European Journal of Human Genetics (1998) 6, 181–184

http://www.stockton-press.co.uk/ejhg

SHORT REPORT

Screening of CFTR mutations in an isolated population: identification of carriers and patients

Ornit Chiba-Falek[1], Malka Nissim-Rafinia[1], Zvi Argaman[2], Adnan Genem[3], Ilana Moran[3], Eitan Kerem[2] and Batsheva Kerem[1]

[1]*Department of Genetics, The Hebrew University of Jerusalem*
[2]*Department of Pediatrics and CF Clinic Shaare Zedek Medical Center Jerusalem*
[3]*Kupat Cholim Clinic, Jerusalem, Israel*

One important application of the identification of disease-causing mutations is carrier screening in the general population. Such a project requires a simple accurate test by which a large proportion of the mutations can be identified. This study describes screening for CFTR mutations in an isolated Israeli Arab village. Two mutations, G85E and ΔF508, accounted for all the CF alleles of these patients. The screening program tested for these two mutations, as well as the 5T allele, which has recently been shown to down-regulate the CFTR expression and cause variable phenotype. The screened population comprised 497 students from one school, which all the children of the village attend. The results revealed high carrier frequency, 8.5%, for the two CFTR mutations, G85E and ΔF508, and a carrier frequency of 12% for the 5T allele. Two compound heterozygotes for the CFTR mutations, ΔF508/G85E and G85E/5T, were identified. Both of these students had not been diagnosed previously as having CF since their disease presentation was not typical of CF. The CF incidence in this village was found to be extremely high, 1:72 life births. The screening results were reported to the physicians of the village to be used, upon request, for genetic counselling. This study emphasizes the importance of such programs for the identification of non-classical patients and for carrier detection.

Keywords: CFTR mutations; genetic disease screening; isolated population; carrier frequency; disease frequency; genetic diagnosis

Correspondence: Dr Batsheva Kerem, Department of Genetics, The Life Sciences Institute, The Hebrew University of Jerusalem, Israel 91904. Tel 972-2-6585689; Fax 972-2-6586975; Email kerem@.leonardo.ls.huji.ac.il
Received 29 May 1997; revised 14 October 1997; accepted 12 November 1997

Journal: European Journal of Human Genetics **Article:** 5200174

Introduction

Cystic fibrosis (CF) is a common severe autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.[1] One important application of the identification of disease causing mutations is carrier screening of the general population. To date in many populations around the world only 80–90% of the CFTR mutations have been identified. Thus screening tests cannot detect all carriers and the implementation of such programs becomes more complex. Isolated populations might be suitable targets for CF carrier screening programs. In such populations the frequencies of particular genetic diseases are often higher than in the general population, causing a major health burden. In addition, in isolated populations a small number of disease-causing mutations is to be expected, thus the screening is simple and cheap.

The Israeli population comprises different Jewish and Arab ethnic groups. The Jews tended to live in relatively closed isolated communities until recently. The majority of the Arab population still lives in closed communities, with a high incidence of consanguineous marriage. Indeed the frequency of CF disease varies considerably among the different Israeli ethnic groups and a unique limited repertoire of CFTR mutations was found in each ethnic group.[2]

This paper describes a screen for CFTR mutations in an Israeli Arab village in which CF patients and infertile males with congenital bilateral absences of the vas deferens (CBAVD) due to mutations in the CFTR gene were already known. Two CFTR mutations, G85E[3] and ΔF508[1] which account for all the CF chromosomes carried by the CF patients were screened. The population was also screened for the 5T allele, which is the major CFTR mutation causing CBAVD[4,5] and can down-regulate the CFTR expression and cause highly variable phenotype.[6,7]

Subjects and Methods

The Screened Population

We studied 497 village school students attending grades 4–12, who belonged to 284 nuclear families. This group included 92% of the students in these grades. The rest did not attend school at the time of sample collection. Prior to the initiation of the project a letter in Arabic, explaining the aim and the process of the study, was sent to all the parents of the school students by the physician of the village (an Israeli Arab). The parents had the option to object to the participation of their children in the study, but no objections were received. In addition to the school students, 89 relatives of CF patients were studied. The study was approved by the health and education ministries.

DNA Extraction

Mouth epithelial cells were collected from all individuals, by mouth wash with 10 ml 0.9% NaCl. The DNA was subsequently extracted as follows. The mouthwash was centrifuged at 2000 rpm for 10 min; the pellet was suspended in 500 μl 0.05M NaOH, and heated to 100°C for 15 min; 100 μl of 1M TRIS (pH 7.7) were then added to each sample.

