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Original article

# Analysis of IVS8 *CFTR* gene polymorphism in asthmatic children in a Tunisian population

## *Analyse du polymorphisme IVS8 du gène CFTR chez une population tunisienne d'enfants asthmatiques*

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### Abstract

**Objective.** – Asthma is a multifactorial disease of genetic origin; its clinical expression depends largely on acquired factors, often related to the environment. It is defined as a chronic inflammatory condition of the airways with mucus obstruction, a feature it shares with cystic fibrosis (CF).

**Material and methods.** – The present work concerns a study, for the first time in Tunisia, of the genotypic and allelic distribution of IVS8 TG<sub>m</sub>T<sub>n</sub> repeat motifs in intron 8 of the *CFTR* gene in an asthmatic population. TG<sub>m</sub>T<sub>n</sub> repeat analysis was performed for 100 asthmatic subjects and 103 controls using a sequencing technique.

**Results.** – The results showed the predominance of the T<sub>7</sub> allele in both patients and controls, as for the TG<sub>11</sub> allele, which represents the major allele of the two groups studied. Our results also showed a significant difference in distribution of the TG<sub>12</sub> allele. In addition, a haplotype study showed predominance of the TG<sub>11</sub>T<sub>7</sub> haplotype in both groups, which is in agreement with the data in the literature.

**Conclusion.** – Our study is one of the first to be carried out in the Tunisian asthmatic population. The results obtained will provide a better understanding of the molecular basis of this multifactorial disease.

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**Keywords:** Asthma; *CFTR* gene; IVS-8 TG<sub>m</sub>T<sub>n</sub>; Tunisian population

### Résumé

**Objectif.** – L'asthme est une maladie multifactorielle d'origine génétique ; son expression clinique est, en grande partie, fonction de facteurs acquis souvent liés à l'environnement. Il a été défini comme un état inflammatoire chronique des voies respiratoires avec obstruction, manifestation clinique commune avec la mucoviscidose.

**Matériel et méthodes.** – Le présent travail consiste à étudier, pour la première fois en Tunisie, la distribution génotypique et allélique du motif répété IVS8 TG<sub>m</sub>T<sub>n</sub> situé au niveau de l'intron 8 du gène *CFTR* au sein d'une population asthmatique. L'analyse du polymorphisme TG<sub>m</sub>T<sub>n</sub> a été réalisée par la technique de séquençage chez 100 sujets asthmatiques et 103 contrôles.

**Résultats.** – Les résultats ont montré la prédominance de l'allèle T<sub>7</sub> chez les patients et les contrôles. De même pour l'allèle TG<sub>11</sub> qui représente l'allèle majeur des deux groupes étudiés. Nos résultats ont également montré une différence significative dans la distribution de l'allèle TG<sub>12</sub>. En outre, l'étude des haplotypes a été menée et a montré la prédominance de l'haplotype TG<sub>11</sub>T<sub>7</sub> dans les deux groupes, ce qui est en accord avec les données de la littérature.

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**Conclusions.** – Notre travail est l'un des premiers travaux menés auprès de la population tunisienne asthmatique. Les résultats obtenus permettront de mieux comprendre la base moléculaire de cette pathologie multifactorielle.

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**Mots clés :** Asthme ; Gène CFTR ; IVS-8 TG<sub>m</sub>T<sub>n</sub> ; Population tunisienne

