Unusual Father-to-Daughter Transmission of Incontinentia Pigmenti Due to Mosaicism in IP Males

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Incontinentia pigmenti (IP; Online Mendelian Inheritance in Man catalog #308300) is an X-linked dominant ectodermal disorder caused by mutations of the inhibitor of κ polypeptide gene enhancer in B cells, kinase γ (IKBKG)/nuclear factor κB, essential modulator (NEMO) gene. Hemizygous IKBKG/NEMO loss-of-function (LoF) mutations are lethal in males, thus patients are female, and the disease is always transmitted from an IP-affected mother to her daughter. We present 2 families with father-to-daughter transmission of IP and provide for the first time molecular evidence that the combination of somatic and germ-line mosaicism for IKBKG/NEMO loss of function mutations in IP males resulted in the transmission of the disease to a female child. We searched for the IKBKG/NEMO mutant allele in blood, urine, skin, and sperm DNA and found that the 2 fathers were somatic and germ-line mosaics for the p.Gln132×mutation or the exon 4–10 deletion of IKBKG/NEMO, respectively. The highest level of IKBKG/NEMO mutant cells was detected in the sperm, which might explain the recurrence of the disease. We therefore recommend careful clinical evaluation in IP male cases and the genetic investigation in sperm DNA to ensure correct genetic counseling and prevent the risk of paternal transmission of IP.

abstract

Incontinentia pigmenti (IP; Online Mendelian Inheritance in Man [OMIM] catalog #308300) is a rare, multisystemic genomic disorder. It is primarily a sporadic condition for which >2000 cases have been documented worldwide.1,2 The disease has an X-linked dominant inheritance and affects the skin and other tissues of ectodermal origin. Skin lesions are the first clinical manifestation and appear in the neonatal period with a vesiculobullous eruption (stage I); they proceed in a 3-stage evolution varying in duration from months to years: the verrucous and hyperkeratotic stage (stage II), the hyperpigmented stage (stage III), and the hypopigmented stage (stage IV), usually continuing throughout life.3 These skin defects, following Blaschko lines, are considered the major criteria for an IP clinical diagnosis.4–6 Systemic involvement include ocular (mainly a retinal vascular involvement), neurologic (seizures, spastic paralysis, mental retardation), and dental (dental shape anomalies, hypodontia, and delayed eruption) abnormalities.6 IP is caused by inhibitor of κ polypeptide gene enhancer in B cells, kinase γ (IKBKG)/nuclear factor κB, essential modulator (NEMO) gene mutations (GenBankNM_003639.3, OMIM #300248).7 IKBKG/NEMO encodes for NEMO/inhibitor of the κB kinase γ (IKKγ), the


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The IP pathogenesis is due to a “functional mosaicism” created by an X inactivation that in females represents an advantage, protecting carriers of heterozygous IKBKG/NEMO mutations from the severe consequences seen in males. Cells expressing the mutated X chromosome are selectively eliminated, so IP females exhibit extremely skewed X inactivation. In contrast, males hemizygous for the IKBKG/NEMO loss of function (LoF) mutation show an extensive apoptosis responsible for the early fetal lethality. Although the classic IP phenotype is almost entirely restricted to females, occasionally 46,XY males present an IP phenotype. These rare cases show the characteristic skin lesions and, in accordance with the pathogenic mosaic model of the disease, they are postzygotic genetic mosaics for the IKBKG/NEMO mutation. IKBKG has also been diagnosed in males with a 47,XXX karyotype (Klinefelter syndrome). Generally IP males are isolated cases without evidence of transmission of IKBKG/NEMO mutation to the offspring, although unexpected types of inheritance, such as father-to-daughter transmission or multiple IP offspring from unaffected parents, have been reported. An unequivocal demonstration of gonadal mosaicism in the affected father as the cause of IP recurrence has not been reported.

CASE REPORT
We report an exceptional type of father-to-daughter transmission for an X-linked dominant disease in 2 families with IP-affected fathers.

The IP patient (Fig 1A; IP-II:1) in Family 1 is a 2-year-old girl presenting with retinopathy and periventricular leukomalacia due to premature birth (27 weeks’ gestation). In her first few months of life, she showed typical IP skin with vesicles on the trunk and limbs (stage I) that progressed to hyperkeratotic warty lesions (stage II) and pigmented spots in a linear and whorled pattern on the trunk and limbs (20 months, stage III). She had woolly hair on the vertex and an incomplete deciduous dentition; there were no alterations in her fingernails. A skin biopsy performed at stage I confirmed a marked dyskeratosis and eosinophilic infiltration consistent with IP (Table 1). Her father was diagnosed with IP (Fig 1A; IP-I:1). He is a 40-year-old man, born from unrelated parents. He presented at birth an IP-like cutaneous rash (stage I) and linear streaks of vesicles following Blaschko lines on the trunk, back, and extremities. A skin biopsy was not performed. An accessory cusp in 1 canine was noted. He had a normal 46,XY karyotype (Table 1).

