

BRIEF REPORT

Inflammatory Skin and Bowel Disease Linked to *ADAM17* Deletion

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SUMMARY

We performed genetic and immunohistochemical studies in a sister and brother with autosomal recessive neonatal inflammatory skin and bowel lesions. The girl died suddenly at 12 years of age from parvovirus B19–associated myocarditis; her brother had mild cardiomyopathy. We identified a loss-of-function mutation in *ADAM17*, which encodes a disintegrin and metalloproteinase 17 (also called tumor necrosis factor α [TNF- α]–converting enzyme, or TACE), as the probable cause of this syndrome. Peripheral-blood mononuclear cells (PBMCs) obtained from the brother at 17 years of age showed high levels of lipopolysaccharide-induced production of interleukin-1 β and interleukin-6 but impaired release of TNF- α . Despite repeated skin infections, this young man has led a relatively normal life. (Funded by Barts and the London Charity and the European Commission Seventh Framework Programme.)

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INFLAMMATORY DISORDERS OF THE SKIN AND GUT, INCLUDING ECZEMA, psoriasis, and celiac disease, have been linked to changes in barrier function and immune responses, by means of genetic and functional studies. Large case-control studies combined with genomewide association studies have identified common genetic risk factors, with low penetrance, for a plethora of human disorders. Such studies have also identified numerous single-nucleotide polymorphisms (SNPs) in genes linked to the regulation of immunity and inflammation affecting epithelial tissues.^{1,2}

High-throughput sequence technology can be used to identify rare but penetrant disease-associated mutations in affected members of families with mendelian conditions.³⁻⁶ We combined this technology with SNP-homozygosity mapping and targeted sequence capture to identify likely causative genes in a syndrome of neonatal-onset inflammatory skin and bowel disease in two siblings.

CASE REPORT

Two of three children born to consanguineous parents (first cousins) of Lebanese origin had the same clinical features involving the skin, hair, and gut. The skin lesions developed on the second day of life, with diarrhea developing in the first week of life. The affected girl died at 12 years of age from fulminant parvovirus B19–associated

myocarditis, and on subsequent investigation, the affected boy was found to have left ventricular dilation. The skin lesions were perioral and perianal erythemas with fissuring and a generalized pustular rash that developed into psoriasiform erythroderma, with flares of erythema, scaling, and widespread pustules (Fig. 1A, 1B, and 1C). The skin of both children, when they were young, was prone to infection with *Staphylococcus aureus*, resulting in recurrent blepharitis and otitis externa. Their hair was short or broken, and their eyelashes and eyebrows were wiry and disorganized. They had thickened nails, with frequent paronychia caused by candida and pseudomonas infections.

The chronic diarrhea in the affected girl was associated, at 4 months of age, with failure to thrive. The diarrhea was predominantly bloody with malabsorptive characteristics, worsening in parallel with increases in the severity of the skin disease and exacerbated by intercurrent gastrointestinal infections. Further details of the clinical history can be found in the Supplementary Appendix (available with the full text of this article at NEJM.org).

METHODS

STUDY PARTICIPANTS

We obtained written informed consent from the family members and controls. Skin samples were obtained from family members as well as control patients undergoing cosmetic (facelift or “tummy tuck”) surgery, and blood specimens for collection of PBMCs were obtained from family members and healthy controls.

SNP MAPPING, SEQUENCE CAPTURE, AND SEQUENCING

The methods used for SNP-homozygosity mapping and targeted next-generation sequencing of the regions of linkage are described in the Supplementary Appendix.

PROTEIN EXPRESSION IN SKIN AND SMALL INTESTINE

We isolated primary keratinocytes from skin-biopsy specimens for Western blotting and carried out immunofluorescence staining on frozen or paraffin-embedded tissue sections from biopsy samples of skin and small intestine from the affected siblings and controls. Details of the antibodies and methods are given in the Supplementary Appendix.

