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ORIGINAL ARTICLE

Case Control Study

NOD2/CARD15 gene mutations in North Algerian patients with inflammatory bowel disease

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Abstract

AIM: To analyse allelic frequency of *NOD2* gene variants

and to assess their correlation with inflammatory bowel disease (IBD) in Algeria.

METHODS: We studied 132 unrelated patients diagnosed with IBD, 86 with Crohn's disease (CD) and 46 with ulcerative colitis (UC). Data was prospectively collected between January 2011 and December 2013. The demographic and clinical characteristics were recorded for all the patients. A group of 114 healthy unrelated individuals were selected as controls. All groups studied originated from different regions of North Algeria and confirmed the Algerian origin of their parents and grandparents. Informed and written consent was obtained from each of the participants. All individuals were genotyped for the three CD-associated *NOD2* variants (p.Arg^{702}Trp, p.Gly908Arg and p.Leu^{1007}fsinsC mutations) using the polymerase chain reaction-restriction fragment length polymorphism method. Allele and genotype frequencies in patients and control subjects were compared by χ^2 test and Fisher's exact test where appropriate. Odds ratios (OR) and 95% confidence intervals (95%CI) were also estimated. Association analyses were performed to study the influence of these variants on IBD and on clinical phenotypes.

RESULTS: The p.Arg^{702}Trp mutation showed the highest frequency in CD patients (8%) compared to UC patients (2%) (P = 0.09, OR = 3.67, 95%CI: 0.48-4.87) and controls (5%) (P = 0.4, OR = 1.47, 95%CI: 0.65-3.31). In CD patients allelic frequencies of p.Gly908Arg and p.Leu^{1007}fsinsC variants compared to HC were 3% *vs* 2% (P = 0.5, OR = 1.67, 95%CI: 0.44-6.34); 2% *vs* 1% (P = 0.4 OR = 2.69 95%CI: 0.48-14.87 respectively). In UC patients, allelic frequencies of p.Gly908Arg and p.Leu^{1007}fsinsC variants compared to HC were 1% *vs* 2% (P = 1, OR = 1.62, 95%CI: 0.17-4.74) and 2% *vs* 1% (P = 0.32, OR = 0.39, 95%CI: 0.05-2.87). The total frequency of the mutated *NOD2* chromosomes was higher in CD (13%), than in HC (8%) and UC (5%). In addition, *NOD2* variants were linked to a particular clinical sub-phenotype in CD in this Algerian cohort. As expected, the three *NOD2* variants showed a significant association with CD but did not reach statistical significance, despite the fact that the allele frequency of *NOD2* variants was in the range found in most of the European populations. This might be due to the non-exposure of the *NOD2* carriers to environmental factors, required for the expression of the disease.

CONCLUSION: Further analyses are necessary to study genetic and environmental factors in IBD in the Algerian population, using larger patient groups.

Key words: Algeria; Crohn's disease; Ulcerative colitis; Inflammatory bowel disease; *NOD2* mutations; Polymerase chain reaction-restriction fragment length polymorphism method

Core tip: We evaluated allelic frequency of *NOD2* variants among 132 inflammatory bowel disease (IBD) patients and 114 unrelated healthy subjects from Algeria. Despite the fact that the frequency of *NOD2* mutant alleles is in the range found in most of the European populations, we failed to demonstrate the association of these *NOD2* variants with IBD susceptibility. This might be due to the non exposure of the *NOD2* carriers to environmental factors, required for the expression of the disease. We can expect in the coming years to see an increased incidence of IBD associated with the spread of Western lifestyle in this region.

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INTRODUCTION

The nucleotide-binding oligomerization domain containing 2 gene (*NOD2*) is 3.1 kb in size and is located on chromosome 16q12.1[1-3]. Consisting of 12 exons and 11 introns, *NOD2* encodes a protein expressed mainly in monocytes, dendritic cells, enterocytes and Paneth cells. NOD2 has an important role in immune system function[4]. In response to bacterial infection, NOD2 acts as an intracellular bacterial receptor in monocytes and activates nuclear factor kappa B (NF-κB) specifically after recognition of the bacterial cell wall component, muramyl dipeptide (MDP)[5] and leads to activation of the inflammatory response[6,7]. *NOD2* mutations are associated with Crohn's disease (CD) as well as other disorders, including Blau syndrome[8,9] and bipolar disorder[10]. *NOD2* mutations have also been associated with a higher incidence of specific types of cancer in affected patients[11].

