



CARD15 Gene Polymorphisms in Algerian Patients with Crohn's Disease: Genotype-Phenotype Correlation

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ABSTRACT

Crohn's disease presents a complex multifactorial etiology with genetic and environmental factors contributing to the disorder. Three common polymorphisms of CARD15: R702W, G908R, and 1007fs are variably associated with Crohn's disease in different ethnic groups. We evaluated the frequencies of these three polymorphisms in patients with CD from East Algeria, and compared it with the healthy control population. A total of 103 patients with Crohn's disease and 144 healthy controls were included in this study. All of these individuals were genotyped for the three CARD15 polymorphisms cited above. Genotyping was performed by PCR-RFLP (restriction fragment length polymorphism). A genotype-phenotype correlation was performed. The control population exhibited frequencies of CARD15 polymorphisms similar to the results of previous studies, 0.35%, 3.5%, and 1.04% for the R702W, G908R, and 1007fs polymorphisms, respectively, whereas Crohn's disease patients had allele frequencies of 1.46%, 5.83%, and 4.85%, respectively. Moreover, in CD patients, R702W and 1007fs polymorphisms were significantly associated with different phenotypes compared to Crohn's disease wild-type patients. This genetic study provides evidence that the two variants alleles, R702W and 1007fs are associated with CD susceptibility in the Algerian population.

Keywords: CARD15; Crohn's disease; genotype-phenotype; Algerian population.

INTRODUCTION

Crohn's disease (CD) is a chronic idiopathic of inflammatory bowel disease (IBD) characterized by a granulomatous inflammation that affects the digestive tract from the mouth to the anus. It is also characterized as an autoimmune disease in which the body attacks itself and causes inflammation. This chronic inflammation of the gastrointestinal tract is caused by an abnormal immune reactivity to the bacterial flora of the intestine, which leads to the tissue damage seen in CD patients.¹

Epidemiological data shows that the incidence and prevalence of CD in northern Europe and North America have increased, and also has major social impact.^{2,3} The prevalence of CD in Algerian population is unknown but one study reports that the incidence of CD in the central region of East Algeria (Constantine) is 5.87/100 000 inhabitants.⁴

The etiopathogenesis of this disease is unknown but epidemiological data have provided evidence that it is a multifactorial disease caused by the interaction of genetic and environmental factors, which underlie disease development and progression.⁵

A number of linkage studies carried out in separate populations have confirmed the genetic linkage of CD to chromosome 16. In the same time Hugot⁶ and Ogura⁷ have independently unraveled the CD associated

nucleotide oligomerisation domain 2 (NOD2) gene, (also called CARD15: caspase activating recruitment domain 15), located on chromosome 16q12 within the area of strongest linkage to CD.

The CARD15 gene has 12 exons and encodes a protein of 1040 amino acids. The protein contains two N-terminal domains (CARDs), a central domain (NOD), and multiple C-terminal leucine-rich repeats (LRR).^{8,9}

CARD15 is an intracellular protein expressed in monocytes, macrophages and Paneth cells. It functions as a sensor for bacterial products, such as lipopolysaccharides (LPS), through the LRR domain. It has been hypothesized that the sensing of bacterial components would, under normal circumstances; result in the activation of nuclear factor-κB (NF-κB).¹⁰⁻¹²

CARD15 may be important for both initial defenses against commensal and pathogenic bacteria and tolerance mechanisms for maintaining controlled activation of the intestinal immune system. Significant progress has been made in defining NOD2 signaling partners and pathways and functional consequences of CARD15 mutations with respect to its activation, expression, signaling, synergistic effects with Toll-like receptor signaling, and antimicrobial effects.

However, CARD15 contributions to human intestinal inflammation are complex and incompletely understood.



Improved understanding of CARD15-mediated pathways may lead to identification of other molecules that can also contribute to the development of Crohn's disease in humans.^{13,14}

More than 60 sequence variants have been identified in the CARD15 gene, two are a single-base pair substitution (Arg702Trp: R702W and Gly908Arg:G908R) and one is frameshift alteration leading to a stop codon and truncation of CARD15 protein (Leu 1007fs insC:3020 insC). These variants affect the structure of LRR domain of the gene product or the adjacent region, with may be the site of binding of microbial pathogens.⁶

The presence of one variant allele increase the risk of developing CD form 1.5- to 4.3-fold, the presence of 2 copies increase the risk to 20- to 40-fold.¹⁵

CD patients with CARD15 mutations exhibit early onset of the disease, mainly ileal involvement and increase risk of fistulas and stenosis.^{11,16}

The accession numbers of these SNPs from the database of the National Center for Biotechnology Information (NCBI) correspond to rs17860491, rs2066845, and rs5743293 (<http://www.ncbi.nlm.nih>).