IMMUNOLOGIC INVESTIGATIONS

We isolated and cultured PBMCs from the affected boy, his unaffected mother, and three age-matched controls. We measured cytokine levels in cell-culture supernatants after stimulation with the use of cytokine-specific enzyme-linked immunosorbent assay kits. Full details of the immunologic studies are given in the Supplementary Appendix.

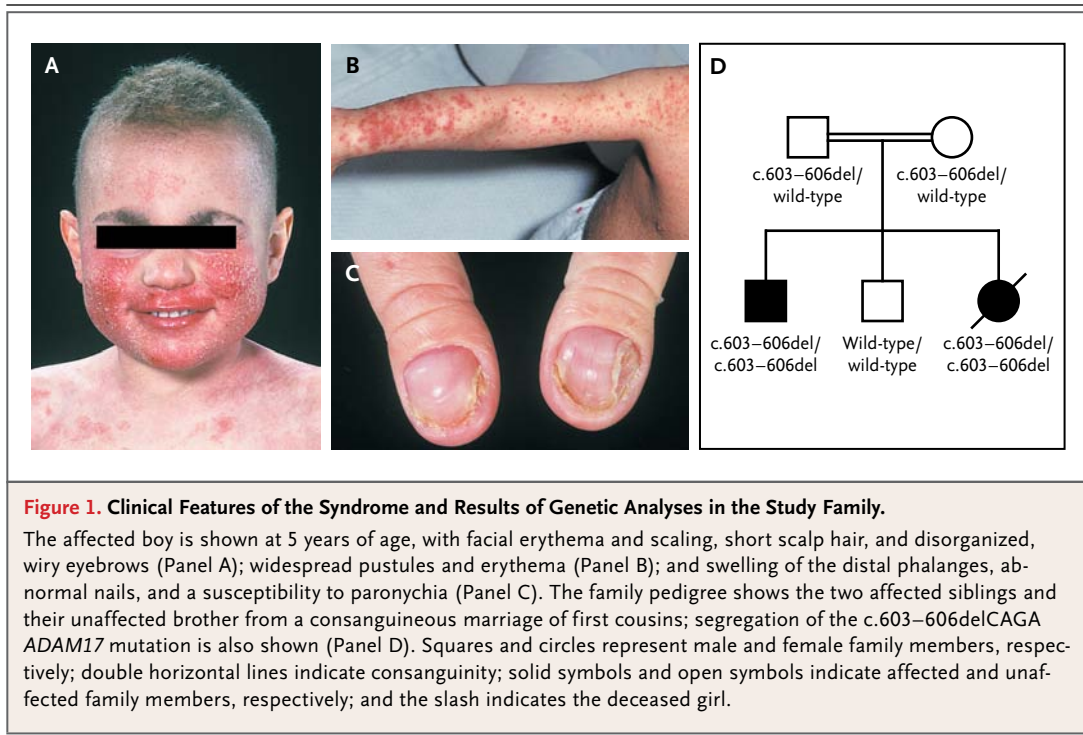
RESULTS

GENETIC ANALYSES

Analysis of the SNP array data revealed putative linkage (maximum lod score of 1.8) to three large stretches of SNP homozygosity, seen in DNA from both affected siblings, on chromosomes 2, 5, and 21 (Fig. 1 in the Supplementary Appendix). After ruling out nine plausible candidate genes by using standard Sanger sequencing (data not shown), we included probes for all exons from these three regions of the genome on a capture array. A total of 1468 exons corresponding to 439 kb of DNA were captured in the affected boy and sequenced. After ruling out known SNPs, we identified 22 nonsynonymous single-nucleotide variants in coding regions. In parallel, we assessed the sequence data for insertion–deletion variations and discovered a new deletion of 4 bp in exon 5 of *ADAM17* (c.603–606delCAGA) on chromosome 2 that segregated with the disease in this family (Fig. 1D, and Fig. 2A in the Supplementary Appendix). The unaffected brother lacked this deletion. We were unable to find rare variants, predicted to result in loss of function, within *ADAM17* in the dbSNP database or the 1000 Genomes database. Bioinformatic analysis predicted that the mutation would introduce a frame shift and a premature stop codon (p.Asp201GlufsX11) separated by 10 codons. Thus, the mutation predicts a severely truncated protein consisting of the signal peptide and prodomain of *ADAM17* and lacking the catalytic domain, disintegrin domain, transmembrane segment, and cytosolic tail (Fig. 2B in the Supplementary Appendix).

