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Differential diagnosis of Norrie disease and X-linked familial exudative vitreoretinopathy (XL-FEVR) based on clinical and molecular evaluation

Kliniczna i molekularna diagnostyka różnicowa choroby Norriego i witreoretinopatii wysiękowej rodzinnej sprzężonej z chromosomem X (XL-FEVR)

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Abstract: Background: Molecular analysis of the *NDP* gene to confirm and precise the clinical diagnosis in two patients with X-linked familial exudative vitreoretinopathy (XL-FEVR).
Material and methods: We report two patients from unrelated families with *NDP* gene mutations: a 14-month-old boy (p1) who was found to have severe exudative vitreoretinopathy and a 4-year-old boy with exudative vitreoretinopathy (p2). An extensive clinical examination of the probands, including slit-lamp examination, B-mode ultrasonography and magnetic resonance imaging was conducted, along with genetic analysis of *NDP* gene.
Results: Clinical findings in patient 1 included no light perception, total retinal detachment and hyperplastic primary vitreous in both eyes. The genetic analysis of the *NDP* gene enabled to identify the novel frameshift mutation c.222_c223insCG in p1 leading to the premature stop codon and production of aberrant norrin protein. In P2, clinical presentation included high myopia with astigmatism, unilateral fibrous bands and retinal detachment. Genetic testing revealed known point mutation c.362G>A leading to amino-acid alteration and improper protein.
Conclusions: Mutation screening of *NDP* gene identified two different mutations in this region, one of which has not been previously reported.

Key words: X-linked familial exudative vitreoretinopathy (XL-FEVR), Norrie disease (ND), *NDP* gene, novel mutation.

Abstrakt: Cel pracy: przeprowadzenie analizy molekularnej w celu potwierdzenia klinicznego rozpoznania witreoretinopatii wysiękowej rodzinnej sprzężonej z chromosomem X u dwóch pacjentów.
Materiał i metody: prezentujemy dwóch pacjentów z niespokrewnionych rodzin z mutacjami w obrębie genu *NDP*: 14-miesięcznego chłopca (p1) z objawami ciężkiej wysiękowej witreoretinopatii oraz 4-letniego chłopca z wysiękową witreoretinopatią (p2). U pacjentów przeprowadzono badania okulistyczne: badanie w lampie szczelinowej, badanie ultrasonograficzne w projekcji B, rezonans magnetyczny oraz analizę molekularną genu *NDP*.
Wyniki: badanie kliniczne u 1. pacjenta wykazało brak poczucia światła, całkowite odwarstwienie siatkówki oraz przetrwałe hiperplastyczne ciało szkliste w obojgu oczach. Badanie molekularne genu *NDP* umożliwiło identyfikację nieopisywanej dotąd mutacji c.222_c223insCG powodującej przesunięcie ramki odczytu, co prowadzi do powstania nieprawidłowego białka. Objawy kliniczne u 2. pacjenta obejmowały wysoką krótkowzroczność, astygmatyzm, jednostronne pasma włókniste na dnie oka oraz jednostronne odwarstwienie siatkówki. Badania genetyczne wykazały znaną mutację punktową c362G>A prowadzącą do zmiany sekwencji aminokwasów w łańcuchu polipeptydowym i nieprawidłowej struktury białka.
Wnioski: badanie genu *NDP* pozwoliło zidentyfikować dwie różne mutacje w tym regionie, z których jedna nie została dotychczas opisana w literaturze medycznej.

Słowa kluczowe: witreoretinopatia wysiękowa rodzinna sprzężona z chromosomem X, choroba Norriego (ND), gen *NDP*, nowa mutacja.

