LETTERS TO THE EDITOR

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

Since the discovery of the CFTR gene,\(^1\,^2\) 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 polycystamide gel electrophoresis (PAGE)\(^3\) and sequencing of PCR products. We also found the ΔS507 to be constantly associated, as already reported by others,\(^4\,^5\) with the D haplotype (XV26/TaqI allele 2, KM19/PstI allele 2). The other common mutations observed in our study with a frequency higher than 1% were: G551D (2.4%), G542X (2.4%), 574delIA (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.\(^6\) 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.

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Allele distribution of a highly polymorphic repeat on chromosome 12 in patients with symptoms of chorea and ataxia

Dentatorubral and pallidolysiasis atrophy (DRPLA) is a progressive neurodegenerative disorder characterised by ataxia, chorea, epilepsy, and dementia. Recently, an unstable CAG expansion CAG repeat in a gene located on chromosome 12 has been identified as causing DRPLA. The repeat size varied from 7 to 23 in normal subjects and one allele is expanded to 49 to 75 in patients.\(^7\) This rare autosomal dominant disorder, almost unknown in Europe, has been described in Japanese pedigrees with prevalence of one per million people. DRPLA has clinical and neurogenetic similarities to Huntington's disease (HD) and spinocerebellar ataxia type 1 (SCA1). As in HD and SCA1, variable age of onset, anticipation of symptoms, and cases with juvenile onset following paternal transmission have been observed in DRPLA families.

HD is an autosomal dominant condition resulting in chorea, cognitive loss, and psychiatric manifestations. A tract of CAG repeats has been identified close to the 5' end of the HD transcript. The nucleotide stretch in the gene IT15 on chromosome 4 varies from 11 to 34 copies on normal chromosomes, whereas patients with HD have repeat units larger than 38 within the mutated gene.\(^8\)

SCA1, another of the numerous autosomal dominantly inherited neurodegenerative disorders, is characterised by ataxia, dysarthria, and variable degree of motor weakness. The neurogenetic findings include selective loss of neurons in the cerebellum, spinal cord, and brain stem. The underlying mutation is an expansion of a CAG trinucleotide repeat in the ataxin gene on chromosome 6. Normal repeat numbers span from 19 to 36 CAG copies, while in SCA1 patients the aberrant repeat is elongated to more than 40 tri-nucleotides.\(^9\)

Patients with DRPLA may have a variety of symptoms overlapping with HD and SCA1 but lacking the appropriate mutations on chromosomes 4 and 6 for length of a CAG repeat on chromosome 12. Normal allele distribution has been confirmed by analyses of 94 control chromosomes.

Blood samples from affected and control persons with a common geographical origin (Germany) were obtained by numerous neurologists asking for direct mutation analyses to confirm or exclude the potential diagnosis. In this study, the neurological criteria, containing the characteristic symptoms of progressive neurodegeneration, chorea, or ataxia or both, were of limited stringency to avoid preselection of the test collective. DNA from blood lymphocytes was examined for the CAG repeat expansion in the DRPLA gene using the PCR assay as previously described.\(^10\)

The number of CAG repeats in the SCA1 and HD genes were determined as precise

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![Allele distribution in the DRPLA gene in the three groups.](https://example.com/allele_distribution.png)
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 monosomy 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, however, in cytogenetic studies where only 5% of cases have Y mosaicism4 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.5 This fact is also a limitation in the use of FISH which has an established role in the identification of fragments.6

Identification of Y chromosomal mosaicism is of clinical relevance because of the high risk of tumour formation in the dysmorphic Turner's syndrome patients with entire or structurally abnormal Y chromosomes present, which may be as high as 30%.7 Page8 has hypothesised the presence of a "gonado-blastoma" 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,9 PCR with one or two Y primer sets10,11 and even Southern blotting of amplified DNA12 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 and also as an expansion of the DRPLA gene in a large unselected cohort of patients with Turner's syndrome to determine the frequency of occult Y mosaicism.

As part of a total ascertaining 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,X in 48, 45,X/46,XX in 45, 45,Xt(X) in 10, 45,46XX in 77, 45,46,XY in three, 45,46,Xi(Y)p in one, 45, 46,XX, +M in nine, and other karyotypes in eight.

DNA was extracted from peripheral lymphocytes of all patients by the same normal female (CEC, karyotype 46,XX) and PCR was carried out with nine sets of primers from the Y chromosome: PABY from the pseudautosomal region which amplifies X and Y specific products of different sizes,13 SRY,14 amelogenin15 which amplifies X and Y specific products with a size difference, Y asex16,17 and Y1.1.8,1819 An additional primer set, sY15,20 was used in patients who were positive with Y1.1. sY14 and sY10 were amplified in a multiplex reaction. All PCR reactions were performed in 100 µL containing 400 ng of DNA and 5% of native female DNA in 20% of the DNA, a mixture of bromide agaroose gels with both normal male and normal female controls on every gel.

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 Y sex, and a fourth, Y510,21 was added when 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 (46,XX karyotype). Separate pipettes were used for setting up the PCR and running gels, autoclaved solutions were used, and all solutions were subjected to UV light for 10 minutes before use. The PCR products were run in a separate laboratory one floor below that used for setting up the PCR. All duplicate samples were run in two different DNA samples extracted from fresh blood samples. In five other cases different DNA samples were used.

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 chromosome was determined either by use of Southern blotting with Southern blotting with Y-specific probes,10 PCR with primers from a repeat sequence in the androgen receptor gene, as previously described.16 Concurrent analysis of X inactivation patterns in patient 9 assisted in the identification of the normal X chromosome in eight.

Nine patients were positive for one or more Y regions on direct visualisation of ethidium bromide stained gels with the results shown in figure 1 along with the results obtained with the primers used. Three of these were already known to have a Y chromosome cytogenetically and were positive for all regions tested (patients 3, 4, and 5, table 1). One of these subjects (patient 3) had a prolactin...