PROTEIN EXPRESSION AND CYTOKINE PRODUCTION

Histochemical analysis of skin specimens from an unaffected, unrelated control, with the use of an antibody that recognizes the prodomain of *ADAM17*, showed expression throughout the epidermis, with a cytoplasmic distribution (Fig. 2A). However, there was a paucity of *ADAM17* expression in skin from the affected boy (Fig. 2B). In



addition, immunofluorescence analysis of biopsy specimens from the small intestine in both affected children revealed a paucity of ADAM17 expression (Fig. 2I and 2J). Western blotting of both PBMCs and keratinocyte lysates obtained from the affected boy showed an absence of ADAM17 expression, in contrast with the findings in the healthy controls and the unaffected mother (Fig. 2N, and Fig. 3 in the Supplementary Appendix). However, the expression of ADAM10, whose substrates overlap with those of ADAM17, was similar in keratinocytes from our patient and from a control patient undergoing cosmetic surgery (Fig. 4 in the Supplementary Appendix).

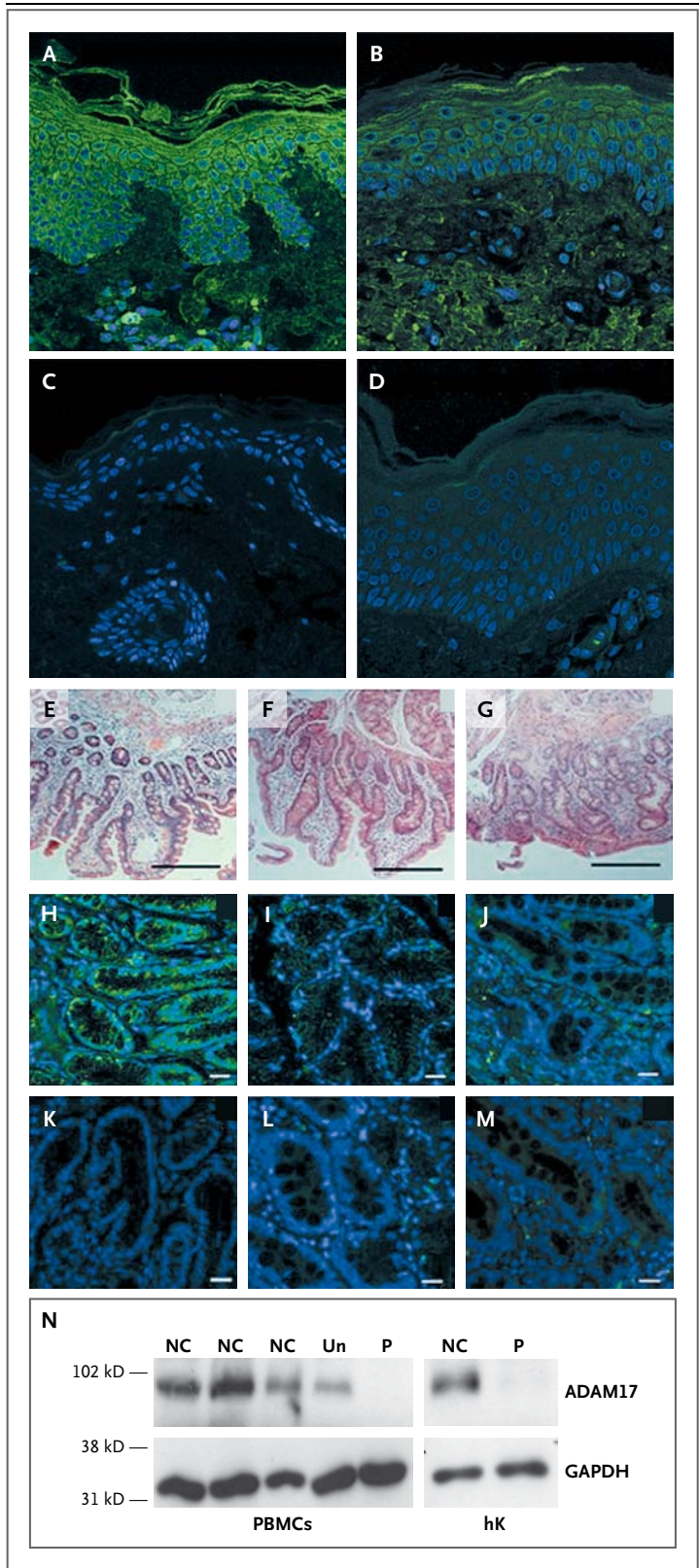
Sheddase enzymes cleave and thereby release many membrane-bound substrates, including desmogleins (DSGs), from the cell surface. Immunofluorescence analysis of skin sections and Western blotting of primary keratinocytes showed that expression of DSG (DSG1, DSG2, or both) was greater in the affected boy than in an unaffected, unrelated control (Fig. 3A, 3B, and 3C), suggesting that DSG is retained on the plasma membrane. We cultured keratinocytes in medium containing fetal-calf serum, which is known to greatly decrease expression of DSG1.⁷ Therefore, although the anti-

