Coll. Antropol. **32** (2008) 4: 1259–1262
Case report

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

Vida Čulić[1], Dragana Gabrić[2], Neira Puizina-Ivić[3], Katja Rozman[4], Borut Peterlin[5] and Jasminka Pavelić[4]

[1] Department for Medical Genetics, Paediatric Clinic, University Hospital Center »Split«, Split, Croatia
[2] University Dental Clinic, University Hospital Center »Zagreb«, Zagreb, Croatia
[3] Department of Dermatology, University Hospital Center »Split«, Split, Croatia
[4] Division of Molecular Medicine, Institute »Ruđer Bošković«, Zagreb, Croatia
[5] Medical Genetics, Gynecology Clinic, University Center Ljubljana, Ljubljana, Slovenia

A B S T R A C T

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-kappaB 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 NEMO Δ4–10 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 teeth[1]. About 25% of the patients have mental retardation, slow motor development, spastic tetraplegia and diplegia[2–4]

More than 95% of the reported cases are females[5]. 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 mutations[6]. 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 TNFα-induced apoptosis. IP cells are highly sensitive to pro-apoptotic signals[8–10]. 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 modulator[3,10,11].

Heterozygous females are gene carriers and have a high frequency of spontaneous abortions.

Received for publication June 1, 2008

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[6]. 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'AGGGCTTAGAGCGCC TGGCTTA-3') and JFT3R (5'-CTCGGAGACACAGGAA CCA GCA-3') which produced a fragment of 1186 bp and diagnostic F and 5R NEMO (5'-CTGCCTTGAGGTTGT CAACGGCTT-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 (10μl) contained 100 ng of genomic DNA, 1xPCR buffer, 1U Taq DNA polymerase, 1.6 mM $MgCl_2$, 0.67 μM of each primer, and 0.2 μM dNTPs. PCR products (10μl) 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.

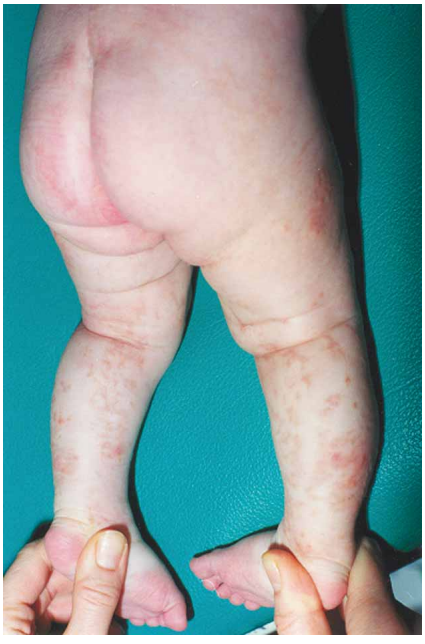

Fig. 1. Clinical feature of infant girl suffering from IP: gluteal and femoral region.

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 confluate 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 (10×10^9/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

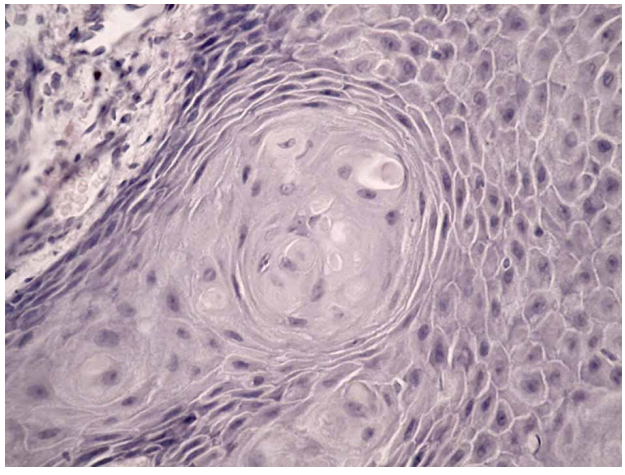

Fig. 2. High-power view of cutaneous IP lesion with characteristic whorls of keratinocytes and eosinophils between epidermal cells; (H&E; original magnification × 400).

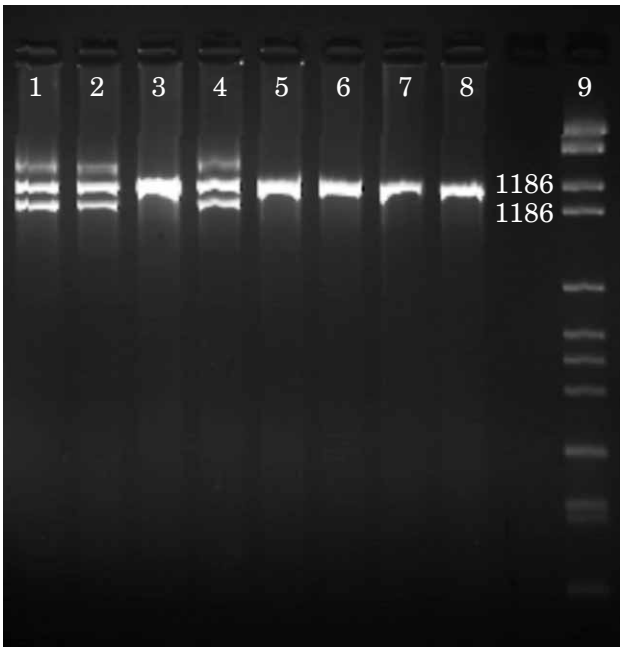

Fig. 3. Separation of the PCR products by electrophoresis in a 1% agarose gel. Template DNA are as follows: lanes 1 and 2: blood DNA from affected child (normal, upper band, at 1186 bp and the lower, with deleted exons, at 1039 bp); lane 3: unaffected mother of diseased child (only normal band is present); lane 4: a known female carrier of NEMO Δ4–10 (positive control); lanes 5 – 8: four healthy female controls; lane 9: molecular weight marker.

