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An investigation into the *MMP1* gene promoter region polymorphism – 1607 2G with recessive dystrophic epidermolysis bullosa disease severity in northeastern Mexican patients

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Abstract

Background Recessive dystrophic epidermolysis bullosa (RDEB) is a severe genetic skin blistering disorder caused by mutations in the gene *COL7A1* encoding type VII collagen. Most of the patients' clinical severity depends in part on the nature and location of the mutations, ranging from the mild form described as RDEB *other-generalized* (RDEB-*O*) to the more aggressive phenotype described as RDEB *severe-generalized* (RDEB-*sev gen*). However, interfamilial and interindividual differences in subjects with identical *COL7A1* mutations suggest the presence of modifier elements, which may influence severity. There is a single nucleotide polymorphism (SNP) at the promoter of the *MMP1* gene-encoding matrix metalloproteinase type 1, which has been studied as a genetic disease modifier in different patient cohorts with different findings.

Methods We tested the SNP in 30 patients with RDEB and 130 controls whose four grandparents were born in northeastern Mexico. Patients were clinically classified as RDEB-*sev gen* and RDEB-*O* by three dermatologists. The SNPStats, RXC, and SPSS software were used to perform statistical testing.

Results The allele frequencies for 2G were 0.607, 0.562, and 0.642 for RDEB-*O*, RDEB-*sev gen*, and the control group, respectively. When the genotype frequencies were compared, there was no significant difference between RDEB-*sev gen* (OR = 0.38, CI 95% 0.12–1.21), RDEB-*O* (OR = 1.03, CI 95% 0.21–4.96), and the control group.

Conclusion We found no significant association in relation to the severity of the study subjects and the SNP at the promoter of the *MMP1* gene.

Introduction

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare severe genetic skin blistering disorder characterized by trauma-induced blister formation and healing with scarring.[1] RDEB is caused by loss-of-function mutations in *COL7A1* gene encoding type VII collagen (Col7), which forms key structures, for dermal–epidermal adherence.[2–4]

Different clinical forms of severity are recognized, ranging from the mild localized form RDEB *other-generalized* (REDEB-*O*) with limited blistering, to the particularly severe and mutilating phenotype observed in the RDEB *severe-generalized* (RDEB-*sev gen*) previously known as Hallopeau–Siemens type.[1]

Patients with RDEB-*sev gen* present with extensive skin erosions and blistering, mutilating scarring, fusion of the extremities, joint contractures, and profound nutritional impairment, which can require gastrostomy. The risk of skin squamous cell carcinoma is also markedly increased in these patients with an accumulative risk of 90.1% by the age of 55 years old.[5] Patients with RDEB-*O* display extensive skin and mucosal blistering but no tendency to fusion of the fingers and toes. The inversa type spares the extremities, affects the folds, and often develops esophageal stenosis. The localized form shows limited blistering in traumatic areas without syndactyly or mucosal involvement.[5]

The clinical severity of RDEB depends in part on the nature and location of the *COL7A1* mutations and on

their consequences on mRNA and protein level.[2,3,6,7] However, establishing genotype–phenotype correlations is not always easy due to significant interfamilial and inter-individual differences in subjects with identical *COL7A1* mutations. Considerable efforts have been made to correlate the clinical presentation with the *COL7A1* genotype.[2–4,6–9] Phenotypic variation has been attributed to genetic modifiers, environmental factors, random events, and interactions between any of these sources in general.[8,10]

Our group recently reported that the level of type VII collagen in the basal membrane in patients with RDEB does not always correlate with clinical severity, measuring the amount of fluorescent immunostaining localized to the basement membrane of skin sections in a cohort of 13 Mexican patients.[11]

Before the *COL7A1* gene was implicated by linkage analysis to the RDEB,[12,13] the *MMP1* gene encoding the matrix metalloproteinase type I (MMP-1) was first implicated, and it has been studied as a candidate gene in RDEB.[14,15]

MMP-1 is the most ubiquitously expressed interstitial collagenase, thereby assigning it a prominent role in collagen degradation. Overexpression of MMP-1 is associated with several pathological conditions, including the irreversible degradation of cartilage, tendon, and bone in arthritis[16] and the degradation of collagens I and III in tumor invasion and metastasis.[17] In the skin, MMP-1 is secreted by basal keratinocytes and dermal fibroblasts and as type VII collagen is one of its substrates,[18,19] an increase in MMP-1 activity could worsen RDEB disease severity through enhanced degradation of type VII collagen molecules.

