

## GENETIC DETERMINANTS

**IL-17A and IL-17F genes variants and susceptibility to childhood asthma in Tunisia**

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**Abstract**

**Objectives:** IL-17A and IL-17F are new pro-inflammatory cytokines implicated in neutrophilic inflammation and thus, involved in the pathogenesis of asthma. We investigated the possible association among asthma and IL-17A -197G/A (rs2275913), IL-17F 7488A/G (rs763780) and IL-17F 7383A/G (rs2397084).

**Methods:** The study was performed in 171 patients with asthma (mean age 9.5 years, 105 boys, and 66 girls) and 171 healthy individuals matched with patients in age and sex. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect genes' polymorphisms.

**Results:** IL-17A -197G/A and IL-17F 7383A/G were associated with asthma in children ( $p = 0.008$ ,  $p = 0.001$ , respectively). No association was found with IL-17F 7488A/G polymorphism. Haplotype analysis revealed a significant association between GA and AG haplotypes and asthma ( $p = 0.004$ ,  $p = 0.02$ ). When patients were stratified according to the atopic status, no significant association was detected with any of the three studied variants.

**Conclusion:** Our results suggested that SNPs in IL-17A and IL-17F confer susceptibility to childhood asthma in Tunisia.

**Keywords**

IL-17, polymorphism, airway inflammation, case-control study

**History**

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**Introduction**

Asthma is a common disease associated with a chronic airway inflammation that is characterized by intense eosinophil and lymphocyte infiltration, mucus hypersecretion, airway hyper-responsiveness (AHR), and airway remodeling. Increased disease severity is often coincided by the additional influx of neutrophils. The role of specific T cells and their cytokines in the pathogenesis of allergic asthma is now well recognized [1]. Accumulating evidence indicates that Th2 cells and their derived cytokines such as interleukin (IL)-4, IL-5, and IL-13 play critical roles in orchestrating and amplifying the allergic inflammation in asthma. However, recent studies have shifted attention toward Th17 cells as being responsible for the pathological processes [2,3].

Th17 cell is a lineage distinct from Th1, Th2, and Treg cells characterized by the production of IL-17. Until now, the IL-17 family contains six members, referred to as IL-17A (also called IL-17), IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F, according to the order in which they were discovered [4]. These molecules have similar molecular weight of 20–30 kDa and have overlapping, but not identical, biological activities. IL-17A and IL-17F are the members of the IL-17 cytokine

family specifically expressed by Th17 and responsible for their pathogenic activity [5]. These two cytokines share the highest protein sequence homology among IL-17 family (approximately 50%) [6], as well as a common receptor (IL-17 receptor A/IL-17 receptor C complex) [7], share the same genes locations on chromosome 6p12 [6], and have similar functions [8,9].

Recent studies have evaluated the expression of IL-17 in chronic inflammatory disorders. An increase in IL-17 levels has been reported in rheumatoid arthritis [10], multiple sclerosis [10], and chronic obstructive pulmonary disease (COPD) [11]. In patients with established asthma, IL-17 mRNA and/or proteins were reported to be increased in the lungs, induced sputum, bronchoalveolar lavage (BAL) fluids, or sera [1,12–15] and the levels of IL-17 correlated with the degree of disease severity [1,14] suggesting the role of this cytokine in airway remodeling. Supporting those observations, functional analysis indicated that both IL-17A and IL-17F are able to stimulate bronchial fibroblasts, epithelial cells, and smooth muscle cells and induce the expression of a variety of cytokines and chemokines including IL-8, IL-6, IL-11, and CXCL1/Gro $\alpha$ , which are important for granulopoiesis and neutrophil recruitment [16]. The ability of IL-17A and IL-17F to induce neutrophils migration suggested that they are involved in severe asthma, in which accumulation of neutrophils in the airways is a major characteristic [17]. The association between IL-17A and IL-17F and neutrophilic

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inflammation was also confirmed by murine asthma models [18,19]. However, the direct regulatory effect and mechanism of this cytokine on eosinophilic airway inflammation are somewhat complex to decipher. Recent studies using murine asthma models showed the role of these two cytokines in enhancing eosinophilic inflammation during the sensitization phase. In fact, mice deficient in IL-17 or IL-17 receptor have a reduced ability to recruit eosinophils into the airways [20,21] due to their low expression of Th2 cytokines such as IL-4 and IL-5 necessary for eosinophils attraction [22]. It appears, therefore, that, at the sensitization phase, IL-17 promotes eosinophilic airway inflammation by mounting allergen-specific Th2 cell responses. These findings were confirmed by the study of Wakashin and colleagues [23] using adoptive transfer experiments. With regard to mild and moderate asthma, in which eosinophilic inflammation is the main feature, IL-17 plays the role of a negative regulator [20], suggesting that high IL-17 levels could inhibit eosinophilic inflammation at the effector phase.

