A common *NYX* mutation in Flemish patients with X linked CSNB

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A common *NYX* mutation in Flemish patients with X linked CSNB

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ABSTRACT

Aims: The Schubert–Bornschein type of complete congenital stationary night blindness (CSNB) is a genetically heterogeneous retinal disorder. It is characterised by a non-progressive disease course, often associated with high myopia and nystagmus. So far, mutations in two genes, *NYX* (nyctalopin) and *GRM6* (metabotropic glutamate receptor 6) have been associated with this form of CSNB. The purpose of this study was to identify the genetic defect in affected male patients from Flemish families with complete CSNB.

Methods: Probands with CSNB from three large Flemish families underwent ophthalmological examination. DNA was extracted from peripheral blood, and the coding region of *NYX* along with parts of the 5′UTR and 3′UTR and intronic regions covering the splice sites were PCR amplified and sequenced.

Results: In the affected individuals of three Flemish families with the complete form of CSNB a novel *NYX* mutation, c.855delG was identified. This deletion is predicted to lead to a frameshift mutation, p.Asp286ThrfsX62 causing a premature stop codon.

Conclusion: Previously, both single families with different mutations in *NYX* as well as different families with an identical mutation, suggestive of a founder mutation, have been described. The c.855delG deletion in *NYX* seems to be a common mutation associated with CSNB in the Flemish population from Belgium. Thus, we suggest performing diagnostic testing for CSNB in the Flemish population initially directed towards the identification of this mutation. Subsequent screening for other mutations in *NYX* or *GRM6* could be performed as a second step.

The Schubert–Bornschein type of congenital stationary night blindness (CSNB)[1] is characterised by a distinctly shaped dark-adapted combined rod–cone electroretinogram (ERG) to a single bright white flash: the amplitude of the a-wave is normal and larger than that of the b-wave, leading to its electronegative aspect. Under psychophysical dark adaptation, rod adaptation is dramatically reduced or completely absent, and cone adaptation may show variable threshold elevations in most patients.[2–4] Miyake and coworkers further divided this type of CSNB into two subtypes, incomplete and complete.[5] The incomplete form of CSNB is characterised by a reduced rod b-wave as well as a substantially reduced cone response, while the complete type of CSNB is associated with either absent or very low amplitudes of the rod b-wave response and normal cone amplitudes. Both types can be inherited as an X linked (XL-CSNB) or autosomal recessive (ar) trait. Mutations in *CACNA1F* (Xp11.23) and *CABP4* (11q13.1) lead to the incomplete form, while mutations in *NYX* (Xp11.4) and *GRM6* (5q35) are associated with the complete form of CSNB.[6–13]

To date, the majority of mutations have been identified in two X linked genes, *CACNA1F* and *NYX*, which are assumed to play a role downstream of the phototransduction cascade in transmitting the signal from the photoreceptors to the adjacent bipolar cells.[14]

NYX comprises three exons and codes for a protein, nyctalopin, which belongs to the small leucine-rich proteoglycan family. Although immunofluorescence and western blot analyses have shown that the protein is attached to the cell surface via a GPI anchor,[15 16] and plays a role in transmitting the biochemical signal from the photoreceptors to the adjacent bipolar cells, the exact role of this protein still has to be discovered.[14] Up to now, 37 different mutations in *NYX* have been associated with the complete type of CSNB.[14] From this total of 37 mutations, 24 single families have been associated with a different mutation. The remaining 13 mutations represent different recurring mutations, most notably a 24 bp deletion, p.Arg29_Ala36del, identified in seven families originating from the USA with a common founder.[9]

The objective of this study was to identify the disease-causing mutation in three apparently unrelated large Flemish families with complete CSNB.

MATERIALS AND METHODS

Patients

Informed consent was obtained from the participants, in accordance with guidelines established by the Declaration of Helsinki.

Clinical assessments of patients

All affected members of the three families underwent an extensive ophthalmological evaluation, including Snellen visual acuity testing, slit-lamp biomicroscopy, funduscopy, Goldmann visual fields, colour vision examination, International Society for Clinical Electrophysiology of Vision standard electroretinography and on–off responses (total flash duration of 200 ms), and infrared, autofluorescence and red-free imaging. Blood was taken from all family members by venesection and genomic DNA extracted according to accepted procedures (Puregene, Gentra, Minneapolis).