In Algeria, until now no CD susceptibility locus has been reported in our population because no genome study has been done.

We decided, therefore, to perform a genetic screening and genotyping to determine whether CARD15 confers susceptibility to CD in East Algeria population.

The present study was undertaken to evaluate the frequency of three polymorphisms (R702W, G908R and 1007fs) of the CARD15 gene in Algerian patients with CD.

In addition, this study aimed to elucidate the importance of CARD15 polymorphisms in this cohort of CD patients and establish a genotype-phenotype correlation for CD.

MATERIALS AND METHODS

Study population

The study population was comprised of 103 patients with CD (45 males and 58 females) and 144 healthy (67 males and 77 females), unrelated controls. These patients were recruited from April 2011 to June 2014 in the gastroenterology service; Constantine, and internal medicine service, Batna, Algeria.

The diagnosis of CD was based on standard clinical, radiologic, endoscopic, and histologic criteria.¹⁷

The patients records were systematically reviewed for the following demographic and clinical characteristics: age, sex, smoking habits, age at diagnostic, disease localization (ileal, colonic, or ileocolonic), disease behavior (inflammatory, stricturing or fistulizing), presence of extraintestinal clinical manifestations (e.g., arthritis, uveitis, erythema nodosum), and familial IBD.¹⁰

All participating subjects gave written, informed consent before the genetic analyses.

Extraction and genotyping of the CARD15

Blood was collected in EDTA tubes by venipuncture from all participants. DNA from whole blood was extracted from peripheral blood leucocytes using standard salting-out procedures.

Each CARD15 mutation was assayed using initial amplification of the DNA sample by polymerase chain reaction (PCR) and subsequent analysis of the PCR products by reaction fragment length polymorphism (RFLP).

PCR conditions were initial denaturation at 94°C for 5mpn, followed by 30 cycles of denaturation at 94°C for 30s, annealing at 61.8°C for 30s and elongation at 72°C for 30s and a final elongation step at 72°C for 7mpn. PCR products were resolved using 2% agarose gel electrophoresis to confirm specific amplification.

RFLP analysis was carried out as described by Helio.⁸ Briefly, fragments corresponding to R702W, G908R and 1007fs were digested with 1U of MspI, HhaI and ApaI (Fermentas), respectively, at 37°C for 16 hours. The digested products were resolved on 3% agarose gel and detected after Ethidium bromide staining.

PCR product size and digestion product size of the three polymorphisms are shown in Table1.

Statistical analysis

The association between a given genotype and phenotypic characteristics of CD was estimated by the odds ratios (OR) with 95% confidence intervals (CI). The genotype frequencies observed in patients and controls were compared with Hardy-Weinberg expectations by using the χ^2 statistics. p-values <0.05% were considered to be significant.

Analysis was carried out using the Epi info 6 versions 6.04dfr – April 2001.

RESULTS

Patient population

In all, 103 patients were recruited in our study. The demographic characteristics and disease location according to the Vienna classification are depicted in Table 2.

The same contingency table was then used to consider each clinical feature (extraintestinal manifestation, familiarity and smoking).

The mean age (and standard deviation) of CD patients was 33.05±12.10 (rang: 15-67). There were more female than male patients in our sample of CD patients.

CARD15 Genotypes

The genotype and allele frequencies of the three common CARD15 variants (R702W, G908R, and 1007fs) in patients



and controls are shown in Table 3. They were all in Hardy-Weinberg equilibrium.

In our study, the association of R702W and 1007fs polymorphisms with CD was statically significant.

The frequencies of mutant's alleles were significantly higher in CD patients compared to controls. (R702W: 4.85% vs. 0.7%, $P<0.05$; OR=7.30; 95% CI, 0.81- 167.61. 1007fs: 9.7% vs. 2.08%, $P<0.05$, OR= 5.05; 95% CI, 1.24-23.83).

Susceptibility allele frequency of the G908R polymorphism observed in CD patients was higher (11.65%) compared with (6.90%) in healthy controls. The results for this polymorphism did not reach statistical significance ($P>0.05$, OR= 1.77; 95% CI, 0.68-4.64).

