REVIEW

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Role of the *CARD15* Gene in the Pathogenesis of Crohn Disease: Phenotypic Classification and Prognostic Implications

Crohn disease pathogenesis is the result of the complex interaction between environmental factors (i.e. micro-flora in the gut), immunologic and genetic predisposition (1, 2). The bacterial pathogenesis hypothesis in Crohn disease is based on experimental data and clinical observation. The first studies in rodent models, such as those in transgenic HLA-B27 rats and many others in mice, demonstrated differences in disease manifestations that lack disease in germ-free environments compared to experimental animals with normal enteric commensal microorganisms (2–5). Evidence for enhanced mucosal permeability, or absorption of potential inflammatory bacterial polymers from the small-bowel system based on overgrowth of luminal bacteria, has been documented (6). These pathogenic or normal luminal bacteria constantly stimulate the mucosal and systemic immune systems and perpetuate the inflammatory cascade (7, 8). Pioneer studies in Oxford by the late Sidney Truelove in the 1960s demonstrated the beneficial effect of diverting the luminal flow in severe colonic disease. Interestingly, the first studies showed better effect in Crohn disease than in ulcerative colitis. In 1983, Harper and co-workers reported an immediate but temporary clinical improvement in colonic Crohn disease patients who had a split or double-barrelled ileostomy (9). When investigating the influence of the faecal stream in the pathogenesis of Crohn lesions in patients with an ileal resection, Rutgeerts and co-workers described important differences between Crohn disease patients with and those without a temporally diverting terminal ileostomy. Patients who underwent reanastomosis after 6 months did not present characteristic inflammatory lesions of Crohn disease in the isolated neoterminal ileum. However, recurrence of disease was observed 6 months after reanastomosis in these patients as well as in those with one-step surgery (10).

The role of bacteria in the pathogenesis of Crohn disease has been the subject of speculation and debate. The fact that abnormal homeostatic mucosal immune responses to normal intestinal flora could predispose individuals to uncontrolled or pathological intestinal inflammation has further enhanced the search for pathogenic microorganisms in Crohn disease (11). One or more specific pathogens have been associated with Crohn disease, but it is clear that the disease is caused by more than just microbes.

Familial aggregation and twin studies have suggested the role of genetics in determining the susceptibility to Crohn disease. In addition, the incomplete concordance for Crohn disease within monozygotic twins, the phenotypic variations and the observed familial pattern of non-Mendelian inheritance suggest the presence and importance of environmental factors in the pathogenesis of this disease or syndrome. Age appears to be a modifying factor and the genetic anticipation of the disease developing at an early age in families with multiple members affected by the disease has been discussed elsewhere (12–14).

In 1996, Hugot (15) reported an association between the *IBD1* locus on the pericentromeric region of chromosome 16 conferring susceptibility to Crohn disease in French families with multiple members affected. Ogura et al. in the United States and Hugot et al. in France (16, 17) identified the relevant gene on chromosome 16q12: *CARD15*. Using linkage disequilibrium mapping analysis on 1272 family members from 235 Crohn disease families, Hugot et al. identified numerous SNPs by sequencing. Subsequently, an association was demonstrated for Crohn disease with three of these identified SNPs—a frameshift mutation and two independent missense mutations—in the *CARD15* gene (16). Ogura et al. independently identified the frameshift mutation in association with Crohn disease. By searching a genomic database for NOD1 homologues, the cDNA encoding the NOD2 protein coded by the *CARD15* gene was identified by the G. Nuñez group in Michigan, USA (18). Sequence analyses predicted the protein and a variant. Transmission disequilibrium tests and case-control analysis on IBD families were used to associate this shorter protein variant with Crohn disease (17). These studies demonstrated that the *CARD15* gene is the first locus causing susceptibility to Crohn disease in a Western population (15–21).

NOD2, a member of the CED4/APAF1 superfamily of apoptosis regulators, is a cytoplasmatic bacterial pattern-recognition receptor expressed primarily in monocytes (18, 22). This protein contains 1040 amino acids and is composed of three motifs: two amino-terminal caspase recruitment domains (CARD), a centrally located nucleotide binding domain (NBD) and 10 carboxy-terminal leucine-rich repeats (LRR) (see Fig. 1) (18).

