Chapter 8

Somatic mosaicism for the COL7A1 mutation p.Gly2034Arg in the unaffected mother of a patient with dystrophic epidermolysis bullosa pruriginosa

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Submitted manuscript
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To the editor,

Dystrophic epidermolysis bullosa (DEB) is a heritable blistering disorder that can be inherited either recessively (RDEB, OMIM #226600) or dominantly (DDEB, OMIM #131750, #131800), with both types being caused by mutations in the type VII collagen gene, COL7A1 (OMIM #120120). The phenotypic spectrum ranges from the most severe ‘RDEB, severe generalized’ phenotype to the mildest ‘DDEB, nails only’ phenotype.1 There is considerable overlap between milder RDEB and DDEB phenotypes, which poses difficulties in differentiating between RDEB and DDEB on clinical grounds alone, especially in early life: does a mild, sporadic case represent an RDEB case due to two mutations that are silent heterozygous, or a DDEB case due to either a de novo dominant mutation or mosaicism for a dominant mutation in one of the unaffected parents? Many presumed de novo dominant mutations have been reported (www.deb-central.org),2 but it is unknown what proportion of these, if any, was actually derived from a mosaic yet unaffected carrier parent.

There are several publications on somatic mosaicism in epidermolysis bullosa (EB), but these are mainly on the phenomenon of revertant mosaicism, in which a somatic mutational event corrects the mutant germline background.3 The number of reports describing ‘forward’ somatic mosaicism, in which a pathogenic mutation arises on a wild-type background and may lead to a disease phenotype, is, however, rather low. Forward mosaicism was demonstrated in a mildly affected mother of an EB simplex family due to the KRT5 mutation c.1649delG;4 paternal germline mosaicism for the recessive LAMB3 mutation p.Arg635X was reported in a family with Herlitz junctional EB;5 and maternal germline mosaicism for the dominant COL7A1 mutation p.Gly2003Arg was proven in a DDEB family.6 However, to the best of our knowledge, somatic mosaicism involving not only the germ cells but also other tissues has not been reported for DEB.

Here we describe a female patient (EB287) with a mild DEB phenotype whose unaffected mother proved to be mosaic for the causative dominant COL7A1 mutation. The index patient presented in our clinic at the age of 36 with pruritic papules and intermittent periods of itching in the groins and on the shins, ankles, forehead, and scalp (Figure 1). She had partly confluent milia on her occipital scalp, umbilicus, and ankles, and in the left auditory canal. Some milia on the dorsum of the hands, feet, and knees had been excised leaving small residual scars. Mild atrophic scarring was observed on her elbows, knees, dorsum of the left foot, and gluteal fold. Pigment alterations on the shins and mild hyperkeratosis on both soles were seen. The toenails were either absent or rudimentary and the 1st-3rd fingernails of both hands were dystrophic. Blistering had occurred from age 1, starting on the heels and later on the hands, elbows, knees, ankles, shins, and feet, but had markedly decreased after the age of 12. One old blister was observed on the right little toe. There were no other disease features. Immunofluorescence staining using the monoclonal antibody LH7:2 (Sigma-Aldrich, Poole, UK) revealed slightly reduced type VII collagen deposition at the cutaneous basement membrane zone and electron
**Figure 1.** DEB-pruriginosa phenotype in the index patient EB287. Clinical photographs of the index patient showing two pruritic lesions on the forehead (A), partly confluent and eroded milia on the occipital scalp (B), dystrophic nails of the 1st-3rd fingers of both hands (C-D), absent or rudimentary toenails and an old blister on the lateral aspect of the right 5th toe (E-F), very mild atrophy of the left elbow (G), erythematous pruritic papules and milia, partly confluent, on the lateral aspect of the right ankle (H), and one milium and atrophic scar on the lateral aspect of the left ankle (I).

**Figure 2.** The mother is somatic mosaic for the p.Gly2034Arg mutation. On the top, the reference sequence (NM_000094.3) for nucleotides 6098-6102, the c.6100G>A transition, and the corresponding translations. Mutation analysis disclosed the c.6100G>A (p.Gly2034Arg) missense mutation in the patient’s lymphocytes (A). The wild-type:mutant allele ratio was estimated to be 50:50, based on the relative heights of the Guanine (black) and Adenine (green) nucleotide peaks, indicating the mutation is present in 100% of cells. Mutation analysis in a blood sample of the patient’s mother revealed the presence of the mutation in a proportion of lymphocytes (B). The mutant allele was also detected to varying degrees in genomic DNA extracted directly from a full-thickness skin biopsy (C), cultured fibroblasts from the same skin biopsy (D), and buccal smear cells (E). A normal control chromatogram is shown for comparison (F). The wild-type:mutant allele ratios were later estimated more precisely by pyrosequencing (Table 1). The results were highly reproducible in up to 10 independent PCR analyses. A green ‘background’ peak at position 6100 was not observed in any of the control analyses.
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Microscopy showed a reduced number of hypoplastic anchoring fibrils in a biopsy from non-lesional skin (obtained after informed consent). The parents were meticulously evaluated but were found to have no DEB features. These data were consistent with a diagnosis of DEB pruriginosa (DEB-Pr), inherited either recessively or de novo dominantly.

Mutation analysis by direct sequencing of genomic DNA from peripheral lymphocytes disclosed two COL7A1 sequence alterations in the index. The first was the c.6100G>A transition in exon 73 leading to the p.Gly2034Arg missense mutation (Figure 2A). This mutation was first reported by Kon et al. and has since been shown to represent a recurrent DDEB mutation in different populations (www.deb-central.org). p.Gly2034Arg has also been described in DDEB-Pr and thus seemed to fully explain the patient’s phenotype. Further supporting this conclusion is the observation that another substitution of the same glycine-2034, p.Gly2034Trp, has also been implicated in DDEB-Pr.

