Title: A novel case series of *NMNAT1*-associated Early Onset Retinal Dystrophy – extending the phenotypic spectrum

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Short Title: *NMNAT1*-associated retinopathy – a novel phenotype

Summary Statement: *NMNAT1*-associated retinopathy has only been described with profound loss of vision from birth associated with characteristic macular atrophy and intraretinal pigmentation. Here we present a novel case series of 2 siblings with an atypical mild, later onset retinal phenotype.

Abstract

Purpose: To report two siblings with *NMNAT1*- associated retinopathy presenting with a later onset and milder phenotype than previously described. **Methods:** Retrospective case series of two siblings. The authors describe two cases of early onset retinal dystrophy caused by disease-causing *NMNAT1* variants. Visual acuity, clinical examination, and retinal imaging including color fundus photography, spectral domain optical coherence tomography, and fundus autofluorescence were performed. Both cases underwent full-field and pattern electroretinography (ERG; PERG) incorporating the International standards. **Results:** Two siblings were found to harbor the variants c.53A>G, p.(Asn18Ser) and c.769G>A, p.(Glu257Lys) in *NMNAT1* following retinal dystrophy panel gene testing. Both had good visual acuity until the ages of 6 and 11 years old respectively, with subsequent gradual worsening into their twenties. At the ages of 10 and 16 years respectively, ERGs indicated generalised rod and cone system dysfunction of moderate severity, with PERG evidence of severe macular involvement. Repeat testing at the ages of 26 and 33 years, revealed only mild worsening of rod photoreceptor function in both. **Discussion:** *NMNAT1*-associated retinopathy has previously only been described as a typical form of Leber Congenital Amaurosis, with poor visual acuity from birth associated with nystagmus, characteristic macular atrophy, and intraretinal pigmentation from birth. Here we present two siblings with a novel, later onset, and far milder phenotype. We suggest this may be due to the two missense *NMNAT1* variants resulting in milder

reduction of NMNAT1 enzymatic activity. These cases extend the phenotypic spectrum associated with *NMNAT1* and further highlight the clinical heterogeneity associated with inherited retinal diseases.

Introduction

Leber Congenital Amaurosis (LCA) was initially described in 1869 and is currently used to describe a group of severe recessively inherited early infantile onset rod-cone dystrophies.[1] Phenotypic variability in this condition has led to the distinction between LCA, as it was first described, and a milder form of the same disease, Early Onset Severe Retinal Dystrophy (EOSRD) or Severe Early Childhood Onset Retinal Dystrophy (SECORD). LCA presents from birth or within the first months of life, and is associated with very poor visual function, nystagmus, poor pupil responses, and, in the majority, with undetectable full field electroretinogram (ERG). In contrast, EOSRD presents as a severe retinal dystrophy, *before* the age of 5 years with better residual visual function and small ERG signals.[1] From 5 years of age, childhood-onset generalised rod-cone dystrophies have been given several names including early-onset rod-cone dystrophy, early-onset retinitis pigmentosa, and early-onset retinal dystrophy.

To date, mutations in 25 genes have been identified as causing LCA/EOSRD.[1] While overlap is noted between the molecular causes of LCA and EOSRD, certain genotypes are almost exclusively associated with an LCA phenotype, including *NMNAT1* associated-LCA. *NMNAT1* is known to encode Nicotinamide mononucleotide adenyltransferase 1, a 279 residue protein which has a role in coenzyme NAD biosynthesis and has been shown to be neuroprotective.[2] *NMNAT1*-associated LCA results in a typical clinically recognizable LCA phenotype, with profound loss of vision and nystagmus from birth, with prominent central macular atrophy and pigment clumping (including nummular pigmentation) throughout the retina.[3] The *NMNAT1*-LCA phenotype is so characteristic that directed molecular screening can be undertaken. Herein, we present two siblings with an unexpected later onset and milder phenotype caused by *NMNAT1* disease-causing sequence variants.

