Epidermolysis bullosa (EB) pruriginosa is an unusual variant of dystrophic EB in which intense itching can lead to striking skin changes resembling acquired skin disorders such as nodular prurigo or hypertrophic lichen planus. The molecular pathology involves mutations in the COL7A1 gene, but the nature of the mutations is similar to those seen in other non-pruritic forms of dystrophic EB. The mechanism of the dramatic phenotypic differences is currently unknown. In this study we assessed the incidence of a common functional polymorphism in the matrix metalloproteinase-1 gene promoter (1G or 2G at nucleotide –1607) in individuals with EB pruriginosa (n = 27) compared with non-itchy dominant dystrophic EB (n = 23), recessive dystrophic EB (n = 25) and normal controls (n = 50). The hypothesis is that the 2G allele, which was previously shown to increase matrix metalloproteinase-1 activity and lead to increased degradation of type VII collagen, could explain the phenotypic heterogeneity encountered in dominant forms of EB, particularly the itchy EB pruriginosa phenotype. The rationale is that increased type VII collagen degradation could trigger an inflammatory response leading to itchy skin characteristic of EB pruriginosa. All 27 individuals with EB pruriginosa were heterozygous for dominant-negative glycine substitution mutations in the COL7A1 gene, six of which have not been reported previously. The frequency of the 2G allele in these subjects (46.3%) was greater than in the controls (42.0%), but less than in non-itchy dominant dystrophic EB (52.2%) or recessive dystrophic EB (62.0%), indicating that variants of a common functional polymorphism in the matrix metalloproteinase-1 gene promoter do not account for the itchy skin phenotype. The pathophysiology of EB pruriginosa remains unexplained. Key words: COL7A1; blister; inherited skin disease; pruritus.

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With regards to the \textit{MMP1} gene promoter, the sequence at the –1607 position can be either 1G or 2G, both of which have been shown to occur with similar frequency in control populations (22, 23). However, the 2G polymorphism creates a new ETS transcription factor binding site, which increases the transcriptional activity of \textit{MMP1}, leading to increased degradation of type VII collagen (Fig. 1). The ETS family of transcription factors play important roles in development, differentiation and proliferation of cells in general and are involved in apoptosis and tissue remodelling. Titeux et al. (18) were able to show that the 2G polymorphism (on either one or both alleles) correlates with disease severity in individuals with recessive DEB and that type VII collagen levels at the dermal-epidermal junction are a more important clinical determinant than the amount of type VII collagen synthesis. Thus, this promoter polymorphism in the \textit{MMP1} gene has been shown to be a potential disease modifier in one variant of DEB. This raises the question as to whether clinical variation in other forms of DEB might also be explained by this mechanism. The aim of our study, therefore, was to assess whether this particular polymorphism might have relevance to the pathophysiology of EBP. The rationale is that increased degradation of type VII collagen could induce secondary inflammatory changes, including expression of cytokines and mediators known to be associated with skin itching (6, 7).

METHODS

Following ethical approval (St Thomas’ Hospital Ethics’ committee; 07/H0802/104) and informed consent, individuals with EBP (n = 27), non-EBP dominant DEB (n = 23), recessive DEB (n = 25) and normal control subjects (n = 50) were recruited for the study through the Robin Eady National Diagnostic EB Laboratory database in London. Each group comprised 70–80% white Caucasians, the remainder were of Middle-Eastern, South American, South-East Asian or Asian origins. There were no major differences between the ethnicity of any of the subject groups, including the controls. In subjects with EBP, clinical and laboratory investigations revealed no underlying cause(s) for the pruritus including atopy, iron deficiency, biochemical or endocrinological abnormalities, although none has yet been screened for mutations in the filaggrin (\textit{FLG}) gene.

