Natural Occurrence of Autoantibodies against Basement Membrane Proteins in Epidermolysis Bullosa

TO THE EDITOR

Epidermolysis bullosa (EB) is a group of genetic blistering diseases characterized by lifelong trauma-induced blistering of the skin and mucosa and extracutaneous manifestations. Autoantibodies to a structural protein of the epidermal basement membrane zone (BMZ) such as dystonin (BP230), plectin, type XVII collagen (COL17/BP180), laminin-332, or type VII collagen (COL7) result in the same level of blister formation as in EB subtypes caused by mutations in their coding gene, such as in EB simplex (DST and PLEC), junctional EB (JEB) (COL7A1, LAMA3, LAMB3, or LAMC2), and dystrophic EB (DEB) (COL7A1) (Goeltz et al., 2017; Has et al., 2020). The innate and adaptive immune systems are designed not to recognize the host’s own cells and proteins owing to natural immunological tolerance and negative selection of host-specific T lymphocytes in the central lymphatic organs. However, the lack of one of the proteins due to inherited mutations can interfere with this process. When the missing protein is introduced later in life, it can be recognized as dangerous, and an immune response can occur (Alberts, 2002; Siprashvili et al., 2016).

Four previous publications presented results of serological tests, ELISA, and indirect immunofluorescence (IIF) on monkey esophagus in patients with EB (DEB and EB simplex) (Annichiarico et al., 2015; Esposito et al., 2016; Tampoia et al., 2013; Woodley et al., 2014). Circulating antibodies against BMZ proteins were present in the serum, and the authors suggested the need for further ex vivo experiments to assess their pathogenicity. Although three publications lacked direct immunofluorescence (DIF) on a skin biopsy specimen and IIF on salt split skin (SSS) for detection of tissue-bound autoantibodies, Woodley et al. (2014) additionally performed DIF and IIF on SSS in patients with DEB with a positive ELISA.

Treatment approaches for EB are being investigated, and progress has been recently made; however, they can be threatened by pre-existent circulating antibodies (Eichstadt et al., 2019; Gaucher et al., 2020; Hirsch et al., 2017). In these studies, their presence was assessed before transplantation only by ELISA, IIF, and western blot. In the study of Eichstadt et al. (2019), DIF was performed but only on the transplanted sites after the transplantation and not before. IgG deposition was found in one of the transplanted sites in one of the treated subjects at 3 months and 2 years after transplantation; however, circulating antibodies were only detectable at months 1 and 3 and until month 6 after transplantation. Therefore, they suggested that the humoral immune response was provoked by the transplantation site rather than that the circulating antibodies were pre-existing. For the diagnosis of pemphigoid diseases, Meijer et al. (2019) recently proposed that DIF and IIF on SSS and not ELISA or blot are essential, and therefore, these techniques should be used to illustrate whether pre-existing antibodies can bind to the skin (Schmidt and Zillikens, 2009). Because these data are missing in the literature, we have investigated skin biopsies and serum of 37 patients with EB with a wide variety of techniques, including DIF and IIF on SSS to assess the presence of circulating antibodies.

Of the 37 patients, 12 were affected with JEB due to mutations in LAMB3 and COL7A1, and 25 were affected with DEB due to mutations in COL7A1 (Table 1). A total of 10 of the 37 included patients had revertant mosaicism (6 with JEB and 4 with recessive DEB [RDEB]) (Supplementary Table S1), that is, healthy, natural skin patches due to correcting somatic mutations that occurred during embryo development or later in life (Pasmooij et al., 2012). We analyzed the already stored punch biopsies from 35 of the 37 patients. Serum samples from all the patients were obtained with permission from medical ethical committees in the Netherlands (University Medical Center Groningen 2013/317) and Spain (Code Hospital Universitario La Paz: PI1359 and PI1595). All patients or their parents provided written informed consent. Furthermore, 14 sera from 13 patients with severe burn wounds were used as the control for ELISA, blotting, and IIF. For a detailed methods description, see previous publications (Groth et al., 2011; Vodegel et al., 2004). The age of the patients at the time of biopsy and serum sampling varied from 0 to 61 years (Supplementary Table S1) for the patients with EB and from 6 to 86 years for the burn wound patients (Supplementary Table S2). DIF was performed on all available skin specimens (1–3 biopsies per patient) to detect human IgG and IgA. Furthermore, we performed IIF on two substrates, monkey esophagus, and SSS; keratinocyte footprint assay for laminin-332 (Giurdanella et al., 2020); and ELISA for COL17 (NC16A), BP230, and COL7. In addition, immunoblot was performed on keratinocyte cell extract.