Results

Patient 1

The younger sister presented aged six years old, with a reported history of a gradual worsening of vision over the preceding three years. On examination, her best-corrected visual acuity (BCVA) was 6/24 in the right eye and 6/12 in the left, with an abnormal macular reflex, and subtle, peripheral pigmentary changes observed on ophthalmoscopy. The discs were noted to appear healthy, with no lens opacity or particulate vitreous debris. Aged 10 years, ERG and pattern ERG (PERG) performed to incorporate the International Society for Clinical Electrophysiology of Vision (ISCEV) standards,[4, 5] indicated generalised rod and cone photoreceptor dysfunction, with additional dysfunction post-phototransduction or at the level of the inner retina. Undetectable pattern ERGs were in keeping with severe macular involvement bilaterally. A slow progression was documented, with her BCVA aged 24 years being 4/60 in the right eye and 6/60 in the left. Retinal examination and retinal imaging revealed macular atrophy, moderate retinal vascular attenuation, and mid-peripheral, intraretinal pigmentation with retinal pigment epithelial mottling (Figure 1). Her refraction was +2.75DS in the right eye and +2.75/-0.50 x 75 in the left eye. Repeat electrophysiological testing (Figure 2) at the age of 26 years showed stable light adapted (LA 3 and LA 30Hz) ERGs, and an approximate 35% reduction in the dark adapted strong flash (DA 10) ERG a-wave compared with baseline, indicating mild worsening of rod photoreceptor function. Additional long duration ERGs were consistent with greater involvement of the cone On- than Off- pathway responses (Figure 2).

Two missense variants, c.53A>G, p.(Asn18Ser) and c.769G>A, p.(Glu257Lys), were identified in the *NMNAT1* gene following targeted Next Generation Sequencing of the coding regions of 176 retina-associated genes from genomic DNA extracted from peripheral blood leukocytes (performed at The Manchester Centre for Genomic Medicine, NHS accredited diagnostic laboratory, Manchester, UK). The two variants segregated appropriately by bidirectional Sanger sequencing of parental DNA. Of note, variants in the following genes were identified, all of which were assessed and deemed not to be disease-

causing: *GNAT2, USH2A, TULP1, CSPP1, USH1C, TSPAN12, ITF140, CEP164, FSCN2, MKKS, GPR179*. The presence of such variants that do not cause disease is common in inherited retinal disease and are not believed to interact with *NMNAT1*.

NMNAT1 c.53A>G, p.(Asn18Ser) has been previously reported in both a compound heterozygous state, with c.472G>C, p.(Asp158His), and homozygous state causing LCA.[6, 7] Furthermore, *NMNAT1* c.769G>A, p.(Glu257Lys) has also been described in the literature in compound heterozygous states, with variants c.817A>G, p.(Asn273Asp), c.619C>T p.(Arg207Trp), c.415G>A, p.(Val151Phe) and c.199G>T, p.(Val67Phe), all causing LCA.[8] Neither has been described together.

Patient 2

The older brother had a BCVA of 6/9 in the right eye and 6/6 in the left at 11 years old, with a refraction of +1.75/+2.00 x 155 in the right eye and +0.50/+1.75 x 25 in the left eye. He noted slow deterioration over time, such that aged 17 his BCVA was 6/24 in either eye, with bilateral macular atrophy, and intraretinal pigmentation. Of note, the patient's optic discs were normal, with no lens opacity and no particulate, vitreous debris. He was last seen aged 33, with further loss of VA, to 6/60 in the right eye and Count Fingers in the left. Patient 2 undertook electrophysiology assessments aged 16 and 33 years (Figure 3). The ERG abnormalities were slightly more severe than in his younger sister (Figure 3), but were also consistent with generalised rod and cone photoreceptor dysfunction, with additional dysfunction post-phototransduction. Pattern ERGs were undetectable, in keeping with severe macular involvement. Repeat ERGs after 16.5 years, revealed reasonably stable LA ERGs, and an approximately 30% reduction in the DA 10 strong flash ERG a-wave, suggesting further mild loss of rod photoreceptor function. The presence of the above *NMNAT1* variants were confirmed in the older brother with bidirectional Sanger sequencing.

Furthermore, detailed clinical examination and OCT imaging revealed no evidence of retinal disease in either the mother, aged 59 years old, or father, aged 58 years old.

