNGA1* and *CNGB1* [3, 5, 6]. Additionally, patients typically exhibit photophobia and compromised colour discrimination [5]. The severity ranges from total colour blindness in complete ACHM to partial colour perception and milder visual acuity diminution, owing to residual channel function, in incomplete ACHM [2, 5]. Both detected *CNGA3* alterations have already been reported in affected patients; however, no clear genotype-phenotype correlations for ACHM exist [5, 7]. The achromat's fundus is usually of normal appearance but may also present a spectrum of abnormalities upon detailed ophthalmologic examination [5]. Significant clinical findings include foveolar hypoplasia of different degrees in approximately 50% of patients and an optically empty space at the fovea in optical coherence tomography imaging; however, in this patient group, it may be difficult to perform, yielding poor-quality scans [1, 2]. Notably, ACHM is considered a clinically stationary disease because visual acuity usually remains stable, while nystagmus and photophobia might even slightly abate with age [5, 6].

The *ABCA4* gene encodes a retina-specific ATP-binding cassette transporter involved in the removal of toxic vitamin A derivatives in both cone and rod photoreceptors [8]. It is a well-established causative gene of autosomal recessive *ABCA4*-related retinopathy, classically juvenile macular dystrophy known as eponymous STGD. However, contingent upon variant deleteriousness and disease duration, it can also present as a more severe phenotype of cone-rod dystrophy or retinitis pigmentosa [8, 9]. *ABCA4* variants have been extensively studied, allowing for the establishment of several genotype-phenotype correlations. Genetic alterations have been grouped into four main categories: 1) mild alleles, including frequent and rare hypomorphs, and p.(Gly1961Glu) variant; 2) moderate; 3) severe; and 4) null variants [8]. Accordingly, STGD clinical prognoses depend on variant configuration and may range from foveal or parafoveal atrophy to widespread lesions involving the entire posterior pole and encroaching upon the mid-periphery [8, 9].

The detected p.(Asn1868Ile) is a frequent hypomorph, with a high minor allele frequency of nearly 7% in non-Finnish Europeans and a total of 2989 homozygotes observed in gnomAD (v4.1.0). Therefore, it has long been regarded as benign [8, 10]. However, currently available genotype-phenotype correlation matrices indicate that it can be clinically penetrant if paired with a deleterious enough variant (i.e. severe or null variant) [8–11]. The second variant, p.(Gly1961Glu), is more explicitly considered pathogenic, albeit mild [8]. It requires at least a moderate allele *in trans* to cause apparent disease, hence suggesting that these two in compound heterozygosity are unlikely to give rise

to the typical STGD phenotype [8]. Furthermore, if present in a penetrant constellation, they result in the mildest prognoses of late-onset and macular-confined phenotypes. The hypomorphic variant is highly abundant in the late-onset STGD cohort, where patients may retain fully functional visual acuity even into the seventh decade of life [8–11]. Notably, the mild manifestations have been reported to be relatively invariant regardless of the second allele severity; hence, these variants are described as “clinically dominant” [8].

Heterozygous *ABCA4* variants have been proposed as a risk factor for age-related macular degeneration (AMD), given the observed co-occurrence of AMD in families affected by classic STGD [12, 13]; however, this association has been disputed and speculated as a possible confusion with a similarly appearing late-onset *ABCA4*-related disease [9, 11, 14]. A longitudinal assessment of STGD patients' carrier parents showed subtle retinal functional and morphological abnormalities, with a slight deterioration over a 5-year follow-up period in some individuals [15]. Although heterozygotes of a recessive disease are not generally expected to exhibit clinical symptoms, this study suggests that at least certain monoallelic variants may confer a risk of developing a phenotype resembling dry AMD [15]. Conversely, another study indicated a normal phenotype of *ABCA4* carriers irrespective of mutation severity [14]. Nevertheless, animal studies of *Abca4*^{+/-} mice support the presence of some abnormalities in carriers on the molecular level, including altered bisretinoid metabolism and increased lipofuscin accumulation [11, 14].

Additionally, the *ABCA4* heterozygosity may not be a homogeneous category, as the disease expressivity hinges strongly on allele severity, complex alleles, *cis*- or *trans*-acting modifiers, intronic variants, and the possibility of mislabelling patients as simple carriers when they are actually compound heterozygotes, harbouring the second variant missed by routine testing [8–11, 15]. Research has shown that many previously unresolved cases, consistent with the STGD phenotype, were indeed later explained by initially unrecognised hypomorphic or deep-intronic variants [10, 11]. This is further emphasised by the absence of phenotypic differences between patients carrying one versus two alleles in a study of late-onset STGD [11]. Moreover, both adverse and protective environmental factors, as well as other genetic modifiers, may contribute to the clinical outcome [10, 11].

Furthermore, the carrier frequency of *ABCA4* variants, including mild alleles, has been estimated at 1: 25, corresponding to an approximated theoretical disease prevalence of 1: 2500, as opposed to the commonly cited 1: 10,000 for STGD [10, 11]. This discrepancy has been hypothesised to stem from reduced penetrance of certain variant assortments, and therefore a lack of perceptible symptoms or misdiagnoses of late-onset STGD as AMD or other phenocopies [9, 11]. Consistently, studies of late-onset STGD lacked the p.(Asn1868Ile) and p.(Gly1961Glu) variant constellation, which may support its likely clinically silent nature [9, 11].

However, the detected *ABCA4* variants cannot be assessed in isolation from the patient's ACHM phenotype related to the biallelic *CNGA3* mutation. Drawing on the above-mentioned considerations, the combination of p.(Asn1868Ile) and p.(Gly1961Glu) appears too mild to diagnose STGD, as the term is used only for patients harbouring the established disease-causing mutation constellations [8, 9]. Furthermore, it is unlikely to result in a clinically perceptible phenotype in this particular case. A postulated association with AMD or phenotypically similar late-onset macular dystrophic changes appears to be of limited relevance for the patient, who is already affected by visually debilitating ACHM. Nonetheless, the presence of concomitant *ABCA4* variants remains relevant for genetic counselling and should not be overlooked.

Conclusion

NGS-based testing is an indispensable diagnostic tool in cases of IN because the most common causes are of genetic aetiology, enabling tailored management. However, most importantly, the present case highlights the challenges of interpreting multiple variants detected via high-throughput methods and cautions against hasty diagnoses. The mere identification of two variants in a recessive disease-associated gene is not always equivalent to a definitive clinical diagnosis. To avoid misdiagnosis, the variants' severity should be judiciously evaluated, also against each other. Additionally, the broader clinical context, such as a primary diagnosis of ACHM in this case, must be considered while determining the clinical significance of genetic findings, underscoring the necessity of an individualised approach to each case. Nevertheless, long-term follow-up of this patient might help clarify the eventual visual outcome and enhance the clinical interpretation of similar cases.

Disclosure

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