Detection of F508del mutation in cystic fibrosis transmembrane conductance regulator gene mutation among Malays

Zilfalil B A, Sarina S, Liza-Sharmini A T, Oldfield N J, Stenhouse S A

ABSTRACT

Introduction: Cystic fibrosis (CF) is one of the common genetic disorders in the western world. It has been reported to be very rare in Asian populations. According to the Cystic Fibrosis Genetic Analysis Consortium, more than 1,000 mutations of the CF gene have been identified. The CF gene, named the cystic fibrosis transmembrane conductance regulator (CFTR), is located on chromosome 7 and composed of 27 exons. This study aims to detect possible CFTR gene mutations in Malays.

Methods: We analysed 50 blood samples from healthy Malays with no symptoms of CF. DNA was extracted from blood using commercially available extraction kits (Eppendorf, Germany). Identification of CFTR gene mutation was performed using the CF OLA (Oligonucleotide Ligation Assay) kit (Applied Biosystems, USA). The PCR-ligation products were electrophoresed on eight percent sequagel using an ABI PRISM® 377 genetic analyser (Applied Biosystems, USA). Electrophoresis data was analysed using the Genotyper® software and a report of the CF genotype for all loci tested was created using the CF Genotyper® Template software. Out of 50, one sample (two percent) was detected to have the F508del mutation (3bp deletion at exon 10), which is one of the most common CFTR gene mutations in Caucasians.

Results: The F508del mutation allele was detected in one subject. This indicates that she was a CF carrier.

Conclusion: We report the finding of a carrier of the F508del mutation of the CFTR gene in the Malay population. Our finding revealed that CF could also affect the Malay population. Larger studies are necessary to determine the exact gene frequency of this population.

Department of Pediatrics
School of Medical Sciences and Human Genome Centre
Universiti Sains Malaysia
Kubang Kerian
16150 Kota Bharu
Malaysia

Zilfalil B A, MBBS, MSc, MMed
Director
Human Genome Centre

Sarina S, BBioMedSc, MSc
Postgraduate Student

Liza-Sharmini A T, MBBS, MMed
Ophthalmologist and Medical Lecturer

Duncan Guthrie Institute of Medical Genetics
Royal Hospital for Sick Children
Glasgow G3 8SJ
The United Kingdom

Oldfield N J, BSc, PhD
Scientist

Stenhouse S A, BA, DipRCPath.
Head, Molecular Genetics Division, Consultant Clinical Scientist and Honorary Senior Lecturer

Correspondence to:
Dr Zilfalil Bin Alwi
Tel: (60) 9 766 3000 ext 4151
Fax: (60) 9 765 8914
Email: zilfalil@kb.usm.my

Keywords: cystic fibrosis, F508del mutation, gene mutation, transmembrane conductance regulator,

Singapore Med J 2006; 47(2):129-133

INTRODUCTION

Cystic fibrosis (CF) is the most common life-threatening autosomal genetic disorder among Caucasians but is less common among the Asian population. CF is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR). The CFTR gene is located on chromosome 7 and composed of 27 exons. According to the cystic fibrosis genetic analysis consortium[1], more than 1,000 mutations of the CF gene have been identified. Deletion of a phenylalanine at amino acid position 508 (F508del) in the first nucleotide binding domain (NBD1) is the most prevalent CF causing mutation in Caucasians and results in defective protein processing and reduced CFTR function leading to chloride impermeability in CF epithelia and heterologous systems. The carrier frequency of CF mutations among Caucasians was reported to be 1:25[2].

To our knowledge, there has been no reported CFTR mutations among the Malaysian population, especially in Malays. Though there are reported cases of CF among Malaysians[3], the diagnosis was made based on clinical presentation and pancreatic enzyme investigation. To our knowledge there has been no reported study conducted on the prevalence of these mutations among the Malay population. We carried out this preliminary study to detect 31 possible CFTR gene mutations, including the F508del mutation and 23 other most common CF mutations detected worldwide, in this population.

METHODS

50 samples of blood were collected from healthy Malay adults with no symptoms suggestive of CF. The healthy volunteers were recruited during the

blood donation programme in the Kelantan state. The potential volunteers were asked a series of questions to rule out any symptoms to suggest CF and to ensure they were healthy. The ethnic origin was traced for at least three generations to ensure they were of Malay descent. Those with a history of mixed marriage and where racial origin was in doubt were excluded. Informed written consent was obtained from the volunteers. This study received approval from the Research and Ethics Committee of the School of Medical Sciences, Universiti Sains Malaysia, in October 2003.

