Brief Report

The coincidence of two ultra-rare hereditary eye diseases: gyrate atrophy and Kjer optic atrophy - a surprising diagnosis based on next-generation sequencing

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SUMMARY   Genetically determined ophthalmic diseases form a numerous and heterogenic group of disorders. Making the accurate clinical diagnosis of genetic eye disease is often a challenge for an ophthalmologist. In many cases, only genetic testing enables the establishment of the proper clinical diagnosis. Here we describe two ultra-rare diseases: gyrate atrophy of the choroid and retina (GACR) and Kjer-type optic atrophy coexisting in a 39-year-old Polish patient with severe visual impairment including a significant reduction of visual acuity and night blindness. Atrophic pigmented changes with large pigment deposits and chorioretinal atrophy with the retina's disturbed structure (with atrophic scarring changes and the epiretinal membrane) of both eyes were observed. Electroretinography (ERG) revealed extinguished responses. A Next-Generation Sequencing (NGS) panel comprising 275 retinal genes revealed a presence of potentially pathogenic variants in two genes: a homozygous variant c.1058G>A (p.Gly353Asp) in the *OAT* gene and a heterozygous variant c.1886C>G (p.Ser629Ter) in the *OPA1* gene. The diagnosis established based on NGS is surprising because initially, several different diagnoses have been made, including high degenerative myopia, choroideremia, Leber congenital amaurosis, and severe, atypical retinitis pigmentosa. This report provides the unquestioned diagnostic value of the combination of chorioretinal imaging and the NGS technique. To our knowledge, this is the first and the only description of the coincidence of gyrate atrophy and Kjer-type optic atrophy.

Keywords   gyrate atrophy of the choroid and retina (GACR), Kjer-type optic atrophy, Next-Generation Sequencing (NGS)

1. Introduction

Gyrate atrophy of the choroid and retina (OMIM#258870, GACR) is an ultra-rare genetic condition inherited in an autosomal recessive manner. The disorder primarily affects the ocular tissues. Symptoms include night blindness, visual field constriction, and myopia, usually starting in the first decade of life, followed by progressive vision loss due to macular affection and cataract formation in the second decade (*1*). The global incidence of GACR is unknown, but the theoretical global incidence is approximately 1 in 1,500,000 births (*2*). The highest prevalence is observed in Finland, with about 1 in 50,000 individuals (*3*). Retinal features of patients with GACR involve sharply demarcated, circular areas of chorioretinal atrophy that start in the mid-peripheral retina in the first decade and spread centrally to the macular region (*1*). It may lead to blindness, at the latest by 40-60 years (*4*). Other symptoms that may occur are neonatal blood hyperammonemia and type II muscle fiber atrophy with tubular aggregates' formation (*5*). Patients with gyrate atrophy generally have normal intelligence. However, minor central nervous system (CNS) abnormalities: degenerative changes in brain magnetic resonance imaging (MRI) and nonspecific electroencephalogram (EEG) abnormalities suggest that the CNS is involved, although no clear clinical correlates have been reported (*6*). Moreover, it was reported that peripheral nervous system abnormalities could also be observed in some gyrate atrophy patients. More than 50% of patients with GA were revealed to have electrophysiologic signs

of peripheral neuropathy. Most of them had mild or no symptoms, but 10% have symptomatic peripheral neuropathy, which was never disabling (*7*).

The disease is caused by a homozygous or compound heterozygous mutation in the *OAT* gene encoding an enzyme: ornithine delta-aminotransferase. OAT is a mitochondrial matrix enzyme that catalyzes ornithine's reversible transamination to glutamate semialdehyde (*8*), which plays a pivotal role in cellular detoxification (*9*). The OAT enzyme binds pyridoxal 5'-phosphate: a derivative of vitamin B6, as a cofactor (*2*). Mutations in the *OAT* gene result in a decrease or absence of the OAT enzyme activity. Deficiency of the OAT enzyme results in a 10 to 20 times increase in the plasma level of the amino acid ornithine, which is toxic to RPE and choroid (*4*). The *OAT* gene is located on chromosome 10q26.13 (*10*). It contains 11 exons and spans over 21 kb (*11*).