These various actions of IL-17 suggest that this cytokine may play different roles in the airways, depending on the context and the timing. The role of IL-17 in eosinophilic inflammation of mild and moderate asthma should be more investigated. Genetic studies using polymorphisms could present a way helping in demonstrating links between certain molecules and diseases like asthma, characterized by interaction between several genes in different chromosomes [24]. Our group has shown an association among genes in regions 12q [25], 11q [26], and 17q [27]. *IL-17A* and *IL-17F* genes are located on chromosome 6p, a genomic region linked to asthma and asthma-related phenotypes in multiple genome scans [28,29]. This region includes also the *VEGF* gene which has been previously demonstrated to be associated with asthma severity in our population [30]. Taken together, these data suggest that the *IL-17A* and *IL-17F* genes could be good candidates for susceptibility to mild and moderate asthma. A number of association studies have previously investigated these genes in different populations and ethnic groups [31–37], but due to their high polymorphic variation, these studies did not replicate the same SNPs.

In our study, we hypothesized that IL-17A and IL-17F could be implicated in mild and moderate asthma. Thus, we sought to investigate the association of one SNP in *IL-17A* (-197G/A, rs2275913) and two SNPs in *IL-17F* gene (7488A/G, rs763780 and 7383A/G, rs2397084) with susceptibility to mild and moderate asthma. The -197G/A polymorphism was selected based on a study by Chen et al [32] that showed an association between this SNP and several asthma-related traits in the Chinese population and also based on its localization in the promoter region of *IL-17A* gene. SNPs affecting the gene's promoter could modify a transcription factor binding site and this modification could influence the transcription rate of the gene. The two other polymorphisms studied in *IL-17F* gene are in the coding region at positions 7383 A/G and 7488 A/G. Both SNPs are localized in exon 3, caused the substitution of adenine to guanine in the DNA sequence, and leading change of amino acid in the protein sequence. The first non-synonymous SNP at position 7383A/G changes glutamic acid (GAG) to glycine (GGG) at amino acid 126 (Glu126Gly), while the second SNP at position 7844A/G

changes histidine (CAT) to arginine (CGT) at amino acid 161 (His161Arg, H161R) and has been demonstrated to be associated with asthma, and with impaired IL-17F signaling *in vitro* [34]. These two SNP were selected based on a study by Paradowska-Gorycka et al. [38].

## Subjects and methods

### Subjects

The study was conducted in a group of 171 patients (105 boys and 66 girls, mean age 9.2 years) with bronchial asthma recruited from the Department of Pediatric and Respiratory Diseases, Abderrahmane Mami Hospital of Chest Diseases, Ariana, Tunisia. Asthma diagnosis was established according to GINA (The Global Initiative For Asthma) recommendations [39], based on clinical asthma symptoms and lung function test. The level of asthma severity was determined on the basis of GINA Report Guidelines. Clinical diagnosis of atopy was determined based on skin sensitivity to specific allergens characterized by a weal of at least 3 mm in diameter, to one or more allergens in the presence of a positive histamine control and a negative uncoated control. Because IgE is a mediator of allergic response, quantitative measurement of IgE, when integrated with other clinical indicators, can provide useful information for the differential clinical diagnosis of atopic and non-atopic asthma. Serum IgE levels were estimated in all the patients (BECKMAN, Access Immunoassay System, FR, Brea, CA) and a cut-off point of 200 IU/ml was used to determine total serum IgE elevation based on a study by Anupama et al. [40]. All patients were given corticosteroids at doses depending on their severity stage.

