Genetic linkage to the type VII collagen gene (COL7A1) in 26 families with generalised recessive dystrophic epidermolysis bullosa and anchoring fibril abnormalities


Abstract
To strengthen the evidence for genetic linkage to COL7A1, we have studied 26 generalised recessive dystrophic epidermolysis bullosa (EB) families of British, Italian, Irish, and South African origin. We chose two linkage markers, a COL7A1 PvuII intragenic polymorphism and a highly informative anonymous microsatellite marker, D3S1100, which maps close to the COL7A1 locus at 3p21.1–3. Diagnosis was established by family history, clinical examination, immunofluorescence, and ultrastructural studies. The PvuII marker was informative in 16 families with a maximum lod score (Zmax) of 3-51 at recombination fraction (θ) = 0. The D3S1100 microsatellite was informative in 24 out of 25 families with

Zmax = 6-8 at θ = 0−0.05 (Z = 4.94 at θ = 0) and no obligatory recombination events. These data strongly suggest that COL7A1 mutations cause EB in these families and, combined with previous studies, indicate locus homogeneity. The importance of anchoring fibrils for dermal-epidermal adhesion is further underlined. D3S1100 may later prove useful in prenatal diagnosis of this disease, if used in combination with other markers.

Epidermolysis bullosa (EB) encompasses a group of inherited diseases characterised by inappropriate blistering of the skin after minor trauma. Clinically it varies from a limited, non-scarring form to a severe mutilating disorder with widespread loss of skin and scarring. Three major types are separated on the basis of the microscopic level of blistering within the skin: (1) EB simplex with cleavage through the basal epidermis; (2) junctional EB with cleavage through the lamina lucida of the basement membrane; and (3) dystrophic EB with cleavage below the lamina densa of the basement membrane. Dystrophic EB has autosomal dominant and recessive forms. Attempts at further sub-categorisation have been based on both clinical and microscopic variation in the phenotype, but there is overlap between categories causing difficulties in precise classification.

COL7A1 is the prime candidate gene for both autosomal dominant and recessive dystrophic EB. Anchoring fibrils, visible on electron microscopy as cross banded filaments, structures extending from the lower lamina densa into the papillary dermis, are composed mainly of type VII collagen and have both qualitative and quantitative abnormalities in dystrophic EB. In generalised recessive dystrophic EB, recognisable anchoring fibrils may be missing and immunofluorescence staining with type VII collagen antibodies is markedly reduced or absent.

Partial cloning of COL7A1 and identification of an intragenic PvuII polymorphism enabled demonstration of tight linkage to dominant dystrophic EB and, in one study of 19 patients from France, to generalised recessive dystrophic EB with Zmax = 3.97 at θ = 0. Recently a homozygous insertion-deletion within the N-terminal non-collagenous (NC1) domain of COL7A1 has been recognised in a generalised recessive dystrophic EB patient and a methionine to lysine substitution within the C-terminal non-collagenous (NC2) domain of a patient with mitis type of recessive dystrophic EB. These data strongly suggest that COL7A1 mutations cause EB in these patients. Following the demonstration of increased synthesis and secretion of collagenase the collagenase genes became strong candidates. However, two independent studies have excluded genetic linkage between it and recessive dystrophic EB.

Informative markers which are tightly linked to a disease locus can be used for prenatal diagnostic testing of fetal DNA obtained from chorionic villus samples at 11 weeks’ gestation. Currently prenatal diagnosis of EB is performed by electron microscopy and immunofluorescence of a fetal skin biopsy, taken at 16 weeks’ gestation. Although there have been reports of prenatal diagnosis with the PvuII RFLP, this marker is only fully
informative in some cases (heterozygosity in
our population was 36%). A highly informative
marker would therefore be very useful for this
purpose.

We have tested linkage to COL7A1 in 26
further generalised recessive dystrophic EB
families to confirm the previous study and to
seek evidence of locus heterogeneity. We chose
the intragenic PvuII COL7A1 RFLP and a
second, microsatellite, repeat marker, D3S1100.25 This marker has a heterozygosity
of 77% and has been mapped close to 3p21.3
by the NIH/CEPH collaborative mapping
group.26 We have also examined the suitability
of this marker for prenatal diagnosis by its
segregation patterns in our families.

Materials and methods

PATIENTS

Families with generalised recessive dystrophic
EB were recruited from clinics in London at
St Thomas's Hospital, Great Ormond Street
Hospital, and through the three charitable
organisations, DEBRA UK, DEBRA Ireland, and
DEBRA Italy. One family was also included
from South Africa. Clinical details were cata-
louged and a skin biopsy, taken from un-
blistered skin after gentle rubbing, was
examined by transmission electron microscopy
using standard methods.27 Indirect immuno-
fluorescence using LH7:2,28 GB3,29 and anti-
type IV collagen antibodies was performed
on UK patients. Blood samples were taken
from all available family members with ethical
committee approval.

