A rare canonical splice-site variant in *VPS13B* causes attenuated Cohen syndrome

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Abstract

Background: To describe a patient with history of obesity, retinal dystrophy, type II diabetes, and mild cognitive impairment; found to harbour biallelic splice-site variants in *VPS13B*.

Materials & methods: A complete ophthalmic evaluation was performed at Moorfields Eye Hospital (London, United Kingdom), consisting of measurement of best corrected visual acuity (BCVA), slit lamp and dilated fundus evaluation, colour, autofluorescence and near-infrared retinal imaging, spectral domain-optical coherence tomography and electroretinogram (ERG). Whole genome sequencing was performed as part of the UK's 100,000 genomes project.

Results: A 26-year-old Pakistani man with normal appearance, stature and head size presented with decreased BCVA and severely constricted visual fields to our Ophthalmic Genetics clinic. He had a history of obesity, type II diabetes, and mild cognitive impairment. His evaluation showed retina-wide severe photoreceptor dysfunction on both eyes, with undetectable scotopic and photopic ERG waveforms. Genomic analysis identified a homozygous rare splice donor variant in the *VPS13B* gene (c.5024+2T>C) that was demonstrated to lead to skipping of the in-frame exon 31 (p.Gln1607_Ser1675delinsHis).

Conclusions: Exon 31 skipping in *VPS13B* may lead to a hypomorphic change, with partial gene function and an incomplete, mild Cohen syndrome-like phenotype.

Introduction

Cohen syndrome (CS, MIM #216550) was first described in 1973 by M. Michael Cohen Jr. and colleagues in United States of America.[1] This multisystemic, autosomal recessive syndrome is characterized by a cheerful disposition, slender limbs, leukopenia, recognisable craniofacial features, growth and developmental abnormalities, persistent hypotonia, enlarged corpus callosum, high myopia, and retinal dystrophy.[2] However, broad clinical heterogeneity has been reported.

At least 200 cases of CS have been reported to date, affecting populations worldwide such as Amish, European, Brazilian, Japanese, and Finnish.[3–6] CS is overrepresented among the latter, with around 17% of all cases diagnosed in Finland.[3,7] Through linkage and haplotype analysis of affected Finnish individuals, the causative gene of CS, *VPS13B* (MIM #607817; also called *COH1*) was discovered 30 years after its original description.[8–10] The longest transcript of *VPS13B* (NM_017890) consists of 62 exons and spans a genomic region of around 864 kb.[9] It encodes a 4022 amino acid residue Golgi apparatus transmembrane protein, which harbors two regions homologous to yeast vacuolar protein sorting-associated protein 13 (VPS13).[11] It is widely expressed and it has been associated with glycosylation and intracellular trafficking of proteins.[12,13] It has been reported that impaired VPS13 function causes decreased neuritogenesis.[11]

Over 230 pathogenic variants have already been described in *VPS13B* (Human Gene Mutation Database -HGMD- Professional 2020.4 - accessed on 20.02.2021), all of which are associated with variants of CS. Founder mutations have been identified in Amish (c.8459T>C, c.9258_9259insT), Irish travelers (c.4471G>T) and Greeks/Mediterranean (c.11564delA), besides the Finnish (c.3348_3349delCT).[14]

Here, we present findings of an individual who was referred to our Ophthalmic Genetics clinic at Moorfields Eye Hospital for evaluation due to retinal dystrophy and was found to have homozygous splice-site variants in *VPS13B*.