## 1. Introduction

Asthma is the most common chronic childhood disease, affecting up to 35% of the population in developed countries [1]. There is limited published literature on the epidemiology of asthma in Sub-Saharan Africa (SSA) and the Middle East and North Africa (MENA), where asthma is under-diagnosed and underestimated [2]. In Tunisia, we noted a prevalence of 6.5% [3]. Asthma is an inflammatory disease of the airways involving respiratory symptoms, such as wheezing and coughing, and reversible airflow limitation. Its severity differs widely between patients, but the majority have a mild form of the disease [4]. Asthma results from an interaction between genetic or host susceptibility and different types of environmental exposure (WHO/NHLBI workshop report, 2009). Genetic variation has major effects on asthma susceptibility; however, these are not restricted to a single gene, but represent the effects of many different genes that are polymorphic (i.e., containing gene mutation or polymorphisms), causing disease risk or severity [5]. Among these genes, we find the *CFTR* (Cystic Fibrosis Transmembrane conductance Regulator) gene which is associated with asthma [6]. This gene encodes a transmembrane channel protein that transports chloride ion into and out of cells that produce mucus, sweat, saliva, tears and digestive enzymes. *CFTR* variants that affect the function of this channel can cause extracellular mucus build-up, following which excessive thick and sticky mucus can obstruct the airways of the lungs as well as ducts in the pancreas [7]. The most common *CFTR* variants are single nucleotide substitutions and small deletions and duplications [8] for which we noted repeated motif IVS8 TG<sub>m</sub>T<sub>n</sub> in intron 8 of the *CFTR* gene [9]. The Poly-T tract is located at the junction of intron 8 (IVS-8) and exon 9 and influences transcription processes, thereby reducing the amount of normal CFTR protein. TG-repeat polymorphisms located on the 5' portion of the Poly-T affect the splicing of exon 9 [11]. This polymorphism was the subject of several studies in CF and CF-related diseases [10,13]. In Tunisia, the IVS-8 TG<sub>m</sub>T<sub>n</sub> marker has been studied in infertile men [14] and a group with congenital bilateral absence of the vas deferens (CBAVD) [15].

The present work consists in studying, for the first time in Tunisia, the genotypic and allelic distribution of the repeated motif IVS8 TG<sub>m</sub>T<sub>n</sub> of the *CFTR* gene in an asthmatic population and in healthy subjects to identify possible association with asthma.

## 2. Subjects and methods

### 2.1. Subjects

A total of 100 unrelated children presenting clinical signs of asthma were investigated. Cystic fibrosis was ruled out following a negative sweat test for all patients ( $[Cl^-] < 60$  mmol/L). Patients (4 months to 17 years old) were enrolled by pediatric departments in the Bechir Hamza Children's Hospital in Tunis. Median age at diagnosis was 72 months.

Asthma was diagnosed in accordance with the American Thoracic Society (ATS) guidelines [16]. In short, ongoing asthma was defined as recurrent wheezing or coughing in the absence of a cold in the preceding 12 months, with a physician's diagnosis and bronchial hyperresponsiveness to methacholine challenge ( $PC_{20} \leq 16$  mg/mL) or at least 12% reversibility of forced expiratory volume in 1 (FEV1) after inhalation of a  $\beta_2$  agonist.

The census information on asthma patients was collected by means of an interview with one or both parents. An information sheet was completed for each analyzed patient containing: patient identification, asthma characteristics (age at diagnosis, number of attacks and hospitalizations), socio-demographic data (origin, address, tobacco and pollution), last prescribed drugs for asthma, family history (history of the disease and consanguinity), coexistence of atopic manifestations (rhinitis, dermatitis and conjunctivitis) and laboratory tests (hemoglobin level and skin test).

At the same time, a control population of 103 healthy subjects was included in our study. Healthy children were age-matched to asthma subjects. They had no clinical symptoms of cystic fibrosis and no history of wheezing, recurrent or chronic diseases or infection over the preceding 2 weeks. None of the subjects had any other disease history, including pancreatic diseases. Written consent was obtained from the parents of all participants before enrollment in the study.

### 2.2. Genotyping of IVS8 TG<sub>m</sub>T<sub>n</sub>

Whole blood was obtained from each subject and genomic DNA was extracted using a salting-out protocol [17]. IVS8 Poly-T TG-repeat genotyping was performed by sequencing. Intron 8 containing the TG<sub>m</sub>T<sub>n</sub> repeat was amplified by PCR using one unit of *Thermus aquaticus* DNA polymerase (Taq DNA Polymerase Recombinant, Invitrogen, USA), 1X buffer, 0.2 mm

dNTP, and 0.04-pmol/ $\mu$ l of each primer. Cycling for reaction was performed as follows: 94 °C for 5 min for initial denaturation, 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by one cycle at 72 °C for 5 min for final extension. Samples of PCR products were checked by electrophoresis on 1.5% agarose gel with Red Safe. PCR products were purified with a Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI) and were sequenced using a Big Dye Terminator Cycle Sequencing Reaction Kit (PE Applied Biosystems, Foster City, California, USA). Each single-stranded product was analyzed on an ABI Prism 310 DNA sequencer (Applied Biosystems, Foster City, CA).