Abbreviations: BMZ, basement membrane zone; COL, collagen; DEB, dystrophic epidermolysis bullosa; DIF, direct immunofluorescence; EB, epidermolysis bullosa; IIF, indirect immunofluorescence; JEB, junctional epidermolysis bullosa; RDEB, recessive dystrophic epidermolysis bullosa; SSS, salt split skin
Table 1. Detailed Data Per Patient of all Tests Conducted for Autoantibodies

| Patient  | Database NR | Mutation (DNA) | Protein Expression in Nonblistered Skin | IIF | ELISA MBP Groningen | ELISA Euroimmune Lübeck | Immunoblot Groningen | Immunoblot Lübeck | LAMB3 Patients | COL7A1 Patients | COL7A1 patients | RDEB-sev | RDEB-sev | RDEB-sev | RDEB-sev |
|----------|-------------|----------------|----------------------------------------|-----|--------------------|------------------------|------------------------|-------------------|-----------------|----------------|----------------|-------------|-------------|-------------|-------------|
| NR       | EB Type     |                |                                        | Biopsy | Direct IF | MO | SSS | KFA Lam332 | BP180 | BP230 | COL7 | BP180 | BP230 | COL7 | BP180 | BP230 | LAD | COL7 | DermExtr | Lam-γ1 |
| 1        | JEB-intermed | EB02501 | COL17A1: c.2237delG | p.Gly746Alafs*53 / p.Gly746Alafs*53 | Negative (COL17) | 18 | 17 | 9 | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 2        | JEB-intermed | EB02601 | COL17A1: c.3676C>T | p.Arg1226*/p.Asp534Alafs*19 | Negative (COL17) | 18 | 17 | 9 | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 3        | JEB-intermed | EB03502 | COL17A1: c.2237delG | p.Gly746Alafs*53 / p.Arg1226* | Negative (COL17) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 4        | JEB-intermed | EB13401 | COL17A1: c.1366delC | p.Ser1166Leufs*6 | Negative (COL17) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 5        | JEB-intermed | EB20801 | COL17A1: c.2237delG | p.Gly746Alafs*53 / p.Gly746Alafs*53 | Negative (COL17) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 6        | JEB-intermed | EB08601 | COL17A1: c.1772-2A>C / c.3327delT | in-frame exon skipping / p.Pro1110Argfs*21 | Negative (COL17) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 7        | JEB-loc     | EB16801 | COL17A1: c.4320delT | p.Gln1442Lysfs*70 / p.Gln1442Lysfs*70 | Strongly reduced (COL17) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 8        | JEB-loc     | EB09801 | COL17A1: c.2251C>T / c.3327delT | p.Gln751* / p.Pro1110Argfs*21 | Strongly reduced (COL17) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 9        | JEB-intermed | EB02901 | LAMB3: c.628G>A / c.628G>A | p.Glu210lys / p.Glu210lys | Strongly reduced (lam332) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 10       | JEB-intermed | EB29901 | LAMB3: c.1106dup / c.1106dup | p.Ala370Serfs*13 / p.Ala370Serfs*13 | Normal (lam332) | -- | -- | Positive | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 11       | JEB-intermed | EB13201 | LAMB3: c.1063T>C / c.1903C>T | p.Cys355Arg / p.Arg635* | Strongly reduced (lam332) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 12       | JEB-loc     | EB25401 | LAMB3: c.628G>A / c.1903C>T | p.Glu210lys / p.Arg635* | Strongly reduced (lam332) | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 13       | RDEB-sev    | EB26501 (P116) | COL7A1: c.887delG / c.6527dup | p.Gly296Valfs*5 / p.Gly2177Trps*113 | Strongly reduced (type XII collagen) | -- | -- | ECS | -- | -- | -- | -- | -- | -- | -- | 24 | 42 | -- | -- | (continued) |
| 14       | RDEB-sev    | EB29701 (P108) | COL7A1: c.2142A>G / c.6527dup | p.Gly296Valfs*5 / p.Gly2177Trps*113 | Negative (COL7) | 18 | 17 | 9 | -- | -- | -- | -- | -- | -- | -- | -- | -- | (continued) |
| 15       | RDEB-sev    | EB02201 | COL7A1: c.3G>T / c.4979dup | Start-lost / p.Pro1668Alafs*4 | Negative (COL7) | 18 | 17 | 9 | -- | -- | -- | -- | -- | -- | -- | -- | -- | (continued) |
| 16       | RDEB-sev    | EB07701 | COL7A1: c.925_944dup / c.1264dup | p.Ile315Metfs*12 / p.Arg422Profs*19 | Negative (COL7) | 18 | 17 | 9 | -- | -- | -- | -- | -- | -- | -- | -- | -- | (continued) |
| 17       | RDEB-sev    | EB09001 | COL7A1: c.4767delA / c.4767delA | p.Asp1590Thrfs*120 / p.Asp1590Thrfs*120 | Negative (COL7) | 18 | 17 | 9 | -- | -- | -- | -- | -- | -- | -- | -- | -- | (continued) |
| 18       | RDEB-sev    | P10 | COL7A1: c.6527dup / c.6527dup | p.Gly2177Trps*113 / p.Gly2177Trps*113 | Strongly reduced (COL7) | Not tested | -- | -- | 10 | 12 | 164 | 30 | 30 | -- | -- | -- | (continued) |