The control group consisted of 171 healthy subjects (106 boys and 65 girls, mean age 9.5 years) matched in age and sex with patients and recruited from the emergency Department of Tunis Children Hospital for only pathologies related to accidents of daily life such as fractures. All controls were free of any infectious symptoms. The inclusion criteria for the control group were as follows: (i) no symptoms or history of asthma, (ii) no symptoms or history of other pulmonary diseases, (iii) no symptoms or history of allergy and (iv) no symptoms or history of any chronic disease. Since spirometry and prick tests were not practiced in emergency department, only a randomized representative sample of 59 children was invited to Abderrahmane Mami Hospital of Chest Diseases to realize these two tests. For the rest of the controls, although no lung function or skin prick tests were available, any allergic disease or asthma was excluded based on the clinical examination and IgE measurements ( $\geq 200$  IU/ml).

The characteristics of the study sample have been presented in Table 1. All participants as well as their parents have given written informed consent. The local ethics committee accepted the project.

### SNP genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the salting out procedure as described [41]. The investigated polymorphisms were analyzed using the polymerase chain reaction-restriction fragment length

polymorphism (PCR-RFLP) method. Primer sequences used for amplification of rs2275913 (*IL-17A*) and rs763780, rs2397084 (*IL-17F*) polymorphisms are listed in Table 2.

The PCR was performed in a 50 µl reaction volume containing 2.4 µM of each primer, 10 mM of each dNTP, 1 × PCR buffer, 1.5 mM MgCl<sub>2</sub>, 2U of Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania), and 100 ng of genomic DNA, diluted to the final volume with H<sub>2</sub>O. The running conditions of PCR were as described previously [32,38]. Amplified fragments were digested with the appropriate restriction enzyme (Fermentas) according to the manufacturer's instructions and visualized on a 3% agarose gel (Table 2). In order to test the quality of genotyping, we performed a control quality test by randomly genotyping 10% of the sample. The results were the same as in the first genotyping.

### Statistical analysis

Hardy–Weinberg equilibrium was tested in cases and controls separately. Association analysis was performed using standard Chi-squared test (Epistat Statistical Package, Epi Info Version 6, Epistat Services, Richardson, TX) and Fisher's exact test to detect differences in genotypes and alleles distribution among our groups. A *p* value of 0.05 or less was considered statistically significant. The strength of a gene

association is indicated by the odds ratio (OR) with a 95% confidence interval (CI). The odds ratio and the 95% confidence intervals (CI) were calculated whenever applicable. Haplotypes analyses were determined on a Bayesian algorithm basis using the “phase” software, and the frequency differences of haplotypes in asthmatic patients and healthy controls were compared using the  $\chi^2$  test with Yates's correction. An adjustment for multiple tests by Bonferroni correction in which the *p* values are multiplied by the number of comparisons was later applied to control the false finding rate.

### Results

#### The association of *IL-17A* and *IL-17F* genes variants with childhood asthma

The distribution of genotypes and alleles frequencies of *IL-17A* and *IL-17F* genes polymorphisms in cases and control subjects is shown in Table 3. The genotype frequencies of the three SNPs were in agreement with Hardy–Weinberg equilibrium. A positive association was detected for -197G/A (*IL-17A*) and 7383A/G (*IL-17F*) polymorphisms with susceptibility to asthma in Tunisian children. It is important to note a dominance of the wild-type allele compared to the mutant allele both in cases and in controls groups. Therefore, the three polymorphisms were tested under the recessive model (homozygous for the wild type allele versus heterozygous and homozygous for the mutant allele).

With regard to the *IL-17A* -197G/A polymorphism, our data showed a significant difference in the distribution of genotypes ( $\chi^2 = 6.84$ , *p* = 0.008) and alleles frequencies ( $\chi^2 = 8.75$ , *p* = 0.003) between cases and controls. The “G” allele was significantly related to the increasing risk of developing childhood asthma (OR = 1.89, 95% CI = 1.21–2.97). In addition, children with homozygous “G” allele were 1.88 times more likely to have asthma than others (95% CI = 1.14–3.10).

For *IL-17F* 7488A/G polymorphism, no difference was observed in genotypes distribution and alleles frequencies between cases and controls.

