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A Novel NYX Mutation Associated with X-Linked Congenital Stationary Night Blindness in a New Zealand Family

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Abstract

Background: Complete Congenital Stationary Night Blindness (CSNB) type 1A is an X-linked condition associated with reduced scotopic vision, myopia, nystagmus and mutations in the *NYX* (nyctalopin) gene. This paper reports a novel mutation identified in this gene associated with X-linked CSNB in a New Zealand Caucasian family.

Methods: A 16 year old male, presenting with night blindness, underwent detailed phenotypic assessment including pedigree construction and electrophysiology testing. Genetic analysis was performed in the Division of Medical Molecular Genetics, University of Zurich, Switzerland. Other family members also underwent clinical examination and genetic testing.

Results: Electrodiagnostic testing has confirmed the diagnosis of Type 1 (complete) CSNB in the proband and his maternal grandfather by identifying a 'negative' rod-mediated waveform and near normal cone responses.

NYX gene testing revealed a novel missense sequence alteration c.425T>G (p.Leu142Arg) in the proband. This has not been detected in control European and New Zealand Caucasian populations and segregates with the disease in this family.

Conclusion: Missense changes account for the majority of the mutations reported in the *NYX* gene so far. A novel missense sequence variant causing complete CSNB has been identified in a New Zealand family. This expands the known mutation spectrum of the *NYX* gene.

Keywords: Congenital stationary night blindness; *NYX*; Mutation

Abbreviations: CSNB: Congenital Stationary Night Blindness; LRR: Leucine Rich Repeats; ISCEV: International Society for Clinical Electrophysiology of Vision

Introduction

Complete congenital stationary night blindness (CSNB) Type 1A (MIM #310500) is an inherited retinal dystrophy characterized by reduced scotopic vision, nystagmus and myopia. It is caused by mutations in the *NYX* (Xp11.4) gene with X-linked inheritance.[1,2] It is non-progressive and characterized by a 'negative' waveform for rod mediated responses with a markedly reduced b-wave and a normal a-wave. Cone responses are normal or only mildly reduced in this type of CSNB.[3]

The nyctalopin protein encoded by the *NYX* gene is a member of the small leucine-rich repeats (LRR) superfamily that plays a role in the neurotransmission between the photoreceptors and the bipolar cells. [1,2]

This paper reports a novel missense mutation identified in a Caucasian New Zealand family with complete X-linked CSNB.

Materials and Methods

Phenotypic assessment

A 16 year old male proband underwent complete ophthalmological examination including detailed history and pedigree construction (Figure 1, IV: 2), visual acuity measurement, slit-lamp and dilated fundus examination, refraction and electro diagnostic testing.

His 70 year old affected maternal grandfather (II: 1), obligate carrier mother (III: 1), aunt (III: 2) and unaffected sister (IV: 1) also underwent clinical examination. In addition, members II: 1 and IV: 2 underwent electrodiagnostic assessment which was recorded according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards using gold foil electrodes and full field Ganzfeld stimulus with Roland-Consult equipment.

This study conformed to the tenets of the Declaration of Helsinki and informed consent was obtained from all participating family members prior to participation.

Mutational analysis

Genetic testing of the proband was undertaken at the Division of Medical Molecular Genetics and Gene Diagnostics, University of Zurich, Switzerland. DNA extracted from peripheral blood was analysed by PCR amplification of the coding region (exons 2 and 3), flanking splice sites and parts of the 5'- and 3'-UTR of the *NYX* gene and the amplicons were sequenced in forward and reverse directions. The data was compared to the *NYX* reference sequence from the National Centre for Biotechnology Information (NCBI) database.

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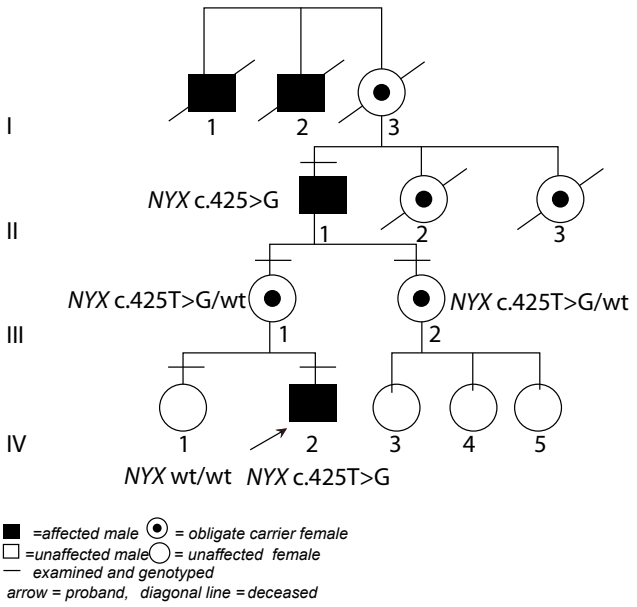