Genomic DNA was extracted from peripheral blood leukocytes using standard methods (24). \textit{COL7A1} mutation screening was performed by direct sequencing of all 118 exons and flanking introns of the \textit{COL7A1} gene, as described previously, using an ABI 3100 sequencer (25). Mutations were verified by bi-directional sequencing and restriction endonuclease digestions, where possible.

To sequence the \textit{MMP1} promoter region corresponding to the predicted 1G/2G polymorphism (–1607bp from the \textit{MMP1} transcription site), the following primers were used: forward primer 5’-gggagactacatgaatctcgac-3’ (–4008 bp to –3988 bp; GenBank No NM002421) and reverse primer 5’-ctgccgcatatctcagag-3’ (–511 bp to –543 bp), with polymerase chain reaction (PCR) amplification conditions as described previously (26). The frequency of the two alleles was compared between the different groups using Fisher’s exact test.

RESULTS

All 27 subjects with EBP (19 families) had dominant DEB resulting from heterozygous glycine substitutions in the type VII collagen triple helix. Six of these mutations have not been reported previously (details are shown in Table I) and thus our mutation screening documents the \textit{COL7A1} molecular pathology in the largest cohort of individuals with EBP (Fig. 2). The \textit{COL7A1} mutations in the 23 non-EBP dominant individuals (20 families) comprised 13 heterozygous glycine substitutions, six unreported (Table I) and thus our mutation screening documented the \textit{COL7A1} molecular pathology in the largest cohort of individuals with EBP (Fig. 2). These cohorts also contained 12 individuals with the same \textit{COL7A1} mutation, p.G2043R, which is the most common mutation found in dominant DEB (27, 28).

Table I. Previously unreported \textit{COL7A1} mutations identified in this study

<table>
<thead>
<tr>
<th>Mutation</th>
<th>cDNA sequence change</th>
<th>Position</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVS37+1G &gt; T</td>
<td>IVS37+1G &gt; T</td>
<td>Intron 37</td>
<td>DDEB</td>
</tr>
<tr>
<td>p.G1483D</td>
<td>c.4448 G &gt; A</td>
<td>Exon 42</td>
<td>BDN</td>
</tr>
<tr>
<td>p.G1770D</td>
<td>c.5309 G &gt; A</td>
<td>Exon 61</td>
<td>EBP</td>
</tr>
<tr>
<td>p.G1773D</td>
<td>c.5318 G &gt; A</td>
<td>Exon 61</td>
<td>DDEB</td>
</tr>
<tr>
<td>p.G1860R</td>
<td>c.5578 G &gt; A</td>
<td>Exon 66</td>
<td>EBP</td>
</tr>
<tr>
<td>p.G1913R</td>
<td>c.5737 G &gt; C</td>
<td>Exon 69</td>
<td>EBP</td>
</tr>
<tr>
<td>p.G2009A</td>
<td>c.6026 G &gt; C</td>
<td>Exon 73</td>
<td>DDEB</td>
</tr>
<tr>
<td>p.G2028E</td>
<td>c.6083 G &gt; C</td>
<td>Exon 73</td>
<td>DDEB</td>
</tr>
<tr>
<td>p.G2067A</td>
<td>c.6200 G &gt; C</td>
<td>Exon 74</td>
<td>EBP</td>
</tr>
<tr>
<td>p.G2159E</td>
<td>c.6476 G &gt; A</td>
<td>Exon 79</td>
<td>EB</td>
</tr>
<tr>
<td>p.G2213R</td>
<td>c.6637 G &gt; A</td>
<td>Exon 83</td>
<td>EB</td>
</tr>
<tr>
<td>p.G2235E</td>
<td>c.6698 G &gt; A</td>
<td>Exon 84</td>
<td>BDN</td>
</tr>
<tr>
<td>p.G2290A</td>
<td>c.6869 G &gt; C</td>
<td>Exon 87</td>
<td>EB</td>
</tr>
</tbody>
</table>

DDEB: dominant dystrophic epidermolysis bullosa; BDN: bullous dermolysis of the newborn; EBP: epidermolysis bullosa pruriiginosa.

