RESEARCH NOTE

New frameshift CF mutation 3729delAinsTCT in a Tunisian cystic fibrosis patient

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Introduction

More than 1800 *CFTR* (cystic fibrosis transmembrane conductance regulator gene) sequence variations have been identified in the cystic fibrosis (CF) mutation database (http://www.genet.sickkids.on.ca/cftr/). For North African populations, however, the nature and frequency of the major *CFTR* mutations remain unclear, although a small number of *CFTR* mutation detection studies have been done in Algeria and Tunisia, which have largely shown European mutations such as 1653del CTT (F508del), 1756 G→T (G542X) and 4036 C→G (N1303K), albeit at different frequencies, which presumably emerged via population admixture with European Caucasians (Messaoud *et al.* 2005; Loumi *et al.* 2008).

In this study, we report identification of a new frameshift mutation, 3729 delA insTCT, in exon 19 of the *CFTR* gene associated with 3442 G→T (E1104X) mutation in a Tunisian CF patient, and its clinical manifestation. Determination of this new mutation enhances the epidemiological data for the Tunisian population. It was helpful in providing genetic counselling to the affected family.

Case report

The patient is the first child of a Tunisian nonconsanguineous couple from southern Tunisia (Zarzis). There is no known case of CF in the family. She was referred to the Regional Hospital in Zarzis, for chronic diarrhoea since early childhood. She is below the average weight (3rd centile) and height (3rd centile) for her age.

At three-months of age, the patient was sent to the Paediatric Department of the Children's Hospital in Tunis. CF was suspected and a sweat test using pilocarpine iontophoresis was recommended. An elevated chloride sweat test was obtained in two cases ([Cl-] = 74 and 80 mmol/L). At five months, she was hospitalized twice for recurrent pneumonia and was treated with antibiotics. At seven months, a daily chest physiotherapy was started together with antibiotic therapy whenever needed. Written consent for the genetic study was obtained from parents.

Methods

DNA was extracted from blood cells of the patient and her parents by the salt precipitation method (Miller *et al.* 1988). Foetal DNA was extracted from amniotic fluid to 18 weeks gestation by addition of a lysis solution then subjected to a temperature of 95°C for 10 min followed by 10 min on ice.

All 27 exons and the flanking intron regions of the *CFTR* gene were amplified by PCR using specific primers as previously described (Fanen *et al.* 1992; Le Maréchal *et al.* 2001). Patient DNA was first screened for the 1653del CTT (F508del) mutation. Sample was further analysed by denaturing gradient gel electrophoresis (DGGE) for exons 5, 11, 19, 20 and 21 (Fanen *et al.* 1992) and by denaturing high pressure liquid phase chromatography (DHPLC), on a Transgenomic WAVE DNA Fragment Analysis System (Transgenomic, Crew, UK.), for the remaining exons (Le Maréchal *et al.* 2001). The DNA samples that showed abnormal profiles were sequenced using the Big Dye terminator cycle sequencing kit (Perkin-Elmer, Applied Biosystems, Foster City, USA) and analysed on an ABI Prism 310 DNA sequencer (Applied Biosystems) according to the manufacture's protocol. The sequencing data were analysed using ABI DNA sequencing analysis software v3.4.1. Each single-stranded product was concentrated using centri-sep columns (Applied Biosystems).