Materials and methods

An individual with retinal dystrophy and his family (GC27438) were involved in this study. They were evaluated at Moorfields Eye Hospital (London, United Kingdom) and consented to have genetic testing, as well as participate in the present study, adhering to the tenets of the Declaration of Helsinki. A complete ophthalmic evaluation was performed, including measurement of best corrected visual acuity (BCVA), slit lamp and dilated fundus evaluation. Additional testing included colour and autofluorescence retinal imaging (Optos Panoramic 200 ultrawide-field retinal imaging device, Optos PLC, Dunfermline, Scotland), near-infrared reflectance and optical coherence tomography (OCT, Spectralis SD-OCT device Heidelberg Engineering, Heidelberg, Germany) and electroretinogram (ERG, commercial electrophysiology system using International Society for Clinical Electrophysiology of Vision -ISCEV- standards).[15,16] External face and hand images were also taken, as well as a full blood count.

He was recruited for whole genome sequencing (WGS) with his unaffected sister as part of the UK's 100,000 genomes project. WGS and rare variant analysis was performed as previously described.[17] Reverse transcription PCR (RT-PCR) was performed on RNA purified from PAXgene stabilized whole blood using oligonucleotide primers (available on request) to amplify a 779bp amplicon from exon 29 to exon 33 followed by direct Sanger sequencing of resulting PCR amplicons.

Clinical Report

The proband was the third child of consanguineous parents (first cousins) of Pakistani descent. He was born at term after an uneventful pregnancy, with no malformations noted at birth and normal newborn hearing screening. At around age 4, his parents noticed he was tripping and having difficulties navigating in dim environments. An optometry evaluation revealed constricted visual fields and further ocular exams led to the diagnosis of retinal dystrophy soon after.

Growing up, mild cognitive impairment caused him to attend a special needs school, where he completed primary and high school education. He started struggling with obesity since late childhood and had a gynecomastia surgery at age 21. He was diagnosed with type II diabetes at age 22, which was managed with diet and

metformin. There was no family history of eye disease and at the time of his evaluation, he was married and had an unaffected 4-year-old daughter.

He first came to Moorfields Eye Hospital as a 26-year-old man with normal appearance, stature and head size (Fig 1A & B). He was already registered sight impaired and reported decreasing central vision since teenage years. His BCVA was 20/2000 (logMAR 1.8) in the right eye (OD) and 20/80 (logMAR 0.6) in the left eye (OS). His refractive error was of mild myopia (spherical equivalent: -0.50 diopters), equal on both eyes (OU). Confrontational visual field testing demonstrated 5-10 degrees' central fields, symmetric OU. His anterior segment exam was positive for cortical blue dot lens opacities, visually non-significant. His posterior segment assessment showed pale optic nerve heads, severe vessel attenuation and peripheral pigmentary changes 360 degrees OU (Fig. 2A). Autofluorescence revealed foveal hypoautofluorescence on both eyes, being the right eye more severely affected (Fig. 2B). Macular OCT showed profound loss of the outer layers on the right eye and a bull's eye pattern on the left eye, with decreased overall macular thickness on both eyes(Fig. 2C). Electrophysiology testing was consistent with a generalized loss of rod and cone function. This was assessed by undetectable pattern, scotopic and photopic ERG OU. A full blood count showed normal platelet, red and white cell count.

Over an 8-year follow up, his BCVA gradually decreased to hand movements OD and 20/125 (logMAR 0.8) OS. His field of view constricted to below 5 degrees and he got registered severely sight impaired. He also developed posterior subcapsular cataracts OU and had phacoemulsification surgery OS, with posterior chamber intraocular lens implant. Given the extent of the retinal dystrophy OD, a lensectomy was not advised. Fundoscopy showed progressive and extensive retinal dystrophy affecting the majority of the fundus OU. No signs of diabetic retinopathy or macular edema were noticed at any point.

