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SHORT COMMUNICATION

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# CSNB1 in Chinese families associated with novel mutations in *NYX*

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**Abstract** X-linked congenital stationary night blindness (CSNB) and *NYX* mutation have not been reported in Chinese. Here, two Chinese families with the complete form of CSNB (CSNB1) are presented. Linkage analysis of one family mapped the disease to Xp11–Xq13 where *NYX* is located. Sequence analysis of *NYX* identified two novel mutations, c.281G > C and c.302T > C, which would result in missense changes of p.Arg94Pro and p.Ile101Thr in the encoded protein. These two mutations were not found in 96 controls. The c.281G > C mutation cosegregated with nyctalopia and myopia. Our results expand the mutation spectrum of *NYX* and enrich the clinical information related to *NYX* mutation. The importance of associated myopia with *NYX* mutations is discussed.

**Keywords** X-linked recessive · CSNB1 · *NYX* · High myopia · Gene mutation

## Introduction

Congenital stationary night blindness (CSNB) is a group of inherited diseases that may be transmitted as autosomal dominant, autosomal recessive, or X-linked recessive traits. To date, ten loci with ten genes have been implicated in CSNB, including *GNAT1*, *PDE6B*, *RHO*, *GRK1*, *GRM6*, *RDH5*, *SAG*, *CACNA1F*, *NYX*, and *RPGR* (Retnet: http://www.sph.uth.tmc.edu/retnet/). Of these, mutations in *NYX* have been reported to cause the complete form of X-linked CSNB (CSNB1, OMIM 310500) (Bech-Hansen et al. 2000; Pusch et al. 2000; Zeitz et al. 2005; Zito et al. 2003). Human *NYX* gene (OMIM 300278) encodes a small leucine-rich protein (nyctalopin) with 481 residues. The exact function of nyctalopin is still unknown (Bech-Hansen et al. 2000; O'Connor et al. 2005; Pusch et al. 2000).

It is of special interest that CSNB1 is always associated with high myopia. Progressive or stationary night blindness have been described in a number of diseases but close association with high myopia has been observed only in a few diseases, such as CSNB1 and RP2 (OMIM 312600). CSNB1 is the only one with functional defects without gross structural abnormalities among those diseases with syndromic high myopia. Understanding the functional properties of *NYX* might provide clues to understanding the molecular mechanism of myopia development.

CSNB1 and *NYX* mutation have not been reported in the Chinese population. We describe two Chinese families with multiple individuals affected with CSNB and high myopia. Clinical and genetic evaluations indicated a phenotype of CSNB1. Linkage analysis for one family mapped the CSNB as well as high myopia to Xp11.4. Sequencing of *NYX* identified two novel mutations.

**Electronic supplementary material** Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s10038-006-0406-5 and is accessible for authorized users.

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## Subjects and methods

### Family and clinical data

CSNB1 was found in two Chinese families of Han ethnicity living in Guangdong, China (Fig. 1). Eighteen individuals in family A and only the proband in family B participated in this study. Informed consent conforming to the tenets of the Declaration of Helsinki was obtained from the participating individuals prior to the study. Standard ophthalmological examination included visual