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.

Discussion

Here we describe a Croatian female with clinical IP phenotype having *de novo* genomic rearrangements in the NEMO gene. Although the mutations of this gene are ascribed to a familial type of IP, it is obvious that in some cases the mutation is not inherited.

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 pathohistolological 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 examine 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[3]. Namely, when IP occurs as a result of *de novo* mutation, the mutation usually occurs in the NEMO gene inherited from the father, just opposite to the inheritance of the familial 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[10].

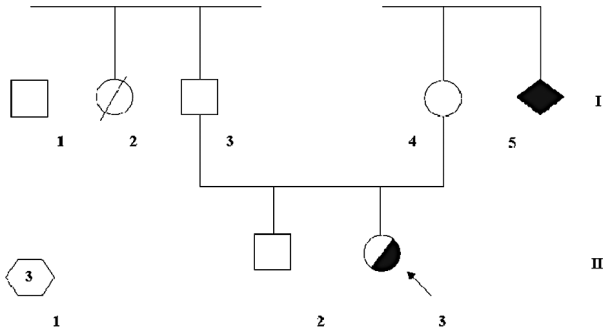

Fig. 4. Pedigree data of the family with de novo NEMO gene mutation causing IP. I and II – generations; 1, 2, 3 etc. – offsprings – birth order within generation; I-1, I-3, II-2 – healthy males; I-4 – healthy females; I-2 – dead female; II-I – 3 spontaneous abortions; II-3 (arrow) proband – affected female.

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 personal, 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

1. SMAHI A, HYDEN-GRANSKOG C, PETERLIN B, VABRES P, HEUERTZ S, FULCHIGNONI-LATAUD MC, DAHL N, LABRUNE P, LE MAREC B, PIUSSAN C, TAIEB A, VON KOSKULL, HORS-CAYLA M C, Hum Mol Genet, 3 (1994) 273. — 2. MIROWSKI GW, CALDEMEYER KS, J Am Acta Dermatol, 43 (2000) 517. — 3. The International Incontinentia Pigmenti (IP) Consortium, Nature, 405 (2000) 466. — 4. HADJ-RABIA S, FROIDEVAUX D, BODAK N, HAMEL-TEILLAC D, SMAHI A, TOUIL Y, FRAITAG S, DE PROST Y, BODEMER C, Arch Dermatol, 139 (2003) 1206. — 5. CARNEY RG, Arch Dermatol, 112 (1976) 535. — 6. BODAK N, HADJ-RABIA S, HAMEL-TEILLAC D, DE PROST Y, BODEMER C, Arch Dermatol, 139 (2003) 201. — 7. LANDY SJ, DONNAI D, J Med Genet, 30 (1993) 53. — 8. ARADHYA S, WOFFENDIN H, JAKINS T, BARDARO T, ESPOSITO T, SMAHI A, SHAW C, LEVY M, MUNNICH A, D'URSO M, LEWIS RA, KENWRICK S, NELSON DL, Hum Mol Genet, 10 (2001) 2171. — 9. MAKRIS C, ROBERTS JL, KARIN M, Mol Cell Biol, 22 (2002) 6573. — 10. FUSCO F, BARDARO T, FIMIANI G, MERCADANTE V, MIANO MG, FALCO G, ISRAEL A, COURTOIS G, D'URSO M, URSINI MV, Hum Mol Genet,13 (2004) 1763. — 11. COURTOIS G, GILMORE TD, Oncogene, 25 (2006) 6831.

J. Pavelić

Institute »Ruđer Bošković«, Division of Molecular Medicine, Bijenička cesta 54, P.O. Box 180, 10002 Zagreb
e-mail: jpavelic@irb.hr

DE NOVO DELECIJA GENA NEMO (Δ4–10) – UZROK NASTANKA INCONTINENTIA PIGMENTI U ŽENSKOG DJETETA: PRIKAZ SLUČAJA

SAŽETAK

Incontinentia pigmenti (IP) je rijetka, nasljedna, multisistemska genodermatoza. Prenosi se kao X-vezano dominantno svojstvo. Bolest je posljedica mutacija gena NEMO (Xq28) koje potpuno dokidaju ekspresiju NF-kappaB. U radu je prikazan slučaj ženskog novorođenčeta (mladih, zdravih roditelja) s klinički dokazanom prvom fazom IP. Delecija gena NEMO (Δ4–10) dokazana je PCR-analizom DNA izolirane iz perifernih limfocita. Promjene na koži vidljive odmah nakon rođenja djeteta upućivale su na prvu, inflamatornu fazu bolesti. Međutim, histopatološka analiza bioptata kože pokazala je da se radi o drugoj fazi bolesti. Genetskim testiranjem dokazana je delecija Δ4–10 u genu NEMO. Međutim, pretpostavka da dijete ima naslijeđeni, obiteljski oblik IP odbačena je nakon što je pokazano da majka ne nosi ovu mutaciju. Ovo mišljenje potkrijepljuje i činjenica da roditelji bolesne djevojčice imaju i zdravo muško dijete, te odsutnost bilo kakvih fenotipskih znakova bolesti u bilo kojem članu obitelji. Prema tome, u djevojčice bolest nije naslijeđena, već je posljedica de novo delecije gena NEMO. U zaključku, u radu opisujemo djevojčicu sa kliničkim znakovima IP i genetski dokazanim de novo dearanžmanom gena NEMO.