Figure 1: Pedigree of the New Zealand Caucasian family with complete X-linked Congenital Stationary Night Blindness. *NYX* genotype beside those tested, wt = wild type.

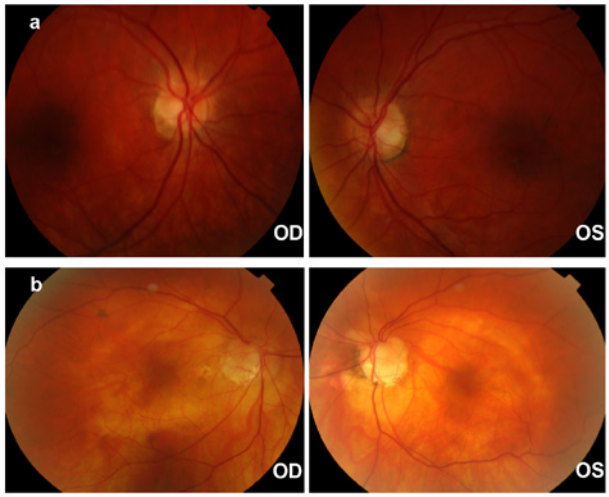

Figure 2: a) Fundus photographs of proband reveal D-shaped discs and normal appearance of maculae. **b)** Fundus photographs of II:1 show D-shaped discs, myopic degeneration and posterior staphyloma in the right eye. Neither individual has pigmentary retinopathy.

PCR and sequence analyses of the mutation containing part of exon 3 were also performed on the DNA extracted from peripheral blood of the remaining family members, to ascertain segregation of the sequence variant identified in the proband.

291 alleles from a Swiss control cohort (Caucasian, no eye disease) and 100 alleles from a clinically unaffected New Zealand Caucasian population were screened for the c.425T>G variant by sequencing.

Results

The proband had impaired night vision since early childhood and a visual acuity of 6/24 OD and 6/36 OS with a myopic refractive error of – 3.00 Dsph / -2.00 Dcyl x 90 OD and -3.00 Dsph / -2.00 Dcyl x 80 OS. Anterior segments and pupil reactions were normal; fundi revealed D-shaped discs, normal appearance of maculae and no evidence of pigmentary retinopathy (Figure 2A), with presence of horizontal jerk nystagmus. Electrophysiology tests identified a 'negative' response on standard flash combined rod-cone ERG with moderately reduced a-wave amplitude (167μV; 60% of normal age-matched median value) and a much reduced b-wave (97μV; 17% of normal age matched median value). Cone-mediated a and b waves revealed normal responses (b wave amplitude 180 V; normal range 140-235μV). Flicker 30 Hz was also within normal limits (Figure 3). All age-matched normal data is established at the Electrodiagnostic Clinic at Greenlane Clinical Centre, Auckland.

II: 1 also had impaired vision and myopia since childhood with a visual acuity of 6/30 unaided OD and 6/12 OS corrected with a - 5.75 Dsph RGP lens. Refraction for OD was not performed, as that eye is amblyopic. He underwent strabismus surgery as a child. Anterior segments and pupil reactions were normal and fundi displayed D-shaped optic discs and myopic degeneration with a posterior staphyloma in the right eye (Figure 2B). There was no history of nystagmus in childhood and it was not identified on examination either. Electrodiagnostic tests revealed unrecordable scotopic b-wave and a reduced a-wave consistent with a 'negative' waveform. A moderately reduced photopic response (a wave amplitude 39μV, age matched normal range 20-40μV; b wave amplitude 79μV, normal range 100-185μV) was noted but he had myopia and posterior staphyloma (Figure 3).

