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CASE REPORT

KERATITIS-ICHTHYOSIS-DEAFNESS SYNDROME WITH HETEROZYGOUS P.D50N IN THE *GJB2* GENE IN TWO SERBIAN ADULT PATIENTS

Kalezić T.[1,*], Vuković I.[2], Stojković M.[1], Stanojlović S.[1], Karanović J.[3], Brajušković G.[3], Savić-Pavićević D.[3]

[*]**Corresponding Author:** Tanja Kalezić, School of Medicine, University of Belgrade; Clinic for Eye Disease, University Clinical Centre of Serbia, Address: Pasterova Street No 2 , Tel. +381638148843, e-mail address: tanjakalezic@gmail.com

ABSTRACT

Purpose: Keratitis-ichthyosis-deafness (KID) syndrome is a rare congenital ectodermal dysplastic syndrome presenting with keratitis, ichthyosis and sensorineural hearing loss. The most common causes of KID syndrome are heterozygous missense mutations in the *GJB2* gene that codes for connexin 26.

Case report: During the ophthalmological examination, two adult females complained of recent worsening of visual acuity in both eyes. Anamnesis revealed that their eyes were red and irritated from early childhood onwards. Both of them had thickening and keratinisation of eyelid margins, lash loss, diffuse opacification of cornea and conjunctiva caused by keratinisation of eye surface, superficial and deep corneal vascularisation and corneal oedema. Partial sensorineural hearing loss and difficulties in speech were also noted along with typical ichthyosiform erythroderma. Genetic testing of the *GJB2* gene revealed a heterozygous p.D50N mutation in both patients.

Patients were treated with a combined topical corticosteroid and artificial tears therapy, with steroid therapy being intensified during the last month. The therapy increased the visual acuity by decreasing corneal oedema and by forming a more regular air-tear interface during the six months follow up. Subsequently, the disease progressed despite the continuation of the therapy.

Conclusion: This is the first report of Serbian patients with KID syndrome. Despite the administration of the combined topical corticosteroid and artificial tears therapy the disease is relentlessly progressive and therapeutic success of ophthalmological signs with local therapeutic modalities used so far had been disappointing.

Key words: *GJB2* gene, keratitis-ichthyosis-deafness syndrome, KID, p.D50N, steroid therapy.

INTRODUCTION

Keratitis-ichthyosis-deafness (KIDAD; MIM #148210) syndrome is a rare congenital ectodermal dysplastic syndrome. Prevalence is unknown, with approximately 100 reported cases to date.[1] KID syndrome presents with the classic phenotypic triad consisting of keratitis, ichthyosiform erythroderma, and sensorineural hearing loss. There is also variability in the clinical presentation among patients. The disease is relentless, with all the therapeutic modalities used so far having been disappointing.[2]

Inheritance of KID syndrome is usually sporadic or autosomal dominant.[3] In most reported cases, KID syndrome is caused by missense mutations in the *GJB2* gene encoding connexin 26 (Cx26).[3] The *GJB2* gene is expressed in a variety of tissues, including several ectodermal epithelia affected in KID syndrome: the corneal epithelium, epidermis of skin, cochlea, and hair follicles. Cx26 is one of 21 connexins coded by the human genome that represents membrane proteins. These consist of four transmembrane domains, linked by one cytoplasmic and two extracellular loops, with cytoplasmic N- and C-terminus.[4] Connexins form homo- or heterohexamers, referred to as hemichannels or connexons, in the endoplasmic reticulum-Golgi pathway. Subsequently, they are arranged in two functionally different structures. Docking of two hemichannels from neighboring cells results in the formation of a gap junction enabling intercellular communication.[5,6] Undocked, or functional, hemichannels serve

[1] School of Medicine, University of Belgrade; Clinic for Eye Disease, University Clinical Centre of Serbia

[2] School of Medicine, University of Belgrade; Clinic for Gynecology and Obstetrics, University Clinical Centre of Serbia

[3] University of Belgrade, Faculty of Biology, Center for Human Molecular Genetics

intracellular–extracellular exchange and signaling across the plasma membrane.[5,6] The *GJB2* gene, harboring missense mutations, encodes for functional Cx26, but with aberrant properties, leading to dysregulated hemichannels that cause syndromic character and heterogeneous phenotypic manifestations, seen in KID syndrome.[7]

Here we report, for the first time, on two adult Serbian patients with KID syndrome, carrying heterozygous p.D50N missense mutation in the *GJB2* gene. The study aims presenting their phenotype-genotype correlation.

PATIENTS AND METHODS

Two unrelated patients with KID syndrome were studied. Clinical diagnosis was based on dermatological, hearing and ophthalmological examinations, and confirmed by molecular genetic analysis of the *GJB2* gene.

