Research Article

Genetic Analysis of Consanguineous Pakistani Families with Congenital Stationary Night Blindness

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Abstract

Introduction: Congenital stationary night blindness (CSNB) is a rare, largely nonprogressive, inherited retinal disorder that can be clinically classified on the basis of fundus and electroretinogram abnormalities. ***Methods:*** We analyzed four large consanguineous families from the Southern Punjab region of Pakistan including multiple individuals affected with CSNB. Exome sequencing was performed in probands of all four families; Sanger sequencing was performed in additional members to test co-segregation of the variants identified. ***Results:*** We identified two novel and likely pathogenic variants in two pedigrees, namely, NM_002905.4:c.668A>C (p.Gln223Pro) in *RDH5* and NM_022567.2:c.908del (p.Gly-303ValfsTer45) in *NYX*. In the two other families, the variants NM_002905.4:c.319G>C (p.Gly107Arg) in *RDH5* and NM_000541.5:c.874C>T (p.Arg292Ter) in *SAG* were identified. These latter mutations have been reported previously, but not in the Pakistani population. ***Conclusions:*** Our findings expand the mutational spectrum of CSNB, in particular within the population of Southern Punjab.

Introduction

Congenital stationary night blindness (CSNB) is a group of heterogenous, largely nonprogressive, inherited retinal disorders that affect bipolar cells, photoreceptor cells, or retinal pigment epithelium cells [1, 2]. Affected individuals often complain of night vision problems or delayed dark adaptation, but some forms are also associated with a decrease in visual acuity, myopia, strabismus, and nystagmus [3]. Based upon fundus appearance, CSNB can be classified into forms with or without fundus abnormalities, with the former including Oguchi disease and fundus albipunctatus (FAP). The characteristic fea-

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ture of Oguchi disease is the presence of a golden-greyish sheen (most prominent in the peripheral retina) when the fundus is exposed to light, while there is no remarkable fundus alteration upon prolonged dark adaptation [2]. The disappearance of the golden-greyish sheen upon prolonged dark adaptation, known as the Mizuo-Nakamura phenomenon, is considered a pathognomonic clinical feature of Oguchi disease [4]. Conversely, FAP is characterized by small white spots in the retina, which are presumed to contain precursors of 11-*cis*-retinal. CSNB with largely normal fundus appearance can be divided into complete or incomplete Schubert-Bornschein-type and Riggs-type, based on distinct ERG findings [5–7].

CSNB may follow an autosomal dominant, autosomal recessive, or X-linked pattern of inheritance, due to mutations in genes having a role in phototransduction or subsequent signal transmission to ganglion cells. More specifically, there are at least 19 genes known to be linked to this condition, including *CABP4*, *CACNA1F*, *CAC-NA2D4*, *GNAT1*, *GPR179*, *GRK1*, *GRM6*, *LRIT3*, *NYX*, *PDE6B*, *RDH5*, *RHO*, *RLBP1*, *RPE65*, *SAG*, *SLC24A1*, *TRPM1*, *GNB3*, and *RIMS2* [1, 8–10]. Recently, mutations in *GUCY2D* have also been associated with congenital night blindness with variable progression to mild retinitis pigmentosa (RP) [11]. Finally, a dual phenotype of congenital night blindness and cone-rod dystrophy was described in two Japanese siblings with coexistence of *GNAT1* and *ABCA4* mutations [12]. Oguchi disease is caused by *GRK1* or *SAG* mutations, and follows an autosomal recessive pattern of inheritance, whereas FAP is mostly associated with recessive mutations in the *RDH5* gene [13–15]. CSNB without fundus abnormalities has greater genetic heterogeneity.

Consanguineous populations represent a powerful tool to study autosomal recessive Mendelian disorders, and the Pakistani population is highly consanguineous, with >50% of marriages occurring between cousins, reaching the value of 80% in some areas [16, 17]. The Southern region of the Punjab Province is one of such areas. Moreover, nearly half of the population is illiterate [18] and essentially unaware of the risks associated with consanguinity.

Many genes and mutations associated with different genetic disorders have been identified in Pakistani pedigrees. However, the majority of these families originated from Central Punjab and the Sindh Province [19, 20]. Here we studied four consanguineous families with CSNB phenotype from South Punjab and identified novel and likely pathogenic variants in known CSNB genes.

