De novo NEMO Gene Deletion (Δ4–10) – a Cause of Incontinentia Pigmenti in a Female Infant: A Case Report

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ABSTRACT

Incontinentia pigmenti (IP) is a rare, inherited, multisystem genodermatosis. It is transmitted as an X-linked dominant trait. The disorder is a consequence of mutations in the NEMO gene (Xq28) that completely abolish expression of the NF-κB essential modulator. Here we present a female infant of healthy nonconsanguinous, young parents with a clinically evident first phase of IP. PCR analysis of patient’s peripheral blood lymphocytes DNA was done for detection of NEMOΔ4–10 deletion. Skin changes present at birth appertain to first inflammatory stage. However, a pathohistological feature of the skin biopsy showed second phase of disease. Genetic testing of diseased child revealed Δ4–10 in NEMO gene. However, the assumption that the female child has familial IP was rejected as PCR performed on the mother’s leukocytes did not record the presence of the same mutation. Moreover, the existence of a healthy male infant of the same mother as well as the lack of any phenotypic signs of the disease in other family members additionally support that IP was not inherited, but it was a consequence of de novo NEMO gene mutation. In conclusion, here we describe a Croatian female with clinical IP phenotype having de novo genomic rearrangements in the NEMO gene.

Key words: incontinentia pigmenti, NEMO gene, mutation, female infant, Croatia

Introduction

The familial Incontinentia pigmenti syndrome (IP) or ‘classical’ incontinentia pigmenti (also called Bloch-Sulzberger syndrome, degenerative melanosis coria and Asboe Hansen disease) is a rare multisystem genodermatosis that segregates as an X inherited dominant condition, usually lethal prenatally in males. In about 80% of presented cases, IP is characterized by a distinctive swirling pattern of the skin, various congenital abnormalities and malformations of the head and neck (microcephaly, ophthalmological defects), skeletal system (kyphoscoliosis, hemivertebrae, and hard palate defects), hair, nails, eyes and teeth1. About 25% of the patients have mental retardation, slow motor development, spastic tetraplegia and diplegia2-4.

More than 95% of the reported cases are females5. Survival of affected men is probably the result of the somatic mosaicism of the X chromosome, or an extra X chromosome, or genetic heterogeneity, or less deleterious mutations6. The IP gene (NEMO or IKK, inhibitor kappaB kinase) localized on Xq28,7 encodes for the NF-κB essential modulator (the regulatory subunit of the IkB kinase complex) which is indispensable for activation of NF-κB transcription factor. When activated, NF-κB controls the expression of multiple genes and protects cells against TNF-α-induced apoptosis. IP cells are highly sensitive to pro-apoptotic signals8-10. The most common mutation responsible for disease development is the 4–10 NEMO gene deletion which completely abolishes the expression of the NF-κB essential modulator8,10,11.

Heterozygous females are gene carriers and have a high frequency of spontaneous abortions.
Early diagnosis of IP is based on the presence of abnormal skin pigmentation often present at birth. It rarely appears after the first two months. The cutaneous lesions evolve through four stages: vesiculobullous, verrucous, hyperpigmentary, and atrophic stage. Their sequence can be irregular, their duration variable and they can overlap. Earlier stages can also occur in utero and do not progress after birth.

Here we present a case of a female infant of healthy nonconsanguinous, young parents with a clinically evident first phase of IP, caused by de novo Δ4–10 deletion in the NEMO gene.

**Methods**

To investigate the NEMO gene deletion (Δ4–10 exons), PCR amplification of DNA from blood leukocytes was performed using two pairs of primers. For the nonmutated gene these were diagnostic F (5’AGGGCTTAGAGCGTGGCTTA-3’) and JFT3R (5’-CTCGGAGACACAGGACAGGAA CA GCA-3’) which produced a fragment of 1186 bp and diagnostic F and 5R NEMO (5’-CTGCCTTGAGGTTGTCAACGGCTT -3’) which amplify the gene when deletion is present (1039 bp). Amplification was performed for 30 cycles (denaturation – 1 min at 94°C, annealing – 1 min at 66 °C, and elongation – 1 min at 72 °C). The PCR mixture (10 μl) contained 100 ng of genomic DNA, 1xPCR buffer, 1.6 mM MgCl₂, 0.67 μM of each primer, and 0.2 μM dNTPs. PCR products (10 μl) were analysed on 1% agarose gel. The band sizes were compared with ones obtained from DNA samples of known female carriers and healthy female controls.

All patients’ samples and clinical details were obtained upon receiving patients’ consent and the local ethical-committee approval.

**Results**

A 3-week-old infant female from the second normal pregnancy was presented to the Department of Dermatology with unusual light brown streaks and whorls with vesicles on the skin of the left arm, trunk and left leg. These changes have been present since birth. The history indicated normal pregnancy and parents were healthy nonsanguinous. The first child was a healthy 2-year-old male child.