Genetic testing results

WGS and virtual gene panel investigation revealed a single rare (gnomAD MAF <0.001) homozygous predicted protein altering variant. The variant (GRCh37 chr8:100,568,883T>C, NM_017890.5 c.5024+2T>C) is found in 2/248632 alleles in

the gnomAD 2.1 dataset and affects the canonical +2 position of the splice donor site of intron 31 and was predicted to abolish the donor site motif. RT-PCR and agarose gel electrophoresis showed a faster migrating band in the patient's lane compared to a control sample. This corresponded to approximately 575bp compared to the wildtype band of 779bp (Figure 3). Direct Sanger sequencing confirmed skipping of the 204bp exon 31 in the patient's sample predicted to lead to deletion of 69 amino acid residues and insertion of a histidine in the encoded protein, p.(Gln1607_Ser1675delinsHis). No other variants that could explain the patient's phenotype were found in a survey of the virtual gene panel.

Discussion

Given the clinical heterogeneity of CS and the vast pleiotropy of *VPS13B*, a delayed diagnosis commonly occurs. It has been postulated that CS may be a frequently underdiagnosed condition, mostly among individuals with unexplained developmental delay or intellectual disability.[18,19]

Several attempts have been made towards delineating diagnostic criteria for CS. The Finnish group that first discovered *VPS13B* proposed eight major characteristics: developmental delay, microcephaly, typical facial gestalt, truncal obesity with slender extremities, overly sociable behavior, joint hypermobility, high myopia and/or retinal dystrophy, and neutropenia. They postulated that patients with six or more of these features should be categorized as true CS, while those with five or fewer could have "Cohen-like syndrome".[20] Horn et al. proposed short stature and hypotonia as two other major criteria.[21] El Chehadeh et al. analyzed a cohort of 14 genetically confirmed CS patients and concluded that the features that should prompt *VPS13B* screening were chorioretinal dystrophy and neutropenia.[22] Rodrigues et al. suggested thinking of CS in infants with microcephaly, early-onset hypotonia, neutropenia, and global developmental delay.[23] Hennies et al. considered that the hallmarks of the condition were the typical facial gestalt, myopia, and developmental delay.[6] Chandler et al. proposed learning difficulties, retinal dystrophy and neutropenia as strong clinical indicators for establishing a diagnosis.[24] A consensus is yet to be built.

Our patient presented with retinal dystrophy, obesity, type II diabetes and mild cognitive impairment. These would only correspond to three features of CS (retinal dystrophy, obesity and developmental delay) and classify him as a Cohen-like syndrome patient. Other differential diagnoses were indeed considered for him: Bardet-Biedl syndrome (the absence of postaxial polydactyly and renal abnormalities was not typical),[25] Diabetes And Deafness, Maternally Inherited (MIDD; it does not present with cognitive impairment and the retinal phenotype corresponds to a pattern macular dystrophy, not a widespread photoreceptor dysfunction as seen in this patient)[26] and Prader-Willi syndrome (retinal dystrophy is not a part of this syndrome).[27]

Facial features of CS (thick hair and eyebrows, low hairline, high-arched and wave-shaped eyelids, long and thick eyelashes, prominent nasal root, high and narrow palate, smooth or short philtrum, and prominent upper central incisors) have been reported absent in the past, and can vary with age and across ethnicities.[3,5,28] Type II diabetes has been associated with CS, however it is still not considered a diagnostic criterion.[29]

Some of the ophthalmic features reported in CS are progressive, high myopia (over 7 diopters by the second decade of life), astigmatism, and chorioretinal dystrophy. The latter has been characterized as a cone–rod dystrophy (with a bull's eye maculopathy pattern) that appears during the first decade of life and evolves to a generalized pigmentary retinopathy with the triad of vessel narrowing, bone spicules and pale optic discs by early adulthood. Children may complain of reduced acuity, night blindness and constricted field.[30] Macular edema and retinoschisis have also been described, as well as early cataracts.[31–33]

