(06)

The first case of Oguchi disease, type 2 in a Polish patient with confirmed *GRK1* gene mutation

Pierwszy w Polsce przypadek chorego na chorobę Oguchiego typu 2. potwierdzony wykryciem mutacji genu GRK1

Anna Skorczyk-Werner[1], Jarosław Kocięcki[2], Anna Wawrocka[1], Katarzyna Wicher[1], Maciej Robert Krawczyński[1,3]

[1] Department of Medical Genetics, Poznan University of Medical Sciences, Poznań, Poland
Head: Professor: Anna Latos-Bieleńska, MD, PhD
[2] Department of Ophthalmology, Poznan University of Medical Sciences, Poznan, Poland
Head: Professor: Jarosław Kocięcki, MD, PhD
[3] NZOZ Center for Medical Genetics GENESIS, Poznan, Poland
Head: Professor: Anna Latos-Bieleńska, MD, PhD

Abstract: Oguchi disease type 2 is a rare autosomal recessive form of congenital stationary night blindness. A typical feature of this disorder is a golden-brown discoloration of the fundus called Mizuo-Nakamura phenomenon, which disappears after prolonged dark adaptation and reappears shortly after the onset of light.

Material and methods: A 13-year-old boy exhibiting the clinical features of congenital stationary night blindness, was examined. Ophthalmic examination including slit-lamp biomicroscopy, perimetry and funduscopy was performed. Additionally, the full-field electroretinography and molecular testing for congenital stationary night blindness using the Single Nucleotide Polymorphism microarray technique were performed.

Results: The ophthalmic examination showed normal visual acuity, normal anterior segment of both eyes and full visual fields. The eye fundus examination showed a typical golden-brownish discoloration of the peripheral retina (disappearing after long dark adaptation) with no pigment deposits. Full-field electroretinography showed reduced amplitudes of both waves under scotopic conditions, while under photopic conditions both shape and parameters of the record were within the normal limits. The Single Nucleotide Polymorphism microarray revealed a homozygous deletion: c.1607_1610delCGGA in *GRK1* gene. This frameshift mutation introduces a stop codon (p.Asp537Valfs*542) and results in deletion of terminal 22 amino acid residues of retinal kinase protein.

Conclusions: This is the first molecular evidence for *GRK1* gene mutation in a Polish patient with Oguchi disease type 2. The identification of the c.1607_1610delCGGA mutation in a patient with Oguchi disease confirms the pathogenicity of this variant.

Key words: Oguchi disease type 2, CSNB, *GRK1* gene, mutations, Single Nucleotide Polymorphism (SNP).

Abstrakt: Choroba Oguchiego typu 2. jest rzadką dziedziczoną autosomalnie recesywnie formą wrodzonej stacjonarnej ślepoty nocnej. Jej charakterystycznym objawem są złotawobrązowe przebarwienia dna oka, nazywane objawem Mizuo-Nakamury, które znikają po długotrwałej adaptacji do ciemności i pojawiają się ponownie krótko po ekspozycji na światło.

Materiał i metody: u 13-letniego chłopca z cechami klinicznymi ślepoty nocnej wykonano badania okulistyczne: badanie w lampie szczelinowej, perymetrię, badanie dna oka, elektroretinografię błyskową oraz test metodą mikromacierzy DNA typu polimorfizmu pojedynczych nukleotydów w celu określenia podłoża molekularnego wrodzonej stacjonarnej ślepoty nocnej.

Wyniki: wyniki badań okulistycznych wykazały prawidłową ostrość wzroku, prawidłowe pole widzenia oraz brak odchyleń od normy w badaniu odcinka przedniego oka. Badanie dna oka ujawniło charakterystyczne złotawobrązowe przebarwienia na obwodzie siatkówki (znikające po długotrwałej adaptacji do ciemności) bez skupisk barwnika. Elektroretinografia błyskowa wykazała obniżone amplitudy obu fal w zapisie skotopowym oraz prawidłowy zapis fotopowy. Badanie z zastosowaniem mikromacierzy DNA typu polimorfizmu pojedynczych nukleotydów pozwoliło zidentyfikować homozygotyczną delecję c.1607_1610delCGGA w obrębie genu *GRK1*. Jest to mutacja typu przesunięcia ramki odczytu, która powoduje powstanie kodonu stop (p.Asp537Valfs*542) i skutkuje delecją ostatnich 22 aminokwasów w białku kinazy rodopsyny.