It has been reported that there is a single nucleotide polymorphism (SNP) in the promoter region of the human *MMP1* gene, – 1607del/insG (rs1799750, designated as 1G 2G) where an additional guanine (G) creates an Ets binding site, 5′GGA-3′.[20] Upon the interaction of adjacent AP-1 site, the promoter activity and hence the transcription level of *MMP1* can be considerably increased.

Studies in non-epidermolysis bullosa patients have shown the SNP in the *MMP1* to be associated with higher cancer invasiveness.[21,22] These observations raised the possibility that increased MMP-1 expression could influence disease severity in patients with RDEB.

Titeux and colleagues examined 31 patients with DEB, and the results shows that two genotypes, homozygous 2G (2G⁄2G) and heterozygous (1G⁄2G), were associated with a more severe RDEB phenotype.[8] However, Kern *et al.*[9] did not find evidence for the involvement of this SNP in a larger cohort of 103 patients with RDEB.

As this presumptive disease modifier could have an important prognostic value and implications for the design of therapy approaches, we evaluated the MMP1 SNP role in a northeastern Mexico cohort of patients with RDEB.

Northeastern Mexico, which includes the states of Nuevo León, Coahuila, Tamaulipas, San Luis Potosí, and Zacatecas, has an area of 432,480 km^2 and has a population of more than 14.4 million inhabitants in 2010.[23] Admixture estimates based on blood group data indicate that the populations of northeastern Mexico are similar in terms of the contribution of Spanish (60%), Amerindian (37%), and African (3%) genes. There is no non-random association of alleles among the genetic marker systems, despite the admixed origin of this Mestizo population.[24–26]

The aim of this case–control study was to find the association of the *MMP1* gene promoter region polymorphism – 1607 2G with patients with RDEB disease in northeastern Mexico.

Materials and methods

Study subjects

From the DEBRA Mexico Association database of 240 patients with EB, subjects were selected whereby four grandparents were born in northeastern Mexico, obtaining a sample of 30 patients whose clinical, morphological, and molecular characteristics were consistent with RDEB. Each patient was examined and classified separately by three dermatologists from the dermatology department of the University Hospital from the Universidad Autónoma de Nuevo León (UANL) in accordance with the Third International Consensus Meeting and classification of EB considering[1]: (i) area of damaged skin; (ii) involvement of nails, mouth, eyes, larynx, and esophagus; (iii) scarring of hands; (iv) skin cancer; (v) chronic wounds; (vi) alopecia; (vii) nutritional compromise; and (viii) delayed puberty among other systemic involvement. In addition, the Birmingham Epidermolysis Bullosa Severity score was performed for each patient.[27] Fourteen patients were diagnosed with RDEB-*O* and 16 patients with RDEB-*sev gen*.

Controls

After completing a questionnaire and discarding any sibling with RDEB, a total of 130 healthy control volunteers whose four grandparents were also born in northeastern Mexico were recruited to determine background allele frequencies in the population.

Following the informed consent to patients and controls, ethylenediamine tetraacetic acid–blood was obtained. The study protocol was approved by the ethics committee of the University Hospital "José E. González" of the UANL, in accordance with the guidelines of the World Medical Association's Declaration of Helsinki.[28]

MMP1 polymorphism

Primers were designed using the software Primer Design 3 (SourceForge, General Public License).[29] The sequences of the primers used to amplify and detect *MMP1* promoter region were 5′-GCACCCTCCCTCTGATGCC TCT-3′ (forward primer) and 5′-GGTGTCTCCCAGCTTTCC CACTGT-3′ (reverse primer); the product amplified is 280 base pairs.

