High incidence of ΔF507 mutation of the CFTR gene in the north west of France

Since the discovery of the CFTR gene,1-3 more than 400 different mutations have been found to be responsible for cystic fibrosis, the most common of them by far being ΔF508. Because the spectrum of mutations in the gene varies with ethnicity of populations, its identification for a given geographical area has major implications in prenatal diagnosis and genetic counselling. In screening for CF mutations in a limited area of the north western part of France (Basse Normandie, an area with 1,400,000 inhabitants), we recently found the ΔF507 mutation to account for 12.3% of the non-ΔF508 chromosomes or 4.2% of the CF chromosomes (seven out of 168 unrelated CF chromosomes). This mutation was identified by detection of heteroduplex molecules obtained after PCR on polyacrylamide gel electrophoresis (PAGE) and sequencing of PCR products. We also found the AI507 to be constantly associated, as already reported by others,24 with the D haplotype (XV2c/TaqI allele 2, KM19/PstI allele 2). The other most common mutations observed in our study with a frequency higher than 1% were: G551D (2.4%), G542X (2.4%), 574delΔ (1.2%), 3659delC (1.2%), and N1303K (1.2%). The ΔF507 was Therefore the second most frequent mutation after ΔF508 (66.3% of CF chromosomes) in the population under study.

Recently, the Cystic Fibrosis Genetic Analysis Consortium has published the population variation throughout the world of common cystic fibrosis mutations. According to that study, the observed mean relative frequency of the ΔF507 mutation in the world is 0.2% and France is the country where the highest frequency is found (a mean of 0.6%, significantly different from the rest of the world). The frequency of ΔF507 observed in the population of Basse Normandie is again significantly higher than in rest of the country (p<0.05). This high frequency and the constancy of association with haplotype D suggest the possible existence of a founder effect in the population under study.

length determinations using the Genescan software on an ABI 373A automatic se-
quencher. Southern blotting of the DRPLA gene was performed with one primer labelled with fluoresce dye.

For the statistical comparison of the distributions of CAG copies for the three samples, the p value from the exact procedure for contingency tables was calculated using StatXact's Monte Carlo approach with 1,000,000 replications. Thus the width of the 99% confidence interval for the p value could be calculated to 0.001 indicating that the p value given is at least to the second decimal.

The distribution of repeat sizes in the DRPLA gene in 47 male and female controls (94 alleles) varies between seven and 25 CAG copies with a maximum for [CAG]_29 in 29% of the chromosomes. In two cases (4%) homozygosity has been observed.

In 35 patients with questionable diagnoses of SCA1 without expansions in the axatin gene, seven to 21 CAG copies in the DRPLA gene have been found, including 26 of 70 alleles with [CAG]_7 (35%). No significant difference in the repeat frequency of the DRPLA gene between controls and patients with ataxia could be ascertained. For seven patients only one allele could be amplified, indicating 20% homozygosity. This restriction is due to the small number of cases. Insufficient amplification of an expanded repeat appears improbable since DNA analyses of two affected persons, kindly provided by T Warner and A Harding, London, have been successful.

Investigating the DNA of 73 patients suspected of having HD but negative for the mutation in the FT15 gene, alleles ranging from [CAG]_7 to [CAG]_30 in the DRPLA gene were identified. Two homozygous DNA samples (3%) were present. Surprisingly, in this collection the second most frequent allele was [CAG]_21, the most frequent being [CAG]_23 (30% of chromosomes). Twenty-six of the 146 chromosomes investigated (18%) contain 10 CAG copies representing about 36% of the patients with symptoms of HD. Controls and patients shared a common ethic background and, therefore, differences between populations can not account for this result. Furthermore, the allele distribution in our controls correlates to data of the white population. Similarly, the allele (CAG)_n is overrepresented in the Japanese population, too.

Allele distributions in the DRPLA gene in the three groups are summarised in the figure. Neither in our patients with chorea nor in patients with symptoms of ataxia could an expansion of the CAG repeat on chromosome 12, to explain the affected status, be found. Our data confirm the low frequency of the DRPLA mutation in the European population. Interestingly, the incidence of allele [CAG]_7 is significantly increased in patients with a questionable diagnosis of HD. The exact p value for the comparison of the distributions of CAG copies is calculated as p = 0.003 which indicates a significant discrepancy between the samples. This result may be because of linkage disequilibrium between the disease and a mutation on chromosome 12 or, possibly, a further gene causing a progressive neurodegenerative disorder is located in the DRPLA region. This hypothesis is supported by polymorphic markers necessary to define a high risk haplotype for this genetically uncharacterised disease. Investigations of pedigrees with affected persons with the allele [CAG]_7 may detect the presence of a gene responsible on chromosome 12.

