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Original article

T300A variant of AT16L1 gene in a cohort of Algerian Crohn disease patients

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ABSTRACT

The T300A variant is among the most Crohn's disease (CD) associated genetic variants. The aim of our study is to bring a first insight about the contribution of the T300A variant in a cohort of Algerian CD. In a case/control design, 118 Algerian CD patients and 161 unrelated healthy subjects were genotyped for the T300A variant using the allelic discrimination test by Applied Biosystems Taqman® genotyping technology. A serological analysis was carried out using Biosystems™ ELISA kit for the assessment of the anti-Saccharomyces cerevisiae antibodies and immunofluorimetry via Luminex® technology for the evaluation of cytokine levels (TNFα, IFNγ, IL-6 and IL-17). The comparison between allelic and genotypic frequencies was performed using the χ^2 test and the exact Fischer test. The odds ratio (OR) was noted adopting confidence interval of 95%. The comparison between the averages was carried out by the Mann-Whitney and Kruskal-Wallis tests. A factorial discriminant analysis and a binary logistic regression were performed as further analyses. The T300A variant showed an increased risk of CD within homozygous variant carriers ($P = 0.027$). Moreover, the carriage of the G allele was associated with the early onset of CD ($P = 0.01$) and a severe CD impairment ($P = 0.045$). We were not able to comfort the association of the T300A variant and ASCA IgA, ASCA IgG and IFNγ levels detected at the univariate analysis. Our results suggest a possible association between the T300A variant and CD in this cohort of Algerian CD patients. Moreover, this variant might be incriminated in the early onset of CD and a severe disease impairment. At the serological study, the univariate and the multivariate analyses yielded contradictory results. Further investigations of larger cohorts of Algerian CD are needed to better assess the suggested associations at the present study.

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1. Introduction

Crohn disease (CD) is a multifactorial chronic inflammatory disorder, which constitutes a subgroup of a large classification of disorders known as inflammatory bowel disease (IBD). To date, CD aetiopathogenesis remains unclear [1], although several genetic and environmental factors are involved in its onset and development.

Among these factors, the autophagic pathway genes have been incriminated. Autophagy is considered to be an important quality control mechanism, involved in the maintenance of cellular homeostasis [2]. Importantly, it also intervenes in both innate and adaptive immunity including mucin and anti-microbial peptide secretion as well as pathogen recognition and clearance [3–5]; considering as well its important role in intestinal cells, notably goblet cells, paneth cells, the gut-resident macrophages [3] and dendritic cells [6], a defective autophagic mechanism may be at the origin of CD development.

Advances in understanding the contribution of genetic variants in CD pathogenesis have demonstrated that deficiency in the

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autophagy-related 16-like 1 (ATG16L1) protein leads to impaired cellular autophagy, bacterial clearance [7] and increased pro-inflammatory cytokines production [8]. ATG16L1 is an adaptor protein composed of an N-terminal ATG5-biding region, a Coiled-coil domain involved in self-dimerization followed by 7 tryptophan-aspartic acid (WD40) repeat domains. It constitutes an essential component of the authophagic pathway involved in the autophagosome formation [2].

Hampe et al. (2007) performed a genome-wide association study of 19,779 non-synonymous single nucleotide polymorphisms (nsSNP) in 735 German CD cases and 368 controls [9]. They investigated 72 SNPs of the successfully genotyped SNPs with $P \leq 0.01$. The allele-based association study of German CD trios and singleton cases in addition to a British case-control study, allowed the identification of a strongly CD-associated nsSNP on the ATG16L1 gene located on chromosome 2q37.1: (rs2241880). This nsSNP encodes a threonine-to-alanine substitution at amino acid position 300 of the protein in the coiled-coil domain.

Promptly after the identification of the T300A variant as a strong CD risk factor, intense investigations have started to evaluate its genetic contribution within different population as well as its molecular mechanism in CD pathogenesis; most of the association studies replicated this finding [10–18], while the lack of association of T300A variant and CD in some populations incriminated cohort sizes and ethnic heterogeneity [19–23].

A recent study demonstrated that the amino-acids 296-299 constitute a Caspase cleavage domain motif in the ATG16L1, an upstream process which inhibits the maturation of the autophagosomal membrane resulting in a cellular stress, apoptotic stimuli and diminished autophagy; a functional crosstalk predisposing to CD [24].

Among all the association studies of the T300A and CD carried out during the last decade, only one study conducted in the North African population has been reported [25]. Therefore, the genetic background of the North African population regarding CD as well as the T300A variant is still obscure.

The first part of the present work is a case-control study, conducted on a modest cohort of Algerian CD for a first evaluation of the genetic variability of the ATG16L1.

