LETTERS TO THE EDITOR

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 more than 265 mutations have been described (CF Genetic Analysis Consortium, 1992). The most common disease causing mutation, $\Delta F508$, occurs in approximately 70% of CF chromosomes and causes moderate to severe disease,² 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 $\Delta F508$ occurring in the other CFTR gene; the compound heterozygotes are mildly affected.³⁻⁶

We have studied a 30 year old French male with sterility owing to congenital bilateral absence of the vas deferens (CBAVD). 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 Sequenase USB 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 CBAVD or CF in his family.

Based on the primary finding of a higher rate of $\Delta F508$ heterozygosity in infertile males,^{7,8} it has recently been suggested that isolated CBAVD might represent a primary genital form of CF.^{9,10} Several males presenting with infertility have been found to be heterozygotes for $\Delta F508$ 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 CBAVD 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 CBAVD, as it may represent a common CF mutation causing very mild disease, with infertility as the only clinical presentation.

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Limb/pelvis/uterus-hypoplasia/aplasia syndrome

I read with great interest the consecutive papers of Farag *et al*¹ from Kuwait and Camera *et al*² from Italy reporting additional patients similar to those we described in 1985 as a new autosomal recessive syndrome.³ This brings the number of cases with limb/pelvis-hypoplasia/aplasia syndrome (LPHAS) to nine (five female and four male). This total includes one case from Brazil⁴ and the three sibs from Israel.⁵ Among these five sibships, the three sets of parents were first cousins or double first cousins.^{1,5} I was delighted to see these reports because they provided further proof (as I believe) that the private syndrome does not exist. Often many 'new' syndromes are referred to as 'private' particularly if they are first described in the third world. So called 'private' syndromes may in fact be previously unrecognised or unreported and yet be 'relatively common' in certain populations. The absence of known parental consanguinity in two families with LPHAS could imply that the gene frequency in the relevant population may not be very low.

I wish to report further data on one of the original patients who was re-evaluated at the age of 18 (in 1990) because of absent menarche. Her breasts and other secondary sexual characteristics had developed by the age of 15 years. She and her parents were not particularly anxious about her fertility because of her severe handicap. They wanted to be sure that there were no life threatening consequences of the disease. Her FSH, LH, and prolactin levels were normal. Ultrasonography showed apparently normal ovaries and absent uterus. This was confirmed by another ultrasonographer. It was not possible to perform pelvic examinations because of virginity. Laparoscopic examination was declined.

These data indicate normal gonadal development in a female and support the finding of Farag *et al*¹ of uterus hypoplasia/aplasia. Such findings in two out of five reported females suggest that it is not fortuitous and is probably a variable manifestation of LPHAS that should be considered in future cases.

From a nosological perspective, LPHAS is an appropriate descriptive designation. However, in the light of the müllerian hypoplasia/aplasia, the term limb/pelvis/uterus-hypoplasia/aplasia may be a more precise name. Three of the five reports have used the respective authors' names for syndrome

identification.^{1,2,5} To avoid confusion, I suggest the use of the name of the first reporting author followed by a brief description of the major components. Considering the expansion in the number of new reported syndromes, this policy would make for easier cataloguing of genetic disorders.

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Molecular characterisation of β thalassaemia heterozygotes in Brazil

At present over 100 different molecular defects producing thalassaemia have been identified. However, a limited number of specific mutations predominate in a given population.¹ Most carriers of β thalassaemia in Brazil are descendants of Italian immigrants among whom the prevalence of β thalassaemia trait has been estimated as 6-4%.² As the molecular basis of thalassaemia in Brazil has not been completely investigated,³ we carried out a study to determine the β globin mutations in patients heterozygous for β 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 (São Paulo State). The diagnosis was based on red cell indices and quantitation of haemoglobin A₂ and F as previously described.⁴