Materials and Methods

Enrollment of Families

All families were identified from the Southern region of the Punjab Province in Pakistan and were enrolled on the basis of the number of affected individuals (at least two affected individuals), presence/absence of consanguinity (only consanguineous pedigrees), and pattern of inheritance (only recessive or X-linked, by pedigree analysis). More specifically, the families described here are part of a larger cohort of patients, all displaying inherited retinal dystrophies, but the four pedigrees analyzed are the only ones with clear-cut CSNB phenotypes. A detailed questionnaire was designed to rule out the presence of a syndromic form of inherited retinal disorders. Written consent was obtained from all the participants of the study or from their parents/legal guardians. This study was approved by the Bioethics Committees of the Islamia University of Bahawalpur, Pakistan, and the Ethikkommission Nordwest- und Zentralschweiz.

Clinical Examination

Clinical examination of three families, namely, PKIURP03, PKIURP06, and PKIURP07, was performed by an ophthalmologist. This examination included the collection of medical history and funduscopy (shown in Fig. 1). Clinical examination of affected individuals from family PKIURP51 was not performed, as they live in a village where the access was difficult for ophthalmologists. In this family, the assessment of the disease was performed by the evaluation of the available medical history and by their descriptive phenotype.

Collection of Blood Samples and DNA Extraction

Ten milliliters of intravenous blood were collected from selected members of the enrolled families, in tubes containing EDTA as an anticoagulant. DNA was extracted by using standard techniques and quantified by spectrophotometry. DNA quality was assessed by gel electrophoresis, on 1% agarose gels.

Exome Sequencing and Segregation Analysis

Exome sequencing (ES) was performed in probands from all four families by using the Twist Comprehensive Exome Panel (Twist bioscience) and sequenced on a HiSeq 4000 instrument (Illumina) with an average coverage of 100–120X at each nucleotide position. Novoalign software (V3.08.00; Novocraft Technologies, Selangor, Malaysia) was used for mapping raw reads to the human genome reference sequence (build hg19). HaplotypeCaller (GATK, v.4.0.3.0) was used for Base Quality score recalibration. Picard (v. 2.14.0-SNAPSHOT) was used for the removal of duplicates. Small InDels and SNVs were detected by using GATK (GATK v4.0) [21]. Homozygosity mapping was performed by the use of Automap [22]. Allele frequencies were checked by the use of the gnomAD database (https://gnomad.broadinstitute.org/), and conservation of variants was performed by using GERP++ [23]. AN-NOVAR [24] and in-house scripts [25] were used for the assessment of the pathogenicity of genetic variants.

Results

Clinical Findings

Thirteen patients from 4 unrelated families were studied. Family PKIURP03 included three patients. Individu-

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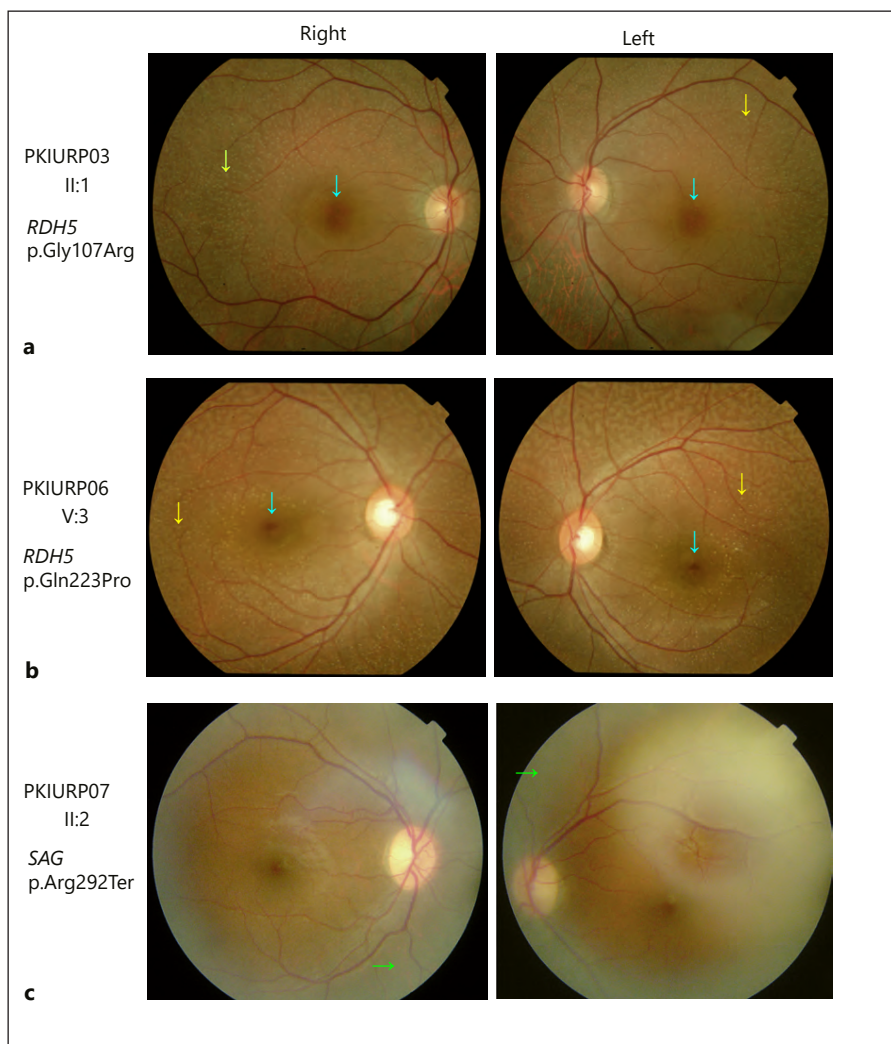Quinodoz/Calzetti/Iqbal/Rivolta