Introduction

NDP-related retinopathy, such as Norrie disease (ND), X-linked familial exudative vitreoretinopathy (XL-FEVR) and Coats disease, involves a spectrum of fibrous and vascular retinal abnormalities (Tab. I). The most severe form of retinopathy is Norrie disease (OMIM 310600), a rare disorder caused by mutations in *NDP* gene, inherited in the X-linked recessive mode. ND is characterized by bilateral retinal detachment and retinal folds. The dysplastic retina presents as pseudoglioma (grayish-yellow mass) with blindness occurring potentially

NDP-related retinopathies/ Retinopatie związane z mutacjami w genie *NDP*	Norrie disease (*NDP*)/ Choroba Norriego	X-linked familial exudative vitreoretinopathy (XL-FEVR)/ Rodzinna witreoretinopatia wysiękowa sprzężona z chromosomem X	Coats disease/ Choroba Coatsa
Clinical findings at birth/ Cechy kliniczne po narodzinach	greyish-yellow fibrovascular masses („pseudoglioma")/ szarawożółte włóknisto-naczyniowe masy, retinal detachment (partial or complete)/ odwarstwienie siatkówki częściowe lub całkowite	peripheral temporal retinal avascular zone/ obwodowa skroniowa strefa awaskularna, retinal angioma/ naczyniak siatkówki, congenital retinal folds/ wrodzone fałdy siatkówki, retained hyaloid vascular remnants/ pozostałości naczyń ciała szklistego, macular ectopia/ przemieszczenie plamki, fibrous tissue band/ pasmo tkanki włóknistej	unilateral retinal telangiectasia/ jednostronne teleangiektazje siatkówki, exudative fibrosis/ włóknienie wysiękowe
Progression of the disease/ Progresja choroby	cataract/ zaćma, posterior synechiae/ zrosty tylne, anterior synechiae/ zrosty przednie, iris atrophy/ atrofia tęczówki, shallowing of anterior chamber/ spłycenie komory przedniej, corneal opacification/ zmętnienie rogówki, band keratopathy/ keratopatia pasmowata, loss of intraocular pressure/ obniżenie ciśnienia wewnątrzgałkowego, shrinking of the globe (phthisis bulbi)/ zanik gałki ocznej vision: light perception impaired or non-existent/ ostrość wzroku: poczucie światła niepełne lub nieobecne 30–50% of males have developmental delay, sensorineural hearing loss is very frequent in males/ u 30–50% mężczyzn stwierdza się opóźnienie rozwoju. Częsta jest również u mężczyzn utrata słuchu odbiorczego	neovascularisation/ neowaskularyzacja, retinal detachment (tractional and/ or exudative; may be unilateral)/ ≤ age 20 yrs/ odwarstwienie siatkówki (trakcyjne i/ lub wysiękowe, może być jednostronne) ≤ 20. roku życia, vitreous haemorrhage/ krwotok do ciała szklistego, secondary glaucoma (neovascular or phacomorphic)/ jaskra wtórna (neowaskularna lub fakomorficzna), phacolytic uveitis/ fakolityczne zapalenie błony naczyniowej vision: impaired/ obniżona ostrość wzroku	progressive vascular leakage/ narastające przecieki z naczyń siatkówki, subretinal exudation and fibrosis/ podsiatkówkowy wysięk lub włóknienie, retinal detachment/ odwarstwienie siatkówki vision: normal to impaired/ ostrość wzroku od prawidłowej do obniżonej

Tab. I. Clinical features of patients with *NDP*-related retinopathies.
Tab. I. Cechy kliniczne pacjentów z retinopatiami związanymi z mutacjami w genie *NDP*.

in early childhood. Familial exudative vitreoretinopathy (OMIM 305390) may be inherited in different modes. It is genetically heterogeneous, and can be caused by mutations in *the NDP* (MIM:300658), *FZD4* (MIM:604579), *LRP5* (MIM:603506), *TSPAN12* (MIM:613138) and *ZNF408* (MIM:616468) genes, whereas only the X-linked FEVR is associated with the mutation in *NDP* gene. FEVR is a vitreoretinal dystrophy with sight-threatening manifestation characterized by macular and vascular traction, retinal folds, retinal neovascularisation, vitreous haemorrhages, tractional retinal detachment and subretinal exudates. The clinical findings may be asymmetrical (1–5).

