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An Incompletely Penetrant Col7a1 Mutation Causes Dystrophic Epidermolysis Bullosa And Epidermolysis Bullosa Pruriginosa

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An incompletely penetrant *COL7A1* mutation causes dystrophic epidermolysis bullosa and epidermolysis bullosa pruriginosa

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ABSTRACT:

An incompletely penetrant *COL7A1* mutation causes dystrophic epidermolysis bullosa and epidermolysis bullosa pruriginosa.

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Epidermolysis bullosa pruriginosa (EBP) is a rare subtype of dystrophic epidermolysis bullosa (DEB) and is characterized by intense pruritus, nodular or lichenoid lesions, and violaceous linear scarring most prominent on the extensor extremities. Remarkably, identical mutations in *COL7A1*, which encodes an anchoring fibril protein present at the dermal-epidermal junction, can cause both DEB and EBP with either autosomal dominant or recessive inheritance. We present one family with both dystrophic and pruriginosa phenotypes of epidermolysis bullosa. The proband is a 19-year-old Caucasian female who initially presented in childhood with lichenoid papules affecting her extensor limbs and intense pruritus consistent with EBP. Her maternal grandmother was followed by a dermatologist for similar skin lesions that developed without any known triggers at age 47 and mostly resolved spontaneously after approximately ten years. The proband's younger brother developed a small crop of pruritic papules on his elbows, dorsal hands, knees, and ankles at age 13. Her second cousin once removed, however, report a mild blistering disease without pruritus consistent with DEB. Genetic sequencing of the kindred revealed a single dominant novel intron 47 splice site donor G>A mutation, c.4668+1 G>A, which we predict leads to exon skipping. Incomplete penetrance is confirmed in her clinically-unaffected mother, who also carries the same dominant mutation. The

wide diversity of clinical phenotypes with one underlying genotype demonstrates that *COL7A1* mutations are incompletely penetrant and strongly suggests that other genetic and environmental factors influence clinical presentation.

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This thesis would not have been made possible without the guidance of Dr. Keith Choate, who took me under his mentorship during my first year of medical school. His exceptional intellect is only surpassed by his brilliant demeanor with his patients. In every possible way, he has exemplified the role of a physician-scientist. Thank you for all of the time you spent assisting my research project and for your continuous encouragement and help in achieving my career aspirations.

Many thanks to all the members of the Lifton lab—in particular, Yin, Carol, Lynn, and Anita—for warmly welcoming me into the lab and for teaching me everything from the details of programming PCR machines to finding the correct reagents unearthed in secret boxes in random freezers.

The patient presented in this thesis was diagnosed by Dr. Richard Antaya, who had the clinical acumen to think of a disease that most people have never heard of. His infectious enthusiasm for childhood skin diseases, his gentle compassion towards his patients, and his unwavering willingness to teach has inspired my own interest in academic pediatric dermatology.

I would like to thank the patient and her family for their willingness to contribute to scientific research. It was an honor to meet such a warm, affectionate family with a lovely sense of humor.

This project was funded in part by the Office of Student Research. I am grateful for Yale's commitment to scholarly pursuits, the opportunities to engage in interesting endeavors, and the endowments available to make research accessible for all students.

And lastly, I wish to express appreciation for my family and friends who have provided endless support for my personal and academic development. I am indebted to my friends who have made New Haven my home away from home these past four years. To my parents—thank you, with my sincerest love and gratitude.

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INTRODUCTION:

The broad term ‘epidermolysis bullosa’ (EB) was first coined by German dermatologist Heinrich Koebner in 1866 to describe a group of inherited trauma-induced blistering disorders. Since its introduction, EB has been stratified into three subtypes depending upon the ultrastructural findings on electron microscopy: 1) EB simplex, denoting non-scarring blistering at the level of the epidermis; 2) junctional EB for blistering at the level of the lamina lucida; and 3) dystrophic EB (DEB) for scarring blisters at the level of the sub-lamina densa. A recent addition to the EB family in 2008 is Kindler's syndrome, a separate entity because its blistering can occur at any of the three cleavage planes (1). EB is a relatively common, prototypical genodermatosis with an estimated prevalence of 8.22 per 1 million people worldwide (2), although some believe this number to be an underestimate that does not capture many individuals falling within the milder spectrum of DEB (3).

DEB is a clinically heterogenous class of diseases that is caused by both dominant and recessive mutations in type VII collagen, the major anchoring fibril located below the basal lamina. Generalized dominant DEB, a term encompassing both Cockayne-Touraine and Pasini-type DEB, tends to produce a mild phenotype of localized blistering at sites of trauma that heal with milia and atrophic scarring. In some patients, the only manifestation of dominant DEB is isolated nail dystrophy or sub-clinical blistering. Severe generalized recessive DEB (RDEB-sev gen, previously Hallopeau-Siemens RDEB), on the other hand, often presents with whole-body blisters and extracutaneous manifestations such as excessive dental caries, esophageal

strictures, and colitis. These patients suffer from painful and mutilating contractures and have a shortened lifespan due to repeated infections or frequent, aggressive squamous cell carcinomas. An intermediate form named recessive DEB generalized other (RDEB-O, previously non-Hallopeau-Siemens RDEB or RDEB-mitis) also demonstrates extensive blistering and extracutaneous involvement but is a milder phenotype than RDEB-sev gen with decreased mortality. In addition to these three common subtypes of DEB, there are at least five other, rarer forms of DEB including epidermolysis bullosa pruriginosa (1).

Epidermolysis bullosa pruriginosa (EBP) was first described by McGrath *et al* in 1994 (4) and is distinguished from other types of DEB by its intense pruritus, lichenified or nodular prurigo-like lesions, and violaceous linear scarring. Blisters and erosions, which are the hallmark features of DEB, are rarely seen in EBP. The extensor extremities, particularly the shins, are commonly affected sites. Excoriations, milia, albopapuloid lesions, and nail dystrophy are often apparent whereas teeth and mucosal membranes are usually unaffected. Extracutaneous manifestations are extremely rare; only one EBP patient with gastrointestinal involvement has been reported in the literature thus far (5). Unlike DEB, which often manifests at birth, many cases of EBP do not present until adulthood (5–10). The oldest reported age of onset is 71 years (11). As such, EBP can be mistaken for acquired disorders such as prurigo nodularis, lichen simplex chronicus, hypertrophic lichen planus, or psychogenic pruritis.