Fig. 1. Fundus images of proband II:1 from family PKIURP03 (**a**), proband V:3 from family PKIURP06 (**b**), and proband II:2 from family PKIURP07 (**c**). Yellow arrows indicate retinal white spots of FAP, while cyan arrows indicate the central macular area, relatively spared from the presence of white spots. Green arrows indicate areas of greyish discoloration of the fundus in Oguchi disease. FAP, fundus albipunctatus.

al II:1 was a male of 29 years, whereas II:2 was a female of 17 years, and individual II:3 was a female of 13 years. All three patients had night vision problems since early ages. Fundus images of the proband (II:1) showed the presence of scattered white spots in the retinal mid-periphery with relative macular sparing, a characteristic of FAP. A localized, symmetrical area of greater choroidal visibility in the nasal inferior fundus was noted in both eyes, but optic disks and retinal vessels were of normal appearance (shown in Fig. 1a).

Family PKIURP06 included four affected individuals in two loops. All affected individuals were of 12 years or younger, and their parents became aware of their nyctalopia when they were toddlers. Fundus photograph of one proband (V:3) showed the presence of scattered whitish spots in the retinal mid-periphery and, to a lesser extent, in the macula of both eyes (shown in Fig. 1b). Based on

these findings, the affected probands of families PKIURP03 and PKIURP06 were given a diagnosis of FAP.

Family PKIURP07 included two patients. Individual II:1 was a female aged 18 years, whereas individual II:2 was a male aged 15 years, both reporting seemingly nonprogressive nyctalopia since early ages. Clinical history could not be further expanded since both parents were deceased. Fundus images of the proband (II:2), taken under light-adapted conditions, showed areas of greyish discoloration beyond the vascular arcades of both eyes without signs of RP, such as bone-spicule pigmentation, optic disc pallor, or vascular attenuation (shown in Fig. 1c), which is compatible with Oguchi disease.