body used recognizes both DSG1 and DSG2, we assume it detected mainly DSG2.

ADAM17 also converts membrane-bound TNF- α into soluble TNF- α . Bearing in mind the inflammatory aspect of the disease phenotype, we investigated cytokine production by PBMCs. Stimulation by lipopolysaccharide (Fig. 3D) or anti-CD3 and anti-CD28 antibodies (Fig. 3E) evoked strong and concentration-dependent TNF- α production in PBMCs from controls and from the patient's unaffected mother, with a smaller increase in TNF- α production in the PBMCs from the affected boy. PBMCs from all samples showed similarly robust production of interleukin-1 β and interleukin-6 after lipopolysaccharide stimulation (Fig. 3D) and high levels of interferon- γ production after stimulation with anti-CD3 and anti-CD28 antibodies (Fig. 3E) (also see the Statistical Analysis section in the Supplementary Appendix). Shedding of transmembrane proteins on immune cells has been shown to be stimulated by activators of protein kinase C, such as phorbol 12-myristate 13-acetate (PMA).⁸ In response to PMA and ionomycin (Fig. 3F), large quantities of interferon- γ were secreted by PBMCs from the affected boy, his mother, and controls, whereas TNF- α production by PBMCs

Figure 2. Expression of ADAM17 in the Skin and Small Intestine.

Immunofluorescence staining with the use of an ADAM17 antibody (green) that reacts with an epitope in the active site of ADAM17 (consisting of a cysteine switch and furin cleavage sites) was performed in paraffin-embedded sections of biopsy samples of skin and small intestine from the affected boy and controls. ADAM17 is expressed throughout the epidermis, with a cytoplasmic staining pattern, in normal skin (Panel A), whereas expression is clearly reduced in the boy's skin (Panel B). Negative-control immunostaining without ADAM17 antibody (blue) was performed on sections of normal skin (Panel C) and the patient's skin (Panel D). Hematoxylin and eosin staining of biopsy specimens of the upper small intestine was performed in a control (Panel E) and the two affected siblings. As compared with the normal findings in the control (Panel E), there was evidence of a mononuclear-cell infiltrate, villus blunting, and lengthening of crypts in the affected girl (Panel F); although the findings were more variable in the affected boy, there was evidence of a mononuclear-cell infiltrate and villus blunting (Panel G). ADAM17 was expressed (green) in the enterocytes of the small intestine in a control (Panel H) but was absent in the small intestine of the affected girl (Panel I) and the affected boy (Panel J). Negative-control staining of the nucleus with 4',6-diamidino-2-phenylindole (DAPI; violet), without the addition of the ADAM17 antibody, is shown for the control (Panel K), the affected girl (Panel L), and the affected boy (Panel M). The scale bar indicates 250 μm in Panels E through G and 20 μm in Panels A through D and H through M. Western blotting of ADAM17, with the use of an antibody that reacts with the C-terminal of ADAM17, in peripheral-blood mononuclear cells (PBMCs) and primary human keratinocyte (hK) lysates shows that ADAM17 expression is absent in cells from the male patient (P) but is present in cells from four normal controls (NC) and the boy's unaffected mother (Un) (Panel N). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to show equal loading of the protein in all samples.



