South African human genes in health and disease — a molecular genetics approach

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Summary

Recombinant DNA technology is playing an increasingly important role in diagnostic confirmation, carrier detection and the prenatal diagnosis of inherited disorders. This article summarizes current progress in the application of this technology to clinically important genetic conditions in South Africa and outlines its potential role in the future practice of medical genetics in this country.

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The terms 'genetic engineering', 'recombinant DNA' and 'genetic manipulation' all pertain to a technology which is devoted to the analysis and characterization of gene structure.[1-5] The analysis of human chromosomal DNA has yielded considerable information which is directly relevant to a number of genetic diseases.[6] The best examples of these are the haemoglobinopathies, where defects in globin synthesis have been elucidated at the nucleotide level.[7,8] Discoveries of this type have a clinical application in diagnostic confirmation, carrier detection and prenatal diagnosis, and in the future will form the basis for gene replacement therapy.[9]

The role of molecular genetics in medical practice has been the subject of several recent articles.[10-12] In this article we outline current progress in the application of recombinant DNA technology to some clinically important genetic disorders in South Africa and indicate the potential role of molecular genetics in the future practice of medical genetics and related disciplines in this country.

Globin gene analysis in South Africa

Haemoglobinopathies and thalassaemias

The direct analysis of genes coding for haemoglobin is fast becoming the method of choice for the diagnosis of sickle cell anaemia and thalassaemia in Europe and the USA.[13] Application of the gene blotting technique to the study of haemoglobinopathies and thalassaemias in South Africa has already proved clinically useful.[14]

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Sickle cell anaemia (Hb SS)

The sickle mutation at $\beta^{6\ Glu \rightarrow Val}$ converts the DNA sequence in this region of the ß-globin gene from CCT-GAG-GAG to CCT-GTG-GAG. The restriction enzyme Mst II cleaves DNA at the sequence CCTNAGG (where N = any of the four bases). The loss of an Mst II cutting site as a consequence of the sickle mutation results in the production of a 1,35 kb ß-globin fragment after digestion of the DNA with this enzyme instead of the 1,15 kb fragment produced from a normal ($ß^A$) gene[13,15] (Fig. 1). The ß-globin fragments detected after Mst II digestion of DNA from local patients with sickle cell anaemia and sickle cell trait are shown in Fig. 2.

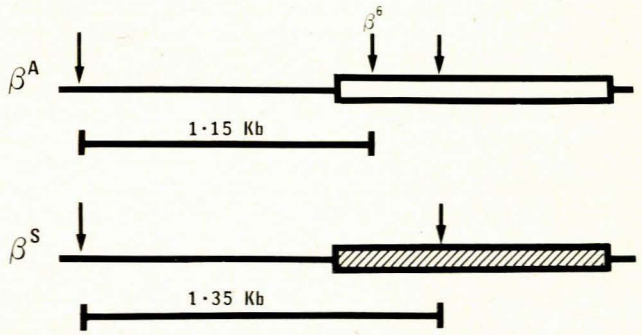

Fig. 1. Diagram of the normal ($ß^A$) and sickle ($ß^S$) ß-globin gene. Mst II cutting sites are indicated by arrows.

It has been suggested that silent third-base polymorphisms could interfere with the Mst II assay. However, all 6 South African sickle genes and 6 $ß^A$ genes that we have studied gave the expected Mst II pattern. The assay can therefore be used locally for the postnatal diagnostic confirmation of sickle cell anaemia and, more importantly, for the antenatal diagnosis of this condition, since sufficient fetal DNA can be obtained from amniotic fluid cells for the analysis. This has recently been accomplished in a South African Indian family (M. Ramsay — personal communication). The test is likely to have limited application locally, however, since the incidence of sickle cell anaemia in South African populations is very low. Carrier rates of 0,3% for South African Blacks,[16] 0,4% for 'Cape Coloureds'[17] and 1% for South African Indians have been reported.[18]

The only other haemoglobinopathy that occurs at a significant frequency in a South African population, and which can cause a severe anaemia, is Hb E. The carrier rate in the 'Cape Coloured' population is 1%.[17] Individuals homozygous for Hb E may have a normal lifespan, but if the $ß^E$ gene is inherited together with a ß-thalassaemia gene (Hb E/ß-thal) the patient may experience severe clinical problems.[19] Although the gene for Hb E cannot be directly detected by DNA analysis, the condition can be diagnosed antenatally using linked restriction enzyme polymorphisms as described below for ß-thalassaemia.

