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Original Article

Molecular Screening of R117H (c.350G>A; p.Arg117His) Mutation in Non Caucasian Cystic Fibrosis Patients from North of Iran

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Cystic fibrosis (CF) is an autosomal recessive disease caused by a wide spectrum of mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. These mutations that correlate with different phenotypes, vary in their frequency and distribution among different populations. In this study, missense mutation R117H (c.350G>A; p.Arg117His) was analyzed in CF patients. Fifty five non relative Iranian CF patients aged between four months to eighteen years old living in the north of Iran, Mazandaran province were screened for clinical presentation and also for R117H mutation in CFTR gene by reverse dot blot method. The most clinical presentation was pulmonary disorder and none of the patients had R117H mutation. Further investigation of this mutation in a larger number of patients and/or infertile male subjects is recommended in this population.

Keywords: Cystic fibrosis (CF), phenotype, R117H, reverse dot blot

Cystic fibrosis (CF) is an autosomal recessive disorder that affects approximately one in 2500 births among most Caucasian populations, though its frequency may vary in specific groups (1). This disease arises from mutations in cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that comprises 27 coding exons, spanning over 250 kb on chromosome 7q31.2 (2, 3). The protein encoded by the CFTR gene constitutes a chloride (Cl^-) channel in the apical membrane of exocrine epithelial cells (4). It comprises 1480 amino acids with a molecular weight of ~170 kDa. The protein's structure indicates that CFTR is part of the ATP binding cassette (ABC) transporter proteins. CFTR protein is found in various cell types including lung epithelium, submucosal gland of intestine, stomach, pancreas, gallbladder, liver, sweat ducts and reproductive tracts. Thus, CF primarily involves epithelial cells in these organs. Poor mucociliary clearance with excessive mucus production causes obstructive lung disease and chronic bacterial infections leading to respiratory failure which is the

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major cause of mortality (1). More than 95% of patients also fail to produce digestive enzymes in pancreas resulting in pancreatic insufficiency (PI) and there is a high level of male infertility (> 95%) caused by absence or obstruction of vas defrens (1). Since *CFTR* gene identification in 1989, more than 1800 mutations have been identified in this gene and their listing is continuously updated within the CF genetic analysis consortium database (http://www .genet.sickkids.on.ca/cftr/). These mutations have different frequencies in different populations. The most common mutation in *CFTR* gene is F508del (ΔF508) which accounts for approximately two third of all CFTR alleles in CF patients. The prevalence of this mutation is decreasing from northwest to southeast of Europe (5-10). Its frequency in Caucasian populations is about 70%(11) but its fre--quency in Arab (12, 13), Indian (14), Iranian (15) and Turkish populations (16) varies between 44% and 13%. The remaining third of alleles are substantially heterogeneous with fewer than 20 mutations occurring at a worldwide frequency of more than 0.1% (17, 18). Only four mutations (p.G542X, p.N1303K, p.G551D and p.W1282X) have overall frequencies greater than 1% (7). Few previous reports of *CFTR* mutations in Iran have been published (15, 19-22). Although CF is the most common autosomal recessive disease in many Cau--casian populations, including those of Europe and the United States, it seems that CF prevalence in Iran isn't rare as the carrier frequency was estimated about 1:40 (19). In the present study, missense mutation R117H (c.350G>A; p.Arg117 His) were analyzed in 55 unrelated northern Iranian CF patients. In addition, the clinical presentations and laboratory findings were studied based on hospital and outpatient records. The R117H mutation that was reported by van Heeckeren et al. (23) is among class IV mutations associated with altered conductance such that the rate of chloride transport is reduced. Thus, mutations in class IV lead to CFTR proteins that can be produced, processed, transported and inserted into the apical membrane, but display a defective conductance. R117H mutation can either result in CF or congenital bilateral absence of vas deferens (CBAVD) by being associated with either 5T or 7T (poly T) allele located within the 3' splice site of intron 8 in the *CFTR* gene, which is associated with a variable efficiency of exon 9 splicing (24). There are three common alleles at this locus, with 5, 7, 9 thymidines (T5, T7, T9 respectively) (25). Among these alleles, the 9T allele is associated with the most efficient usage of the intron 8 splice acceptor site (25). R117H association with 7T allele may result in CBAVD while its association with 5T may result in CF. this mutation is more frequently observed in patients with CBAVD. In this study R117H mutation was screened using reverse dot blot (RDB) assay for CF patients.

Table 1. Primers for exon 4 amplification of *CFTR* gene

Primers	Primer sequence
CF4-F	5'-Biotin-TCACATATGGTATGACCCTC-3'
CF4-R	5'-Biotin-TTGTACCAGCTCACTACCTA-3'

Materials & methods

Patients

Fifty five CF patients (28 males and 27 females) aged between 4 months and 18 years were diagnosed based on principle clinical evaluations (pulmonary complications and pancreatic insuffic--ency) and sweat chloride values (from borderline to >60 mEd/l).

The patients were recruited from pediatric hospital of Babol medical university and all subjects were from the north of Iran, Mazandaran province.

