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Relative Frequencies of Three Cystic Fibrosis Mutations in North Jordan; "F508, W1282X, and N1303K

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ABSTRACT 120 Patients from North Jordan were screened for three cystic fibrosis mutations; "F508, W1282X, and N1303K. This study identified "F508 (23.75%) and W1282X (15%) only. N1303K was not detected, although it was reported earlier from Jordan in low frequency.

INTRODUCTION

Cystic fibrosis (CF) is the most common lethal genetic disorder among Caucasian population; it affects 1 in 2500 live births (Kambouris 2000). It is an autosomal recessive diseases that arises due to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7q 31.2 (Welsh et al. 1995). Those mutations produce abnormal cystic fibrosis transmembrane conductance regulator, which functions as a chloride channel. This impairs water movement across epithelia and lead to formation of viscous mucus that obstructs the airways of the lungs and ducts of the pancreas (Cutting et al. 1990). On the other hand, dysfunction of the CFTR elevates sodium and chloride concentration in sweat, and almost all males are infertile due to bilateral absence of vas deferens (Jacobson et al. 1993).The first CF mutation described is known as "F508, this is the most common mutation in Northern European derived populations including the United States and accounts for 70-75% of CF chromosomes (Kerem et al. 1989; Lemna et al. 1990).

CF shows high heterogeneity among patients; some phenotypic variations correlated with the type of mutation present in CFTR gene. Moreover, it was shown that frequencies of CF mutations are variable among different ethnic population (Eitan Kerem et al. 1995)

According to CF mutation database, more than 1000 mutations have been identified until now. The most common one is "F508, which includes deletion of phenylalanine at positions 508 of protein containing 1480 amino acids and account for 67 % of Caucasian CF chromosome world-wide (Welsh et al. 1995). It has been reported that CF occurs in non-Caucasian populations but is less common than Caucasian (Cutting 1997). Although CF is considered very rare in many races including Arabs, this is probably due to a lack of careful surveys and screening programmers (Nazer 1986; Ginsberg et al. 1994).

The aim of this study was to identify the frequency of three common CF mutations ("F508, W1282X, N1303K) among Jordanian CF patients in North Jordan population for possible use in a nation wide screening program for CF.

MATERIAL AND METHODS

Peripheral blood samples from 120 CF patients (sweat chloride > 60 mmol/L diagnostic of CF and clinical symptoms characteristic of CF) in King Abdullah university hospital and Princes Rahmah Teaching Hospital in Irbid (North Jordan) were collected in EDTA tubes. All patients were fully informed that their blood would be used for molecular investigation of the CFTR gene mutations and consent was obtained from each patient or his/her guardians.

DNA extraction was carried out using salt precipitation method with purification kit provided by Promega, USA (Miller et al. 1988). Samples were screened for mutations using ARMS PCR (Amplification Refractory Mutation System- PCR). This method is rapid, reliable, nonisotopic and results can be easily obtained

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within one working day. For each mutation there are three primers (a common primer, a normal primer and the mutant primer). The forward primer is the common primer (C), whereas the reverse primer is designed to either anneal and to amplify the mutant (M) or the wild type in allele. A typical ARMS test consists of two complementary reactions: the first reaction contains an ARMS primer specific for the normal DNA sequence and cannot amplify mutant DNA at a given locus. Similarly, the second reaction contains a mutant specific primer and does not amplify normal DNA. Table 1 includes primers which were used for mutations detection (Ferrie et al. 1992; Newton et al. 1989). DNA bands stained with ethidium bromide and localized using Polaroid camera (Polaroid 677).

The genotype of each individual was determined by analysis of the amplification product. A normal individual generate PCR product only in normal reaction; a heterozygous gives products in both reaction, and the homozygous mutant does so only in the mutant reaction.

RESULTS

120 CF patients were screened for CF mutations; ("F508, W1282X, and N1303K). The frequency of "F508 was the most common mutation among Jordanian CF patients with a frequency of 23.75% followed by W1282X with a frequency of 15 % where as N1303K was not detected in our samples. Table 2 summarizes the frequencies of those mutations.

The genotype of each individual was determined based on the analysis of the amplification product. Table 3 summarizes the genotype result.