Mechanisms of action of the NOD2 protein

The NOD2 protein can induce apoptosis (23, 24) and trigger the NFκB signalling pathway (18). Both processes are initiated, respectively, through a CARD-CARD interaction with caspase-9 (involved in apoptosis) and intracellular

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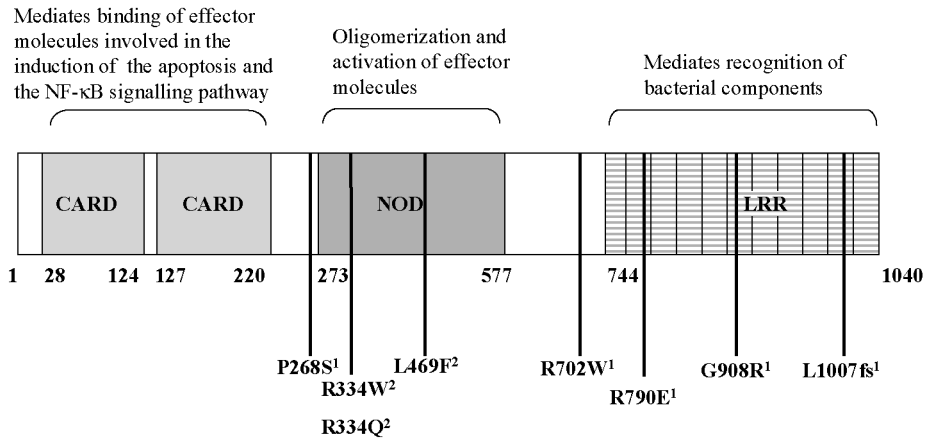

Fig. 1. Allelic variants associated with disease. [1]Denotes variants related to Crohn disease; [2]denotes variants related to Blau syndrome.

signalling mediated by the serine/threonine kinase RIP2 (also named RICK or CARDIAK) (22, 24, 25). This signal transduction pathway is preceded by the interaction of bacterial lipopolysaccharide to the leucine-rich repeats domain of NOD2. Transcription of the NFκB responsive genes includes cytokines and genes coding for cellular adhesion and procoagulant molecules (see Fig. 2). Kobayashi et al. (25) reported that the effect of a mutation in RIP-2 results in a reduced IL2 production, low proliferation of T-cell receptors and a low differentiation to the T_H1 cells.

Significance of leucine-rich repeats

The leucine-rich repeats domains are present in many different proteins found in a variety of organisms with diverse functions and cellular locations. Their primary function appears to be to provide a versatile structural framework for the formation of protein–protein interaction (26, 27) and subsequent signal transduction (28). Bacterial leucine-rich repeats containing proteins are found in *Listeria monocytogenes*: InlA and InlB, internalins with a critical role in the phagocytosis induced by this pathogen in mammalian cells and that promote invasion of permissive cells (29). Leucine-rich repeats containing proteins have also been identified in *Yersinia pestis* (YopM a plague virulence protein), *Shigella flexneri* (IpaH) (30), *Salmonella typhimurium* (SspH1 and SspH2) (31) and *Burkholderia* (*Pseudomonas*) *solanacearum* (Hrp) (32). Among eukaryotes, leucine-rich repeats domains are found in proteins encoded by the disease-resistant genes of the plant immune system and by the toll and toll-like genes of *Drosophila* and mammals, which are involved in innate immune responses (26, 27, 33).

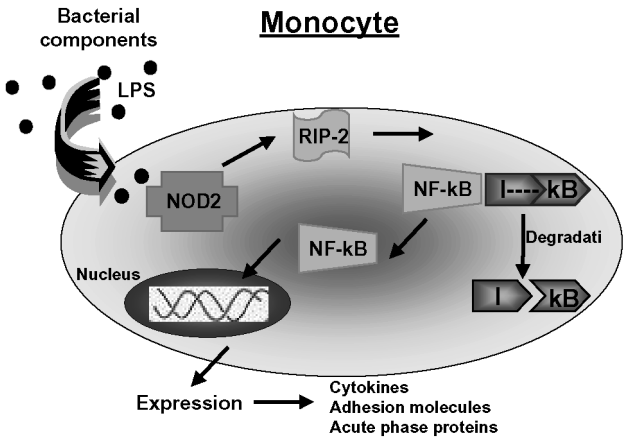

Fig. 2. Mechanism of NFκB pathway activation. Adapted from van Heel et al. (11).