(continued)
| Patient | EB Type | Database NR | Mutation (DNA) | Mutation (Protein) | Protein Expression in Nonblistered Skin | Biopsy | IIF | ELISA MBP Groningen | ELISA Euroimmune Lübeck | Immunoblot Groningen | Immunoblot Lübeck | Lam-γ1 |
|---------|---------|-------------|----------------|-------------------|----------------------------------------|---------|-----|---------------------|------------------------|-----------------------|------------------------|--------|
| 19      | RDEB-sev | P31         | COL7A1: c.6527dup / c.6527dup | p.Gly2177Trpfs*113 / p.Gly2177Trpfs*113 | Strongly reduced (COL7) | Direct IF | MO | SSS 16 | BP180 | BP230 | COL7 | BP180 | BP230 | COL7 | BP180 | BP230 | LAD | COL7 | DermExtr |
| 20      | RDEB-sev | P46         | COL7A1: c.325_326insCG / c.327-1G>C | p.Gln109Ala*39 / Altered splicing resulting in out-of-frame transcripts | Strongly reduced (COL7) | Direct IF | MO | SSS 48 | 35 | 14 | | Positive | | |
| 21      | RDEB-sev | P52         | COL7A1: c.7756dup / c.7390-1G>C | p.Glu258Profs*12 / In-frame exon skipping | Negative (COL7) | Direct IF | MO | SSS 39 | 30 | 64 | | EBA/p200 | | |
| 22      | RDEB-sev | P104        | COL7A1: c.6618+1G>A / c.6618+1G>A | p.K2206_G2207insMSL_E220Gfs*86 | Strongly reduced (COL7) | Direct IF | MO | SSS 96 | 40 | 60 | 83 | 64 | | |
| 23      | RDEB-sev | P14         | COL7A1: c.6527dup / c.336C>G | p.Gly2177Trpfs*113 / p.Tyr112* | Negative (COL7) | Direct IF | MO | SSS 25 | | | | | | | | | | | |
| 24      | RDEB-sev | P18         | COL7A1: c.6527dup / c.2984dup | p.Gly2177Trpfs*113 / p.Gly996Trpfs*44 | Negative (COL7) | Direct IF | MO | SSS 25 | 73 | | | | | | | | | | | |
| 25      | RDEB-sev | P61         | COL7A1: c.6527dup / c.6527dup | p.Gly2177Trpfs*113 / p.Gly2177Trpfs*113 | Strongly reduced (COL7) | Direct IF | MO | SSS 96 | | | | | | | | | | | | |
| 26      | RDEB-sev | P104.1      | COL7A1: c.6618+1G>A / c.6618+1G>A | p.K2206_G2207insMSL_E220Gfs*86 | Strongly reduced (COL7) | Direct IF | MO | SSS 24 | 20 | | | | | | | | | | | |
| 27      | RDEB-sev | P78         | COL7A1: c.6527dup / c.5130_5131insTCATAC | p.Gly2177Trpfs*113 / p.Thr1711Ala*132 | Negative (COL7) | Direct IF | MO | SSS 25 | 73 | | | | | | | | | | | | |
| 28      | RDEB-sev | EB26001 (P17) | COL7A1: c.6527dup / c.6527dup | p.Gly2177Trpfs*113 / p.Gly2177Trpfs*113 | Strongly reduced (COL7) | Direct IF | MO | SSS 30 | 60 | | | | | | | | | | | | |
| 29      | RDEB-sev | EB24001     | COL7A1: c.6508C>T / c.6508C>T | p.Gln2170* / p.Gln2170* | Negative (COL7) | Direct IF | MO | SSS 27 | | | | | | | | | | | | |
| 30      | RDEB-sev | EB06401     | COL7A1: c.1573C>T / c.6508C>T | p.Arg525* / p.Gln2170* | Negative (COL7) | Direct IF | MO | SSS 9 | 11 | | | | | | | | | | | | |
| 31      | RDEB-intermed | EB0901 | COL7A1: c.2699G>A / c.7237G>A | p.Trp900* / p.Gly2413Arg | Minimally reduced (COL7) | Direct IF | MO | SSS 22 | 12 | | | | | | | | | | | | |
| 32      | RDEB-intermed | EB14701 | COL7A1: c.5272G>A / (unknown) | p.Gly1758Arg / unknown | Minimally reduced (COL7) | Direct IF | MO | SSS 36 | | | | | | | | | | | | |
| 33      | RDEB-intermed | P36 | COL7A1: c.6527dup / c.7300G>A | p.Gly2177Trpfs*113 / p.Gly2434Arg | Reduced (COL7) | Direct IF | MO | SSS 10 | | | | | | | | | | | | |
| 34      | RDEB-intermed | P204 | COL7A1: c.2722_2723delCA / c.5188 C>T | p.Gln908Valfs*45 / p.Arg1730* | Reduced (COL7) | Direct IF | MO | SSS 43 | | | | | | | | | | | | |

(continued)
to detect antibodies against BP230, COL17, and LAD-1 (Groningen, The Netherlands) (Pas, 2001), on dermal extract to detect antibodies against COL7, and on the recombinant C-terminus of laminin γ1 (Lübeck, Germany) to detect antibodies against the p200 protein. ELISA for the NC16A domain of COL17, BP230, and COL7 were performed in two different laboratories in Groningen (The Netherlands) and in Lübeck (Germany) using MBL and Euroimmun kits, respectively.