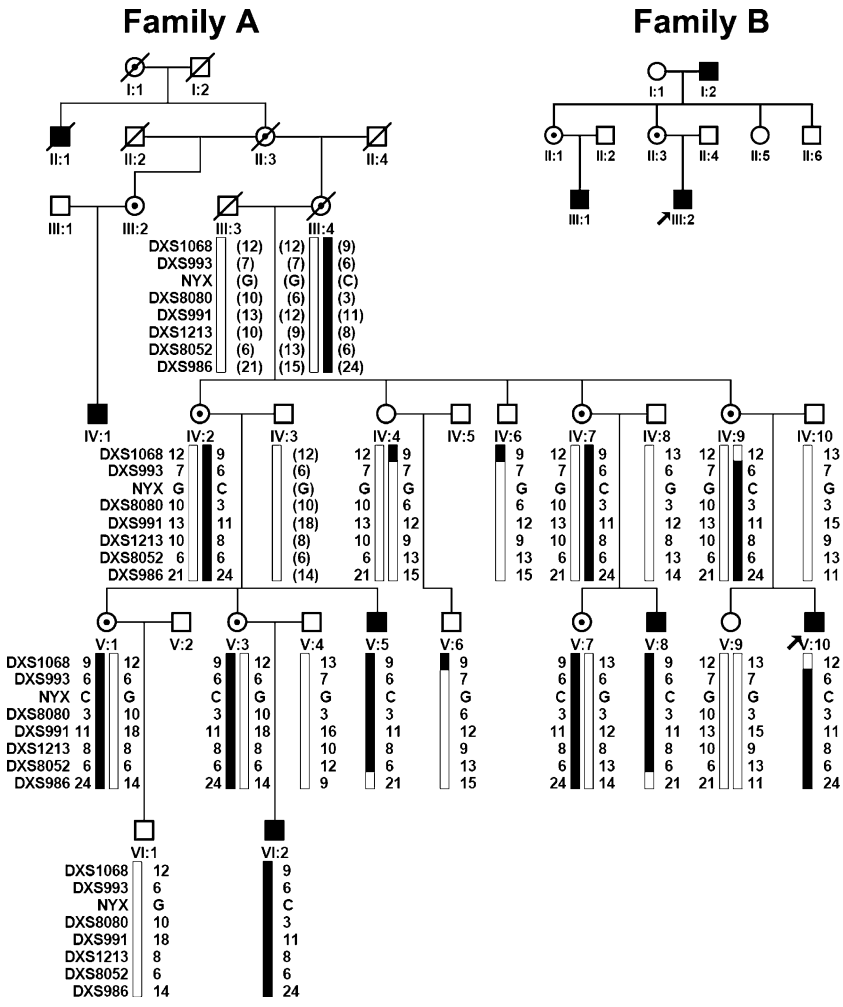


**Fig. 1** Family pedigrees and haplotype diagram. *Black squares* indicate individuals affected with X-linked congenital stationary night blindness (*CSNB1*). *Circles* with a *central black dot* indicate carriers. *Black bars* represent the disease allele inherited from ancestor

acuity, slit-lamp, and funduscopic examinations by ophthalmologists (Q.Z. and X.G.). Refractive errors were measured by retinoscopy. Electroretinogram (ERG) responses for selected members were consistent with ISCEV standards.

### Genotyping and linkage analysis

X-chromosome-wide linkage scan was performed for family A. Genotyping and linkage analysis was carried out as previously described (Guo et al. 2006; Zhang et al. 2006). CSNB1 in the family was analyzed as an X-linked recessive trait with full penetrance and a disease allele frequency of 0.0001.

### Mutation screening of candidate genes

Five pairs of primers (Table 1) were used to amplify the two coding exons and the adjacent intron sequence of the *NYX* gene (NCBI human genome build 35.1, NC_000023 for genomic DNA, NM_022567 for mRNA, NP_072089 for protein). The amplicons were sequenced with the ABI BigDye Terminator cycle sequencing kit v3.1, according to the manufacturer's instructions, on an ABI 3100 Genetic Analyzer. Sequencing results from affected and unaffected individuals as well as *NYX* consensus sequences from the NCBI Human Genome Database were imported into the SeqManII program of the Lasergene package (DNASTAR) and then aligned to identify variations.

## Results

### Clinical findings

Multiple individuals had CSNB1 in each of the two Chinese families (Fig. 1, Table 2). The disease in these families was transmitted as an X-linked recessive trait. All affected individuals in the families had had nyctalopia and myopia since early childhood. By the time of examination, all individuals with nyctalopia had high myopia (Table 1) except one who had moderate myopia (VI2 in family A). Funduscopic observation of all affected individuals in both families revealed myopic fundus changes typical of high myopia (Fig. 2), without

**Table 1** Primers used for PCR amplification and sequencing of *NYX*

| Primers | Sequence (5'–3') | Product length (bp) | Anneal Tm |
|---|---|---|---|
| NYX1F | TGGGGAGCTTCTGATTTTCTGTTG | 443 | 62 |
| NYX1R | ATCCCCACCACCTGCTGTTTTCTT | | |
| NYX2AF | CCCGGACAGGCAGGATTTTT | 590 | 58 |
| NYX2AR | CGGCACGCGGCGGAACAGG | | |
| NYX2BF | GTCGCTGCGCCACAACAACC | 651 | 65 |
| NYX2BR | TGCAGCGCGAGGAGACCCGAGAGG | | |
| NYX2CF | GCTGGCGCCTTCGGGGACTGTGG | 545 | 65 |
| NYX2CR | TCTGGGGACGGGCCTGGACTGGAC | | |
| NYX2DF | CCCGTGGTGCTGCGACTGC | 530 | 68 |
| NYX2DR | ATTTTCACCTCTGCCCTCCATTCC | | |