### 2.3. Statistical analysis

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 for Windows. A  $\chi^2$  test was used to compare genotypes and allele distribution. Differences were considered statistically significant at  $P < 5\%$ .

## 3. Results

### 3.1. Retrospective study of asthma patients

The clinical and epidemiological characteristics of the 100 asthmatic patients were analyzed. Most Tunisian regions were represented in our patient population; 54.1% of the patients were from the Northwest and 18.4% from the Northeast. The sex ratio was 1.85 in favor of boys. 29.5% of patients were from consanguineous couples. This relatively high rate of consanguinity is characteristic of the Tunisian population [18]. Half of the patients (53.3%) had a family history of asthma. The skin test performed for all patients revealed that 60.78% had an allergy, of which 67.74% was due to dust and pollen. Screening for associated diseases showed that 55.17% of patients presented allergic rhinitis, 20.68% conjunctivitis, 13.79% atopic dermatitis and 12% anemia. We also noted that 61.9% of patients were passive smokers.

### 3.2. Genotype and allele frequencies of $TG_mT_n$ in the asthma and non-asthma groups

To investigate the association between IVS8  $TG_mT_n$  and asthma, a case-control study was performed for 100 patients.

The genotypic and allelic distribution  $TG_mT_n$  marker in the non-asthma and asthma groups is summarized in Tables 1 and 2. In our two study populations we described the presence of three allelic forms of poly  $T_n$ :  $T_5$ ,  $T_7$  and  $T_9$ , with a predominance of the  $T_7$  allele in both patients and controls. However, it should be noted that the  $T_7$  allele was expressed more prominently in patients vs. controls (80.5% vs. 68.75% respectively,  $P = 0.0049$ ). Regarding poly  $TG$ , we found four allelic forms:  $TG_9$ ,  $TG_{10}$ ,  $TG_{11}$  and  $TG_{12}$ . It should be noted that the  $TG_9$  allele was described for the first time in a patient from the South-West (Sidi Bouzid) with the genotype  $TG_{10}T_9/TG_9T_9$ . The  $TG_{11}$  allele was the main allele in the two populations (49% in patients and 48.05% in controls). Our results also showed a significant difference in distribution of the  $TG_{12}$  allele in both groups: 12.5% for patients vs. 6.79% for controls ( $P < 0.0001$ ). The genotypic distribution of poly  $TG_mT_n$  showed a predominance of genotype  $T_7/T_7$  in the asthmatic and healthy groups with a significant difference (66% and 48.54% respectively,  $P = 0.0152$ ), and likewise for genotype  $TG_{10}/TG_{11}$  (32% in patients vs. 46.6% in controls).

Haplotyping of  $TG_mT_n$  marker was also performed (Table 3) and showed predominance of the  $TG_{11}T_7$  haplotype in both groups (42% in asthmatics and 35.93% in controls) followed by  $TG_{10}T_7$  (27% in patients and 27.18% in controls).

## 4. Discussion

The poly (T) sequence, located in the splicing acceptor site of intron 8, has three variants with five, seven or nine thymidines ( $T_5$ ,  $T_7$  and  $T_9$  alleles) [19].  $T_7$  and  $T_9$  alleles generate a predominantly normal mRNA transcript, whereas the  $T_5$  variant affects splicing efficiency and results in reduced levels of normal mRNA due to deletion of exon 9 [20]. The protein product of the *CFTR* transcript lacking exon 9 is devoid of cyclic adenosine monophosphate-activated chloride conductance, and the  $T_5$  allele is thus currently considered a mild mutation with incomplete penetrance [20]. Previous studies have shown the disease penetrance of  $T_5$  to be dependent on the copy-number of its adjacent  $TG$  repeats [21]. The number of  $TG$  repeats immediately adjacent to  $T_5$  is associated with variable splicing efficiency for exon 9 [22]. The different alleles at  $TG_mT_n$  polymorphic loci at the 3' end of human *CFTR* intron 8 determine splicing efficiency for exon 9 [23]. This marker has been studied in subjects with *CFTR*-associated disease, mainly cystic fibrosis (CF) [10], congenital bilateral absence of the vas deferens (CBAVD) [9,15],