was weak in the boy but strong in the controls and the unaffected mother.

Further immunohistochemical characterization showed an infiltrate of T cells (CD3+) around the skin follicles and in the epithelium. We observed CD4+ T cells in the perifollicular region and CD8+ T cells in the epithelium at the neck of the follicle (Fig. 5 in the Supplementary Appendix). There were very few B cells (CD20+), natural killer cells (CD56+), or neutrophils (elastase-positive) present, and levels of dendritic cells (S100-positive) were within normal limits (data not shown). Of the

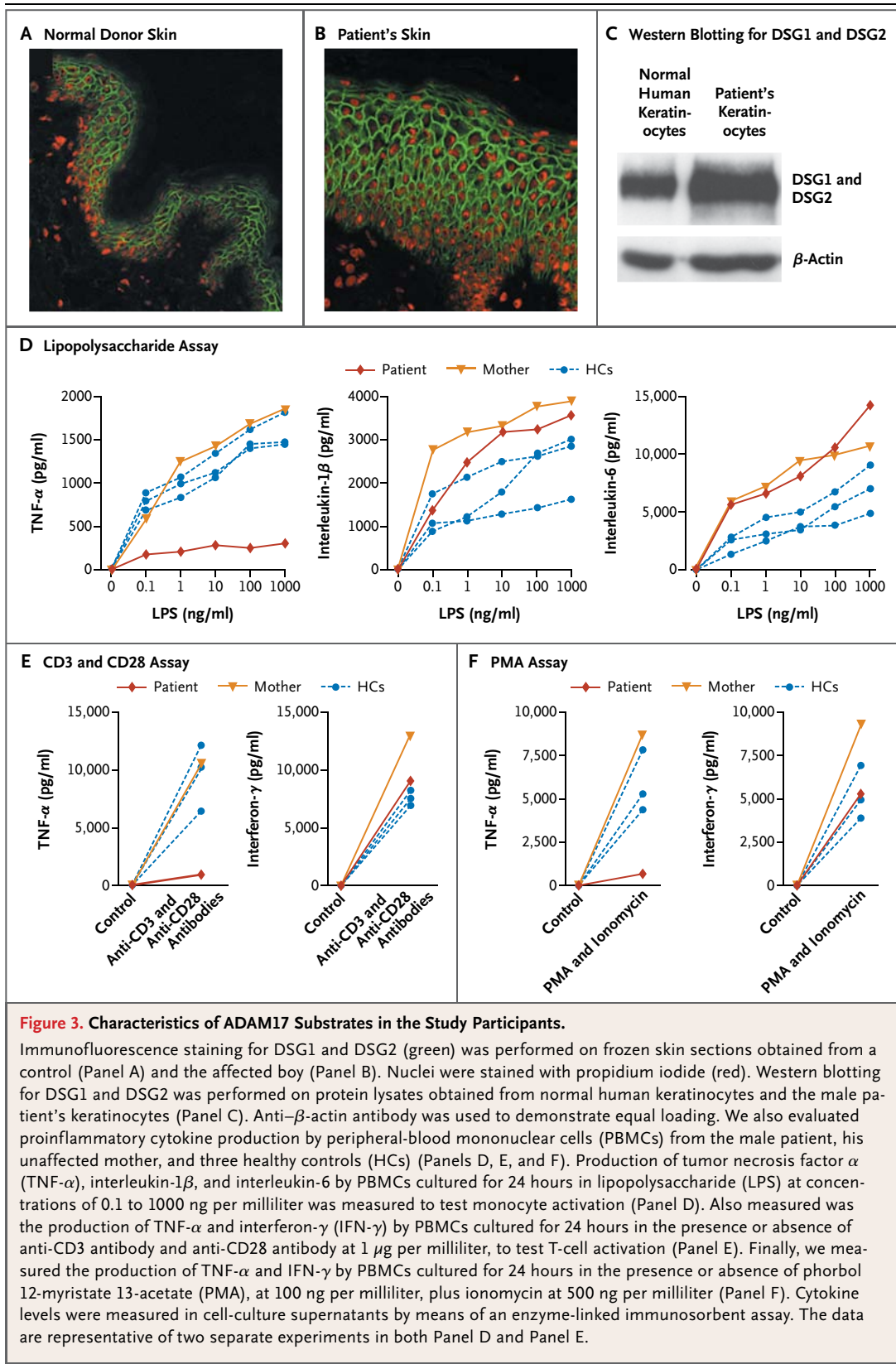


Figure 3. Characteristics of ADAM17 Substrates in the Study Participants.

Immunofluorescence staining for DSG1 and DSG2 (green) was performed on frozen skin sections obtained from a control (Panel A) and the affected boy (Panel B). Nuclei were stained with propidium iodide (red). Western blotting for DSG1 and DSG2 was performed on protein lysates obtained from normal human keratinocytes and the male patient's keratinocytes (Panel C). Anti- β -actin antibody was used to demonstrate equal loading. We also evaluated proinflammatory cytokine production by peripheral-blood mononuclear cells (PBMCs) from the male patient, his unaffected mother, and three healthy controls (HCs) (Panels D, E, and F). Production of tumor necrosis factor α (TNF- α), interleukin-1 β , and interleukin-6 by PBMCs cultured for 24 hours in lipopolysaccharide (LPS) at concentrations of 0.1 to 1000 ng per milliliter was measured to test monocyte activation (Panel D). Also measured was the production of TNF- α and interferon- γ (IFN- γ) by PBMCs cultured for 24 hours in the presence or absence of anti-CD3 