Letters to the Editor

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Female Patient Showing Hypohidrotic Ectodermal Dysplasia and Immunodeficiency (HED-ID)

To the Editor:

Amorphic mutations of the *NEMO* gene (Smahi et al. 2000) lead to incontinentia pigmenti (IP [MIM 308300]), an X-linked dominant disorder in which heterozygous female patients exhibit skin lesions along the Blaschko lines, cicatrization of the retina, and hypodontia (Landy and Donnai 1993). The majority of hemizygous male fetuses die in utero. In the December 2000 issue of the *Journal,* Zonana et al. (2000) reported that hypomorphic mutations in the *NEMO* gene lead to an apparently novel X-linked recessive entity, hypohidrotic ectodermal dysplasia and immunodeficiency (HED-ID). HED-ID is characterized by features—ectodermal dysplasia and recurrent infections accompanied by dysgammaglobulinemia—distinct from those of IP. Zonana et al. (2000) stated that HED-ID affects only males and that female heterozygotes do not exhibit clinical manifestations of immunodeficiency. Independently of Zonana et al., in the March issue of the *Journal,* Aradhya et al. (2001) reported that hypomorphic mutations in *NEMO* lead to an "atypical form of IP in males" whose clinical features, including immunodeficiency, overlap those of HED-ID. We report a female patient with features of HED-ID and a heterozygous hypomorphic *NEMO* mutation to show that female heterozygotes can be overtly affected and that the wide phenotypic spectrum of female heterozygotes should be taken into account when counseling families with HED-ID.

The proband presented with hyperpigmented macules in a streaky configuration on the trunk and extremities at age 3 wk. Histologic examination revealed irregular basal melanosis and a slight increase in the number of melanocytes in the epidermis, but there was no evidence of the pigmentary incontinence typically observed in IP. The areas of skin involvement stabilized by age 5 mo. At that time, the proband and the mother had been reported as the first familial cases of "linear and whorled nevoid hypermelanosis" (Akiyama et al. 1994). At 4 years of age, the patient began to experience recurrent infections, including respiratory-tract infections, cervical lymphadenitis, otitis media, cellulitis, and soft-tissue abscesses. The patient had a history of chronic lung infections with secondary bronchiectasis and pulmonary hypertension at 8 years of age and developed renovascular hypertension at 10 years of age. Immunoglobulin determinations at age 10 years showed abnormally high levels of IgD, 1,520 mg/dL (normal range 1.8–8.5 mg/dL), and IgE levels elevated to 17,600 IU/mL (normal range <250 IU/mL). At 11 years of age, she exhibited eczematous, dry skin with hyperpigmentation along the Blaschko lines; decreased sweating; periorbital wrinkling of the skin; thin scalp hair and sparse eyebrows; conical incisors; and hypodontia with four permanent teeth missing. The patient died shortly after cardiac catheterization at 11 years of age. The proband's mother manifested hyperpigmented macules in a streaky configuration on the trunk and the extremities at several weeks of age. The mother, 44 years of age, never developed clinical manifestations of immunodeficiency. The younger sister of the proband developed hyperpigmentation along the Blaschko lines in infancy but is otherwise healthy at 7 years of age.

After obtaining informed consent, a genomic DNA sample from the proband's peripheral blood leukocytes was examined. Direct sequencing of the entire coding region of the *NEMO* gene (Smahi et al. 2000) revealed insertion of a cytosine (dupC1161) within a wild-type run of seven cytosines. This insertion resulted in a novel amino acid at codon 390–393 and a premature stop at codon 394. This mutation truncates the protein and deleted a putative zinc-finger domain. X-inactivation analysis of the proband's peripheral blood leukocytes based on the methylation status of the androgen-receptor gene (Parrish et al. 1996) revealed that the normal and the mutated chromosomes were randomly inactivated. We were unable to perform molecular analyses of the mother and the younger sister.