Fig. 1. The 1G/2G polymorphism in the matrix metalloproteinase 1 gene promoter is located at nucleotide –1607 from the \textit{MMP1} transcription site. The 2G polymorphism creates an ETS transcription factor binding site, which increases the transcriptional activity of \textit{MMP1}; this leads to increased degradation of type VII collagen.
ticular glycine substitution was EBP in four cases and non-EBP dominant DEB in the other eight. Thus, the same mutation can underlie both clinical subtypes, as illustrated in Fig. 3.

Genotyping of the MMP1 polymorphism in the control group showed that the major allele was 1G (58.0%) and the minor allele was 2G (42.0%). In the EBP group, sequencing showed that the frequency of 1G was 53.7% and of 2G was 46.3%, whereas in the non-EBP dominant DEB individuals the frequency of 1G was 47.8% and of 2G was 52.2%. For the recessive DEB group the allele frequencies were 38.0% for 1G and 62.0% for 2G. These data are shown in more detail in Table II. No significant difference in allelic frequency was found between EBP and controls ($p = 0.61$), between EBP and non-EBP dominant DEB ($p = 0.56$), or between non-EBP dominant DEB and controls ($p = 0.25$). However, a significant difference in the incidence of allelic frequency was found between recessive DEB group and controls ($p = 0.02$): the frequency of the 2G allele is clearly higher in the recessive DEB subjects.

With respect to the MMP1 genotype in individuals with the COL7A1 mutation p.G2043R, 10 out of 12 individuals expressed the 2G variant on at least one allele but the two subjects who were homozygous for 1G had non-EBP dominant DEB. Differences between the 2 phenotypes with respect to the 1G/2G alleles were not significant. Inter-familial phenotypic heterogeneity was also observed for the COL7A1 mutation p.G1522E. Individuals who were heterozygous for this glycine substitution had either EBP or bullous dermolysis of the newborn (OMIM 131705), but this considerable phenotypic disparity was not explained by the MMP1 polymorphism as the 2G allele was detected in both clinical variants. Intra-familial variability in phenotype was noted in one family with EBP that was heterozygous for the COL7A1 mutation p.G2251E. Clinicopathological details of this family have been reported previously (11). However, there was no correlation between the presence or absence of the 2G allele and the EBP phenotype.

Table II. Incidence of the 1G/2G polymorphism at the –1607 position of the MMP1 gene promoter in non-EBP DDEB, dominant EBP, RDEB and controls in this study

<table>
<thead>
<tr>
<th></th>
<th>EBP</th>
<th>Non EBP-DDEB</th>
<th>RDEB</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total patient no.</strong></td>
<td>27</td>
<td>23</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td><strong>Total allele no.</strong></td>
<td>54</td>
<td>46</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>1G/1G</td>
<td>7</td>
<td>6 (26.1%)</td>
<td>2 (8.0%)</td>
<td>16 (32.0%)</td>
</tr>
<tr>
<td>1G/2G</td>
<td>15</td>
<td>10 (43.5%)</td>
<td>15 (60.0%)</td>
<td>26 (52.0%)</td>
</tr>
<tr>
<td>2G/2G</td>
<td>5</td>
<td>7 (30.4%)</td>
<td>8 (32.0%)</td>
<td>8 (16.0%)</td>
</tr>
<tr>
<td><strong>Total 2G alleles</strong></td>
<td><strong>25/54</strong></td>
<td><strong>24/46</strong></td>
<td><strong>31/50</strong></td>
<td><strong>42/100</strong></td>
</tr>
</tbody>
</table>

* $p=0.02$ compared with controls.