Mutation Analysis

Analysis of CFTR mutations was performed by polymerase chain reaction (PCR) using oligonucleotide primers for the amplification of exons 3, 10 and 9[8] to identify the mutations G85E, ΔF508 and 5T respectively. Mutation detection was performed as described elsewhere: G85E,[3] ΔF508[9] and 5T.[7]

Results and Discussion

The screened population descended from one original family, which had founded the village near Jerusalem in the 16th century. Today some 5500 individuals reside in the village. There is a high prevalence of consanguineous marriage in the village. There is only one school in the village and its students represent the village gene pool. We assumed therefore that the frequency of CF carriers in the population of the village would be reflected by the school students.

The group studied was tested for the ΔF508 and the G85E mutations which accounted for all the CFTR mutations carried by the CF patients known in this village.[10] Of the 497 students, we found that 41 (8.3%) were carriers of the G85E mutation and one (0.2%) was a carrier of the ΔF508 mutation. Thus, the total carrier frequency was 1:12 (8.5%) (Table 1). Since the students at the school are members of 284 nuclear families, this carrier frequency could have been overestimated by the identification of carriers from the same nuclear family. Therefore, we recalculated the carrier frequency according to nuclear families. This analysis revealed that 22 (7.7%) of the nuclear families had 1–4 carriers of the G85E mutation and one family (0.4%) had a ΔF508 carrier. Thus, the estimated total carrier frequency was 8.1% (Table 1). There was no significant difference in the carrier frequencies calculated by analysing the students as individuals or according to nuclear families (Table 1). This carrier frequency is higher than that of entire population of Israel (2.8%) and of the European population in general (4%).[2,11]

Random samples of 203 students were tested for the 5T mutation. Twenty-four of them (12%) were found to carry this allele. These 203 students were members of

161 nuclear families, in 21 (13%) of which heterozygotes for the 5T allele were found (Table 1). Again, the two analyses revealed no significant difference in the carrier frequencies (Table 1). Thus, the overall CF carrier frequency (for the ΔF508, G85E and the 5T allele) is very high, > 20%, indicating that CF disease is a major health problem in this village.

In the screening we identified two students who were compound heterozygous for CFTR mutations. One of the students was compound heterozygous for the G85E and the ΔF508 mutations. This was a 10-year-old child who was considered healthy and rarely visited the doctor's clinic. On subsequent evaluation he was found to be in a good nutritional state, in the 80th percentile for weight and the 75th for height. A chest X-ray revealed minimal central bronchiectesis and pulmonary function studies were normal (FEV_1– 110% predicted). The 72 hour stool fat collection revealed borderline steatorrhea and the sweat chloride was elevated (110 meq/L), diagnostic for CF. This child had a brother who had died from CF at 6 years of age. Another student was initially found in the screening program to be heterozygous for the G85E mutation. He suffered from recurrent respiratory infections and chronic sinusitis. Following detection of the mutation he was found to have bilateral bronchiectasis, and severe nasopulmonary disease. His sweat chloride level was 52 mmol/L. Subsequently he was found to carry the 5T allele on the other chromosome. Haplotype and mutation analysis and sequencing of the entire coding region of the 5T allele in this family confirmed that the 5T allele is his second CFTR mutation.[7] Among the school students two females were found to be homozygous for the 5T allele. One was an 18-year-old female, who had never suffered from any significant symptomatology. Her sweat chloride level was 37 mmol/L and her pulmonary function was normal. The other did not agree to further clinical evaluation, but according to her medical records she had never suffered from any significant medical problems. These results further support previous observations that although cystic fibrosis is a severe lethal disease, there is a significant minority of patients with a milder phenotype.[11] The definite diagnosis of such cases can often only be made after genotype analysis is available. For these patients, diagnosis of CF and earlier introduction of therapy might improve their prognosis. The identification of these patients in the screening study underscores the importance of population screening for CFTR mutations, as it not only detects carriers but also identifies patients with atypical disease.

We estimated the frequency of CF disease in the village. Seven CF patients were known to have been born in the last three decades. Five of them were born between 1975 and 1984, the period during which the students who participated in the screen were born. In the course of the screening program two additional CF patients were identified (ΔF508/G85E and G85E/5T as mentioned above). Between 1975 and 1984 there were 502 live births in the village, hence the CF incidence was approximately 1:72. This is a minimal estimation, since it is possible that there were more CF patients who died undiagnosed. This is an extremely high incidence, compared to that of the entire population of Israel (1:5,000)[2] and to the general Caucasian population (1:2,500).[11] It is about eight times higher than the calculated frequency (1:560), assuming random mating, in a population with 8.5% carrier frequency. This strong deviation from the Hardy-Weinberg calculation reflects the extent of consanguineous marriage[12] which is characteristic of Arab villages in Israel.[13] We also screened 89 relatives of the CF patients for the G85E and ΔF508 mutations, aiming to identify additional carriers and patients. Forty-four (49.5%) were found to