any signs of retinal degeneration. Such fundus changes and high myopia were not observed in unaffected individuals and obligate carriers (Table 1). An ocular A-scan of individual V10 in family A at 11 years of age recorded an axial length of 26.72 (OD) and 26.65 mm (OS). Ocular B-scan of V10 showed a comparatively normal shape of the eyeballs (Fig. 2). Keratometric measurement of V10 was 43.50/43.00D (OD) and 43.00/44.25D (OS). ERG recording of the proband in family A showed waves typical of CSNB1 (Fig. 3). ERG recording for individual III2 in family B showed changes comparable to those of CSNB1 (supplementary Fig. 6). Microcornea, cataracts, nystagmus, and strabismus were not observed in the affected individuals in these two families.

### Molecular genetic analysis

Upon X-chromosome-wide linkage analysis, nyctalopia and myopia in family A were mapped to Xp11–Xq13 between DXS1068 and DXS986, with the highest lod score of 2.66 for DXS991 at theta = 0. All six markers inside the linked region gave positive lod scores. Haplotypes of markers in this region support the linkage results (Fig. 1). The *NYX* gene, located in this region, has been shown to cause CSNB1 when mutated.

Sequence analysis of *NYX* identified two novel mutations, c.281G > C in family A and c.302T > C in family B (Fig. 4). These two mutations should result in missense changes of the encoded protein: p.Arg94Pro and p.Ile101Thr. The c.281G > C mutation cosegregated with nyctalopia and myopia in family A (Fig. 1). Besides the proband, genomic DNA from other family members of family B was not available. These two mutations were not detected in 96 control individuals (57 males and 39 females).

## Discussion

To date, 39 mutations in *NYX*, including two described here, have been reported (Bech-Hansen et al. 2000; Miyake 2002; Pusch et al. 2000; Zeitz et al. 2005; Zito

**Table 2** Clinical data of available individuals in the two Chinese families

| ID | Family | Gender | Age | Unaided visual acuity (corrected) | Refractive error | | Nyctalopia | ERG |
|---|---|---|---|---|---|---|---|---|
| | | | | | OD | OS | | |
| IV2 | A | Female | 53 | 20/20; 20/25 | N/A | N/A | No | |
| IV3 | A | Male | 58 | 20/20; 20/20 | 0 | 0 | No | |
| IV4 | A | Female | 51 | 20/20; 20/20 | 0 | 0 | No | |
| IV6 | A | Male | 44 | 20/20; 20/20 | 0 | 0 | No | |
| IV7 | A | Female | 38 | 20/20; 20/20 | 0 | 0 | No | |
| IV8 | A | Male | 39 | 20/15; 20/15 | 0 | 0 | No | |
| IV9 | A | Female | 35 | 20/20; 20/20 | 0 | 0 | No | |
| IV10 | A | Male | 44 | 20/20; 20/20 | 0 | 0 | No | |
| V1 | A | Female | 32 | 20/20; 20/20 | 0 | 0 | No | |
| V3 | A | Female | 31 | 20/20; 20/20 | 0 | 0 | No | |
| V4 | A | Male | 31 | 20/20; 20/20 | 0 | 0 | No | |
| V5 | A | Male | 25 | 20/200; 20/200 (20/60; 20/60) | −18.00D | −15.00D | Yes | |
| V6 | A | Male | 20 | 20/40; 20/15 (20/20; 20/15) | −1.00D | 0 | No | |
| V7 | A | Female | 12 | 20/50; 20/40 (20/20; 20/20) | −1.50D | −1.00D | No | |
| V8 | A | Male | 7 | 20/60; 20/60 (20/30; 20/30) | −6.00D | −6.50D | Yes | |
| V9 | A | Female | 8 | 20/20; 20/20 | 0 | 0 | No | |
| V10 | A | Male | 11 | 20/300; 20/500 (20/40; 20/40) | −9.50D | −9.25D | Yes | Yes |
| VI1 | A | Male | 5 | 20/20; 20/20 | 0 | 0 | No | |
| VI2 | A | Male | 11 | 20/200; 20/200 (20/40; 20/40) | −4.50D | −5.00D | Yes | |
| III2 | B | Male | 4 | N/A | −6.00D | −6.00D | Yes | Yes |