The most important finding of our study is that only two patients (2 of 35, 5.7%) showed linear binding of IgG along the BMZ in DIF. Both patients, #23 and #29, have severe RDEB and were negative for COL7 staining, although patient #29 also had a proven revertant patch. Patient #23 was negative for all serological tests except for one of the ELISA's for COL7 (Table 1). Because patient #23 was negative for the COL7 protein with LH7.2 in the skin, the IgG in the DIF was either not directed to COL7 or it is possible that the patient expresses small amounts of truncated COL7 protein to which the IgG is directed. Patient #29 DIF showed 3+ IgG staining to the BMZ in the revertant skin and negative in the mutant skin. Serology revealed dermal binding of IgG in SSS and positive anti-COL7 autoantibodies in both ELISAs, consistent with a diagnosis of EB acquisita (Figure 1 and Table 1). Both patients did not report any noticeable change of skin phenotype that would indicate the manifestation of EB acquisita, and in the case of patient #29, his revertant skin patch did not blister, even after inducing mechanical trauma (minimal skin rub test) (Figure 1b). This suggests that his general blistering was caused by RDEB and not by circulating autoantibodies as in a case published by Guerra et al. (2018), where EB acquisita occurred in a patient with a mild DEB phenotype.

In 22 of the 37 patients (59.5%), we found at least one positive serological test (Table 1), and in three other patients, we found at least one serological test that was doubtful, meaning that 67.5% (25/37) of our cohort had circulating antibodies against BMZ proteins. Interestingly, the proportion of patients with at least one positive or doubtful serological test was highest in the severe RDEB subgroup (83%, 14/18

---

**Table 1. Continued**

| Patient | NR | EB Type | Database | Mutation (DNA) | Mutation (Protein) | Protein Expression in Nonblistered Skin | Direct IF | IIF | KFA | Lam-Extr | Immunoblot | ELISA | Immunoblot | ELISA | ELISA | ELISA | ELISA | ELISA | ELISA | ELISA | ELISA |
|---------|----|---------|----------|--------------|------------------|----------------------------------------|---------| --- | --- |---------|-----------|-------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|
| 35      | RDEB-int | NR120 | C.5760_5761del / C.4012G > A | p.Lys1858fs*12 / p.Gly1338Arg | Strongly reduced (COL7) | e e e e e e e e e e ee | 16 | 154 | 16 | - | - | - | - | - | - | - |
| 36      | RDEB-int | EB04701 | COL7A1: c.8083G > A / c.8083G > A | p.Gly2695Ser / p.Gly2695Ser | Minimally reduced (COL7) | e e e | - | - | - | - | - | - | - | - | - | - |
| 37      | RDEB-int | P50 | COL7A1: c.6182G > T / c.4803G > A | p.Gly2061Val / p.Gly2061Val | Reduced (COL7) | e ee e e e e ee | - | - | - | - | - | - | - | - | - | - |

Abbreviations: BMZ, basement membrane zone; COL, collagen; DDEB, dominant dystrophic epidermolysis bullosa; DermExtr, dermal extract; EB, epidermolysis bullosa; ECS, extracellular space; IF, immunofluorescence; IIF, indirect immunofluorescence; JEB-intermed, junctional epidermolysis bullosa intermediate; JEB-loc, junctional epidermolysis bullosa localized; KFA, keratinocyte footprint assay; Lam, laminin; MO, monkey esophagus; NR, number; RDEB-intermed, recessive dystrophic epidermolysis bullosa intermediate; RDEB-sev, recessive dystrophic epidermolysis bullosa severe; SSS, salt split skin. Only positive results above the cut-off value are given. Cut-off values as used and evaluated by our diagnostic laboratories: For MBP, COL17 NC16A: > 9; COL7: > 9; BP230: > 9. Positive results are indicated in bold, and doubtful results are indicated in italic.
Figure 1. Clinical presentation of patient #29 and immunofluorescent linear staining of the patient’s revertant patch. (a) Clinical presentation of patient #29 with RDEB due to homozygous COL7A1: c.6508C>T mutation. (b) Detail of revertant patch due to natural correction with a second-site mutation c.6510G>T, which removed the termination codon. (c–e) Immunofluorescent linear staining of revertant patch with LH7.2 for (c) COL7 (green; Alexa 488), and (f) IgG3 (green, Alexa 488), and (g) antihuman IgG (red, Alexa 586), (e) antihuman IgG3 (green, Alexa 488), and (h) antihuman C3c (green, Alexa 488) along the basement membrane zone in a u-serration pattern. (g) Indirect immunofluorescence on the salt-split skin from a healthy donor, with patient #29’s serum showing dermal IgG (green) binding to the floor of the split. (h) Indirect immunofluorescence with patient #29’s serum on MO showing positive IgG binding (green) to the basement membrane zone. Bar = 20 mm. The patient consented to the publication of the images. COL, collagen; IIF, indirect immunofluorescence; MO, monkey esophagus; RDEB, recessive dystrophic epidermolysis bullosa; SSS, salt split skin.