A. Molecular processing of collagen VII

All forms of DEB, including EBP, are caused by mutations in the gene encoding for type VII collagen, *COL7A1*. The *COL7A1* gene, located on chromosome 3p21, contains 32 kilobases (12). The gene is transcripted into an 8.9 kb mRNA product, which is then translated into a 2944 amino acid proα1 polypeptide (13). The protein consists of three domains: a large non-collagenous (NC-1) domain from exon 1 to 28, a triple helical glycine-X-Y repeat domain from exon 29 to 111 with several short interruptions, and a smaller non-collagenous (NC-2) domain from exon 112-118. In the extracellular matrix, three proα1 polypeptides assemble into a collagen VII homotrimer. Two homotrimers form an antiparallel dimer and an aggregate of dimers assemble into anchoring fibrils with intermolecular disulphide bonds and transglutaminase cross-links for stabilization (14). The NC-1 domain on both ends of the anchoring fibrils adheres to the basement membrane above and the collagen IV fibrils in the dermis below (12,13).

Over 300 mutations in *COL7A1* have been identified in patients with DEB (12,15) and there appears to be some genotype to phenotype correlation. Typically, missense mutations and in-frame splice site mutations, deletions, and insertions are dominantly inherited while mutations resulting in premature termination codons (PTCs) are recessively inherited. Dominant DEB, which has milder clinical phenotypes, usually shows normal quantity and/or appearance of anchoring fibrils on biopsy. These mutations cause qualitative changes such as disrupting the correct processing of collagen VII or decreasing the stability of the anchoring fibrils. Recessive DEB, however, is more severe, with decreased amounts of collagen VII apparent on electron

microscopy and immunofluorescence staining. Patients with RDEB-sev gen typically possess two PTCs leading to no functional protein whereas RDEB-O contain one PTC and one missense mutation or two missense mutations causing malfunctioning or absent anchoring fibrils at the dermal epidermal junction (12). Hence, as expected, the severity of the DEB depends on the amount of collagen VII present.

Comparatively, over 50 mutations in *COL7A1* have been reported in EBP. The majority of the mutations are dominant missense alterations in the amino acid glycine within the triple helical domain, although exceptions exist (5,6,9). The immunofluorescence pattern in EBP typically resembles that of DDEB, with staining similar to normal skin or slightly decreased. No clear pattern of mutations leading to EBP emerges, but exon 87 skipping in particular has been suggested as predictive for EBP (10,16–18). At least six mutations either within the splice site donor or within exonic regulatory sequences have been identified in families with EBP (9–11,16–20). However, exon 87 skipping has also been described in dominant DEB (21) and has more recently been suggested to be prognostic for a mild phenotype of DEB rather than specifically EBP (22).

B. Hypotheses regarding the etiology of the pruritus in EBP

The cause of pruritus in EBP is unknown. Remarkably, the identical mutation in *COL7A1* can cause DEB in one patient and EBP in another (5–9,23). Alternate hypotheses regarding the origin of pruritus have included elevated serum IgE levels (9,20,24), concurrent filaggrin mutations leading to atopy (5), increased expression of

matrix metalloproteinase 1 (*MMP1*) causing imbalance of collagen degradation (10,25), and increased cytokine IL-31 (26). None of these hypotheses uniformly accounts for pruritus observed in EBP. A more thorough discussion of these theories is presented below.

i. Elevated serum IgE levels

Elevated serum IgE levels initially seemed like a promising correlation with the hallmark pruritus of EBP. Early case reports had noted elevated IgE levels in their patients (24,27). In one study, seven out of nine patients studied had elevated IgE levels, several with values threefold higher than the upper limit of normal (20). However, in a 2006 study by Drera *et al*, only two out of seven EBP patients had elevated IgE levels (9). Furthermore, in a control group of patients with non-pruritic DEB, two out of six also had elevated IgE and thus the marker proved to be poorly specific for EBP (20). In subsequent reports, it quickly became apparent that the link between hyper-IgE levels and EBP was tenuous at best, as the overwhelming majority of patients with EBP have normal IgE levels (5,16,17).

ii. Co-existing pruritic disorder

The initial correlation between IgE levels and EBP induced a discussion of whether these patients possessed an atopic diathesis. In the initial report by McGrath *et al*, only one of the seven patients presented had a history of eczema, asthma, or rhinoconjunctivitis (4). Among the patients with elevated IgE levels reported in other

case series, several had either a personal or family history of atopic disease (27), but most were unexplained (9,20,28). Yet, in many instances, adequately treating the atopy with corticosteroids induced improvement in the patient's cutaneous lesions of EBP as well (9,27).

Other etiologies of pruritus such as thyroid dysfunction, cholestasis, end stage renal disease, iron deficiency, and Hodgkin's lymphoma are routinely screened for and rarely found (4,9,20). However, many patients recall mild DEB that evolve into EBP, often attributing this to the onset of pruritus due to an external cause such as pregnancy, thyroid cancer, diabetes mellitus, or even varicella virus infection (5,6,8,11) although correction of these diseases did not lead to improvement of their cutaneous lesions. An interesting observation is the onset of pruritus with onset of puberty, suggesting a possible modifying role for the hormonal cascades associated with adolescence acting as a trigger (9,29). However, many others report improvement of their lesions during puberty (6,18) and thus again, no clear pattern emerges.

iii. Filaggrin mutations

For years, mutations in filaggrin, a protein integral to an intact skin barrier, was suspected to predispose children to atopic dermatitis and ichthyosis vulgaris (30), but further characterization of this association proved elusive due to the highly repetitive sequences precluding sequencing until 2007 (31). This seminal paper documented two filaggrin variants with a prevalence of approximately 9% in the European population

and three additional less common variants that cumulatively accounted for another 2.6% (30,31). Schumann *et al* postulated that mutations in filaggrin may further compromise the skin barrier and thus correlate with the pruritus of EBP (5). They screened seven patients with EBP for the two most common filaggrin variants in the European population, R501X and 2282del4, but found neither in all of the patients.

iv. Matrix metalloproteinase-1 expression

Matrix metalloproteinase-1 (MMP1) degrades extracellular proteins such as type VII collagen and is integral to tissue remodeling and wound healing. Titeux *et al* described a polymorphism in the gene promoter of MMP1 that increases its expression, leading to more severe RDEB than those who did not carry the polymorphism (25). The addition of a nucleotide G creates a new ETS transcription binding site in the promoter, thus enhancing the expression of MMP1. Almaani *et al* postulated that the increased degradation of type VII collagen by MMP1 could lead to secondary inflammatory changes causing pruritus (32). They screened a cohort of 27 patients with EBP, 23 patients with DDEB, 25 patients with RDEB, and 50 control subjects for the MMP1 promoter polymorphism, rs1799750: (-)>G. Although the researchers found a higher frequency of the 2G allele in RDEB group, there was no significant difference between the 1G and 2G MMP1 alleles among the EBP, DDEB, and control groups (32).

v. Immunomodulatory factors

Given the success of immunomodulatory agents such as cyclosporine and tacrolimus in the treatment of EBP, a hypothesis regarding the effect of cytokines and other immunomodulatory factors has been proposed (9,33). Overexpression of cytokine interleukin-31 (IL-31) has been found to be upregulated in patients with atopic dermatitis and also to induce severe itching and eczema in mice (34,35). Polymorphisms within cytokine IL-31 has been linked to increased stimulation of T-cell antibodies and hence, eczema susceptibility (36). Using the same cohort of EBP, DDEB, RDEB, and unaffected patients described above in 'iv' (32), the group tested the IL-31 haplotype and again found no statistically significant differences in allele frequencies. However, the authors readily admit the limitations of their study, which only examined the genetic sequence and did not measure the amount of IL-31 present in patients' serum.

