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A Novel Frameshift Mutation of the *IKBKG* Gene Causing Typical Incontinentia Pigmenti

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SUMMARY

Introduction Incontinentia pigmenti (IP) is a rare X-linked dominant genodermatosis. Mutations of the *IKBKG* gene are responsible for IP. A deletion of exons 4–10 can be found in 80% of patients with IP. There are 69 different mutations of the *IKBKG* gene that have been reported.

Case Outline A proband, female patient from a family without previously diagnosed IP is reported. She had skin and dental changes typical of IP. The diagnosis was made according to updated IP criteria. Pathohistological and ultrastructural analysis of skin biopsy confirmed the diagnosis. However, the common deletion of exons 4–10 in the *IKBKG* gene could not be detected. Sequencing revealed the indel (deletion/insertion) mutation c.641_647delGCATGGAinsAT (p.Arg214HisfsX38) in exon 5 of the *IKBKG* gene. Because this mutation could not be detected in the unaffected mother of the proband, it seems to be a *de novo* mutation.

Conclusion The registered novel frameshift *IKBKG* mutation c.641_647delGCATGGAinsAT (p.Arg214HisfsX38) can be considered to be the cause of IP in this case.

Keywords: incontinentia pigmenti; *IKBKG* gene; frameshift mutation; genodermatosis; diagnosis

INTRODUCTION

Incontinentia pigmenti (IP) is a rare X-linked dominant genodermatosis that appears almost exclusively in females and is usually lethal *in utero* for males [1]. The *IKBKG* (*inhibitor of kappa-B kinase gamma*, previously *NEMO*) gene is the only gene known to be associated with IP [2]. Mutations of the *IKBKG* gene are responsible for IP. A deletion of exons 4–10 in the *IKBKG* gene can be found in 80% of IP patients [1]. To date, in IP patients 69 different mutations in the *IKBKG* gene have been reported [3, 4, 5]. These mutations originate from different molecular mechanisms [6]. The *IKBKG* gene product NEMO/IKKγ is required for activation of NF-κB (nuclear factor kappa-B) transcription factor. As a consequence of the *IKBKG* gene mutation, its accurate product does not arise and NF-κB activation does not occur [1]. At the skin level, NF-κB appears to have a dual role in cell growth and apoptosis. The phenotypic expression of *IKBKG* gene mutation is highly variable [1]. No genotype–phenotype correlation is apparent from the comparison of patients with different loss-of-function mutations [7].

It is noteworthy that some hypomorphic mutations in the *IKBKG* gene, reducing but not eliminating NF-κB activation, were found in surviving male patients. These males are affected by a different disease, named hypohidrotic ectodermal dysplasia associated with severe immunodeficiency (EDA-ID) or occasionally associated with osteopetrosis and lymphoedema (OL-EDA-ID) [7].

CASE REPORT

In this study, a female patient from a family without previously diagnosed IP is reported. IP diagnosis was made according to updated criteria [8]. The family pedigree was constructed, and routine laboratory findings for the proband and the mother were obtained. The investigation protocol followed the guidelines of the Helsinki Declaration and was approved by the Clinical Center of Serbia Ethics Committee. Written informed consent was obtained from all participants or their parent/guardian.

The pedigree analysis revealed that there were no other family members with IP stigmata. The proband's mother had two sisters. One died one month after birth (of unknown reason), and the other was healthy. The proband from clinically healthy nonconsanguineous parents was born at term by Caesarean section. She was the first child from a first normal pregnancy. At birth she had vesiculo-bullous lesions, typical for IP stage 1, grouped along Blaschko's lines. The lesions were located on the extremities, trunk, and back, with more on the left side. A skin biopsy was taken, and skin samples were prepared for light and electron microscopic investigation in a routine way [9]. Pathohistologically intraepidermal vesicles with eosinophils, apoptotic keratinocytes, and eosinophils infiltrating the epidermis and dermis were found, indicating IP stage 1 [10]. On light microscopy, apoptotic keratinocytes are characterized by a condensed and basophilic nucleus and eosinophilic homogenization of the cytoplasm, which sometimes contains

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irregular basophilic materials. Ultrastructural analysis revealed keratinocytes and dermal cells in the process of apoptosis. Eosinophilia of 29% was registered. After a couple of months the skin lesions evolved through stages 2 and 3.

The proband was 32 months old. Some of the skin changes already evolved into stage 4. In addition to hyper- and hypopigmented macules, proband had conical teeth, and her dentition had been delayed. There were no abnormal neurological and ophthalmological findings. To confirm IP diagnosis, molecular genetic testing for *IKBKG* gene mutation was performed.