Since the *CARD15* gene is involved in the regulation of immuno-inflammatory processes and is located at a known susceptibility locus for Crohn disease, this gene provides a clear link between the onset of inflammatory immune response to enteric bacteria and the development of the disease (3, 4, 11, 22, 34, 35).

Allelic variants associated with CD

As stated above, Ogura et al. and Hugot et al. (16, 17, 19) have reported a frameshift mutation in nucleotide 3020 in exon 11 of the *CARD15* gene by a cytosine insertion. This mutation in the 10th leucine-rich repeat results in a truncated protein with 1007 amino acids instead of 1040 amino acids. This variant has been shown to be defective in vitro in activation of the NFκB pathway modifying the response to lipopolysaccharide. Defects in sensing lipopolysaccharide might give rise to an abnormal innate and adaptive immune response (2, 4, 24, 35, 36).

In exons 4 and 8 another two missense mutations reported by Hugot et al. give rise to non-conservative amino-acid substitutions in either leucine-rich repeats (G908R) or an adjacent region (R702W) (16, 37). Lesage et al. described all these variants as potential disease causing mutations (DCM). Ninety-three percent of the described mutations in the *CARD15* gene, including other less frequent variants, carry

Table I. Frequencies of the R702W, G908R and L1007fs *CARD15* variants in different populations

Author	Population	*n*	Allele frequencies (%)			Genotype frequencies (%)			
			R702W	G908R	L1007fs	Mutant homozygous simple	Mutant heterozygous simple	Mutant compound heterozygous	At less one variant
Hugot J (16)	Not specified	468 CD	11.0	6.0	12.0	6.0	28.4	8.5	
		159 UC	3.0	0.0	1.0	0.6	8.1	0	
		103 HC	4.0	1.0	2.0	0	14.0	0	
Ogura Y (17)	Caucasian/Ashkenazi Jews (USA)	416 CD			8.2				
		287 HC			4.0				
Hampe J (19)	British/German	304 CD			16.0				
		165 UC			3.1				
		272 HC			4.4				
Murillo L (56)	Caucasian (Dutch)	130 CD		4.3	8.5				
		152 HC		3.0	5.9				
Vermeire S (62)	Caucasian (Quebec, Canada)	231 CD	12.9	5.2	10.3	5.2		6.5	45.0
		71 HC	4.2	0.7	0.7				9.0
Lesage S (38)	Caucasian (European)	453 CD	10.8	6.1	10.5				
		159 UC	3.1	0.3	1.3				
		103 HC	4.4	1.0	1.9				
Inoue N (46)	Japan	350 CD	0	0	0				
		272 UC	0	0	0				
		292 HC	0	0	0				
Cuthbert A (39)	Caucasian (European)	429? CD	9.1	3.4	6.6	3.8	27.8	4.2	
		566? UC	3.7	1.6	1.5	0.8	13.2	0.3	
		290 HC	3.5	0.6	2.1	0	13.6	0	
Vermeire S (63)	Caucasian (Belgium)	245 CD	10.0	4.7	5.3	2.4	35.0	4.9	32.6
		95 HC							15.0
Ahmad T (64)	Caucasian (UK)	244 CD	12.5	3.3	9.4	2.9	26.6	9.0	
		349 HC	5.2	1.4	1.6	0.3	15.2	0.3	
Radlmayr M (65)	No specified	97 CD				13.9			
		97 UC				2.1			
		120 HC				1.7			
Sugimura M (47)	Japan	188 CD	0	0	0				
		192 HC	0	0	0				
Vermeire S (66)	Canada	146 CD			12.3				
		72 HC			0.7				
Zhou Z (67)	Azhkenazi Jewish (USA)	312 CD sporadic		5.9					
		169 CD familial		12.7					
Vavassori P (68)	Italy	133 CD			11.7				
		81 HC			1.2				
Folwaczny C (69)	Germany	97 CD			13.9				
		97 UC			2.1				
		120 HC			1.7				
Van der Linde K (70)	The Netherlands	61 CD			13.9				
		32 UC			1.6				
		81 HC			1.9				
Yamazaki K (50)	Japan	483 CD	1	0	0				
Abreu M (71)	Caucasian/Jews and non-Jews (two cohorts)	142CD	16.9	12.0	11.3			4.0	36.6
		59 CD	15.3	10.2	11.9				32.2
		175 UC	5.7	1.7	3.4			0	10.9
Crichton D (72)	Scotland	176 CD			4.1				
		104 UC			0.7				
		141 HC			1.8				
Vemiere S (73)	Canada/Belgium?	137CD							43.8

a DCM located in the distal third of the gene (38). P268S, a fourth variant adjacent to NBD region exon 4, has been described in the literature (39). This variant has strong linkage disequilibrium with the three main variations described above in the association with Crohn disease, forming three independent disease haplotypes (39). Defects in the protein function were shown to result in a decreased NFκB activation for R702W and G908R compared with wild-type NOD2 and NOD2 P268S by Bonen et al., at the American Congress of Gastroenterology (AGA) in 2002 (40).