Genomic DNA was extracted from blood using commercially available extraction kits (Eppendorf, Germany). DNA was brought to the Duncan Guthrie Institute of Medical Genetics in Glasgow, UK for the analysis of CF gene mutations. Duncan Guthrie Institute is a well established, CPA (UK) fully accredited diagnostic lab which have been providing diagnostic services for CF gene mutation detection in the west of Scotland. CF mutation identification system, oligonucleotide ligation assay (OLA) kit (Applied Biosystems, Foster City, CA, USA, was used to detect the CF gene mutation. CF assay technology employs a rapid, single-tube, PCR/OLA multiplex, followed by four-colour electrophoresis to identify CFTR normal and mutant alleles. GeneScan® Analysis and Genotyper® are general purpose software programs that analyse fluorescent electrophoresis data.

The CF mutation detection assay starts with a simple boil of DNA samples. DNA samples were added to the microcentrifuge tubes containing the mixtures of buffer for DNA and the sterile de-ionised water. These mixtures were heated at 97ºC for 40 minutes before PCR amplification was carried out. Using 0.2 ml microcentrifuge tubes, 2.5 μl of these mixtures and 2.5 μl of OLA PCR reagent (supplied with the kit) were added. After vortexing these tubes, PCR amplification was performed in an automatic thermal cycler GENEAMP 9700 E (Perkin Elmer, USA). The programme used was an initial denaturation at 94ºC for 12 minutes, followed by five cycles of 98ºC for 15 seconds, 58ºC for 30 seconds and 72ºC for one minute 30 seconds. The following 22 cycles of denaturation (94ºC for 15 seconds, annealing (58ºC for two minutes) and extension (72ºC for five minutes) were performed. The final denaturation step was performed for 30 minutes at 99ºC. Both the ligase enzyme and the OLA reagent supplied with the kit were then added. 5 μl of this mixture was added directly to each of the PCR products from the previous step. These mixtures were vortexed and proceeded with the second PCR amplification of 32 cycles. The conditions for this round consisted of denaturation at 90.5ºC for five seconds and annealing at 46.5ºC for 45 seconds.

Table I. Type of mutations detected by CF OLA version 2 kit 31 mutations.

MUTATIONS
R553X
G551D
1507 del
F508 del
1717-1 G>A
G542X
R560T
R347P
W1282X
R334W
1078 Del T
3849 + 10KB C>T
R1162X
N1303K
3659 Del C
A455E
R117H
2183 AA>G
2789+5 G>A
1898 +1 G>A
621+1 G>T
711+1 G>T
G85E
S549N
S549R
V520F
Q493X
R347H
3849 +4 A>G
3905 INS T
Y122X

Detection of the CFTR gene mutations was performed by Sequagel analysis using ABI PRISM® 377 Genetic Analyzer (Applied Biosystems, USA). Complete loading buffer for each sample was prepared containing formamide, loading dye (Blue Dextran) and OLA-TAMRA size standard. For 8% Sequagel analysis of the CFTR gene, 2 μl of PCR-ligation product was mixed with 5 μl of loading buffer and heat-denatured at 95ºC for two minutes. The denatured products were then immediately cooled on ice and 2 μl of this mixture were loaded onto the 8% Sequagel. The OLA programme was set up utilising the GeneScan®