Additional cutaneous examination revealed linear macular hyperpigmentation and linear accumulations of clear vesicles and tight papules, which confluence in some areas. They were associated with smooth, red plaques, of irregular location on the lower extremities, sides of the trunk and around the perigenital region. The slight hyperpigmentation followed the lines of Blaschko (Figure 1).

Routine physical examination revealed a child of normal stature without any stigma. She displayed normal gross motor development. Associated congenital anomalies, which are characteristic for IP, were not present. Hematological analysis showed marked blood eosinophilia (29%) and leukocytosis (10 × 10⁹/L). The biochemical analyses as well as the CT scan were under normal limits. The brain ultrasound and ophthalmological findings as well as the EEG examination were within physiological limits. Blood samples were obtained for karyogram and DNA analysis.

Pathohistological examination revealed the second phase of the disease based on the findings of basketwave hyperkeratosis and slight acanthosis with interepidermal invasion of eosinophils and whorls of keratinocytes with scattered dyskeratotic cells. The basal cells showed

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**Fig. 1.** Clinical feature of infant girl suffering from IP: gluteal and femoral region.

**Fig. 2.** High-power view of cutaneous IP lesion with characteristic whorls of keratinocytes and eosinophils between epidermal cells; (H&E; original magnification x 400).
vacuolisation. In dermis, a mild perivascular chronic inflammatory infiltrate was found. Collagen tissue was normal. Leukocytosis and eosinophilia, characteristic for IP were also found (Figure 2).

A cytogenetic examination yielding normal results (46, XX), was performed to exclude chromosomal aberrations.

PCR analysis revealed deletion of exons 4–10 in NEMO gene in the child, but not in any other family member, including the mother (Figure 3). Moreover, the child’s mother has never had any skin or other manifestation which could indicate IP. Only the infant’s grandmother has had one spontaneous miscarriage of unknown gender of the fetus. All these findings indicate that de novo deletion of the NEMO gene appeared in this particular young girl (Figure 4).

On the basis of the typical hyperpigmentation of blaschkoid pattern, vesicles and histological examination as well as the results of PCR analysis, the diagnosis of incontinentia pigmenti was confirmed.

Skin changes present at birth made this patient highly suspective to IP. Clinical manifestations of the skin appertain to first, inflammatory stage. However, the pathohistological feature of the skin biopsy showed a second, progressive verrucose phase of disease, with an absence of pigment in the basal cells and a large quantity of melanin in the melanophages in the upper dermis. Such disproportion between clinical and pathohistological findings regarding the phase of IP disease, is quite unusual. Similar data in available literature could not be found. However, it might be expected that with the course of time the skin changes will get worse and finally equalize with pathohistological findings that will categorize IP to the second phase of disease.

Genetic testing of diseased child revealed Δ4–10 in the NEMO gene, further supporting the clinical diagnosis, incontinentia pigmenti. However, the assumption that the female child has familial IP was rejected as the PCR performed on the mother’s leukocytes did not reveal the presence of the same mutation as found in the child. Moreover, the existence of a healthy male infant of the same mother, as well as the lack of any phenotypic signs of the disease in other family members additionally support the conclusion that IP was not inherited, but is a consequence of de novo NEMO gene mutation.

However, after the final, comprehensive, medical examination of all family members we can say with noticeable certainty that the disease was caused by de novo mutation in the NEMO gene typical for familial IP type.

The cause of this de novo mutation is unknown. One of the possible explanations could be that it was caused by gene rearrangement during paternal meiosis. Namely, when IP occurs as a result of de novo, the usually occurs in the NEMO inherited from the father, just opposite to the inheritance of the form of the disease which is mostly inherited from the mother.

Some other de novo NEMO gene changes in cases of sporadic IP, such as frameshift, missense and nonsense mutations, have been described as well.

After the disease was proven by genetic testing, the genetic counseling for the parents was provided. They were informed about the nature, inheritance, and implications of this disorder with special emphasis on per-
sonal, cultural, and ethical issues that their child might face. It was stressed to them that their child descendants will follow the X linked pattern of heredity i.e, half of the male conceptuses (those with the mutant) will be miscarried. Thus, at delivery the expected sex ratio of offspring will be: 33% females; 33% females; 33% males.

Clinical and pathohistological diagnosis together with molecular analysis are a part of a conventional procedure for every congenital skin disorder. In this particular case the genetic testing, by which the disease was finally proven, gave the basis for family counseling.

Acknowledgement

This work was supported by the Croatian Ministry of Science, Education and Sports, grant number 098-0982464-2394.

REFERENCE


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DE NOVO DELECIJA GENA NEMO (∆4–10) – UZROK NASTANKA INCONTINENTIA PIGMENTI U ŽENSKOG DJETETA: PRIKAZ SLUČAJA

SAŽETAK