Genomic DNA was extracted from peripheral blood leukocytes in all members of 2 trios (child/children, mother, and father) and from the skin, urine, and sperm of the affected fathers. The IKBKG/NEMO gene deletion (NEMOdel4-10), the risk alleles for IP (NEMO pseudogene [NEMOP] del4-10), and (duplication of MEdium Reiterated 67B [MER67B] repeat sequences [MER67Bdup]), and the mutational
analysis of the IKBKG/NEMO exons were performed by polymerase chain reaction (PCR) as previously described.\(^\text{22}\)

In Family 1 we identified a novel, nonsense mutation in exon3 (NM_003639:c.394C>T; NP_003630.1:p.Gln132\(^{\times} \)) of the IKBKG/NEMO gene in IP female II:1 (Fig 1A), predicted to produce a premature stop codon in position 132 in the coiled-coil 1 (CC1) domain and an inactive NEMO protein. The mutation was absent in the IKBKG/NEMO gene and pseudogene in the DNA from the peripheral blood of her IP father (Fig 1A; I:1) and of her clinically unaffected mother (Fig 1A; I:2). We excluded an event of gene conversion from the pseudogene to the IKBKG/NEMO gene that can occur, albeit rarely, in the parent germ line.\(^\text{10,12}\) Because the father (IP-I:1; 46,XY) presented the clinical features of IP (Table 1), we investigated whether he was a somatic and germ-line mosaic for NEMOc.394C>T, identified in his IP-female child. We screened for NEMOc.394C>T the DNA from an adult biopsy of the affected skin (hypopigmented, stage IV) and from the urine and sperm. Sequence analysis revealed the heterozygous NEMOc.394C>T mutation in the skin, urine, and sperm. However, we were unable to reveal the mutation in DNA from the fibroblast culture derived from the same skin biopsy (Fig 1B). One possible explanation is that the fibroblasts carrying the LoF NEMOc.394C>T mutation are susceptible to cell death during continuous culture in vitro.

FIGURE 1
DNA sequencing and analysis of the mosaicism in Family 1. (A) The sequence analysis of the c.394C>T in exon 3 of the IKBKG/NEMO gene on DNA from peripheral blood of each member of IP trios and (B) from different tissues of the IP-male father (I:1) is shown. The new heterozygous mutation NEMOc.394C>T is present in the IP proband (II:1) (A) and in affected skin, urine, and sperm DNA samples from the IP father (I:1) (B), whereas only the normal wild-type sequence is present in the peripheral blood of the mother (I:2) and father (I:1) (A) and in fibroblast cell lines derived from affected skin biopsy of the father (I:1) (B). The percentage of cellular mosaicism for the NEMOc.394T allele (C) in DNA from peripheral blood, fibroblast cell lines, affected skin biopsy, urine, and sperm samples of the IP-male father (I:1) is expressed as the mean of at least 3 experiments performed in triplicate (see Supplemental Tables 2 and 3).
In Family 1, DNA from peripheral blood cells, the mutated NEMOdel4–10 allele was detectable only in sperm DNA (Fig 2B). In Family 2, we detected the NEMOdel4–10 mutation in the 2 IP sisters (Fig 2A; IP-II:1; IP-II:2), which was absent in DNA from the blood of the parents (Fig 2A; I:1; I:2). We then established the degree of mosaicism in the IP patient I:1 DNA from different tissue sources by quantifying the copy number variations in the IP locus (Fig 2D) (Supplemental Tables 4 and 5) by quantitative PCR using probes previously described.10 The percentage of cells carrying the NEMOdel4–10 allele in DNA from the urine was 25%, whereas in the germ line it was 35% (Fig 2C). We did not detect the NEMOdel4–10 mutant cells in the peripheral blood and skin lesions of the IP father (Fig 2C), although some IKBKG/NEMO mutant cells in the affected skin would have been expected, as observed in the IP father of Family 1 (Fig 1C and Supplemental Tables 4 and 5).
Overall these findings suggest the following:

1. Peripheral blood is not the appropriate tissue to reveal the somatic mosaicism in the IP male, although it represents the main source of DNA in IP routine diagnosis.

2. We recommend genetic investigation in sperm DNA because the gonadal cells carrying the IKBKG/NEMO LoF mutation are able to survive differently from other cells (fibroblasts and blood cells), which instead disappear because of a selective disadvantage due to the lack of NEMO protein.

The combined somatic and germ-line mosaicism in IP males, suggests a nonclonality of the germ line in which the mosaicism arose at a totipotent cell stage of development within the first few cell divisions of the embryo, probably by postzygotic mutation. Thus, the timing of the mutation should affect the abundance of the mutant cells, the presence of the mutation in the germ cells, and, by extension, the potential recurrence risk for the same mutation to be transmitted to multiple offspring.

CONCLUSIONS

The 2 cases reported here highlight that mosaicism should be considered...
as a possible inheritance pattern in genetic counseling with a recurrence risk estimation in families with male IP cases. In males with suspected IP, the causative IKBKG/NEMO mutation should be identified in sample DNA from a biopsy of the affected skin in childhood and in germ-line cells in adulthood.

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REFERENCES


ABBREVIATIONS

del4-10: deletion exon 4-10
IP: incontinentia pigmenti
IKBKG: inhibitor of κ polypeptide gene enhancer in B cells, kinase γ
LoF: loss of function
MER67Bdup: duplication of MER67B repeat sequences
NEMO: nuclear factor κB, essential modulator
NEMOP: NEMO pseudogene
NF-κB: nuclear factor κB
OMIM: Online Mendelian Inheritance in Man
PCR: polymerase chain reaction

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