Wnioski: w tym artykule opisujemy pierwszy w Polsce przypadek mutacji w genie *GRK1* u chorego na chorobę Oguchiego typu 2. Identyfikacja mutacji c.1607_1610delCGGA u chorego na chorobę Oguchiego potwierdza patogenność tego wariantu.

Słowa kluczowe: choroba Oguchiego typu 2., wrodzona stacjonarna ślepota nocna CSNB, gen *GRK1*, mutacje, polimorfizm pojedynczego nukleotydu (SNP – Single Nucleotide Polymorphism).

Introduction

Oguchi disease type 2 (MIM 613411) is a rare form of congenital stationary night blindness (CSNB), which shows autosomal recessive inheritance. A typical feature of this disorder is a golden-yellow discoloration of the fundus called Mizuo-Nakamura phenomenon, which disappears after prolonged dark adaptation and reappears shortly after the onset of light (1). Patients with Oguchi disease have normal visual acuity, full visual fields and color vision. Full-field electroretinograms (ERGs) show the absence of rod b waves after 30 minutes of dark adaptation with extremely reduced rod b waves and nearly normal a waves in standard combined responses. Cone responses are usually within normal limits under photopic conditions (2). There are two types of Oguchi disease: type 1 and type 2, each related to different genetic background. Mutations in arrestin gene (*SAG*, S-antigen) were first discovered in patients of Japanese origin (Oguchi disease type 1) (3), while more recently, the *GRK1* gene was implicated in patients of European ancestry (Oguchi disease type 2) (4). Both genes encode photoreceptor proteins involved in recovery of rhodopsin after photoactivation. G-rhodopsin kinase gene designed *GRK1* (G-protein-coupled receptor kinase 1) (MIM 180381) contains 7 exons, spanning 4296 bp and encodes 563-amino acid polypeptide. The gene was mapped to 13q34 (5). *GRK1* is a specialized G-protein-coupled receptor kinase expressed in retina. This rod cytosolic enzyme works with arrestin (SAG gene) in shutting off rhodopsin after it has been activated by a photon of light (4). Rhodopsin kinase catalyses the phosphorylation of photoactivated rhodopsin, thereby initiating the sequence of biochemical events that shut off phototransduction. The phosphorylated photoactive rhodopsin is then bound by a regulatory cytosolic protein arrestin, and is thereby uncoupled from the G protein transducin downstream in the phototransduction process (5).

Material and methods

This study was conducted in accordance with the Declaration of Helsinki. A 13-year-old boy exhibiting the clinical features of congenital stationary night blindness was examined. Voluntary informed consent for genetic examination was obtained from his parents. Ophthalmic examination, including slit-lamp biomicroscopy, perimetry, funduscopy, and full-field electroretinography (ERG), was performed. Genomic DNA was extracted from peripheral blood using standard salting-out procedure (6). Molecular testing for congenital stationary night blindness was performed using the single nucleotide polymorphism (SNP) microarray technique based on the APEX approach (Asper Biotech Ltd.). This test can be used to screen the total of 159 mutations in 11 genes: *GRK1*, *SAG, RHO, PDE6B, GNAT1, CABP4, GRM6, NYX, CACNA1F, CACNA2D4* and *TRPM1*. Seven mutations were analyzed in *GRK1* gene using the CSNB SNP microarray test: c.614C>A, c.827+623_883del1118, c.971delT, c.1139T>A, c.1172C>A, c.1411_1412delCC and c.1607_1610delCGGA.

