Linkage of epidermolysis bullosa simplex to keratin gene loci

K E McKenna, A E Hughes, E A Bingham, N C Nevin

Department of Dermatology, Royal Victoria Hospital, Grosvenor Road, Belfast BT12 6BA, Northern Ireland.
K E McKenna
E A Bingham

Department of Medical Genetics, The Queen's University Belfast, Belfast City Hospital, Belfast, Northern Ireland.
A E Hughes
N C Nevin

Correspondence to Dr McKenna.

Received 28 January 1992.
Accepted 13 February 1992.

Abstract

Epidermolysis bullosa simplex (EBS) is an autosomal dominant disorder characterised by intraepidermal blistering of the skin. Two families with Weber-Cockayne EBS have been analysed for linkage to keratin gene loci. In the first family, linkage was found to chromosome 17 markers flanking the keratin 14 gene (*D17S74*: $Z_{\max} = +2.45$, $\theta = 0.10$; *COL1A1*: $Z_{\max} = +0.97$, $\theta = 0.00$) and markers near the keratin 5 gene on chromosome 12 were excluded (*D12S17*: $Z < -2.0$, $\theta = 0.08$; *COL2A1*: $Z < -2.0$, $\theta = 0.13$). In contrast, the second family showed linkage to the region containing the keratin 5 gene (*D12S17*: $Z_{\max} = +1.37$, $\theta = 0.08$; *COL2A1*: $Z_{\max} = +0.33$, $\theta = 0.15$) and was not linked to the keratin 14 gene (*D17S74*: $Z < -2.0$, $\theta = 0.14$). The Weber-Cockayne form of EBS is genetically heterogeneous with linkage to different keratin gene loci.

Epidermolysis bullosa simplex (EBS) is a non-scarring blistering disorder of the skin, characterised by autosomal dominant inheritance.^{1,2} Blister cleavage is intraepidermal with basal cell cytolysis. The most common forms of EBS present as blistering either localised to the hands and feet (Weber-Cockayne or EBS-WC) or with more generalised blistering (Koebner or EBS-K). Abnormal epidermal keratin filament formation with associated basal cell cytolysis has recently been found in transgenic mice expressing a mutant keratin 14 gene.³ This suggests the importance of keratin filaments in maintaining epidermal cytoskeletal stability. Abnormal organisation of keratin intermediate filaments in patients with EBS has also been recognised⁴⁻⁶ and clumping of keratin tonofilaments in the Dowling-Meara form of EBS (EBS-DM) is a characteristic feature.⁷

These findings prompted us to study whether two families with EBS-WC showed linkage to the keratin gene clusters on chromosomes 12 and 17. Previous authors^{8,9} have also suggested that EBS-WC and EBS-K are linked to chromosome 1 loci. We studied one of our families to determine whether their disease was linked to these loci.

Patients and methods

PATIENTS

Family 1 (fig 1A) consists of four living generations with 14 affected members who complain of recurrent blistering of the hands and feet. The blistering mainly affects the feet and is more common during the summer months. The onset of blistering varied from 3 months to 7 years. In this family there is no mucosal blistering or dental or nail involvement. Family 2 (fig 1B) comprises three living generations with 11 affected subjects. Their blistering is confined to the soles of the feet. Onset of blistering was in infancy. There is no other manifestation of the disorder. Electron microscopic examination of skin from affected members of each family confirmed the presence of epidermal basal cell cytolysis. Blood samples were obtained from affected and unaffected members of each family. Genomic DNA was extracted from these samples and Duffy status was established by blood group analysis in family 1.

SOUTHERN HYBRIDISATION

DNA was digested with several restriction enzymes and electrophoresed through 1%

Figure 1 Pedigrees of EBS-WC families 1 (A) and 2 (B).

agarose gels at 65 V overnight. After electrophoresis, DNA fragments were depurinated, denatured, and transferred to nylon membranes (Hybond N+, Amersham International). DNA probe hybridisation and autoradiography were used to identify polymorphisms as listed in table 1.

Table 1 Polymorphisms on chromosomes 1, 12, and 17 analysed by Southern blotting.