More than 40 non-conservative mutations were identified on the *NOD2* gene[12]. The most common are two missense mutations, p.Arg^{702}Trp (SNP8, C/T) in exon 4, leading to substitution of arginine in position 702 by tryptophan, p.Gly908Arg (SNP12,G/C) in exon 8, leading to substitution of glycine in position 908 by arginine and an insertion mutation of a C in exon 11, a frame shift resulting in a truncated NOD2 protein at position 1007, p-Leu^{1007}fsinsC (SNP13, insC). Independent groups have reported the association of these three mutations with increased susceptibility to CD[12-14]. These mutations affect the structure of

either the carboxy-terminal leucine-rich repeat (LRR) domain of the protein or the adjacent region. The activating functions of NF-κB are regulated by the LRR domain, which has an inhibitory role and acts as an intracellular receptor for components of microbial pathogens[12]. In particular, these *NOD2* mutations are associated with the phenotypes of CD that involve the ileum, and with fibrostenosing disease[15]. In Caucasian populations, the contribution of the *NOD2* gene mutations to CD has been studied. The risk of CD has been evaluated to be 1.5-3-fold for heterozygous carriers and 10-44-fold for homozygous/compound heterozygous carriers[15-19]. However, the background prevalence of *NOD2* mutations depends on ethnicity. In Asian populations such as Chinese, Korean and Japanese, the three previously described major variants of the *NOD2* gene were not found in CD patients and controls[20,21], indicating that although ethnically divergent populations may present identical phenotypes, they do not necessarily share the same set of predisposing genes. In African populations, very few studies have been performed to investigate the influence of genetic factors in the development of CD. Gasche *et al*[22], screened the three *NOD2* SNPs in a collection of 1064 DNA samples from 52 worldwide populations, including seven sub-Saharan populations and one North African population composed of 30 Algerian Mozabites. They likewise found no *NOD2* mutations in African populations except for a single positive case of p.Arg^{702}Trp mutation in the Algerian Mozabite population. They concluded that the three CD-associated single nucleotide polymorphisms (SNPs) were almost exclusively found in Europe and are absent in native populations from Africa. In North African populations, two previous genotyping analyses in Morocco and Tunisia[23,24] revealed that the *NOD2* allele's frequencies are very low when compared to the frequencies seen in Caucasians of European origin. More recently, the contribution of *NOD2* polymorphisms to CD has been studied for the first time in an Algerian population[25] and a strong association between CD and *NOD2* variants was reported. However, p.Leu^{1007}fsinsC variant was not investigated in this study even it showed the strongest association with CD in previous studies[15,17]. The objectives of the present study were thus to determine allelic frequency of the three major *NOD2* gene variants and to investigate whether they determined specific phenotypes of IBD among North Caucasian Algerians.

MATERIALS AND METHODS

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the ethics committee of the Agence Thématique de la Recherche Scientifique en Santé, (ATRSS, ex ANDRS). All patients signed an informed consent form for this investigation.

Patients with IBD

A total of 132 unrelated patients diagnosed with IBD (86 Crohn's disease (CD), 46 ulcerative colitis (UC)) reported in this study were recruited from Maillot Hospital, Algiers, Algeria, between January 2011 and December 2013 and represented an independent cohort not studied before in any IBD genetic studies. Diagnosis of IBD was based on standard clinical, radiological, endoscopic and histological criteria. The following demographic and clinical characteristics were recorded for all the patients: geographical origin, gender, age, age at diagnosis, disease location, presence of extra intestinal manifestations, surgery and familial or sporadic disease (familial disease was considered if one first or second degree relative had IBD). In addition, we carried out an evaluation of the incidence of IBD in our monocentric study, since no published epidemiological data on IBD in Algeria are available. Data were obtained from the registry of IBD of the Department of Gastroenterology at the Maillot University Hospital of Algiers. We have considered all patients permanent residents of Algiers, consulted for the first time between January 1988 and December 2008, for symptoms consistent with a diagnosis of IBD.

