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Multimodal characterization of a novel mutation causing vitamin B6-responsive gyrate atrophy

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Abstract

Purpose: Gyrate atrophy (GA) is a rare chorioretinal degeneration that results in the deterioration of night and peripheral vision, eventually leading to blindness. The disorder is caused by mutations in the gene encoding ornithine amino transferase (OAT), causing increased levels of plasma ornithine. Treatment revolves around lowering plasma ornithine levels, with vitamin B6 supplementation being the preferred treatment. Nevertheless, most patients do not respond to this therapy. Here we report a rare case of vitamin B6-responsive GA caused by a novel mutation in OAT and characterize the presentation with multimodal imaging.

Methods: This is a single-patient case report with a clinical diagnosis based on history, multimodal retinal imaging, laboratory findings, and DNA sequencing analysis. We include a 3D structure prediction of the novel mutant protein.

Results: DNA sequencing analysis demonstrated that there is a homozygous, novel variant c.473A>C: p.Y158S in *OAT*. Upon undergoing two weeks of vitamin B6 supplementation, the patient exhibited a 28.5% reduction in plasma ornithine levels. In a follow-up visit two years later, plasma ornithine levels were reduced by 24.1% from the levels at initial presentation and disease progression was retarded based on clinical findings.

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AUTHOR CONTRIBUTIONS

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DECLARATION OF INTEREST STATEMENT

The authors declare no conflicts of interest.

Conclusion: One novel homozygous missense mutation in *OAT* was identified and considered to be pathogenic in a patient with GA. The response for the vitamin B6 supplementation was positive, which is rare in all the GA cases reported in the literature. Our data suggests that further studies regarding the relationship between genotype and responsiveness to vitamin B6 should be conducted.

Keywords

gyrate atrophy; vitamin B6; ornithine; OCT-A; structural modeling

Introduction

Gyrate atrophy (GA) is a rare disorder that leads to progressive degeneration of the retinal pigment epithelium (RPE), choroid, and photoreceptors. Patients usually present in the first decade of life with nyctalopia and high myopia, followed by progressive vision loss and visual field constriction in the later decades of life, and many also develop posterior subcapsular cataracts¹. Most patients have isolated ocular findings, but systemic manifestations such as thin and rare hairs, muscle weakness from atrophied type II fibers, mental retardation with diffuse brain atrophy, peripheral nervous system abnormalities, and hyperammonemia in newborns have been reported^{2–4}.

GA is a recessively inherited disorder caused by mutations in the gene encoding ornithine aminotransferase (*OAT*, OMIM #613349)⁵. A defective *OAT* enzyme leads to plasma ornithine concentration levels that are 10 to 20 times higher than those of normal subjects, which creates a problem as ornithine is toxic to the RPE⁶. Treatment methods aim to lower ornithine levels via a protein- or arginine-restricted diet, as arginine is a precursor to ornithine, or vitamin B6 supplementation, as pyridoxine is a co-factor of the *OAT* enzyme. Multiple studies have reported that an arginine-restricted or low-protein diet may slow the progression of chorioretinal lesions and visual loss^{7–11}. The response to vitamin B6 supplementation, however, varies among patients, with most not responding to this therapy^{12, 13}.

Many studies have characterized different *OAT* mutations in both human and animal models. Studies involving mouse models have shown that *OAT*^{−/−} mice exhibit hyperornithemia, chorioretinal degeneration, and death within 48 hours of birth, while *OAT*^{+/-} mice undergo delayed growth, defective hair growth, and premature death¹⁴. In humans, a total of 66 *OAT* mutations have thus far been reported in the Human Gene Mutation Database (HGMD, <http://www.hgmd.cf.ac.uk/>). More than 50 of these mutations have been described as pathogenic. The highest incidence of GA is in Finland, in which 1 in 50,000 individuals carry homozygous *OAT* mutations^{13, 15}.