*ERG* Electroretinogram, *N/A* not available

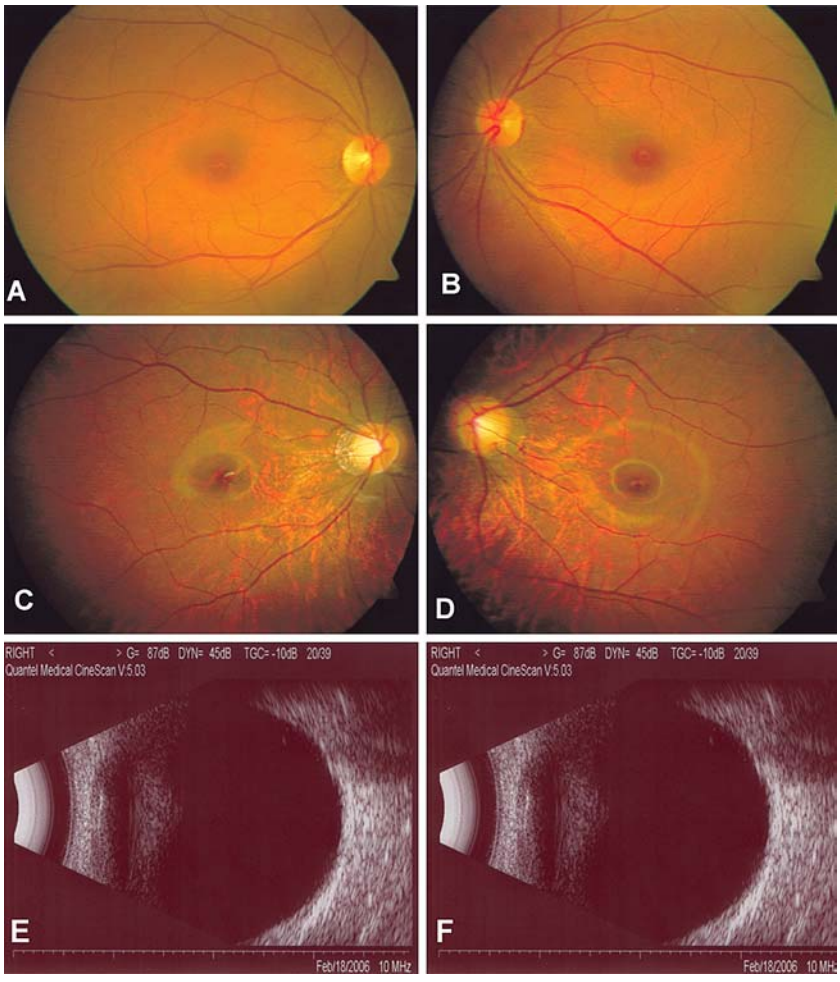


**Fig. 2a–f** Fundus and B-scan photos. **a** Right eye and **b** left eye photos of normal fundus from individual IV9 in family A (carrier). **c** Right eye and **d** left eye fundus photos from individual V10 in family A with high myopia, where optic nerve head crescent and "tigroid" appearance of posterior retina are shown. **e** Right eye and **f** left eye photos of B-scan from individual V10 of family A

et al. 2003) (two mutations in Japanese are not included because of limited information available) (Fig. 5). Of the 39, 21 were missense mutations and 17 were other types including deletion, insertion, splicing site, or nonsense. These mutations were distributed in or around the whole coding region of *NYX*. The novel c.281G > C and c.302T > C mutations identified in two Chinese families were located in the second and third leucine rich repeat (LRR). Mutation in the second LRR motif has not been reported before. The c.302T > C mutation occurred at a site where an inframe deletion (I101del) had been described.