Table 2: Frequencies of three mutations detected in CF Patients in North Jordan

Mutation	*No. of chromosomes carry CF allele*	*%*
"F508	57/240	23.75
W1282X	36/240	15.00
N1303K	0/240	0.00
unknown	147/240	61.25
Total	240/240	100.00

Table 3: Frequency of the observed genotypes (total 120) cystic fibrosis patients in North Jordan

Genotype	*No. of patients*	*%*
"F508/"F508	13	10.83
"F508/unidentified	33	27.5
"F508/W1282X	11	9.17
W1282X/unidentified	25	20.83
Unidentified/unidentified	38	31.66
Total	120	100.00

DISCUSSION

The first report about cystic fibrosis in Jordan appeared in 1984, in which 12 patients were diagnosed after postmortem examination (Nazer 1984). In neonatal screening including 7682 neonates for CF from 10 hospitals using meconium albumin method, the incidence was calculated as 1 per 2560 live births (Hisham M. Nazer 1992). The present study demonstrates the prevalence of mutations in north Jordan by determining the frequency of CF mutation mainly in affected individuals in contrast with the previous study that was a population based study.

Our findings indicate that, "F508 mutation is the most common among CF patients in North Jordan (23.75%) followed by W1282X mutation with 15% where as N1303K was not detected in our samples. The distribution of mutations has

Table 1: Sequences of primers used in ARMS reactions

Mutation	*Sequence 5N—3N—*	*Size bp*
"F508		
Common primer	GACTTCACTTCTAATGATGATTATGGGAGA	
Normal primer	GTATCTATATTCATCATAGGAAACACCACA	160
Mutant primer	GTATCTATATTCATCATAGGAAACACCATT	157
W1282X		
Common primer	CCCATCACTTTTACCTTATAGGTGGGCCTC	
Normal primer	CCTGTGGTATCACTCCAAAGGCTTTCCAC	178
Mutant primer	CCTGTGGTATCACTCCAAAGGCTTTCCAT	178
N1303K		
Common primer	CTCAATTTCTTTATTCTAAAGACATTGG	
Normal primer	GATCACTCCACTGTTCATAGGGATCCAAG	328
Mutant primer	GATCACTCCACTGTTCATAGGGATCCAAC	328

Table 4: Frequencies of CF mutation in the neighboring countries.

	"F508	*W1282X*	*N1303K*	*Reference*
Jordan	23.75%	15%	0.00%	Present study
Israel	29.40%	-	2.70%	Orgad et al. 2001
Turkey	27%	0.60%	1.80%	Onay et al. 1998 & 2001
Lebanon	37.50%	15.60%	9.40%	Desgeorges et al. 1997
Tunis	17.90%	2.50%	6.40%	Taieb et al. 1996
Iran	16.20%	4.00%	-	Jalalirad M et al. 2004
Saudi Arabia	15%	3.00%	-	Desgeorges et al. 1997

now been established in many populations of various ethnic groups, where as "F508 was the most common mutation with a frequency of 66% world-wide. Its relative frequency varies between north and south of Europe ("F508 account for 90% of CF chromosomes in Denmark, and 30% in Turkey) [Cystic Fibrosis Genetic Analysis Consortium, pers.commun].

Our results are slightly different from those reported from neighboring countries for the three mutations ("F508, W1282X, and N1303K) as seen in Table 4. CF mutations and their relative frequencies observed in our samples are within the range as reported from other neighboring countries which various for the "F508 mutation from 37.5% among Lebanese patients (Desgeorges et al. 1997) to 15% observed in Saudi Arabia (Desgeorges et al. 1997), from 15.6% in Lebanese patients (Desgeorges et al. 1997) to 0.6% in Turkish patients (Onay et al. 1998, 2001) for W1282X mutation and form 9.4% in Lebanese patients to 1.8% (Desgeorges et al. 1997) in Turkish patients (Onay et al. 1998, 2001) for N1303K mutation. These differences could be partially explained by variation in the diagnostic criteria among each study (Gerardo et al. 2002), and evidence of declining frequency of these mutations in a north-west to south-east direction Europe has been mapped (Yilmaz et al. 1990; European Working Group on CF Genetics 1990) which may applied to the our region (middle east) which is located to south-east of Europe.

CONCLUSION

Further studies of larger samples from different geographical locations of Jordan should be undertaken to better understand the prevalence of CF among the Jordanian population.

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REFERENCES

Cutting GR, Kasch LM, Rosenstein, Tsui LC, kazazian H, Antonarakis SE 1990. Two patients with cystic fibrosis, nonsense mutations in each cystic fibrosis gene, and mild pulmonary disease. *New Eng J Med,* **323**: 1685- 1689.

Cutting GR 1997. Cystic fibrosis. In: DL Rimoin, JM Connor, RE Pyeritz (Eds.): *Emery and Rimoin's Principles and Practice Medical Genetics.* London: Churchill-Livingston, pp. 2685-2717.