In this study, we present a patient with a diagnosis of GA. DNA sequencing of the patient's *OAT* gene revealed a novel, homozygous mutation, c473A>C:p.Tyr158Ser, and multimodal imaging modalities were used to characterize the phenotype created by this novel mutation. Subsequent follow-up visits revealed that the patient demonstrated a rare case of retarded disease progression as a response to continuous vitamin B6 supplementation.

Case report

A 67-year-old woman with a history of hypothyroidism, dementia, and type II diabetes presented to the clinic for worsening vision due to retinal degeneration, as the patient carried a diagnosis of retinitis pigmentosa. The patient complained of progressively constricting visual fields in both eyes and nyctalopia. Ocular history is significant for bilateral cataract extraction two years earlier. At the time of presentation, the patient was taking levothyroxine for hypothyroidism and metformin and glipizide for diabetes. Her most recent hemoglobin A1c level was 7.6% six months before presentation.

Family history for the patient is significant for consanguinity as her parents are first cousins (Figure 1A). The patient grew up in Honduras and she is the only sibling of the marriage. The patient is married with two daughters that have no significant medical history. No family members, including her parents and daughters, have vision problems similar to the patient's or other vision problems that cannot be remedied with corrective lenses.

Visual acuity could be best corrected to 20/25 in the right eye and 20/50 in the left eye. On dilated fundus examination there was preservation of the RPE around the macula bilaterally with distinct borders. There was also preservation of RPE islands in the periphery, along with some intraretinal pigment migration. The preserved RPE islands had very distinct borders that coalesced into a "scalloped" pattern (Figure 1B).

Short-wavelength fundus autofluorescence (FAF) and spectral-domain optical coherence tomography (SD-OCT) were obtained (Spectralis HRA + OCT device; Heidelberg Engineering, Heidelberg, Germany). FAF imaging showed bilateral RPE atrophy that encroached from the periphery and surrounds the central macula. The boundary between the spared and atrophied RPE was sharply demarcated (Figure 1D). SD-OCT showed extensive choroidal sclerosis and degeneration in the areas of RPE atrophy, as seen by the increased transmittance of signal. Retinal degeneration is evident in areas of RPE atrophy with thinning of the outer nuclear layer (ONL) (Figure 1C). Full-field electroretinogram (ffERG) was performed using DTL recording electrodes and Ganzfeld stimulation according to standards outlined by the International Society for Clinical Electrophysiology of Vision (ISCEV). Scotopic rod-specific and maximal responses were extinguished. Photopic 30 Hz-flicker amplitudes were diminished to 5.6 μ V in the right eye and 3.7 μ V in the left eye. Peripheral whole blood lymphocytes were obtained and sent for DNA sequencing analysis of the *OAT* gene at the Casey Eye Institute of Oregon Health and Science University (Portland, Oregon). A sample of urine was obtained and sent for quantification of amino acids, which revealed an elevated level of ornithine, 1906 μ mol/g (N: 0–24). The patient's history, imaging, and laboratory findings were consistent with a diagnosis of gyrate atrophy.

The patient presented for follow-up and genetic counseling six months later with no new changes in vision. DNA sequencing analysis of the *OAT* gene was performed by PCR amplification and sequenced in two directions of all coding exons and exon/intron boundaries. The analysis revealed a homozygous, novel variant in *OAT*, c.473A>C: p.Y158S. Therapeutic options of vitamin B6 supplementation, arginine-restricted diet, and low-protein diet were discussed with the patient. To minimize the impairment on quality of

life, the decision was made to start on vitamin B6 supplementation 400 mg/day. Baseline plasma amino acid levels were obtained and the ornithine level was 586 $\mu\text{mol/L}$ (N: 20–135). After two weeks of treatment with vitamin B6, plasma ornithine levels were re-evaluated and decreased to 419 $\mu\text{mol/L}$. The patient was encouraged to continue treatment with vitamin B6 in order to avoid an arginine/protein-restricted diet and retard disease progression.