Family PKIURP51 included four affected brothers and three unaffected sisters. The eldest patient (II:1) was 30 years old at the time of analysis. Two affected individuals were available at the time of sampling, II.1 and II.2 (28
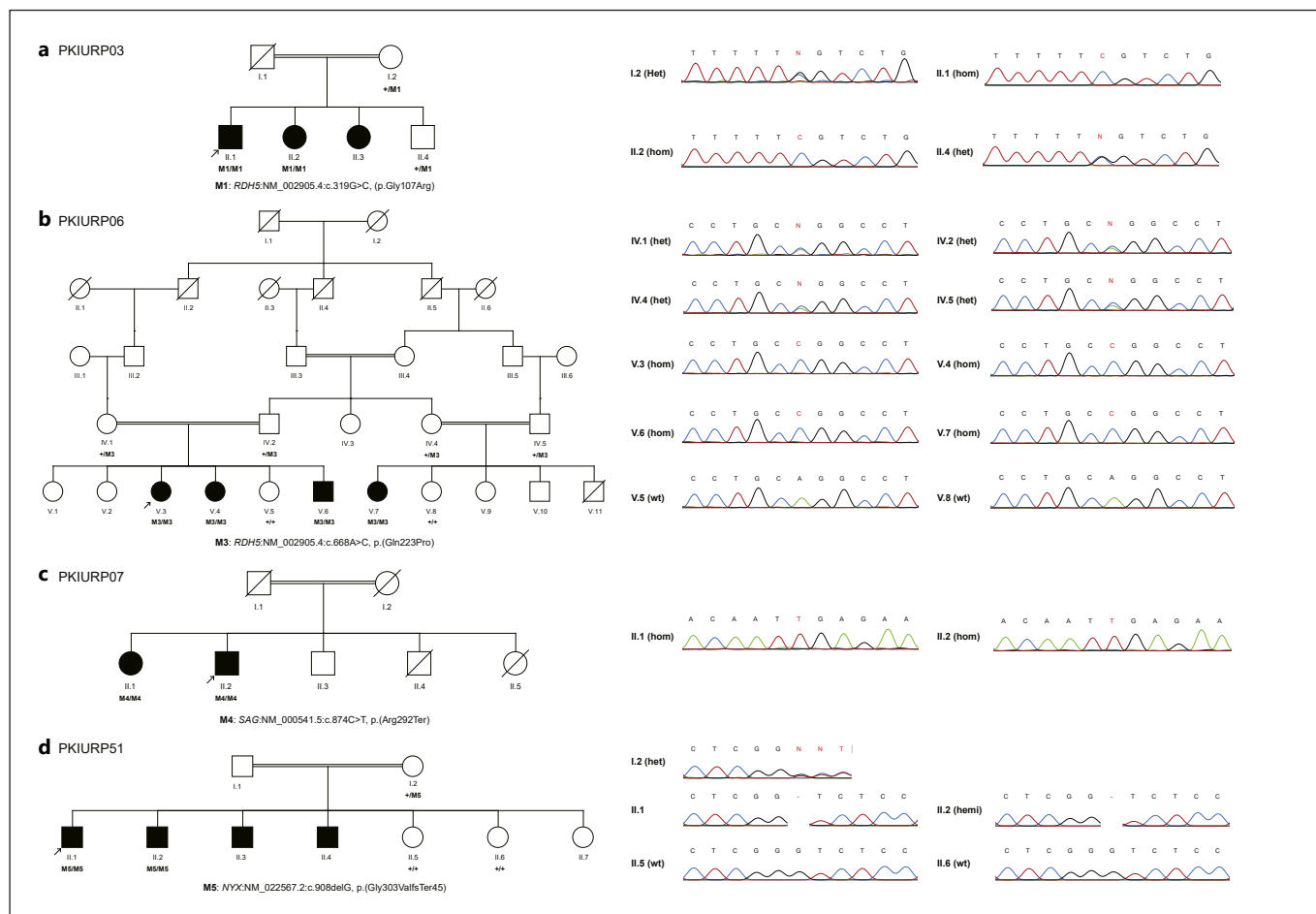
Fig. 2. a–d Segregation analysis of the variants identified within the pedigrees analyzed. Probands from every family are indicated by the arrow sign. In total, there were 13 patients (black squares and circles); however, only 10 were genetically analyzed (mutations are reported below each patient's symbol). In the electropherograms, the red nucleotide corresponds to the variant, "N" to heterozygous calls, and "-" to deleted nucleotides. In subject I.2 of family PKIURP51, the hetrozygous deletion of one base pair results in the shifting of the sequence; therefore, there are multiple "N" calls. het, heterozygote; hom, homozygote; hemi, hemizygote; wt, wild type.

years old), both reporting nonprogressive difficulties in seeing at night associated with high myopia. No additional clinical data were available.

Molecular Findings

Ten patients were genetically analyzed (Fig. 2). ES analysis of probands from all four families (PKIURP03, PKIURP06, PKIURP07, and PKIURP51) revealed the presence of four variants in three different genes already linked to CNSB.

In family PKIURP03, we identified the homozygous variant NM_002905.4:c.319G>C (p.Gly107Arg) in the *RDH5* gene, which was absent from the gnomAD database. This variant segregated with the condition within the family (shown in Fig. 2). The effect of this variant on the three-dimensional structure and on the function of the retinol dehydrogenase enzyme (encoded by *RDH5* gene) was predicted by HOPE [26] to be likely pathogenic because of the larger size and the positive charge of arginine with respect to glycine.

Family PKIURP06 carried a novel homozygous variant, NM_002905.4:c.668A>C (p.Gln223Pro) in the *RDH5* gene. This variant, again, co-segregated with disease in a Mendelian recessive fashion in 10 individuals (4 affected and 6 unaffected individuals, shown in Fig. 2). This missense variant was not present in gnomAD, and HOPE

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predicted pathogenicity based on the larger size of glutamine with respect to proline and the presence of this amino acid residue within the alpha helical region of retinol dehydrogenase.