Desgeorges M, Megarbane A, Guittard C, Carles S, Loiselet J, Demclaustres M 1997. Cystic fibrosis in Lebanon: distribution of CFTR mutations among Arab communities. *Hum Genet,* **100**: 279.

Eitan Kerem, Yoram M. Kalman, Yaacov Yahav et al. 1995. Highly variable incidence of cystic fibrosis and different mutation distribution among different Jewish ethnic groups in Israel. *Hum Genet,* **96**: 193-197

European Working group on CF Genetics 1990. *Hum Genet,* **85**: 391-393.

Ferrir RM, Schwartz MJ, Robertson NH, et al.1992. Development, Multiplexing, and applications of ARMS tests for common mutations in the CFTR gene. *Am J Hum Genet,* **51**: 251-261.

Gerardo L, Isabel A, Beatriz C, et al. 2002. Cystic fibrosis in Uruguay. *Genetics & Molecular Research,* **1**: 32-38.

Ginsberg G, Roland G, Noble G et al.1994. Administration of an adenovirus containing the human CFTR cDNA to the respiratory tract of individuals with cystic fibrosis. *Nature Genetics,* **8**: 42-51.

Jacobson E, Rave-Harel N, Madgar I, Goshen R, Nissim-Rafnia M, Ziadni A, Rahat A, Chiba O, Kalman Y, Brautbar C, Levinson D, Augarten A, Kerem E 1993. CFTR haplotype analysis reveals genetic heterogeneity in the etiology of congenital bilateral aplasia of the vas deferens. *Am. J. Hum Genet,* **56**: 1359-1366.

Jalalirad M, Houshmand M, Mirfakhraie R, et al. 2004. First study of CF mutations in the CFTR gene of Iranian patients: detection of "F508, G542X, A120T, R117H, and R347h mutations. *J Trop Pediatr,* **50**: 359-361.

Kambouris M, Banjar H, Moggari I, Nazer H, Al-Hamed M, Meyer BF 2000. Identification of novel mutations in Arabs with cystic fibrosis and their impact on the cystic fibrosis transmembrane

regulator mutation detection rate in Arab population. *Eur J Pediatr,* **159**: 303-309.

Kerem BS, Rommens JM, Buchanan JA, et al. 1989. Identification of the cystic fibrosis gene: genetic analysis. *Science,* **245**: 1073-1080.

Lemna WK, Feldman GL, Kerem BS, et al. 1990. Mutation analysis for hetrerozygote detection and the prenatal diagnosis of cystic fibrosis. *N Engl J Med,* **322**: 291-296.

Miller SA, Dykes DD, Poleskys HF 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res,* **16**: 1215.

Nazer H, Kamal MF 1984. Cystic fibrosis in Jordan: a pilot study. *Ann Trop Paediater,* **4**: 1-4.

Nazer H 1986. Cystic fibrosis in Jordan: incidence and prevalence. *Trans Roy Soc Trop Med,* **80**: 348.

Nazer H 1992. Early Diagnosis of Cystic Fibrosis in Jordanian Children. J Trop Pediatr, **38**: 113-115.

Newton CR, Graham A, Heptinstall LE, Powell SJ, Summers C, Kalsheker N, Smith JC 1989. Analysis of any point mutation in DNA: the amplification refractory mutation system (ARMS). *Nucleic Acids Res,* **17**: 2503.

Orgad S, Neumann S, Loewenthal R, et al. 2001. Prevalence of cystic fibrosis in mutations in Israeli Jews. *Gen updtaesin Biotech,* **5**: 47-52.

Onay T, Topaloglu O, Zielenski J 1998. Analysis of the CFTR gene in Turkish cystic fibrosis patients: identification of three new mutations (3172delAC, P1013L, and M1028I). *Hum Genet,* **102**: 224-230.

Taieb M, Claudine V, Isabelle Q et al. 1996. Distrebution of CFTR mutations in cystic fibrosis patients of Tunisian origin identification of two noval mutations. *Eur J Hum Genet,* **4**: 20-24.

The Cystic fibrosis Genetic Analysis Consortium 1994. Population variation of commom cystic fibrosis mutations.*Hum Mutat*, **4**: 167-177.

Welsh MJ, Tsui L-C, Boat TF, Beaud et al. 1995. Cystic fibrosis. In: CL Scriver, AL Beaudet., WS Sly, Valle D (Eds.): *The Metabolic Basis of Inherited Disease.* 7th ed. New York, NY: McGraw-Hill, pp. 3799-3879.

Yilmaz E, Erdem H, Ozgue M et al.1995. Study of 12 mutations in Turkish cystic fibrosis patients. *Hum Hered,* **45**: 175-177.