Table 1  
Allelic distributions of the Poly  $TG_mT_n$  repeat in asthmatic patients and healthy controls.

| Populations (2n) | Allelic frequencies of poly T and TG |             |            |         | 2n (%)     |            |           |
|------------------|--------------------------------------|-------------|------------|---------|------------|------------|-----------|
|                  | $T_5$                                | $T_7$       | $T_9$      | $TG_9$  | $TG_{10}$  | $TG_{11}$  | $TG_{12}$ |
| Asthmatic (200)  | 6 (3)                                | 161 (80.5)  | 33 (16.5)  | 1 (0.5) | 76 (38)    | 98 (49)    | 25 (12.5) |
| Healthy (206)    | 16 (7.76)                            | 141 (68.44) | 49 (23.78) | 0       | 93 (45.14) | 99 (48.05) | 14 (6.79) |
| P                | 0.0520                               | 0.0049      | 0.0617     | 0.4924  | 0.0809     | 0.0585     | <0.0001   |

P-values < 0.05 are considered to indicate statistical significance. n: number.

Table 2  
Genotypic distributions of the Poly T<sub>n</sub>TG<sub>m</sub> repeat in asthma patients and healthy controls.

| Populations ( <i>n</i> ) | Genotypic frequencies of poly T and TG |                                |                                |                                |                                |                                |                                   | <i>n</i> (%)                       |                                    |                                    |                                    |                                    |                                    |
|--------------------------|----------------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-----------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
|                          | T <sub>5</sub> /T <sub>5</sub>         | T <sub>5</sub> /T <sub>7</sub> | T <sub>5</sub> /T <sub>9</sub> | T <sub>7</sub> /T <sub>7</sub> | T <sub>7</sub> /T <sub>9</sub> | T <sub>9</sub> /T <sub>9</sub> | TG <sub>9</sub> /TG <sub>10</sub> | TG <sub>10</sub> /TG <sub>10</sub> | TG <sub>10</sub> /TG <sub>11</sub> | TG <sub>10</sub> /TG <sub>12</sub> | TG <sub>11</sub> /TG <sub>11</sub> | TG <sub>11</sub> /TG <sub>12</sub> | TG <sub>12</sub> /TG <sub>12</sub> |
| Asthmatic (100)          | 1 (1)                                  | 5 (5)                          | –                              | 66 (66)                        | 23 (23)                        | 5 (5)                          | 1 (1)                             | 18 (18)                            | 32 (32)                            | 7 (7)                              | 27 (27)                            | 12 (12)                            | 3 (3)                              |
| Healthy (103)            | 0                                      | 15 (7.28)                      | 1(0.97)                        | 50(48.54)                      | 26(25.24)                      | 11 (10.67)                     | –                                 | 22 (21.35)                         | 48 (46.60)                         | 1 (0.97)                           | 19 (18.44)                         | 13 (12.62)                         | –                                  |
| <i>P</i>                 | 0.4924                                 | 0.0303                         | 0.5104                         | 0.0152                         | 0.9603                         | 0.1420                         | 0.5104                            | 0.5479                             | 0.0341                             | 0.0588                             | 0.1475                             | 0.8762                             | 0.1865                             |

*P*-values < 0.05 are considered statistically significant. *n*: number.

Table 3  
Different TG<sub>m</sub>T<sub>n</sub> patterns.

| Populations (2n) | Frequency of TG <sub>m</sub> T <sub>n</sub> patterns |                                 |                                 |                                | 2n (%)                          |                                 |                                 |                                 |                                 |                                 |
|------------------|------------------------------------------------------|---------------------------------|---------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                  | TG <sub>11</sub> T <sub>7</sub>                      | TG <sub>10</sub> T <sub>7</sub> | TG <sub>11</sub> T <sub>9</sub> | TG <sub>9</sub> T <sub>9</sub> | TG <sub>10</sub> T <sub>5</sub> | TG <sub>11</sub> T <sub>5</sub> | TG <sub>12</sub> T <sub>5</sub> | TG <sub>12</sub> T <sub>7</sub> | TG <sub>12</sub> T <sub>9</sub> | TG <sub>10</sub> T <sub>9</sub> |
| Asthmatic (200)  | 84 (42)                                              | 54 (27)                         | 13 (6.5)                        | 1 (0.5)                        | 2 (1)                           | 3 (1.5)                         | 2 (1)                           | 22 (11)                         | 1 (0.2)                         | 18 (9)                          |
| Healthy (206)    | 74(35.92)                                            | 56(27.18)                       | 18(8.73)                        | –                              | 4(1.94)                         | 7(3.39)                         | 5(2.42)                         | 11(5.33)                        | 1(0.48)                         | 30(14.56)                       |
| P                | 0.209                                                | 0.966                           | 0.395                           | 0.309                          | 0.431                           | 0.217                           | 0.296                           | 0.036                           | 0.983                           | 0.082                           |