The *NDP* gene maps to the chromosome Xp11.3, spans over 28kb, consists of 3 exons, but only exons 2 and 3 are translated (6). The *NDP* gene encodes the norrin protein, a 133-amino-acid secretory growth factor, that promotes vascular development and maintenance in the retina. Norrin contains a cystein-knot motif that activates the Wnt/beta-catenin pathway. The protein forms disulfide-linked oligomers in the extracellular matrix (7, 8). To date, more than 100 pathogenic *NDP* gene mutations were detected and described as associated with various inherited retinopathies (9).

Material and methods

Two patients from two unrelated families of Polish origin, diagnosed with vitreoretinopathy were enrolled. Ocular assessment including slit-lamp examination, eye fundus examination, B-mode ultrasonography and magnetic resonance imaging (MRI) were performed. The study conforming to the Declaration

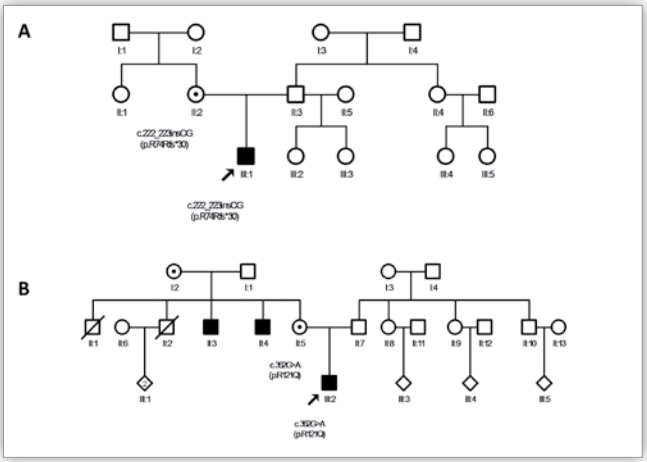

Fig. 1. Pedigrees and genotyping results of families with X-linked familial exudative vitreoretinopathy (A – patient's p1 family, B – patient's p2 family). White squares and circles represent unaffected males and females, respectively. Black squares represent affected males. White squares with a dot inside the symbol represent female carriers of the mutation detected in their children. Molecular status on DNA and protein level is referred to below. Arrows point to probands.

Ryc. 1. Rodowody oraz wyniki genotypowania rodzin z witreoretinopatią sprzężoną z chromosomem X (A – rodzina pacjenta p1, B – rodzina pacjenta p2). Białe kwadraty i koła wskazują odpowiednio zdrowych mężczyzn i zdrowe kobiety. Czarne kwadraty wskazują chorych mężczyzn w rodzinie. Białe kwadraty z kropką umieszczoną w środku symbolu wskazują nosicielki mutacji. Wykryte mutacje na poziomie DNA oraz białka umieszczono poniżej symboli. Strzałki wskazują probantów.

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of Helsinki was approved by the Institutional Review Board at the Medical University in Poznan. Written informed consent was obtained from patients' legal guardians.

Genomic DNA was extracted from venous blood samples using a standard salting-out procedure. The genetic analysis of the patients included PCR amplification of genomic DNA and sequencing of coding exons (2 and 3) and flanking intronic sequences of the *NDP* gene. PCR products were separated on an ABI 3130xl Capillary Sequencer (Applied Biosystems). The sequences were verified by comparing them to the human reference sequence of the *NDP* (GenBank _ NM_000266.3) and screened for mutations. Identified sequence variants were referred to the Human Gene Mutation Database (HGMD) and the Exome Variant Server (NHLBI Exome Sequencing Project ESP). Segregation analysis for the presence of mutant allele was performed in patient's p1 asymptomatic mother. Analysis was done by sequencing exon 3 of the *NDP* gene. Pedigrees of the families with mutations in the *NDP* gene are presented in Figure 1.

Results

Clinical features

Patient 1.