STATEMENT OF PURPOSE

In order to further elucidate the clinical heterogeneity of DEB and EBP, we studied one kindred with both of these phenotypes (Figure 1). The proband is a 19-year-old female of Italian descent who was referred to Yale Dermatology Associates at age five with new-onset pruritic lesions on her right medial malleolus and chin. Over time, the patient's pruritus gradually worsened with extension of lesions to her extensor extremities bilaterally, the peri-umbilicus, the sacrum, and the upper mid-back. No triggers were reported. She was otherwise healthy, without a history of atopy. Her parents are non-consanguineous and neither reported any history of atopy, skin fragility, or pruritic dermatoses. The proband's grandmother developed similar lesions at age 47, which persisted for approximately ten years before mostly resolving spontaneously. The proband's 15-year-old brother also developed a few pruritic papules at age 13, but was much less extensively affected. Meanwhile, her second cousins once removed and their father report only trauma-induced blistering. The objective of this study was to better characterize the genetic complexity of *COL7A1* mutations causing DEB and EBP in this kindred.

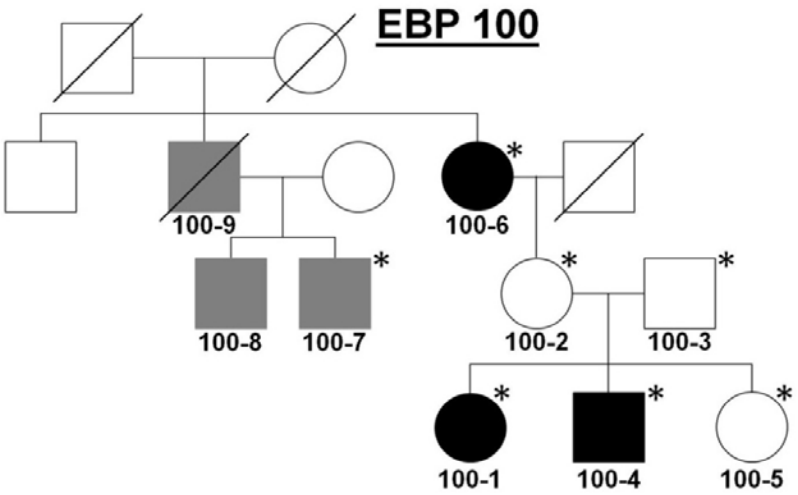

Figure 1. DEB and EBP phenotypes independently segregate in one kindred with incomplete penetrance. (A) Individuals in EBP kindred 100 show either an epidermolysis bullosa pruriginosa phenotype (black symbols) or a dominant dystrophic epidermolysis bullosa phenotype (grey symbols). * indicates genetic analysis has been performed.

METHODS:

This study protocol was approved by the Human Investigation Committee at Yale University. The proband (100-1), her immediate family members (100-2-5), and two members from her extended family (100-6, 100-7) were clinically examined by dermatologists between 1997 and 2011. Two family members reported to have DEB were unable to participate in this study (100-8, 100-9).

In the proband, clinical diagnosis was verified with skin biopsies of lesional sites performed with local anesthetic. The biopsy specimens were embedded for light and electron microscopy by Yale Dermatopathology Labs (New Haven, CT). Immunofluorescence staining for collagen IV, VII, and keratin 14 was performed by Beutner Laboratories (Buffalo, NY). Complete blood count, IgE levels, thyroid function, renal function, AST/ALT, bilirubins, iron, and ferritin were tested in the proband to exclude alternative causes of pruritus.

Genomic DNA was extracted from peripheral blood lymphocytes in the proband and immediate family members using phenol-chloroform extraction. Primer sets representing all 118 exons of *COL7A1*, including intron-exon boundaries, were generated with Primer3 (http://frodo.wi.mit.edu/primer3/) using the hg18 version of the genome masked to exclude DNA sequence repeats (GenomeMasker: http://bioinfo.ebc.ee/snpmasker/) as the template. We also generated primers for the five most common filaggrin mutations in European populations (R501X; 2282del4; 3702delG; R2447X; and S3247X) (31) and for the previously reported promoter SNP

in MMP1 (rs1799750: (-)>G) hypothesized to account for the pruritus of EBP (25). Polymerase chain reaction was carried out on 50 ng of DNA using KAPA2G Fast Polymerase (Kapa Biosystems; Woburn, MA) and then sequenced. The sequences were compared to the human reference sequence (hg18, NCBI) utilizing Sequencher (Gene Codes; Ann Arbor, MI). A single sequence variant in *COL7A1* was identified which was not found in SNP databases and in sequencing of 95 ethnically-matched unrelated controls without history of skin disease.

Review of the literature was performed using PUBMED search term "epidermolysis bullosa pruriginosa" between June 2009 and October 2011 and results are summarized in Table 1. Forty-two papers were identified; thirteen papers were excluded either because the case presented was not affected with epidermolysis bullosa pruriginosa or because no genetic analysis was performed. Additional mutations were found using the International Dystrophic EB Patient Registry database (http://www.deb-central.org). Each mutation was then surveyed using the Human Gene Mutation Database (http://www.hgmd.org/) and recent reviews of *COL7A1* gene (12,15) to determine if it had been previously reported to cause DEB. Any discrepancies between sources were resolved by referring to the original article reporting the mutation in question.

Statement of contributions of authors:

Anita Farhi was actively involved in patient recruitment, in acquiring HIC approval for this study, and in obtaining peripheral blood samples from the subjects of this

study. Carol Nelson-Williams extracted the DNA from the blood samples. Drs. Richard Antaya and Keith Choate performed the clinical characterization of all the subjects examined and the skin biopsies when appropriate. Dr. Earl Glusac performed the histopathological analysis of the skin biopsies. Dr. Michael Kashgarian performed the electron microscopy readings of the proband's biopsy. I designed and executed the study, performed all of the PCRs, and analyzed all of the DNA sequence readings.

RESULTS:

On full skin examination, the proband displayed excoriated, lichenoid papules with milia coalescing into confluent plaques (Figure 2). There was no nail dystrophy and ancillary lab tests to evaluate for pruritus were all within normal limits. Skin tests for common food allergens and standard inhalants were negative, except for an allergic reaction to penicillin at age 9. Histopathology of her lesions showed a subepidermal cleft with milia and electron microscopy showed a subepidermal cleft with lysis and separation of collagen fibrils in the reticular dermis. There was no evidence of amyloid deposition. Direct immunofluorescence showed normal quantities and immunolocalization patterns of collagen IV, collagen VII, and keratin 14 (Figure 3). Altogether, these findings supported a diagnosis of EBP.