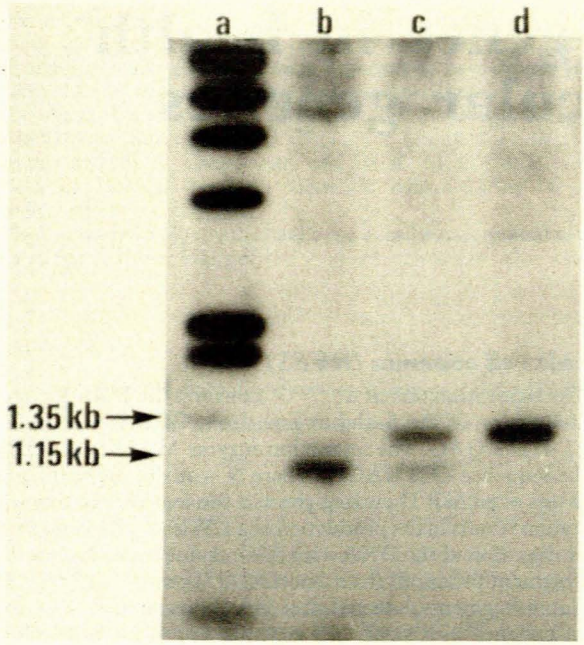

Fig. 2. DNA prepared from white blood cells of 3 individuals was digested with Mst II, electrophoresed and blotted onto nitrocellulose. This was incubated with a ^{32}P-labelled ß-globin clone[50] and the restriction fragments containing ß-globin sequences were detected by autoradiography (a = Hind III digested lambda DNA molecular weight marker; b = normal control; c = sickle cell trait; d = homozygous sickle cell anaemia).

Beta-thalassaemia

Direct detection of ß-thalassaemia by restriction mapping is generally not possible. The molecular lesion is usually only a single base change in the entire sequence of the gene which does not fall within the recognition sequence of a known restriction enzyme.[19] Antenatal diagnosis can be performed, however, since restriction fragment length polymorphisms (RFLPs) at the ß-globin locus can be used as genetic markers to follow the inheritance of faulty genes in families.[10–12] The existence of these RFLPs in the South African population and their use in tracing ß-thalassaemia alleles in a Cape Malay family have recently been described.[20] The validity of this approach has been underlined by a large American study in which 86% of pregnancies at risk for sickle cell disease or ß-thalassaemia were successfully diagnosed using DNA polymorphisms.[21]

Little published information is available on the incidence of ß-thalassaemia in South African populations. The prevalence of heterozygous ß-thalassaemia in the Greek community of Cape Town is 9,2%, with a gene frequency of 0,046.[22] Bird *et al.*[23] have shown that of 627 'Cape Coloured' children with microcytic anaemia, 45 were carriers of the ß-thalassaemia gene. The frequency of the disorder in the South African Black and Indian populations is not known. Studies on other African Black populations have reported frequencies of 0,2 - 1,7% (reviewed by Jenkins and Dunn[24]). The founder effect is evidently operative, since in India the frequency ranges from 1% to 17%, depending on the community which is sampled.[19]

In the light of the foregoing, the antenatal diagnosis of ß-thalassaemia using DNA polymorphisms is likely to be applied mainly in South Africa's Greek and 'Coloured' populations.