Optic atrophy type 1 (OMIM#165500, OPA1, Kjer-type optic atrophy), also known as autosomal dominant optic atrophy (ADOA), is a neuro-ophthalmic condition characterized by bilateral optic nerve pallor associated with an insidious decrease in visual acuity in early childhood, visual field defects, and color vision defects. The most typical symptoms are centrocoecal visual field scotoma found in the vast majority of patients affected with OPA1 and tritanopia (*12,13*). The disease causes bilateral degeneration of the optic nerves affecting primarily the retinal ganglion cells (RGC) and their axons forming the optic nerve (*14,15*). It leads to a moderate to severe loss of visual acuity. A considerable degree of inter- and intra-familial phenotypic variability was observed in ADOA (*12*). Moreover, the disease shows incomplete penetrance. It was reported to be as low as 43% (*16*). The clinical picture of optic atrophy type 1 may also include (in 20% of patients) some extraocular symptoms (s.c. ADOA plus syndrome, OMIM#125250), such as auditory neuropathy resulting in sensorineural hearing loss, mild peripheral myopathy, neuropathy, or less commonly: progressive external ophthalmoplegia, spastic paraparesis and multiple sclerosis-like illness (*13,15,17*).

Kjer-type optic atrophy is inherited in an autosomal dominant manner, and is caused by heterozygous variants in the *OPA1* gene. The prevalence of ADOA is 1:30,000-1:50,000 births and is much higher in Denmark (1:10,000 births) (*15*). The *OPA1* gene encodes ubiquitously expressed mitochondrial dynamin-like GTPase. The protein is associated with the inner mitochondrial membrane. It is required to maintain cristae integrity and play an essential role in mitochondrial fusion and maintaining mitochondrial DNA stability. It controls many processes, including energy metabolism and apoptosis (*14,15*). The *OPA1* gene is located on chromosome 3q28. It contains 31 exons, including the alternatively spliced exons: 4, 4b, 5b, and spans more than 100 kb (*14*).

2. Patient and Methods

2.1. Clinical data and analysis

A 39-year-old man of Polish origin was referred to a genetic clinic in 2019, due to severe visual impairment, including a significant reduction of visual acuity and night blindness. Initially, several differential diagnoses have been made in the proband including high degenerative myopia, choroideremia, Leber congenital amaurosis, and severe, atypical retinitis pigmentosa. Written informed consent was obtained from all subjects: the patient, his healthy mother and son, the patient's sister showing ADOA symptoms, and her three sons. This study was conducted in accordance with the tenets of the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology (ARVO) statement on human subjects.

The patient was born at term from an uneventful pregnancy. His psychomotor development was normal. The parents were unrelated. The mother is still healthy and shows normal vision, but the father died at 37 due to a heart attack, and there is no information regarding his ophthalmological status. The patient has two older sisters and a younger brother. One sister and her son show ADOA symptoms (Figure 1).

Severe visual impairment has been observed in the patient since childhood. When the subject was 4-years-old, his parents noticed his low visual acuity and night blindness. At the age of 8, the patient's visual acuity
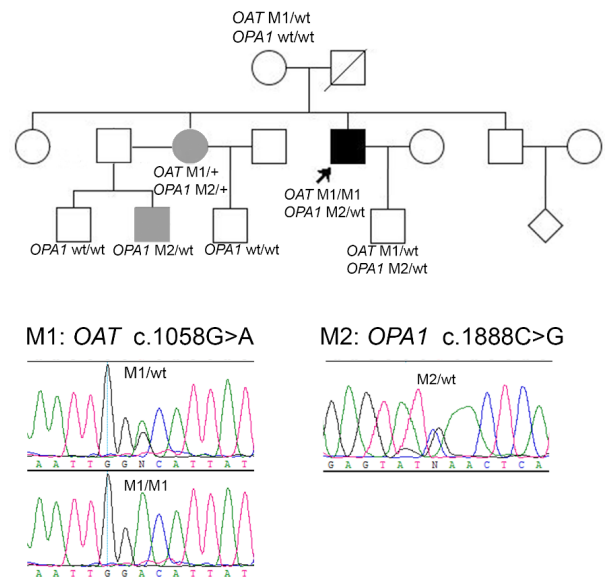

Figure 1. Pedigree of the examined family together with the segregation analysis results, and chromatograms of the identified variants. The upper panel shows the pedigree. Symbols filled with grey indicate individuals affected with optic atrophy type 1, the proband (affected with GA and ADOA) is marked with an arrow and a square filled with black. Unfilled symbols indicate unaffected individuals. A slash indicates a deceased person. The bottom panel shows chromatograms of the identified variants.

was 0.3, and he had high myopia (-11,0 D). In 2010, at the age of 29, the patient underwent bilateral cataract extraction with posterior chamber intraocular lens implantation. In the next years, biodegradation and subluxation of intraocular lenses (IOLs) to the vitreal cavity were noted. In 2017 the pars plana vitrectomy with removal of both IOLs was done at the age of 36. A significant deterioration of vision has been observed. Presently, the best-corrected (+6.0 D) visual acuity is reduced to 0.05 and 0.063 in the right and left eye, respectively. The patient is aphakic now and shows massive keratopathy. No extraocular symptoms were observed.