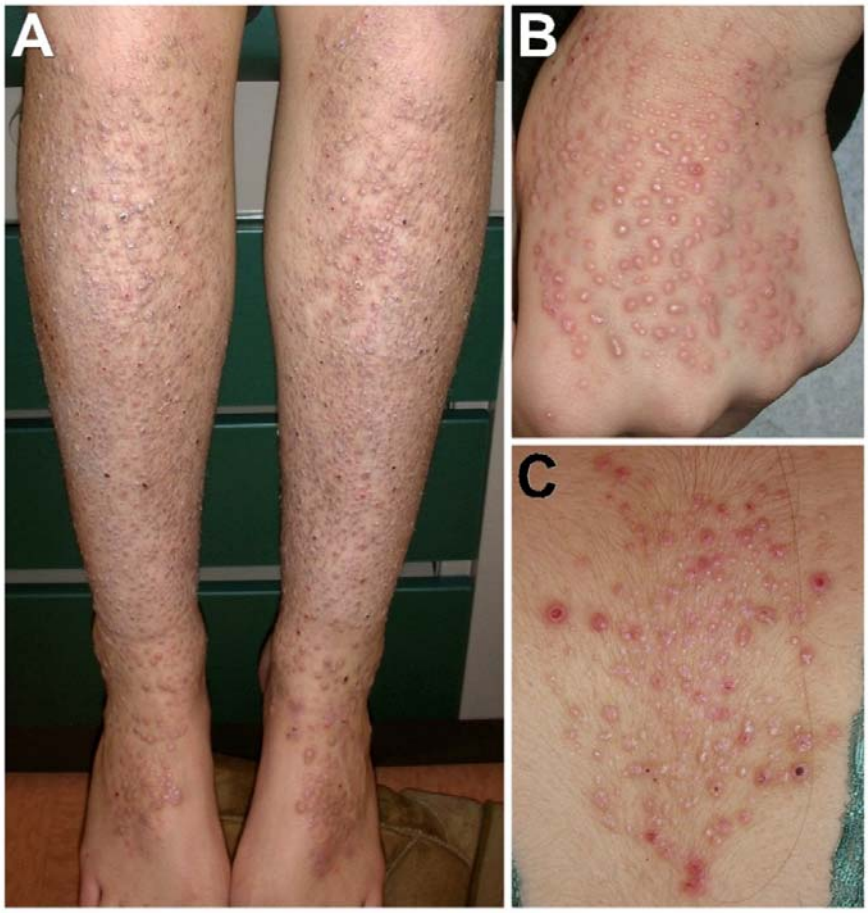

Figure 2. Clinical features of EBP in proband, 100-1. (A-C) Lichenoid papules and nodular prurigo-like lesions on shins bilaterally (A), dorsal hand (B), and upper back (C). Many papules are excoriated.

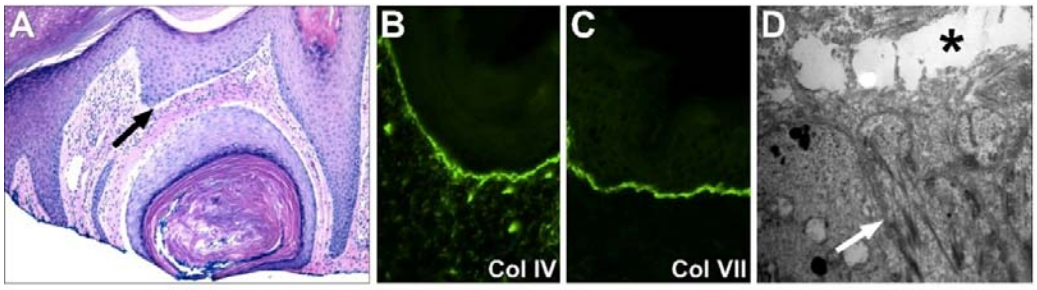

Figure 3. Histologic features of EBP in proband, 100-1. EBP is characterized by subepidermal clefting and disruption of anchoring fibrils. (A) Hematoxylin and eosin stain of affected skin shows subepidermal clefting (black arrow) and milium, 20X. (B, C) Direct immunofluorescence staining shows normal pattern and amount of collagen IV and VII. (D) Electron microscopy of skin adjacent to a nodular lesion shows lysis and separation of collagen fibrils in the reticular dermis (white arrow). * indicates subepidermal cleft.

The only remnants of EBP in the propositus' grandmother were macular scars on her upper back and intermittent clusters of pruritic papules on her upper extremities (Fig. 4A). The proband's brother had few, isolated pruritic papules on his dorsal hands, elbows, knees, and ankles (Fig. 4B). The proband's second cousin once removed exhibited trauma-induced blistering, atrophic scarring, and few milia on his extensors (Fig. 4C). Her mother had no skin findings on full skin examination.

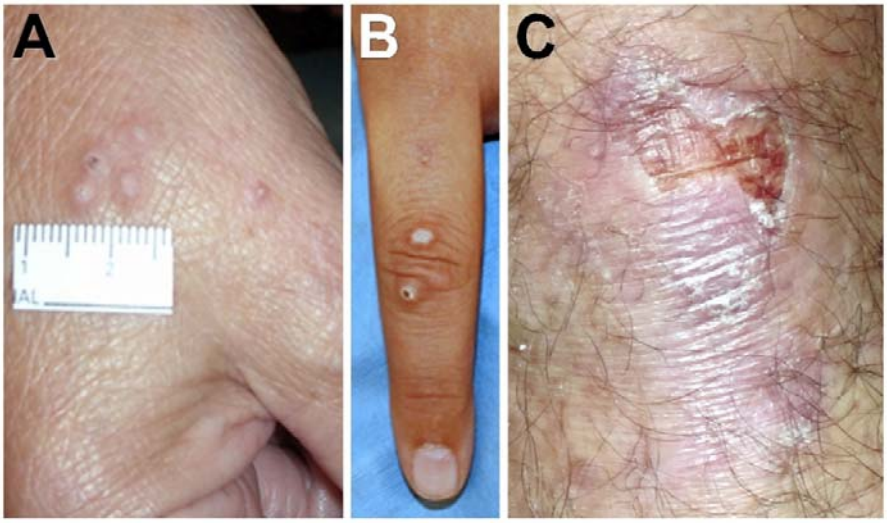

Figure 4. Clinical heterogeneity of EBP and DEB in kindred EBP 100. (A) Cluster of papules on the right hand of the proband's maternal grandmother, 100-6. (B) Lichenoid papules on the left index finger of the proband's 15-year-old brother, 100-4. (C) Blister and atrophic scarring on knee of the proband's second cousin once removed, 100-7.