A total of 25.24% patients with CD carried a single copy of a CARD15 risk allele. At least one variant of risk was present in 9.72% of controls ($p=0.001$, OR= 3.24, 95% CI = 1.47-6.77)

All individuals genotypes with each variant were either homozygotic for the normal allele or heterozygotic, and none homozygotic form for the mutated alleles had been detected.

Genotype-Phenotype Correlations

In the next step a detailed genotype/phenotype correlation was performed in our patients with CD. The percentages of CD patients carrying at least one CARD15 variant were correlated to demographic data, disease localization and behavior (Table 4).

In particular, the 1007fs risk allele was present in 9.70% patients and showed a significant positive association with younger age of onset (A1) ($P=0.0026$). However, the sex of patients did not have any effect.

In increased frequency of stricturing form (15%) and ileocolon localization (18.60%) was positively related with 1007fs allele in the CARD15 gene ($P=0.03$, $P=0.022$, respectively).

Table 1: Primers, PCR product sizes, restriction enzymes and restriction digest product sizes for the three CARD15 mutations

Mutation	Forward and reverse primer sequences	PCR product size	Restriction enzyme	Digestion product size
R702W	5'TCA GCC TGA TGA CAT TTC3' 5'CGG GAT GGA GTG GAA GT3'	449bp	MspI	Wild type: 329, 66 and 54 bp Heterozygous: 329, 66, 54 and 120 bp Mutant:329 and 120 bp
G908R	5' CAC TGA CAC TGT CTG TTG ACT C 3' 5' AAG ACC TTC ATA AGT GGC CCC 3'	163bp	HhaI	Wild type: 163 bp Heterozygous: 27, 136 and 163 bp Mutant:27 and 136 bp
1007fs	5'CCT CAA AAT TCT GCC ATT CC3' 5'GGC AGA AGC CCT CTG CAG GGC G3'	151bp	ApaI	Wild type: 151 bp Heterozygous: 20, 131 and 151bp Mutant: 20 and 131 bp

Table 2: Clinical features of CD Patients

Clinical features	Total n (%)
Sex	
Male	45(43.7%)
Female	58(56.3%)
Smoking	
Never	42(40.78%)
Ever	61(59.22%)
Family history	29(28.1%)
Age at diagnostic (Mean±SD)	33.05 ±12.10
Age at onset	
<40 years (A1)	80(77.7%)
≥40 years (A2)	23(22.3%)
Disease behavior	
Nonstricturing, nonpenetrating (B1)	23(22.3%)
Stricturing (B2)	60(58.2%)
Penetrating (B3)	20(19.5%)
Location of the disease	
Terminal ileum (L1)	33(32.1%)
Colon (L2)	24(23.3%)
Ileocolon (L3)	43(41.7%)
Upper gastrointestinal tract (L4)	3(2.9%)
Extraintestinal clinical manifestations	43(41.7%)



Table 3: Genotype and allele frequencies of CARD15 in Algerians patients with Crohn's disease and healthy controls

Polymorphisms	Genotypes		Minor Allele frequency (%)		Percentage		†P-value	*Odds Ratio (95% CI)
	CD	Controls	CD	Controls	CD	Controls		
R702W								
WT/WT (C/C)	98	143						
WT/WM(C/T)	5	1	1.46	0.35	4.85	0.7	0.036	7.30
WM/WM(T/T)	0	0						
G908R								
WT/WT(G/G)	91	134						
WT/WM(G/C)	12	10	5.83	3.5	11.65	6.94	0.20	1.77
WM/WM(C/C)	0	0						
1007fs								
WT/WT	93	141						
WT/WM	10	3	4.85	1.04	9.71	2.08	0.008	5.05
WM/WM	0	0						

*Odds ratio for the minor allele; † P-value (chi-square test) for allele frequencies

Table 4: Genotype-Phenotype Correlations in CD Patients with susceptibility Alleles of the CARD15 Gene