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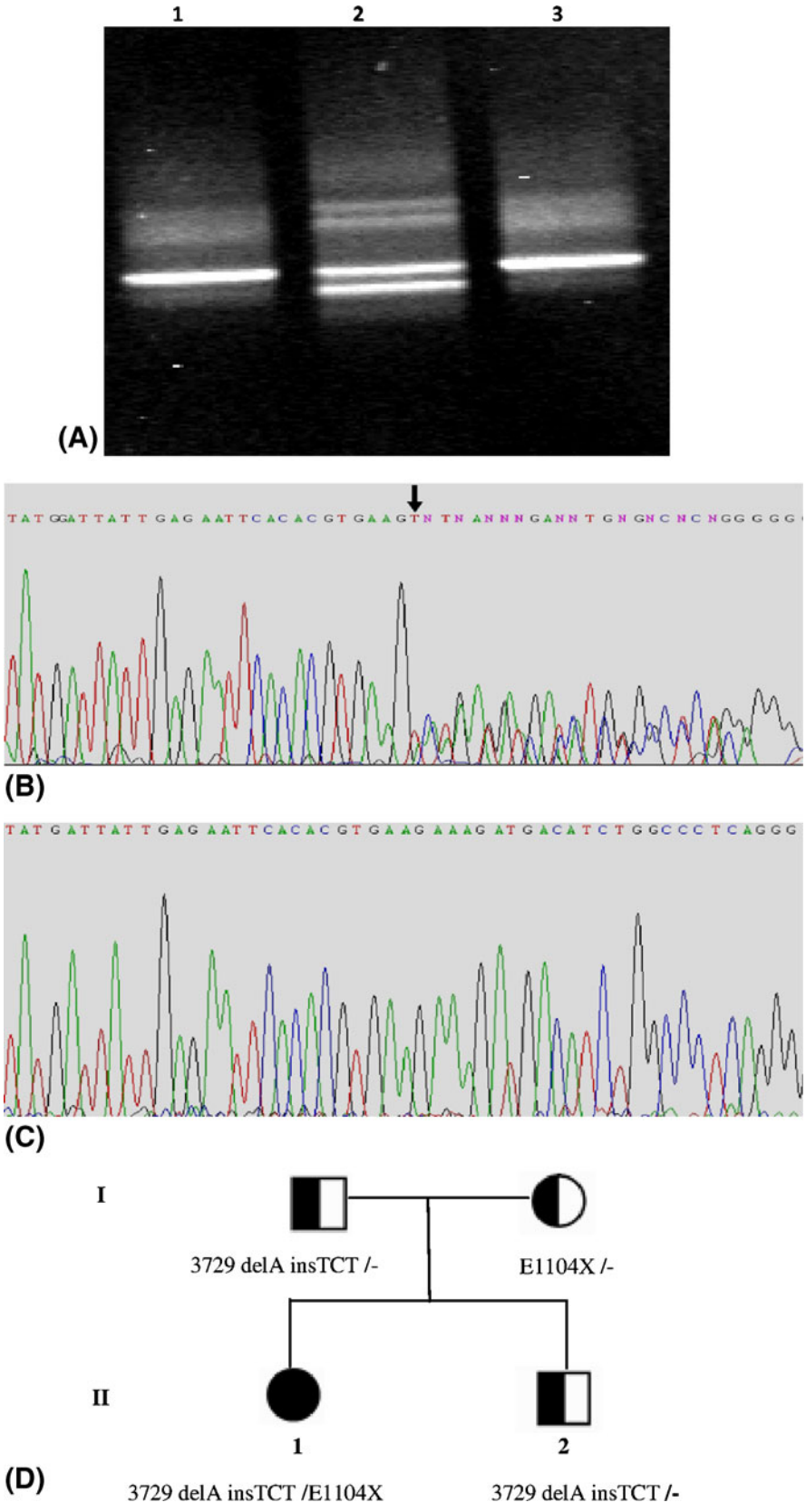

Figure 1. Mutation detected by DGGE and sequence confirmation of 3729delAinsTCT mutation. (A) DGGE analysis of exon 19 detects and abnormal pattern of the patient. Lane 2, patient; lanes 1 and 3 normal controls. (B) Sequence analysis to show 3729delAinsTCT mutation. (C) wild-type sequence (D) Pedigree of the studied family.

Mutation nomenclature

Nucleotide numbers are derived from cDNA CFTR sequences (GenBank accession no. NM_000492). Mutations are named according to the numbers used in the CFTR Mutation Database (http://www.genet.sickkids.on.ca/cftr/).

