Role of FOXP3 gene polymorphism in the susceptibility to Tunisian endemic Pemphigus Foliaceus

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ABSTRACT

Objective: Forkhead box P3 (*FOXP3*) is an essential and crucial transcription factor of regulatory T-cells. Genetic polymorphisms in the promoter region of *FOXP3* gene may alter the gene expression level and, therefore, contribute to several autoimmune diseases susceptibility. We aimed to investigate the possible role of genetic variants of four SNPs (rs3761547, rs3761548, rs3761549 and rs2294021) and a $(GT)_n$ microsatellite located in *FOXP3* gene in the susceptibility to Tunisian Pemphigus Foliaceus (PF).
Method: A case-control study was conducted on 98 patients with different clinical features of PF and 182 matched healthy controls using PCR-RFLP method.
Results: According to the epidemio-demographic features of the disease, patients were classified into two groups: an endemic group (n = 33, mean age = 31 [18–48]) versus a sporadic one (n = 65, mean age = 36 [19–84]). In the whole population, rs3761548, rs3761549 and rs2294021 were associated with the susceptibility to PF. Interestingly, significant differences of gene distributions between the two sub-groups of patients were observed. In the endemic group, all associations observed in the whole population were maintained and reinforced and a new association was revealed with rs3761547; while in the sporadic group, only the association with rs3761549 was conserved. Further, the haplotype analysis showed that the G-A-C-15-C risk haplotype was significantly much more expressed in PF patients and specially in the endemic group. The phenotype-genotype correlation revealed that the rs3761548 > AA genotype was significantly correlated with the severity of the disease including Nickolsky sign, generalized form of the disease and the earliest age onset.
Conclusion: These results underline the particular genetic background of the Tunisian endemic PF and suggest the implication of *FOXP3* gene in the susceptibility and the clinical course of the disease.

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1. Introduction

Pemphigus foliaceus (PF) is an autoimmune bullous skin disease, typically characterized by skin lesions in the upper epidermis. The pathogenesis of PF involves pathogenic auto-antibodies (-Abs) targeting an essential inter-keratinocyte adhesion protein, desmoglein 1 (Dsg1), leading to acantholysis and subsequent blister formation [1]. The production of anti-Dsg1 auto-Abs is dependent, not only on B lymphocytes, but also on Dsg1 specific Th lymphocytes and Th2 like cytokine profile [2]. Furthermore, recent evidence suggests the involvement of Th17 cells in the development of PF [3]. Indeed, a recent study has demonstrated an imbalance of Th17 and Treg cells in Pemphigus patients [4]. The aberrant function of Treg cells has also been described in the pathogenesis of several autoimmune diseases(AIDs) such as Pemphigus Vulgaris [5].

Treg cells are a unique subpopulation of CD4+T cells which play a crucial role in the maintenance of immune homeostasis. They are able to keep tolerance to self-antigens and to repress

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excessive immune responses by secreting suppressive cytokines or through a direct cell contact-dependent mechanism [6,7]. The phenotype of Treg cells is generally known as CD4+CD25hiFOXP3+ CTLA4+GITR+CD45RO+CD45RA−CD69−Ki67− [8,9].

Forkhead Box Protein3 (*FOXP3*),a member of transcription factor winged-helix family, is considered as a specific marker to distinguish Treg cells from the other T cells subpopulations [10,11]. It plays a key role in the control of Treg cells development and function. Several studies demonstrate that the continued expression of *FOXP3* in mature Treg cells is absolutely essential for keeping a dominant tolerance [12,13]. FOXP3 can also act as both a transcriptional activator and a repressor on *FOXP3*+ T cells through modulating the expression of *FOXP3*–bound genes [14,15].

The *FOXP3* gene is located on chromosomeXp11.23 within an area of AIDs linkage, thus strengthening its role as a putative susceptibility gene in those diseases; particularly disorders with a female predominance [16–18]. Sequence analysis reveals the *FOXP3* promoter as a highly conserved region. Thus, polymorphism in this region may change the binding specificity of this transcription factor leading to an aberrant expression of FOXP3 and nonfunctional Treg cells [19–21]. In fact, genetic polymorphism in *FOXP3* gene has been widely studied and proved to be implicated in the pathogenesis of several AIDs such as Systemic Lupus Erythematosus [18], but no study has been interested in PF.

In the light of these data, we aimed to analyze the polymorphism of *FOXP3* gene and evaluate its potential role in Tunisian PF. Four SNPs, rs3761547, rs3761548, rs3761549 and rs2294021and a (GT)$_n$ microsatellite polymorphism located in the promoter or in the intronic region of *FOXP3* gene were studied.