DDEB: dominant dystrophic epidermolysis bullosa; RDEB: recessive dystrophic epidermolysis bullosa; EBP: epidermolysis bullosa pruriginosa.
DISCUSSION

This study has identified six new heterozygous glycine substitutions in type VII collagen as the molecular basis for EBP. It also identifies another 6 novel heterozygous glycine substitutions underlying non-pruritic dominant DEB (Table I). These findings expand the total COL7A1 glycine substitution mutation database to 133 mutations (71 dominant, 62 recessive) (29–31), but demonstrate that there is nothing atypical in the nature or location of the EBP mutations compared with other dominant or recessive forms of DEB resulting from glycine substitution mutations in type VII collagen (29–33).

In addition, our data on the 1G/2G MMP1 gene promoter polymorphism show that there is no association between the 2G allele and the EBP phenotype. Thus, the striking inflammatory and itchy skin phenotype of EBP does not appear to be explained by this potential genetic modifier.

Nevertheless, MMPs may contribute to various pathophysiological processes in DEB, such as cancer progression (squamous cell carcinoma) and wound healing (34–37). For example, MMP7 has the capacity to cleave surface molecules such as E-cadherin and syndecan-1, as well as extracellular matrix proteins including fibrillin and type VII collagen, thereby facilitating cancer progression (34, 35). Furthermore, varying clinical severity in three siblings affected with recessive DEB has been shown to correlate with increased levels of MMP2, MMP3 and MMP9 and reduced levels of the tissue inhibitor of metalloproteinase-1 (TIMP1) (36). In contrast, however, our study has demonstrated no correlation between specific clinical phenotypes of dominant DEB and a functional polymorphism in the MMP1 gene promoter. The possibility of genetic variants in these other MMPs contributing to phenotypic expression awaits further study.

EBP can be a difficult disorder to treat. Previous treatments have included topical and systemic corticosteroids, topical tacrolimus, ciclosporin and thalidomide. In our 27 subjects with EBP, treatment with topical tacrolimus in eight individuals provided significant symptomatic relief from the pruritus in only one subject, and partial relief in three others. Similar responses to topical steroids were also noted. Systemic therapies were tried in too few of these particular individuals to comment on their efficacy.

Based on the data from Titeux et al. (18), novel anti-proteolytic therapies may emerge as useful treatment options for some individuals with DEB, especially perhaps for recessive DEB, but our findings (albeit based on findings in genomic DNA) do not suggest that EBP subjects will specifically benefit from this form of treatment compared with other individuals with DEB.

Our findings in recessive DEB, however, add support to the published data showing that the 2G polymorphism may be associated with greater clinical severity (18). But there may also be other clinical implications, perhaps relevant to the pathophysiology of squamous cell carcinoma, which is a frequent complication in this subtype of DEB (37). Within the recessive DEB group that we assessed, six subjects had developed cutaneous squamous cell carcinoma before the age of 30 years. Of note, each of these individuals had the 2G variant on one or both MMP1 alleles. This observation is of interest, given the potential role of MMPs in cancer invasion through degradation of the extracellular matrix (20–23).

Indeed, in other studies, the 2G polymorphism has been associated with an increased risk of developing colorectal, oesophageal, bladder, breast, lung and head and neck cancers (38–42). Furthermore, for some of these malignancies the expression level of MMP1 has been found to correlate negatively with survival in affected patients (43–46). These findings led to the possibility of trying to modify cancer progression by using pharmacological inhibitors of MMPs. Despite promising data in animal models, however, the lack of specificity of the currently available inhibitors, and the adverse effects noted in clinical trials, have so far failed to yield significant benefits for patients (47). Nevertheless, specific anti-cancer agents targeting MMPs, TIMPs or genetic regulators of MMPs such as the tumour suppressor gene RECK, may have future relevance to the management of squamous cell carcinoma in recessive DEB.

With regards to the EBP phenotype, however, our study has shown that the unusual inflammatory clinical features seen in this condition are not explained by a common genetic variant in the MMP1 gene promoter and the pathophysiology of this subtype of DEB therefore remains unresolved.

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MMP1 promoter polymorphisms and dystrophic EB


Acta Derm Venereol 89