Phenotype/genotype	R702W sus. Allele	G908R sus. Allele	1007fs sus. allele	One sus. Allele
N/Total (%)	5/103(4.85%)	12/103(11.6%)	10/103(9.70%)	26/103(25.24%)
Sex				
Male	2/45(4.44%)	4/45(8.9%)	6/45(13.33%)	12/45(26.66%)
Female	3/58(5.17%)	8/58(13.8%)	4/58(6.9%)	14/58(24.14%)
Smoking				
Never	2/42(4.76%)	<u>1/42(2.4%)</u>	<u>1/42(2.4%)</u>	<u>4/42(9.52%)</u>
Ever	3/61(4.92%)	11/61(18.03%)	<u>9/61(14.75%)</u>	22/61(36.06%)
Family history	1/29 (3.45%)	1/29(3.45%)	2/29(6.9%)	4/29(13.8%)
Age at onset				
<40 years (A1)	3/80(3.75%)	10/80(12.5%)	<u>4/80(5%)</u>	17/80(21.25%)
≥40 years (A2)	2/23(8.62%)	2/23(8.7%)	6/23(2%)	10/23(34.48%)
Disease behavior				
Non stricturing, nonpenetrating (B1)	1/23(4.35%)	2/23(8.7%)	1/23(4.35%)	4/23(17.4%)
Stricturing (B2)	4/60(6.66%)	7/60(11.6%)	<u>9/60(15%)</u>	19/60(31.66%)
Penetrating (B3)	0/20(0%)	3/20(15%)	0/20(0%)	3/20(15%)
Location of the disease				
Terminal ileum (L1)	0/33(0%)	3/33(9%)	0/33(0%)	3/33(9.09%)
Colon (L2)	1/24(4.16%)	3/24(12.5%)	1/24(4.16%)	5/24(20.83%)
Ileocolon (L3)	4/43(9.30%)	6/43(14%)	<u>8/43(18.60%)</u>	<u>17/43(39.53%)</u>
Upper gastrointestinal tract (L4)	0/3 (0%)	0/3(0%)	1/3(33.33%)	1/3(33.33%)
Extraintestinal clinical manifestations	2/43(4.6%)	4/43(9.3%)	<u>1/43(2.32%)</u>	<u>7/43(16.27%)</u>

†Susceptibility allele; *Statistically significant positive associations are underlined; † Statistically significant negative associations are indicated



Table 5: Comparison of allele frequencies of CARD15 susceptibility variants from different populations

Population [reference]	Cohort P/C	Allele frequencies (%)		
		R702W	G908R	1007fs
Spanish [16]	183/172	7.6	3.0	4.6
		3.4	1.1	2.0
Brazilian [11]	187/255	9.63	1.93	3.48
		2.75	1.64	0.78
Italy [38]	133/103	9.8	4.5	9.8
		2.4	4.3	2.9
Iranian [37]	40/100	16.25	5	2.5
		1	1	1
Greece [18]	364/539	9.1	10	/
		5.47	6.77	/
Indian [29]	82/149	0	0	0
		0	0	0
Turkish [46]	56/100	0.9	8	1.8
		0.5	0	1
Saudian [36]	46/50	5.22	4.22	4.56
		3.4	3.2	4
African American [34]	124/601	1.7	0	/
		5.4	1.5	/
Tunisian [5]	130/90	1.9	4.9	1
		0.5	2.7	0
Morocco [27]	101/100	0.49	6.43	0.99
		0.46	2.80	0.00
Present study	103/144	1.46	5.83	4.85
		0.35	3.5	1.04

By univariate analysis, the presence of one risk allele was significantly associated with ileocolon form ($p=0.017$) and Terminal ileum (0.009).

Interestingly, the smoking, an environmental factor involved in the modulation of the phenotype was directly associated with CD patients (1007fs: $p=0.03$, G908R: $p=0.014$, and one variant of risk: $p=0.002$).

In CD, the estimated chance of finding at least one variant, and the 1007fs susceptibility allele was significantly increased among patients with the extra-intestinal clinical manifestation ($P=0.025$, $P=0.032$, respectively).

After adjusting, none of three variants of CARD15 gene alone was significantly associated with sex and family history.

DISCUSSION

Since 2001, several independent studies of different ethnic and racial groups have demonstrated that the CARD15 gene variants contribute to a significant extent to the risk of CD.

In the present study, we report the first analysis of genetic susceptibility to CD in East Algerian population (Constantine and Batna).

In our CD patients, we observed significantly higher frequencies than in the control population. Moreover, as expected, no individuals with two susceptibility variants



were found in our cohort, as most other studies have reported.¹⁶⁻¹⁸

Several studies were carried out to investigate the role of the CARD15 gene in CD susceptibility in different population. The three polymorphisms of CARD15 gene displayed the strongest association in European,^{6-8,19-21} American,^{10,22} Australian²³ and Arabic population.²⁴ Nevertheless, some studies have reported a variable association of CARD15 with CD. No disease association with CARD15 variants was documented in Asia and Africa patients.^{5,25-28} These results confirm the complexity of the origin of CD and provide evidence for the genetic variability of the CARD15 gene in different populations.