At a presentation two years later, the patient reported no changes in vision. Best corrected visual acuity was 20/40 and 20/60 in the right and left eye, respectively. Dilated fundus exam was unchanged from the previous encounters. FAF imaging showed minimal disease progression as suggested by the area of RPE atrophy as compared to the previous visits (Figure 1D). Optical coherence tomography angiography (OCT-A) with the Angioplex Cirrus HD-OCT 5000 (Carl Zeiss Meditec Inc., Dublin, USA) was used to image the retinal vasculature. Automated segmentation of full-thickness retina scans into superficial capillary plexus (SCP), deep capillary plexus (DCP), and choriocapillaries (CC) was performed and images with an 8×8 mm area centered on the macula were obtained. Given that OCT-A cannot visualize individual CC due to their small size and small intersinusoidal spacing, the CC appear homogenous with bright areas representing blood flow, whereas darker areas have less or are devoid of flow¹⁶. The 8×8 mm images showed spared RPE around the central macula with paramacular areas of RPE atrophy (Figure 1E). The CC appearance in the spared RPE is homogenous as expected, but the areas of RPE atrophy show the individual larger choroidal vessels from Sattler's and Haller's layers, which become visible as the overlying RPE and CC atrophy. The plasma ornithine level obtained at this visit was 445 $\mu\text{mol/L}$.

We performed structural modeling and used a prediction software to gain insight into the novel c.473A>C: p.Y158S *OAT* mutation. PolyPhen-2, a software used to predict the damaging effects of missense mutations, characterized this mutation as “probably damaging” with the highest score of 1.0¹⁷. Structural modeling was done with PyMOL¹⁸. Other *in silico* prediction software also suggest a damaging effect of this mutation on protein function. PROVEAN predicted the mutation to be “deleterious” (score of −8.52, cutoff of −2.5), while SIFT predicted the mutation to be “damaging” (score of 0.006, cutoff of 0.05)^{19, 20}.

Discussion

Based on the Ensembl genome browser, the *OAT* c.473A>C: p.Tyr158Ser mutation is novel. Studies have characterized the structure of the OAT enzyme as a dimer of two chains, and this dimer interacts with another OAT dimer to create a tetrameric quaternary structure²¹. At the dimer-dimer interface, an important residue is Arg217, as it is placed in an optimal position to hydrogen bond with the backbone carbonyl moiety of either Ser186* or Ser188* (* denotes a residue belonging to another dimer). Furthermore, the hydrocarbon portion of the Arg217 lies in a hydrophobic pocket, interacting with Tyr194*, Tyr166*, and Phe200*. Another important interaction at the interface involves the interaction of Glu216 with the amino group of Lys165*. Structural modeling shows that in the wild-type OAT enzyme, the Tyr158 residue lies in a hydrophobic pocket, with the aromatic moiety of the side chain

interacting with the residues Ala168, Ile163, Tyr166, the hydrocarbon moiety of the Lys165 side chain, and Gly196 on the other chain of the dimer (Figure 2A). The mutation Tyr158Ser replaces a tyrosine with serine, a smaller amino acid whose side chain does not contain the aromatic moiety in tyrosine (Figure 2B). The lack of the aromatic moiety and the increased distance between the residues would weaken the interactions of serine with the aforementioned residues in the hydrophobic pocket, altering the stabilization of the dimer and impacting the activity of the OAT enzyme. We predict that this is the structural basis for the pathogenicity of this mutation.