Numerous studies have investigated the relationship between the T300A variant, autoimmunity and tissue inflammation in CD [7,26,27]. To our knowledge, only two studies have evaluated the effect of the T300A variant on systemic autoimmunity and systemic inflammation [27,28]. In this context, a second part of our work focused on the assessment of the association between this variant and the serum levels of the anti-saccharomyces cerevisiae antibodies (ASCA IgA and ASCA IgG) and the pro-inflammatory cytokines notably Tumor necrosis factor α (TNFα), Interferon γ (IFNγ), Interleukin 6 (IL-6) and Interleukin 17 (IL-17).

2. Materials and methods

2.1. Patients

In a case-control design, 118 Algerian-born CD patients were recruited randomly from two gastro-enterology departments in Algiers: Army Central Hospital and Mustapha Bacha University Hospital, the recruitment period extended from January 2014 to May 2015. Crohn's disease diagnosis was established according to the clinical, endoscopic, radiologic and histological criteria [29].

Demographical and clinical data were collected and confirmed CD patients were classified according to the recommendations of Montreal classification system [29].

2.2. Controls

One hundred and sixty-one unrelated healthy subjects, with no personal and/or family history of chronic inflammatory disorders, were recruited from the Immunology Laboratory staff and blood donation volunteers of Blood Transfusion Center of the Army Central Hospital.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent was obtained from all patients and control subjects for being included in the study.

2.3. DNA extraction

EDTA peripheral blood samples were obtained from both patients and controls. Genomic DNA was isolated using "QuickGene DNA whole blood kit (DB-S)" on Nucleic Acid Isolation System QuickGene Mini-80 (Kurabo industries LTD, Okasa/Tokyo, Japan) according to the manufacturer's instructions.

DNA quantification and quality control were assessed using Thermo Scientific NanoDrop™ 2000 Spectrophotometer.

2.4. Genotyping

The Genotyping procedure for the T300A was performed using the allelic discrimination assay by Applied Biosystems Taqman® genotyping technology (Applied Biosystems, Warrington, United Kingdom) based on the MGB specific allelic probes (code C__9095577_20) following the manufacturer's protocol. Polymerase chain reactions were carried out in 96-well plates on 7500 Real Time PCR System thermocycler for amplifying and detecting specific SNP alleles in purified genomic DNA samples. Allelic discrimination was performed automatically using 7500 software v2.3 system.

2.5. Serological analysis

The anti-Saccharomyces cerevisiae antibodies (ASCA IgA and ASCA IgA) were evaluated using Biosystems™ enzyme-linked immunosorbent assay (ELISA) kit and expressed in ELISA units (EU/ml); ASCA IgA and ASCA IgG levels higher than 20 EU/ml were considered positive.

Cytokines levels were assessed by immunofluorimetry via Luminex® technology on Luminex®100 automate, using FIDIS™ Human cytokine TNFα, FIDIS™ Human cytokine IFNγ, FIDIS™ Human cytokine IL-6 and FIDIS™ Human cytokine IL-17 for respectively: TNFα, IFNγ, IL-6 and IL-17. Cytokine levels were expressed in (pg/ml).

2.6. Statistical analysis

In order to measure the distribution of the polymorphisms, observed and expected frequencies were calculated and the Hardy-Weinburg Equilibirium test was performed separately for control group and CD. The comparisons between genotypic and allelic frequencies were performed using the Chi-square test (χ^2) or Fischer's exact test. Odds ratio were noted adopting a confidence interval of 95% and analyzed using binary logistic regression as a further analysis of the T300A associated odds. The comparisons between means were performed using Mann-Whitney test or Kruskal-Wallis test. A discriminant analysis was also carried out to evaluate the discriminant effect of clinical and demographical parameters associated with the T300A variant. The variables with a P value lesser than 0.05 were considered to indicate a statistically significant difference. Statistical analyses were performed using GraphPad Prim version 7 (GraphPad Software, San Diego, California, United States) and IBM® SPSS® Statistics V22.0 (IBM, United States, Software).

3. Results

3.1. Epidemiologic data

One hundred and eighteen Algerian-born CD patients were recruited within two gastro-enterology departments in Algiers: 50 (42.37%) patients from Central Hospital of Army with a sex-ratio M/F of 36/14 and 68 (57.62%) patients from University Hospital Mustapha Bacha with a sex-ratio M/F of 42/26. The classification of CD patients in terms of clinical and demographical parameters is shown on (Table 1). Our cohort comprises 78 (66.10%) males versus 40 (33.90%) females, with an average age of 34 ± 12.46 years old. The disease evolves since at least 5 years with an average age at diagnosis of 29.45 ± 11.08 years old. Additionally, we enrolled 161 unrelated healthy subjects, with an average age of 31.26 ± 10.74 years old and a sex-ratio M/F of 110/51.

3.2. The hardy-Weinburg equilibrium

All genotype frequencies of the ATG16L (T300A) variant, for both control subjects and Crohn disease patients, were consistent with the Hardy-Weinburg Equilibrium ($P > 0.05$).

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