P-values < 0.05 are considered statistically significant. n: number.

idiopathic bronchiectasis [12], and non-obstructive azoospermia [13], and also in the general population (healthy subjects) [24]. Few studies have been conducted in asthmatic patients concerning genotypes and allelic distribution of IVS8 TG<sub>m</sub>T<sub>n</sub>. The present work is the first study to investigate the association between IVS8 repeats and asthma in Tunisian children. The allelic distribution showed predominance of the T<sub>7</sub> allele in both patients and controls with a significant difference in favor of patients. The study by Ping Wang et al. also noted predominance of the T<sub>7</sub> allele in the Chinese population, but no difference was found between the two study groups [11]. The T<sub>5</sub> allele is involved in abnormal splicing of exon 9 and low levels of normal CFTR and it is the major CFTR mutation in patients with CBAVD, in association with another mutation in the other CFTR allele [25]; it has also been found to be common in patients with bronchiectasis and aspergillosis [26]. The small number of T<sub>5</sub> alleles in asthma patients suggests that the possible involvement of CFTR gene in asthma is not via reduction of CFTR levels. Consequently, we rejected a predominant role of the T<sub>5</sub> variant in asthmatic patients, despite its presence in 3% of our patients. This result is in concordance with Munthe-Kaas et al., who found that only 3.7% of Norwegian children with asthma carried the IVS8-T<sub>5</sub> polymorphism [27].

Concerning the allelic distribution of poly TG in the control group, we noted the presence of two major allelic forms, TG<sub>10</sub> and TG<sub>11</sub>, with similar percentages (45.14% and 48.05% respectively), whereas in asthma patients, we observed a predominance of the TG<sub>11</sub> allele (49%). We also noted a significant difference in the allelic distribution of the TG<sub>12</sub> allele in both groups. Indeed, this allele was more extensively expressed in patients than in controls ( $P < 0.0001$ ). However, a study of an asthmatic population in Korea showed no significant difference between the allelic and genotypic distribution of TG<sub>m</sub>T<sub>n</sub> [28]. The particularity of our study is the description of the TG<sub>9</sub> allele in one asthmatic patient in Tunisia and in the world.

The most common TG<sub>m</sub>T<sub>n</sub> haplotypes observed in our analyzed populations were TG<sub>11</sub>T<sub>7</sub> and TG<sub>10</sub>T<sub>7</sub>. TG<sub>11</sub>T<sub>7</sub> was the most common haplotype in our population (42% and 35.92% in asthma patients and healthy subjects respectively), echoing the findings of a Greek study by Tzetzis et al. [29]. No association was found between patients and controls regarding haplotype distribution of TG<sub>m</sub>T<sub>n</sub> except for the TG<sub>12</sub>T<sub>7</sub> haplotype, which, according to the literature, has no effect on the splicing of exon 9. These results are consistent with those described in a Norwegian asthmatic population and a Chinese population [11,23].

Furthermore, a linkage study conducted in Iceland confirmed that no interaction exists between the CFTR gene and asthma occurrence [30]. Conversely, studies in Greece, Denmark and Spain have reported a positive association between asthma and the CFTR gene that may represent a genetic linkage between the two conditions [31]. Based on our findings, there is likely to be no significant association of TG<sub>11</sub>T<sub>7</sub> in Tunisian bronchial asthma patients. Since the TG<sub>11</sub>T<sub>7</sub> haplotype could have provided protection against reduced lung function [27], we might speculate whether these modulating polymorphisms exert an effect on lung physiology in general rather than on lung function specifically seen in subjects with asthma.