Discussion

NMNAT1-associated retinopathy has previously only been reported to cause a characteristic LCA phenotype with onset at birth. Here we describe 2 siblings harboring *NMNAT1* disease-causing variants, that present with a childhood-onset rod-cone dystrophy phenotype, who have retained moderate and good visual acuities until the ages of 6 years and 11 years old, respectively. Furthermore, detailed electrophysiological assessment revealed milder rod- and cone- mediated ERG abnormalities compared with typical *NMNAT1* cases, with substantial function being retained into the third and fourth decades of life. There was evidence of only mild worsening of rod function over 16.5 years in both cases. We propose that the presence of two missense variants, rather than a missense variant associated with a more severe variant, produces a milder reduction in coenzyme NAD biosynthetic function and a thereby milder phenotype, with a later onset of visual loss and far slower rate of progression. Such variant dependent enzymatic activity, has also previously been described *in vitro,* with NMNAT1 enzyme rates shown to vary from 18.9% to 99.5% activity, depending on the variant (different to the ones described herein).[9] These cases extend the phenotypic spectrum associated with *NMNAT1*, further highlight the bountiful clinical heterogeneity associated with inherited retinal diseases, and demonstrate the utility of next generation sequencing of large panels of retina-associated genes.

Clinical trials in LCA have shown safety and varying efficacy of gene replacement therapy and pharmacological intervention to overcome biochemical blockade in *RPE65*-associated LCA and *LRAT*-associated LCA.[10-12] Due to its small size, *NMNAT1* is an attractive target for gene therapy and furthermore, its role in neuroprotection suggests pharmacological intervention may also be possible. The potential for such clinical intervention has led to the identification of a mouse model, which demonstrates rapidly progressive photoreceptor degeneration and subsequent chorioretinal disease, similar to that seen in humans, and is therefore appropriate for preclinical investigations of potential therapies.[13] As such the identification of two siblings with a milder phenotype of *NMNAT1*-associated retinopathy is valuable; both

to inform clinicians of this milder phenotype and also such patients with a milder phenotype may provide a wider window of opportunity for future potential therapeutic intervention.

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Figure Legend

Figure 1: (A and B) Montaged fundus photos of the right (A) and left (B) eyes of patient 1, aged 24 showing macular atrophy, generalised retinal degeneration, and intraretinal mid-peripheral pigmentation. (C and D) Fundus autofluorescence images of the right (C) and left (D) eyes of patient 1, aged 25 showing marked hyperfluorescence centrally, with a surrounding ring of hypofluorescence, and subsequent hyperfluorescence. (E and F) Spectral-domain optical coherence tomography of patient 1, aged 26, showing marked loss of central outer retina. (G and H) Fundus photos from a typical *NMNAT1*-LCA subject demonstrating marked macular atrophy, widespread chorioretinal atrophy, and dense, intraretinal pigmentation.

Figure 2: Electrophysiology for patient 1 (aged 26 years). The dark-adapted (DA) ERGs are shown to flash strengths of 0.01 and 10.0 cd.s.m^{-2}; light-adapted (LA) ERGs to flash strengths of 3.0 cd.s.m^{-2} (30Hz and 2Hz). The DA 0.01 ERG is markedly subnormal, and the DA 10 ERG has a subnormal b:a ratio, with additional significant a-wave reduction. The LA 30Hz and LA 3 ERGs are markedly delayed and subnormal. Pattern ERG (PERG) is recorded to an alternating checkerboard and is undetectable bilaterally. The On-Off ERG (light duration 200ms) shows a-wave and b-wave reduction, with relative preservation of the Off response. Right eye (RE), left eye (LE) and representative normal (N) traces are shown for comparison. Note the 10ms (DA0.01 ERG) or 20ms pre-stimulus delay in single flash full-field ERG recordings and different scaling of normal traces. Patient traces are superimposed to demonstrate reproducibility. Broken lines replace blink artefacts for clarity.

Figure 3: Electrophysiology for patient 2 (aged 33 years). The DA and LA ERGs show similar but slightly more severe abnormalities compared with patient 1 (see Figure 2 caption and text). PERG P50 component is undetectable. Right eye (RE), left eye (LE) and representative normal (N) traces are shown for

comparison. Note the 10ms (DA0.01 ERG) or 20ms pre-stimulus delay in single flash full-field ERG

recordings and different scaling of normal traces. Patient traces are superimposed to demonstrate

reproducibility. Broken lines replace blink artefacts for clarity.