Molecular genetic testing for *GJB2* mutations was done for both patients and their unaffected first-degree relatives (mother and sister), while samples of both fathers were unavailable for analysis. Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Kit (Qiagen, Germany). Exon 2 of the *GJB2* gene was amplified by polymerase chain reaction (PCR) using the following primers: forward (5' GGTGAGGTTGTGTAAGAGTTGG 3') and reverse (5' TGGGTTTTGATCTCCTCGAT 3'), which were designed by opensource Primer3 software.[8] Bidirectional Sanger sequencing was performed by BigDye® Terminator v3.1 Cycle Sequencing kit (Life Technologies, USA) and sequencing data was analyzed using the BioEdit Sequence Alignment Editor.[9]

Detailed ophthalmological examination and follow up for both patients were done in April 2020 at the Clinic for Eye Disease, University Clinical Center of Serbia, Belgrade, Serbia. Both patients were treated with topical steroids (Prednisolone 0.5% q.i.d.) and artificial tears (HPMC 0.3% preservative free q.h.) for three months, and the therapy was intensified with topical Prednisolone 0.5% q.h. per day during the fourth month. Patients were followed up for six months.

RESULTS

Patient 1

Patient 1 was a 40 year old female. Although no previous documentation was available, the patient's mother reported that a diagnosis for the patient of an ichthyosiform skin lesion with hearing impairment was established shortly after birth, while corneal signs appeared in the second year of life, leading to the diagnosis of KID syndrome. Severely slurred and slow speech was noticeable from early childhood. Family history revealed that the father and paternal side grandfather had strikingly dry and scaly skin, with minimal visual symptoms. Due to red and irritated eyes from the second year of life, therapy with artificial tears was applied during the patient's lifespan, but it has not been continuous.

The patient was referred to the Clinic for Eye Disease, University Clinical Center of Serbia due to recent worsening of visual acuity, grittiness, tearing, and photophobia. She presented with hypotrichosis of the whole body, with multiple scars on the capillitium. Skin was in total xerosis, with lichenification and ichthyasiform skin lesions. Nails were dystrophic and dark colored, some nails were missing. The patient had no obvious problems in hearing the questions and commands uttered in a normal voice. Regardless of the obvious problems in communication, Patient 1 seemed unusually cooperative and outspoken. Without any formal education and with the help of her mother, she developed good reading and writing skills. When an intelligence test was performed, her IQ measured, with Raven's progressive matrices, at 130. Genetic testing revealed a heterozygous missense mutation, c.148G>A, in exon 2 of the *GJB2* gene (Fig. 1: A1), which results in amino acid change from aspartic acid (Asp) to asparagine (Asn) at codon 50 (p.D50N). This mutation was not found in the unaffected mother (Fig. 1: A2).

Thickening and keratinization of eyelid margins, lash loss, diffuse opacification of cornea, loss of conjunctival luster caused by keratinization of eye surface, superficial as well as deep corneal vascularization, and corneal edema were noticed during the ophthalmological examination (Fig.1: A2). Esotropia with amblyopia in the right eye were detected. Best corrected visual acuity (BCVA) was counting fingers in the right eye and 0.2 to 0.3 using Snellen chart in the left eye. Central corneal thickness (CCT) using tomography (Orbscan 2z, Baush and Lomb) was 462 μm for the right and 584 μm for the left eye.

The four month regime of topical therapy with corticosteroids and artificial tears, with intensified topical corticosteroid therapy during the last month, resulting in improved vision in the left eye of Patient 1. There was an increase in BCVA in follow up time of six months and it was better in the left eye (from 0.3 to 0.7, Snellen chart). No intraocular pressure increase was noticed throughout the course of therapy.

Patient 2

Patient 2 was 34 year old females at the time of the ophthalmological examination. According to Patient 2's history, skin lesions were present from birth. The patient manifested difficulty since walking and, up to 10 years of age, she walked on her tiptoes. Her whole skin, including