Sanger sequencing of *COL7A1* in this kindred revealed a novel heterozygous splice site mutation in intron 47, c.4668+1 G>A, in the proband, her affected family members, and her unaffected mother (Figure 5). This mutation abolishes the intron 47 splice donor; similar mutations frequently lead to exon skipping or use of cryptic splice sites nearby (9,16,18,19). Fifty-six mutations causing EBP are reviewed in Table 1 and Figure 6. All of the dominant mutations, including c.4668+1 G>A, interfere with the consecutive Gly-X-Y repeats in the triple helical domain of the protein. Only ten recessive cases were identified.

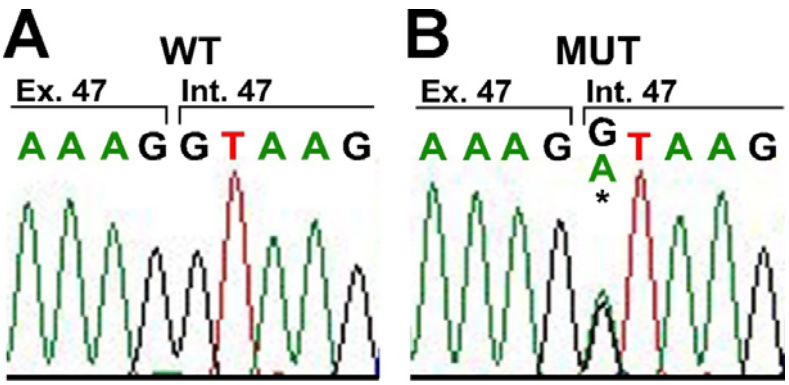

Figure 5. DEB and EBP phenotypes result from the same *COL7A1* mutation with incomplete penetrance. Sanger sequencing of exons and intron/exon boundaries of *COL7A1* was performed. (A) WT junction between exon 47 and intron 47 shows position of normal splice donor. (B) A heterozygous c.4668+1 G>A mutation abolishes the intron 47 splice donor in all individuals with EBP (100-1, 4, 6), in a single individual with DEB (100-7), and in the unaffected mother of the index case (100-2). Wild type sequence was present at this position in the unaffected father (100-3) and unaffected sibling (100-5) of the index case.

Mutation	Exon	Effect	Previously reported to cause DEB?	Age of onset	Ethnicity	Sex	IgE level	Other affected family members?	Reference
p.R28G/ p.G2366A	1/92	Missense/ Missense	G2366N: RDEB-O	DEB since 8 y/o; EBP developed after first pregnancy	Italian	F	Normal	Brother with nail-dystrophy-only DEB	Pruneddu, 2010
p.R51G/ p.R2492X	2/98	Missense/ PTC	c.7474C>T: RDEB-O	13 y/o	Italian	M	Normal	None	Drera, 2006
c.425A>G/ c.7344G>A	3/95	Splice PTC/ Splice PTC	Both: RDEB-O	12 y/o	Italian	F	Normal	None	Drera, 2006
c.425A>G/ p.E2736K	3/110	Splice PTC/ Missense	c.425A>G: RDEB-O	DEB since 6 y/o; EBP developed at 53 y/o	Caucasian	M	Normal	None	Schumann, 2008
c.497insA/ p.G1347W	4/34	PTC/ Missense	c.497insA: RDEB-sev gen G1347N: RDEB-O	8 y/o	Caucasian	F	Normal	None	Schumann, 2008
c.4668+1 G>A	47	In-frame exon skipping	No	5 y/o	Italian	F	Normal	Multiple family members w/ DDEB spectrum	This report
p.G1572A/ c.7787delG	48/104	Missense/ PTC	No	NA	NA	NA	NA	NA	Almaani, 2011
p.R1630X/ c.7344G>A	51/95	Nonsense/ Splice PTC	R1630X: RDEB-sev	8 y/o	Italian	F	Normal	None	Drera, 2006

			gen c.7344G>A: RDEB-O						
p.R1630X	51	Nonsense	RDEB-sev gen	1 y/o	Caucasian	F	Normal	None	Schumann, 2008
c.5399+1G>C	In. 55	In-frame exon skipping	No	6 y/o	Caucasian	M	NA	Mother and sister w/ toenail dystrophy-only DEB	Tey, 2011
p.G1755D	59	Missense	No	NA	NA	NA	NA	NA	Posteraro, 2005
p.G1755D	59	Missense	No	39 y/o	Italian	F	Normal	None	Drera, 2006
p.G1755D	59	Missense	No	8 y/o	Turkish	F	Normal	Mother with nail-dystrophy-only DEB	Schumann, 2008
p.G1770D	61	Missense	RDEB	NA	NA	NA	NA	NA	Almaani, 2009
p.G1773R	61	Missense	DDEB	9 y/o	Chinese	M	Normal	Mom and maternal grandfather w/ mild phenotype	Jiang, 2011
p.G1791E	61	Missense	No	Infancy	Caucasian	F	NA	None	Mellerio, 1999
c.5532+1G>A/ c.7786delG	In 64/ Ex104	In-frame exon skipping/ PTC	c.7786delG: RDEB-O	Infancy	Caucasian	F	NA	None	Mellerio, 1999
p.G1860R	66	Missense	No	NA	NA	NA	NA	NA	Almaani, 2009
p.G1913R	69	Missense	No	NA	NA	NA	NA	NA	Almaani,

									2009
p.Q1924P/ c.6619-2A>T	Ex 69/ In 82	Missense/ In-frame exon skipping	No	NA	Middle Eastern	NA	NA	NA	Abu Sa'd, 2006
p.G2028R	73	Missense	DDEB and RDEB	20 y/o	Japanese	F	NA	Father, aunt, and paternal grandmother with EBP	Murata, 2000
p.G2028R	73	Missense	DDEB and RDEB	DEB since 1 y/o; EBP developed at 18 y/o	Caucasian	F	Normal	Grandmother, brother, and daughter with DEB	Schumann, 2008
p.G2034R	73	Missense	DDEB	NA	Chinese	NA	NA	NA	Chen, 2000
p.G2034W	73	Missense	DDEB	DEB since birth; EBP developed at 7 y/o	Caucasian	F	Normal	Grandmother, father, and sister have DEB	Schumann, 2008
p.G2037E	73	Missense	DDEB	NA	NA	NA	NA	NA	Unpublished
p.G2040D	73	Missense	DDEB	Infancy	Caucasian	F	Elevated	None	Ozanic, 2005
p.G2073V	75	Missense	RDEB-O	10 y/o	Italian	F	Elevated	Father with EBP	Drera, 2006
p.G2079R	75	Missense	No	NA	NA	NA	NA	NA	Abu Sa'd, 2006
p.G2159E	79	Missense	No	NA	NA	NA	NA	NA	Almaani, 2009
p.G2210V/ p.G2791W	83/113	Missense/ Missense	G2791W: DDEB	NA	Aboriginal	NA	NA	Multiple family members w/ DDEB-Pr and	Dang, 2007