Healthy control subjects

One hundred and fourteen healthy unrelated individuals (mainly students, blood donors and hospital employees) with a mean age of 25.21 ± 8.66 were selected as controls on the basis of a lack of personal or family history of IBD or any other autoimmune or immune diseases.

Stastical analysis

A comparison of genotype frequencies between different groups was evaluated using the χ^2 test or Fisher's exact test. Odds ratios (OR) were noted with a 95%CI. Expected and observed heterozygosity and Hardy-Weinberg equilibrium (HWE) were calculated. Variables were considered to indicate a statistically significant difference for a *P* value less or equal to 0.05. We used GraphPad prism version 6.01 (GraphPad Software, San Diego, California, United States).

DNA extraction

DNA was extracted from blood samples by the phenol-chloroform method according to standard protocols[26].

We screened samples for the presence of p.Arg^{702}Trp, p.Gly908Arg and p.Leu^{1007}fsinsC mutations of the *NOD2* gene by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Primers and PCR reactions were previously described[27]. Primers used were synthesized by Invitrogen (Invitrogen, Life Technologies, Carlsbad, California, United States) and PCR reactions were carried out using a T100 thermal cycler (BIO-RAD, Richmond CA, United States). PCR products were analysed on 1% agarose gels and stained with 1 mg/mL ethidium bromide. The digestion reaction

Table 1 Demographic, clinical data and genotype-phenotype correlations in Crohn's disease patients *n* (%)

Variables	All subjects	Non carriers	Carriers	*P* value
HC	114	102 (89.47)	12 (10.52)	0.120
CD patients	86	70 (81.39)	16 (18.6)	0.103
Sex (F/M)	46/40	40/30	6/10	0.155
Age at diagnosis	25.28± 14.85	25.70 ± 15.55	22.12 ± 10.66	
A1	28 (32.5)	22	6	0.809
A2	43 (50)	35	8	
A3	15 (17.5)	13	2	
Location				
L1: Ileum	25 (29)	23	2	0.020
L2: Ileo-colon	49 (57)	35	14	
L3: Colon	12 (14)	12	0	
Resective	37 (43)	31	6	0.620
Surgery				
EIM	24 (28)	18	6	0.343
Positive family history for CD	23 (27)	17	6	0.281

A1 < 16, A2 = 16-40, A3 > 40 years according Montreal classification. CD: Crohn's disease; HC: Healthy controls; EIM: Extra intestinal manifestations.

Table 2 Demographic, clinical data and genotype-phenotype correlations in ulcerative colitis patients *n* (%)

Variables	All subjects	Non carriers	Carriers	*P* value
HC	114	102 (89.47)	12 (10.52)	0.120
UC patients	46	42(91.3)	4(8.7)	0.726
Sex (F/M)	30/16	27/15	3/1	0.667
Age at diagnosis	36.86 ± 12.30	36.92 ± 11.34	36.25 ± 22.60	
A1	1 (2.1)	1	0	0.916
A2	31 (67.4)	28	3	
A3	14 (30.5)	13	1	
Location				
E1: proctitis	9 (20)	9	0	0.115
E2: distal colitis	19 (41.5)	16	3	
E3: extensive colitis	18 (39)	17	1	
Resection	4 (9)	3	1	0.225
Surgery				
EIM	11 (24)	11	0	0.240
Positive family history for UC	11 (24)	10	1	0.957

A1 < 16, A2 = 16-40, A3 40 years according Montreal classification. UC: Ulcerative colitis; HC: Healthy controls; EIM: Extra intestinal manifestations.

and restriction enzyme digestion conditions were taken from the manuals included with the restriction enzymes MspI, HhaI purchased from Promega (Promega, Madison, wisconsin, United States) and NlaIV purchased from New England Biolabs (New England Biolabs, Massachusetts, United States). Digestion products were loaded on polyacrylamide gel electrophoresed under 120 volts for 60 min, coloured with 1 mg/mL ethidium bromide. Images were captured with a Gel Doc EZ imager analyser (BIO-RAD, Richmond CA, United States).