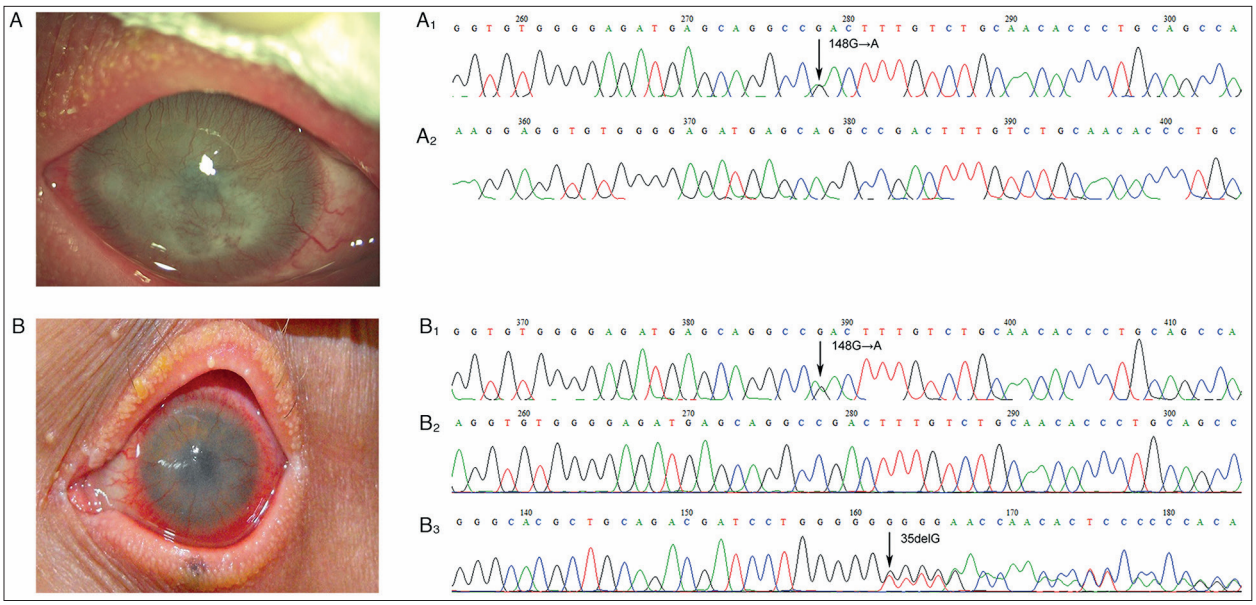

Figure 1. Mutation analysis of *GJB2* gene region associated with KID in two unrelated female patients. (A) Patient 1, thickening and keratinization of eyelid margins, lash loss, cornea showing a rim of abnormal epithelium with superficial neovascularization peripheral, significant stromal scarring with extensive neovascularization. (A1) Sequencing chromatograms showing the *GJB2* heterozygous transition c.148G>A in first patient. (A2) Unaffected mother of patient 1. (B) Patient 2, thickening and keratinization of eyelid margins, lash loss, diffuse opacification of cornea and loss of conjunctival luster. (B1) Sequencing chromatograms showing the same c.148G>A heterozygous transition in patient 2. (B2) Unaffected mother of patient 2. (B3) Sister who is carrying c.35delG heterozygous mutation of patient 2.

the face, was affected from the second year of life. At times, she had red circles on the capillitium. Her diagnosis of KID syndrome was set at 16 years of age. Family members were with no remarkable ophthalmologic nor skin problems. Patients 2's mother and father had difficulties with hearing.

Patient 2 was referred to the Clinic for Eye Disease, University Clinical Center of Serbia due to the blurred vision that was worse in the left eye. Her skin was with typical ichthyasiform erythroderma and lash loss. Facial skin was xerotic with local lichenification. The extensor sides of the arms and hull of the body also had lichenification and darker pigmented zones of the skin. The dorsal side of the hands and feet were covered with diffuse, yellow hyperkeratosis. Nails on the hands were normal while, on the feet, they were yellow colored. Speech pattern and hearing for Patient 2 was severely impaired. The same heterozygous missense mutation c.148G>A (p.D50N) in exon 2 of *GJB2* gene, as in Patient 1, was identified in the Patient 2 (Fig. 1: B1). This mutation was not found in the unaffected mother and sister (Fig. 1: B2 and B3). However, the sister had heterozygous deletion c.35delG in the *GJB2* gene, a particularly common mutation associated with autosomal recessive non-syndromic hearing loss (Fig. 1: B3) (Tsukada et al. 2015).[10]

On ophthalmological examination for Patient 2, BCVA, using the Snellen chart, was counting fingers in both eyes. Corneal opacification and loss of corneal and conjunctival luster was found. CCT using tomography (Orbscan 2z, Baush and Lomb) were 555 µm for the right and 620 µm for the left eye. Local corticosteroid therapy during follow up time of six months did not improve vision in Patient 2 (Fig. B: B1). Still, her eyes were less irritated with blood vessels slightly less engorged and corneal surfaces had more luster then upon presentation. Corneal thickness was steady.

DISCUSSION

This is the first report of two Serbian patients with KID syndrome, caused by heterozygous p.D50N mutation in the *GJB2* gene.