								Pasini subtype	
p.G2213R	83	Missense	RDEB	37 y/o	Caucasian	F	NA	NA	Almaani, 2009
c.6652-2A>G	83	In-frame exon skipping	No	NA	NA	NA	NA	NA	Abu Sa'd, 2006
p.G2239D	85	Missense	No	NA	NA	NA	NA	NA	Tamai, 1998
p.G2239V	85	Missense	No	NA	NA	NA	NA	NA	Almaani, 2011
p.G2242E	85	Missense	No	NA	NA	NA	NA	NA	Tamai, 1998
p.G2242E	85	Missense	No	12 y/o	Japanese	F	NA	Son w/ bullous dermolysis of the newborn	Murase, 2011
p.G2242R	85	Missense	No	Teens	Chinese	F	NA	Father, brother, aunt, and cousin affected with mild EBP	Lee, 1997
p.G2242R	85	Missense	No	7 y/o	Caucasian	M	NA	Father with mild EBP	Mellerio, 1999
p.G2242W	85	Missense	No	10 y/o	Chinese	M	Elevated	Father, grandfather, and paternal uncle with EBP	Shi, 2009
p.G2251E	86	Missense	DDEB	25 y/o	Chinese	F	Normal	4 siblings and mother with EBP; 2 unaffected siblings with mutation	Ee, 2007
p.G2251E	86	Missense	DDEB	1 month old	Japanese	M	NA	Unaffected father	Takayashi,

								w/ mutation	2011
c.6846G>C	87	In-frame exon skipping	No	3 kindreds	Danish	NA	NA	Multiple family members w/ blistering and nail dystrophy	Covaciu, 2011
p.G2287R	87	Missense	DDEB	71 y/o	Japanese	M	Normal	None	Hayashi, 2011
c.6863del16	87	PTC	No	11 y/o	Hispanic	M	NA	Daughter with mild EBP; 5 generation family with DEB	Mellerio, 1999
p.G2290A	87	Missense	No	40s	Caucasian	F	NA	NA	Almaani, 2009
c.6899+2A>G	87	In-frame exon skipping	No	< 35 yo	Chinese	M	NA	18 other family members with EBP	Jiang, 2002
c.6900+1G>T	In. 87	In-frame exon skipping	DEB	2 month old	Chinese	M	Normal	11 other family members with EBP	Ren, 2007
c.6900+1G>C	In. 87	In-frame exon skipping	No	20 y/o	Chinese	M	Normal	Multiple affected family members	Jiang, 2011
c.6900+2delTGAT	In. 87	In-frame exon skipping	No	30 y/o	Italian	F	Elevated	16 members in 5 generations with skin lesions	Drera, 2006
c.6900+4A>G	In. 87	In-frame exon skipping	RDEB-sev gen	38 y/o	Italian	F	Normal	5 other affected members in 4 generations with skin lesions	Drera, 2006
p.G2360R	92	Missense	No	NA	NA	NA	NA	NA	Almaani,

									2011
p.G2366V	92	Missense	RDEB-O	20s	Chinese	F	Normal in proband; elevated in son	Son and daughter with EBP	Chuang, 2004
p.G2369S	93	Missense	RDEB	17 y/o	Pakistani	M	NA	None	Mellerio, 1999
p.G2508D	100	Missense	No	NA	NA	NA	NA	NA	Almaani, 2011
p.G2517D	100	Missense	No	NA	NA	NA	NA	NA	Almaani, 2011
p.G2623V	105	Missense	RDEB-O and DDEB	8 y/o	Caucasian	M	Normal	Brother with DEB	Schumann, 2008
p.G2626D	106	Missense	No	2 y/o	Chinese	F	NA	4-generation family with skin lesions	Wang, 2007
p.G2680D	108	Missense	No	NA	NA	NA	NA	NA	Almaani, 2011
p.G2701W	109	Missense	No	25 y/o	Chinese	F	Normal	Unaffected mother w/ mutation	Jiang, 2011
p.G2713R	110	Missense	DDEB	29 y/o	Caucasian	F	NA	Father and nephew with DEB	Mellerio, 1999
p.G2713R	110	Missense	DDEB	27 y/o	Turkish	F	NA	None	Broekaert, 2006
p.G2719D	110	Missense	No	6 y/o	Caucasian	F	NA	Brother and mother	Riedl, 2009

Table 1. Reported EBP mutations organized by position within *COL7A1*. We found 61 reports of EBP. In 27 cases, other family members were affected with DEB and its variants. Of the 56 different mutations found, only 34 mutations were not previously reported to cause either DDEB or RDEB. All probands have unifying features of pruritus, lichenoid papules, and/ or prurigo-like nodules most commonly on the shins.

Many additionally presented with milia and nail dystrophy. Missense mutations are denoted using protein nomenclature while other mutations consisting of, but not limited to, splice site mutations, insertions, and deletions are denoted using the coding sequence. AR = autosomal recessive; AD = autosomal dominant; DDEB = dominant dystrophic epidermolysis bullosa; RDEB-sev gen = severe generalized recessive dystrophic epidermolysis bullosa; RDEB-O = generalized other recessive dystrophic epidermolysis bullosa; In = intron; X = termination codon; N = any protein; NA = not available; ND = not detected.

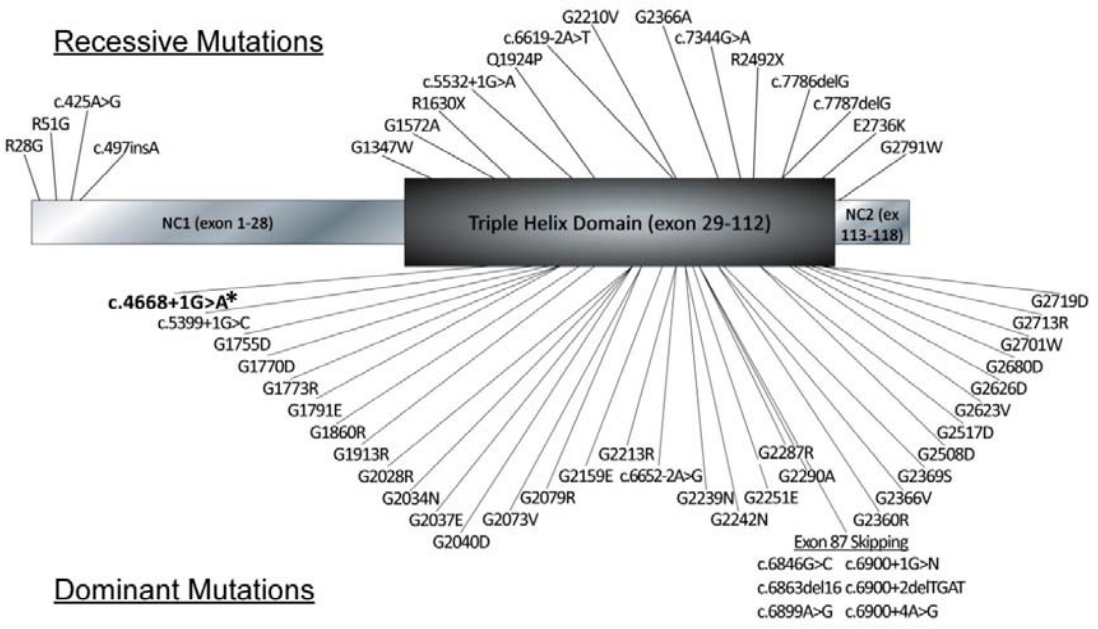

Figure 6. *COL7A1* mutations reported to cause EBP. Fifty-six mutations are represented. Missense and nonsense mutations are represented using the protein sequence whereas splicing mutations, deletions, and insertions are represented using the cDNA sequence. The mutation identified in this family is bolded and denoted by an asterisk (*). Recessive mutations appear above the protein schematic and dominant mutations appear below.