position and 1/18 doubtful) than in patients with JEB (50%, 4/12 positive and 2/12 doubtful) and in patients with other types of DEB (57%, 4/7 positive). However, the number of patients with JEB and other types of DEB was limited. Furthermore, besides patient #29, none of the other patients showed binding of IgG to the BMZ in SSS, whereas only two patients showed IgA binding (patient #6, positive and patient #27, doubtful), thereby resulting in 3 of 37 (8.1%) with positive or doubtful IIF on SSS. These positive findings on DIF and/ or SSS, although only in three patients, are in contrast with the findings of Woodley et al. (2014). In their RDEB cohort, 11 of 22 patients had a positive ELISA. However, none of these 11 patients had a positive DIF or SSS. Finally, it is remarkable that several of the patients with circulating autoantibodies against the protein that they are thought to be lacking owing to their mutations. This might indicate that patients are never truly null and that these patients still express a very small amount of the deficient protein, albeit in a truncated or altered form. An alternative explanation could be that these patients have revertant areas, which have not yet been identified.

In our cohort, we found positive Euroimmun ELISA for NC16A in 5 of 37 patients (13.5%) and positive MBP ELISA for NC16A in 12 of 37 patients (32.4%). van Beek et al. (2014) described that ELISA for NC16A in the elderly (aged >70 years) was positive in about 6.5% and 3.5% for Euroimmun and MBP ELISAs, respectively. This suggests more positive reactions in the elderly group.

An important question is why circulating autoantibodies are found so frequently in patients with EB and especially in patients with severe RDEB, which do not seem to be clinically relevant. Esposito et al. (2016) showed that patients with EB and especially those with RDEB have higher levels of proinflammatory cytokines than the levels in the control population. In addition, a recent review by Huijtena et al. (2021) states that there is evidence that patients with RDEB have an underlying immune defect. The high number of positive ELISAs in patients with EB may thus be caused by exposure to self-antigens due to repeated skin damage combined with a chronic immunological response or underlying immune defect because all serological tests were negative in 13 patients with severe acute burn used as controls (data not shown). However, the exact reason is still unknown and warrants further investigation.

To summarize, the clinical relevance of autoantibodies in EB is disputable, especially those detected by ELISA or immunoblot because in the majority of patients, no in vivo binding of antibodies could be shown. Furthermore, because more than half of our cohort had a positive serological test without apparent clinical meaning, an exclusion for the therapy trials for EB based on ELISA causes a risk of omitting possible candidates. We suggest DIF combined with IIF on SSS because these methods have a better diagnostic and prognostic value. However, in EB, the clinical significance of reactivity even in DIF and/or IIF on SSS remains uncertain.

Data availability statement
No datasets were generated or analyzed during this study.

ORCIDs
Agnieszka Gostyński: http://orcid.org/0000-0002-1
0001-8225-0035
Gilles F.H. Diercks: http://orcid.org/0000-0001-8
053-216X
Maria-Jose Escamez: http://orcid.org/0000-0002-
5434-1885
Nisha Suyien Chandran: http://orcid.org/0000-
0001-8225-0035
Antoni Gostyński: http://orcid.org/0000-0002-1
0001-8225-0035
Raid de Lucas: http://orcid.org/0000-0001-
7587-267X
Adela Garcia-Martín: http://orcid.org/0000-0001-
7054-7907
CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
This study was funded by the Dutch Butterfly Child Foundation (Stichting Vlinderkind). We gratefully acknowledge all the patients and families for their participation in this study. In addition, we are thankful for the great assistance provided by the research technicians from the University Medical Center Groningen (The Netherlands): Miranda Nijenhuis, Ducu Kramer, Connie Meijer, and Janny Zuiderveen. MFJ, founder of the Center of Blistering Diseases in Groningen, is deceased (14 January 2019).

AUTHOR CONTRIBUTIONS
Conceptualization: AG, AMGP, GFHD, HHP, MFJ, NSC; Data Curation: AG, AGMP, GFHD, HHP, MFJ, NL; Formal Analysis: AG, GFHD, NSC; Investigation: AG, AGMP, GFHD, HHP, MFJ, NL; Methodology: AG, AMGP, GFHD, HHP, MFJ, NSC; Supervision: AG, AMGP, GFHD, HHP, NL; Validation: AG, AMGP, GFHD; Writing - Original Draft Preparation: AG, AGMP, GFHD, HHP, MFJ, NL; Writing - Review and Editing: AG, AM, AMGP, GFHD, HHP, JB, MCB, MFJ, MFJ, NSC, SGL.

