Maternal Germline Mosaicism in Dominant Dystrophic Epidermolysis Bullosa

Peter B Cserhalmi-Friedman[*], Maria C Garzon[*], Edwin Guzman[‡], Amalia Martinez-Mir[*], Wendy K Chung[‡], Kwame Anyane-Yeboa[‡] and Angela M Christiano[*,†]

[*]Department of Dermatology, New York, New York, U.S.A.

[†]Department of Genetics and Development, New York, New York, U.S.A.

[‡]Department of Pediatrics, Division of Clinical Genetics, College of Physicians and Surgeons, Columbia University, New York, New York, U.S.A.

Correspondence: Dr Angela M. Christiano, Department of Dermatology, College of Physicians & Surgeons, Columbia University, 630 West 168th Street VC-1526, New York, NY 10032 Email:amc65@columbia.edu

To the Editor:

The dystrophic form of epidermolysis bullosa (DEB) can be inherited in both an autosomal dominant or a recessive fashion (Christiano and Uitto, 1996). The recessive form can range from a very severe condition (Hallopeau-Siemens DEB) to a relatively mild disease, clinically indistinguishable from the dominantly inherited mild form of DEB, (DDEB) (Fine *et al*, 2000). A new case of mild DEB, therefore, always presents the problem of differentiating recessively inherited vs. dominantly inherited DEB due either to *de novo* mutations or to germline mosaicism. The latter was previously suggested in the junctional form of epidermolysis bullosa, which originates from mutations in the genes encoding laminin 5 (Kivirikko *et al*, 1996), but has not been demonstrated in the case of DEB.

Here, we report a family with one child affected by mild DEB. At birth, blistering and denuded areas were present together with the syndactyly of the second and third toes. By the age of 11 mo, blistering was significantly reduced but the blisters healed with hyperpigmented and hypopigmented scars and milia. Both parents and an older sibling were clinically unaffected, and there was no family history of consanguinity or of a blistering disorder or skin fragility.

Screening for mutations in the COL7A1 gene in the patient using previously described techniques (Christiano *et al*, 1997) revealed a single glycine substitution in the triple helical domain of the type VII collagen gene (G2003R), which has previously been associated with Bart's syndrome, a form of DDEB (Christiano *et al*, 1996). This mutation, however, was not found in the peripheral blood DNA of the clinically unaffected parents and the sibling **Figure 1**. As nonpaternity based on parental interviews was unlikely, we concluded that the mutation was most likely a *de novo* event in the patient, and would be associated with little risk of recurrence. Nevertheless, the parents were appropriately counselled that the possibility of having another affected child existed, if the mutation

existed as a germline mosaic transmitted from either parent – a possibility that was considered remote.

Shortly after completing the mutation analysis, the mother became pregnant and the parents requested prenatal diagnosis. DNA isolated from the amniotic fluid sample was used as template for polymerase chain reaction amplification and automated sequencing. Unexpectedly, the mutation G2003R was also present in the fetus, suggesting that the initial occurrence was in fact not a *de novo* event. We could only explain the second occurrence of the identical mutation in this family by invoking the possibility of germline mosaicism. To further investigate the parental transmission of the mutation, we performed haplotype analysis using chromosome 3 microsatellite markers **Figure 1**, synthesized based on publicly available oligonucleotide sequences (Cooperative Human Linkage Center and Genome Database). Polymerase chain reaction products containing microsatellite markers for chromosome 3 were analyzed on a 6% nondenaturing polyacrylamide gel. We determined that all three offspring carried the same maternal chromosome 3 haplotype. The clinically unaffected sister of the patient and the affected fetus shared the same paternal chromosome; however, the patient carried the other paternal chromosome. Thus, we concluded that the mutation is most likely present in a percentage of cells in the germline of the mother.

To our knowledge, this study represents the first documented case of germline mosaicism in epidermolysis bullosa and serves as a reminder that germline mosaicism should be considered in every case when a mutation is found in the offspring but not in the parents. In the event of a *de novo* mutation, the family can be counselled that the likelihood of having another affected child is the same as in the general population. In the case of germline mosaicism, however, the likelihood of having another affected child is much higher, depending on the ratio of mutant to wild-type germ cells. This possibility has a significant impact on genetic counselling, and unfortunately, exact determination of the transmitting parent is not always possible. In the case of paternal transmission, a semen sample can be used for further studies to determine the percentage of mutant germ cells, and thus calculate an accurate risk of recurrence for the family. These findings heighten our awareness of the existence of unusual modes of inheritance in epidermolysis bullosa, which should be taken into account when counselling families about the recurrence risk for future offspring.

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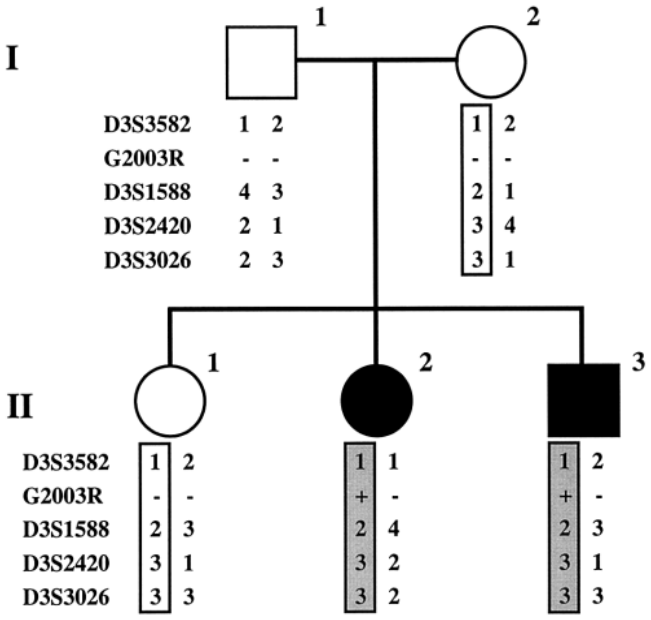
Figures



Haplotype analysis of a pedigree with DEB. The figure shows the haplotype of the family members, listed according to the microsatellite markers indicated on the left. The mutation G2003$ is also listed. The figure demonstrates that all three offspring (individuals II-1 to II-3) carry the same maternal (individual I-2) chromosome 3 haplotype. The two affected individuals (individuals II-2 and II-3) display different paternal chromosome 3 haplotype. The mutation G2003 is present only in individuals II-2 and II-3 (gray shading), suggesting that it is only contained in a percentage of the maternal chromosomes.