Recognizing that filaggrin mutations could modify the phenotypes observed in this kindred (30,31), we performed sequencing of the five most common *FLG* variants in the European population in our patient and found none. We also addressed the findings of another group which suggested that a promoter SNP in *MMP1* leads to an imbalance of collagen degradation secondary to overexpression of matrix metalloproteinase 1 with ensuing inflammation and pruritus (25,32). Sequencing of the proposed transcription factor binding site (rs1799750: (-)>G) of *MMP1*, however, did not reveal any sequence differences between the proband and her mother.

DISCUSSION:

Intra-familial differences in clinical manifestations of EBP is well described in the literature (8,18,37,38). One member of the family might suffer from severe EBP while another from a milder EBP (17,20,39), nails-only DDEB (5,6,40), generalized DDEB (5,18,20), rarer variants of DEB (29,38), or even no phenotype at all (8,17,37). For EBP, this phenomenon has been attributed to a presumed adult onset, and attempts have been made to predict a phenotype in infants or children based on their genetic mutation and family history (8,21,29). However, the pattern is not consistent and others report affected children and a clinically unaffected parent carrying the mutation (37). In our case, although the proband and her brother presented in childhood, their grandmother developed EBP at age 47 and their 46-year-old mother currently has no skin findings despite carrying the same dominant mutation.

The heterogeneity of phenotypes even within one family can be partially explained by the incomplete penetrance of *COL7A1* gene mutations. In accordance with previous reports, no clear pattern emerged from our attempt to correlate *COL7A1* gene mutations with disease phenotype. Most of the pathogenic mutations in EBP are dominant and within the triple helix, thus altering the Gly-X-Y amino acid triplet integral for collagen stability and tensile strength. Of the ten recessive cases found in our review of the literature, seven contain a combination of loss-of-function/gain-of-function mutation, suggesting that the gain-of-function mutation is in reality the causative allele while the other allele includes a silent mutation or SNP (Table 2). Clinically, the mild blistering phenotype of EBP usually resembles that of DDEB

more than RDEB and only rarely is quantitative or qualitative changes in type VII collagen noted upon biopsy.

Case	Mutation 1 (Effect)	Mutation 2 (Effect)
1	p.R28G (missense)	**p.G2366A** (missense)
2	**p.R51G** (missense)	p.R2492X (PTC)
3	c.425A>G (splice PTC)	**c.7344G>A** (splice PTC)
4	c.425A>G (splice PTC)	**p.E2736K** (missense)
5	c.497insA (PTC)	**p.G1347W** (missense)
6	**p.G1572A** (missense)	c.7787delG (PTC)
7	p.R1630X (nonsense)	**c.7344G>A** (splice PTC)
8	**c.5532+1G>A** (in-frame exon skipping)	c.7786delG (PTC)
9	p.Q1924P (missense)	c.6619-2A>T (in-frame exon skipping)
10	p.G2210V (missense)	**p.G2791W** (missense)

Table 2. Reported mutations causing recessive EBP. The majority of these cases have one mutant allele predicted to result in loss of function and a second gain of function mutant allele expected to contribute to disease phenotype, giving essentially mono-allelic expression. Exceptions include cases 1, 9, and 10 in which two gain-of-function mutations are present. The mutations c.425 A>G, c.497insA, and R2492X are predicted to undergo nonsense-mediated decay and thus would not be expressed (41). In Case 10, the authors postulated that previously reported G2791W was the relevant mutation and that G2210V is a polymorphism found in Australian Aboriginals (38). The putative causative mutations are bolded, based on prior reports of the same mutation causing EBP or presumed deleterious effect on the encoded protein.

Overwhelmingly, the EBP patients presented in the research literature and summarized in Table 1 are from institutions that can afford genetic sequencing, that is, those located in Western Europe, China and Japan, Australia, and the USA. Only a few case reports have been published from India, but none offer a genetic analysis and some did not perform the necessary biopsies to rule out other causes of the lesions such as lichen amyloidosis or prurigo nodularis (42,43). There is a dearth of genetic information known about populations in the Middle East, Africa, and South America, although these kindreds often present with unique *COL7A1* mutations that may

perhaps lead to new insights regarding the phenotypic variability in this genodermatosis (44).

The mutation identified in this study, c.4668+1 G>A, is located at the conserved splice site donor of intron 47, which we predict leads to in-frame exon 47 skipping based upon previous studies investigating splice site mutations (20,40,44). Although we did not confirm the altered protein product with reverse transcriptase PCR, we hypothesize that two mRNA products would be isolated from the proband and all other carriers of the heterozygous mutation. Exon 47 contains 11 amino acids and is located within the triple helix. Exclusion of this exon is expected to alter the stability of collagen VII anchoring fibrils in the papillary dermis.

Notably, affected individuals can transition from a DEB to an EBP phenotype; most attribute this to the development of pruritus, which then, in turn, causes the lesions of EBP (5,6,9,24,27). This finding lends support to the question of whether EBP is a unique disease entity or whether the phenotype is due to pruritus superimposed on DEB (4,11,29). Although some patients report an initiating incident such as thyroid dysfunction or chickenpox infection, most EBP cases did not improve when the pruritic disease resolved and the majority of EBP patients do not remember an inciting factor (5). Thus, there may be an element of recall bias in these case reports, but larger trials are difficult to conduct given the rarity of EBP. In our kindred, neither the index case nor her brother is atopic, and although their grandmother developed hypothyroidism in her 30s, she was clinically euthyroid at the time of her EBP

eruption nearly fifteen years later. Yet, the question of whether itch is a symptom of EBP versus integral to the pathogenesis of EBP is still being debated.