Identification of the β thalassaemia mutations was made by hybridising PCR amplified DNA with ³²P labelled synthetic oligonucleotide probes. The primers for amplification, the sequence of the probes, and the dot blot hybridisation procedures were as previously described.^{4,5} Probes for four mutations were used: codon 39 (C→T), β IVS-1-nt 110 (G→A), β IVS-1-nt 6 (T→C), and β IVS-1-nt 1 (G→A). The presence or absence of β IVS-2-nt 745 (C→G) mutation was detected on agarose gels after digestion of the amplified DNA with *Rsa*I. For this reaction we used a pair of primers which amplified a fragment from β IVS-2-nt 684 to codon 132 of exon III.¹

Hybridisation of amplified DNA from the samples with the four oligonucleotide probes allowed the characterisation of 68 out of 70 (97.1%) β thalassaemia chromosomes. The distribution and frequencies of the mutations are listed in the table. The mutation β IVS-2-nt 745 (C→G) was tested for but not observed among the patients. From our

The frequencies of four different β thalassaemia alleles in a Brazilian population.

Mutation	Type	No of chromosomes	%
(1) CD 39 (C-T)	β^0	45	64.3
(2) IVS-1 110 (G-A)	β^+	14	20.0
(3) IVS-1 6 (T-C)	β^+	5	7.1
(4) IVS-1 1 (G-A)	β^0	4	5.7
(5) Unknown		2	2.9

results, the four most common alleles account for 97.1% of the disease genes in Brazil. Two mutations, namely the nonsense mutation at codon 39 (C→T) and the β IVS-1-nt 110 C→A, account for 84.3% of the thalassaemia alleles. Although approximately 31 thalassaemia mutations have been reported in (Mediterranean populations,² the allele frequencies vary greatly from one country to another. Comparison of our data on the frequencies of β thalassaemia 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.⁶ This was to be expected based on the origin of Italian immigrants in Brazil.⁷ Most of them originated from northern Italy (Veneto, Piemonte, Lombardia, and Emilia Romagna) and to a lesser extent from southern Italy (Campania and Calabria).⁶ Interestingly, the frequencies of different mutations reported among thalassaemia heterozygotes from central Portugal,⁸ where the β IVS-1-nt 6 and β 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 thalassaemia genes in Brazil originated from Italian immigrants and a limited spectrum of mutation is found. They enable us to design a simple and accurate approach to prenatal diagnosis of β thalassaemia 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.¹ 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.*²

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 Smith³ and more recently by Lander and Botstein.⁴ Morton⁵ 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:

$$\text{Elod} = \log_{10} \left[\frac{qrF + (1-F)q^2r^2}{\{qF + (1-F)q^2\}\{rF + (1-F)r^2\}} \right]$$

Figure 1 Effect on the lod score under complete linkage ($\theta=0$) 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 (F_p) at $\theta=0$ on the lod score for a single affected offspring of first cousins for marker allele frequency (r) and disease allele frequency $q=0.02$.

The effect of variation in the disease allele frequency (q), the marker allele frequency (r) will affect the power of this approach in individual pedigrees (fig 1).

Detailed enquiry into the family history of affected offspring of ostensible first cousin matings from ethnic groups in which consanguineous marriage is common usually shows the consanguinity to be much more complex than at first enquiry. In addition, ostensibly unrelated affected subjects from that population are often found to be related but in different sibships within the same pedigree.^{6,7} Efficient use of linkage information from such complex consanguineous families requires conventional linkage analysis.⁸

When pooling linkage information from different complex consanguineous families, the possibility of genetic heterogeneity must be considered. It is likely, however, that a limited number of genes will be responsible for a particular autosomal recessive disorder in an individual isolated population in which complex consanguinity is common.⁹

In populations in which consanguineous marriage is common, it has often been the usual pattern of marriage for a number of generations. It has been argued that long term inbreeding will reduce the power of this approach.¹⁰ Two factors will affect the usefulness of this approach in this situation. Prior or remote long term inbreeding (F^p) combines with the 'bottleneck' of close inbreeding (F^c) for a particular pedigree as $F^p + (1-F^c)F^c$, which essentially reduces to $F^p + F^c$. The effect of substituting this in the above formula is to increase the apparent power of this approach. Another consequence of long term prior inbreeding is to 'redistribute' the disease and marker alleles with a reduction in the proportion of heterozygotes and increase the proportion of the two homozygotes in the population.¹¹ The effect of this can be