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Original Article

Asthma and Pulmonary Function Abnormalities in Heterozygotes for Cystic Fibrosis Transmembrane Regulator Gene Mutations

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Abstract: The aim of our study is to evaluate the association between CFTR gene mutations with asthma and pulmonary function abnormalities. For this purpose, 214 mutation carriers were compared to 185 non-carriers. Although the relative risk of asthma did not differ between groups (OR=0.61, 95% CI: 0.23-1.61, p=0.32), the values of FEV1, and FEV1/FVC ratio were lower in carriers (p=0.001, and p<0.001, respectively). This may imply that heterozygosity may be related with a silent obstructive pulmonary profile.

Key Words: Asthma, cystic fibrosis transmembrane regulator gene mutations, cystic fibrosis heterozygosity

Introduction

Asthma results from a complex interaction between genetic susceptibility and environmental risk factors and it is one of the most common chronic diseases globally [1]. On the other hand, cystic fibrosis (CF) is the most common autosomal, recessive, lethal disease among white people [2]. It is caused by mutations in the cystic fibrosis transmembrane regulator (CFTR) gene, which is responsible for encoding chloride channels at the surface of epithelial cells [3]. There are over 1000 known mutations, with the most prevalent being ΔF508 [4]. Amongst the Greek general population 5% are carriers of CFTR gene mutations [5].

Carriers of CFTR gene mutations (heterozygotes) show no symptoms of CF, although heterozygosity has been associated in some studies with male infertility [6] and chronic pancreatitis [7, 8]. Studies that tried to assess the association between asthma and CFTR gene mutations heterozygosity have led to conflicting results claiming, either that there is a positive association between mutations and asthma [9, 10] or, on the contrary, that mutations protect heterozygotes from bronchial asthma [11]. The aim of this study was to investigate the association of CFTR gene mutations heterozygosity with asthma and spirometric pulmonary function abnormalities among the Greek population.

Materials and Methods

Study population was of Greek descent and was comprised of two groups. The first one consisted of heterozygotes of CFTR gene mutations (carriers) who were parents of children with CF. The other group of subjects who served as controls, consisted of non-carriers of CFTR gene mutations, the majority of whom were relatives of CF children. All participants had been checked with sweat chloride test and reviewed for the most common genetic mutations of the CFTR gene

Table 1. Characteristics of CFTR gene mutations carriers and non-carriers

Characteristics	Participants		p-value
	Carriers (N=214)	Non-carriers (N=185)	
Men/Women	92/122	71/114	0.35
Mean age (years, range)	36.32 (20-63)	32.32 (19-48)	<0.001
Height (cm, ±SD)	164.1 ± 9.5	167.7 ± 9.6	0.001
Weight (Kg, ±SD)	73.9± 15.9	71.1±17.1	0.28
Current smokers (%)	89 (41.5)	91 (49.1)	0.36
Light / heavy smokers	49/40	49/42	0.87
Ex-smokers (%)	7 (3.3)	9 (4.8)	0.18
Asthma (%)	9 (4.2)	14 (7.6)	0.15
Allergy (except asthma) (%)	33 (15.4)	29 (15.7)	0.94

in the Greek population.

Clinical histories were taken with the help of a detailed questionnaire, and pulmonary function tests were performed on all participants. More specifically, subjects were meticulously asked about the presence of chronic pulmonary symptoms, smoking habits, coexistence of atopic manifestations (i.e., allergic rhinitis, conjunctivitis, and dermatitis), chronic diseases, or use of any drugs. If they were on any medication for bronchial asthma, we recorded for how long, how often, and when was the last time it was taken. Finally, hospital admissions, emergency department visits, or outpatient attendance with the diagnosis of asthma were recorded. Subjects with symptoms of chronic bronchitis were excluded from the study.

According to their smoking habits, participants were classified as non-smokers, ex-smokers and smokers. The latter were divided further in "light" and "heavy" smokers (up to 20 or more than 20 cigarettes per day, respectively).

We used a functional definition of asthma for the purposes of the study, and as asthmatics were considered those who in the last 2 years were given one or more inpatient or emergency department diagnoses of asthma, or two or more outpatient diagnoses, or two or more prescriptions for short-acting β-agonists, or one or more prescriptions for a preventive medication for asthma [12].

Pulmonary function tests included measurements of forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and FEV1/FVC ratio. Measurements of FEV1, and FVC, were expressed as percentage of predicted values (Spirometer: Morgan type of Flexiflo). In all cases, measurements were performed if participants were in good health and were able to produce technically acceptable flow-volume curves according to the standards of the American Thoracic Society [13]. Values of percent predicted FEV1 and FVC higher than 80%, and FEV1/FVC ratio higher than 0.7, were defined as normal. The project was approved by the Ethics Committee of "Aghia Sophia" Children's Hospital and informed consent was obtained from all participants.

Statistical analysis: We used Student's t-test and Pearson's $\chi 2$ for univariate analysis. In multivariable analysis we assessed CFTR gene mutations heterozygosity as an independent predictor of asthma using multiple logistic regression, and as an independent predictor of FEV1, FVC, and FEV1 /FVC using ANCOVA. All multivariable models were adjusted for potential confounders namely, age, sex, presence of allergy (except asthma), and smoke. We used backward elimination procedures for selecting the best models.