Antoni Gostyński1,2,3, Gilles F.H. Diercks4,5,6, Nisha Suyien Chandran4,8,9, Raúl de Lucas10, Adela García-Martín5,6,7, Marcela Del Rio4,5,6, Jeroen Bremer1, María C. Bolling1, Alvaro Meana4,9,11, Sara G. Llames4,11, Enno Schmidt12,13, Ralf Ludwig1,2,7, Marcel F. Jonkman1, Hendri H. Pas1 and Anna M.G. Pasmooij1,2
1Center for Investigation Biomédica en Red de Enfermedades Rasas, Instituto de Salud Carlos III (CIBERER-ISCI), Madrid, Spain; 2Department of Bioengineering, Universidad Carlos III de Madrid (UC3M), Madrid, Spain; 3Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain; 4Instituto investigación Sanitaria Fundación Jimenez Díaz (IIS-FJD), UAM, Madrid, Spain; 5Division of Dermatology, Department of Medicine, National University Hospital, Singapore, Singapore; 6Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; 7Department of Dermatology, Hospital Universitario La Paz, Madrid, Spain; 8Tissue Engineering Unit, Comité Centro de Sangre y Tejidos del Principado de Asturias, Oviedo, Spain; 9Lübeck Institute of Experimental Dermatology (LIED), University of Lübeck, Lübeck, Germany; and 10Department of Dermatology, University of Lübeck, Lübeck, Germany.
*Corresponding author e-mail: a.m.g. pasmooij@umcg.nl

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2021.10.030.

REFERENCES
Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. 4th ed. New York City, NY: Garland Publishing Science; 2002.
Annichiarico G, Morgese MG, Esposito S, Lopalco G, Lattarulo M, Tampoia M, et al. Proinflammatory cytokines and antiinflammatory autoantibodies in patients with inherited epidermolysis bullosa. Medicine (Baltimore) 2015;94:e13528.
Eichstadt S, Barriga M, Ponakala A, Teng C, Nguyen NT, Siprashvili Z, et al. Phase 1/2 clinical trial of gene-corrected autologous cell therapy for recessive dystrophic epidermolysis bullosa. JCI Insight 2019:4:e130554.
Esposito S, Gueez S, Orenti A, Scuvera G, Corti L, et al. Autoimmunity and cytokine imbalance in inherited epidermolysis bullosa. Int J Mol Sci 2016;17:1625.
Gaucher S, Lwin SM, Titeux M, Abdul-Wahab A, Eichstadt S, Barriga M, Ponakala A, Teng C, Lewis J, Raff M, Roberts K, Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. 4th ed. New York City, NY: Garland Publishing Science; 2002.
Annichiarico G, Morgese MG, Esposito S, Lopalco G, Lattarulo M, Tampoia M, et al. Proinflammatory cytokines and antiinflammatory autoantibodies in patients with inherited epidermolysis bullosa. Medicine (Baltimore) 2015;94:e13528.
Eichstadt S, Barriga M, Ponakala A, Teng C, Nguyen NT, Siprashvili Z, et al. Phase 1/2 clinical trial of gene-corrected autologous cell therapy for recessive dystrophic epidermolysis bullosa. JCI Insight 2019:4:e130554.
Esposito S, Gueez S, Orenti A, Scuvera G, Corti L, et al. Autoimmunity and cytokine imbalance in inherited epidermolysis bullosa. Int J Mol Sci 2016;17:1625.
Gaucher S, Lwin SM, Titeux M, Abdul-Wahab A, Eichstadt S, Barriga M, Ponakala A, Teng C, Nguyen NT, Siprashvili Z, et al. Phase 1/2 clinical trial of gene-corrected autologous cell therapy for recessive dystrophic epidermolysis bullosa. JCI Insight 2019:4:e130554.
Esposito S, Gueez S, Orenti A, Scuvera G, Corti L, et al. Autoimmunity and cytokine imbalance in inherited epidermolysis bullosa. Int J Mol Sci 2016;17:1625.
Gaucher S, Lwin SM, Titeux M, Abdul-Wahab A, Eichstadt S, Barriga M, Ponakala A, Teng C, Nguyen NT, Siprashvili Z, et al. Phase 1/2 clinical trial of gene-corrected autologous cell therapy for recessive dystrophic epidermolysis bullosa. JCI Insight 2019:4:e130554.
Esposito S, Gueez S, Orenti A, Scuvera G, Corti L, et al. Autoimmunity and cytokine imbalance in inherited epidermolysis bullosa. Int J Mol Sci 2016;17:1625.
Gaucher S, Lwin SM, Titeux M, Abdul-Wahab A, Eichstadt S, Barriga M, Ponakala A, Teng C, Lewis J, Raff M, Roberts K, Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular biology of the cell. 4th ed. New York City, NY: Garland Publishing Science; 2002.
Annichiarico G, Morgese MG, Esposito S, Lopalco G, Lattarulo M, Tampoia M, et al. Proinflammatory cytokines and antiinflammatory autoantibodies in patients with inherited epidermolysis bullosa. Medicine (Baltimore) 2015;94:e13528.
### Supplementary Table S1. Included patients with age at the time of serum and biopsy sampling, and identification of revertant mosaicism