RESULTS

In our study, we have analysed two series of data. We have obtained data retrospectively from the Department of Gastroenterology's register. A group of 770 IBD patients resident in Algiers, were consulted between January 1988 and December 2008. Based on these data, we evaluated the incidence rate for IBD. It ranges from 0.5 to 2 cases/10^5 per year, with an average rate of 1.2. The second series of data was obtained prospectively between January 2011 and December 2013. Our cohort was composed of 132 IBD patients (86 CD and 46 UC). The mean age of the patients at diagnosis was 25.28 ± 14.85 and 36.86 ± 12.30 years for CD and UC, respectively. The clinical characteristics and phenotype data are reported in Tables 1 and 2. Family history in a first- or second-degree relative was found in 27% and 24% of the CD and UC patients studied, respectively, which is higher than reported before in CD patients in the same population[25]. There was a slight female gender predominance in IBD patients (57%). PCR-RFLP was employed to detect p.Arg^{702}Trp, p.Gly908Arg and p.Leu^{1007}fsinsC mutations of the *NOD2* gene in 132 IBD patients and 114 healthy control subjects. Allele frequencies of mutant alleles of each *NOD2* variant are shown in Table 3. The p.Arg^{702}Trp mutation showed the highest frequency in CD patients (8%) compared to UC patients (2%) (P = 0.09, OR = 3.67, 95%CI: 0.48-4.87) but its frequency was also high in controls (5%) (P = 0.4, OR = 1.47, 95%CI: 0.65-3.31). Likewise, p.Gly^{908}Arg and p.Leu^{1007}fsinsC mutations showed similar frequency in CD patients and in controls (3% *vs* 2%, P = 0.5, OR = 1.67, 95%CI: 0.44-6.34; 2% *vs*1%, P = 0.4, OR = 2.69, 95%CI: 0.48-14.87, respectively). In UC patients, allelic frequencies of p.Gly^{908}Arg and p.Leu^{1007}fsinsC variants compared to HC were 1%*vs* 2%, (P = 1, OR = 1.62, 95%CI: 0.17-4.74) and 2% *vs* 1% (P = 0.32, OR = 0.39, 95%CI: 0.05-2.87)). As expected, the total frequency of the mutated *NOD2* chromosomes was higher in CD (13%), than in HC (8%) and UC (5%). However, this difference was not statistically significant (CD *vs* HC, P = 0.12, OR = 1.71, 95%CI: 0.88-3.3; UC *vs* HC, P = 0.63, OR = 0.67, 95%CI: 0.24-1.86). In addition, the comparison of the total allelic frequencies between CD and UC patients did not show any statistical difference (P = 0.08, OR = 0.39, 95%CI: 0.14-1.07).

When considering the carriers of at least one copy of mutant alleles in any variant and the carriers of two copies (Table 4), a similar result was observed. 18.6% of CD patients and 8.7% of UC patients carried at least one mutant allele in any of the three considered variants, *vs* 10.52% of controls (P = 0.103, P = 0.726, respectively). Two copies of mutant alleles were

Table 3 *NOD2* Allele frequencies in Crohn's disease, ulcerative colitis and healthy control groups