Conversely, there was a lack of mutant variants in the Asian populations of India,²⁹ China,^{30,31} Korea³² and Malaysia.³³

The R702W frequency observed in our Algerian CD patients (1.46%) is consistent with those generally reported in tunisian and african-american cohorts.^{5,34}

In the present study the frameshift mutation 1007fs showed a decreased allele frequency (4.85%), compared with the range of 6%-16% in the majority of study populations in Europe and America.^{6,7,33} In addition, studies from Spain,¹⁶ Brazilian,¹¹ and Saudian population have also reported similar low allele frequencies for 1007fs.³⁶

The frequency of G908R is higher in CD patients (5.83%) compared to controls (3.5%), but no significant association with CD was established.

These results were in agreement with some European,⁸ Iranian,³⁷ Tunisian,⁵ and Moroccan studies,²⁷ where no significant association was observed. G908R mutant allele frequency in CD patients covered a wide range regarding different ethnic groups (0.6%-14.2%).³⁷

Regarding the different results in allele frequencies between the studies, can be explained by larger regional and ethnic differences in genotypes, and the relatively small numbers of cases included in most studies (Table 5).

Moreover, in our study, the risk of developing CD with a more aggressive course was increased in compound heterozygotes. In other population, stronger associations have been reported for homozygotes.³⁸

Some studies have suggested that familial and sporadic CD, are two distinct entities because of the differences in disease localization and behavior between the two groups³⁹. Our patients were constituted only of sporadic cases, no familial cases were found. European and American studies have revealed that the familial as well as the sporadic cases were used to show the association between CARD15 and CD.^{6,7,10,19,20}

We found that different risk alleles might be associated with different clinical features; in particular, the 1007fs allele seems to be associated with age of onset <40 years. We observed a significant association in patients with one

susceptibility allele toward a younger age of onset; this association was replicated in Spanish, North American and Italian population.^{21,35,40} However, in previous studies of the Mediterranean population, there is substantial heterogeneity between populations, and no association was noted in one Spanish cohort.⁴¹ This a priori contradiction could be explained by the different demographic features of this region.

The 1007fs allele seems to correlate with ileocolan localization and stricturing form. These data suggest a more aggressive course of the disease in carriers of risk alleles. These patients with one variant were also found to show an association with (simply remove : the structuring form and) ileocolan localization, as was also reported by Lesage,²⁰ De Diego¹⁶ and Bhullar.²³

The strongest observed effect for ileal location is consistent with the proposed involvement of ileal Paneth cells in the pathophysiology of NOD/CARD15-mediated disease susceptibility.³⁸

NOD/CARD15 mutations may, thus, abrogate normal Paneth cell behavior, explaining preferential involvement of the terminal ileum.⁴²

Tobacco factor is associated with the CD specifically with G908R and 1007fs. These results are consistent with studies which were carried out on the Spanish⁶, French¹⁶ and Italian population.³⁸

The analysis of genotype-phenotype correlations revealed an increased chance of mutation positivity (1007fs) in CD patients and extraintestinal manifestations, in agreement with most previous studies,^{3,43} and disagreement with other studies.^{16,23,40}

These contrasting results can be ascribed to the different ethnic background of the various CD populations studied.

CONCLUSION

Our study is the first to determine the prevalence of CARD15 gene mutations and correlation genotype-phenotype in east Algerian patients with disease.

This genetic study provides evidence that the two major CARD15 variant (R702W, 1007fs) alleles are associated with Crohn's disease susceptibility in Algeria; however, there is no evidence that the G908R polymorphism has any association.

Moreover, our data confirm a correlation between genotype (1007fs) and phenotype (younger age of onset, ileocolan localization and stricturing form). Indeed, an eventual goal in the genomic study of Crohn's disease is to identify this association and to apply them to clinical practice.