The proband of family PKIURP07 with Oguchi disease showed an already-reported pathogenic homozygous variant in the *SAG* gene NM_000541.5:c.874C>T (p.Arg292Ter) [27–30]. This mutation is a stopgain and has an allele frequency 0.000359 in gnomAD South Asia; the variant segregated with disease in both patients from this pedigree (shown in Fig. 2).

Pedigree analysis of family PKIURP51 suggested an X-linked pattern of inheritance for CSNB. Exome data of the proband identified a deletion in the *NYX* gene NM_022567.2:c.908del (p.Gly303ValfsTer45), on chromosome X, which was also absent from gnomAD. Segregation analysis of this novel deletion in the family confirmed indeed an X-associated inheritance of the mutation (shown in Fig. 2).

Discussion

In this study, we analyzed four Pakistani families with CSNB by performing ES and segregation studies. Two families (PKIURP03 and PKIURP06) had FAP, one (PKIURP07) had Oguchi disease, and the fourth family (PKIURP51) had *NYX*-associated CSNB.

Families PKIURP03 and PKIURP06, with the phenotype of FAP, were found to carry the homozygous variants p.Gly107Arg and p.Gln223Pro in *RDH5*, respectively. *RDH5* encodes retinol dehydrogenase 5, an essential enzyme for the visual cycle [31]. This enzyme causes the catalytic conversion of 11-*cis*-retinol to 11-*cis*-retinal chromophore in the retinal pigment epithelium [32]. The variant p.Gly107Arg identified in family PKIURP03 has already been reported in 2 compound heterozygous [33, 34] and 3 homozygous [35, 36] Japanese patients with FAP and retinal degenerative changes, respectively. Interestingly, one homozygous patient showed sectorial retinochoroidal degenerative changes in the inferonasal quadrant resonating with the fundus findings in our proband [36]. The p.Gln223Pro variant identified in the second *RDH5* family is instead a novel variant.

Family PKIURP07 was found to carry a homozygous variant (p.Arg292Ter) in the *SAG* gene. This gene encodes the arrestin 1 protein [37, 38]. It is specifically expressed in rods and has a regulatory function within the phototransduction cascade. Mutations in *SAG* have been reported in association with Oguchi disease as well as autosomal recessive RP [39]. The homozygous variant p.Arg292Ter has been previously identified only in association with Oguchi disease [27, 29], which is in line with the findings in our proband.

Male patients of family PKIURP51 carried the hemizygous variant p.Gly303ValfsTer45 in *NYX*. This frameshift deletion is located in the second and last exon of the gene, therefore likely leading to an aberrant mRNA possibly escaping nonsense-mediated mRNA decay. The *NYX* gene encodes nyctalopin, a 481-amino acid protein involved in the localization of the TRPM1 protein in depolarizing bipolar cells [40]. Mutations in *NYX* result in the indirect mislocalization of TRPM1, which in turn causes X-linked CSNB [40–42]. Affected individuals from family PKIURP51 had night blindness with high myopia, similar to the phenotype described by Yip et al. [43]. Many reports suggest that mutations in the *NYX* gene are a common cause of X-linked CSNB in the Chinese and in other populations [43–46]. However, to the best of our knowledge, this is the first report of a *NYX* mutation in a Pakistani pedigree.

One important limitation of this study is the clinical characterization of the patients, including the lack of electrophysiological assessment and other analyses of visual function or retinal structure, such as optical coherence tomography and fundus autofluorescence [47]. Furthermore, we could not document the Mizuo-Nakamura phenomenon in the proband of family PKIURP07, and we based the clinical diagnosis only on medical history and questionnaire in the patients from family PKIURP51. The nonprogressive nature of night blindness supported a clinical diagnosis of CSNB rather than other progressive phenotypes such as RP. In families PKIURP03, 06, and 07 fundus photographs were consistent with phenotypes of FAP or Oguchi disease, showing no typical signs of RP such as bone spicules, vascular attenuation, or optic disk waxy pallor. Finally, our molecular findings correlated with the available clinical phenotype in all the examined probands. In conclusion, following the molecular analysis of four Pakistani families with CSNB, we identified two novel and likely pathogenic variants in the *RDH5* and *NYX* genes, broadening the mutational spectrum of this rare retinal condition.

Acknowledgments

Statement of Ethics

Conflict of Interest Statement

Funding Sources

Author Contributions

Data Availability Statement

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