III: 1 had a mildly amblyopic right eye with a visual acuity of 6/9 OD and 6/6 OS. She had an otherwise normal ocular examination. IV: 1 had a visual acuity of 6/6 OU with normal ocular examination. She

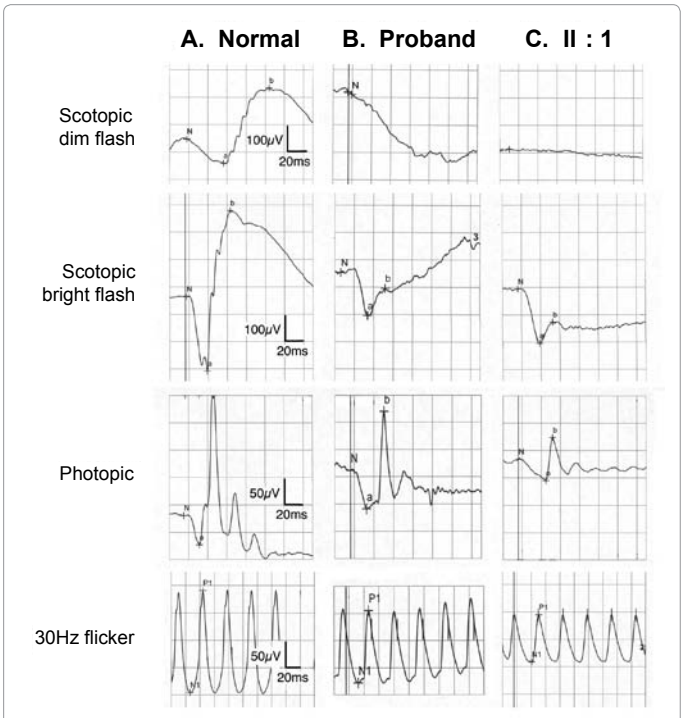

Figure 3: Electrophysiology tests of the proband and II:1 in comparison with a normal control. Both proband and II:1 display a 'negative' waveform on standard ISCEV flash testing with near normal a-waves and reduced b-waves. Photopic ERG and 30 Hz flicker are near normal in the proband. II:1 has a moderately reduced photopic response.

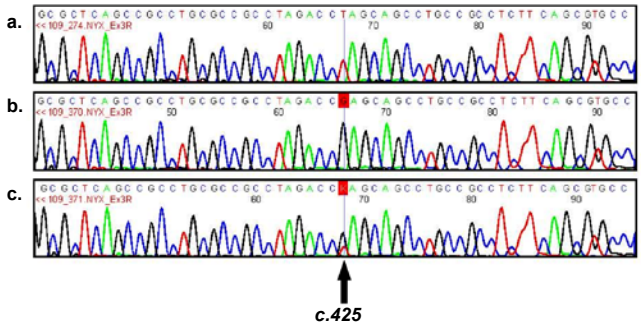

Figure 4: Electropherograms of NYX exon3 demonstrating. **a)** Unaffected unrelated control, wildtype G/G. **b)** c.425T>G, p.Leu142Arg, hemizygous affected male proband **(**IV:2). **c)** c.425T>G, p.Leu142Arg, heterozygous obligate female carrier (III:1).

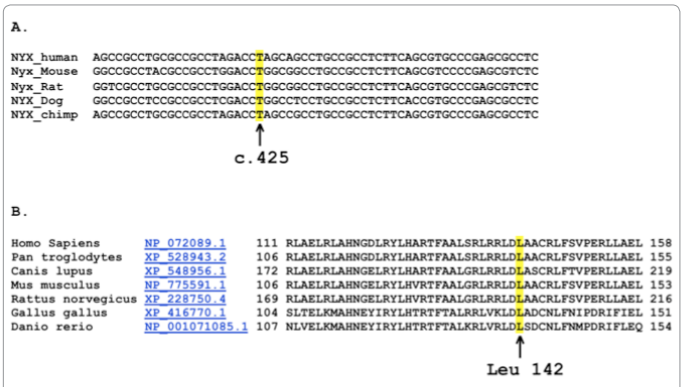

Figure 5: Multiple sequence alignment of **a)** *NYX* gene confirms that the nucleotide T at position c.425 is highly conserved. **b)** The protein sequence coded by the NYX gene also shows conservation of the Leu aminoacid through different species.

had a well-defined waveform on pattern ERG and normal rod and cone mediated responses on flash ERG.