Polymorphisms		Allele frequency (%)											
		CD (n = 86)	UC (n = 46)	HC (n = 114)	IBD (n = 132)	*P* value[1]	OR (95%CI)	*P* value[2]	OR (95%CI)	*P* value[3]	OR (95%CI)	*P* value[4]	OR (95%CI)
p.Arg[702]Trp (C/T)	C	92	98	95	94	0.09	3.67	0.40	1.47	1.00	0.92	0.36	2.5
	T	8	2	5	6		0.48-4.87		0.65-3.31		0.42-2.01		0.54-11.4
p.Gly[908]Arg (G/C)	G	97	99	98	98	0.66	2.72	0.50	1.67	0.75	0.76	1.00	1.62
	C	3	1	2	2		0.31-23.69		0.44-6.34		0.21-2.75		0.17-14.74
p.Leu[1007]fsinsC (WT/insC)	WT	98	98	99	98	1.00	1.07	0.40	2.69	0.29	0.38	0.32	0.39
	insC	2	2	1	2		0.19-5.9		0.48-14.87		0.07-1.9		0.05-2.87
Total frequency (%)		13	5	8	10	0.08	0.39	0.12	1.71	0.43	1.32	0.63	0.67
							0.14-1.07		0.88-3.3		0.71- 2.48		0.24-1.86

[1]The *P* value for CD patients *vs* UC patients; [2]The *P* value for CD patients *vs* HC group; [3]The *P* value for IBD patients *vs* HC group; [4]The *P* value for UC patients *vs* HC group. (%) represents allele frequency. CD: Crohn's disease; UC: Ulcerative colitis; IBD: İnflammatory bowel disease (CD + UC patients); HC: Healthy controls; WT: Wild type allele; insC: Insertion mutation of a C.

Table 4 Number of different genotypes observed in Crohn's disease/ulcerative colitis/healthy controls groups

Variant	p.Arg[702]Trp	p.Gly[908]Arg	p.Leu[1007]fsinsC	WT
p.Arg[702]Trp	2/0/4	1/0/1	2/1/1	6/1/2
p.Gly[908]Arg		0/0/0	1/0/0	3/1/3
p.Leu[1007]fsinsC			0/0/0	1/1/1
WT				70/42/102

In each cell of the table, the genotype is obtained by combining the two genetic variants indicated in the corresponding row and column. No patients homozygous for *NOD2* gene p.Gly908Arg and p.Leu1007fsinsC mutations were found in this study. CD: Crohn's disease; UC: Ulcerative colitis; HC: Healthy controls; WT: Wild type.

Table 5 Hardy-Weinberg equilibrium and the *P* value

Groups	Genotypes Frequencies	*NOD2* polymorphisms											
		p.Arg[702]Trp				p.Gly[908]Arg				p.Leu[1007]fsinsC			
		CC	CT	TT	*P* value	GG	GC	CC	*P* value	WT/WT	insC/WT	insC/insC	*P* value
HC	Observed	92.98	3.51	3.51	0	96.49	3.51	0	0.8488	98.25	1.75	0	0.9247
	Expected	89.75	9.97	0.28		96.52	3.45	0.03	NS	98.25	1.74	0	NS
CD	Observed	87.21	10.46	2.33	0.0117	94.19	5.81	0	0.7813	95.35	4.65	0	0.8252
	Expected	86.92	12.62	0.46		94.27	5.64	0.08	NS	95.41	4.54	0.05	NS
IBD	Observed	90.15	8.33	1.52	0.0106	95.45	4.55	0	0.7893	95.45	4.55	0	0.7893
	Expected	88.96	10.72	0.32		95.51	4.44	0.05	NS	95.51	4.44	0.05	NS

CD: Crohn's disease; HC: Healthy controls; IBD: İnflammatory bowel disease; NS: Non-significant *P* value; WT: Wild type; insC: Insertion mutation of a C.

carried by 7% of the CD subjects (either homozygous for one variant or compound heterozygous), *vs* 5.26% in the control group (P = 0.613). Only a single subject was compound heterozygous for p.Arg[702]Trp and p.Leu[1007]fsinsC variant in the UC group (2.17%) (P = 0.397). No subjects were homozygous for p.Gly908Arg or p.Leu[1007]fsinsC variants in the whole sample of CD. This reveals the risk conferred by the possession of more than one of these mutations.

When analysing the genotype data, the HWE test P value was not significant for any of the SNPs except for p.Arg[702]Trp (Table 5). The observed excess of p.Arg[702]Trp homozygous genotypes in the HC sample was significant and could be due to consanguinity.