Alpha-thalassaemia

The majority of cases of α-thalassaemia have been shown to result from substantial deletions of DNA at the α-globin locus.[19] The human α-globin gene is duplicated on chromosome 16; a normal diploid cell therefore has a total of 4 α-globin genes. Deletion of 1 - 4 of these genes produces manifestations ranging from the clinically asymptomatic carrier state to the hydrops fetalis syndrome. The various possibilities are illustrated diagrammatically in Fig. 3A. Because these deletions remove several kilobases of DNA they are readily detectable by the restriction mapping technique (Fig. 3B). Deletions which remove both α-globin genes from the chromosome (Fig. 3A, lanes d and f) cannot be identified with an α-globin gene probe, since no α-globin restriction fragments are detected. However, the deletion is so large that the restriction map around the adjacent zeta (embryonic)-globin gene is perturbed and the affected chromosome can therefore be detected using a zeta-globin gene probe.[19]

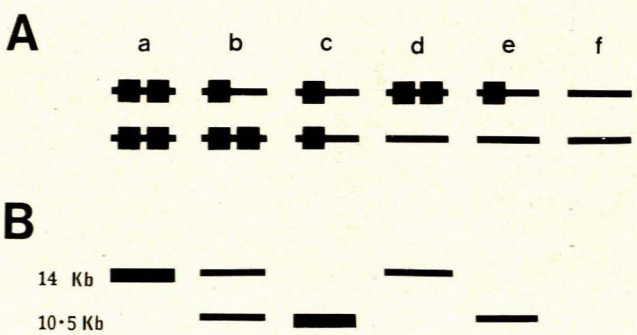

Fig. 3. A — diagram of possible α-globin genotypes showing deletion of 0 (a) to 4 (f) α-globin genes; B — expected restriction fragment patterns for the various genotypes after digestion with Bam HI and hybridization to a probe specific for the α-globin gene.

A study of α-thalassaemia in the Western Cape has shown that almost all cases result from α-globin gene deletions and that chromosomes with the single (-α/) and with the double (--/) deletion occur in the local population.[14] The gene mapping technique can therefore be applied in at least two clinical situations. Firstly, an unequivocal postnatal diagnosis of α-thalassaemia can be made in cases of persistent hypochromic microcytic anaemia. Furthermore, carrier detection and antenatal diagnosis can be made available to families in which the Hb Bart's hydrops fetalis syndrome has occurred.

The widespread occurrence of α-thalassaemia in Africa, India and South-East Asia[19] suggests that these genes will be found in most South African populations. However, it is unlikely that Hb H disease or Hb Bart's hydrops fetalis will be encountered in the Indian and Black populations since the double gene deletion (--/) is extremely rare in Africa and India.[19] This deletion does occur at a significant frequency in China and Malaysia, and the more serious α-thalassaemia syndromes can therefore be expected to occur in the South African Chinese and 'Cape Coloured' populations.

In summary, DNA analysis can be used for the postnatal diagnosis of sickle cell anaemia and α-thalassaemia, and for the prenatal diagnosis (via amniocentesis) of sickle cell anaemia, α-thalassaemia and ß-thalassaemia.

Human collagen gene analysis in South Africa

The collagens are a family of proteins of at least five different types. Type I collagen is the most abundant and represents the major collagen found in tissues such as skin and tendon. Although these proteins constitute one-half of the total body protein in fully developed adult organisms,[25] it is only recently

that the structure of genes coding for some of the collagen types has been elucidated. Schafer *et al.*[26] and Boyd *et al.*[27] were the first to demonstrate the very complex arrangement of coding and intervening sequence DNA within the gene coding for sheep pro α_2 (type I) procollagen. More recently this structure has been confirmed in chick, mouse and human DNA.[28-30] Restriction maps of human type I collagen gene sequences have now been used to commence a study of the molecular events responsible for abnormalities known to occur in a number of inherited disorders of connective tissue. Prockop *et al.* have shown, for example, that a lethal form of osteogenesis imperfecta is due to a deletion in the middle of the pro α_1 (type I) collagen gene. A related approach that A. Grobler-Rabie and co-workers (personal communication) have taken is the determination of the incidence of RFLPs in the human type I collagen gene family (RFLPs have been defined and discussed in a number of recent reviews[31,32] including an earlier review by one of us in this journal[5]). This approach has the advantage of establishing RFLPs as genetic markers in this gene family for any trait with which they may co-segregate, in addition to inherited disorders of connective tissue where restriction fragment length variants may occur within the collagen gene family.