Mutation analysis

Four PCR fragments containing the exons 1–3 of *NYX* were amplified with primers previously described,[17] using DNA polymerase (HotFirePol,

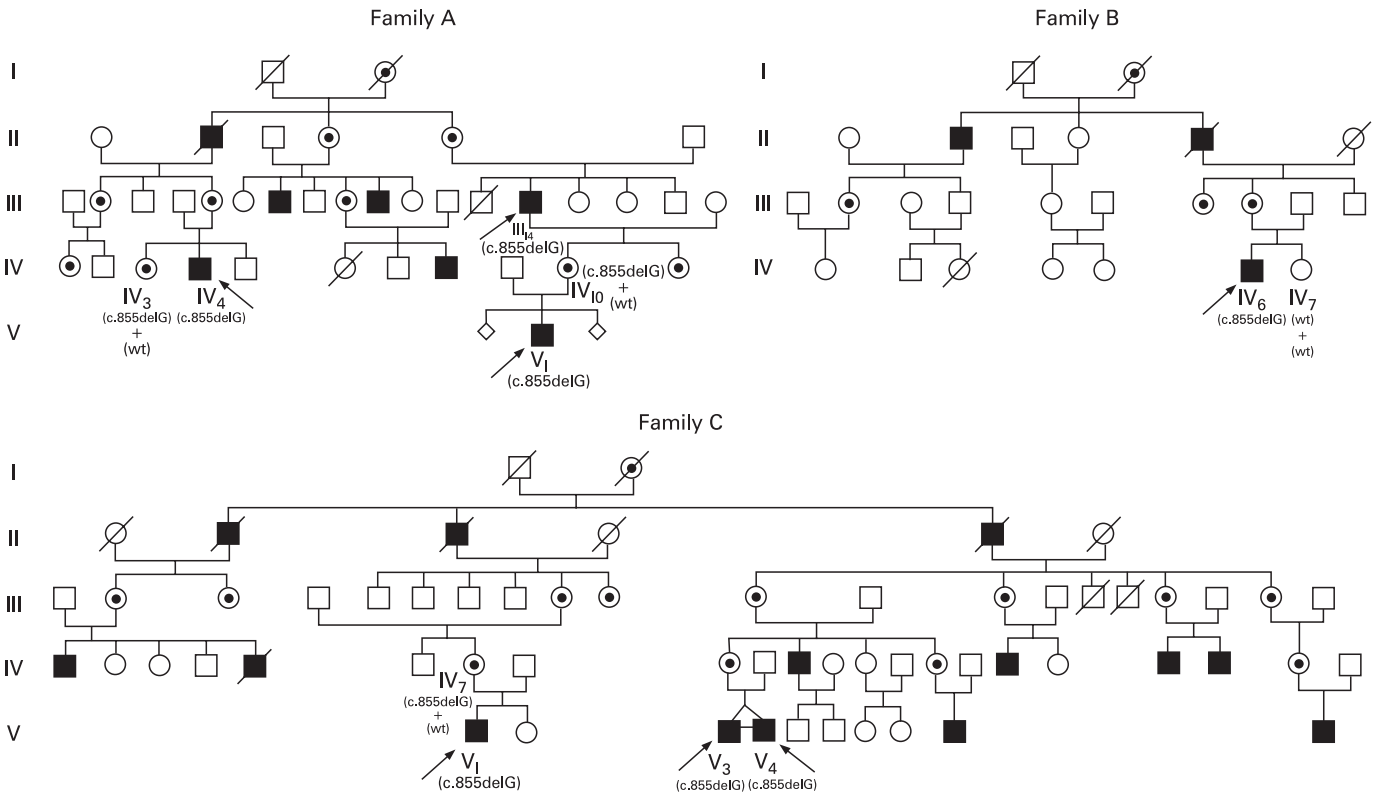

Figure 1 Pedigrees of patients showing a c.855delG mutation in the *NYX* gene, which is predicted to lead to a frameshift mutation, p.Asp286ThrfsX62. Males are represented by boxes, females by circles. Filled boxes indicate that the patient is affected; dots indicate a carrier status; "wt" means "wild type"; arrows indicate patients examined.