Finally, the presented data suggest a need for further work and larger studies to determine other genes (TLR4, IL23R, R381Q, STAT3, IRGM, eQTLs, P268S, JW1 and N852S) and environmental factors influencing Crohn's disease and behavior in the Algerian population.^{44,45}



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REFERENCES

- Rogler G, The effects of NOD2/CARD15 mutations on the function of the intestinal barrier. *Journal of Crohn's and Colitis*, 1, 2007, 53-60.
- Kappelman MD, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, Finkelstein JA, The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States, *Clin Gastroenterol Hepatol*, 5, 2007, 1424-1429.
- Burisch J, Munkholm P, Inflammatory bowel disease epidemiology, *Curr Opin Gastroenterol*, 29, 2013, 357-362.
- Hammada T, Lemdaoui C, Boutra F, Zoughailech D, Asselah H, Epidemiological aspect of inflammatory bowel disease in a Algerian population, *J Afr Hépatol Gastroentérol*, 5, 2011, 293-302.
- Zouiten-Mekki L, Zaouali H, boubaker J, Karaoui S, Fekih M, Matri S, Hamzaoui S, Filali A, Chaabouni H, Hugot JP, CARD15/NOD2 in a Tunisian population with Crohn's disease. *Dig Dis Sci*, 50, 2005, 130-135.
- Hugot JP, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Bindert V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M and Thomas G, Association of CARD15 leucine-rich repeat variants with susceptibility to Crohn's disease, *Nature*, 411, 2001, 599-603.
- Ogura Y, Bonen DK, Inohra N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kischner BS, Hanauer SB, Nunez G and Cho JH, A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease, *Nature*, 411, 2001, 603-606.
- Helio T, Halme L, Lappalainen M, Fodstad H, Paavola-Sakki P, Turunen U, Färkkilä M, Krusius T and Kontula K, CARD15/NOD2 gene variants are associated with familiarly occurring and complicated forms of Crohn's disease, *Gut*, 52, 2003, 558-562.
- King K, Sheikh MF, Cuthbert AP, Fisher S, Onnie CM, Mirza M, Pattni RC, Sanderson J, Forbes A, Mansfield J, Lewis CM, Roberts RG and Mathew CG, Mutation, selection, and evolution of the Crohn's disease susceptibility gene CARD15, *Human Mutation*, 27, 2006, 44-54.
- Vermeire S, Wild G, Kocher K, Kocher K, Cousineau J, Dufresne L, Alain Bitton, Langelier D, Pare P, Lapointe G, Cohen A, Daly MJ and Rioux JD, CARD15 genetic variation in a Quebec population: prevalence, genotype-phenotype relationship, and haplotype structure, *Am J Hum Genet*, 71, 2002, 74-83.
- Baptista ML, Amarante H, Picheth G, Sdepanian LV, Peterson N, Babasukumar U, Lima HC and Kugathasan S, CARD15 and IL23R influences Crohn's Disease susceptibility but not disease phenotype in a Brazilian population, *Inflamm. Bowel. Dis*, 14, 2008, 674-679.
- Maev IV, Andreev DN, Role of Mutations in NOD2/CARD15, ATG16L1, and IRGM in the Pathogenesis of Crohn's Disease, *Inter J Bio Medicin*, 4, 2014, 7-10.
- Brand S, Konrad A, Crispin A, Göke B, Lohse P and Ochsenkühn T, The role of toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms and CARD15/NOD2 Mutations in the susceptibility and phenotype of Crohn's disease, *Inflamm Bowel Dis*, 11, 2005, 645-652.