Results

The 13-year-old boy was referred to the ophthalmic clinic due to night blindness identified in early childhood. The patient has a younger brother, but the brother and the parents had no eye problems. The ophthalmic examination of the patient showed normal visual acuity, normal anterior segment of both eyes and full visual fields. The eye fundus examination showed a typical golden-brownish discoloration of the peripheral retina (Fig. 1) with no pigment deposits, which disappeared after long dark adaptation. Full-field electroretinography

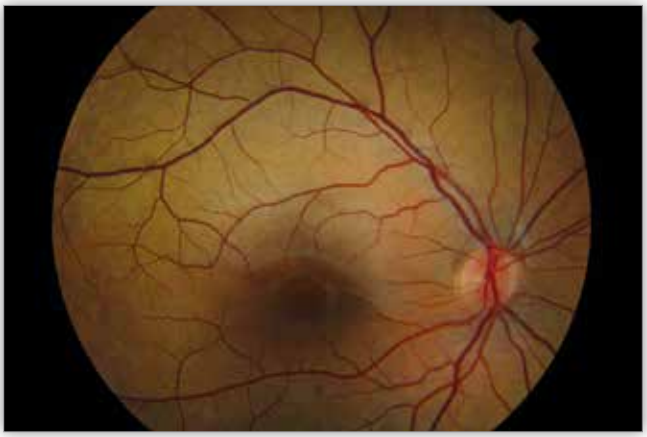

Fig. 1. The eye fundus photograph showing characteristic golden-brownish discoloration of the peripheral retina with no pigment deposits.

Ryc. 1. Fotografia dna oka z charakterystycznym złotobrązowym przebarwieniem w obrębie obwodowej części siatkówki bez skupisk barwnika.

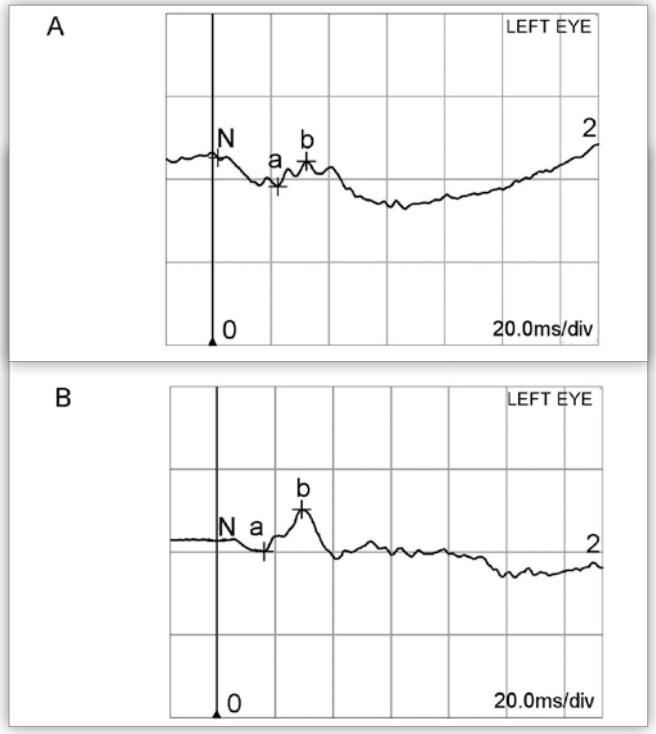

Fig. 2. Full-field electroretinography of the left eye. A – scotopic record. Amplitudes of both waves are reduced, wave a is bigger than wave b (electronegative record characteristics); a-wave 86.5 μV; b-wave 76.6 μV; b/a 886 mV. B – photopic record showing normal shape and parameters; a-wave 32.1 μV; b-wave 128 μV.

Ryc. 2. Elektroretinografia błyskowa całopolowa oka lewego. A – odpowiedź skotopowa. Obniżenie amplitud obu fal, fala a jest większa niż fala b (zapis elektroujemny); fala a 86,5 μV; fala b 76,6 μV; b/a 886 mV. B – odpowiedź fotopowa: prawidłowy kształt i parametry fal; fala a 32,1 μV; fala b 128 μV.

ISSN 0023-2157 Index 362646

showed reduced amplitudes of both waves under scotopic conditions, wave *a* was bigger than wave b (electronegative record characteristics) (Fig. 2a), while under photopic conditions the shape and parameters of the record were within the normal limits (Fig. 2b). Initially, the complete form of CSNB was diagnosed clinically, but after the molecular microarray analysis, the Oguchi disease type 2 diagnosis was finally determined. Molecular analysis helped identify a deletion of four nucleotides: CGGA in exon 7 of *GRK1* gene. The mutation was described according to transcript (ENST00000335678) and protein (ENSP00000334876) reference sequence from Ensembl Genome Browser database. The SNP microarray test revealed the mutation c.1607_1610delCGGA in homozygous form. This frameshift mutation causes a change from T**CGGA**CG to TΔCG in codons 536-537. It leads to an alteration of amino acid residues 537-542 and introduces a stop codon after the amino acid valine at the position 542 (p.D537Vfs*542). This change results in deletion of terminal 22 amino acid residues of retinal kinase protein.

