Identification of a cystic fibrosis mutation W19X in Tunisia

Identification de la mutation mucoviscidosique W19X en Tunisie

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Abstract. Cystic fibrosis (CF) is a common and serious condition with autosomal recessive inheritance. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR). The frequencies of mutations vary according to the ethnic origin of populations. We describe in this study a patient with cystic fibrosis. She was homozygous for a new nonsense mutation identified for the first time in Tunisia: W19X, which expected to cause significant morbidity. This mutation appears to be specific to Tunisian population, although, it has identified only in CF Tunisian patients. The information provided by our study contributes to defining the molecular spectrum of CF in Tunisia, to improve genetic testing and prenatal diagnosis.

Key words: cystic fibrosis, Tunisian patient, CF mutation, W19X

Résumé. La mucoviscidose encore appelée fibrose kystique du pancréas, est la plus fréquente des maladies autosomiques récessives graves. Elle est causée par des mutations au niveau du gène CFTR. Les fréquences des mutations mucoviscidosiques varient selon l’origine ethnique des populations. Nous décrivons dans cette étude, une patiente atteinte de mucoviscidose. Elle était homozygote pour une mutation non-sens identifiée pour la première fois en Tunisie : la W19X, qui a entraîné une morbidité importante. Cette mutation semble être spécifique à la population tunisienne, puisqu’elle n’a été identifiée que chez des patients d’origine tunisienne. Les informations fournies par notre étude contribuent à définir le spectre de mutations de la mucoviscidose en Tunisie, afin d’améliorer le dépistage génétique et le diagnostic prénatal.

Mots clés : gène CFTR, patient tunisien, mutation mucoviscidosique, W19X

Cystic fibrosis (CF) is a common and serious condition with autosomal recessive inheritance. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR) on chromosome 7q31 and is characterized by malfunction of chloride ion channels and of transport pathway regulation [1, 2]. CFTR protein is primarily expressed in the apical membrane of exocrine epithelial cells. Classic CF is characterized by failure to thrive, recurrent bacterial endobronchitis, progressive decline of lung function, exocrine pancreatic dysfunction [3]. The phenotype is variable; it ranges from mild with limited manifestations to rapid deterioration and death within the first year of life. The severity of clinical manifestations depends on the set of CFTR mutations, modifying genes, and other variables. To date, more than 1,800 sequence variants have been reported to the cystic fibrosis mutation database but the type and the frequency of the mutation change according to the geographic and ethnic origin. In the present study, we report a W19X mutation in a Tunisian CF patient and her clinical presentations.

Studied case

One patient was examined in this study, which have cystic fibrosis clinical manifestations. The proband CF patient was two years old female infant; she is the first child of second-degree consanguineous parents from Mareth located at the region of Gabes in South-Est of Tunisia. She was born at term (38 week of gestation) with a birth weight of 2.850 g. She was firstly admitted to the regional hospital of Gabes for anemia and generalized edema with hypoproteinemia.
Then, she was repeatedly hospitalized for severe dehydration and dyspnea but without chronic diarrhea. The spittle cytology examination revealed *Pseudomonas aeruginosa* and the radio chest showed a thoracic distension. Later, she was referred to the Children hospital in Tunis for a sweet test. A high chloride concentration was obtained on two occasions. Her parents were healthy and have not any clinical manifestation of CF.

**Methods**

10 mL of venous blood samples of the proband mucoviscidosis patient and her parents were obtained. Genomic DNA was extracted according to salting-out extraction method [4]. Samples were collected after obtaining the informed consent from parents.

Fetal DNA was extracted from amniotic fluid by the addition of a lysis solution subsequently subjected to a temperature of 95°C for 10 min followed by 10 min on ice.

Amplification of all *CFTR* exons analyzed, including flanking intronic regions, was performed using *CFTR* gene specific primers, which are commonly used in many studies for *CFTR* analysis [5, 6]. The PCR products were analyzed by DGGE (denaturing gradient gel electrophoresis) [7] for the exons (5, 11, 17b, 19, 20 and 21) and by DHPLC (denaturing high performance liquid chromatography) for the remaining exons [6]. Samples with altered variation, were sequenced on an ABI Prism 310 Genetic Analyzer (Applied BioSystems, USA) using a Big-Dye-Terminator cycle sequencing Ready Reaction Kit. Both forward and reverse strands were sequenced [8].

**Results**

The 27 coding exons of the *CFTR* gene and their intron-exon junctions, of the studied patient, were PCR amplified and screened by DHPLC and/or DGGE. Based on the DHPLC elution profiles of the heterozygous parents and the normal control, the only detectable difference was noted in the 189 pb region encompassing the exon 2 (figure 1). Patterns of wild-type genotype and CF mutant genotypes could not be distinguished. However, only by additional heteroduplex-based assay of mixing the proband sample and the standard wild-type control, similar profile to those observed in heterozygous parents were noted. These results clearly indicate that proband was effectively homozygous for this variation. Nucleotide direct sequencing of the PCR-amplified region in both forward and reverse strands revealed the presence of a homozygous substitution of a guanine (G) by an adenine (A) at nucleotide position 189, which causes the conversion of the tryptophan to a stop codon, this mutation is W19X (figure 2).

The family of our patient benefited of a prenatal diagnosis performed on amniotic fluid collected at 14 SA showed that the fetus is heterozygous for the W19X mutation (figure 3).

**Discussion**

The W19X mutation appears to be severe, since the patient associates infection by *Pseudomonas aeruginosa* at an early age, while 80% of CF patients are colonized with *Pseudomonas aeruginosa* by eight years of age [9], dyspnea and severe hypotrophy <4 DS.
This nonsense mutation is rare because it has been identified only by Bozon in 1998, in one CF case whose parents are from Tunisia (cystic fibrosis mutation database). Indeed, in two Tunisian studies involving 368 CF patients, the W19X mutation was not identified [10, 11]. At the southern region of Tunisia (Gabes, Mednine and Tataouine), 3 mutations have been identified N1303K, G542X and F508del. The latter one, is the most common and accounts for 68.75% [11]. The W19X mutation, and given its identification in one patient born in Gabes, it has not been identified.

Few works have been developed on the molecular study of the CFTR gene in Tunisia. 5 new mutations have been identified in our population whose the 2766 del8 mutation appears to be specific to the Tunisian patients and accounts for 1.85% [10]. The results obtained in this study serve to broaden the spectrum of mutation in our population.

**Conclusion**

This is the first genotype–phenotype correlation study on the W19X mutation. Our results showed that patient carrying the W19X allele in a homozygous status have a severe CF phenotype. Determination of the common CFTR
Current practice

mutations in a specific population allows confirming the clinical diagnosis and facilitates the development of a rapid and accurate assay for prenatal diagnosis and carrier detection.

Conflicts of interests: none.

References