Chromosome	Locus	Probe	Enzyme	Alleles (kb)
1q21-q23	<i>MUC1</i>	pMUC7	<i>HinfI</i>	VNTR 2.5-7.0
1q21	<i>SPTA1</i>	3021E1	<i>MspI</i>	A1 16.2, A2 10.0, 6.2
1q	<i>D1S42</i>	L1054	<i>MspI</i>	A1 9.3, A2 6.8, A3 4.7, 3.9, A4 4.7
12q13-24.1	<i>D12S17</i>	pYNH15	<i>MspI</i>	A1 4.0, A2 3.2, A3 2.6
17q	<i>D17S74</i>	pCMM86	<i>HinfI</i>	VNTR 1.0-3.5

Table 2 Polymorphisms on chromosomes 1, 12, and 17 analysed by PCR.

Locus	Map location	Alleles (bp)
<i>AT3</i>	1q23	272 & 198
<i>AT3</i> (<i>PstI</i>)	1q23	270 & 188 + 82
<i>AT3</i> (<i>DdeI</i>)	1q23	127 & 53 + 74
<i>APOA2</i>	1q21-q23	(CA) _n 131-145
<i>COL2A1</i>	12q13.1-q14.3	VNTR 600-720
<i>COL1A1</i> (<i>RsaI</i>)	17q21.3-q22.15	1000 & 800 + 200

Table 3 Pairwise lod scores between EBS and chromosome 1q, 12q, and 17q loci.

Locus	Lod score at θ of					Zmax/ θ
	0.01	0.05	0.1	0.2	0.3	
Family 1						
<i>MUC1</i>	-6.21	-2.88	-1.58	-0.51	-0.10	—
<i>SPTA1</i>	-2.73	-1.36	-0.80	-0.35	-0.15	—
<i>Fy</i>	+1.01	+1.00	+0.96	+0.80	+0.59	+1.01/0.01
<i>APOA2</i>	-6.22	-2.92	-1.65	-0.57	-0.12	—
<i>AT3</i>	-5.84	-3.11	-2.11	-1.10	-0.52	—
<i>D12S17</i>	-5.53	-2.90	-1.88	-0.99	-0.53	—
<i>COL2A1</i>	-6.52	-3.73	-2.55	-1.41	-0.76	—
<i>D17S74</i>	+1.22	+2.29	+2.45	+2.10	+1.40	+2.45/0.10
<i>COL1A1</i>	+0.95	+0.87	+0.77	+0.56	+0.36	+0.97/0.00
Family 2						
<i>D12S17</i>	+0.75	+1.27	+1.34	+1.15	+0.82	+1.37/0.08
<i>COL2A1</i>	-0.52	+0.09	+0.28	+0.32	+0.22	+0.33/0.15
<i>D17S74</i>	-8.72	-4.60	-2.92	-1.37	-0.60	—

Figure 2 Southern blot of *HinfI* digests of DNA from part of family 1 probed with pCMM86 (*D17S74*). Subjects are numbered above each lane as in fig 1A and those affected are marked with an asterisk. Alleles are labelled 1 to 6. The disease gene is transmitted with allele number 6. Subject III-9 is a recombinant.

Figure 3 PCR amplification of *COL2A1* polymorphisms in part of family 2. Subjects are numbered above each lane as in fig 1B and those affected are marked with an asterisk. Alleles are labelled 1 to 3. The disease gene is transmitted with allele number 2.

POLYMERASE CHAIN REACTION (PCR)

PCR was used to analyse polymorphisms at the *AT3*, *APOA2*, *COL2A1*, and *COL1A1* loci (table 2). PCR was performed on a thermocycler (Techne) using *Taq* polymerase (Cambio) according to conditions described by previous authors.⁹⁻¹³ PCR products were analysed by gel electrophoresis with previous enzyme digestion when necessary.