Fig. 4 shows one of the first RFLPs observed within the human pro α_2 (type I) collagen gene which was detected with a pro α_2 cDNA clone (a cloned partial copy of human pro α_2 mRNA). The radioactively labelled cDNA ('gene probe') was hybridized to total human DNA that was initially digested with the restriction enzyme Msp I and then transferred to nitrocellulose paper following the procedure developed by Southern.[33] The 2,0 kb Msp I restriction fragment (indicated with an arrow) represents an RFLP associated with the pro α_2 gene that occurs in the population at a frequency high enough to make this RFLP a useful marker for future linkage studies (A. Grobler-Rabie — personal communication). It should permit diagnosis of any trait segregating with the 2,0 kb Msp I restriction fragment. While this Msp I RFLP falls within the pro α_2 human procollagen gene, disorders of connective tissue, where abnormalities in collagen gene structure or function are implicated, may well segregate with DNA sequence variation present outside of the connective tissue genes themselves. A recent example of this situation is a large family with osteogenesis imperfecta in which the condition segregates with an RFLP near the growth-hormone gene.[34] The genes for both pro α_1 (I) procollagen and growth hormone are located on chromosome 17, and it could therefore be suggested that there is genetic linkage between this polymorphism and the disorder in question.

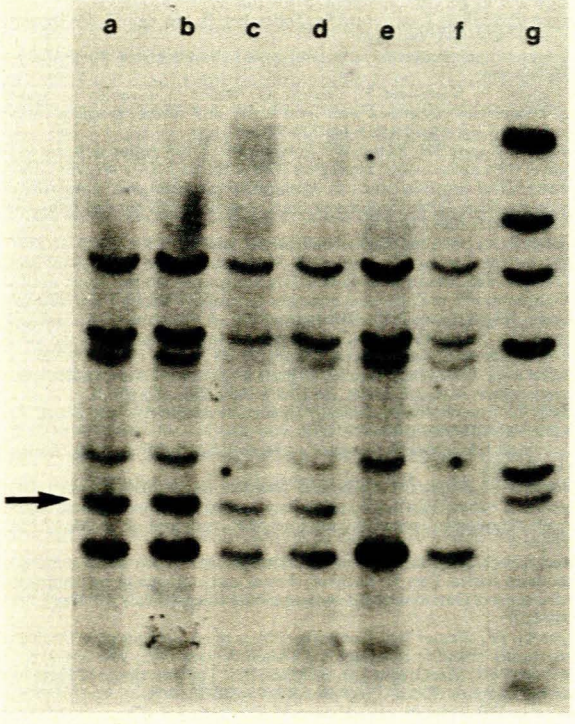

Fig. 4. DNA from apparently normal Afrikaner individuals digested with Msp I and probed for the pro α_2 (I) collagen gene. The probe used was Hf-32, a 2,2 kb pro α_2 collagen cDNA clone kindly provided by Darwin Prockop.[51] (a - d show the variant 2,0 kb Msp I fragment indicated by an arrow; e - f = normal pattern; g = molecular weight marker.)

