Short communications

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Direct detection of haemoglobin E with *Mnl*I

The development of DNA restriction endonuclease mapping has been an important step in the study of genetic diseases as it allows the prenatal detection of mutations by analysis of fetal DNA obtained by chorionic villus biopsy in the first trimester. It is preferable to identify mutations directly rather than indirectly by linkage analysis to restriction fragment length polymorphisms (RFLPs), since this removes the need for informative family pedigrees and is not prone to diagnostic errors caused by non-paternity or recombination. A mutation can be identified directly by synthetic oligonucleotide probe hybridisation or by restriction analysis if it involves a cleavage site for a restriction enzyme. The latter approach has not been possible for the detection of some mutations, as the DNA fragments generated are too small (<1 kb) to be detected reliably by routine gene mapping techniques. However, we now show that this limitation can be overcome by improved techniques, which involve the use of nylon membranes which bind small DNA fragments more efficiently and the hybridisation to DNA probes ^{32}P labelled to a high specific activity by the recent technique of random hexanucleotide priming.[1]

We report the successful application of this approach to the direct detection of haemoglobin E (Hb E), a very common haemoglobin variant in south-east Asia and parts of the Indian subcontinent. Hb E results from a single amino acid substitution of glutamic acid to lysine due to a point mutation (GAG to AAG) in codon 26 of the β globin gene. The Hb E mutation destroys a cleavage site for the restriction enzyme *Mnl*I[2] which cleaves DNA at the sequence 3′-GGAG-5′ (G=guanosine, A=adenosine, C=cytosine, T=thymine).

		Codon	
	25	26	27
β^A	5′ GGT	GAG	GCG-3′
β^E	5′ GGT	AAG	GCG-3′

Fig 1 shows the map of the β globin gene and the recognition sites for *Mnl*I. When normal ($\beta^A\beta^A$) DNA is digested with *Mnl*I and hybridised with the 430 base pair (bp) *Nco*I/*Bam*HI β gene probe, fragments of 60, 170, 120, and 680 bp are seen. Removal of the *Mnl*I cleavage site by the Hb E mutation increases the 170 bp fragment to 230 bp.

Genomic DNA (10 μg) was digested with 20 units of *Mnl*I (New England Biolabs). The DNA fragments were separated through electrophoresis in a 2·2% agarose gel at 110 V for four hours and transferred by Southern blotting onto Pall 'Biodyne' nylon membrane (0·2 μmol/l) which was then baked at 80°C for one hour. The *Nco*I/*Bam*HI β gene fragment, prepared from the *Pst*I β plasmid, was labelled by random hexanucleotide priming[1] using [α^{32}P] cytidine triphosphate (specific activity $>10^9$ cpm μg^{-1} DNA) and hybridised to the membrane in 5×SSPE, 7·5% dextran sulphate, 1·0% SDS, 5 × Denhardt's, and 100 μg/ml salmon sperm DNA at 65°C overnight. The membrane was washed in 0·1 × SSC at 65°C and autoradiographed for one to three days at −70°C with intensifying screens.

Fig 2 illustrates the results of this analysis in three normal subjects and three others, one shown previously to be heterozygous and the other two homozygous for Hb E. The normal subjects (β^A/β^A) are homozygous for the smaller 170 bp fragment, while the two who are homozygous for Hb E (β^E/β^E) are homozygous for the abnormal 230 bp fragment, the smaller 170 bp fragment being clearly absent. The subject who is heterozygous for Hb E ($\beta^A\beta^E$) shows both the 170 and the 230 bp fragments.