Groman et al. reported an association of TG<sub>12</sub>T<sub>5</sub> and TG<sub>13</sub>T<sub>5</sub> haplotype with congenital bilateral absence of vas deferens (CBAVD) [32], while Tzetzis et al. reported an association between TG<sub>11</sub>T<sub>5</sub>, TG<sub>12</sub>T<sub>5</sub> and TG<sub>13</sub>T<sub>5</sub> and pulmonary diseases [29]. Furthermore, Ping et al. found that the frequency of the TG<sub>12</sub>T<sub>5</sub> allele in bronchial asthma patients (7.6%,  $P < 0.05$ ) was significantly higher than in controls (3%) [11]. In the present study, 5 asthmatic subjects had TG<sub>11</sub>T<sub>5</sub> and TG<sub>12</sub>T<sub>5</sub> with no significant association. The variability of the allelic distribution of the IVS8 TG<sub>m</sub>T<sub>n</sub> variant in the literature can be explained by the geographic origin and demographic history of the study populations in question.

The low frequency of T<sub>5</sub> observed in our patients suggests a lack of correlation between this polymorphism and asthma. A further study in a larger population is necessary to confirm the results obtained.

## 5. Conclusion

Our study of the IVS8 TG<sub>m</sub>T<sub>n</sub> polymorphism of the CFTR gene is one of the first to be carried out in the Tunisian asthmatic population. We conclude that this study failed to show that TG<sub>m</sub>T<sub>n</sub> significantly influences the clinical expression of asthma in the Tunisian population. Nevertheless, the results obtained will provide a better understanding of the molecular basis of this multifactorial disease.

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## Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or with comparable ethical standards.

## Informed consent

Informed consent was obtained from all individual participants included in the study.

## Disclosure of interest

The authors declare that they have no competing interest.