antibody and anti-CD28 antibody at 1 μ g per milliliter, to test T-cell activation (Panel E). Finally, we measured the production of TNF- α and IFN- γ by PBMCs cultured for 24 hours in the presence or absence of phorbol 12-myristate 13-acetate (PMA), at 100 ng per milliliter, plus ionomycin at 500 ng per milliliter (Panel F). Cytokine levels were measured in cell-culture supernatants by means of an enzyme-linked immunosorbent assay. The data are representative of two separate experiments in both Panel D and Panel E.

skin-barrier proteins assessed, only transglutaminase 1 showed reduced expression in the skin of the affected boy (Fig. 6 in the Supplementary Appendix).

DISCUSSION

We suggest that the deletion mutation in *ADAM17* that was present in the homozygous state in both affected children caused their disease. The mutation is predicted to result in a protein that lacks all functional domains, including the catalytic domain required for the sheddase function of *ADAM17*.

The mouse orthologue of *ADAM17* was knocked out in mice over a decade ago, with most mutant mice dying before or soon after birth.⁸ A few mice survived for some weeks after birth but had eye degeneration, altered hair, and impaired epithelial-cell maturation in multiple organs, including the intestine.⁸ This extensively abnormal phenotype has been attributed to the effects of *ADAM17* not only on the membrane-bound form of *TNF- α* but also on a plethora of other molecules, including transforming growth factor α (*TGF- α*) and epidermal growth factor (*EGF*).⁸ Indeed, the hair and epithelial defects observed in mice lacking *Adam17* are similar to those reported in mice lacking *TGF- α* or *EGF* receptor.⁹⁻¹¹