The 5′ terminal promoter region of MMP1 (ENSEBL: ENSR00000568021) was directionally sequenced using ABI BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI Prism 3100 Avant Genetic Analyzer (Applied Biosystems) following the manufacturer's instructions. Sequence Analysis 5.2 software (Applied Biosystems) was used to help analyze the raw data. Assembled contigs were aligned to the MMP1 reference sequence (ENSEBL: ENSR00000568021) using Geneious 4.7.[30]

Statistical analyses

For the *MMP1* SNP, genotype and allele frequencies, tests for Hardy–Weinberg equilibrium, analysis of association, odds ratio, and multiple inheritance model (co-dominant) were carried out using the SNPStats software (http://bioinfo.iconcologia.net/SNPstats). The comparison of the genotype and allelic profiles of patients and controls with 50,000 simulations was carried out using the RXC software package of Miller*. $P < 0.05$ was considered significant.

Results

MMP1 single nucleotide polymorphism in controls

In order of frequency, the genotypes 1G/1G, 1G/2G, and 2G/2G (12.6, 41.4, and 46%) were present in our control group.

Table 1 MMP1 single nucleotide polymorphism genotype and allele frequency

Genotype	Controls	RDEB-*O*	RDEB-*sev gen*	Hispanic[a]
1G/1G	19 (12.6%)	2 (14.3%)	5 (31.2%)	17.4%
1G/2G	55 (41.4%)	7 (50.0%)	4 (25.0%)	39.1%
2G/2G	56 (46.0%)	5 (35.7%)	7 (43.8%)	43.5%
Total patient number	130	14	16	23
1G allele frequency	0.3577	0.3929	0.4375	0.370
2G allele frequency	0.6423	0.6071	0.5625	0.630

O, *other generalized*; RDEB, recessive dystrophic epidermolysis bullosa; *sev gen*, *severe generalized*.

[a]Twenty-three individuals of self-described Hispanic heritage (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1799750).

*Guo SW, Thompson EA. Technical Report No. 187. Departmental of Statistics, University of Washington; 1989.

The distributions of the three genotypes are shown in Table 1. The major allele in our control group was 2G (64.2%), similar to that reported in the NCBI SNP database for another control Hispanic population (63%) (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1799750).

MMP1 single nucleotide polymorphism in patients with recessive dystrophic epidermolysis bullosa

The 2G *MMP1* SNP was consistent with the Hardy–Weinberg equilibrium in patients and controls. The major allele in our patient groups was 2G (58.4%), showing less frequency compared with controls, yet this decrease was not statistically significant ($P > 0.05$). The allele frequencies for 2G were 60.7 and 56.2% for RDEB-O and RDEB-*sev gen* groups, respectively.

MMP1 single nucleotide polymorphism clinical association with recessive dystrophic epidermolysis bullosa *other-generalized* and *severe-generalized* groups

When the genotype frequencies were compared, there was no significant difference between the RDEB-*sev gen* (OR = 0.38, CI 95% 0.12–1.21), RDEB-O (OR = 1.03, CI 95% 0.21–4.96), and control group (see Table 2).

Discussion

The regulation of the *MMP1* gene depends on expression levels and regulations of the endogenous tissue inhibitors of metalloproteinase; this homeostasis system occurs in normal wound healing and because in patients with RDEB the skin is repeatedly disrupted and there is a chronic wound situation that could lead to disturbances of the balance between MMP-1 and its inhibitors.

The 2G *MMP1* SNP has a significantly higher transcriptional activity in MMP-1 expression, increasing the collagenase activity, and therefore has shown to be associated with higher cancer invasiveness, arthritis, and other diseases where collagen degradation is involved.

It has been proposed that this genetic variant, which increases MMP1 expression, could have a modulator role of disease severity, which is an important prognostic value and implications for the design of molecular therapy approaches in patients with RDEB.[8]

Table 2 *MMP1* single nucleotide polymorphism clinical association with RDEB-O and RDEB-*sev gen* groups

	χ^2	OR	CI 95%	*P*-value
Controls vs. RDEB-*O*	0.00	1.03	[0.21–4.96]	0.97
Controls vs. RDEB-s*ev gen*	2.87	0.38	[0.12–1.21]	0.09

O, *other generalized*; RDEB, recessive dystrophic epidermolysis bullosa; *sev gen*, *severe generalized*; OR, odds ratio; CI, confidence interval

Kern *et al.*[3] suggested that given the relatively small numbers of patients available and the likely low magnitude of effect size, replication of results in independent populations is essential.