Arginine-restricted diet, low protein diet, and vitamin B6 supplementation have all been proposed as treatments that can slow the progression of GA. Of the three, vitamin B6 supplementation is the most convenient therapy to administer as it minimally impairs the patient's quality of life, as opposed to a strict arginine-restricted or low protein diet. Most studies, however, show that patients with GA do not respond to vitamin B6 supplementation. In a clinical trial of vitamin B6 supplementation with 7 patients, only 3 responded to oral vitamin B6 supplements with over 50% reduction in plasma ornithine¹⁴. Of the approximately 70 cases of GA reported in Finland, zero patients have responded to vitamin B6²². From additional studies worldwide, a total of 9 patients have been reported to respond to vitamin B6^{13, 23}. Thus, the patient presented in this study is a rare case that responded to vitamin B6 supplementation. Within two weeks of starting administration of 400 mg vitamin B6 per day, plasma ornithine levels decreased by 28.5% from 586 $\mu\text{mol/L}$ to 419 $\mu\text{mol/L}$. On her presentation to clinic two years later, plasma ornithine levels were 445 $\mu\text{mol/L}$, a decrease of 24.1% from the initial presentation. A study similar to ours described another case of GA that responded to 300 mg vitamin B6 supplementation per day for six months, reducing ornithine levels by more than 50% from 629 $\mu\text{mol/L}$ to 293 $\mu\text{mol/L}$ ¹³. In another study presenting three cases from Japan, two failed to respond to 300 mg vitamin B6 per day, while in the remaining case there was a maximum of 60% reduction in ornithine levels after administration of 300 mg vitamin B6 per day for 1 week followed by 600 mg per day of vitamin B6¹². A different study in Japan reported that 300 mg vitamin B6 supplementation per day for 6 months reduced ornithine levels by 20–30%²⁴. As seen in this case presented, vitamin B6 supplementation maintained the levels of plasma ornithine at a constant range while simultaneously retarding the progression of visual impairment and RPE atrophy. Future studies should be geared towards investigating a relationship between vitamin B6 responsiveness and the different mutations in the *OAT* gene, though the rarity of the disease presents a challenge to larger studies. Given that vitamin B6 has been shown to retard the progression of visual impairment and blindness in some GA patients, it will be important to characterize the molecular basis of the different responses observed in patients with GA towards vitamin B6.

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Figure 1.

Clinical characterization of the phenotype caused by the novel *OAT* mutation. (A) The patient presented is the only family member with a congenital ocular disease and family history is significant for consanguinity, as the patient's parents are first cousins. (B) Dilated fundus examination shows preservation of RPE around the macula with distinct borders. On the periphery, there is also preservation of RPE islands along with intraretinal pigment migration. The preserved RPE islands also have very distinct borders that coalesce into a "scallop" pattern. (C) SD-OCT shows extensive choroidal sclerosis and degeneration in

the areas of RPE loss, as seen by the increased transmittance of signal. The vertical green line marks the border of the spared macular RPE and the atrophied peripapillary RPE. A higher degree of signal transmittance can be detected on the area of peripapillary atrophy as compared to the area of spared macular RPE, as indicated by the red arrow. **(D)** FAF shows bilateral RPE atrophy that encroached from the periphery and surrounds the central macula, with a clear demarcation between healthy and atrophied RPE. The top row corresponds to FAF done at presentation, while the bottom row corresponds to FAF done at a follow-up visit two years later. FAF imaging showed minimal disease progression as suggested by the area of RPE atrophy as compared between visits. **(E)** OCT-A imaging shows a homogeneous appearance of the CC in the RPE-spared area around the macula, while the individual larger choroidal vessels can be seen in the areas of RPE atrophy.

Figure 2.

Structural modeling with PyMOL of the Y158S *OAT* mutation. (A) In the wild-type *OAT* enzyme, the Tyr158 residue lies in a hydrophobic pocket. The aromatic moiety of Tyr158 is at an appropriate distance to interact with the nonpolar moieties of Ala168, Ile163, Tyr166, Lys165, and Gly196 on the other chain of the dimer. (B) In the mutated Tyr158Ser *OAT* enzyme, tyrosine is replaced by serine, which does not contain an aromatic moiety and is smaller in dimension. The Tyr158Ser mutation would thus weaken serine's interactions with the aforementioned residues. (C) Surface representation of the wild-type *OAT* protein. (D) Surface representation of the Tyr158Ser *OAT* enzyme. The surface representations show a decrease in size between tyrosine in the wild-type protein compared to serine in the defective *OAT* enzyme, as seen by the smaller area of red.