

Absence of cystic fibrosis mutations in a large Asian population sample and occurrence of a homozygous S549N mutation in an inbred Pakistani family

Ann Curtis, R J Richardson, J Boohene, A Jackson, R Nelson, S S Bhattacharya

Abstract

The occurrence of cystic fibrosis is very rare in the Asian population. Carriers of the mutations most commonly found in Caucasians were not detected in a large Asian population sample of almost 900 chromosomes. However, an affected Pakistani child born to consanguineous parents was further investigated and shown to be homozygous for the mutation S549N (G→A). Molecular and clinical data are presented which may improve our understanding of the phenotypic effects of the S549N mutation in the CFTR gene.

(J Med Genet 1993;30:164-6)

Cystic fibrosis (CF) is reported to be one of the most common severe recessive disorders in the Caucasian population.¹ The carrier frequency among Caucasians averages about 1/25.

A mutation has been identified in the CF gene whose frequency increases in a south-east to north-west gradient from 30% in Turkey to 87% in Denmark.² The mutation is a deletion of CTT in exon 10 which results in the removal of a phenylalanine residue at amino acid position 508 (Δ F508) in the encoded protein, the CF transmembrane conductance regulator (CFTR).³

The number of further mutations which have been identified in the CF gene is now about 200. However, there are a cluster of mutations in exon 11 which account for a further 4 to 5% of the defective Caucasian CF chromosomes.^{4,5}

The disease incidence of CF in other ethnic groups is very low, for example 1/10 000 in Pakistanis in England, 1/17 000 in blacks in Washington, and 1/90 000 in orientals in Hawaii,¹ and there are fewer than 10 confirmed cases of CF in African blacks.⁶ Devoto *et al*⁷ found no Δ F508 carriers among an unrelated sample of 98 Chinese, 50 Dravidian Indians, 68 Malaysians, and 85 black Africans from Zimbabwe. However, these authors did not exclude possible occurrence of other CF mutations in these populations in view of the increasing molecular heterogeneity of CF and the gradient distribution of Δ F508 in Europe.

In this study, we have analysed 443 unaffected Asians (400 unrelated Indians, 43 unrelated orientals) and 222 unrelated Caucasians from north-east England for the presence of exon 10 Δ F508 mutations and 200 members

of the same Asian sample for the exon 11 mutations G551D, R553X, and S549.⁴ In addition, we have identified a Pakistani child with CF with consanguineous parents who is homozygous for the exon 11 mutation R549N.

Owing to the rare occurrence of most of the non- Δ F508 mutations, correlations between the variable CF phenotype and genotype have been difficult to catalogue. Some non- Δ F508 compound heterozygotes have been reported,^{8,9} but their small numbers make meaningful analysis difficult. The functions of the several domains of the CFTR protein may be better understood from a study of the phenotypes of subjects who are shown to be homozygous for rare mutations when it may be simpler to assess the pulmonary and gastric contributions of the various mutations. Such homozygotes are likely to be extremely rare, but some useful cases have been identified, such as a G85E homozygote¹⁰ and an R553X¹¹ homozygote, in addition to the S549N case described here. Significantly all three of these patients were born to consanguineous parents.

Materials and methods

SAMPLES

DNA was extracted by standard methods from 10 ml frozen blood samples from the parents and three children of a Pakistani family, of which one child was affected with CF, and from 400 unrelated Indians, collected at a blood transfusion centre in Bombay, from 43 unrelated orientals, and from 222 unrelated Caucasians living in the north-east of England.

CASE REPORT

The proband was born by caesarean section at term, birth weight 3118 g. She presented with chronic cough and failure to thrive at the age of 4 months. Stools were offensive and contained numerous fat globules on microscopy. Serum immune reactive trypsin was increased, and sweat tests were diagnostic of cystic fibrosis (sweat sodium 85 μ mol/l, sweat chloride 85 and 95 μ mol/l).

She continued to have a poor appetite and poor growth and weight gain, despite efforts to supplement her calorie intake as an outpatient and inpatient. Current height and weight are below the 3rd centile. Chronic progressive chest infections have continued despite many courses of antibiotics. Her lungs have been infected by mucoid strains of *Pseudomonas*

Division of Human Genetics, University of Newcastle upon Tyne, 19/20 Claremont Place, Newcastle upon Tyne NE2 4AA.

A Curtis
R J Richardson
J Boohene
A Jackson
S S Bhattacharya

Northern Region Genetics Service, Royal Victoria Infirmary, Newcastle upon Tyne.

A Curtis
R J Richardson
A Jackson
S S Bhattacharya

Department of Paediatrics, Royal Victoria Infirmary, Newcastle upon Tyne.
R Nelson

Correspondence to Dr Curtis.

Received 4 June 1992.
Revised version accepted 30 July 1992.

aeruginosa since the age of 5 years. Pulmonary function test (FVC, FEV1, and PEFR) are reduced to less than 50% expected values for her height and age. She has had several minor haemoptyses and chest x rays show progressive changes of hyperinflation and bronchiectasis of both bases.