DATA ANALYSIS

Linkage analyses were carried out using the LINKAGE computer program, version 5.04.¹⁴

Results

Polymorphisms on chromosomes 12 and 17 were analysed in both families and also on chromosome 1 in family 1 (table 3). Linkage to markers flanking the region containing the keratin 14 gene (chromosome 17q12-q21) was found in family 1. The *D17S74* locus was analysed using the probe pCMM86 (fig 2) giving a maximum lod score (Zmax) of +2.45 at recombination fraction (θ) = 0.10 and the less informative *COL1A1* locus by PCR (Zmax = +0.97, θ = 0.00). Multilocus analysis gave a Zmax of 3.6 at the *COL1A1* locus. Exclusion of linkage to markers flanking the region containing the keratin 5 gene (12q11-q13) was found in this family (*D12S17*: $Z < -2.0$, θ = 0.08; *COL2A1*: $Z < -2.0$, θ = 0.13). In contrast, family 2 was not linked to the *D17S74* locus ($Z < -2.0$, θ = 0.14) but showed probable linkage to the *D12S17* locus using the probe pYNH15 (Zmax = +1.37, θ = 0.08). PCR analysis of the *COL2A1* locus (fig 3) gave a Zmax = +0.33 at θ = 0.15. These data provide evidence that in these families EBS-WC is linked to different keratin gene loci on chromosomes 12 and 17.

Family 1 was studied for possible linkage to chromosome 1 markers. Analysis of haplotypes of three polymorphisms within the *AT3* gene excluded close linkage to this locus ($Z < -2.0$, θ = 0.10). Possible linkage between the EBS-WC gene and *Fy* is indicated; however, this can be discounted on the basis of exclusion from the more informative *APOA2* locus (Zmax = -2.0 at θ = 0.075) which maps very close to the *Fy* locus. Multilocus analysis

(data not shown) indicates that the gene causing EBS-WC in family 1 does not lie in the *MUC1-AT3* region of chromosome 1q, thus excluding the *EBS2*⁹ locus.

Discussion

Keratins are major structural proteins of the epidermis and form an extensive network of intermediate filaments. Keratins can be divided into two different classes, type 1 and type 2.¹⁵ One member of each type is required for intermediate filament formation. In the basal cells of the epidermis the type 1 keratin K14 and the type 2 keratin K5 are expressed.¹⁶ These basal cell keratins are encoded by keratin gene clusters on chromosomes 17 and 12 respectively.^{17,18}

There is now considerable evidence to implicate the keratin genes in the pathogenesis of EBS. In all forms of EBS cytotoxicity occurs in the basal layer of the epidermis and there is abnormal keratin intermediate filament formation.⁴⁻⁷ Transgenic mice expressing a mutant K14 gene also develop these features of the disease.³ Different point mutations have been found within the K14 gene in two patients with EBS-DM¹⁹ and a third point mutation in this gene has also been identified in a family with EBS-K.²⁰ Recently, a family with EBS which was initially generalised and subsequently mainly localised to the hands and feet has shown linkage to loci near the K5 gene.²¹ One family with EBS-WC is also reported to be linked to this region.²⁰

We have found one family with EBS-WC affecting the hands and feet linked to the keratin 14 gene on chromosome 17. This is the first report of a family with EBS-WC showing linkage to this locus. Our second family with disease confined to the feet shows probable linkage to the region of the keratin 5 gene on chromosome 12. We hope to elucidate the nature of the mutations responsible for the disease in these two families.

This work was supported by a grant from the dystrophic epidermolysis bullosa research association (DEBRA). Dr K E McKenna is grateful to the Eastern Health and Social Services Board for a research grant from Endowment Funds of the Royal Victoria Hospital which has enabled him to carry out this work. We thank the Collagen Genetics Group, Oxford, for supplying COL1A1 primers

(funded by the EC Concerted Action on Heritable Connective Tissue Disease) and the Human Genome Mapping Project (HGMP), London for supplying probes pYNH15 and 3021E1 (donated by Drs Y Nakamura, R White, and B Forget). The authors thank Dr Kevin Ennis for helpful advice.