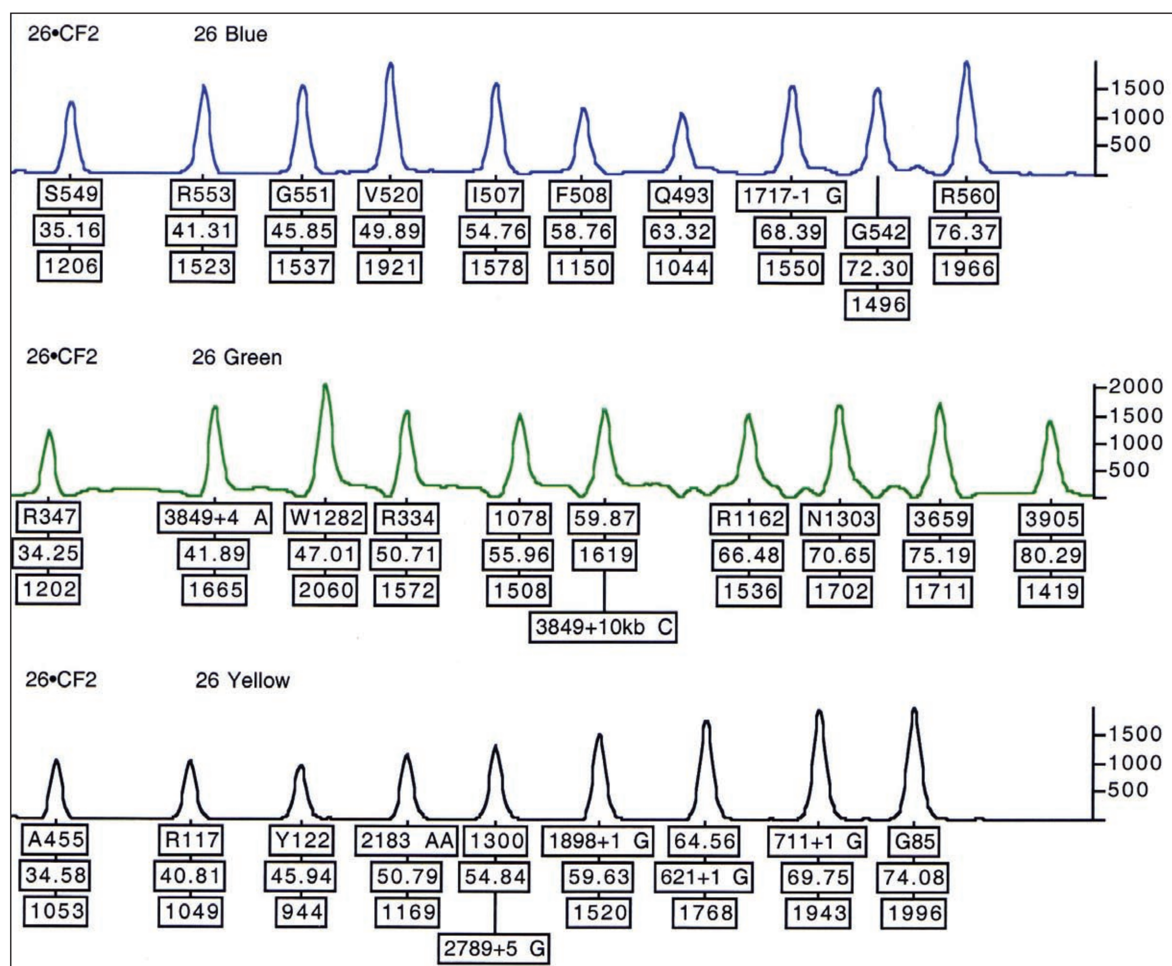

Fig. 1 Electropherograms show the normal profile using the Genotyper(r) software analysis. There are three dye profiles for each sample: blue, green and yellow (black) traces. The software has labelled ten blue, ten green and nine yellow traces with the name eg. S549, the size (35.16 base pairs) and the peak height (1206).

software before running the gel electrophoresis in 1X TBE using ABI PRISM® 377 Genetic Analyzer (Applied Biosystems, USA) for 45 minutes. Electrophoresis data was analysed using the Genotyper® software and a report of the CF genotype for all loci tested was created using the CF Genotyper® Template software.

RESULTS

The ABI OLA has been applied in detecting the mutation status of the CFTR gene among 50 healthy Malays with no symptoms of CF. All the DNA samples were successfully screened for CFTR gene mutations using ABI PRISM® 377 Genetic Analyzer. Out of 50, only one sample (2%) was detected to have the F508del mutation (3bp deletion at exon 10), which is one of the most common CFTR gene mutations in Caucasians. These results showed that this subject carried one mutant allele and was therefore a carrier of cystic fibrosis. The CF Mutation Identification System using the OLA kit is able to screen for 31 possible mutations, including the 24 most common CF mutations worldwide, as identified by the CF Consortium (Table I). Electrophoresis data was analysed using GeneScan® and CF Genotyper® template software. The electropherogram results for CFTR gene mutation screening interpreted by the software are shown in Figs. 1 & 2.

DISCUSSION

In 1989, the gene responsible for CF was identified[4]. Since then, more than 1,000 sequential alterations of the CFTR gene have been tabulated by the CF Genetic Analysis Consortium. F508del mutation by far is the commonest (approximately 70%) mutation identified, while the other mutations are less than 1%. CF is known to be a Caucasian disease, rarely found among Asians. Several studies[5-7] that found CFTR gene mutations among Asians have shown that the Asian CF patients tend to have more novel mutations of CFTR gene,