For *IL-17F* 7383A/G polymorphism, the frequency of “GG” and “AG” genotype in the asthmatic group was 18.1%, which was significantly higher than the corresponding value for the control group (7%) ( $\chi^2 = 9.6$ , *p* = 0.001). The OR was calculated, and carriers with one or two copies of “G” allele were 2.93 times as likely to develop asthma (OR = 2.93, 95% CI = 1.39–6.31). In addition, the alleles

Table 1. Clinical characteristics of the study sample.

|                                | Asthmatics    | Healthy controls |
|--------------------------------|---------------|------------------|
| Number of subjects             | 171           | 171              |
| Mean age (range)               | 9.2 (4–16)    | 9.5 (5–16)       |
| Sex ratio (girls/boys)         | 0.62 (66/105) | 0.6 (65/106)     |
| Total frequency of females (%) | 38.6%         | 38%              |
| Total frequency of males (%)   | 61.4%         | 62%              |
| Atopic status                  |               |                  |
| Atopics ( <i>n</i> , %)        | 107 (69.5%)   | –                |
| Non-atopics ( <i>n</i> , %)    | 47 (30.5%)    | 171              |
| Total IgE (IU/ml)              |               |                  |
| In atopics                     | 637.4         | –                |
| In non-atopics                 | 59.8          | 62.3             |
| Severity                       |               |                  |
| Mild ( <i>n</i> , %)           | 97 (56.7%)    | –                |
| Moderate ( <i>n</i> , %)       | 65 (38%)      | –                |
| Severe ( <i>n</i> , %)         | 9 (5.3%)      | –                |
| FEV1%, predicted               |               | 102.7            |
| Mild asthma                    | 85.75         |                  |
| Moderate asthma                | 71.25         |                  |
| Severe asthma                  | 52            |                  |

FEV1, forced expiratory volume in 1 s; IgE, immunoglobulin E.

Table 2. Different loci selected within the *IL-17 A/F* sequence and their corresponding primers.

| Gene   | SNP                            | PCR primer                                                          | PCR product (bp) | Restriction enzyme      | RFLP products (bp)                                                           |
|--------|--------------------------------|---------------------------------------------------------------------|------------------|-------------------------|------------------------------------------------------------------------------|
| IL-17A | -197 G/A (rs2275913)           | F: 5'-CAGAAGACCTACATGTTACT-3'<br>R: 5'-GTAGCGCTATCGTCTCTCT-3'       | 344-bp           | Xmn-I (Pdm-I) (37 °C)   | GG: 344 bp<br>GA: 344, 213, and 131 bp<br>AA: 213 and 131 bp                 |
| IL-17F | 7488 A/G His161Arg (rs763780)  | F: 5'-GTGTAGGAACCTGGGCTGCATCAAT-3'<br>R: 5'-AGCTGGGAATGCAAACAAAC-3' | 470-bp           | NlaIII (Hin1II) (37 °C) | AA: 288, 130, and 52 bp<br>AG: 418, 288, 130, and 52 bp<br>GG: 418 and 52 bp |
|        | 7383 A/G Glu126Gly (rs2397084) |                                                                     |                  | AvaII (Eco47I) (37 °C)  | AA: 470 bp<br>AG: 470, 395 and 75 bp<br>GG: 395 and 75 bp                    |

frequencies were significantly different, indicated by a 10.2% prevalence of “G” allele in cases and 3.5% in controls ( $p=0.0005$ ). The odd ratio was calculated, and children carrying “G” allele are 3.14 times more at risk to develop asthma (OR = 3.14, 95% CI = 1.54–6.51) than healthy individuals.

### Haplotype distribution of IL-17F genetic variants

To achieve a comprehensive genetic association analysis, we determined the linkage disequilibrium (LD) pattern within *IL-17F*. A significant LD was detected between the two SNPs tested,  $D' = 0.47$  and  $r^2 = 0.09$  in patients and  $D' = 0.45$  and  $r^2 = 0.08$  in controls. We have also estimated haplotypes for the *IL-17F* polymorphisms and haplotypes frequencies are depicted in Table 4. The AA haplotype in *IL-17F* gene was present with the highest frequency, but the difference between asthmatics and controls was not statistically significant ( $p=0.54$ ). However, the GA haplotype was more frequently detected in asthmatics than in controls (7.6% and 2%, respectively) ( $p=0.004$ ) and thus represent the risk haplotype. In contrast, the AG haplotype was more frequently detected in controls than asthmatics ( $p=0.02$ ) and thus represent the protective haplotype. Interestingly, these results support our genotypic results, since A and G alleles of His161Arg polymorphism had no effect on the asthmatic state; G allele of Glu126Gly SNP is the risk allele and lead to a risk haplotype GA, and A allele is protective and leads to a protective haplotype AG.