- Abraham C, Cho JH, Functional Consequences of NOD2 (CARD15) Mutations, *Inflamm Bowel Dis*, 12, 2006, 641-650.
- Niess JH, Klaus J, Stephani J, Pflüger C, Degenkolb N, Spaniol U, Mayer B, Lahr G and von Boyen GBT, NOD2 Polymorphism Predicts Response to Treatment in Crohn's Disease—First Steps to a Personalized Therapy, *Dig Dis Sci*, 57, 2012, 879-886.
- De Diego C, Alcantara M, Valle J, Pérez-grueso MJ, Muñoz-Rosas C, Carrobbles JM and Martínez-Castro P, Frequency of CARD15 polymorphisms in patients with Crohn's disease from Toledo, Spain: Genotype-Phenotype Correlation, *Genetic Testing*, 10, 2006, 178-85.
- Alvarez-Lobos M, Arostegui JI, Sans M, Tassies D, Plaza S, Delgado S, Lacy AM, Pique JM, Yagüe J and Panés J, Crohn's disease patients carrying Nod2/CARD15 gene variants have an increased and early need for first surgery due to stricturing disease and higher rate of surgical recurrence, *Ann Surg*, 242, 2005, 693-700.
- Gazouli M, Pachoula L, Panayotou L, Mantzaris G, Chrousos G, Anagnou NP and Roma-Giannikou E, NOD2/CARD15, ATG16L1 and IL23R gene polymorphisms and childhood-onset of Crohn's disease, *World J Gastroenterol*, 16, 2010, 1753-1758.
- Hampe J, Grebe J, Nikolaus S, Solberg C, Croucher P JP, Mascheretti S, Jahnsen J, Moum B, Klump B, Krawczak M, Mirza MM, Foelsch UR, Vatn M and Schreiber S, Association of NOD2 (CARD15) genotype with clinical course of Crohn's disease: a cohort study, *Lancet*, 359, 2002, 1661-1665.
- Lesage S, Zouali H, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M and Jannot AS, CARD15/NOD2 Mutational Analysis and Genotype-Phenotype Correlation in 612 Patients with Inflammatory Bowel Disease, *Am J Hum Genet*, 70, 2002, 845-857.
- Nunez C, Barreiro M, Dominguez-Munoz JE, Lorenzo A, Zapata C and Pena S, CARD15 mutations in patients with Crohn's disease in a homogeneous Spanish population, *Am J Gastroenterol*, 99, 2004, 450-456.
- Alvarez-Lobos M, Pizarro DP, Palavecino CE, Espinoza A, Sebastián VP, Alvarado JC, Ibañez P, Quintana C, Díaz O, Kalergis AM and Bueno SM, Role of Salmonella enterica exposure in Chilean Crohn's disease patients, *World J Gastroenterol*, 19, 2013, 5855-5862.
- Bhullar M, Macrae F, Brown G, Smith M, Sharpe K, Prediction of Crohn's disease aggression through NOD2/CARD15 gene sequencing in an Australian cohort, *World J Gastroenterol*, 20, 2014, 5008-5016.
- Al-Sulaiman R, Ismail M, Yasawy M, Al-Ateeq S, Abdelrashid M, Hussameddin AM, Al-Nafie A, Al-Ali AK, Akhtar MS and Al-Quorain AA, Association of NOD2/CARD15, DLG5, OCTN1 and Toll-like Receptor 4 Gene Polymorphisms with Inflammatory Bowel Disease: A University Hospital Experience, *Saudi Journal of Medicine & Medical Sciences*, 2, 2014, 81-85.
- Yamazaki K, Takazoe M, Tanaka T, Kazumori T, Nakamura Y, Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease, *J Hum Genet*, 47, 2002, 469-472.
- Ouyang Q, Tandon R, Goh K, Ooi CJ, Ogata H and Focchi C, The emergence of inflammatory bowel disease in the Asian Pacific region, *Current Opinion in Gastroenterology*, 21, 2005, 408-413.



