

Short report

Use of type VII collagen gene (COL7A1) markers in prenatal diagnosis of recessive dystrophic epidermolysis bullosa

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Abstract

Generalised recessive dystrophic epidermolysis bullosa (EB) is a severe inherited disease in which patients suffer from blistering and scarring of the skin and mucous membranes after minor mechanical trauma. Tight genetic linkage has been established to the type VII collagen gene (COL7A1) at 3p21, with no evidence of locus heterogeneity. Several COL7A1 mutations have now been identified in recessive dystrophic EB patients. Prenatal diagnosis has been performed by examination of a fetal skin biopsy taken at about 16 weeks' gestation, and relies on identification of characteristic ultrastructural and immunohistochemical changes. We have now achieved a first trimester prenatal diagnosis using intragenic and flanking COL7A1 markers in a pregnancy at risk for recessive dystrophic EB. Segregation of the informative markers predicted the baby would be an unaffected carrier. The pregnancy continued to term and a healthy baby was born, confirming this result.

(*J Med Genet* 1995;32:749-750)

Recessive dystrophic epidermolysis bullosa is an inherited disorder of skin and mucous membranes characterised by blistering and scarring after minor mechanical trauma. Mutilating deformities and squamous cell carcinoma may occur, and patients frequently die in the third or fourth decade. Electron microscopy has shown abnormalities of anchoring fibrils, attachment structures composed of type VII collagen.¹ Genetic linkage studies have implicated the type VII collagen gene (COL7A1) at 3p21. The combined maximum lod score (Zmax) from studies of generalised recessive dystrophic EB using an intragenic *PvuII* restriction fragment length polymorphism (RFLP) is 7.48 at a recombination fraction (θ) of zero.^{2,3} None of the families showed any evidence of recombination, firmly supporting locus homogeneity for this disease. Pathogenic mutations

on COL7A1 have now been identified, confirming the role of COL7A1 in this disease.⁴⁻⁷

Prenatal diagnosis is often requested by parents of children with severe recessive dystrophic EB. Fetal skin biopsy allows prenatal diagnosis at 16 weeks' gestation.⁸ Although this is an accurate and reliable method, the necessity of a late midtrimester termination is disadvantageous. We have recently achieved first trimester prenatal diagnosis in a pregnancy at risk for severe recessive dystrophic EB using intragenic and flanking COL7A1 markers.

The consanguineous parents of a child with generalised recessive dystrophic EB requested prenatal diagnosis in the 10th week of a subsequent pregnancy. A chorionic villus sample was obtained at 10½ weeks' gestation using transcervical biopsy forceps and DNA was extracted using standard methods after elimination of maternal tissue contamination. The polymerase chain reaction (PCR) was used to amplify DNA fragments containing the markers, and PCR products were then digested with the appropriate restriction enzymes.^{3,9} The *PvuII* polymorphism, which lies within the NC-1 coding domain of COL7A1,² was fully informative, and predicted that the fetus would be an unaffected heterozygous carrier (figure A). A second polymorphic *MspI* site flanking COL7A1 (D3S2) was partially informative, and indicated fetal inheritance of the maternal allele carrying the mutation (figure B). The absence of the (-) allele from the fetal sample helped to exclude maternal contamination. The pregnancy was continued to term and a healthy baby was born, confirming the result.

The gene encoding type VII collagen is highly complex with 118 exons and 9.2 kb cDNA.¹⁰ Furthermore the mutations so far described have been largely unique to individual families and do not cluster.⁴⁻⁷ Without common mutations and in the context of a large and complex gene, it may not be feasible to identify the pathogenic mutations in all families requesting prenatal diagnosis. Appropriate polymorphic markers within and tightly flanking COL7A1 will therefore be important for prenatal diagnosis. The speed and accuracy of DNA based testing coupled with the ability to obtain fetal

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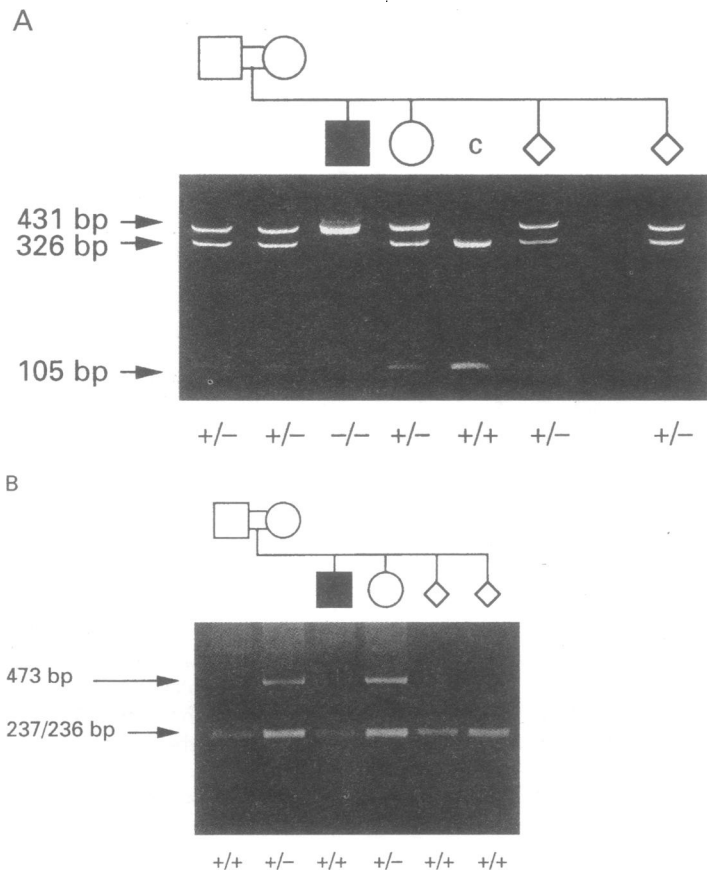
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Received 23 February 1995
Revised version accepted for publication 12 May 1995



Marker results. Open box, unaffected male; closed box, affected male; open circle, unaffected female; open diamond, fetal sample. (A) The *PvuII* marker. The affected child is homozygous for the (-) allele. The fetal sample was run in duplicate and is heterozygous (+/-), indicating unaffected carrier status. A control sample, C, known to be homozygous (+/+) was also run in the 5th lane. (B) The *D3S2* flanking marker. The mother is heterozygous (+/-) and the affected child is homozygous (+/+), indicating segregation of the maternal mutation with the (+) allele. The fetus is homozygous (+/+) showing inheritance of the maternal mutation.

DNA from chorionic villus in the first trimester mean that this method will supersede fetal skin biopsy in the future.

Note added in proof

After we submitted our manuscript for publication, Hovnanian *et al*¹¹ published a report on prenatal diagnosis of generalised recessive dystrophic EB based on genotype analysis with *COL7A1* polymorphic markers or direct mutation detection.

We would like to acknowledge Miss J Denyer for her help in counselling the family and Dr Alain Hovnanian for his kind advice. We thank DEBRA UK and the MRC for partial funding of laboratory work. MGSD is supported by a DEBRA research fellowship.

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