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Detection of Y mosaicism in patients with Turner's syndrome

The very high intrauterine loss rate in Turner's syndrome and the observation that there is a higher ratio of mosaic karyotypes to monosity X in liveborn compared with aborted fetuses has led to the speculation that all liveborn patients are mosaic for a critical cell line.1

Experimental evidence has shown that 70 to 80% of patients with Turner's syndrome retain the maternal X chromosome.2 Therefore there is the theoretical possibility of Y mosaicism occurring in 35 to 40% of Turner's syndrome patients.3 This has not been shown in cytogenetic studies where only 5% of cases have Y mosaicism and another 3% have unidentified markers. However, low level mosaicism may be missed by conventional cytogenetic techniques and over 100 cells would need to be examined to exclude 5% mosaicism with 99% confidence.4 This fact is also a limitation in the use of FISH which has an established role in the identification of fragments.5

Identification of Y chromosomal mos-
aicism is of clinical relevance because of the high risk of tumour formation in the dysgenetic gonads. In patients with entire or structurally abnormal Y chromosomes present, which may be as high as 30%.6 Page19 has hypothesised the presence of a "gonado-
brastoma" locus which is probably located in interval 48b–5.

Several studies on relatively small numbers of patients have investigated Y mosaicism in Turner's syndrome using Southern blotting with Y specific probes,7,8,9,10 and even Southern blotting of amplified DNA11 and have suggested that occult Y mosaicism occurs in 0 to 33% of patients. In view of this lack of consensus, we have used PCR with a panel of Y primers from the whole length of the Y chromosome amplified from DNA in a large unselected cohort of patients with Turner's syndrome to determine the frequency of occult Y mosaicism.

As a part of a total ascertainment study, 100 patients with Turner's syndrome were recruited from the four growth clinics and from adult Turner's syndrome clinics in Scotland. The patients had the following karyotypes: 45,XX, Turn(X) in 10, 45,XX, X(X) in seven, 45,XX,XY in three, 45,XX,X(y)(p) in one, 45,XX,XY in one, and other karyotypes in eight.

DNA was extracted from peripheral lymphocytes of all patients by the same normal female (CEC, karyotype 46XX) and PCR was carried out in nine sets of primers from the Y chromosome: PABY from the pseudoautosomal region which amplifies X and Y specific products of different sizes,12 SRY,13,14 amelogenin15 which amplifies X and Y specific products with a size difference, Y chromosome16; Y; Y1.1; Y1.11,17 Y1.1.1. An additional primer set, y159,17 was used in patients who were positive with Y1.1. S146 and y160 were amplified in a multiplex reaction. All PCR products were resolved on 1–2% agarose gels extracted from fresh blood samples. In five other cases different DNA samples were used.

Southern blotting of amplified DNA was also carried out on 84 of the patients who had been shown to be negative on direct visualisation of PCR products. Three primer sets were used initially, SRY1, Y centromere, and XYS, and a fourth SYIL for the SRY gene was used on positive cases. Great care was taken to prevent contamination either by PCR products or genomic DNA when setting up the PCR reaction or running gels. All the experiments were carried out by the same female (46XX karyotype). Separate pipettes were used for setting up the PCR and running gels, autoclaved solutions were substituted for water which was used throughout, and all solutions were subjected to UV light for 10 minutes before use. The PCR controls were run in a separate laboratory one floor below that used for setting up the PCR. All duplicate controls were repeated in two DNA samples extracted from fresh blood samples. In five other cases different DNA samples were used.

Forty-six female controls were also screened using the SRY reverse and probe and 40% of these the DNA was extracted by female laboratory staff and in six by a male scientist. Probes used for the Southern blotting were gene cleaned PCR products amplified from normal males. These were labelled with 32P dCTP by the random labelled method.

Parental origin of the normal X chro-
mosome was determined either by use of Southern blotting with Y specific probes,7,8,9,10,11,12,13 by PCR with primers from a repeat sequence in the androgen receptor gene, as previously described.12 Concurrent analysis of X inactivation patterns in patient 9 assisted in the identification of the normal X chromosome.

Nine patients were positive for one or more Y regions on direct visualisation of ethidium bromide stained gels with the results shown in table 1 along with the position of the primers used. Three of these were already known to have a Y chromosome cytogenetically and were positive for all ranges tested (patients 3, 4, and 5, table 1). One of these subjects (patient 3) had a prepubertal
Allele distribution of a highly polymorphic repeat on chromosome 12 in patients with symptoms of chorea and ataxia.

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