27. Hama I, Ratbi I, Reggoug S, Elkerch F, Kharrasse G, Errabihi, Ouazzani H and Sefiani A, Non-association of Crohn's disease with NOD2 gene variants in Moroccan patients, *Gene*, 4994, 2014, 121-123.
28. Cavanaugh J, NOD2: Ethnic and geographic differences, *World J Gastroenterol*, 12, 2006, 3673-3677.
29. Pugazhendhi S, Amet A, Balamurugan R, Subramanian V and Ramakrishna B, Common NOD2 mutations are absent in patients with Crohn's disease in India, *Indian J Gastroenterol*, 27, 2008, 201-203.
30. Leong WL, Armuzzi A, Ahmad T, Wong ML, Tse P, Jewell DP and Sung JYI, NOD2/CARD15 gene polymorphisms and Crohn's disease in the Chinese population, *Aliment Pharmacol Ther*, 17, 2003, 1465-1470.
31. Li M, Gao X, Guo CC, Wu KC, Zhang X and Hu PJ, OCTN and CARD15 gene polymorphism in Chinese patients with inflammatory bowel disease, *World J Gastroenterol*, 14, 2008, 4923-4927.
32. Croucher PJ, Mascheretti S, Hampe J, Huse K, Frenzel H, Stoll M, Lu T, Nikolaus S, Yang SK, Krawczak M, Kim WH and Schreiber S, Haplotype structure and association to Crohn's disease of CARD15 mutations in two ethnically divergent populations, *Eur J Hum Genet*, 11, 2003, 6-16.
33. Chua KH, Ng CC, Hilmi I, Goh KL, Co-inheritance of variants/mutations in Malaysian patients with Crohn's disease, *Genet Mol Res*, 11, 2012, 3115-3121.
34. Kugathasan S, Loizides A, Babusukumar U, McGuire E, Wang T, Hooper P, Nebel J, Koftman G, Noel R, Broeckel U and Tolia V, Comparative phenotypic and CARD15 mutational Analysis among African American, Hispanic, and White children with Crohn's disease, *Inflamm Bowel Dis*, 11, 2005, 631-638.
35. Ananthakrishnan AN, Huang H, Nguyen DD, Sauk J, Yajnik V and Xavier RJ, Differential Effect of Genetic Burden on Disease Phenotypes in Crohn's Disease and Ulcerative Colitis: Analysis of a North American Cohort, *Am J Gastroenterol*, 109, 2014, 395-400.
36. Azzam N, Nounou H, Alharbi O, Aljebreen A and Shalaby M, CARD15/NOD2, CD14 and Toll-Like 4 receptor gene polymorphisms in Saudi patients with Crohn's disease, *In J Mol Sci*, 13, 2012, 4268-4280.
37. Derakhshan F, Naderi N, Farnood A, Firouzi F, Hbibbi M, Rezvany MR, Javeri A, Bahari A, Balaii H, Ghaffarzadeh MR, Aghazadeh R and Zali MR, Frequency of three common mutations of CARD15/NOD2 gene in Iranian IBD patients, *Indian J Gastroenterol*, 27, 2008, 8-11.
38. Rigoli L, Romano C, Caruso RA, Lo Presti M A, Bella CD, Procopio V, Giudice GL, Amorini M, Costantino G, Sergi MD, Cuppari C, Calabro GE, Gallizi R, Salpietro CD and Fries W, Clinical significance of NOD2/CARD15 and Toll-like receptor 4 gene single nucleotide polymorphisms in inflammatory bowel disease, *World Gastroenterol*, 14, 2008, 4454-4461.
39. Barmada MM, Brant SR, Nicolae DL, Achkar JP, Panhuysen C, Bayless T, Cho J and Duerr RH, A genome scan in 260 inflammatory bowel disease-affected relative pairs, *Inflamm Bowel Dis*, 10, 2004, 15-22.
40. Giachino D, Duist MM, Regazzoni S, Gregori D, Bardessono M, Salacone P, Scaglione N, Sostegni R, Sapone N, Bresso F, Sambataro A, Gaia E, Pera A, Astegiano M and Marchi MD, Analysis of the CARD15 variants R702W, G908R and L1007fs in Italian IBD patients, *European Journal of Human Genetics*, 12, 2004, 206-212.
41. Mendoza JL, Murillo LS, Fernández L, Peña AS, Lana R, Urcelary E, Cruz-Santamaría DM, Concha EG, Díaz-Rubio M and García-Paredes J, Prevalence of mutations of the NOD2/CARD15 gene and relation to phenotype in Spanish patients with Crohn disease, *Scand J Gastroenter*, 38, 2003, 1235-1240.
42. Ahmad T, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, De Silva A, Cook JT, Cullen S, Welsh KI and Jewell DP, The molecular classification of the clinical manifestations of Crohn's disease, *Gastroenterology*, 122, 2002, 854-866.
43. Bunning C, Genschel J, Buhner S, Kruger S, Kling K, Dignass A, Baier P, Bochow B, Ockenga J, Schmidt HJ and Lochs H, Mutation in the NOD2/CARD15 gene in Crohn's disease are associated with ileocecal restriction and are a risk factor for reoperation, *Aliment pharmacol ther*, 19, 2004, 1073-1078.
44. Liu J, Anderson C, Genetic studies of Crohn's disease: Past, present and future, *Best Practice & Research Clinical Gastroenterology*, 28, 2014, 373-386.
45. Long WY, Chen L, Zhang CL, Nong RM, Lin MJ, Zhan LL and Lv XP, Association between NOD2/CARD15 gene polymorphisms and Crohn's disease in Chinese Zhuang patients, *World J Gastroenterol*, 20, 2014, 4737-4744.
46. Uyar FA, Over-Hamzaolgu H, Ture F, Gul A, Tözün N, Saruhan-Direskeneli G, Distribution of common CARD15 variants in patients with sporadic Crohn's disease case from Turkey, *Digestive Disease and Sciences*, 51, 2006, 706-710.

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