Results

Two heterozygous mutations were found following the screening of the entire coding sequence of the *CFTR* gene. A DNA sequence change 3442 G→T (E1104X) was found in exon17b. This mutation was detected by DHPLC and confirmed by direct sequencing. In the analysis of exon 19, we detected an abnormal banding pattern on DGGE. Direct sequencing showed deletion of an A and insertion of TCT at position 3729 in exon 19 (3729delAinsTCT) (figure 1). This results in a premature stop codon 31 nucleotides downstream of the deletion/insertion. The novel mutation 3729delAinsTCT was confirmed by genotyping of the parents. In fact, the father carries this mutation and the mother carries the E1104X mutation. To verify the new mutation, 100 alleles (50 Tunisian normal blood donors, aged one month to 20 years) were analysed, and no 3729delAinsTCT was found. This mutation has been reported to the CF Mutation Database. Prenatal diagnosis was offered to the parents during the second pregnancy. The molecular study showed that the foetus was a healthy carrier.

Discussion

The analysed patient was compound heterozygous for truncation mutations in exons 17b and 19 (3442 G→T (E1104X) and 3729delAinsTCT). This is consistent with null CFTR protein and a severe clinical phenotype. It is also consistent with an earlier study which reported that most patients carrying E1104X are severely affected (Fredj *et al.* 2009).

Stop mutations in the *CFTR* gene are known to disrupt gene function as they can lead to a severe reduction or total absence of cytoplasmic CFTR mRNA. The nonsense mutation E1104X is the second most common *CFTR* mutation in the Tunisian population with a mean frequency of 16.18%. The geographical distribution of CF mutations showed that the E1104X mutation is observed in most regions of the country, with a high frequency in the southwestern region (28.57%) but this is the first described case in southeast Tunisia (Fredj *et al.* 2009). Eighteen micro-insertions and/or micro-deletions have been reported involving exon 19 (Cystic Fibrosis Mutation Database). However, none of these frameshift mutations has been identified in North African and Arab countries. Few studies concerning CF, have looked at North African populations in general and only three studies have been conducted on the Tunisian population. According to these reports, F508del occurs in more than 40% of all CF chromosomes. Several other mutations described in our population have never been described elsewhere (2766del8, 3629 T→G (F1166C), 1811+5 G→T and 4268+2T→G) (Fredj *et al.* 2009; Messaoud *et al.* 1996, 2005).

Conclusion

The CF proband carries two CF heterozygous mutations (E1104X and 729delAinsTCT). 729delAinsTCT mutation is a new mutation which is not reported previously. This information is important as it helps in confirming the clinical diagnosis and allows for the option of accurate prenatal diagnosis.

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References

Fanen P., Ghanem N., Vidaud M., Besmond C., Martin J., Costes B. *et al.* 1992 Molecular characterization of cystic fibrosis: 16 novel mutations identified by analysis of the whole cystic fibrosis conductance transmembrane regulator (CFTR) coding regions and splice site junctions. *Genomics* **13**, 770–776.

Fredj S. H., Messaoud T., Templin C., des Georges M., Fattoum S. and Claustres M. 2009 Cystic fibrosis transmembrane conductance regulator mutation spectrum in patients with cystic fibrosis in Tunisia. *Genet. Test. Mol. Biomarkers* **13**, 577–581.

Le Maréchal C., Audrezet M. P., Quere I., Raguenes O., Langonne S. and Ferec C. 2001 Complete and rapid scanning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene by denaturing high performance liquid chromatography (D-HPLC): major implications for genetic counselling. *Hum. Genet.* **108**, 290–298.

Loumi O., Ferec C., Mercier B., Creff J., Fercot B., Denine R. and Grangaud J. P. 2008 CFTR mutations in the Algerian population. *J. Cyst. Fibros.* **7**, 54–59.

Messaoud T., Verlingue C., Denamur E., Pascaud O., Quéré I., Fattoum S. *et al.* 1996 Distribution of CFTR mutations in cystic fibrosis patients of Tunisian origin: identification of two novels mutations. *Eur. J. Hum. Genet.* **4**, 20–24.

Messaoud T., Bel Haj Fredj S., Bibi A., Elion J., Férec C. and Fattoum S. 2005 Epidémiologie moléculaire de la mucoviscidose en Tunisie. *Ann. Biol. Clin.* **63**, 627–630.

Miller S. A., Dykes D. D. and Polesky H. F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16**, 1215.

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