| Patient | Database NR | Mutation (DNA) | Mutation (Protein) | Protein Expression in Nonblistered Skin | Serum Sampling | Biopsy Sampling | Revertant Mosaicism Identified |
|---------|-------------|----------------|--------------------|-----------------------------------------|----------------|----------------|---------------------------------|
| **COL17A1 Patients** | | | | | | | |
| 1 | JEB-intermed EB02501 | COL17A1: c.2237delG/c.2237delG | p.Gly746Alafs*53/p.Gly746Alafs*53 | Negative (type XVII collagen) | 61 | 59 | Yes |
| 2 | JEB-intermed EB02601 | COL17A1: c.3676C>T/c.1601delA | p.Arg1226* / p.Asp534Alafs*19 | Negative (type XVII collagen) | 46 | 44 | Yes |
| 3 | JEB-intermed EB03502 | COL17A1: c.2237delG/c.3676C>T | p.Gly746Alafs*53/p.Arg1226* | Negative (type XVII collagen) | 55 | 48 | Yes |
| 4 | JEB-intermed EB13401 | COL17A1: c.1365delC/c.3600_3601delCT COL17A1: c.1260delC/c.3495_c.3496delCT | p.Thr421Leufs*72/p.Ser1166Leufs*6 | Negative (type XVII collagen) | 11 | 8 | Yes |
| 5 | JEB-intermed EB20801 | COL17A1: c.2237delG/c.2237delG | p.Gly746Alafs*53/p.Gly746Alafs*53 | Negative (type XVII collagen) | 52 | 47 | Yes |
| 6 | JEB-intermed EB08601 | COL17A1: c.1772-2 A/c.3327delT | in-frame exon skipping/p.Pro1110Argfs*21 | Negative (type XVII collagen) | 14 | 0 | No |
| 7 | JEB-loc EB16801 | COL17A1: c.4320delT/c.3327delT | p.Gln1442Lysfs*70/p.Gln1442Lysfs*70 | Strongly reduced (type XVII collagen) | 46 | 37 | No |
| 8 | JEB-loc EB09801 | COL17A1: c.2251C>T/c.3327delT | p.Gln751*/p.Pro1110Argfs*21 | Strongly reduced (type XVII collagen) | 47 | 34 | No |
| **LAMB3 patients** | | | | | | | |
| 9 | JEB-intermed EB02901 | LAMB3: c.628G>A/c.628G>A | p.Glu210Lys/p.Glu210Lys | Strongly reduced (lam332) | 71 | 71 | Yes |
| 10 | JEB-intermed EB29901 | LAMB3: c.1106dup/c.1106dup | p.Ala370Serfs*13/p.Ala370Serfs*13 | Normal (lam332) | 61 | 57 | No |
| 11 | JEB-intermed EB13201 | LAMB3: c.1063T>C/c.1903C>T | p.Cys355Arg/p.Arg635* | Strongly reduced (lam332) | 38 | 39 | No |
| 12 | JEB-loc EB25401 | LAMB3: c.628G>A/c.1903C>T | p.Glu210Lys/p.Arg635* | Strongly reduced (lam332) | 0 | 0 | No |
| **COL7A1 Patients** | | | | | | | |
| 13 | RDEB-sev EB26501 (P116) | COL7A1: c.887delG/c.6527dup | p.Gly296Valfs*5/p.Gly2177Trpfs*113 | Strongly reduced (type XII collagen) | 26 | 25 | Yes |
| 14 | RDEB-sev EB29701 (P108) | COL7A1: c.2142A>G/c.6527dup | Altered splicing resulting in out-of-frame transcript p.Gly2177Trpfs*113 | Negative (type VII collagen) | 25 | 25 | Yes |
| 15 | RDEB-sev EB02201 | COL7A1: c.3G>T/c.4997dup | Start-lost/p.Pro1668Alafs*4 | Negative (type VII collagen) | 21 | 17 | No |
| 16 | RDEB-sev EB07701 | COL7A1: c.925_944dup/c.1266dup | p.Ile315Metfs*12/p.Arg422Profs*19 | Negative (type VII collagen) | 35 | 20 | No |
| 17 | RDEB-sev EB09001 | COL7A1: c.4767delA/c.3277-1G | p.Asp1590Thrfs*12/p.Asp1590Thrfs*120 | Negative (type VII collagen) | 16 | 2 | No |
| 18 | RDEB-sev P10 | COL7A1: c.6527dup/c.6527dup | p.Gly2177Trpfs*113/p.Gly2177Trpfs*113 | Strongly reduced (type VII collagen) | 9 | Not tested | No |
| 19 | RDEB-sev P31 | COL7A1: c.6527dup/c.6527dup | p.Gly2177Trpfs*113/p.Gly2177Trpfs*113 | Strongly reduced (type VII collagen) | 21 | 20 | No |
| 20 | RDEB-sev P46 | COL7A1: c.325_362insCG/c.3277-1G>C | p.Glu109Alafs*39/Altered splicing resulting in out-of-frame transcripts | Strongly reduced (type VII collagen) | 48 | 48 | No |
| 21 | RDEB-sev P52 | COL7A1: c.7756dup/c.7910-1G>C | p.Gln2586Profs*12/in-frame exon skipping | Negative (type VII collagen) | 50 | 45 | No |
| 22 | RDEB-sev P104 | COL7A1: c.6618+1G>A/c.6618+1G>A | p.K2206_G2207insMSL_E220Gfs*86/Altered splicing resulting in out-of-frame transcripts | Strongly reduced (type VII collagen) | 28 | 28 | No |
| 23 | RDEB-sev P14 | COL7A1: c.6527dup/c.336C>G | p.Gly2177Trpfs*113/p.Tyr112* | Negative (type VII collagen) | 7 | 7 | No |
| 24 | RDEB-sev P18 | COL7A1: c.6527dup/c.2984dup | p.Gly2177Trpfs*113/p.Gly996Trpfs*44 | Negative (type VII collagen) | 13 | Not tested | No |