The disease in the Chinese families was identified as the complete form of CSNB (CSNB1) according to the suggestion initiated by Miyake (2002) and Miyake et al. (1987). All patients had had stationary night blindness and myopia since early childhood. ERG recording demonstrated typical rod and cone responses (Fig. 3). Linkage results, mutation identification, and analysis of controls indicate that the disease in the two families is caused by mutations in the *NYX* gene. Our results, in agreement with those previously reported (Bech-Hansen et al. 2000; Miyake 2002; Miyake et al. 1987; Nakamura and Miyake 2004; Pusch et al. 2000), support the association of CSNB1 with *NYX* mutation, although one report did not establish this phenotype–genotype association (Allen et al. 2003). Clinical diagnosis is very important in the initial step in correlating phenotype with genotype. Subdivision of CSNB into CSNB1 and CSNB2 (incomplete type) through ERG patterns, first described by Miyake et al. (1987), played an important role in identification of different loci and of causative genes for families with CSNB as shown in this study and many others. Careful clinical observation of diseases occasionally provides unique, useful clues in elucidating etiology and molecular mechanisms.

Moderate to high myopia was present in all five affected individuals examined (the other four affected individuals were described as having myopia similar to the five examined). Of the five, four had high myopia, and the other one (individual VI2 in family A) is expected to advance to high myopia according to his fundus changes as well as his myopia progression. Individual VI2 had fundus changes similar to V10. He had myopia of −2.25D/OD and −2.5D/OS at 4 years of age, −3.5D/OD and −4D/OS at 8 years of age, and −4.5D/OD and −5.0D/OS at 11 years of age. The environmental effect on myopia development in these