- 1 Gedde-Dahl T Jr. Sixteen types of epidermolysis bullosa: on the clinical discrimination, therapy and prenatal diagnosis. *Acta Dermatol Venereol (Suppl)* 1981;95:74-87.
- 2 Fine JD, Bauer EA, Briggaman RA, et al. Revised clinical and laboratory criteria for subtypes of inherited epidermolysis bullosa: a consensus report by the Subcommittee on Diagnosis and Classification of the National Epidermolysis Bullosa Registry. *J Am Acad Dermatol* 1991;24:119-35.
- 3 Vassar R, Coulombe PA, Degenstein L, et al. Mutant keratin expression in transgenic mice causes marked abnormalities resembling a human genetic skin disease. *Cell* 1991;64:365-80.
- 4 Haneke E, Anton-Lamprecht I. Ultrastructure of blister formation in epidermolysis bullosa hereditaria. V. Epidermolysis bullosa simplex localisata type Weber-Cockayne. *J Invest Dermatol* 1982;78:219-23.
- 5 Kitajima Y, Inoue S, Yaoita H. Abnormal organization of keratin intermediate filaments in cultured keratinocytes of epidermolysis bullosa simplex. *Arch Dermatol Res* 1989;281:5-10.
- 6 Ito M, Okuda C, Shimizu N, et al. Epidermolysis bullosa simplex (Koeber) is a keratin disorder. Ultrastructural and immunohistochemical study. *Arch Dermatol* 1991;127:367-72.
- 7 Anton-Lamprecht I, Schyder UW. Epidermolysis bullosa herpiformis Dowling-Meara: report of a case and pathomorphogenesis. *Dermatologica* 1982;164:221-35.
- 8 Mulley JC, Nicholls CM, Probert DN, et al. Genetic linkage analysis of epidermolysis bullosa simplex, Koeber type. *Am J Hum Genet* 1984;35:573-7.
- 9 Humphries MM, Sheils D, Lawler M, et al. Epidermolysis bullosa: evidence for linkage to genetic markers on chromosome 1 in a family with the autosomal dominant simplex form. *Genomics* 1990;7:377-81.
- 10 Daly ME, Perry DJ. DdeI polymorphism in intron 5 of the ATIII gene. *Nucleic Acids Res* 1990;18:5583.
- 11 Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am J Hum Genet* 1989;44:388-96.
- 12 Wu S, Seino S, Bell GI. Human collagen, type II, alpha 1, (COL2A1) gene: VNTR polymorphism detected by gene amplification. *Nucleic Acids Res* 1991;18:3102.
- 13 Baker R, Lynch J, Ferguson L, et al. PCR detection of five restriction site dimorphisms at the type I collagen loci COL1A1 and COL1A2. *Nucleic Acids Res* 1991;19:4315.
- 14 Lathrop GM, Lalouel JM, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Natl Acad Sci USA* 1984;81:3443-6.
- 15 Fuchs E, Coppock SM, Green H, Cleveland DW. Two distinct classes of keratin genes and their evolutionary significance. *Cell* 1981;27:75-84.
- 16 Nelson W, Sun TT. The 50- and 58-kdalton keratin classes as molecular markers for stratified squamous epithelia: cell culture studies. *J Cell Biol* 1983;97:244-51.
- 17 Rosenberg M, Raychaudhury A, Shows TB, et al. A group of type 1 keratin genes on human chromosome 17: characterization and expression. *Mol Cell Biol* 1988;8:722-36.
- 18 Rosenberg M, Fuchs E, Lebeau MM, et al. Three epidermal and one epithelial keratin gene map to human chromosome 12. *Cell Cytogenet* 1991;57:33-8.
- 19 Coulombe PA, Hutton ME, Letai A, et al. Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. *Cell* 1991;66:1301-11.
- 20 Bonifas JM, Rothman AL, Epstein EH Jr. Epidermolysis bullosa simplex: evidence in two families for keratin gene abnormalities. *Science* 1991;254:1202-5.
- 21 Ryyanen M, Knowlton RG, Uitto J. Mapping of epidermolysis bullosa simplex mutation to chromosome 12. *Am J Hum Genet* 1991;49:978-84.