No significant associations were found with *NOD2* mutations for family history, presence of extra intestinal manifestations or surgery, even when p.Arg[702]Trp/p.Gly908Arg/p.Leu[1007]fsinsC polymorphisms were considered together or separately or when only p.Gly908Arg/p.Leu[1007]fsinsC were considered.

The stratification of CD according to age at diagnosis showed that CD patients carrying one or two copies of any rare variant had a mean age at diagnosis lower than non-carrier CD patients. However, this

Table 6 Comparative study of *NOD2* allele frequencies in North African populations

Population	Number		Allele frequency (%)							
			p.Arg^{702}Trp		p.Gly^{908}Arg		p.Leu^{1007}fsinsC		Total allele	
	CD	HC	CD	HC	CD	HC	CD	HC	CD	HC
North Algeria (this study)	86	114	8	5	3	2	2	1	13	8
Algeria[25]	204	201	5	0.50	3	0.5	ND	ND	ND	ND
Morocco[23]	101	107	0.49	0.46	6.43	2.8	0.9	0	8	3.2
Tunisia[24]	130	90	2	0.60	5	3	1	0	8	3.6

CD: Crohn's disease; HC: Healthy controls; ND: Not determined.

association was not significant (P = 0.809).

One positive association was shown between *NOD2* mutations and location of disease. In the CD group, 59% of patients had combined small bowel and colonic involvement, 30% had isolated small bowel involvement and 11% had an isolated colonic disease. *NOD2* carriers with any risk allele showed ileal or ileocolonic CD and none of them had an isolated colonic CD. There were significant differences between allele frequencies and location of disease (P = 0.020). In our study, *NOD2* alleles were associated with a particular CD sub-phenotype. This result is in agreement with previous studies in which ileal disease was found associated with *NOD2* variants[15].

In the UC group, no significant associations were found among the *NOD2* mutations and age at diagnosis, presence of extra intestinal manifestations, surgery or family history. When considering extent of disease, 9 (20%) patients had proctitis, 19 (41.5%) distal colitis and 18 (39%) extensive colitis. Interestingly, none of the UC patients with *NOD2* risk alleles had E1 disease location, suggesting a potential association between *NOD2* mutations carrier status and disease severity. However, the frequency of *NOD2* mutations did not achieve statistical significance (P = 0.115), probably because of the small number of patients in this sub-group (only 4).

DISCUSSION

Allele frequency of the three major *NOD2* gene variants was assessed in this analysis. As expected, the total allele frequency was higher in CD patients than in UC patients and HC. The frequencies of the three *NOD2* variants range from 4% to 5%, 1% to 2% and 2% to 3% for p.Arg^{702}Trp, p.Gly908Arg and p.Leu^{1007}fsinsC, respectively, in healthy Caucasian populations[28]. The corresponding frequencies among Caucasian CD patients range from 9% to 13%, 3% to 6% and 7% to 16%, respectively[29]. In UC patients, the frequencies of these three variants are lower, ranging from 4% to 6%, 2% and 2.5% to 3.3%[30-32]. In our cohort of IBD and controls, the observed frequency of *NOD2* mutant alleles is in agreement with a high Caucasian component of the targeted population and is in the range found in most European populations, except for p.Leu^{1007}fsinsC frequency which was lower. Much lower frequency was also found for the frameshift mutation in neighbouring populations from Morocco and Tunisia, with similar sample size (Table 6). Interestingly, another study in a North Tunisian population has shown that p.Leu^{1007}fsinsC mutation was strongly associated with susceptibility to CD with an allelic frequency value of 15%[33], indicating that significant differences might be obtained for the same population.

The p.Arg^{702}Trp allelic frequency obtained on 114 North Algerian healthy controls is likewise significantly different from that recently determined on 201 controls (P = 0.0002, OR = 11.11)[25]. This divergence indicates that the control samples used in the two studies are significantly different. Previous studies described the population of North Africa as extremely heterogeneous, composed of a mosaic of sub-populations with significantly different genetic structures and with high endogamy rates[33,34]. This is confirmed by the Hardy-Weinberg analyses performed in our study. Thus, recruitment from different geographic origins in Algeria may lead to different *NOD2* frequencies. Taken together, all these data from the Algerian population suggested that genetic variations of *NOD2* gene are not homogeneously distributed.