(continued)
### Supplementary Table S1. Continued

| Patient | Age | Serum Sampling | Biopsy Sampling | Revertant Mosaicism Identified | Protein Expression in Nonblistered Skin |
|---------|-----|----------------|----------------|-------------------------------|----------------------------------------|
| 25 RDEB-sev | 6   | 0              | No             |                               | Strongly reduced (type VII collagen)   |
| 26 RDEB-sev | 35  | 32             | No             |                               | Strongly reduced (type VII collagen)   |
| 27 RDEB-sev | 5   | 0              | No             |                               | Negative (type VII collagen)           |
| 28 RDEB-sev | 44  | 43             | Yes            |                               | Strongly reduced (type VII collagen)   |
| 29 RDEB-sev | 25  | 27             | Yes            |                               | Negative (type VII collagen)           |
| 30 RDEB-sev | 29  | 27             | No             |                               | Negative (type VII collagen)           |
| 31 RDEB-intermed | 30 | 26           | No             |                               | Minimally reduced (type VII collagen) |
| 32 RDEB-intermed | 43 | 43             | No             |                               | Minimally reduced (type VII collagen) |
| 33 RDEB-intermed | 14 | 8              | No             |                               | Reduced (type VII collagen)            |
| 34 RDEB-intermed | 22 | 21             | No             |                               | Reduced (type VII collagen)            |
| 35 RDEB-intermed | 4  | 0              | No             |                               | Strongly reduced (type VII collagen)   |
| 36 RDEB-inversa | 27 | 9              | No             |                               | Minimally reduced (type VII collagen) |
| 37 DDEB     | 17  | 17             | No             |                               | Reduced (type VII collagen)            |

Abbreviations: DDEB, dominant dystrophic epidermolysis bullosa; DEB, dystrophic epidermolysis bullosa; EB, epidermolysis bullosa; JEB, junctional epidermolysis bullosa; JEB-intermed, junctional epidermolysis bullosa intermediate; JEB-loc, junctional epidermolysis bullosa localized; NR, number; RDEB-intermed, recessive dystrophic epidermolysis bullosa intermediate; RDEB-inversa, recessive dystrophic epidermolysis bullosa inversa; RDEB-sev, recessive dystrophic epidermolysis bullosa severe.

The age at serum and biopsy sampling is indicated per patient with EB. In 10 patients, revertant mosaicism was identified (6 JEB and 4 DEB). In patients in which revertant mosaicism was not identified, it cannot be completely excluded that these patients may have a revertant area that has remained unnoticed until now.
## Supplementary Table 2. The Age at Serum Sampling Per Patient with Burn Wounds

| Burn Wound Patient NR | Percentage of Body Surface Affected | Age Serum Sampling |
|-----------------------|--------------------------------------|--------------------|
| 38                    | 65                                   | 70                 |
| 39                    | 80                                   | unknown            |
| 40                    | 24                                   | 67                 |
| 41                    | Unknown                              | 20                 |
| 42                    | 80                                   | 76                 |
| 43                    | Unknown                              | unknown            |
| 44                    | 50                                   | 38                 |
| 45                    | 62                                   | 6                  |
| 46                    | 70                                   | 41                 |
| 47                    | 90                                   | 45                 |
| 48                    | 90                                   | 53                 |
| 49                    | 90                                   | 86                 |
| 50                    | 95                                   | 46                 |

Abbreviation: NR, number.

In addition, the percentage of affected skin area is indicated.