As described by Salas-Alanís and McGrath,[31] we also found that mutation 2470insG located in exon 18/19 of the *COL7A1* gene was most prevalent in our patients. It is expected that patients with this mutation have a homogeneous phenotype, but as we can see in Table 3, not all patients with the mutation 'x 18/19, 2470insG of *COL7A1* has the same clinical features. However, no relationship was found with the MMP1 gene SNP, assuming that other disease-modifying factors are involved.

Our study analyzed 130 control individuals where grandparents were specifically from northeast Mexico, a genetically homogeneous population in all Mexico, and compared with 30 patients with the same geographic background.

The 2G SNP allele frequencies in the Hispanic population is different from the Caucasian population where the major allele found was 1G (56.7%) in contrast to the Hispanic population where 2G is the major allele (63%).

The higher allele frequency of the SNP in the Hispanic population may suggest greater clinical severity in patients with RDEB; however, regardless of the type of mutation in the *COL7A1* gene, the group of patients with less severe RDEB showed higher frequency of the presence of SNP compared with the group of patients with RDEB-*sev gen*.

Although we cannot rule out a small amount of bias, we did not find significant differences between the RDEB-*sev gen* group and the control group. It will be important, in the future, to increase the sample size and investigate other RDEB populations to determine the contribution, if any, of the MMP1 gene SNP.

Conclusion

There was no association between the clinical severity of patients with RDEB and the SNP 2G *MMP1* gene 'promoter in patients with RDEB in our northeastern

Table 3 RDEB phenotypes, COL7A1 mutations, and MMP1 SNP

Code	Age	MUT1	MUT2	Predicted phenotype	Actual phenotype	BEBS score	Mmp1 SNP
GEP1062	38	'x 56, G1703E	'x 69, 5772insT	O	O	15.6	2G/2G
GEP1061	52	'x 18/19, 2471insG	'x 56, G1703E	O	SG	23.5	2G/2G
GEP1095	35	'x 56, G1703E	'x 69, 5772insT	O	O	24.1	2G/2G
GEP1057	32	'x 18/19, 2471insG	'x 18/19, 2471insG	SG	SG	23.4	2G/2G
GEP1074	37	'x 56, G1703E	ivs23-1G>A	O	SG	20.5	2G/2G
GEP1060	42	'x 56, G1703E	ivs23-1G>A	O	SG	20.75	2G/2G
GEP1072	19	'x 73, 6026 G>C; G2009A	N/A	O	SG	20.75	2G/G
GEP1133	42	'x 18/19, 2471insG	'x 18/19, 2471insG	SG	O	26.75	2G/G
GEP1076	32	'x 18/19, 2471insG	'x 18/19, 2471insG	SG	O	27	2G/G
G0847[a]	62	'x 18/19, 2471insG	'x 18/19, 2471insG	SG	O	29.5	2G/G
GEP1055	29	'x 56, G1703E	'x 84, c.6696insC	O	O	15.5	2G/G
GEP1068	36	'x 18/19, 2471insG	'x 18/19, 2471insG	SG	O	8.75	G/G
GEP1066	35	'x 18/19, 2471insG	'x 18/19, 2471insG	SG	O	15.5	G/G
GEP577	27	'x 18/19, 2471insG	'x 32, 3948insT	SG	SG	19.5	G/G
GEP1067	37	'x 18/19, 2471insG	'x 18/19, 2471insG	SG	SG	20	G/G
GEP1065	16	'x79 6501G>A	N/A	O	SG	30	G/G
GEP1073	16	N/A	N/A	N/A	SG	59.75	G/G
GEP1048	5	NA	NA	N/A	O	12	2G/2G
GEP1064	11	N/A	N/A	N/A	SG	19.6	2G/2G
GEP1069	9	N/A	N/A	N/A	O	20.75	2G/2G
GEP1075	16	N/A	N/A	N/A	SG	29	2G/2G
GEP1093	16	N/A	N/A	N/A	SG	31	2G/2G
GEP1053	25	N/A	N/A	N/A	O	15.5	2G/2G
GEP1058	6	N/A	N/A	N/A	O	6.7	2G/G
GEP1056[a]	10	NA	NA	N/A	SG	15	2G/G
GEP1059	16	N/A	N/A	N/A	SG	23.4	2G/G
GEP946	39	N/A	N/A	N/A	SG	34.5	2G/G
GEP1047	5	N/A	N/A	N/A	O	15.5	2G/G
GEP1049[a]	19	N/A	N/A	N/A	O	6.7	2G/G
GEP1071	6	N/A	N/A	N/A	SG	18.5	G/G

