Research Letter

Incontinentia Pigmenti in a Female Conceived by In Vitro Fertilization

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To the Editor:

Incontinentia pigmenti (IP) also known as Bloch-Sulzberger syndrome is an X-chromosome-linked genodermatosis caused by mutations of nuclear factor (NF)-κB essential modulator (NEMO) gene at Xq28. The vast majority of NEMO mutations involve identical deletion of exons 4–10 (ΔNEMO) causing IP [Aradhya et al., 2001]. The NEMO locus contains an inversely oriented, truncated and nonfunctional NEMO pseudogene that plays an essential role in de novo recurrent formation of the ΔNEMO mutation [Bardaro et al., 2003]. The NEMO protein is a regulatory component of a 1kB (NF-κB inhibitors) kinase complex required to activate the NF-κB pathway [Karin and Ben-Neriah, 2000]; NEMO gene mutations abolish protein function and eliminate NF-κB activity [Aradhya et al., 2001]. NF-κB is found in all cell types and is involved in activating an exceptionally large number of genes in response to infections, inflammation, and other stressful situations that require rapid reprogramming of gene expression and prevention from apoptosis [Karin and Ben-Neriah, 2000]. IP is usually fatal in male fetuses and thus most IP patients (>95%) are female [Cohen and Kurzrock, 1995]. The expression of IP in female patients can be modulated by selection against the X-chromosome with ΔNEMO gene causing skewed X-chromosome inactivation (XCI) [Aradhya et al., 2001]. The clinical spectrum of IP is broad and highly variable, with the skin lesions being the most prevalent features [Kim et al., 2006]. They are caused by the incontinence of melanin from the superficial epidermis into the dermis where it is cleared by macrophages [Cohen, 1994]. The most significant medical problems in IP are blindness, immunodeficiency and central nervous system defects, which can cause mental retardation and/or seizures. Other characteristics are blood eosinophilia, and dental, auricular, musculoskeletal, and cardiovascular anomalies [Kim et al., 2006]. The current report focuses on an in vitro fertilization (IVF) newborn girl who manifested IP due to a paternally inherited pathological NEMO gene. Informed consent for scientific publication was obtained from both parents.

The proposita is the only child of nonconsanguineous parents born after frozen mixed IVF and intracytoplasmic sperm injection embryo transfer. The mother and father were 34 and 39 years old, respectively, and had experienced infertility due to teratozoospermia for 11 years. The uneventful pregnancy ended in a term delivery. The birth weight of the female newborn was 3,600 g (0 standard deviations (SD)), length 48 cm (−1 SD), and the occipitofrontal circumference was 34 cm (−3 SD). At birth, vesicular lesions of the skin were detected covering arms, legs, and buttocks, continuing along the lateral side of trunk following Blaschko’s lines;
thus IP was suspected. Ultrasonography detected normal brain structure, but pre-retinal hemorrhage was diagnosed by an ophthalmologist. The chromosomal analyses from peripheral blood lymphocyte cultures of the patient and her parents were normal.

PCR-based screening of NEMO exons 4–10 deletion (Fig. 1a) on DNA from the parents’ blood samples yielded only the normal 733 bp product (wild-type gene) (Fig. 1b). Amplification of DNA from patient’s blood and father’s sperm cells revealed an additional 1,045 bp product characteristic of the NEMO mutation (Fig. 1b). In order to verify the presence of a mutation in the NEMO gene rather than in the pseudogene, deletion-specific PCR was performed using forward F3 and reverse R primers (Fig. 1a) [Bardaro et al., 2003]. The results confirmed the presence of ΔNEMO in the patient’s blood leukocytes and father’s sperm cells (data not shown). The XCI pattern was assessed by Eco147I restriction fragment length polymorphism (RFLP) analysis of androgen receptor gene exon 1 G/A (rs6152) SNP with and without methylation-sensitive endonuclease HpaII digested patient’s DNA. The results revealed GA heterozygosity and an extremely skewed paternal XCI at the age of 1 month. Following Eco147I-RFLP with HpaII digested DNA, the maternal unmethylated/active G allele was undetectable (HpaII-cleaved), while PCR amplified only the paternal methylated/inactive A allele.

At 1 month of age, the patient had a secondary infection of the skin with Methicillin-resistant Staphylococcus aureus (MRSA). Laboratory analyses revealed blood leukocytosis with eosinophilia while no other abnormal findings in blood count or serum albumin, liver enzymes, and C-reactive protein were seen. After a week of anti-bacterial treatment, T-cell function was evaluated and repeated at the age of 8 months. Lymphocyte subpopulations were almost within the normal range. Only absolute count and relative frequency of CD8+ T-cells were increased at 1 month, with the relative frequency staying slightly elevated by the end of 8 months. In vitro T-cell function in response to S. aureus enterotoxin B improved but a deficit in up-regulation of CD69 + CD4+ T-cells after mild phytohemagglutinin (10 μg/ml) stimulation persisted in the patient at 8 months.

At 8 months, no primary teeth had developed (expected between 6 and 10 months of age); the patient did not crawl but sat well without support (expected to develop at 6–10 and 6–7 months of age, respectively). The patient did not suffer from any severe systemic or local infections of the skin between 1 and 8 months of age. Skin lesions healed, but left hyper-pigmented patches.
This case illustrates that in vitro methods of reproduction may inadvertently result in genetic disorders in the offspring by overcoming infertility related to genetic diseases in a parent.

In this report of a newborn girl with IP who was born to her IVF-treated parents that had been infertile for 11 years, the patient carried the pathological ΔNEMO gene inherited from her father, who was a germ line mosaic. The patient manifested classic IP symptoms.

ΔNEMO gene mutations that cause IP can originate by intra-chromosomal misaligment in the paternal germine during meiosis or less frequently from inter- and intra-chromosomal exchange in the maternal germine [Aradhya et al., 2001]. It can also be inherited from a carrier mother with nonrandom XCI [Kenwrick et al., 2001] or could be a de novo mutation of mitotic origin in either of the parental X-chromosomes of a female child [Martinez-Pomar et al., 2005]. We report on an additional way of inheritance by paternal germine ΔNEMO which probably developed in the pre-meiotic stage of spermatogenesis. Our suggestion is supported by the observation that ΔNEMO persisted in father's sperm cells without a mutation being detected in peripheral blood cells. Therefore, since the father has teratozoospermia, which could be associated being a carrier of the mutation, it can be interpreted that it was not the IVF procedure itself that caused the problem but rather that it made it possible to pass on the gene to an offspring.

The fact that ΔNEMO in our patient was an inherited mutation could explain the development of extremely skewed XCI during the pre- or early postnatal period, allowing female fetuses to survive due to the negative selection of cells possessing X-chromosomes with the gene defect. Clinical manifestations, but also compensation for IP, seem to occur earlier in cases of inherited ΔNEMO germ-line transmission than in patients who acquired the mutations during the embryonic or fetal period [Martinez-Pomar et al., 2005].

In conclusion, although IVF treatment did not cause the gene defect in the present case, physicians and consultants in the field of infertility treatment should be aware that IVF may enable someone carrying a previously unrecognized genetic mutation to conceive and thereby pass on the genetic abnormality to their child. The couple in the current study may be offered prenatal diagnosis in another pregnancy.

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