A 7-month-old boy (p1), was referred to the ophthalmic clinic due to suspected eye problems. At the age of 3 months the ocular asymmetry, horizontal nystagmus and photophobia were noticed. During the ophthalmic assessment at the University Hospital of the Medical University of Silesia the patient showed questionable light perception, no pupil light reaction and failure to track the light in both eyes. Horizontal corneal diameters were slightly different: 11.0 mm in the right and 12.0 mm in the left eye, while biometry revealed an axial length around 20.0 –21.0 mm in both eyes without significant differences. The right cornea was opacified while the left one was clear. Bilaterally, marked iris rubeosis was shown, caused by neovascularisation. The right eye presented with changes in anterior chamber, iris synechiae and congenital cataract. The lens of the left eye was displaced posteriorly and yellow masses were visible in vitreous. B-scan ultrasonography demonstrated bilateral hyperplastic primary vitreous accompanied by retinal detachment in both eyes, which was also confirmed by MRI. Fundus examination revealed complete retinal detachment and hyperplastic primary vitreous. Flash Visual Evoked Potentials (FVEP) testing was performed, with no response elicited bilaterally. The decision to carry out pars plana vitrectomy together with lensectomy and reconstruction of the anterior chamber in the right eye was made at the age of 5 months. The postoperative B-scan ultrasonography confirmed retinal reattachment in the upper quadrants of the right eye, additionally revealing retinal detachment forming closed funnel-like configuration (PVR grade D) in the left eye. Based on the ocular findings, Norrie disease was suspected and the genetic testing was scheduled.

Patient 2.

A 4-year old boy (p2). Family history revealed high myopia in a mother (-9,0 D) and blindness in two uncles (maternal brothers). At the age of 2, the boy presented with decreased visual acuity was observed and diagnosed with high myopia and astigmatism. Additionally, fibrous bands covering optic disc and extending towards the periphery were shown in the right eye. In the left eye, changes typical of high myopia were present only. In the MRI, hyperintense lesion in the right eye was found, described as focal retinal elevation sized 2.0 x 7.0 mm. Two years after the first examination, at the age of 4, he was diagnosed with retinal detachment with exudates, vitreoretinal proliferations and neovascularisation in the right eye.

Molecular findings

Sequence analysis of 2 coding exons of the *NDP* gene in the proband p1 revealed a two-nucleotide insertion in exon 3, position c.222_223insCG (p.R74Rfs*30) (Fig. 2). This mutation has not been previously reported and it causes a premature stop codon in *NDP* gene. Another, previously described missense mutation c.362G>A (p.R121Q) in exon 3, was found in patient p2 (Fig. 2). The identified mutation causes amino acid change from arginine to glutamine at position 121 of the mature peptide.

Segregation analysis revealed that the patient's p1 mother is a heterozygous carrier of the mutation detected in her son (Fig. 1).

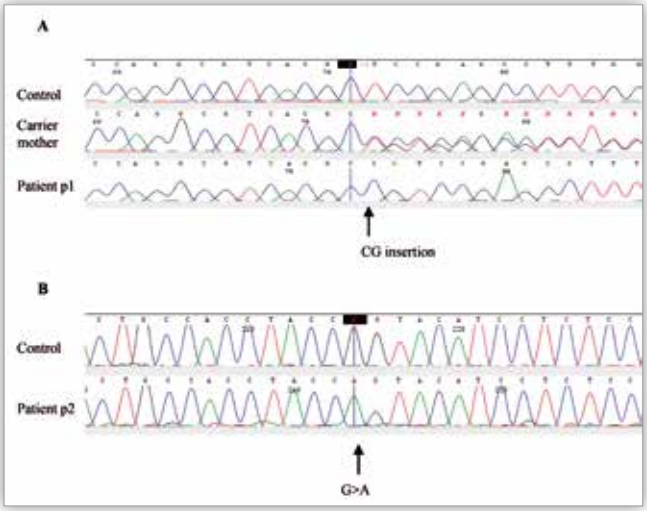

Fig. 2. Chromatograms showing two mutations identified in the *NDP* gene of patients with X-linked familial exudative vitreoretinopathy. A – sequence trace of part of exon 3 in a control DNA (upper panel), carrier mother (middle panel) and affected individual p1 carrying a mutation c.222_223insCG (lower panel). B – part of exon 3 in a control DNA (upper panel) and in an affected individual p2 carrying a mutation c.362G>A (lower panel).