Table 1 Carrier frequencies in the village school

	Students n=497		*Nuclear families n=284*	
Mutation	*No. of carriers*	*Carrier frequency (%)*	*No. of families with carriers*	*Carrier frequency (%)*
G85E	41	8.3	22	7.7
F508	1	0.2	1	0.4
Total	42	8.5	23	8.1
5T[a]	24	12	21	13

[a]The size of the group which was screened for the 5T allele was $n=203$ for the students and $n=161$ for the nuclear families.

be carriers. Forty carried the G85E mutation and four carried the ΔF508 mutation. No further CF patients were found among this group. The high incident of CF found in this village shows that CF is a major health burden there and explains the high cooperation of the citizens of the village with the screening program.

The screening results of the ΔF508 and the G85E mutations were reported to the physicians of the village. The results are confidential and given only to those who request them, and who can then be referred for genetic counselling. The information about the identified carriers for the 5T allele was not reported to the physician of the village since this allele is associated with variable disease expression and genetic counselling is complicated. Considering the high rate of consanguineous marriage in the village, the screening information could be used to identify couples at risk of having a CF child. Furthermore, as matchmaking is customary in the village, the screening results could be referred to the families prior to suggesting a match. CF genetic information is being used in this manner in ultra-orthodox Jewish communities around the world.[14] This study led to increased awareness and openness on the part of the village population and physicians to genetic counselling.

Acknowledgements

This work was supported by a grant from the Israel Ministry of Health to B Kerem.

References

1 Kerem B, Rommens JM, Buchanan JA *et al*: Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; **245**: 1073–1080.
2 Kerem E, Kalman YM, Yahav Y *et al*: Highly variable incidence of cystic fibrosis and different mutation distribution among different Jewish ethnic groups in Israel. *Hum Genet* 1995; **96**: 193–197.
3 Zielenski J, Bozon D, Kerem B, Markiewicz D, Durie P, Rommens JM, Tsui L-C: Identification of mutations in exon 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 1991; **10**: 229–235.
4 Chillon M, Casals T, Mercier B, Bassas L, Lissens W, Silber S, Romey M-C: Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *N Engl J Med* 1995; **332**: 1475–1480.
5 Jarvi K, Zielenski J, Wilschanski M *et al*: Cystic fibrosis transmembrane conductance regulator and obstructive azoospermia. *Lancet* 1995; **345**: 1578.
6 Rave-Hare N, Kerem E, Nissan-Rafinia M *et al*: The molecular basis of partial penetrance of splicing mutations in cystic fibrosis. *Am J Hum Genet* 1997; **60**: 87–94.
7 Kerem E, Rave-Harel N, Augarten A *et al*: A CFTR splice variant with partial penetrance associated with variable cystic fibrosis presentation. *Am J Respir Crit Care Med* 1997; **155**: 1914–1920.
8 Zielenski J, Rozmahel R, Bozon D *et al*: Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 1991; **10**: 214–228.
9 Rommens JM, Kerem B, Greer W, Chang P, Tsui L-C, Ray P: Rapid nonradioactive detection of the major cystic fibrosis mutation. *Am J Hum Genet* 1990; **46**: 395–396.
10 Kerem E, Nissim-Rafinia M, Argaman Z *et al*: A missense cystic fibrosis transmembrane conductance regulator mutation with variable phenotype. *Pediatric* 1997; **100**: 1–6.
11 Welsh MJ, Tsui L-C, Boat TF, Beaudet AL: Cystic fibrosis.. In Scriver CL, Beaudet AL, Sly WS, Valle D (eds): *The Metabolic Basis of Inherited Disease, 7th edn*. MacGraw-Hill, New York, 1995; pp 3799–3876.
12 Bittles AH, Neel JV: The costs of human inbreeding and their implications for variations at the DNA level. *Nat Gen* 1994; **8**: 117–121.
13 Jaber L, Bailey-Wilson JE, Haj-Yehia M, Hernandez J, Shohat M: Consanguineous matings in an Israeli–Arab community. *Arch Pediatr Adolesc Med* 1994; **148**: 412–415.
14 Abeliovich D, Quint A, Weinberg N, Verechozon G, Lerer I, Ekstein J, Rubinstein E: CF heterozygote screening in the orthodox community of the Ashkenazi Jews: the 'Dor Yesharim' approach and heterozygote frequency. *Eur J Hum Genet* 1996; **4**: 338–341.