DETECTION OF MUTATIONS

Exon 10 and exon 11 of the CF gene were amplified through 30 cycles of the polymerase chain reaction (91°C for 30 seconds, 52° for 30 seconds, 72°C for one minute) in reaction mixes containing 0.5 µmol/l of each exonic primer.

Exon 10F: GTTTTCCTGGATTATGCCTGGCAC.

Exon 10R: GTTGGCATGCTTTGATGACGCTTC.

Exon 11F: AGACATCTCCAAGTTTGACA.

Exon 11R: CTTGCTAAAGAAATTCTTGC.

Heterozygotes for the Δ F508 mutation were identified after electrophoresis of the exon 10 PCR products on 10% neutral polyacrylamide gels when heteroduplex bands were clearly visible after ethidium bromide staining.

The S549 mutation was detected by the removal of a *Dde*I site and G551D and R553X mutations were identified by the creation of an *Mbo*I site and the removal of a *Hinc*II site respectively in the exon 11 PCR product.

DNA SEQUENCING

PCR products for sequencing were prepared in reaction mixes containing one 5' biotinylated primer and one normal primer. The double stranded product was denatured in 0.15 mol/l NaOH and the biotinylated strand was separated from the non-biotinylated strand using magnetic streptavidin coated beads (Dyna-beads, DYNAL UK Ltd). The biotinylated strand was sequenced from the original non-biotinylated PCR primer by the dideoxy chain formation method using a USB sequenase version 2.0 DNA sequencing kit and ³⁵S dATP as the radioactive label.

Results

Thirteen CF heterozygotes (5.8%) were detected among the 222 unrelated Caucasians from the north-east of England. Eleven of these carried the Δ F508 mutations and two had the G551D mutation. None of the unaffected Asian samples tested (400 unrelated Indians, 43 unrelated orientals) were Δ F508, G551D, R553X, or S549 carriers.

The five members of the Pakistani family tested negative for the Δ F508, G551D and R553X mutations, but the affected child appeared homozygous for the rare exon 11 S549 mutation when the PCR product failed to digest with *Dde*I. This was confirmed when both parents were shown to be S549 heterozygotes as presented in fig 1. The exon 11 PCR products from both of her unaffected brothers, however, digested completely with *Dde*I. Sequencing results of PCR products from the

mother, the affected child, and one unaffected child are shown in fig 2. These show a G to A substitution at cDNA nucleotide position 1778 which creates the S549N (Ser→Asn) mutation in the CFTR protein previously described by Cutting *et al.*⁴

Discussion

Cystic fibrosis has rarely been described in subjects from the Asian population. No carriers of the commonest Caucasian CF mutation (Δ F508) or of the less common G551D, R553X, and S549N mutations were detected in an Asian population of 443 unrelated subjects, even though the frequency of 5.8% in our population was slightly greater than expected. However, the presence of other CF mutations cannot be excluded when the emerging molecular heterogeneity of CF is considered.

Schwartz *et al.*¹² reported six affected Pakistani children, all with consanguineous parents, of whom three were homozygous for Δ F508 and three had unidentified mutations. The affected Pakistani child studied here is homozygous for a rare CF mutation, S549N. Homozygosity is not unexpected considering the close relationship of her parents. The detection of this mutation allowed carrier testing for her two unaffected brothers, neither of whom were shown to carry the S549N mutation (figs 1 and 2).

Some 80 to 85% of CF patients have the severe pancreatic insufficient form of the disease. If these patients are homozygous for 'severe' alleles which are recessive to the putative 'milder' alleles associated with pancreatic sufficiency, then the frequency of severe alleles must be 90 to 92%. However, compound

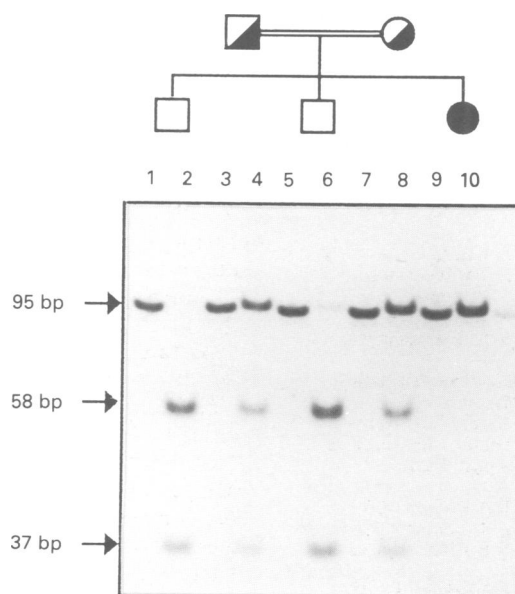


Figure 1 Lanes 1, 3, 5, 7, and 9 show the 95 bp undigested CFTR exon 11 PCR product. Lanes 2, 4, 6, 8, and 10 show the PCR product after *Dde*I digestion which produces fragments of 58 bp and 37 bp. The lanes correspond to the subjects shown in the pedigree directly above. Both parents are heterozygous for the presence of the *Dde*I restriction site, both unaffected sons are homozygous for the presence of the site, and the affected daughter is homozygous for absence of the site.