The second alteration was the c.4889G>A transition in exon 51 that introduces a p.Arg1630Gln amino acid change. This variation has not been reported as pathogenic mutation, but it was found in the 1,000 Genomes project with a minor allele frequency of 0.001 and marked as a single nucleotide variation with unknown clinical significance (rs201196696, dbSNP, www.ncbi.nlm.nih.gov/projects/SNP/). p.Arg1630Gln is located in a conserved portion of the collagenous triple-helix domain, but the chemical differences between arginine and glutamine are small (SIFT, http://sift.jcvi.org/; PolyPhen, http://genetics.bwh.harvard.edu/pph/) and several other arginine substitutions in the triple-helix domain have been reported as neutral single nucleotide polymorphism (dbSNP). Mutation analyses in parental blood samples subsequently showed that the unaffected father carried this variation. Altogether, these data suggest that p.Arg1630Gln is a neutral variation, although we cannot fully exclude a mild contribution to our patient’s phenotype yet.

Unexpectedly, the mother carried the p.Gly2034Arg mutation, but only in a proportion of her lymphocytes, indicating somatic mosaicism (Figure 2B, Table 1). By using pyrosequencing, the mutation could be detected in ~66% of her lymphocytes. Since she did not express a disease phenotype, we wondered whether the mutation was present in her skin. Pyrosequencing of DNA extracted directly from a 4 mm punch biopsy, obtained after informed consent, revealed that the mutation was present in ~23% of her skin biopsy cells (keratinocytes and fibroblasts) (Figure 2C, Table 1). The mutation was detected in ~35% of fibroblast cultured from the same biopsy (Figure 2D, Table 1) and ~55% and 58% of buccal swab cells and a saliva sample, respectively (Figure 2E, Table 1). Unfortunately, we were not able to determine directly the proportion of keratinocytes carrying the p.Gly2034Arg mutation, but our skin biopsy data indicate that this is likely to be in the order of 20% (although up to ~50% cannot be excluded, as the mutational load in buccal mucosa cells, also of ectodermal origin, was ~27%). As the mutation was found in tissues of different embryological origins, it must have occurred early during embryonic development.
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Table 1. p.Gly2034Arg mutational load in different tissues.

<table>
<thead>
<tr>
<th></th>
<th>Sanger sequencing</th>
<th>Pyrosequencing</th>
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<tbody>
<tr>
<td></td>
<td>No. of tests a</td>
<td>% mutant alleles (normalized) b</td>
</tr>
<tr>
<td>Index patient (EB287)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral lymphocytes</td>
<td>12</td>
<td>50%</td>
</tr>
<tr>
<td>Unaffected mother</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral lymphocytes</td>
<td>10</td>
<td>21%</td>
</tr>
<tr>
<td>Skin biopsy (direct DNA isolation)</td>
<td>10</td>
<td>&lt;8%</td>
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<tr>
<td>Cultured fibroblasts</td>
<td>8</td>
<td>18%</td>
</tr>
<tr>
<td>Buccal mucosa cells</td>
<td>3</td>
<td>~8%</td>
</tr>
<tr>
<td>Saliva</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Negative control</td>
<td>4</td>
<td>1%</td>
</tr>
<tr>
<td>Positive control</td>
<td>5</td>
<td>50%</td>
</tr>
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| a Number of independent sequencing reactions performed.  
| b Average mutational load (%) of the independent sequencing reactions. ND, not determined.  
| c Deduced from mutational load.  

If a DDEB patient carries a dominant COL7A1 mutation in 100% of skin cells, 50% of the pro-α1(VII) procollagen chains will contain the mutation, which will result in disease. In the mother, ~10-25% of her pro-α1(VII) procollagen chains will harbor the mutation, which is apparently too low to result in disease. The threshold to develop DDEB must therefore be higher than 10-25% mutant pro-α1(VII) procollagen, with the exact threshold likely differing per mutation. These data are consistent with in vitro studies that showed that an increase in the ratio of wild-type:mutant pro-α1(VII) chains gradually increased type VII collagen thermostability20 and could have important implications for therapeutic strategies aiming to increase the wild-type:mutant pro-α1(VII) chains ratio.

Our case emphasizes that mutation analysis should always be performed in the parents of sporadic DDEB patients to confirm the diagnosis of DDEB and the de novo status of the mutation. This will ultimately reveal the frequency of true de novo mutations and somatic mosaicism in carrier parents, which has important implications for genetic counseling. Although the proportion of the parent’s germ cells that harbor the mutation in mosaic parents is difficult to determine, it is obvious that at least some cells do, as in our case, and the recurrence risk could therefore be as high as 50%, instead of ‘low’, as in the case of true de novo mutations. In the EB simplex family described by Nagao-Watanabe et al. the causative mutation could not be detected in the mother’s peripheral lymphocytes, which led to the erroneous conclusion of a de novo event in her first child.4 After a second affected child with the same mutation was born, the mother was shown to be somatic mosaic for the mutation in buccal smear and hair bulb cells.
We therefore recommend always performing a mutation analysis in a buccal swab, once the mutation has been excluded in parental blood samples. If the mutation is not found in either sample, the recurrence risk is likely low. A small probability of parental germline mosaicism should nonetheless always be kept in mind and the option of prenatal diagnosis should always be discussed.\textsuperscript{5,6} If the mutation is found in one of the parents, it might be worth checking other tissues in order to understand why the carrier parent is unaffected.

**ACKNOWLEDGEMENTS**

We thank the family for their participation in this study and Jackie Senior for editing the manuscript. This work was supported by the Netherlands Organization for Health Research and Development (ZonMw) grant 92003541 and the Dutch *Vlinderkind* (Butterfly Child) Foundation.
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