Genetic analysis of the *NYX* gene in the proband has identified a novel sequence variant c.425T>G causing the amino acid change p.Leu142Arg in exon 3, and two SNPs in the 5' untranslated region (5'-UTR, rs3013121 and rs3013122). The exon 3 missense sequence variant has also been identified in family members II: 1, III: 1 and III: 2 but is absent in IV: 1 (Figure 4). It has not been detected in a normal control New Zealand and European Caucasian population. Multiple sequence alignment of the *NYX* gene confirms that the nucleotide T at position c.425 is highly conserved through mammalian species showing homology of sequences (Figure 5A). The protein sequence thus coded also shows conservation of the Leucine aminoacid through different species (Figure 5B).

Discussion

CSNB is a non-progressive retinal dystrophy that demonstrates genetic heterogeneity with autosomal dominant, autosomal recessive and X-linked patterns of inheritance.[1,2,4-10] The autosomal dominant forms are caused by mutations in the *RHO* (CSNBAD1, MIM #610445), *PDE6B* (CSNBAD2, #163500) and *GNAT1* (CSNBAD3, #610444) genes.[5-7] The X-linked and autosomal recessive forms are further divided into complete and incomplete sub-types on the basis of electroretinogram findings of markedly reduced or absent rod b-waves but retained cone function in the complete type and residual rod function with reduced cone amplitudes in the incomplete form.[3]

The autosomal recessive complete forms are associated with mutations in *GRM6* (CSNB1B, MIM #257270) and *TRPM1* (CSNB1C, MIM #613216) and incomplete forms with *CABP4* (CSNB2B, MIM #610427) genes. [9,10,11] *CACNA1F* is implicated in the incomplete form of X-linked CSNB (CSNB2A, MIM #300071) whereas *NYX* mutations cause complete X-linked CSNB.[1,2,4] Mutations in *CACNA2D4* have been found in a family with recessive retinal cone dystrophy RCD4. This however caused slowly progressive reduction in visual acuity with near normal fundal appearances, although the electroretinograms had suggested incomplete CSNB. [12]

There are 42 mutations reported in the *NYX* gene so far (The Human Gene Mutation Database - http://www.hgmd.cf.ac.uk). Of these, the majority are missense changes (59%), with small insertions or deletions accounting for 19%, gross insertions or deletions 12%, complex rearrangements and splice site mutations for 2% of changes each.

The missense sequence variant identified in this family occurs within the fourth leucine rich repeat region (LRR) of the gene and appears to be highly conserved through the species. As it segregates with the disease in the family, has not been identified in a control population and homology of sequences identifies conservation of this residue throughout mammalian species, it is most likely to be a pathogenic mutation rather than a benign polymorphism. The NYX protein encodes an extracellular membrane anchored protein with 13 LRRs, and previously described missense mutations occur in many of these LRRs in highly conserved amino acids, as observed with this mutation. [1,2] Polyphen protein prediction (http://genetics.bwh.harvard.edu/pph/) suggests this change is predicted to be possibly damaging, on the basis of alignment, with a PSIC score of 1.885. Another pathogenic missense mutation has been previously described in the adjoining codon p.Ala143Pro (c.427G>C). [1]

As many of the previously identified *NYX* mutations are private, it is expected that several novel mutations will still be identified. As sequence analysis of genes is expensive, CSNB genotyping microarray with arrayed primer extension technology (APEX) has been developed for rapid identification of known mutations.[13] Asper Biotech in Estonia (www.asperbio.com) offers microarray testing for CSNB, screening 159 mutations in 11 genes. The authors would recommend that the novel mutation recognised in this family be added to this microarray. However, direct sequencing will remain important for *NYX* testing when the microarray fails to identify any pathologic sequence change.

Some phenotypic variability in the degree of myopia and the presence of nystagmus has been noted in the affected members of this family. Although the difference in myopia is apparently not dramatic between proband and his affected grandfather, this maybe an underestimation, as the severity of myopia in the amblyopic eye of the grandfather is unknown. This phenotypic heterogeneity has also been previously described in another family with a different *NYX* mutation where nystagmus or strabismus were not observed but the degree of myopia differed greatly between affected individuals, ranging from -4.50D to -18.0D and was not associated with age at examination. [14]

Genetic analysis in this family has enabled the sister of the proband to be reassured that she is not a carrier of the disorder. As she is in the reproductive age-group, this has positive implications on her future family.

In conclusion, identification of this novel mutation in the *NYX* gene expands the known mutation spectrum of this gene. Specifically in this pedigree, molecular analysis has permitted informed family planning decisions and genetic counseling. This family further illustrates the phenotypic heterogeneity associated with *NYX* mutations.

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