2. Material and methods

2.1. Subjects

A case-control study was performed on a total of 98PF patients and 182 healthy controls (HC). Since *FOXP3* gene is located on the X-chromosome, male subjects were excluded. The diagnosis of PF was confirmed by histopathology and direct immunofluorescence. All patients were found to be positive for circulatory anti-Dsg1 Abs.PF patients were recruited at the department of Dermatology in Hedi Chaker University Hospital of Sfax and were matched by age (±5 years), sex and geographical origin to HC who did not suffer from any autoimmune or inflammatory disease.

All samples were collected after obtaining a written informed consent which was approved by the ethical committee of Habib Bourguiba University Hospital of Sfax (protocol number of ethical committee, 4/12).

2.2. SNP selection

Tag Single Nucleotide Polymorphisms (tag SNPs) in the *FOXP3* gene were selected according to their association with the susceptibility to other AIDs and using the genotyping data from the CEU available from the International Hapmap project (Table 1). Selection was undertaken using minor allele frequency (MAF: greater than 5%) in Caucasian and sub-Saharian populations.

Four candidate SNPs; rs2294021 in the intronic region, rs3761549 (−2383C/T), rs3761548 (−3279C/A), rs3761547 (−3499A/G) and a (GT)$_n$ microsatellite in the promoter region, were selected (Table 1).

2.3. FOXP3 genotyping

Genomic DNA was extracted from whole blood samples using a standard proteinase K digestion and phenol/chloroform extraction procedure.

Genotyping was performed using PCR-RLFP method. The PCR amplification was carried out in a volume of 25 μl including 1× buffer,2 mM MgCl$_2$, 0.2–0.4 μmol of each primer,0.12 mM dNTP (Invitrogen®, CA, USA),1U Taq polymerase (Invitrogen®, CA, USA) and 50ηg of DNA template. Enzymatic digestion was performed in a total of 10 μl mixture reaction containing 1× buffer, 0.1× BSA and 2U restriction enzyme (Promega®, WI, USA). Primers were designed using primer3 software (http://primer3.ut.ee/). Restriction enzymes were selected using the NEBcutter software (http://nc2.neb.com/NEBcutter2/). All primers and enzymes used in this study are presented in Table 1.

The microsatellite locus was amplified with specific primers (Table 1) extracted from the genome browser (https://genome.ucsc.edu/). Forward primer was labeled with 6-FAM fluorescent labels. Amplified products were run on ABI prism DNA sequencer (Perkin–Elmer®, CT, USA) and the output file was analyzed using GeneScan softwares analysis.

2.4. Statistical analysis

A case-control analysis was performed using SHESIS software (http://analysis.bio-x.cn) for each SNP and haplotype. Hardy–Weinberg equilibrium (HWE) of each SNP was assessed in cases and controls separately using a χ2 test with one degree of freedom. A threshold P < 0.05 was regarded to indicate deviation from HWE. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for each allele using 2 × 2 contingency tables to estimate the magnitude of association. p ≤ 0.05 was considered statistically significant.

The linkage disequilibrium (LD) coefficients D' = D/Dmax and r^2 values for the pair of the most common alleles at each site were also estimated and high values of LD were defined as r^2 > 0.33 and D' > 0.7. SPSS 20.0 (IBM SPSS Inc., IL, USA) was used to determine the associations between SNP genotypes and different clinical features.

3. Results

3.1. Study populations

Ninety-eight PF patients with a mean age of 35 years (range, 18–64) matched to 182 HC with a mean age of 38 years (range, 14–73) were recruited at the department of Dermatology in the Hedi Chaker University Hospital of Sfax since 2002. All patients and controls originated from the center and south of Tunisia. In a previous study on epidemiologic, immunological and genetic features of Tunisian PF, we clearly showed that the characteristic endemic form occurs in the rural regions of the south and predominantly in the three localities of Moknessy (Sidi Bouzid), Jebenièna (Sfax) and Mereth (Gabès) [22,23]. Taking into account these data, we divided our patients in 2 groups: those coming from one of the three endemic localities (endemic group) and those coming from other regions (sporadic group) (Table 2).

3.2. SNP analysis

The genotypic and allelic distributions of the various polymorphisms in *FOXP3* among patients and controls, as well as their associations with the risk to PF are shown in Table 3.

Considering the whole population, no significant differences were found in the allelic and genotypic distribution for the rs3761547A > G polymorphism; while for the rs3761548A > C, the A allele was slightly overexpressed in patients (51.1%) compared to HC (42.1%) (p = 0.04, OR = 1.43, 95%CI [1–2.05]). Concerning the rs3761549C > T polymorphism, the C allele and its homozygote genotype exhibited a susceptibility role to PF (p = 0.01, OR = 2.06,