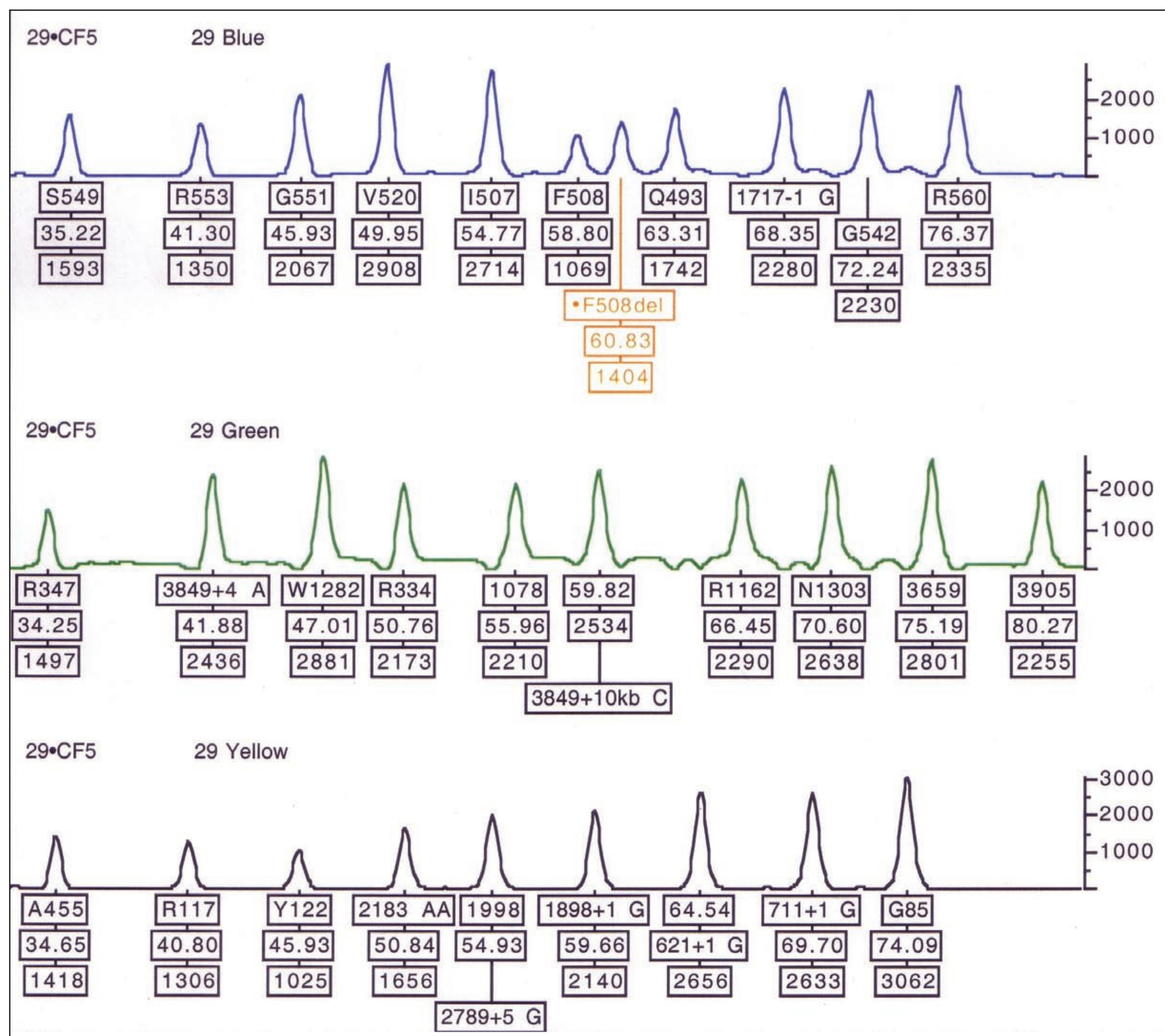

Fig. 2 Electropherograms show the mutation of CFTR gene F508del in heterozygote sample using Genotyper(r) software analysis. Profile of F508del heterozygote - the software labels both normal and mutant peaks. In the blue trace, the normal runs at 58.80 base pairs and the mutation at 60.83 base pairs.

compared to Caucasians, and the F508del mutation seems to be uncommonly found.

Anzai et al[5] found three missense mutations (W216X, G1349S, Q1352H) in seven CFTR alleles and 5T allele was positive in 11 of 38 CFTR alleles among Japanese patients with congenital bilateral absence of the vas deferens (CBAVD). The novel mutations found in Asian populations include a missense mutation A1081P in CFTR gene reported on a Loatian patient with CBAVD[6], two novel mutations, E7X and 989-992insA, in a Taiwanese cystic fibrosis patient[7] and three Asian mutations, K166E, L568X and 3121-2A→G (in homozygosity), reported by Macek et al[8]. Based on the review done by Suwanjutha et al[9], splicing mutations at 1898+1G→T and 1898+5G→T were also found to be more common in East Asian CF patients.