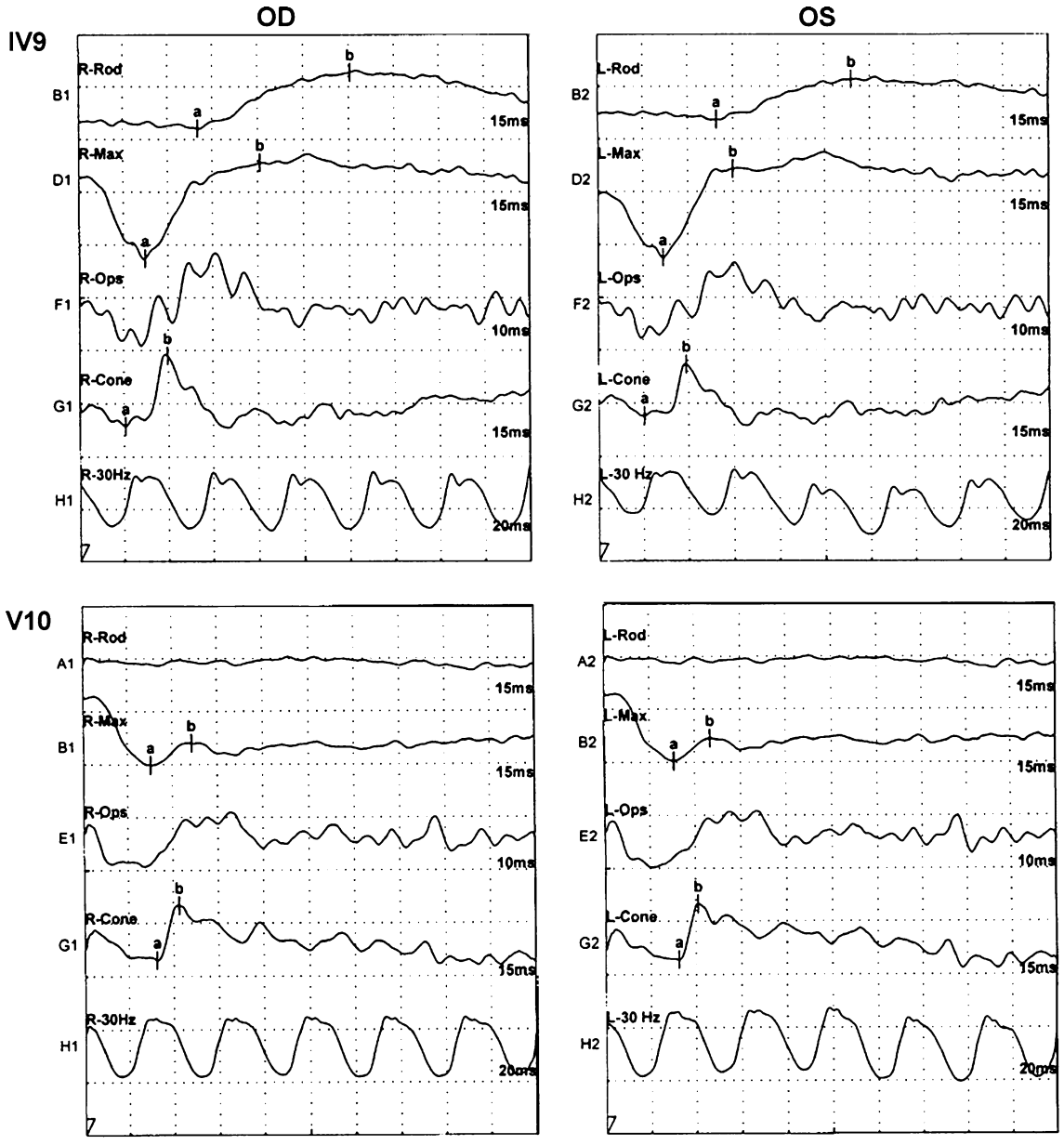


**Fig. 3** Electroretinogram (ERG) recording under standard conditions for individual IV9 (*top*) and V10 (*bottom*). Normal ERG in IV9 and ERG changes typical of X-linked congenital stationary night blindness (*CSNB1*) in V10 are shown. Individual V10 had diminished rod responses, a negative waveform under bright white, and an essentially normal cone amplitude

two families would be limited, as they lived in the countryside and most of the unaffected family members did not have myopia except a few with mild myopia (Table 2). A clear gap for myopia, at least −3D (V7 vs VI2), was observed between affected and unaffected individuals in family A (Table 2). Such a gap might be helpful in distinguishing genetic contribution from environmental impact. In addition, the degree of myopia varied among affected individuals, and was not associated with the age at examination.

Impaired visual acuity has been documented in four affected individuals of family A (V5, V8, V10 and VI2) (Table 2). Such a finding is present in Caucasian patients with *NYX* mutation (Jacobi et al. 2002; Zeitz et al. 2005) as well as patients with other diseases, such as X-linked myopia (Haim et al. 1988; Young et al. 2004; Zhang et al. 2006) and CSNB2 (OMIM 300071). Functional analysis identified that retinal ON-pathway dysfunction is involved in CSNB1, CSNB2 and GRM6 mutations (Dryja et al. 2005; Khan et al. 2005; Langrova et al. 2002). As a number of cases of high myopia are not accompanied by impaired visual acuity, it would be interesting to know the association among high myopia, impaired visual acuity and retinal ON-pathway dysfunction. Analysis of retinal ON-pathway dysfunction for those individuals with X-linked