Ryc. 2. Na chromatogramach wskazano dwie mutacje w genie *NDP* wykryte u pacjentów. A – sekwencja fragmentu eksonu 3. u badanych z grupy kontrolnej (górny panel), u matki nosicielki (środkowy panel) oraz u pacjenta p1 z mutacją c.222_223insCG (dolny panel). B – sekwencja fragmentu eksonu 3. u badanych z grupy kontrolnej (górny panel) oraz u pacjenta p2 z mutacją c.362G>A (dolny panel).

Discussion

In this study, we present a clinical description and molecular analysis of two patients, from two unrelated families with XL-FEVR. Genetic analysis of the *NDP* gene in two patients revealed one novel and one previously reported mutation. Both mutations found in our patients are located in cysteine knot-like

domain of norrin protein, which spans from codon 39 to codon 132 (according to the Human Protein Reference Database).

A novel mutation c.222_223insCG (p.R74Rfs*30) in exon 3 identified in patient p1 results in a frame-shift and premature termination codon, truncating norrin protein after 30 aberrant amino acids. Truncating mutations are one of the most frequent aberrations detected in *NDP* gene. In 1996, Berger and collaborators used gene targeting technology to generate Ndp mutant mice. Human norrin protein and its murine homologue shared 94% of the amino acid sequences. Genetic construct was carrying a deletion of the exon 2 fragment. It was a truncating mutation which removed 56 amino acids from the N-terminal part of the norrin protein. Hemizygous mice carrying a mutation developed ocular symptoms consistent with ocular findings observed in ND patients, such as developing retrolental structures in the vitreous body and disorganization of the retinal ganglion cell layer. This experiment allowed to create an animal model for Norrie disease and confirmed a deleterious effect of the truncating mutation on protein (10).

A known mutation c.362G>A (p.R121Q) was identified in p2. Replacement of arginine at residue 121 by glycine, leucine or glutamine amino acids were previously reported in Norrie and FEVR diseases (11–13). A missense mutation may affect norrin protein by reducing its acitivity, while a nonsense mutation leading to a frameshift in the coding sequence may result either in a producing truncated mRNA which activates the nonsense-mediated decay (NMD) process or in a reduction of biological activity of norrin protein.

The truncating mutation c.222_223insCG (p.R74Rfs*30) detected in p1, causes more severe symptoms of FEVR as compared to a missense mutation c.362G>A (p.R121Q) identified in p2. This finding seems to be consistent with research showing that mutation p. R121Q which changes the positively charged arginine to neutral glutamine, causes a less severe phenotype of Norrie disease. Moreover, it further supports the ideas of other researchers, who stated that truncating mutations tended to be more severe than missense mutations in the C-terminus region of cysteine knot-like domain of norrin protein (11, 14).

To conclude, this is the first report of molecular analysis of the *NDP* gene in Polish patients with XL-FEVR. We expand the mutational spectrum associated with XL-FEVR by describing a novel mutation of the *NDP* gene. The overlapping clinical features result in diagnostic challenge. It can be important to consider the age of onset, hearing difficulties or CNS deficits. Mutations in the same locus have been associated with different diseases affecting the retina: XL-FEVR or ND, therefore, screening in the *NDP* gene cannot definitely discriminate between these two diseases. Molecular analysis with knowledge of clinical phenotype and family history can provide important information for disease diagnosis and genetic counselling. While the phenotypes in FEVR or ND may overlap, we should emphasize the importance of using gene-based nomenclature such as NDP-associated vitreoretinopathy rather than clinical nomenclature such as ND or XL-FEVR, which might be easily mistaken.

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