## References

- [1] Beasley R, Keil U, Von Mutius E, Pearce N. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. *Lancet* 1998;351:1225–32.
- [2] Ahmed R, Robinson R, Mortimer K. The epidemiology of noncommunicable respiratory disease in sub-Saharan Africa, the Middle East, and North Africa. *Malawi Med J* 2017;29(2):203–11.
- [3] Toujani S, Mjid M, Ouahchi Y, et al. Prevalence of asthma and rhinitis in a Tunisian population. *Clin Respir J* 2018;12(2):608–15.
- [4] Laitinen LA. Comparative clinical data in mild asthma populations. *Eur Respir Rev* 1997;7:313–5.
- [5] Meyers DA, Larj MJ, Lange L. Genetics of asthma and COPD similar results for different phenotypes. *Chest* 2004;126:105S–10S.
- [6] Maurya N, Awasthi S, Dixit P. Association of CFTR gene mutation with bronchial asthma. *Indian J Med Res* 2012;135:469–78.
- [7] Wang Y, Wrennall JA, Cai Z, Li H, Sheppard DN. Understanding how cystic fibrosis mutations disrupt CFTR function: from single molecules to animal models. *Int J Biochem Cell Biol* 2014;52:47–57.
- [8] The Clinical and Functional TRanslation of CFTR (CFTR2). <http://cftr2.org>. Accessed: 15th December 2015.
- [9] Groman JD, Hefferon TW, Casals T, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. *Am J Hum Genet* 2004;74(1):176–9.
- [10] Giselda MK, Cabello Pedro H, Cabello Juan C, Llerena JR, Octavio F. Polymorphic Markers Suggest a Gene Flow of CFTR Gene from Sub-Saharan/Arabian and Mediterranean to Brazilian Population. *J Hered* 2006;97(4):313–7.
- [11] Wang P, Satoru N, Hong Y, et al. The susceptibility of T5-TG12 of the CFTR gene in chronic bronchitis occurrence in a Chinese population in Jiangsu province, China. *J Biomed Res* 2012;26(6):410–7.
- [12] Milosevic K, Nikolic A, Divac Rankov A, Ljubic M, Nestorovic B, Radojkovic D. Analysis of CFTR gene variants in idiopathic bronchiectasis in Serbian children. *Pediatr Allergy Immunol Pulmonol* 2013;26(2):93–8.
- [13] Jiang L, Jin J, Wang S, et al. CFTR gene mutations and polymorphism are associated with non-obstructive azoospermia: From case-control study. *Gene* 2017;626:282–9.
- [14] Ghorbel M, Baklouti-Gargouri S, Keskes R, et al. Screening of DF508 mutation and IVS8-poly T polymorphism in CFTR gene in Tunisian infertile men without CBAVD. *Andrologia* 2012;44:376–82.
- [15] Boudaya M, Fredj SH, Haj RB, et al. Cystic fibrosis transmembrane conductance regulator mutations and polymorphisms associated with congenital bilateral absence of vas deferens in a restricted group of patients from North Africa. *Ann Hum Biol* 2012;39(1):76–9.
- [16] American Lung Association. Lung Disease Data: 2008. New York, NY: American Lung Association; 2008. [http://www.lung.org/assets/documents/publications/lung-disease-data/LDD\\_2008.pdf](http://www.lung.org/assets/documents/publications/lung-disease-data/LDD_2008.pdf). Accessed 7 January 2012.
- [17] Miller SA, Dyk DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16(3):1215.
- [18] Romdhane L, Abdelhak S. Research Unit on Molecular Investigation of Genetic Orphan Diseases Collaborators. Genetic diseases in the Tunisian population. *Am J Med Genet* 2011;155A:238–67.
- [19] Kieseewetter D, Macek M, Davis C, et al. A mutation in CFTR produces different phenotypes depending on chromosomal background. *Nat Genet* 1993;5:274–8.
- [20] Chu CS, Trapnell BC, Currstin S, Cutting GR, Crystal RG. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet* 1993;3:151–6.
- [21] Chiang HS, Lu JF, Liu CH, Wu YN, Wu CC. CFTR (TG)m(T)n polymorphism in patients with CBAVD in a population expressing low incidence of cystic fibrosis. *Clin Genet* 2009;76:282–6.
- [22] Niksic M, Romano M, Buratti E, Pagani F, Baralle FE. Functional analysis of cis-acting elements regulating the alternative splicing of human CFTR exon 9. *Hum Mol Genet* 1999;8:2339–49.
- [23] Radpour R, Gourabi H, Gilani MA, Dizaj AV. Molecular study of (TG)m(T)n polymorphisms in Iranian males with congenital bilateral absence of the vas deferens. *J Androl* 2007;28:541–7.
- [24] Jin CX, Fujiki K, Song Y, Ping Z, et al. CFTR polymorphisms of healthy individuals in two chinese cities-changchun and nanjing. *Med Sci* 2012;74:293–301.
- [25] Chillón M, Casals T, Mercier B, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of vas deferens. *N Engl J Med* 1995;332:1475–80.
- [26] Weiner-Miller P, Hamosh A, Macek M, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 1996;59:45–51.
- [27] Munthe-Kaas MC, Lødrup Carlsen KC, Carlsen KH, et al. CFTR gene mutations and asthma in the Norwegian Environment and Childhood Asthma study. *Respir Med* 2006;100:2121–8.
- [28] Kyung Won K, Ji Hyun L, Min Goo L, Kyung Hwan K, Myung Hyun S, Kyu-Earn K. Association between cystic fibrosis transmembrane conductance regulator gene mutations and susceptibility for childhood asthma in Korea. *Yonsei Med J* 2010;51(6):912–7.
- [29] Tzetzis M, Efthymiadou A, Strofalis S, et al. CFTR gene mutations - including three novel nucleotide substitutions and haplotype background in patients with asthma, disseminated bronchiectasis and chronic obstructive pulmonary disease. *Hum Genet* 2001;108:216–21.
- [30] Hakonarson H, Bjornsdottir US, Ostermann E, Arnason T, et al. Allelic frequencies and patterns of single-nucleotide polymorphisms in candidate genes for asthma and atopy in Iceland. *Am J Respir Crit Care Med* 2001;164:2036–44.
- [31] Dahl M, Nordestgaard BG, Lange P, Tybjaerg-Hansen A. Fifteen-year follow-up of pulmonary function in individuals heterozygous for the cystic fibrosis phenylalanine-508 deletion. *J Allergy Clin Immunol* 2001;107:818–23.
- [32] Groman JD, Hefferon TW, Casals T, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. *Am J Hum Genet* 2004;74:176–9.