EBP is notoriously challenging to treat. Therapeutics reported to be successful in the literature include high-dose topical and oral corticosteroids (4,9), cyclosporine (33,40), topical tacrolimus (10,45), and thalidomide (46). However, none of these therapeutics work universally for patients with EBP. In a cohort of eight patients on tacrolimus, only one patient reported significant relief and three others reported partial relief (32). Corticosteroids has a similar, if not higher, failure rate (4,9) and the number of patients treated with cyclosporine is too few to gauge its efficacy. The exact mechanisms by which these treatments affect EBP is still unknown, but the common endpoint among them is the dissolution of pruritus with improvement of the cutaneous lesions manifesting several months later (9,32,46). This observation leads to the possibility of new medications directed at novel targets as we learn more about the pathophysiology of pruritus.

Our patient was treated with numerous medications including topical and systemic corticosteroids, anti-histamines, anti-opiates, capsaicin, TNFα inhibitors, and phototherapy with minimal success. Oral cyclosporine 5 mg/kg mildly improved her pruritus, but was discontinued after five months due to marked gingival hyperplasia. Low-dose thalidomide has been documented to improve EBP, most likely via immunomodulation of the production of TNFα, IFNγ, and other cytokines (46). Our patient was started on 100 mg/day thalidomide and reported decreased pruritus and

flattening of her lesions within two months. She developed mild peripheral neuropathy after four months of treatment so the dose was decreased to 50 mg/day with resolution of her paresthesias and continued efficacy. After nine months of thalidomide treatment, the proband's pruritus is well-controlled and her lesions continue to flatten leaving hypopigmented macules and milia (Figure 7).

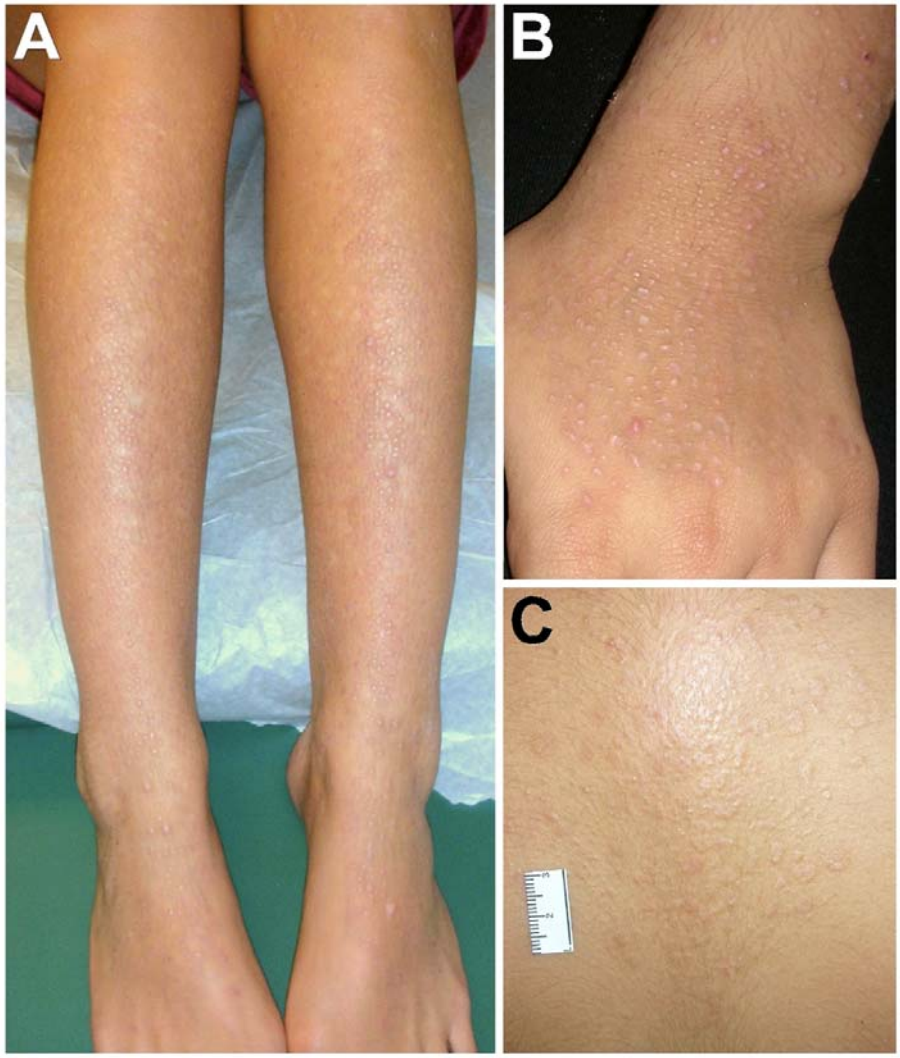

Figure 7. Improvement of skin lesions with systemic thalidomide administration. The index case was started on thalidomide 100 mg daily and improved gradually with most apparent results at two months. Shins (A), left dorsal hand (B), and mid-upper back (C) show flattening of papules with less excoriation after six months of treatment.

Mutational analysis of *COL7A1* gene in patients suspected of DEB is not currently used for diagnosis, but rather only for the purposes of research or for genetic counseling. As genetic sequencing becomes easier and more cost-effective, it is plausible that it will become more routinely used in clinical practice but the practical implications remain unclear. For severe RDEB, the gene has been identified as a possible target for modulation through allogeneic bone marrow transplantation or induced pluripotent stem cells and a few small clinical trials have shown improvement (47,48). However, the technology of correcting genetic mutations is still nascent, and the risk of morbidity and mortality greatly outweigh any potential benefit for patients with DDEB or EBP. In medical genetics, prenatal diagnosis has been heralded as a new area of intervention. This endeavor has been attempted for expectant parents with relatives affected by severe variants of EB such as RDEB and junctional EB and has been mostly accurate in identifying the mutation using current prenatal sampling and mutation detection methods (1,49). Yet, the variability of clinical severity in DEB displayed in our kindred and in others, even within RDEB, (18,25,38) suggest caution is needed in interpreting the results of mutational analysis.

This case demonstrates the phenotypic complexity and incomplete penetrance of COL7A1 mutations in an extended kindred with DEB and EBP. The same mutation in intron 47, c.4668+1 G>A, resulted in a mild EBP in one individual, a severe but time-limited course in another, and no effect in yet another, but caused severe EBP in the proband that responded to thalidomide treatment after more than ten years of trying alternative therapies. Additionally, the same mutation caused classic DEB with solely trauma-induced blistering in another branch of this family. Clinical studies and DNA

sequencing for five common European variants of filaggrin and an *MMP1* promoter SNP failed to reveal the etiology of the proband's pruritus. Given that the observed mutation is predicted to affect splicing, one might speculate that alternative splice isoforms are expressed in different members of this kindred, though this would not explain the phenotypic variability seen in the majority of EBP kindreds which carry missense mutations. We expect that genetic and/or environmental factors are involved in the initiation and progression of EBP, although the exact mechanisms remain to be determined. Further elucidation may become permissible with broader availability of whole genome and transcriptome sequencing and better understanding of common polymorphisms within the population that may contribute to the pathogenesis of pruritus and to EBP.

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