DNA ANALYSIS

Standard methods were used for phenol/chlo-
roform extraction of lymphocyte genomic
DNA. Two variable DNA markers were used.
(1) PvuII RFLP. The PCR was used to amplify
a 431 bp fragment containing the relevant
PvuII site. (Primer 1: 5'GTGGCCAG-
GAACAGTCGCCGGTCC3'; primer 2:
5'CGAGGGTCCACCACGTTAGTTCC3').
After five minutes denaturing at 95°C, 30 cycles
of denaturing at 95°C, annealing at 68°C and
elagination at 72°C were performed. Amplified
fragments were digested with PvuII (North-
embria Biologicals Limited) and separated on
2% agarose gels (fig 1A). (2) D3S1100 mi-
crosatellite. PCR amplification of this micro-
satellite using 32P end labelled primer 1
(5'GGTTTCTATACATCAATTCCAC3')
and cold primer 2 (5'GTACACCATCG-
AGAGCTCTGG3') was performed.30 PCR
product (10 µl) and 4 µl of loading buffer (98%
formamide, 1% xylene cyanol, 1% bro-
mophenol blue) were heated to 95°C for five
minutes, cooled on ice, and the single stranded
products were immediately electrophoresed on
6% polyacrylamide gels containing 8 mol/l urea
at 37 W for three to four hours. The dried gel
was autoradiographed at −70°C for two to
seven days (fig 1B).

LINKAGE ANALYSIS

Two point lod scores were calculated using the
MLINK programme in the LINKAGE
package.30 Allelic frequencies were calculated
on the basis of 47 unrelated persons from our
study population. Autosomal recessive inher-
ance and complete penetrance were
assumed.

Results

CLINICAL AND ULTRASTRUCTURAL FINDINGS

The structure, consanguinity, and origins of
the 26 families are shown in table 1 and fig 2.
All patients had extreme skin fragility with
blistering, scarring, and milia formation from
birth. Nail loss, pseudosyndactyly, con-

Figure 1  Marker results. Pedigrees are shown with subjects above their corresponding lane. Open box: unaffected male; open circle: unaffected female; filled box: affected male; filled circle: affected female. (A) PvuII RFLP in family 12. The affected child is homozygous for the + allele and shows two digested fragments of 326 bp and 105 bp. (B) D3S1100 microsatellite in family 12. (C) D3S1100 microsatellite in family 15. Arrows show the number of repeats (rpt).
Table 1  Structure, consanguinity, and origin of families with generalised recessive dystrophic epidermolysis bullosa

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SA = South Africa. * Pedigrees for the two families with four parents were identical (fig 2).

DNA ANALYSIS AND LINKAGE

The lod scores for the PvuII RFLP and the microsatellite marker are shown in table 2(A) and (B) respectively. All families were informative for at least one of the markers. The 16 informative families for the PvuII RFLP gave a combined maximum lod score of 3·51 at θ = 0 with no obligatory recombinations. Our observed allelic frequencies were 0·28 (+ allele) and 0·72 (− allele). Heterozygosity in 50 unrelated persons was 36%. Zmax for the microsatellite was 6·8 at θ = 0·05 with Z = 4·94 at θ = 0. Although there were no obligatory recombinations, in two consanguineous families (15 and 25) the affected patients were heterozygous for the marker, suggesting a probable recombination event in the grandparents or great grandparents (fig 1C). The calculated two point lod score between the two markers estimates the proximity of D3S1100 to COL7A1 (full lod table not shown). Thirteen families were informative for both markers with 28 informative meioses and one obligatory recombination. The disease showed no clustering of any particular allele for either marker.

Discussion

The lod score of 3·52 for the PvuII RFLP in our generalised recessive dystrophic EB families is highly significant and confirms the results of Hovnanian et al. The PvuII polymorphism lies in the coding sequence for a fibronectin-like domain (FN-8) in the NC-1 region of the type VII collagen molecule. The combined evidence of positive gene linkage, abnormalities of anchoring fibrils and type VII collagen protein expression in epidermal basement membrane, and the observation of two recessive dystrophic EB patients with separate COL7A1 mutations strongly suggests that all generalised recessive dystrophic EB families have allelic mutations.

Since the PvuII marker was only informative in 16 out of 26 families we used the microsatellite repeat D3S1100 at 3p21.1–3.29,32 This was informative for 24 out of 25 families tested. There were two possible recombinations.
is families who help, J Hovnanian for his kind DEBRA is consistent with observations of other diseases particular type produce in independent selection, we that the marker site showed flank of maximum distance of 5 cM between COL7A1 and D3S1100. If used alone for prenatal diagnosis this predicts a 5% recombination (misdiagnosis) rate which we consider unacceptably high. However, in combination with a second suitable microsatellite located at the opposite flank of COL7A1, it could form the basis of an accurate and informative test for the majority of families.

Other workers have tested the hypothesis that the collagenase gene participates in the pathogenesis of recessive dystrophic EB, but two independent reports have excluded gene linkage. Finally, despite strict criteria for clinical selection, we have noted significant interfamily variation of clinical severity. Very probably this is related to the site and type of mutation within COL7A1. The lack of linkage disequilibrium for particular RFLP genotypes supports the hypothesis that many differences COL7A1 mutations produce the EB clinical phenotype and is consistent with observations of other diseases such as osteogenesis imperfecta, Ehlers-Danlos syndrome type IV, and certain chor drosplasias.

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3 Sakai LY, Keene DR, Morris MP, Burgess RE. Type VII collagen is a major structural component of anchoring fibrils. Cold Spring Harb Symp Quant Biol 1986;51:1777-86.
8 Leigh IM, Eady RAJ, Heagerty AHM, Purks PE, Whitehead PA, Burgess E. Type VII collagen is a normal component of epidermal basement membrane, which shows altered expression in recessive dystrophic epidermolysis bullosa.

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