Although there are some similarities between the phenotypes of the children we describe here and the mutant mice, particularly in relation to the hair,^{8,12} it appears that humans have mechanisms that compensate for the lack of *ADAM17*. In the two affected children, we observed a marked reduction of *ADAM17* expression in the skin and small intestine, and although both children had skin and gut problems, one child developed normally until the age of 12 years and the other remains healthy. Two of the most striking features of mice lacking *Adam17* are the hypoplastic crypts in the small bowel and the very low rate of epithelial production, which are almost certainly due to the fact that *ADAM17* is needed to cleave the epithelial-cell mitogen, *TGF- α* , from the cell membrane.^{9,10} Both children had early-onset diarrhea, which may have an origin similar to that of the diarrhea in mice lacking *Adam17*. However, later in life, biopsy revealed that the intestines of the affected children were substantially normal. This finding also supports the presence in humans of compensatory mechanisms or tethered ligand sufficient to maintain the intestinal epithelial renewal in the absence

of *ADAM17*. The cause of the gut problems in both children has never been satisfactorily resolved and requires more investigation.

The affected boy's keratinocytes expressed *ADAM10*, which cleaves some of the same substrates as *ADAM17*.¹³ *Desmoglein 2*, expressed in the less-differentiated layers of the epidermis and hair follicle, has been shown to be a direct target of both *ADAM17* and *ADAM10*.¹⁴ The increase in *DSG2* expression that we observed in the boy's skin and keratinocytes suggests that *ADAM17* regulation of *DSG2* availability at cell junctions is important in these tissues.¹⁵ *DSG2* is the predominant desmoglein expressed in cardiac myocytes, and *DSG2* mutations are associated with arrhythmogenic and dilated cardiomyopathies.^{16,17} The relationship between myocarditis and possible early-onset arrhythmogenic cardiomyopathy is well recognized,¹⁸ and impaired *DSG2* regulation by *ADAM17* may be responsible for the cardiac manifestations in the affected siblings. In addition, the lack of *TNF- α* may have been partly responsible for the affected girl's death, given the cardioprotective role of the molecule in acute myocarditis.¹⁹

PBMCs from the affected boy had impaired *TNF- α* production after stimulation with lipopolysaccharide, phorbol 12-myristate 13-acetate, or anti-CD3 and anti-CD28 antibodies, probably as a consequence of the *ADAM17* mutation. The low level of *TNF- α* in these cells is most likely due to molecules such as *ADAM10*. The scant production of *TNF- α* may have been a determinant of the affected boy's increased susceptibility to opportunistic infections of the skin, such as otitis externa from *S. aureus* and paronychia from candida. Given the redundancy in the immune system, however, other pathways were probably operating in the patient's skin and gut to limit infection and inflammation. The observation of marked T-cell infiltration in affected areas of the skin, in the absence of an acute inflammatory response, is consistent with this hypothesis.

It seems reasonable to conclude that the disease seen in the two siblings we studied is due to functional ablation of *ADAM17*. This enzyme is a highly attractive drug target for the treatment of chronic inflammatory diseases resulting from excess *TNF- α* production.^{20,21} However, the dramatic phenotype affecting the gut, lung, eyes, and hair of mice with *Adam17* deficiency has dampened enthusiasm for this therapeutic approach in humans. Our study suggests that loss of *ADAM17* is not

incompatible with human survival. Perhaps it would be worthwhile to re-examine ADAM17 as a therapeutic target in patients with chronic diseases such as psoriasis, rheumatoid arthritis, and cancer, with the understanding that ADAM17 inhibitors may have deleterious effects, including hair loss, cardiomyopathy, and myocarditis. However, further studies are needed to determine whether

short-term inhibition of the ADAM17 pathway has effects similar to those observed in the absence of the pathway in our patients.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES