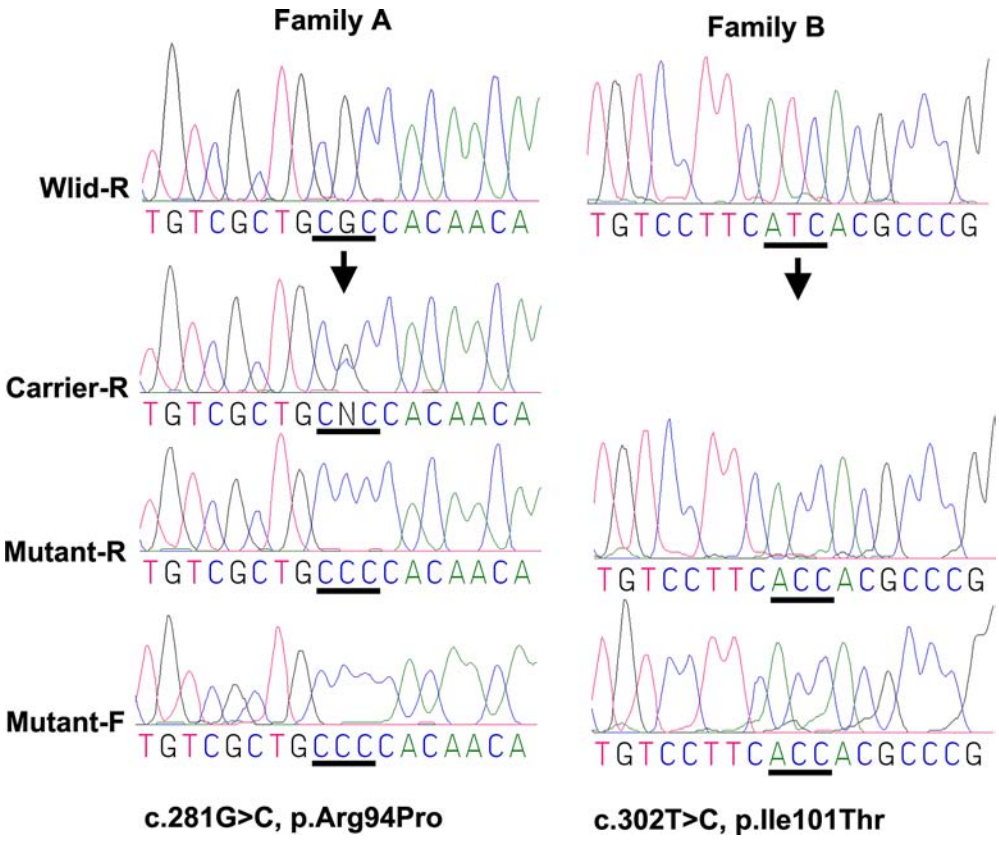


**Fig. 4** Sequence results of the *NYX* mutation. *Wild-R* reverse sequencing of *NYX* gene fragments in normal control. *Carrier-R* reverse sequencing of *NYX* gene fragment for individual IV9. *Mut-R* or *Mut-F* reverse or forward sequencing of *NYX* gene fragments for individual V10 in family A (*left column*) and for individual III2 in family B (*right column*). The c.281G > C mutation cosegregated with X-linked congenital stationary night blindness (*CSNB1*) in family A as shown in Fig. 1. *Underlining* below each sequence highlights the codon triplet where mutations occurred

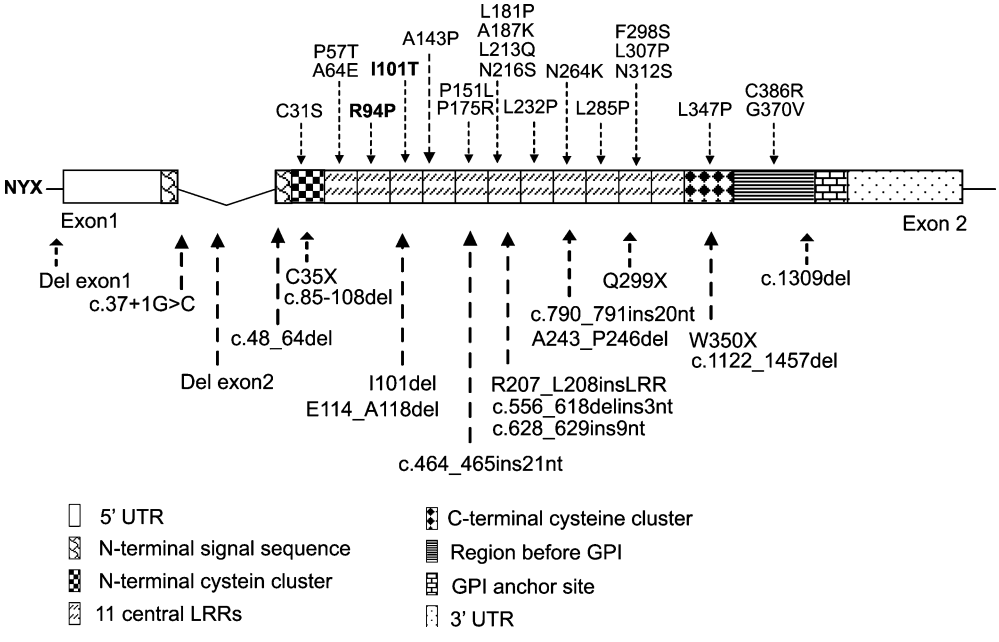


**Fig. 5** Schematic representation of *NYX* gene and its mutations. Protein motifs are shown with *boxes* filled with *different patterns*. Missense mutations are shown on top of the gene and other mutations below the gene. The 39 mutations shown in this figure include 37 from published literature (Bech-Hansen et al. 2000; Miyake 2002; Pusch et al. 2000; Zeitz et al. 2005; Zito et al. 2003) and two described here

high myopia alone may provide useful clues in disclosing such an association.

In summary, two Chinese families with CSNB1 were described. The disease in these families is associated with novel c.281G > C and c.302T > C mutations in *NYX*.

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