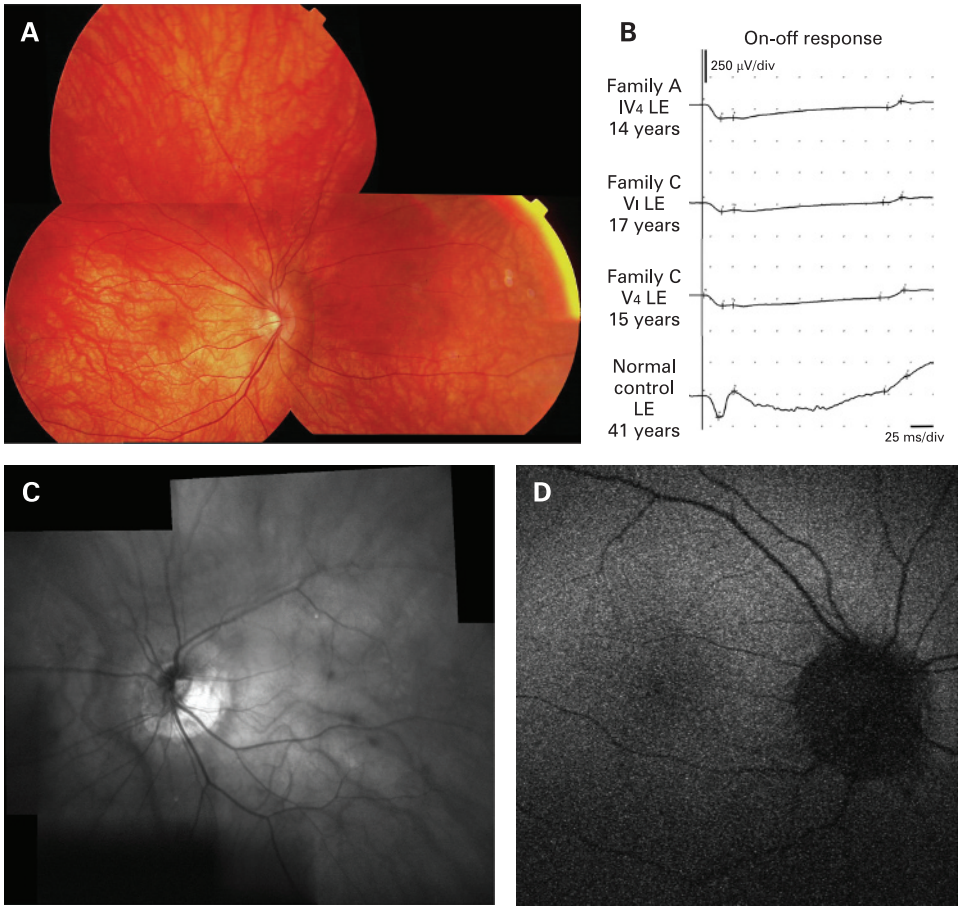

Figure 2 (A) Composite fundus image of the right eye of individual IV_6 of family B: note the tilted optic disc with temporal peripapillary chorioretinal atrophy and macular hypoplasia with increased visibility of the choroidal vasculature. (B) On–off responses of three affected individuals as indicated on left (LE, left eye; RE, right eye); representative normal control at bottom for purposes of comparison; note the absence of an on-response in all patients, with preservation of the off-response. (C) Composite infrared image of the fundus of the left eye of individual III_{14} of family A: note the tilted disc, pronounced peripapillary atrophy and macular hypoplasia. (D) Autofluorescence image of the fundus of the right eye of individual III_{14} of family A: background autofluorescence is normal.