GJB2 p.D50N mutation is the most commonly identified mutation associated with KID syndrome The mutation has been described in both sporadic and familial cases with autosomal dominant inheritance[1,3,7]. According to family history, KID syndrome in Patient 1 may be familial with reduced penetrance in her father and grandfather, who had strikingly dry and scaly skin with minimal visual symptoms. Patient 2 seems to be sporadic, since none of the parents were clinically affected and the mother was proven not to carry the p.D50N mutation in her blood cells. In this family, the *GJB2* p.D50N mutation could have arisen *de novo*, or one of the parents could be germline mosaic. Nevertheless, the unavailability of the father's DNA sample in

both examined families did not allow us to unambiguously conclude whether KID syndrome was sporadic or familial.

KID syndrome is considered a dysregulated hemichannel disorder because a common feature of *GJB2* missense mutations appears to be an aberrant gain of function of hemichannels, leading to perturb voltage gating and the control of hemichannels by extracellular Ca^{2+}.[6] Nevertheless, a genotype–phenotype correlation has emerged among KID patients. Patients with *GJB2* p.D50N mutation live into adulthood,[1,11] while infant death occurred in almost all patients with *GJB2* p.G45E and p.A88V.[12]

An affected Austrian family, mother and child showed mild-to-moderate hearing loss and the keratitis was mild to absent, while in a 12 year old Austrian, sporadic patient hearing loss was profound, and the keratitis was therapy-resistant.[11] Findings on our patients confirm the variability in the clinical course of the disease caused by *GJB2* p.D50N mutation. A common feature of patients described by Janecke et al.[11] and ours, was an appearance of skin lesions shortly after birth, although of different severity, and normal psychomotor development and intellectual ability. Cerebellar and neuromuscular defects, extremely rarely reported in KID syndrome, was described in patients with *GJB2* p.D50N mutation. A Portuguese boy had nystagmus, generalized hypotonia, spastic tetraparesia, and hypoplasia of the cerebellar vermis revealed by MRI.[13] Of note, both of our patients had slurred and slow speech, while Patient 2 walked on her tiptoes until 10 years of age. Described phenotype variability implies that the effect of *GJB2* p.D50N mutation can likely be modified by the genetic background of the patients. However, studying genetic modifiers is challenging in an extremely rare disease like KID syndrome.

Mental retardation was described in about 10% of patients with KID syndrome.[14,15] In addition, developmental delay is characteristic of autosomal recessive form of Kid syndrome (KIDAR; MIM#242150) caused by homozygous or compound heterozygous mutations in the *AP1B1* gene on 22q12.2. To the best of our knowledge, our Patient 1 is the first one reported to have a higher-than-normal IQ, which is indirect proof that IQ is probably distributed among KID population in the same manner as in the general population.

Although KID syndrome is a rare condition, it is both a therapeutic and a diagnostic challenge. Severe infections of the skin lesions and septicemia may have a fatal course during early childhood.[16] Patients with *GJB2* p.D50N mutation frequently develop squamous cell carcinomas.[1,17]

No therapy reported so far seems to offer long-term visual recovery. Pharmacotherapeutic strategies successes were reported to be mostly limited, due to the decrease in irritative symptoms, but not in regard to visual rehabilitation.[18] The treatment of ocular manifestation includes ocular lubricants, autologous serum, tetracycline, and anti-inflammatory agents, including topical corticosteroids and topical cyclosporine A. Several studies have found that topical immunosuppressive therapy of corticosteroids and topical cyclosporine A improves ocular surface disease. Both patients have less irritable eyes and feel satisfied with therapy. Subsequently the disease progressed after discontinuation of the local corticosteroid therapy. The use of gas-permeable contact lenses may enhance visual acuity and quality of life in advanced cases with corneal neovascularization. Patients treated with subconjunctival bevacizumab, with partial response of corneal neovascularization and symptomatic improvement, have been described in the literature.[19] Systemic treatment with retinoids such as isotretinoin may worsen the ocular surface disease. However, mild vision and hearing improvement have been reported with acitretin.[19,20]

There were some attempts in doing surgery for KID syndrome, such as corneal opacification. Results on limbal stem cell transplantation, superficial keratectomy and penetrating keratoplasty were not successful, and they lead to the recurrence of the disease. Finally, the only possibility for better visual outcome may be the Boston Keratoprosthesis.[20]

CONCLUSION

Two Serbian KID patients expand our knowledge of the phenotype variability among patients carrying *GJB2* p.D50N mutation.

Controlled studies on local steroid therapy administration in KID syndrome are needed in order to improve our knowledge about this form of therapy.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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