Discussion

In this study, we report the first evidence for mutation in *GRK1* gene in a Polish patient. The 4-bp deletion identified in our patient was previously reported in a white woman of European ancestry (4, 7). Yamamoto and his research team identified this mutation in a patient, who was a compound heterozygote of this 4-bp deletion and a missense mutation in exon 5 c.1139T>A (p.V380D). It was suggested that the possible effect of 4-bp deletion in a heterozygous form was interferation with the normal isoprenylation of the carboxy terminus (4). C-terminal domain is thought to be involved in regulating rhodopsin activity (8). Isoprenylation is a post-translational modification essential for the full function of rhodopsin kinase (4). Khani and his research team showed that the both mutant proteins: 4-bp deletion and p.V380D exhibited decreased catalytic activity of the rhodopsin kinase protein (8).

The results of the microarray analysis in our patient indicate a homozygous c.1607_1610delCGGA deletion. However, the APEX microarray technique is not a quantitative method, so we cannot exclude that the proband may be a compound heterozygote of the 4bp deletion and a bigger deletion encompassing a part or even a whole *GRK1* gene. Nevertheless, to the best of our knowledge deletions of the entire *GRK1* gene have not been reported. The assumption that the proband is a homozygote for c.1607_1610delCGGA mutation can be supported by the fact that the maternal grandparents and paternal grandfather of the patient lived in two villages located just 15 km one from another, what can suggest that the proband's parents can be related. As our patient has either two *GRK1* alleles with 4-bp deletion affecting C-terminal domain of the protein or a bigger deletion in one allele, he has no functional rhodopsin kinase protein.

Although our patient shows normal ERG cone response, it was confirmed by in vitro experiments that null mutations in *GRK1* gene slow the rod recovery kinetics, but also cone phototransduction in men (9). There is a variability in the cone electroretinographic responses, but most patients reported to date presented normal or mildly abnormal cone ERG response (2). On the other hand, experiments on mice revealed that mice null for *GRK1* (*GRK1–/–* mice) do exhibit severe defects in cone recovery (10, 11). This difference between humans and mice was explained by the discovery that humans express both *GRK1* and another rhodopsin kinase: *GRK7* in cones. When *GRK1* is missing, there is partial compensation by *GRK7* (12, 13).

To date, only several mutations in *GRK1* gene have been identified in patients with Oguchi disease. Most of them have been proved to affect the functionally important catalytic domain. Three mutations have been found in European patients: a homozygous deletion (that encompasses exon 5 beginning in intron 4, approximately 2.5 kb downstream of exon 4), the previously mentioned missense substitution c.1139T>A (p.V380D) and the c.1607_1610delCGGA deletion (4, 8) described in this study.

Three mutations have been identified in Japanese patients: a homozygous substitution: c.1172C>A (p.P391H) found in two patients (2), and two deletions: c.971delT and c.1411_1412delCC (p.P470fs) (14).

Moreover, two mutations also affecting the catalytic domain have been described in Pakistani families: a deletion encompassing exon 3: c.827+623_883del (p.Gln277fsX6) (15) and c.614C>A (p.S205X) in exon 1 of rhodopsin kinase gene (16).

In this report, we described the first molecular evidence for *GRK1* gene mutation in a Polish patient with Oguchi disease type 2. The 4-bp deletion (p.D537Vfs*542) identified in our patient was previously reported in one patient of European ancestry. The identification of the c.1607_1610delCGGA mutation in a patient with Oguchi disease confirms the pathogenicity of this variant.

Acknowledgements
This study was partially supported by a grant from the Polish Ministry of Science and Higher Education (806/N-NIEMCY/2010/0).