Blood samples were collected and used to extract DNA using standard protocols. Molecular genetic testing was done at Diagenos, Center for Medical Genetics, Osnabrueck, Germany. For testing a modified polymerase chain reaction (PCR) protocol was performed [1]. However, the common deletion of exons 4–10 in the *IKBKG* gene could not be detected. Sequencing revealed the indel (deletion/insertion) mutation c.641_647delGCATGGAinsAT (p.Arg214HisfsX38) in exon 5 of the *IKBKG* gene, a heterozygous frameshift mutation with a premature termination signal. This mutation could not be detected in the unaffected mother of the proband.

DISCUSSION

The proband developed skin and dental changes typical for IP, and with an unambiguous clinical diagnosis she met updated IP diagnostic criteria [8]. Slightly higher expression of skin lesions on the left side was consistent with literature data [10]. Pathohistological findings corresponded to the stage 1 of IP and confirmed the diagnosis [11]. Ultrastructural analysis revealed apoptotic changes of keratinocytes that are typical for IP [1]. The mutation c.641_647delGCATGGAinsAT (p.Arg214HisfsX38) that was found in the proband has not been described as a causative mutation in the previous literature or in mutation databases (HMGD, Cardiff) [3]. The mutation resulted in an altered amino acid sequence beginning at position 214 and subsequently in a premature termination signal. Because this mutation could not be detected in the unaffected mother of the proband, it seems to be a *de novo* mutation. The local high frequency of micro/macro-homologies, tandem repeats, and repeat/repetitive sequences makes the *IKBKG* gene locus susceptible to novel pathological IP alterations [12]. The novel mutation has probably been generated by *de novo* events during parental gametogenesis, whose origin could be due to the peculiar genomic architecture of the *IKBKG* gene locus [6, 12]. However, gonadal mosaicism can't be ruled out – either maternal or paternal.

The phenotypic expression of *IKBKG* gene mutation is highly variable, even among related patients with the same mutation [1]. In contrast, patients with different *IKBKG* mutations may have the same clinical phenotype [1]. The presented patient has a typical IP phenotype with an accelerated course of skin changes but novel *IKBKG* gene mutation. Variability of the IP phenotypic expression was likely to be the result of the skewed X-chromosome inactivation [1], the pleiotropic role of the NEMO/IKKγ [6], or dimer-specific regulatory mechanisms within the NF-κB family of transcription factors [12, 13].

A large scale of different deletions of exons 4–10 has been identified in the *IKBKG* gene [10]. The presence of common *IKBKG* exons 4–10 deletion in six Serbian IP patients has been reported [14]. This mutation corresponds to the majority (80%) of *IKBKG* mutations in IP [1, 10]. In the remaining 20% of patients with IP, the mutation is hidden by the second copy of the *IKBKG* gene and the presence of a highly homologous *IKBKG* pseudogene [10]. In cases of hidden mutations [10], when no large deletion is identified in the gene, while phenotypical expression of the disease is highly suggestive of an *IKBKG* gene anomaly, a microrearrangement can be searched for using direct sequencing of the coding regions [7]. Besides the 69 different *IKBKG* gene mutations published, in the presence of a single IP minor criterion when other IP major criteria are absent, the newly detected novel mutation c.641_647delGCATGGAinsAT (p.Arg214HisfsX38) would be acceptable for making a diagnosis among female first-degree relatives [3, 8].

In conclusion, in the proband with typical IP skin and dental phenotype the novel *IKBKG* gene mutation c.641_647delGCATGGAinsAT (p.Arg214HisfsX38) was registered. This novel *IKBKG* frameshift mutation can be considered to be the cause of IP in this case.

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Нова *frameshift* мутација гена *IKBKG* као узрок инконтиненције пигменти

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КРАТАК САДРЖАЈ

Увод Инконтиненција пигменти (ИП) је ретка генодерматоза која се наслеђује доминантно везано за *X*-хромозом. За појаву ИП одговорне су мутације гена *IKBKG*. Код 80% болесника са ИП нађена је делеција на егзонима 4–10 гена у *IKBKG* гену. Досад је код болесника са ИП утврђено 69 различитих мутација на овом гену.

Приказ болесника Пробанд је била девојчица из породице у којој досад није дијагностикована ИП. Она је на кожи и зубима имала промене типичне за ИП. Дијагноза је постављена применом унапређених критеријума за ИП. Дијагнозу су потврдиле патохистолошка и ултраструктурна анализа биопсије коже. Код пробанда није откривена делеција егзона 4–10 гена *IKBKG*. Секвенционирањем је показано присуство indel (deletion/insertion) мутације *c.641_647delGCATGGAinsAT (p.Arg214HisfsX38)* егзона 5 на гену *IKBKG*. Пошто ова мутација није откривена код мајке пробанда, изгледа да је у питању мутација *de novo*.

Закључак Новооткривена *frameshift* мутација гена *IKBKG c.641_647delGCATGGAinsAT (p.Arg214HisfsX38)* може се сматрати узрочном ИП.

Кључне речи: инконтиненција пигменти; ген *IKBKG*; *frameshift* мутација; генодерматоза; дијагноза

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