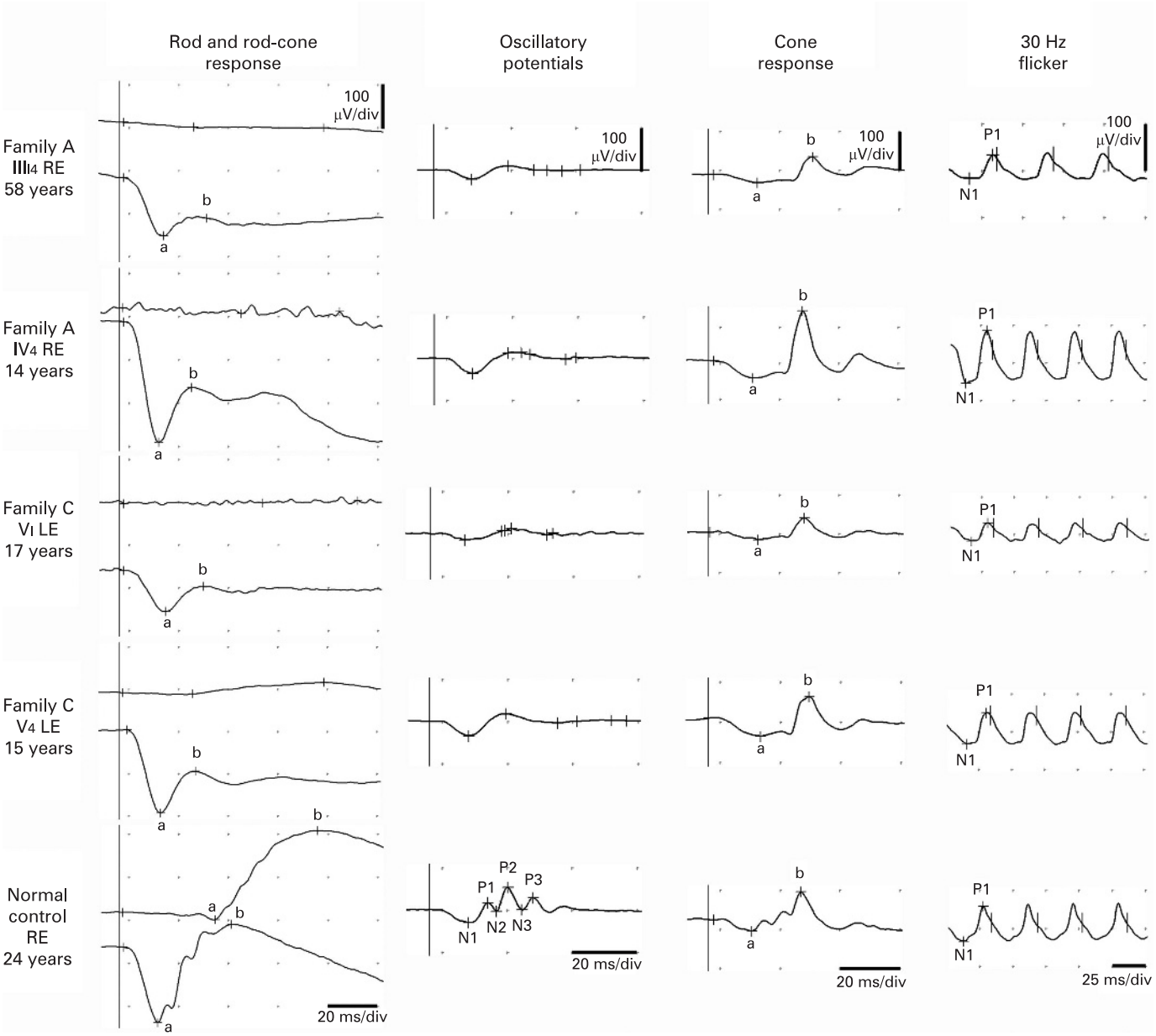

Figure 3 International Society for Clinical Electrophysiology of Vision standard full-field flash electroretinography (ERG) of four representative congenital stationary night blindness patients (LE, left eye; RE, right eye) as indicated on the left, and representative normal control at the bottom: note the typical Schubert–Bornschein complete type abnormalities with: absent or residual rod-specific responses, electronegative mixed rod–cone responses; absent scotopic oscillatory potentials, cone-specific responses to transient flash with normal amplitudes but broadened a-wave due to loss of photopic oscillatory potentials (slight delay in individual III_{14} of family A), and (near-) normal cone responses to 30 Hz flickering stimulus.

Soli Biodyne, Tartu, Estonia), 1.5 mM $MgCl_2$ (exon 1–2, fragments B and C of exon 3) or 2.5 mM $MgCl_2$ (fragment B of exon 3) and an annealing temperature of 56°C (exons 1–2) or 58°C (all fragments of exon 3) in the presence of Q-solution (Qiagen, Hombrechtikon, Switzerland). Purification and sequencing were performed with the appropriate primers as described earlier.[12] The DNA of 43 Flemish females and 51 Flemish unaffected males served as controls for this study.