Table 2. Percentage predicted FEV1 and FVC, and FEV1/FVC ratio in CFTR gene mutations carriers and non-carriers

	Carriers (N=214)	Non-carriers (N=185)	p-value
Mean FEV1 % ± SD (range)	125.00 ± 17.26 (69-163)	128.10 ± 12.27 (101-156)	0.043
Mean FVC% ± SD (range)	121.80 ± 16.32 (73-159)	123.7 ± 12.62 (95-155)	0.20
Mean FEV1/FVC ± SD (range)	0.87 ± 0.06 (0.63-0.98)	0.88 ± 0.05 (0.76-0.98)	0.012

Results

Two hundred and fourteen carriers and 185 non-carriers of CFTR gene mutations were recruited. ΔF508 mutation was found in 118 (55.1%) carriers. Nine (4.2%) carriers and 14 (7.6%) non-carriers were characterized as asthmatics. Of the 9 carriers with asthma, 5 (55.5%) had the ΔF508 mutation. The characteristics of the study subjects are shown in **Table 1**.

In univariate analysis, no significant difference was observed in the prevalence of asthma between carriers and non-carriers (p=0.15, **Table 1**). A similar, non-significant result was also obtained from multivariable analysis. More specifically, the odds ratio (OR) for asthma in CFTR gene mutations carriers was found to be 0.61 (95% CI: 0.23-1.61, p=0.32). When we restricted our analysis to ΔF508 carriers, results remained virtually the same (OR= 0.52, 95% CI: 0.15-1.71, p=0.28).

Spirometric results are shown in **Table 2**. Values lower than normal limits for FEV1, FVC, and FEV1/FVC ratio were found only amongst heterozygotes. Totally, 9 out of 214 carriers had at least one pathological spirometric value. FEV1 and FEV1/FVC were significantly lower in heterozygotes (p=0.043 and p=0.012, respectively). None of them had reported bronchial asthma or other allergic manifestations or any other chronic disease, whereas 2 were classified as heavy smokers.

On ANCOVA models, CFTR gene mutations heterozygosity was found to be an independent predictor of FEV1 (p=0.001), and FEV1/FVC (p<0.001). No statistically significant association was found between heterozygosity and FVC (p=0.35). We also found similar results after restricting our analysis to ΔF508 carriers subgroup (p=0.042 and p=0.015 for FEV1 and FEV1/FVC, respectively).

Discussion

We investigated the association of CFTR gene mutation heterozygosity with asthma and spirometric pulmonary function in Greece. Previous studies have led to conflicting results. Dahl and colleagues, studying a Danish population, claimed that there is a positive association between ΔF508 mutation carriers and asthma. They found that asthma prevalence was 9% in a sample of 250 ΔF508 mutation carriers compared to 6% for non-carriers and the OR was 2.0 (95% CI: 1.2-3.5) [9]. Lowenfels, et al conducted a multinational survey in 1113 obligate CFTR gene mutation heterozygotes and 688 controls and they found that prevalence of asthma in CF heterozygotes was 9.6%, which is similar to that reported by Dahl, et al, but the OR was only slightly raised. They also observed similar results when they restricted their analysis to ΔF508 carriers [14]. On the contrary, Schroeder, et al from the United States, claimed that carriers of ΔF508 are somewhat protected against asthma and in their study found the OR for asthma in carriers of ΔF508 to be 0.31 (95% CI: 0.107-0.909) [11]. Finally, the EGEA study in France showed that 3.2% of asthma cases and 2.9% of controls were ΔF508 heterozygotes. This difference was not proved to be statistically significant [15]. Our results showed that the group of CFTR gene mutation carriers as well as the sub-group of

ΔF508 carriers did not have any significant difference in prevalence of asthma compared to non-carriers. Although we cannot exclude the possibility of error in recording asthma, a systematic bias is unlikely since diagnosis was set either in hospital emergency departments or from family doctors, independently from our study.

As far as spirometric values are concerned, it has been shown that ΔF508 carriers have lower values of FEV1 and FVC compared to non-carriers, although no difference in annual decline in lung function was observed between the two groups [10]. However, an earlier study had not found significant differences in spirometric values between CFTR gene mutations carriers and non-carriers and concluded that if any pulmonary function abnormalities existed in carriers they would be small and clinically insignificant [16]. In our study, CFTR gene mutations carriers, as well as the subgroup of ΔF508 carriers, had significantly lower values in FEV1, and FEV1/FVC from non-carriers although they did not differ in FVC.

Abnormal values for FEV1, FVC, or FEV1/FVC were found in 9 obligate heterozygotes; all of them were free of respiratory symptoms and 7 out of 9 did not have any other obvious factors that could have contributed to obstructive pulmonary disease. Abnormal values for these variables were not found in the control group. Although a fully justifiable explanation cannot be given, these findings might be attributed to CFTR gene mutation heterozygosity suggesting that a number of carriers may have a detectable silent obstructive pulmonary profile of unclear clinical significance. We are not able to predict how these abnormalities will evolve with increasing age and whether they could become clinically significant. Further follow-up of these individuals in future studies is needed to clarify this matter.

Drawbacks of our study are the use of a functional and not a strict definition of asthma and the lack of bronchial provocation tests, which might be helpful in making our results more clear.

In conclusion, our results indicate that CFTR gene mutations heterozygotes do not have different prevalence of bronchial asthma compared to non-carriers. However, according to pulmonary function testing, heterozygosity may be related with a silent obstructive pulmonary profile.

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