### Stratification according to atopic status

We also analyzed the correlation of these polymorphisms with atopic status (Table 5). The genotypes and the alleles frequencies of the three *IL-17* polymorphisms did not differ

significantly between the atopic and non-atopic asthmatic groups ( $p=0.21$  for *IL-17A* -197 G/A,  $p=0.36$  for *IL-17F* 7488 A/G and  $p=0.83$  for *IL-17F* 7383 A/G).

### Discussion

Inflammation is the main feature of asthma, characterized by an increased function of immune and resident cells, which released various proinflammatory cytokines such as IL-13, IL-6, IL-18, IL-8, and TNF- $\alpha$ , and contributed to the development of diverse clinical manifestations in airways. Polymorphisms in these cytokines may be associated with susceptibility to asthma and thus, cytokines genes present good candidates for the investigation. In this case-control study, we attempted to clarify the association between -197G/A polymorphism in *IL-17A* and 7488A/G, 7383A/G polymorphisms in *IL-17F* and childhood asthma. A summary of the literature verifying the association between single nucleotide polymorphisms (SNPs) in *IL-17A* and *IL-17F* genes and asthma is presented in Table 6.

With regard to *IL-17A* gene, our study detected a significant association between -197G/A variant and asthma but failed to detect any association with atopy. This result is consistent with that reported in the Chinese population [32] but not in the Taiwanese one [37] despite the common genetic background shared by the two populations. The discrepancy may be explained by some intra-ethnic variations. Further studies are needed to clarify the association of this polymorphism in Caucasians. Interestingly, other SNPs in *IL-17A* gene were demonstrated to be associated with asthma in different populations [31,33,37].

With regard to *IL-17F* gene, we were unable to detect any association between 7488A/G polymorphism and asthma in children. The implication of this polymorphism in

Table 3. Frequency of alleles and distribution of genotypes of *IL-17A* and *IL-17F* polymorphisms in asthmatic and control subjects.

| SNP             | Genotypes         |                 |          |         |                  | Alleles           |                 |          |         |                  |
|-----------------|-------------------|-----------------|----------|---------|------------------|-------------------|-----------------|----------|---------|------------------|
|                 | Asthmatics, n (%) | Controls, n (%) | $\chi^2$ | p Value | OR 95% CI        | Asthmatics, n (%) | Controls, n (%) | $\chi^2$ | p Value | OR 95% CI        |
| IL-17A -197 G/A |                   |                 |          |         |                  |                   |                 |          |         |                  |
| GG              | 132 (77)          | 110 (64.3)      | 6.84     | 0.008   | 1.88 (1.14–3.10) | G 303 (88.6)      | 275 (80)        | 8.75     | 0.003   | 1.89 (1.21–2.97) |
| GA              | 39 (23)           | 55 (32.2)       |          |         |                  | A 39 (11.4)       | 67 (20)         |          |         |                  |
| AA              | 0 (0)             | 6 (3.5)         |          |         |                  |                   |                 |          |         |                  |
| IL-17F 7488 A/G |                   |                 |          |         |                  |                   |                 |          |         |                  |
| AA              | 155 (90.6)        | 145 (84.8)      | 2.71     | 0.09    | –                | A 326 (95)        | 313 (91.5)      | 4.02     | 0.06    | –                |
| AG              | 16 (9.4)          | 23 (13.4)       |          |         |                  | G 16 (5)          | 29 (8.5)        |          |         |                  |
| GG              | 0 (0)             | 3 (1.8)         |          |         |                  |                   |                 |          |         |                  |
| IL-17F 7383 A/G |                   |                 |          |         |                  |                   |                 |          |         |                  |
| AA              | 140 (81.9)        | 159 (93)        | 9.6      | 0.001   | 2.93 (1.39–6.31) | A 307 (89.8)      | 330 (96.5)      | 12.09    | 0.0005  | 3.14 (1.54–6.51) |
| AG              | 27 (15.8)         | 12 (7)          |          |         |                  | G 35 (10.2)       | 12 (3.5)        |          |         |                  |
| GG              | 4 (2.3)           | 0 (0)           |          |         |                  |                   |                 |          |         |                  |

Table 4. Haplotypes frequencies of *IL-17F*.

| Haplotype | Cases (n, %) | Controls (n, %) | OR (95% CI)      | p Value | p Value c |
|-----------|--------------|-----------------|------------------|---------|-----------|
| AA        | 300 (87.7)   | 306 (89)        | –                | 0.54    | –         |
| GA        | 26 (7.6)     | 7 (2)           | 3.7 (1.6–8.5)    | 0.001   | 0.004*    |
| AG        | 7 (2)        | 23 (7)          | 3.29 (1.42–7.59) | 0.005   | 0.02*     |
| GG        | 9 (2.7)      | 6 (2)           | –                | 0.6     | –         |

\*Signification, the number of tests for Bonferroni correction is 4.