Copy number variants (CNV) and particularly intragenic deletions have been reported as an important cause of CS.[34,35] We found that the rare homozygous splice-site variant in *VPS13B* led to exon 31 skipping. This variant transcript is not likely to undergo nonsense mediated decay since there is not a reading frame shift and therefore no resultant premature termination codon. Thus, it is likely to lead to a mature protein lacking the 68 residues encoded by exon 31 (deletion of 69 residues and insertion of a histidine). We hypothesize that the mutant protein may retain some biological function because the functional domains of VPS13B are not lost due to

skipping of exon 31 and the patient's extra-ocular phenotype is mild compared to the biallelic loss of function (LOF) disease seen in typical CS patients. Thus, the protein may retain enough function to mitigate the impact on extra-ocular tissues. However, the true functional effect on the protein is yet to be elucidated and the patient presented with a severe ophthalmic phenotype, therefore it is alternatively possible that a distinct role of VPS13B exists in the retina or that the skipped exon encodes a domain essential and specific to retinal function.

Most of the patients with CS carry variants that result in premature termination.[36] The pathogenicity of missense changes and exon skipping is yet to be clarified. The latter mechanism has been reported in *VPS13B*, associated with different phenotypes. The variant c.2934+1_2934+2delGT led to skipping of exon 20 (out of frame) and, in trans with an intragenic deletion, represents a biallelic LOF genotype causing a complete CS phenotype.[36] The splice-site mutation c.6940+1G>T generated exon 38 skipping (out of frame) and (in compound heterozygosity with a frameshift deletion) was seen in a Chinese patient with developmental delay, obesity, high myopia and dysmorphic facial features.[12] Interestingly, skipping of the in frame exon 57 in trans with a second splicing mutation (c.5983+2dupT, shown to reduce transcript level) has been associated with a mild form of CS, showing only neutropenia and retinopathy.[37] Gueneau et al. related the incomplete phenotype of this patient with a possible residual effect of VPS13B protein. Moreover, skipping of exons 8 to 15 (out of frame), 32 and 33 (out of frame) resulted in a mild phenotype with intellectual disability and hypotonia in two young Japanese siblings.[38]

In conclusion, we report an individual of Pakistani origin, homozygous for a rare splice-site mutation in *VPS13B* that leads to in frame skipping of exon 31. He presented with features from CS spectrum (retinal dystrophy, developmental delay and obesity), no other plausible variants to explain his phenotype were found in a survey of the virtual gene panel, and mild forms of CS have been reported in individuals with residual levels of VPS13B. Therefore, we propose *VPS13B* as the causative gene of his phenotype, possibly through a hypomorphic mechanism, and report an additional case in which exon skipping in *VPS13B* can lead to an attenuated syndrome. This case adds to the understanding of this complex gene and the delineation of genotype-phenotype

correlations. We suggest considering the possibility of biallelic non-LOF variants in *VPS13B* in patients whose disease partially fulfils CS phenotype.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Figure captions

Figure 1: Facial (A) and hands (B) images of the proband. Typical facial features of CS such as low hairline, wave-shaped eyelids and smooth or short philtrum are absent. Hands are also within normal limits.

Figure 2: Fundus evaluation of the proband. Ultra-wide field colour retinal images (A) show pale optic nerve heads, severe vessel attenuation and peripheral pigmentary changes 360 degrees symmetric OU. Autofluorescence imaging (B) depicts hypoautofluorescence on the mid-periphery and peripheral pigmented deposits bilaterally. Right foveal atrophy is seen as a well circumscribed hypoautofluorescent defect. Macular OCT (C) corresponds to severe foveal outer layers loss on the right eye and a bull's eye pattern on the left eye, with decreased overall macular thickness.

Figure 3: RT-PCR aberrant transcript analysis. (A) RT-PCR agarose gel electrophoresis showing the wildtype (779bp) and variant (575bp) bands. L: Ladder, N: negative, C: control (wildtype), P: Patient sample. (B) Sanger sequencing showing the exon 31 skipping in the patient sample. (C) Partial sequence alignment of VPS13B amino acid residues, showing (-) the amino acids absent in the patient compared to reference sequence (NP_060360) and, in red and blue the Gln to His change.

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