Spondyloarthropathy and CARD15 gene

The term spondyloarthropathy covers spondylitis ankylopoetica, undifferentiated spondyloarthropathy, psoriatic arthritis and reactive arthritis. The frequency of this category of diseases is between 0.5% and 1.0% and the pathogenesis is thought to be a combination of endogenic/genetic factors

(HLA-B27 and other) and exogenic factors (microorganisms such as *Chlamydia*, *Yersinia*, *Shigella* and *Salmonella*). Spondyloarthropathies are a group of inflammatory rheumatic diseases with recognized extra-articular manifestations and are associated with inflammatory bowel disease, Crohn disease as well as ulcerative colitis, suggesting a common pathogenic-genetic background between these two clinical entities. Approximately 10%–15% of inflammatory bowel disease patients have complications such as spondylitis ankylopoetica or other forms of spondyloarthropathy. Interestingly, Crane et al. demonstrated that the P268S *CARD15* variant was inversely associated with ulcerative colitis-spondylitis ankylopoetica, and the carriership of this SNP was associated with a greater disease severity as assessed by the Bath Ankylosing Spondylitis Disease Activity Index (41). However, no association has been found between the other described *CARD15* variants and primary spondylitis ankylopoetica and between ulcerative colitis and Crohn disease spondylitis ankylopoetica (41–44).

Chronic granulomatous disease and CARD15 gene

In granulomatous disease, the Blau syndrome, a rare genetic autosomal-dominant disorder characterized by granulomatous arthritis, uveitis and skin lesions, several mutations in the *CARD15* gene have been found. This disease is clearly different from Crohn disease, but both are characterized by granuloma formation. Three missense mutations (R334Q, R334W and L469F) located in the NBD region were found in families with this syndrome (see Fig. 1). The heterozygosity pattern that was found in the affected subjects was consistent with the autosomal-dominant inheritance characterized in this disorder (45).

Epidemiological and clinical studies

It is now clear that *CARD15* gene variations predispose some but not all populations to Crohn disease. Table I summarizes frequencies of the *CARD15* variants in different populations from peer-reviewed publications and from data presented at the AGA Congress in 2002. In Western populations, including North American and European groups, an increased susceptibility to Crohn disease is reported. People with at least one of the three frequent variants (R702W, G908R and L1007fs) were more likely to have Crohn disease. Hugot et al. observed a gene dose-effect for Crohn disease susceptibility in this study with European populations. Individuals homozygous for a variant or with a compound heterozygosity trait had an increased risk of Crohn disease (16, 34).

In the Japanese population, *NOD2/CARD15* variants were not found in Crohn disease, ulcerative colitis or controls (46–47), which suggests additional susceptibility loci for Crohn disease that may be located in other regions on chromosome 16 or on other chromosomes (48–50). Bonen et al. found lower allele frequencies of the three Crohn disease associated variants (R702W, G908R and L1007fs) in African-Americans as compared to Caucasian cohorts. These authors in the United States identified another variant (R790E) solely present in African-Americans (51).

Adams et al. have reported a similar frequency of the L1007fs in familial Australian Crohn disease to that previously reported in other populations (52). Ashkenazi Jew populations (ancestors from Eastern or Central Europe) are found all around the world and many are intermarried, ensuring a large genetic isolation. In this population a particular association was observed between *CARD15* gene variants and Crohn disease. Although a positive linkage was described for the frameshift mutation L1007fs and Crohn disease in this group, no significant relation between Crohn disease and the R702W and/or the G908R missense mutations was found (40, 53). Unfortunately, frequencies of the *CARD15* variants in other non-Ashkenazi Jews remain unknown to date.

