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ORIGINAL ARTICLE

NOD2/CARD15 and IL23R genetic variability in 204 Algerian Crohn's disease

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Summary NOD2/CARD15 and IL23R gene variants play an important role in the susceptibility to Crohn's disease (CD). Studies of genotype-phenotype relationship suggest that these variants are associated with the development of the disease and specific phenotype. Preliminary reports analyzing the association between these variants have never been made on Algerian CD's. In a case-control design, 204 Algerian with CD diagnosed for at least 5 years and 201 controls were included were genotyped for single nucleotide polymorphisms (SNP) in the NOD2/CARD15 gene R702W (SNP8, rs2066844), G908R (SNP12, rs2066845) and IL23R R381Q (rs11209026) gene variants were determined using the TaqMan SNP genotyping assays. NOD2/CARD15 908R was carried by 3% of the patients and none in control subjects ($\chi^2 = 8.6$, $P_c = 0.003$, OR = 13.20). NOD2/CARD15 702W was associated to CD outcome ($\chi^2 = 17.2$, $P_c = 0.00003$, OR = 12.5) and early onset of disease (group A1, $\chi^2 = 19.3$, $P_c = 1.10^{-5}$, OR = 14.05, $P_{M-H} = 2.10^{-6}$). IL23R 381Q variants was more frequent in CD's patients than controls ($\chi^2 = 8$, $P_c = 0.005$, OR = 3.48), it was associated to earlier onset (group A1, $\chi^2 = 7.1$, $P_c = 0.007$, OR = 1.04, $P_{M-H} = 0.002$), extra-intestinal manifestations (EIM) outcome ($\chi^2 = 10.6$, $P_c = 0.001$, OR = 1.05, $P_{M-H} = 0.002$) and ileocolonic location ($\chi^2 = 6.8$, $P_c = 0.009$, OR = 1.05, $P_{M-H} = 0.001$). In this Algerian cohort, NOD2/CARD15 and IL23R variants were associated with CD's outcomes and linked to a particular clinical phenotype.

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Introduction

Crohn's disease is a chronic inflammatory disease that can affect any portion of the digestive tract but it is most common in the ileum. It is also characterized as an autoimmune disease in which the body attacks itself and causes inflammation. CD is most prevalent in North America and Europe, and least prevalent among African Americans and Asians [1]. The underlying basis of pathogenesis in inflammatory bowel disease (IBD) is not yet clear but may involve persistent bacterial infection, a defective mucosal barrier, or an imbalance in the regulation of the immune response [2]. Recent research has indicated specific genetic variations as a direct cause of CD [3]. The genetic aspects of CD have been linked by observing familial clustering of IBD cases [4,5]. Genetic variations in the NOD2/CARD15 have strongly been linked to CD [6—8]. Three variants of NOD2/CARD15 gene R702W (SNP8), G908R (SNP12), L1007fsinsC (SNP13), present in 30 to 50% of CD patients, are implicated in the susceptibility to CD [8]. Since a number of genotype-phenotype studies have suggested that NOD2/CARD15 variants are associated with ileal disease and development of intestinal strictures [9—13]. Otherwise, highly significant association between Crohn's disease and the IL23R gene on chromosome 1p31, which encodes a subunit of the receptor for the pro-inflammatory cytokine interleukin-23 was highlighted [14]. An uncommon coding variant (rs11209026, c.1142G>A, R381Q) confers strong protection against CD [14].

In this report, we examine the contribution of 2 mutations in the NOD2/CARD15 gene and 1 mutation in the IL23R gene to CD susceptibility in cohorts of Algerian CD cases. We also investigate the pathogenic effect of these mutations through stratification by clinical phenotype and variants combination.

Materials and methods

Patients and controls

In a case-control design, we studied 204 Algerian-born CD patients and compared them with 201 unrelated healthy Algerian control subjects. The patients were recruited from March 2007 to October 2008 using the standard clinical, radiological, endoscopic and histological criteria at three departments of Gastroenterlogy (Center Algiers Hospital, University Hospital Bab El Oued and Central Hospital of Army), and their clinical characteristics are detailed (Table 1). The 201 controls were recruited from blood transfusion centre of Central Hospital of Army. Control subjects with any chronic medication, abnormal basic biochemical workup, individual or familial IBD history were excluded. Patients and healthy controls were informed and given the written consent. The study was approved by hospital ethics committee of participant departments.

Table 1 Demographic and clinical characteristics of CD patients $n = 204$ (%).

Age of onset (%)	
A1; < 40 years	158 (78)
A2; > 40 years	46 (22)
Location (%)	
L1; ileal	46 (22)
L2; colonic	49 (24)
L3; ileocolonic	105 (51)
L4; upper	4 (2)
Behaviour (%)	
B1; inflammatory	55 (26)
B2; structuring	133 (65)
B3; penetrating	16 (8)
p; perianal injuries	46 (23)
Age[a]	$40,3 \pm 13.4$
Follow-up (yearq)[b]	9 ± 4.2
Family history[c] (%)	31 (15)
EIM (%)	
Musculoskeletal	62 (75)
Dermatologic	22 (26)
Ophthalmologic	16 (19)

EIM: Extra-intestinal manifestations.
[a] Mean ± SD.
[b] Mean (extremes).
[c] Antecedents of IBD in 1st or 2nd degree relative.

Methods

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes of affected individuals and controls using chloroformed isoamyl procedures. Genotyping of NOD2/CARD15 and IL23R variants were performed using Applied Biosystems Taqman assays (Applied Biosystems, Warrington, United Kingdom) according to the manufacturer's protocol (Life Technologies, Carlsbad, CA). PCR reactions were carried out in 96-well plates on 9700 Applied Biosystems Thermocyclers, and allelic discrimination was achieved using the ABI7500 and Sequence Detection System. All control genotypes were in Hardy—Weinberg equilibrium.

Statistical analysis

Categorical variables were compared using the χ^2 test, applying the Yates correction when necessary. We used univariate analysis to study the association between clinical characteristics and NOD2/CARD15, IL23R genotypes. Odds ratio was noted with a CI (confidence index) of 95%. Variables reaching a P value of less or equal than 0.05 were considered to indicate a statistically significant difference. Analysis was carried out using the SPSS v.16 (IBM SPSS Statistics Core System).

Results

Frequency distribution of NOD2/CARD15 and IL23R genotypes

The genotype frequencies of the two NOD2/CARD15 and IL23R SNPs were in accordance with Hardy—Weinberg equilibrium. NOD2/CARD15 and IL23R gene variants displayed highly significant associations with CD (Table 2).