The patient underwent ophthalmological examinations, including visual acuity testing, fundus photography, spectral optical coherent tomography (SOCT), fundus autofluorescence (FAF), and electroretinography (ERG).

2.2. Molecular analysis

Blood samples from the patient were obtained for genetic examination. Later, blood samples were also obtained from his healthy mother and son, the sister showing ADOA symptoms, and her three sons (Figure 1). Genomic DNA was extracted from peripheral blood leukocytes using standard protocols. Due to the clinical suspicion of Leber congenital amaurosis NGS (Next Generation Sequencing), a diagnostic panel for 20 LCA genes (Asper Biogene, Asper Biotech Ltd., Tartu, Estonia) was firstly performed on the patient. The names of the genes analyzed in the LCA panel are listed in Supplementary material 1a (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=75*). The analysis results have not revealed any potentially pathogenic variants in the analyzed genes, so the patient's DNA sample was subjected to panel NGS of 275 inherited retinal disease-associated genes (Genomed, Warsaw, Poland). The names of the genes analyzed in the retinal panel are listed in Supplementary material 1b (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=75*). The NGS analysis was performed using SeqCap EZ HyperCap protocol and molecular probes NimbleGen SeqCap EZ (Roche) on a NextSeq 500 Illumina sequencing system.

3. Results and Discussion

Here we report an unusual case of a Polish patient with ocular symptoms of atypical severe retinal dystrophy and night blindness. The patient underwent ophthalmological examinations, including visual field testing, fundus photography, spectral optical coherent tomography (SOCT), fundus autofluorescence (FAF), and electroretinography (ERG). Retinal changes: atrophic pigmented changes with large pigment deposits and chorioretinal atrophy were present in both eyes'
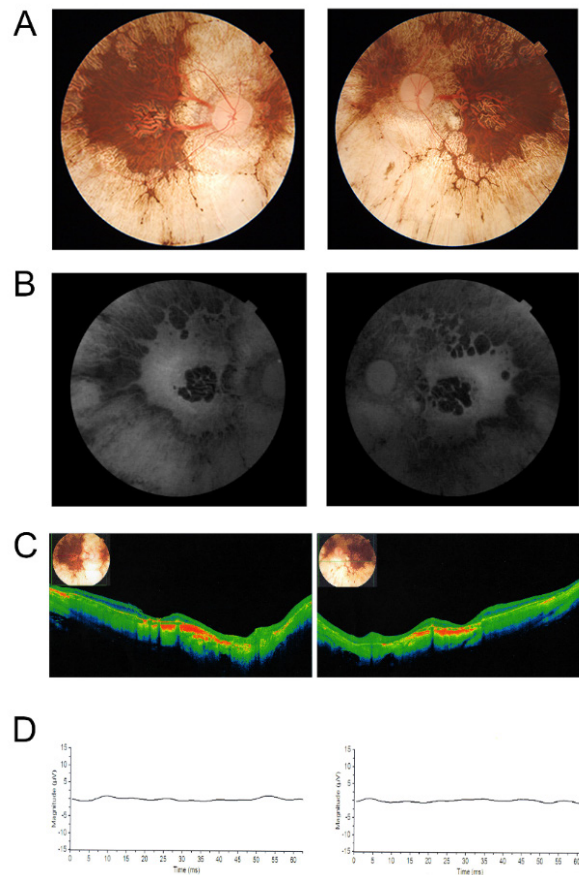

Figure 2. Retinal features of the patient. Right eye photographs are shown on the left and left eye - on the right. (A) Eye fundus photographs; **(B)** Eye fundus autofluorescence; **(C)** SOCT macular scan of the eye; **(D)** Photopic ERG.

retinas. SOCT revealed a totally disturbed structure of the retina with atrophic scarring changes and the epiretinal membrane. Pattern VEP showed no responses. Full-field ERG revealed totally extinguished photopic responses. Figure 2 shows the results of funduscopy (Fig.2A), fundus autofluorescence imaging (Fig.2B), SOCT (Fig.2C), and ERG (Fig.2D).