The dupC1161 mutation has been documented in three other pedigrees (Zonana et al. 2000; Aradhya et al. 2001). The phenotypic spectrum of the obligate carrier females in these families, including the one in this report, is very broad: HED-ID in the proband of the family described here, linear and whorled skin hyperpigmentation in the mother and the younger sister, "typical IP skin-pigmentation and retinal abnormalities" in families XL267 and XL344 (Aradhya et al. 2001), and "normal" (HED-ID-4, II-2; Zonana et al. 2000).

Variable phenotype in females with the same mutation could be explained by variations in expression due to differences in X inactivation among families, as suggested by Aradhya (2000). If so, an X-inactivation study could be helpful in predicting phenotypic outcome in a female heterozygote with the mutation. However, the proband in the family described here (HED-ID phenotype) and the female heterozygote in family XL344 (IP-like phenotype; Aradhya et al. 2001) yielded the same random X-inactivation pattern. We therefore suspect that an X-inactivation study would have limited predictive value.

To summarize, hypomorphic mutations in the *NEMO* gene can lead to the devastating HED-ID phenotype in female heterozygotes as well as males. The very broad phenotypic spectrum of female heterozygotes, ranging from normal to overt immunodeficiency, poses a challenge during counseling of families with HED-ID.

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Reply to Kosaki et al.

To the Editor:

The letter from Kosaki et al. (2001 [in this issue]) reports the first instance of immunodeficiency in a female heterozygous for a hypomorphic mutation of the X-linked *NEMO* gene. *NEMO* hypomorphic mutations result, in males, in the disorder hypohidrotic ectodermal dysplasia with immunodeficiency (HED-ID) (Zonana et al. 2000; Aradhya et al. 2001; Doffinger et al. 2001; Jain et al. 2001). In the 17 families previously reported, only males have had clinical immunodeficiency. As expected, owing to Lyonization, carrier females either were normal or had defects of their teeth, skin, or hair. A single female carrier, heterozygous for the same mutation reported in the family studied by Kosaki et al., had an elevated immunoglobulin A level (Zonana et al. 2000) but did not have immunodeficiency.

Null mutation of the *NEMO* gene generally results in prenatal lethality in hemizygous males and in incontinentia pigmenti in heterozygous females. Affected females are immunologically normal, presumably owing to preferential survival and proliferation of cells expressing the normal allele (skewed X inactivation). Female carriers with hypomorphic mutations have shown both random and skewed X inactivation, indicating that at least some of the mutations do not affect T- and B-cell survival or proliferation (Aradhya et al. 2001; Doffinger et al. 2001; Jain et al. 2001). Rarely, females can fully manifest X-linked recessive disorders, including other X-linked immunodeficiencies, such as Wiskott-Al-

drich syndrome (Puck and Willard 1998). However, in contrast to the patient reported by Kosaki et al., cases not due to cytogenetic abnormalities demonstrate skewed X inactivation, presumably of cells expressing the mutant allele. In these instances, the mutations do not impair cell proliferation or survival but still do cause cellular dysfunction.

It is difficult to reconcile Kosaki et al.'s finding of random X inactivation in the peripheral blood leukocytes with their patient's clinical manifestations. We would expect to find skewed X inactivation in which the X chromosome expressing the mutant allele is predominant. It is possible that a functionally important subset of B or T cells (Jain et al. 2001) would have displayed skewed X inactivation if Kosaki et al. had sorted the leukocytes into separate populations before analysis. Another remote possibility is that, unlike the patient's presumably heterozygous mother and sister, who had ectodermal findings but no immunodeficiency, the patient had a loss of her normal *NEMO* allele. The existence of a highly homologous *NEMO* pseudogene on the X chromosome makes it very difficult to distinguish hemizygosity from true heterozygosity.

Kosaki et al.'s findings are unique, but, as in other examples, fully manifesting females with X-linked recessive disorders—such as Duchenne muscular dystrophy and hemophilia B—usually represent rare events (Puck and Willard 1998). Families should still be counseled that generally, in HED-ID, only males manifest clinically significant immunodeficiency. Further cases of immunodeficient females with HED-ID must be described before such counseling should be altered.

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