Patients' parents were informed of the nature of the research and consented to participate in this

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Table 2. Specific probes for R117H mutation detection

Probe name	Location in the *CFTR* gene	*CFTR* probe sequence
R117H-N	E4	5'-NH2-ATAGAGCGTTCCTCCT3'
R117H-M	E4	5'-NH2-ATAGAGTGTTCCTCCTT3'

N: normal allele; M: mutant allele.

study.

Molecular analysis

DNA was prepared from peripheral blood leukocytes using salting out method. DNA of exon 4 of CFTR gene was amplified by PCR using 5' biotinylated primers (Table 1). PCR amplifications were performed in 50 µl reaction volumes contain - -ning approximately 250 µM dNTPs , 2 mM MgCl2 , 200 nM each forward and reverse biotinylated primers , 1.5 unit Taq DNA polymerase. All PCR reagents were from Roche Company, Germany except primers which were from Pioneer Company, Korea. The amplification conditions included initial denaturation at 94 ºC for 3 minutes, followed by 30 cycles, each consisting of a 1 min denaturation at 94 ºC, annealing at 58 ºC for 45 s and extension at 72 ºC for 45 s, followed by final extension at 72 ºC for 10 min. PCR products were visualized after electrophoresis on 1.5% agarose LE gel under UV transilluminator. R117H mutation detection was performed by RDB reaction according to Lappin et al. (26). In this method, biotinylated PCR products were used for hybridization with specific probes (Table 2) fixed on biodyne C membrane.

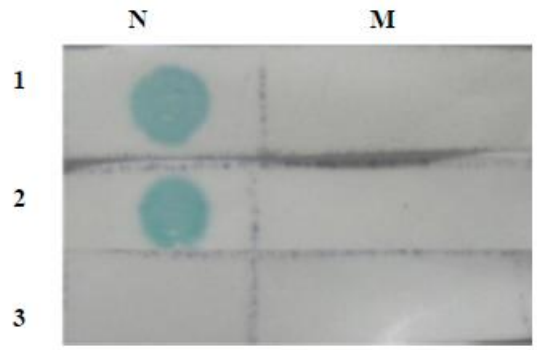

Figure 1. Analysis of R117H mutation by RDB method. Lanes 1 and 2 represent patients with normal alleles and lane 3 is a blank without PCR product. N: normal; M: mutant.

Results

Phenotype analysis

In the present study, 41.8% of the patients were borne from consanguineous marriage (mostly between first cousins). 96.4% of patients showed acute or persistent respiratory symptoms. Most of patients (63.6%) had malnutrition and abnormal stool (63.6%). Other manifestations were observed in a small number of patients. None of the patients had a previous family history of CF. The phenotype of patients and their frequencies are presented in (table 3).

Molecular analysis

R117H mutation screening of the *CFTR* gene in 110 alleles by PCR and RDB hybridization method showed that none of mutant alleles had this mutation. (Figure 1) shows the results of RDB method in detecting R117H mutation in *CFTR* gene.

Table 3. Phenotype of CF patients and their frequencies

Clinical presentations	Frequency %
Pulmonary disorder	96.4
Malnutrition	63.6
Abnormal stool	63.6
Meconium ileus	14.5
Electrolyte abnormality	10.9
Rectal prolapse	5.5
Nasal polypes	1.8
Liver/gallbladder problem	14.5
Edema	7.3
Others (dark blindness, diabetes...)	20

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Discussion

There are only a few reports that describe the distribution and frequency of *CFTR* gene mutations in Iran (15, 19-22). In the present study, Fifty five CF patients originating from the north of Iran, Mazandaran province, were analyzed for R117H mutation using RDB. RDB method is a simple, rapid and reliable method which allows simultaneous detection of many different mutations in a single hybridization assay (27). R117H has diverse frequencies in the world and table 4 summarizes the frequencies of this mutation in different populations of the world (28).

R117H was studied almost in CBAVD subjects because *CFTR* mutations commonly assoc--iated with male infertility are F508del, R117H, and the IVS8_(5T) polymorphism (28). R117H mutation severity is modulated in cis by the poly thymidine tract (9T, 7T and 5T) in intron 8. R117H-T7 genotype is associated with milder phenotype such as CBAVD and most of the time even absence of symptoms with normal or borderline sweat test, while the R117H-T5 genotype can cause sweat chloride elevation and severe clinical CF symptoms. Because of the need for accuracy in genetic counseling and detection of the correlation between genotypes and phenotypes in CF patients, diagnosis and follow up of R117H may be necessary. However, many mutations occuring in *CFTR* gene may cause variable clinical phenotypes and may not be predictable due to the interaction of environment and modulator genes. The absence of R117H mutation detection in the present study, may be due to the relatively low number of studied subjects. Further investigation of this mutation in a larger number of patients and/or patients presenting CBAVD in this population is recommended.

Table 4. R117H mutation distribution in the world

Country region	R117H frequency %
Greece	1.2
Ireland	2.4
Norway	3
Sweden	0.6
United kingdom (N.Ireland)	1.5
United kingdom (Scotland)	1.4
United kingdom (Wales)	0.5
Canada	0.9
Mexico	0.5
United states	0.7
Australia	0.6

Conflict of interests

The authors declared no conflict of interests.

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