BEBS, the Birmingham Epidermolysis Bullosa Severity score; O, other generalized; RDEB, recessive dystrophic epidermolysis bullosa; SG, severe generalized; SNP, single nucleotide polymorphism.
[a]Deceased during the study.

Mexican cohort. Given the low incidence of the disease, RDEB studies in other populations are needed to assess the real impact of this polymorphism on the clinical severity of these patients. Some of the limitations of the study were that controls were not matched to each case; however, the ratio of controls to patients was 3 : 1 to give a proper statistical weight to the study. In addition, we only performed a disease severity score and not a disease activity index; therefore, we suggest that future studies take into account different clinical parameters to assess the correlation between them and the genetic modifiers. Future analysis of the complex balance between proteases and their inhibitor could clarify the role of the MMPs in EB and help to find new therapeutic approaches to increase the quality of life in our patients with RDEB.

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References

1 Fine J, Eady R, Bauer E, *et al.* The classification of inherited epidermolysis bullosa (EB): report of the third international consensus meeting on diagnosis and classification of EB. *J Am Acad Dermatol* 2008; **58**: 931–950.
2 Gardella R, Zoppi N, Zambruno G, *et al.* Different phenotypes in recessive dystrophic epidermolysis bullosa patients sharing the same mutation in compound heterozygosity with two novel mutations in the type VII collagen gene. *Br J Dermatol* 2002; **147**: 450–457.
3 Kern J, Kohlhase J, Bruckner-Tuderman L, *et al.* Expanding the COL7A1 mutation database: novel and recurrent mutations and unusual genotype-phenotype constellations in 41 patients with dystrophic epidermolysis bullosa. *J Invest Dermatol* 2006; **126**: 1006–1012.
4 Varki R, Sadowski S, Uitto J, *et al.* Epidermolysis bullosa. II. Type VII collagen mutations and phenotype-genotype correlations in the dystrophic subtypes. *J Med Genet* 2007; **44**: 181–192.
5 Fine JD, Johnson LB, Weiner M, *et al.* Epidermolysis bullosa and the risk of life-threatening cancers: the national EB registry experience, 1986–2006. *J Am Acad Dermatol* 2008; **60**: 203–211.
6 Shimizu H, McGrath J, Christiano A, *et al.* Molecular basis of recessive dystrophic epidermolysis bullosa: genotype/phenotype correlation in a case of moderate clinical severity. *J Invest Dermatol* 1996; **106**: 119–124.
7 Wessagowit V, Kim S, Woong OhS, *et al.* Genotype-phenotype correlation in recessive dystrophic epidermolysis bullosa: when missense doesnt make sense. *J Invest Dermatol* 2005; **124**: 863–866.
8 Titeux M, Pendaries V, Tonasso L, *et al.* A frequent functional SNP in the MMP1 promoter is associated with higher disease severity in recessive dystrophic epidermolysis bullosa. *Hum Mutat* 2008; **29**: 267–276.
9 Kern J, Grüninger G, Imsak R, *et al.* Forty-two novel COL7A1 mutations and the role of a frequent single nucleotide polymorphism in the MMP1 promoter in modulation of disease severity in a large European dystrophic epidermolysis bullosa cohort. *Br J Dermatol* 2009; **161**: 1089–1097.
10 Bodemer C, Tchen SI, Ghomrasseni S, *et al.* Skin expression of metalloproteinases and tissue inhibitor of metalloproteinases in sibling patients with recessive dystrophic epidermolysis and intrafamilial phenotypic variation. *J Invest Dermatol* 2003; **121**: 273–279.
11 Garza-Gómez J. Level of type VII collagen at the basement membrane does not always correlate with clinical severity in a cohort of 13 Mexican patients with recessive dystrophic epidermolysis bullosa. Annual meeting of the society of investigative dermatology, Montreal, Canada. *J Investig Dermatol* 2009; S87.
12 Hovnanian A, Duquesnoy P, Amselem S, *et al.* Exclusion of linkage between the collagenase gene and generalized recessive dystrophic epidermolysis bullosa phenotype. *J Clin Invest* 1991; **88**: 1716–1721.
13 Colombi M, Gardella R, Zoppi N, *et al.* Exclusion of stromelysin-1, stromelysin-2, interstitial collagenase and fibronectin genes as the mutant loci in a family with recessive epidermolysis bullosa dystrophica and a form of cerebellar ataxia. *Hum Genet* 1992; **89**: 503–507.
14 Bauer E, Eisen A. Recessive dystrophic epidermolysis bullosa. Evidence for increased collagenase as a genetic characteristic in cell culture. *J Exp Med* 1978; **148**: 1378–1387.
15 Stricklin G, Welgus H, Bauer E. Human skin collagenase in recessive dystrophic epidermolysis bullosa. Purification of a mutant enzyme from fibroblast cultures. *J Clin Invest* 1982; **69**: 1373–1383.
16 Vincenti MP, White LA, Schroen DJ, *et al.* Regulating expression of the gene for matrix metalloproteinase-1 (collagenase): mechanisms that control enzyme activity, transcription, and mRNA stability. *Crit Rev Eukaryot Gene Expr* 1996; **6**: 391–411.
17 Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst* 1997; **89**: 1260–1270.
18 Seltzer J, Eisen A, Bauer E, *et al.* Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin. *J Biol Chem* 1989; **264**: 3822–3826.
19 Ala-aho R, Kähäri V. Collagenases in cancer. *Biochimie* 2005; **87**: 273–286.