The correlation of *NOD2* genotypes with phenotypic expression of IBD was assessed in this analysis. IBD is a heterogeneous disorder characterized by the presence of different clinical sub-phenotypes. In our study, we have established different sub-groups, after the stratification of IBD patients according to demographic and phenotypic data. In CD patients, no significant association was found between *NOD2* variants and phenotypic data (Tables 1 and 2). However, this association was significant with disease location, as described previously. In the UC group, our data suggests that *NOD2* mutations appear to be associated with a more aggressive course of UC. Similar findings have been recently reported on UC in the Portuguese population[35]. To our knowledge, this study is the first one performed to investigate the influence of genetic factors in the development of UC in the Algerian population. The small number of UC patients with *NOD2* risk alleles is the limitation in our study and a larger sample is certainly needed to clarify

the role of *NOD2* gene variants in phenotype severity of UC in Algeria.

Finally, our results clearly show an influence of *NOD2* gene variants on specific CD clinical sub-groups. Since only 18.6% of CD patients and 8.69% of UC patients carry at least one mutation in the *NOD2* gene, it is possible that other polymorphisms in the *NOD2* gene or in other genes, in combination with environmental factors, are involved in IBD susceptibility. Indeed, despite the fact that the observed frequency of *NOD2* mutant alleles is in the range found in most European populations, the association of these polymorphisms on IBD susceptibility in our studied population could not be demonstrated. This might be due not only to the statistical test power failure but also to an environmental effect. *NOD2* carriers do not express the disease, because they are not exposed to environmental factors required for the expression of the disease and are probably protected because they lead a lifestyle that is not at risk. In these conditions, the OR values attributed to the *NOD2* mutations are proportional to the population exposure. Our study showed that the incidence rate for IBD increased from 0.5 to 2 cases/10^5 per year, between 1988 and 2008. This incidence rate is lower than that of other population groups and is certainly underestimated because it is evaluated only in a monocentric study. However, it is interesting to note that over these two decades the population in Algeria became predominantly urban. Thus, the increased incidence of IBD observed in the targeted population might be associated with the change in lifestyle. This is in agreement with a previous study demonstrating that urbanization of society is an important risk factor for the development of IBD. Soon *et al*[36], demonstrated that living in an urban society was positively associated with the development of IBD. Since change in lifestyle is relatively recent in Algeria, an increased incidence in IBD associated with the spread of Western lifestyle in this region can be expected in the coming years.

In conclusion, the data we obtained are relevant to estimate the *NOD2* gene variants in the Algerian population. Our study confirmed that the *NOD2* gene is significantly associated with a specific clinical sub-phenotype in CD. The results obtained up to now in Algeria have shown that the *NOD2* gene is involved in IBD susceptibility and have suggested a heterogeneous distribution of *NOD2* mutations across Algerian populations. Further analyses are necessary to study genetic and environmental factors in IBD in the Algerian population, using larger patient groups.

COMMENTS

Background

Very few studies have been performed to investigate the influence of genetic factors in the development of Crohn's disease (CD) in Algeria.

Research frontiers

The authors have explored *NOD2*/*CARD15* gene mutations in North Algerian patients with inflammatory bowel disease (IBD).

Innovations and breakthroughs

This is a novel study in which we determine, for the first time, the allelic frequency of the three major *NOD2* gene variants among the North Caucasian Algerians and investigate whether they determine specific phenotypes of IBD. The authors clearly showed an influence of *NOD2* gene variants on specific CD clinical sub-groups. The authors also proposed that an increased incidence of IBD observed in the targeted population might be associated with the change in lifestyle.

Applications

Investigating other genetic determinants, but also environmental factors, in IBD patients could help us to understand the pathogenic pathway of disease both in CD and ulcerative colitis (UC).

Peer-review

This is an original work in which a genetic study has revealed the importance of the environment on the expression of IBD. The format of the manuscript was slightly revised.

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