References:

1. Mizuo G: *A new discovery in dark adaptation in Oguchi's disease [in Japanese].* Nippon Ganka Gakkai Zasshi. 1913; 17: 1148–1450.
2. Hayashi T, Gekka T, Takeuchi T, Goto-Omoto S, Kitahara K: *A novel homozygous GRK1 mutation (P391H) in 2 siblings with Oguchi disease with markedly reduced cone responses*. Ophthalmology. 2007; 114: 134–141.
3. Fuchs S, Nakazawa M, Maw M, Tamai M, Oguchi Y, Gal A: *A homozygous 1-base pair deletion in the arrestin gene is a frequent cause of Oguchi disease in Japanese*. Nat Genet. 1995; 10: 360–362.
4. Yamamoto S, Sippel KC, Berson EL, Dryja TP: *Defects in the rhodopsin kinase gene in the Oguchi form of stationary night blindness*. Nat Genet. 1997; 15: 175–178.
5. Khani SC, Abitbol M, Yamamoto S, Maravic-Magovcevic I, Dryja TP: *Characterization and chromosomal localization of the gene for human rhodopsin kinase.* Genomics. 1996; 35: 571––576.
6. Lahiri DK, Bye S, Nurnberger JI Jr., Hodes ME, Crisp M: *A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine*

other methods tested. J Biochem Biophys Methods. 1992; 25: 193–205.
7. Carr RE, Gouras P: *Oguchi's disease*. Arch Ophthalmol. 1965; 73: 646–656.
8. Khani SC, Nielsen L, Vogt TM: *Biochemical evidence for pathogenicity of rhodopsin kinase mutations correlated with the Oguchi form of congenital stationary night blindness*. Proc Natl Acad Sci USA. 1998; 95: 2824–2827.
9. Cideciyan AV, Zhao X, Nielsen L, Khani SC, Jacobson SG, Palczewski K: *Null mutation in the rhodopsin kinase gene slows recovery kinetics of rod and cone phototransduction in man*. Proc Natl Acad Sci USA. 1998; 95: 328–333.
10. Chen CK, Burns ME, Spencer M, Niemi GA, Chen J, Hurley JB, et al.: *Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase*. Proc Natl Acad Sci USA. 1999; 96: 3718–3722.
11. Lyubarsky AL, Chen C-K, Simon MI, Pugh EN Jr.: *Mice lacking G-protein receptor kinase 1 have profoundly slowed recovery of cone-driven retinal responses*. J Neurosci.2000; 20: 2209–2217.
12. Cideciyan AV, Jacobson SG, Gupta N, Osawa S, Locke KG, Weiss ER, et al.: *G-protein-coupled receptor kinase 1 (GRK1) and GRK7 expression and cone deactivation kinetics in enhanced S-cone syndrome (ESCS) caused by mutations in NR2E3*. Invest Ophthalmol Vis Sci. 2003; 44: 1268–1274.
13. Osawa S, Weiss ER: *A tale of two kinases in rods and cones*. Adv Exp Med Biol. 2012; 723: 821–827.
14. Oishi A, Akimoto M, Kawagoe N, Mandai M, Takahashi M, Yoshimura N: *Novel mutations in the GRK1 gene in Japanese patients With Oguchi disease*. Am J Ophthalmol. 2007; 144: 475–477.
15. Zhang Q, Zulfiqar F, Riazuddin SA, Xiao X, Yasmeen A, Rogan PK, et al.: *A variant form of Oguchi disease mapped to 13q34 associated with partial deletion of GRK1 gene.* Mol Vis. 2005; 11: 977–985.
16. Azam M, Collin RW, Khan MI, Shah ST, Qureshi N, Ajmal M, et al.: *A novel mutation in GRK1 causes Oguchi disease in a consanguineous Pakistani family*. Mol Vis. 2009; 15: 1788–1793.

The study was originally received 01.04.2014 (890785)
Praca wpłynęła do Redakcji 01.04.2014 r. (890785)
Accepted for publication 09.03.2015/
Zakwalifikowano do druku 09.03.2015 r.

Adres do korespondencji (Reprint requests to):
Anna Skorczyk-Werner, PhD
Department of Medical Genetics, Poznan University of Medical Sciences
ul. Rokietnicka 8, 60-806 Poznań, Poland
e-mail: aniaskorczyk@poczta.onet.pl

ISSN 0023-2157 Index 362646