Table 1 Haplotype analysis of the patients with the c.855delG mutation in *NYX*

		Alleles of patients						
Marker	**Position cM**	**$AIII_{14}$**	**AV_1**	**AIV_4**	**BIV_6**	**CV_1**	**CV_3**	**CV_4**
DXS993	63.90	307	307	307	307	307	307	307
NYX		[c.855delG]	[c.855delG]	[c.855delG]	[c.855delG]	[c.855delG]	[c.855delG]	[c.855delG]
DXS6810	66.88	222	222	222	222	222	222	222
DXS8035	70.00	138	138	138	138	138	138	138

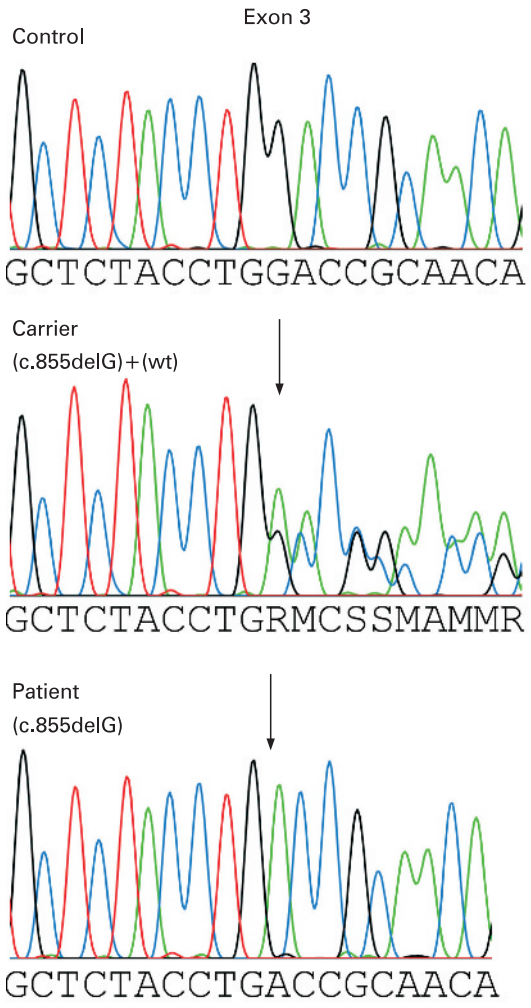

Figure 4 Electropherogram showing the hemizygous c.855delG mutation in a male patient and the heterozygous carrier state in the patient's mother, compared with a control.

Genotyping

Four polymorphic microsatellite markers were used for genotyping the affected individuals of families A, B and C (fig 1). Products of PCR assays with fluorescently labelled primers were analysed by automated capillary genotyping on an ABI 3730 DNA Analyser (Applied Biosystems, Foster City, California) and scored using the GeneMapper analysis software (Applied Biosystems).

RESULTS AND DISCUSSION

All seven affected family members of the three Flemish families have been diagnosed as having complete CSNB (fig 1). The diagnosis was based on a combination of congenital night blindness without photophobia, subnormal best-corrected visual acuity with nystagmus but (near-) normal visual fields, a documented personal or family history of stationary disease, fundus changes limited to myopia with macular hypoplasia, absence of hyperautofluorescence on autofluorescence imaging (fig 2) and Schubert–Bornschein complete type ERG abnormalities (figs 2, 3 and supplementary table 1). All patients are males and have a family history compatible with X linked inheritance. The three families are apparently unrelated (fig 1). They had all been diagnosed in their first few years of life, after failing to fix and follow at the age of 3 months, in the presence of nystagmus. Normal fundus autofluorescence imaging, which was performed in six patients, further supported the stationary nature of the disease.

The DNAs from seven male patients from the three different families were screened for mutations in the *NYX* gene. In all of them, a c.855delG was identified (fig 4).

Additionally, also the carrier status of four unaffected females of these families has been investigated (A-IV_3, A-IV_{10}, B-IV_7, C-IV_7) (fig 1). Only B-IV_7 did not carry the mutation. These studies clearly showed that the mutation c.855delG co-segregates within these families. None of the 137 control alleles from the same population revealed this mutation. The deletion is predicted to lead to a shift in the open reading frame, p.Asp286ThrfsX62, and causes a premature stop codon. Microsatellite screening of markers near the *NYX* locus revealed that all affected individuals of the three Flemish families share the same haplotype in the 6.1 cM region limited by the markers *DXS993* and *DXS8035* (see table 1). The haplotypes of a close located marker *DXS1068* at position 60.25 cM are distinct in the different families but also within the same family, indicating that recombination occurred (data not shown).

These studies suggest that a common founder mutation of *NYX* is associated with the complete form of CSNB in the Flemish population of Belgium. Consequently, molecular testing in Flemish patients with a suspected diagnosis of complete CSNB should be directed first towards identification of this common mutation by sequencing only the respective part of exon 3 of *NYX*. After exclusion of this common mutation, screening for other mutations in either *NYX* or *GRM6* can be performed as a second step.

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Patient consent: Obtained.

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