Linkage of autosomal dominant dystrophic epidermolysis bullosa in three British families to the marker D3S2 close to the COL7A1 locus

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Abstract

Linkage of the anonymous marker D3S2 at 3p21 has been shown in three British families with dominant dystrophic epidermolysis bullosa with a combined lod score of 6.75 at $\theta=0$. This locus is close to the collagen type VII locus implying that abnormalities of this gene cause dominant dystrophic epidermolysis bullosa.

(DDEB) with a *PvuII* RFLP of the COL7A1 gene has already been clearly established.⁸ When our studies began the COL7A1 cDNA sequence was unavailable. We therefore elected to test linkage of DDEB in our British families to 3p21 with the anonymous marker D3S2 for which an *MspI* dimorphism has been described.⁹

Epidermolysis bullosa (EB) comprises a group of heterogeneous hereditary cutaneous disorders characterised by inappropriate blistering and erosions after mild trauma. Although up to 23 subtypes may exist,¹ they form three main categories: simplex, junctional, and dystrophic. Dystrophic EB has autosomal dominant and recessive forms.²

Classifications of dominant dystrophic EB have been rather arbitrary but include Cockayne-Touraine and Pasini variants. Although the latter is recognised by allopapuloid lesions their specificity is questionable.³ Very probably the two disorders are allelic. The blistering and erosions of all forms of dystrophic EB are followed by atrophic cutaneous scarring and milia formation. Whereas changes in the autosomal recessive form can be widespread with severe mutilation, the autosomal dominant variety is much milder with lesions virtually limited to the extensor aspects of the limbs. Nail dystrophy is also a consistent feature. In all forms the histological level of blistering lies just beneath the lamina densa of the epidermal basement membrane. There are variable abnormalities of anchoring fibrils as identified by electron microscopy. Additionally, immunostaining of this region using antibodies against different epitopes of the type VII collagen molecule mostly shows abnormal staining in the recessive disease.⁴ Furthermore, anchoring fibrils contain type VII collagen.⁵ These observations strongly imply that molecular abnormalities of the COL7A1 gene underlie at least some forms of dystrophic EB.

In 1976, Anton-Lamprecht and Hashimoto⁶ originally suggested that a mutation in the structural gene for anchoring fibrils caused Pasini type autosomal dominant epidermolysis bullosa dystrophica.

The recent localisation of the gene for type VII collagen in chromosome 3p21 and the publication of a partial cDNA sequence encoding the 5' end (1.9 kb) of the 10 kb type VII mRNA⁷ now allows the testing of this hypothesis. Linkage of one Finnish family with dominant dystrophic epidermolysis bullosa

Clinical details

Three large British families were ascertained from either the Institute of Dermatology Epidermolysis Bullosa Clinic (RAJE) or with the help of the Dystrophic Epidermolysis Bullosa Association (DEBRA). The families were individually visited, interviewed, and examined by one of us (FMP) and found to contain 67 affected patients with 101 unaffected relatives. Affected subjects were identified by a combination of specific physical features including dystrophic or missing finger and toenails, characteristic atrophic scarring with milia formation over the dorsum of the hands and feet and extensor aspects of shins, knees, and elbows, and typical milia over the dorsum of the fingers. Occasional patients showed small painless intraoral bullae which were sometimes haemorrhagic. There were no allopapuloid lesions.

Methods

DNA PREPARATION

Blood samples were collected into dextrose/citrate anticoagulant (FMP) and DNA prepared by standard methods from dextran sedimented leucocytes. In some instances, saliva or hair samples were obtained for DNA preparation.

DNA MARKERS

The *MspI* polymorphism at D3S2 has a population frequency of 0.70 for the (−) allele and 0.30 for the (+) allele.⁹ This region was amplified from genomic DNA using the oligonucleotide primers described by Ganly and Rabbitts.¹⁰ The PCR product was restricted with *MspI* and analysed by agarose gel electrophoresis (fig 1).

Results

Analysis of three British DDEB families with the anonymous marker D3S2 at 3p21 shows clear segregation of the disease with the (+)

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Figure 1 Agarose gel electrophoresis of PCR amplified D3S2 fragments digested with *MspI*. The more common genotype indicates the higher molecular weight uncleaved fragment, while the rare + marker is digested to 237 and 236 bp products. The outside left and right tracks contain λ HindIII and 0X174 HaeIII markers respectively.

Figure 2 Part of the pedigree of the largest British family showing haplotypes of D3S2 markers.

allele with no observed recombination. A representative portion of the largest pedigree is shown in fig 2. The combined lod score was 6.75 at $\theta=0$ indicating strong linkage of the marker to the disease. (Individual families

gave scores of 3.86, 2.37, and 0.52, respectively.)

When combined with several other important pieces of evidence this information strongly suggests that mutations of COL7A1 cause autosomal dominant dystrophic EB. The other data include the co-localisation of the COL7A1 gene to 3p21,⁷ and the reduction of anchoring fibrils in DDEB skin samples, which immunostain normally. Our data also confirm the linkage of a COL7A1 marker to DDEB in a Finnish family⁸ and imply locus homogeneity for this disease.

In each of the three families tested here the disease has segregated with the (+) allele of D3S2 (DOSLC9). This was the rarer allele in the American study of 28 normal subjects in which this RFLP was originally described.⁹ We note that the lod score varies slightly if the population frequency of the (+) and (-) alleles is altered. This is because DNA from certain subjects has been unobtainable. For instance if the allele frequencies were 0.5 the lod score would fall to 5.41. We also cannot exclude the possibility that all three British families have a common founder, although there is no apparent connection between the available family names over six generations.

The recent publication of a partial cDNA sequence for the type VII collagen gene⁷ will soon allow the identification of a full length cDNA sequence. This will lead to the mutational analysis of DDEB using various gene mapping techniques such as chemical cleavage, denaturing gradient gel electrophoresis (DGGE), or single stranded conformational analysis, which has already been successfully exploited in other inherited connective tissue diseases such as osteogenesis imperfecta, Ehlers-Danlos syndrome type IV, and Marfan syndrome.

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