Kabra et al[10] conducted a study on 120 Indian, Pakistani and Afghan children diagnosed as CF and found that the prevalence of F508del mutations in these populations was 19%, much lower than the Caucasian population. He also observed that the patients who originated from Pakistan expressed higher prevalence of F508del (56%) as compared to those from India (12%). However, Curtis et al[11] and Wang et al[12] reported the absence of F508del mutation after screening almost 900 Asians in United Kingdom and 100 reported cases of CF among Japanese patients, respectively.

The OLA version 2 kit has been used over the last five years at the molecular genetics lab of Duncan Guthrie Institute of Medical Genetics to provide diagnostic services and it is run using the appropriate controls. Hundreds of clinical reports have been issued per year and this kit has been

proven to be accurate in detecting the CF mutation gene. Even though the product has been discontinued, the reason was to establish a kit that works on the newer sequencing platforms and is no reflection on the accuracy and efficiency of the old kit. Moreover, as the test was conducted in an accredited diagnostic laboratory using appropriate internal quality control measures and control samples, we are confident with our findings.

We detected one subject with F508del mutation in this preliminary study on 50 healthy Malay volunteers. Compared to previous studies on the detection of the CFTR gene mutation[5-10,11] that was conducted on either clinically-diagnosed CF or CBAVD Asian patients, our study was conducted on normal healthy volunteers. This result suggests that the incidence of CF in our population may not be as rare as previously thought. A larger scale study among the Malay population will provide us with the prevalence rate of F508del mutation in both symptomatic patients and the general population. It is important to create awareness of this fatal autosomal recessive multisystem disorder among doctors and medical personnel in our population as the delay in diagnosis or wrong diagnosis in patients presenting with CF will lead to an increase in the mortality rate, especially among children.

In conclusion, we report the finding of carriers of the F508del mutation of the CFTR gene in our preliminary study on the Malay population. Our findings revealed that the Malay population is also at risk from cystic fibrosis. Larger studies are necessary to determine the exact gene frequency of this population.

REFERENCES

1. Cystic Fibrosis Mutation Database (online). Available at: www.genet.sickkids.on.ca/cftr/. Accessed June 1, 2005.
2. Boat TF, Welsh MJ, Beaudet AL. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic Basis of Inherited Disease. 6th ed. New York: McGraw-Hill, 1989: 2649-80.
3. Norzila MZ, Azizi BH. Cystic fibrosis in Malay children – a report of three cases. Singapore Med J 1996; 37: 273-4.
4. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245:1066-73.
5. Anzai C, Morokawa N, Okada H, et al. CFTR gene mutations in Japanese individuals with congenital bilateral absence of the vas deferens. J Cyst Fibros 2003; 2:14-8.
6. Ngukam A, Jacquemont ML, Souveille I, et al. A novel missense mutation A1081P in the cystic fibrosis transmembrane conductance regulator (CFTR) gene identified in a Loatian patient with congenital bilateral absence of the vas deferens. J Trop Pediatr 2003; 50:239-40.
7. Alper OM, Shu SG, Lee MH, et al. Detection of novel CFTR mutations in Taiwanese cystic fibrosis patients. J Formos Med Assoc 2003; 102:287-91.
8. Macek M, Mercier B, Mackova A, et al. Sensitivity of the denaturing gradient gel electrophoresis technique in detection of known mutations and novel Asian mutations in the CFTR gene. Hum Mutat 1997: 9:136-47.
9. Suwanjutha S, Huang NN, Wattanasirichaigoon D, et al. Case report of a Thai male cystic fibrosis patient with the 1898+1G →T splicing mutation in the CFTR gene: a review of East Asian cases. Mutations in brief no. 196. [online]. Hum Mutat 1998; 12:361.
10. Kabra SK, Kabra M, Lodha R, et al. Clinical profile and frequency of delta F508 mutation in Indian children with cystic fibrosis. Indian Pediatr 2003; 40:612-9.
11. Curtis A, Richardson RJ, Boohene J, et al. Absence of cystic fibrosis mutations in a large Asian population sample and occurrence of a homozygous S549N mutation in an inbred Pakistani family. J Med Genet 1993; 30:164-6.
12. Wang W, Okayama H, Shirato K. Genotypes of cystic fibrosis (CF) reported in the world and polymorphisms of cystic fibrosis transmembrane conductance regulator (CFTR) gene in Japanese. Nippon Rinsho 1996; 54:525-32.