Table 5. Distribution of different genotypes and alleles for the *IL-17A* and *IL-17F* polymorphisms in the atopic and non-atopic groups of asthmatic children.

| SNP             | Genotypes                              |                                           |          |         | Alleles                     |                                 |          |         | OR | 95% CI |
|-----------------|----------------------------------------|-------------------------------------------|----------|---------|-----------------------------|---------------------------------|----------|---------|----|--------|
|                 | Atopic asthmatics,<br>n (%)<br>N = 107 | Non-atopic asthmatics,<br>n (%)<br>N = 47 | $\chi^2$ | p Value | Atopic asthmatics,<br>n (%) | Non-atopic asthmatics,<br>n (%) | $\chi^2$ | p Value |    |        |
| IL-17A -197 G/A |                                        |                                           |          |         |                             |                                 |          |         |    |        |
| GG              | 83 (77.6)                              | 32 (68)                                   | 1.55     | 0.21    | G 190 (89)                  | 79 (84)                         | 1.33     | 0.24    | –  |        |
| GA              | 24 (22.4)                              | 15 (32)                                   |          |         | A 24 (11)                   | 15 (16)                         |          |         |    |        |
| AA              | 0 (0)                                  | 0 (0)                                     |          |         |                             |                                 |          |         |    |        |
| IL-17F 7488 A/G |                                        |                                           |          |         |                             |                                 |          |         |    |        |
| AA              | 99 (92.5)                              | 41 (87)                                   | –        | 0.36    | A 206 (96)                  | 88 (94)                         | –        | 0.37    | –  |        |
| AG              | 8 (7.5)                                | 6 (13)                                    |          |         | G 8 (4)                     | 6 (6)                           |          |         |    |        |
| GG              | 0 (0)                                  | 0 (0)                                     |          |         |                             |                                 |          |         |    |        |
| IL-17F 7383 A/G |                                        |                                           |          |         |                             |                                 |          |         |    |        |
| AA              | 88 (82)                                | 38 (81)                                   | 0.04     | 0.83    | A 191 (89)                  | 85 (90.5)                       | 0.1      | 0.75    | –  |        |
| AG              | 15 (14)                                | 9 (19)                                    |          |         | G 23 (11)                   | 9 (9.5)                         |          |         |    |        |
| GG              | 4 (4)                                  | 0 (0)                                     |          |         |                             |                                 |          |         |    |        |

Table 6. A summary of the literature concerning the association between single-nucleotide polymorphisms (SNPs) in *IL-17A* and *IL-17F* genes and asthma.