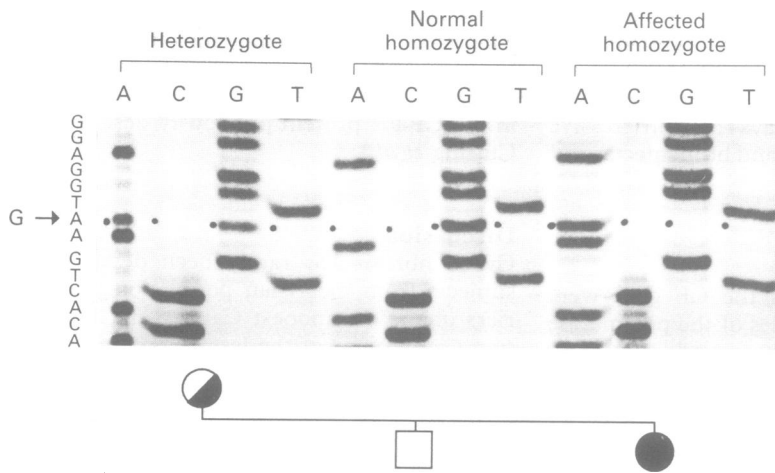


Figure 2 Sequence of CFTR nucleotides 1771–1784 showing the substitution of a G by an A residue (indicated by the dots) at position 1778 in the carrier mother. This removes a DdeI restriction site and mutates the amino acid position 549. This substitution has been inherited by the affected girl in the homozygous state, but not by her normal brother.

heterozygotes may present a complex clinical picture and, therefore, homozygotes may be more informative for determining the biological effects of different mutations. It is also possible that the absence of a CFTR product altogether, as in the homozygous R553X patient,¹¹ may be less severe than a substitution or deletion mutation, since complete absence of CFTR may be more compatible with cell function than an altered membrane protein. Exon 11 mutations affect the first nucleotide binding fold of CFTR and thus, in general, are expected to have a severe phenotypic effect. The mutations S549I (Ser→Ile) and S549R (Ser→Arg) have been reported as severe.¹³

The homozygous S549N (Ser→Asn) child described here shows a severe clinical course with malnutrition, growth failure, and advanced pulmonary disease, despite diagnosis during early infancy, aggressive treatment of the pancreatic steatorrhea and chest infection, and calorie supplements.

- 1 Boat TF, Welsh MJ, Beaudet AL. Cystic fibrosis. In: Scriver CL, Beaudet AL, Sly S, Valle D, eds. *Metabolic basis of inherited disease*. New York: McGraw-Hill, 1989.
- 2 European working group on CF genetics. Gradient of distribution in Europe of the major CF mutations and of its associated haplotype. *Hum Genet* 1990;85:436–41.
- 3 Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterisation of the complementary DNA. *Science* 1989;245:1066–73.
- 4 Cutting GR, Kasch LM, Rosenstein BJ, et al. A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis and conductance regulator protein. *Nature* 1990;346:366–9.
- 5 Kerem BS, Zieleski J, Markiewicz D, et al. Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. *Proc Natl Acad Sci USA* 1990;87:8447–51.
- 6 Cutting GR, Antonarakis SE, Buetow KH, et al. Analysis of DNA polymorphism haplotypes linked to the cystic fibrosis locus in North American Black and Caucasian families supports the existence of multiple mutations of the cystic fibrosis gene. *Am J Hum Genet* 1989;44:307–18.
- 7 Devoto M, Castagnola S, Saha N, et al. Screening for the major cystic fibrosis mutations in non-caucasian populations. *Am J Hum Genet* 1991;49:903–4.
- 8 Curtis A, Nelson R, Porteous M, Burn J, Bhattacharya SS. Association of less common cystic fibrosis mutations with a mild phenotype. *J Med Genet* 1991;28:34–7.
- 9 Schloesser M, Arieth S, Lenz U, Botele RM, Reiss J. A cystic fibrosis patient with the nonsense mutation G542X and the splice site mutation 1717–1. *J Med Genet* 1991;28:878–80.
- 10 Chalkley G, Harris A. A cystic fibrosis patient who is homozygous for the G8SE mutation has very mild disease. *J Med Genet* 1991;28:875–7.
- 11 Bal J, Stuhmann M, Schloesser M, Schmidtke J, Reiss J. A cystic fibrosis patient homozygous for the nonsense mutation R553X. *J Med Genet* 1991;28:715–7.
- 12 Schwarz MJ, Super M, Wallis C, et al. Delta F508 testing of the DNA bank of the Royal Manchester Children's Hospital. *Hum Genet* 1990;85:426–30.
- 13 Karen BS, Zielenski J, Markiewicz D, et al. Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. *Proc Natl Acad Sci USA* 1990;87:8447–51.