We have shown that it is possible to detect the Hb E mutation directly using the enzyme *Mnl*I. This

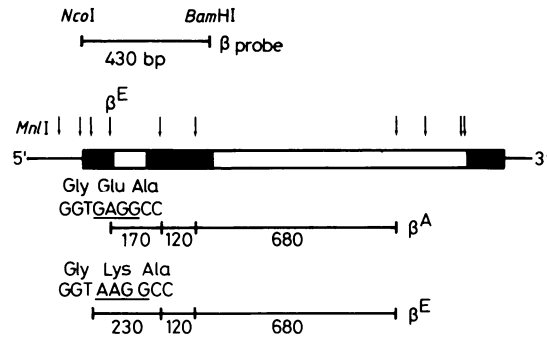

FIG 1 *Map of the β globin gene showing the position of the cleavage sites of MnlI. DNA digested with MnlI and hybridised with the NcoI/BH1 (BamHI) fragment probe prepared from the PstI β plasmid shows a 170 bp fragment in β^A (normal) chromosomes and a 230 bp fragment in B^E chromosomes.*

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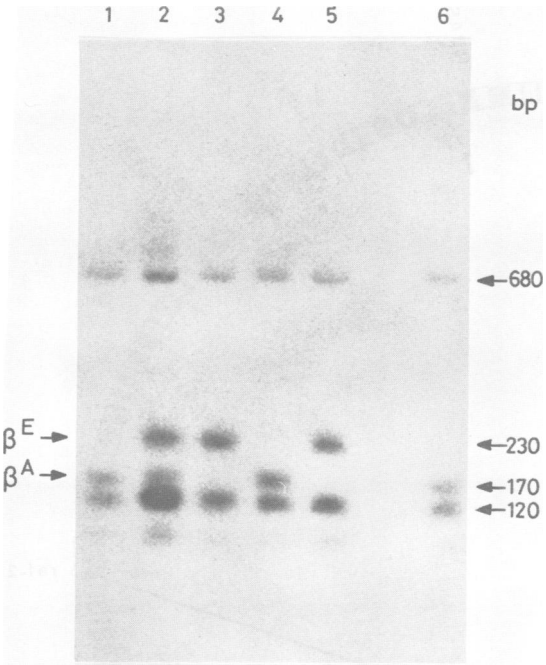

FIG 2 *Autoradiograph showing genomic DNA samples digested with MnlI and hybridised to the NcoI/BamHI β fragment probe. Lanes 1, 4, and 6=normal subjects (β^A/β^A), lane 2=subject heterozygous for Hb E (β^A/β^E), lanes 3 and 5=subjects homozygous for Hb E (β^E/β^E).*

method requires no modification of routine gene mapping procedures and is directly applicable to prenatal diagnosis of Hb E by fetal DNA analysis. Studies have shown that the majority of thalassaemic children in south-east Asia result from the interaction of Hb E and β thalassaemia.[3] Thus, detection of the Hb E mutation alone offers a 50% chance of excluding compound heterozygosity for Hb E thalassaemia in a large proportion of Asian couples at risk of producing thalassaemic children. The isolation of restriction enzymes with different cleavage sites should permit more mutations to be directly detectable by this approach, since detection of small DNA fragments should no longer be a limiting factor.

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Translocation chromosome map of oncogenes

Recent progress in medical genetics has shown that chromosomal reciprocal translocation is one of the most frequent and consistent mechanisms of genetic disease and human malignancies.[1 2] Human gene maps currently used to identify gene loci are based on a classical Denver idiogram which is unable to display the connections between chromosome breakpoints.[1] Consequently, chromosome maps representing oncogene loci, breakpoints, and reciprocal translocations are confusing. In order to solve this problem, I propose to use a special circular translocation chromosome map ordered by size which allows translocation lines to be traced in the area of the circle.

The figure shows a circular idiogram which contains 24 human chromosomes, including the X and Y, in order of size. The outer circumference shows gene loci symbols of proto-oncogenes[2] and the lines represent translocation tracks between chromosome breakpoints (arrows). Associated malignant diseases are displayed along the translocation lines. Relative chromosome lengths are approximate to those of McKusick's map.[1]

This new circular idiogram has considerable advantages over the classical Denver model for displaying a general overview of oncogenic reciprocal translocation. This new model is preferable to other designs, such as oval or bicycle chain models, because it allows identification of oncogenic loci on the standard scale of 360° of the circumference of the circle; for example, proto-oncogene *abl* is located at 200° and proto-oncogene *sis* at 346°. These

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