| Gene   | SNP ID                 | Chromosome position | Population |         |     |           |          |           |           |           |
|--------|------------------------|---------------------|------------|---------|-----|-----------|----------|-----------|-----------|-----------|
|        |                        |                     | Tunisian   | Saudian | NHS | Taiwanese | Japanese | Chinese 1 | Chinese 2 | Chinese 3 |
| IL-17A | rs17878530             | 52054045            | –          | NS      | –   | –         | –        | –         | –         | –         |
|        | rs17880588             | 52054024            | –          | <0.05   | –   | –         | –        | –         | –         | –         |
|        | rs4711998              | 52050353            | –          | –       | –   | NS        | –        | –         | –         | NS        |
|        | rs8193036              | 52050493            | –          | –       | –   | <0.05     | –        | –         | –         | NS        |
|        | rs3819024              | 52050786            | –          | –       | –   | NS        | –        | –         | –         | <0.05     |
|        | rs2275913 <sup>a</sup> | 52051033            | <0.05      | –       | –   | NS        | –        | <0.05     | –         | –         |
|        | rs3819025              | 52051274            | –          | –       | –   | NS        | –        | –         | –         | NS        |
|        | rs8193038              | 52051382            | –          | –       | –   | NS        | –        | –         | –         | –         |
|        | rs3804513              | 52053197            | –          | –       | –   | NS        | –        | –         | –         | NS        |
|        | rs1974226              | 52055335            | –          | –       | –   | NS        | –        | –         | –         | NS        |
|        | rs3748067              | 52055339            | –          | –       | –   | NS        | –        | –         | –         | –         |
|        | rs1889570              | 52110734            | –          | –       | –   | –         | NS       | –         | –         | –         |
|        | rs1266828              | 52108062            | –          | –       | NS  | –         | NS       | –         | –         | NS        |
|        | rs722323               | 52106860            | –          | –       | NS  | –         | –        | –         | –         | –         |
|        | rs7771511              | 52104182            | –          | –       | –   | –         | <0.05    | –         | –         | –         |
| IL-17F | rs766748               | 52102899            | –          | –       | –   | –         | NS       | –         | –         | –         |
|        | rs2397084 <sup>a</sup> | 52101844            | <0.05      | NS      | –   | –         | –        | –         | –         | –         |
|        | rs11465553             | 52101758            | –          | NS      | NS  | –         | –        | –         | –         | –         |
|        | rs763780 <sup>a</sup>  | 52101739            | NS         | NS      | NS  | –         | <0.05    | –         | <0.05     | –         |
|        | rs641701               | 52101165            | –          | –       | NS  | –         | –        | –         | –         | –         |
|        | rs13209590             | 52099651            | –          | –       | –   | –         | –        | –         | NS        | NS        |
|        | rs669161               | 52121015            | –          | –       | –   | –         | –        | –         | –         | <0.05     |

<sup>a</sup>SNP investigated in this study.

Tunisian: this study (171 asthmatic children + 171 healthy children). Saudian: Bazzi et al. [31] (100 asthmatics + 102 healthy individuals). Nurses' Health Study (NHS): Ramsey et al. [36] (511 asthmatics and 516 controls). Taiwanese: Wang et al. [37] (546 asthmatic children + 481 controls). Japanese: Kawaguchi et al. [42] (432 asthmatics (mean age = 47.16 years) from Hokkaido + 435 controls). Chinese 1: Chen et al. [32] (168 asthmatic children from Chongqing (mean age = 5.23 years) + 205 controls). Chinese 2: Qian et al. [35] (318 asthmatics (aged 14–75 years) from Nanjing + 352 controls). Chinese 3: Wang et al. [33] (197 allergic rhinitis with comorbid asthma from Beijing China (mean age = 24.6 years) + 281 controls).

susceptibility to asthma varies between different ethnic groups. Caucasian populations [31,36] which investigated this SNP failed like as to demonstrate any association with the disease; however, studies in some Asiatic populations [34,35] reported significant association. The inter-ethnic discrepancy may be due, not only to differences in the genetic background but also to differences in environmental exposure. Gene–environment interactions most likely differ between populations. For 7383A/G polymorphism, our results indicated that G allele was positively associated with asthma. Children with G allele are 3.14 times more likely to have asthma than others.

Moreover, GA haplotype is associated with an increased risk of asthma with an OR of 3.7 (CI = 1.6–8.5). These findings showed that this *IL-17F* variant may be incriminated in the development of the disease and should be investigated in other association studies. Although the Saudian and the European American (NHS) populations failed to detect any association between some investigated *IL-17F* variants and asthma [31,36], a positive correlation was reported with rs7771511 in the Japanese population and with rs669161 in the Chinese population. Further replication studies will be more informative. Interestingly, our study shows similarity in

the genetic background of the Tunisian population with Caucasian and even with Asiatic populations which prove that Tunisians, like the north-Africans, are a mosaic of subpopulations.

Genetic polymorphisms in coding or non-coding regions may have important functional consequences. These variations are able to influence either the expression or the activity of the resulting protein and thus, the susceptibility or severity of a number of disorders will be influenced by possession of specific alleles of polymorphic genes.