- Dubois PCA, Trynka G, Franke L, et al. Multiple common variants for celiac disease influencing immune gene expression. *Nat Genet* 2010;42:295-302. [Erratum, *Nat Genet* 2010;42:465.]
- Liu Y, Helms C, Liao W, et al. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet* 2008;4(3):e1000041.
- Ng SB, Bigam AW, Buckingham KJ, et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet* 2010;42:790-3.
- Volpi L, Roversi G, Colombo EA, et al. Targeted next-generation sequencing appoints C16orf57 as clericuzio-type poikiloderma with neutropenia gene. *Am J Hum Genet* 2010;86:72-6. [Erratum, *Am J Hum Genet* 2010;87:445.]
- Rehman AU, Morell RJ, Belyantseva IA, et al. Targeted capture and next-generation sequencing identifies C9orf75, encoding taperin, as the mutated gene in nonsyndromic deafness DFNB79. *Am J Hum Genet* 2010;86:378-88.
- Kahrizi K, Hu CH, Garshasbi M, et al. Next generation sequencing in a family with autosomal recessive Kahrizi syndrome (OMIM 612713) reveals a homozygous frameshift mutation in SRD5A3. *Eur J Hum Genet* 2011;19:115-7.
- Denning MF, Guy SG, Ellerbroek SM, Norvell SM, Kowalczyk AP, Green KJ. The expression of desmoglein isoforms in cultured human keratinocytes is regulated by calcium, serum, and protein kinase C. *Exp Cell Res* 1998;239:50-9.
- Peschon JJ, Slack JL, Reddy P, et al. An essential role for ectodomain shedding in mammalian development. *Science* 1998; 282:1281-4.
- Luetke NC, Qiu TH, Peiffer RL, Oliver P, Smithies O, Lee DC. TGF α deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell* 1993;73:263-78.
- Mann GB, Fowler KJ, Gabriel A, Nice EC, Williams RL, Dunn AR. Mice with a null mutation of the TGF α gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell* 1993;73:249-61.
- Miettinen PJ, Berger JE, Meneses J, et al. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 1995;376:337-41.
- Chalaris A, Adam N, Sina C, et al. Critical role of the disintegrin metalloprotease ADAM17 for intestinal inflammation and regeneration in mice. *J Exp Med* 2010;207:1617-24.
- Le Gall SM, Bobé P, Reiss K, et al. ADAMs 10 and 17 represent differentially regulated components of a general shedding machinery for membrane proteins such as transforming growth factor alpha, L-selectin, and tumor necrosis factor alpha. *Mol Biol Cell* 2009;20:1785-94.
- Bech-Serra JJ, Santiago-Josefat B, Esselens C, et al. Proteomic identification of desmoglein 2 and activated leukocyte cell adhesion molecule as substrates of ADAM17 and ADAM10 by difference gel electrophoresis. *Mol Cell Biol* 2006;26:5086-95.
- Klessner JL, Desai BV, Amargo EV, Getsios S, Green KJ. EGFR and ADAMs cooperate to regulate shedding and endocytic trafficking of the desmosomal cadherin desmoglein 2. *Mol Biol Cell* 2009; 20:328-37.
- Posch MG, Posch MJ, Geier C, et al. A missense variant in desmoglein-2 predisposes to dilated cardiomyopathy. *Mol Genet Metab* 2008;95:74-80.
- Syrris P, Ward D, Asimaki A, et al. Desmoglein-2 mutations in arrhythmogenic right ventricular cardiomyopathy: a genotype-phenotype characterization of familial disease. *Eur Heart J* 2007;28: 581-8.
- Delmar M, McKenna WJ. The cardiac desmosome and arrhythmogenic cardiomyopathies: from gene to disease. *Circ Res* 2010;107:700-14.
- Wada H, Saito K, Kanda T, et al. Tumor necrosis factor-alpha (TNF-alpha) plays a protective role in acute viral myocarditis in mice: a study using mice lacking TNF-alpha. *Circulation* 2001;103: 743-9.
- Saftig P, Reiss K. The "A Disintegrin And Metalloproteases" ADAM10 and ADAM17: novel drug targets with therapeutic potential? *Eur J Cell Biol* 2011;90: 527-35.
- Arribas J, Esselens C. ADAM17 as a therapeutic target in multiple diseases. *Curr Pharm Des* 2009;15:2319-35.

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