We have recently initiated a related approach to the study of DNA sequence variance in a number of South African skeletal dysplasias. Sclerosteosis is a unique autosomal recessive disorder in which progressive skeletal overgrowth leads to gigantism, distortion of the head and face, compression of cranial nerves and elevation of intracranial pressure.[35] Affected persons are severely incapacitated and sudden death in adulthood is frequent. About 50 patients have been identified in South Africa; all are members of the Afrikaner community and the minimum frequency in this group is about 1 in 60 000. Approximately 1 in 120 Afrikaners carries the defective gene and there are more than 20 000 asymptomatic heterozygotes in this population.

In view of the lack of effective therapy and the serious nature of the condition, antenatal diagnosis of 'at risk' pregnancies with a view to termination if the fetus is shown to be affected would be fully justified. However, the biochemical defect has not been elucidated and at present couples who have declared themselves to be at risk by virtue of having produced an affected child cannot be aided in this way. In this condition our approach will be confirmation of whether abnormalities in connective tissue biosynthesis are involved. If they are, or should an RFLP associated with the collagen gene family or any other unique genomic sequence co-segregate with the disease, an understanding of the molecular defect and prenatal diagnosis may be possible.

Spondylo-epimetaphyseal dysplasias are a heterogeneous group of inherited skeletal disorders.[36] A condition in this category, in which joint laxity is a major component, occurs with significant frequency in the Afrikaner population. It is a potentially lethal disorder and is being investigated in an analogous manner.

There is a paucity of information concerning the molecular events responsible for many of these and other inherited disorders of connective tissue. However, the approach outlined in this discussion, which has been successfully applied to the inherited haemoglobinopathies, should in the near future yield a clearer picture of the detailed biochemical defects in these skeletal disorders.

Unique sequences of unknown coding function

DNA sequence variations in several groups of genes which produce related products (i.e. gene families) are presently under investigation. These include not only the genes coding for globin and collagen, but also those directing, for example, the synthesis of insulin[37] and the major histocompatibility complex.[38] However, study of RFLPs has far wider implications.

Botstein *et al.*[31] have used random, unique sequences of DNA, complementary only to polymorphic regions of the genome. They have proposed that there would be sufficient polymorphism in all of the human chromosomes to facilitate construction of an entire genetic linkage map of DNA sequence variation and biochemical and genetic markers. Bowcock[39] has taken this approach, using isolated random copy 'probes', and has demonstrated population-specific polymorphisms in DNA isolated

from Venda and Bushman communities. Chromosomal assignment of these random markers will facilitate recognition of genetic linkage between these polymorphisms and genetic markers (HLA types, for example). This will be of importance not only in population studies but also in the pre- and postnatal diagnosis of common and severe diseases where little is known concerning the fundamental biochemical defects (cystic fibrosis is such an example). Similarly, an X chromosome-linked 'probe' has recently been shown[40] to partially segregate with Duchenne's muscular dystrophy, which is a well-known X-linked disorder.

Comment

We have attempted to summarize some of the molecular approaches presently being used in South Africa in order to facilitate diagnosis of a wide range of inherited disorders prevalent in the Republic. These possibilities of early detection at the gene level have extensive applications and will be of enormous value in other conditions which are common in this country. These include familial colonic polyposis,[41] hypercholesterolaemia,[42] porphyria variegata[43] and Gaucher's disease.[44] In addition, many other less common inherited disorders occur with high frequency in the various populations of southern Africa,[45,46] and these will also eventually be amenable to the new technology.

A perspective of the rapidity of development in this field is provided by the fact that during the few weeks in which this article was being prepared, close linkage was found between a polymorphic DNA probe and the Huntington's disease gene.[47] If this finding is confirmed it will have a dramatic impact on affected families. More than 100 persons with the condition and 500 potentially affected relatives have been studied in the Department of Human Genetics at the University of Cape Town in recent years,[48,49] and the new technology could soon play a crucial role in their medical and genetic management.

The application of RFLPs is not restricted to diseases with clear patterns of inheritance, but can possibly be extended to early detection of genetic predisposition to a variety of disorders. It is clear that molecular techniques will play an increasingly important role in the continued improvement of health care, not only in the rest of the world but also in southern Africa.

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