Regarding IL-17F activity, the induction of the IL-17F/IL-17F receptor axis needs that IL-17F forms a dimer complex with either IL-17A or IL-17F, binds with high affinity to its receptor (IL-17RA/IL-17RC), and finally induces the activation of the signaling pathway involving Raf-1, mitogen-activated protein kinase (MEK) 1 and 2, and extracellular signal-regulated kinase (ERK) 1 and 2 [42]. Any defect in this axis may alter IL-17F activity. Using recombinant wild-type and mutant IL-17F proteins, Kawaguchi et al. [34] revealed functional consequences of the H161R substitution which is caused by 7488A/G polymorphism. They demonstrated that compared to the wild-type IL-17F, the mutant variant was unable to activate the signaling pathway and to induce the production of cytokines and chemokines such as IL-6, IL-8, and GM-CSF in bronchial epithelial cells. Since IL-17RA and IL-17RC have different tissue expressions, the roles of IL-17F may be tissue-specific and the impact of this polymorphism may consequently change in other IL-17F target cells like fibroblasts or vein endothelial cells. In contrast, there has been no study evaluated the functional impact of 7383A/G or -197G/A polymorphism on the pro-inflammatory activity of IL-17F or IL-17A.

Since IL-17 levels were reported to be increased in many diseases, alleles associated with elevated IL-17 levels may be considered as risk alleles. However, the functional influence of IL-17 variants on IL-17 production is still unclear. Considering IL-17F, Bazzi et al. [31] revealed that in Saudian asthmatic patients, carriers with 7488AA genotype express high IL-17F levels (750.295 pg/ml) than heterozygotes (42.336 pg/ml) ( $p < 0.002$ ) suggesting that the A allele could be considering as a risk allele. However, this result is not consistent with their findings in the healthy group (850.144 pg/ml for AA genotype versus 479.212 pg/ml for AG genotype,  $p > 0.05$ ) and with their genetic result where the 7488A/G polymorphism was not associated with asthma. The other investigated SNP (IL-17F 7383A/G) does not have any influence in the IL-17F levels. Considering IL-17A, the impact of -197G/A variant on IL-17A production is under debate. While Chen et al. [32] found that -197G/A polymorphism did not affect IL-17 expression level in induced peripheral blood mononucleated cell (PBMC), Espinoza et al. [43] reported that PBMCs with GG genotype express low IL-17 levels than cells with AA and AG genotypes. Interestingly, our results showed that GG genotype is a risk genotype for asthma. Taken together, these results suggest that patients with mild-to-moderate asthma and having GG genotype will have low IL-17 levels which enhance eosinophilic inflammation. Recently, Won Kim et al. [44] explained for the first time the mechanisms by which IL-17A variants

influence the expression of IL-17A. They found that IL-17A variants have allele-specific effects on the gene expression by modifying their affinities for transcription factor complexes or their CpG methylation profiles. However, the -197G/A polymorphism did not show any affinity for transcription factor complexes or methylation status suggesting that it may simply be a "linked SNP" rather than an SNP with its own functional consequences.

Recently, Wang et al. [33] draw attention to the importance of polymorphisms in the intergenic region between *IL-17A* and *IL-17F* gene. Results of his study support the indication that this region may confer genetic susceptibility for allergic rhinitis and allergic rhinitis associated with asthma in the Chinese population. Further investigations in other ethnicities will be useful to understand the role of these regions in asthma pathogenesis.

The eventual role of IL-17F in atopic asthma has been studied *in vitro* and *in vivo*. Kawaguchi et al. [6] found that cells from BAL fluid of asthmatic patients express IL-17F after allergen stimulation in contrast to saline-challenged cells. In addition, Oda et al. [19] demonstrated that the over expression of the *IL-17F* gene in an animal model of asthma provides an additive effect on Ag-induced allergic inflammatory responses, characterized by an increase in the levels of neutrophils and an increase in goblet cell hyperplasia and mucin gene expression. These findings suggest that IL-17F may have a role in atopic asthma, but not in non-atopic asthma. However, IL-17F-induced cytokines and chemokines attract neutrophils to the inflammation site and this neutrophilic inflammation is a characteristic of both atopic asthma in severe stages and non-atopic asthma [45]. In our study, we failed to demonstrate any association between studied variants in *IL-17A* and *IL-17F* and atopy, in contrast to the Japanese study which demonstrated that 7488A/G variant was significantly associated with both asthma and COPD, especially among atopic subjects [46]. Unfortunately, this study has not specified the kind of the implicated allergen. In Tunisia, acarins are the most common allergens detected in atopic asthmatics, but it does not mean that *IL-17F* or *IL-17A* polymorphisms increase the risk of allergy to those allergens.

Our results suggested that SNPs in *IL-17A* and *IL-17F* confer susceptibility to childhood asthma.

## Declaration of interest

The authors declare no conflicts of interest

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