Male infertility as the only presenting sign of cystic fibrosis when homozygous for the mild mutation R117H

Since the identification of the cystic fibrosis gene (CFTR),1,2 more than 265 mutations have been described (CF Gene Analysis Consortium, 1992). The most common disease causing mutation, 

\[ \Delta F 508 \text{,} \]

occurs in approximately 70% of CF chromosomes and causes moderate to severe disease,3 with variable prevalence in populations of different ethnic origins. Among the numerous rare mutations, R117H (a G to A transition at nucleotide 482) produces a missense amino acid substitution (arginine to histidine) in the first transmembrane domain of CFTR. It has only been reported in the heterozygous state, usually with 

\[ \Delta F 508 \text{ occurring in the other CFTR gene; the compound heterozygotes are mildly affected.4} \]

We have studied a 30 year old French male with sterility owing to congenital bilateral absence of the vas deferens (CBVD). He is homozygous for the R117H CFTR mutation, which was detected by DGGE screening and characterised by direct sequencing of PCR amplified DNA from exon 4 using the Sequenex ES kit. The subject has no respiratory or pancreatic involvement and has a normal sweat electrolyte value. His parents are not consanguineous and there are no other cases of CBVD or CF in his family.

Based on the primary finding of a higher rate of AF508 heterozygosity in infertile males,5,6 it has recently been suggested that isolated CBVD might represent a primary genotypic form of CF.7,8 Several males presenting with infertility have been found to be heterozygotes for AF508 and other known mutations and on investigation have mild CF with normal or raised sweat electrolytes and subclinical lung disease. However, this is the first report of homozygosity for R117H. It results in a clinical presentation of CBVD cystic fibrosis completely devoid of the classical symptoms of CF.

Among the reported cases of rare alleles of CFTR found in compound heterozygotes, the R117H mutation seems to be highly represented. It should be systematically screened for in all patients with CBVD, as it may represent a common CF mutation causing very mild disease, with infertility as the only clinical presentation.

**LETTORS TO THE EDITOR**

Molecular characterisation of \( \beta \) thalassaemia heterozygotes in Brazil

At present over 100 different molecular defects producing thalassaemia have been identified. However, a limited number of specific mutations predominates in a given population.1 Most carriers of \( \beta \) thalassaemia in Brazil are descendants of Italian immigrants among whom the prevalence of \( \beta \) thalassaemia trait has been estimated as 6-4%.2 As the molecular basis of thalassaemia has not been completely investigated,3 we carried out a study to determine the \( \beta \) globin mutations in patients heterozygous for \( \beta \) thalassaemia in south eastern Brazil.

High molecular weight DNA was prepared from peripheral leucocytes of 70 unrelated thalassaemia heterozygotes from the region of Campinas in south eastern Brazil (Sao Paulo State). The diagnosis was based on red cell indices and quantitation of haemoglobin A and F as previously described.4 Identification of the \( \beta \) thalassaemia mutations was made by hybridising PCR amplified DNA with \( 5^\prime \) labelled synthetic oligonucleotide probes. The primers for amplification, the sequence of the probes, and the dot blot hybridisation procedures were as previously described.4 Probes for four mutations were used: \( 5^\prime \) GGGGAGCGAAAGGCCAGG (T+C), \( \beta \) IVS-1 nt 110 (G→A), \( \beta \) IVS-1 nt 6 (T→C), and \( \beta \) IVS-1 nt 1 (G→A). The presence or absence of \( \beta \) IVS-2 nt 745 (C→G) mutation was determined by digestion of the amplified DNA with RsaI. For this reaction we used a pair of primers which amplified a fragment from \( \beta \) IVS-2 nt 684 to codon 132 of exon 2.

Hybridisation of amplified DNA from the samples with the four oligonucleotide probes allowed the characterisation of 64 patients (97.1%) \( \beta \) thalassaemia chromosomes. The distribution and frequencies of the mutations are listed in the table. The mutation \( \beta \) IVS-2 nt 745 (C→G) was not observed among the patients. From our
results, the four most common alleles account for 97.1% of the disease alleles in Brazil. Two mutations, namely the nonsense mutation at codon 39 (C→T) and the IVS-1-110 C→A, account for 84.3% of the thalassemia alleles. Although approximately 31 thalassemia mutations have been reported in Mediterranean populations, the allele frequencies vary greatly from one country to another. Comparison of our data on the frequencies of β thalassemia alleles with other Mediterranean populations shows a close similarity between the allele distribution in south eastern Brazil and northern Italy, mainly the regions of Milan and Lombardia. This was to be expected based on the origin of Italian immigrants in Brazil. Most of them originated from northern Italy (Veneto, Piemonte, Lombardia, and Emiglia Romagna) and to a lesser extent from southern Italy (Campania and Calabria). Interestingly, the frequencies of different mutations reported among thalassemia heterozygotes from central Portugal, where the IVS-1-nt 6 and IVS-1-nt 1 mutations reach high frequencies, are different from those observed in our study. In conclusion, the results presented here indicate that most of the thalassemia genes in Brazil originated from Italian immigrants and a limited spectrum of mutation is found. The ability to design a simple and accurate approach to prenatal diagnosis of β thalassemia in the country.

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Autozygosity mapping, complex consanguinity, and autosomal recessive disorders

Mapping of autosomal recessive disorders is more problematical than for autosomal dominant or X linked disorders. Many autosomal recessive disorders are individually rare, making it difficult to collect sufficient numbers unless this is done on an international collaborative basis. In addition, in most parts of the world family sizes are limited, with it being uncommon for families to have more than three to four children and therefore making it unusual for there to be more than two affected sibs within a sibship.

Mathematical analysis of the power of nuclear families with autosomal recessive disorders has shown that in order to have a high likelihood of showing linkage in gene mapping studies, inordinate numbers of families, preferably with multiple affected sibs, are required as described by Wong et al.

Use of homozygosity mapping with affected offspring of first cousins has been advocated as far fewer families are needed to have the same likelihood of showing linkage, an approach originally suggested by Smith and more recently by Lander and Botstein. Morton has reminded us that this approach is more correctly called autozygosity mapping.

An estimate of the lod score under complete linkage to determine rapidly the potential usefulness of various consanguineous pedigrees for a single affected offspring can be derived by the use of the formula:

\[
lod = \log \left[ \frac{qF^2 + (1-F)^2}{q^2 + (1-q)^2} \right]
\]

\[
q = \frac{fF}{fF + (1-F)^2}
\]

\[
F = \frac{F}{F + (1-F)^2}
\]

Figure 1. Effect on the lod score under complete linkage (0-0) of the disease allele (q) and marker allele (r) for an affected offspring of first cousins.

Figure 2. Effect of various prior inbreeding coefficient of the population (Fp) at 0-0 on the lod score for a single affected offspring of first cousins for marker allele frequency (r) and disease allele frequency q = 0-02.
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