NGS on the retinal panel revealed presence of potentially pathogenic variants in two genes: a homozygous variant c.1058G>A (p.Gly353Asp) in the *OAT* gene (NM_000274.4) and a heterozygous variant c.1886C>G (p.Ser629Ter) in *OPA1* gene (NM_130837.3). Both variants are classified as pathogenic according to ACMG (American College of Medical Genetics and Genomics). Moreover, the *in silico* predictions of the (p.Gly353Asp) substitution potential pathogenicity with the use of SIFT (Sorting Intolerant from Tolerant, *https://sift.bii.a-star.edu.sg*) and Poly-Phen-2 (Polymorphism Phenotyping v.2, *http://genetics.bwh.harvard.edu/pph2*) indicated that the variant is probably damaging (the score 1.0 for PolyPhen-2 and 0.00 for SIFT). These results indicate a coincidence of two ultra-rare hereditary eye diseases: gyrate atrophy of the choroid and retina (GACR) and optic atrophy type 1 (Kjer-type optic atrophy). Segregation analysis for the

presence and independent inheritance of two identified altered alleles with Sanger sequencing of the appropriate *OAT* (exon 9), and *OPA1* (exon 20) gene fragments was performed. The primers used for amplification and sequencing as well as the Polymerase Chain Reaction (PCR) conditions are available upon request. The PCR products were bidirectionally sequenced using dye-terminator chemistry (v3.1BigDye® Terminator, Life Technologies). The sequencing products were separated on an ABI 3130xl capillary sequencer (Applied Biosystems). The segregation analysis revealed the presence of the heterozygous c.1886C>G *OPA1* variant in the patient's 40-year-old sister and her 8-year-old son, which confirmed the Kjer optic atrophy diagnosed in these individuals. The substitution was also identified in the patient's 10-year-old, asymptomatic son. The c.1058G>A variant in the *OAT* gene was tested in the patient's mother, the sister affected with Kjer optic atrophy, and the patient's son. The variant was identified in a heterozygous state in all these three patient's relatives. The segregation analysis results together with the pedigree of the family and chromatograms of the identified variants are shown in Figure 1.

The c.1058G>A (p.Gly353Asp) variant identified in the *OAT* gene was a previously reported rare variant (*18*) and it was identified as a heterozygous variant in GnomAD Browser (*19*) in 10 out of 113,298 analyzed alleles in healthy individuals. Based on *in silico* predictions of potential pathogenicity, the c.1058G>A variant is predicted to be damaging. Moreover, it causes a substitution of conserved glycine to aspartic acid at the amino acid position 353, localized within the C-terminal domain. The C-terminal domain and the N-terminal segment contribute to generating the gateway to the enzyme's active site (*2,9*). The OAT enzyme is expressed in most tissues, but the harmful consequences are confined mainly to the visual system. Our patient doesn't present any non-ocular symptoms or muscle fiber atrophy that can be observed in some gyrate atrophy patients.

The heterozygous variant c.1886C>G in the *OPA1* gene results in an introduction of the premature stop codon (p.Ser629Ter) in the protein's dynamin central region. It has been suggested that haploinsufficiency rather than the truncated protein's improper function may represent a major pathomechanism for dominant optic atrophy (*20*). The segregation analysis performed in the affected family revealed the p.Ser629Ter variant in the proband's sister and her son, showing symptoms of optic atrophy type 1, which was previously not confirmed by genetic diagnosis. The patient's mother, who has no ophthalmological problems, does not carry the mutation. Still, there is no information about the patient's father's vision, who died at 37 due to a heart attack. We cannot exclude the possibility that he also carried the *OPA1* variant, especially considering the high phenotypic variability of the ADOA and incomplete penetrance of

the gene.

The diagnosis made based on the NGS retinal panel is surprising because several different diagnoses have been previously suggested. Moreover, the clinical picture of the visual impairment observed in our patient did not allow us to diagnose any of these two identified diseases due to overlap of their symptoms. In the GACR, the pace of vision deterioration is not so fast as in our patient, while patients suffering from ADOA do not show retinal changes observed in the proband.

The appropriate molecular diagnosis in patients with genetic eye diseases is crucial, considering that the possibility of treatment with gene therapy has recently emerged for some of these disorders (*21*). In patients with GACR, pharmacological treatment may help to moderate the rate of chorioretinal atrophy progression. The treatment includes a low-protein, arginine-restricted diet, which may slow the progression of the disease (*22*) and administration of vitamin B6 (pyridoxine) - the precursor of the OAT cofactor that may help to reduce by 50% the level of serum ornithine in a subset of patients and slow down the chorioretinal atrophy (*2*). Proper genetic counseling also plays a crucial role, especially from the point of view of family planning.

4. Conclusion

To our knowledge, this is the first and the only description of the coincidence of gyrate atrophy and Kjer-type optic atrophy. The cooperation between ophthalmologists and geneticists is indispensable in making an accurate clinical diagnosis and planning treatment. The use of the NGS technique is beneficial, especially in unique, unclear cases. In most cases, the use of NGS panels enables a proper diagnosis, which is the basis of genetic counseling, and nowadays, in some cases, it gives a chance for an effective treatment. This report provides the unquestioned diagnostic value of the combination of retinal imaging and the NGS technique.

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