20 Rutter JL, Mitchell TI, Butticè G, *et al.* A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. *Cancer Res* 1998; **58**: 5321–5325.
21 Ghilardi G, Biondi M, Mangoni J, *et al.* Matrix metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness. *Clin Cancer Res* 2001; **7**: 2344–2346.
22 Ye S, Dhillon S, Turner S, *et al.* Invasiveness of cutaneous malignant melanoma is influenced by matrix metalloproteinase 1 gene polymorphism. *Cancer Res* 2001; **61**: 1296–1298.
23 I.N.E.G.I Census, 2010. Instituto Nacional de Estadística Geografía e Informática(http://www.INEGI.gob.mx).
24 Cerda-Flores RM, Kshatriya GK, Barton SA, *et al.* Genetic structure of the populations migrating from San Luis Potosi and Zacatecas to Nuevo León in Mexico. *Hum Biol* 1991; **63**: 309–327.
25 Cerda-Flores RM, Villalobos-Torres MC, Barrera-Saldaña HA, *et al.* Genetic admixture in three Mexican Mestizo populations based on D1S80 and HLA-DQA1 loci. *Am J Hum Biol* 2002; **14**: 257–263.
26 Cerda-Flores RM, Budowle B, Jin L, *et al.* Maximum likelihood estimates of admixture in Northeastern Mexico using 13 short tandem repeat loci. *Am J Hum Biol* 2002; **14**: 429–439.
27 Moss C, Wong A, Davies P. The Birmingham epidermolysis bullosa severity score: development and validation. *Br J Dermatol* 2009; **160**: 1057–1065.
28 Rits IA. Declaration of Helsinki. Recommendations Guidins doctors in clinical research. *World Med J* 1964; **11**: 281.
29 Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, eds. *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Totowa, NJ: Humana Press, 2000: 365–386.
30 Kearse M, Moir R, Wilson A, et al. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 2012; **28**: 1647–1649.
31 Salas-Alanís JC, McGrath JA. 2470insG, represents the commonest mutation in Mexican patients with dystrophic bullous epidermolysis. A study of 21 families. *Gac Med Mex* 2006; **142**: 29–34.