A few groups have investigated the phenotypic effect of these mutations in the clinical subtypes and course of Crohn disease. Table II gives the associations of *CARD15* gene mutations found according to the major subgroups of the Vienna Classification (54, 55). We reported the first study of the frequencies of the mutations in *CARD15* G2722 and L1007fs. The sample size did not allow a significant association with the disease with any subgroup of Vienna Classification in the Dutch population (56). The different conclusions that can be drawn from the review of the literature and from our own observations may be the result of ethnic differences of disease classification particularly when the length of follow-up of the patients is taken into account. In general, the studies have demonstrated an increased risk to ileum-terminal lesions, an early onset and a less frequent colonic involvement in Western Europe's people with at least one of the mutations. The variability in the phenotype-genotype relations reported does not allow definitive conclusions to be drawn. Efforts should be directed toward building a molecular classification based on genotypes, a classification which could help predict a better prognosis, a response to therapy, outcome of Crohn disease, and also whether surgery was necessary.

Burenhult et al. have suggested that, according to human history, modern human beings appeared between 200,000 and 150,000 years ago in Africa. From there, they dispersed to Europe and Southwest Asia (57). This divergence in evolution implies a remarkable ability to adapt to new ecosystems, which potentially could require different responses to new stimuli. Both European and Asiatic ancestors developed different genetic and immunologic response characteristics. This genetic divergence may explain the lack of mutations of the *CARD15* gene in eastern populations with Crohn disease and suggest that these mutations are relatively late in the evolution. The same clinical picture may be caused by different evolutionary responses to the environment. The European stem required a modification in the immune

Table II. Associations described of *CARD15* variants according to the major subgroups of the Vienna Classification in Crohn disease

Author	Population	Association	A1	A2	B1	B2	B3	L1	L2	L3	L4	Notes
Hampe J (74)	Caucasian: Germans Norwegians (Prospective study)	Haplotypes associations*							X			No information about the risk effect of the significant relations. Previous resection too significant by retrospective study
	Retrospective: Germans					X		X	X			
Murillo L (56)	Caucasian (Dutch)	Carriage *CARD15* variants 1007fs, 908Arg										*CARD15* variants not related with a particular form of CD
Cuthbert A (39)	Caucasian (European)	Carriage of compound genotypes*						X		X		*CARD15* variants increase risk for L1 and L3
Radlmayr M (65)	No specified	Carriage 1007fs			X	X	X					*CARD15* variant increases risk for B2, B3 and ileocaecal resection and is negatively correlated with B1.
Ahmad (64)	British	1007fs carriership	X					X				*CARD15* 1007fs increase risk for A1 and B3
		Carriage *CARD15* variants*					X	X				*CARD15* variants increase risk for L1 and have a protective association for B3
Vermeire S (63)	Caucasian (Belgium)	Carriage *CARD15* variants*										*CARD15* not predictive of treatment outcome.
Vermeire S (62)	Caucasian (Canada)	Carriage *CARD15* variants*						X				
Lesage S (38)	Caucasian (European)	Carriage *CARD15* DCMs				X						*CARD15* DCMs increase risk for B2
		Double dose mutation of *CARD15* DCMs†	X			X			X			*CARD15* DCMs increase risk for A1 and B2 and have a protective association for L2
Hugot J (37)	France	Double dose mutation of *CARD15* DCMs†	X			X			X			*CARD15* variant increases risk for A1, B2, and is negatively correlated with L2
Hampe J (75)	Germany	Carriage *CARD15* variants*						X				*CARD15* variant increases risk for B2, B3 and Ileocecal resection and is negatively correlated with L2.
Louis E (76)	France	Carriage 1007fs					X	X				*CARD15* variant increases risk for B3 and L1
		Carriage Arg702Trp							X			*CARD15* variant increases risk for L1
		Carriage *CARD15* variants*	X					X				*CARD15* variant increases risk for A1 and L1
Vermeire S (66)	Canada	Carriage *CARD15* variants*							X			*CARD15* variant is negatively correlated with L2
Annese V (77)	Italy	Carriage *CARD15* variants*	X			X		X				*CARD15* variant increases risk for A1, B2, and L1
Vermeire S (78)	France	Carriage *CARD15* variants*						X				*CARD15* variant associated with ASCA+ and risk for L1
Vavassori P (68, 79)	Italy	Carriage 1007fs				X		X	X			*CARD15* variant increases risk for B2, L1 and is negatively correlated with L2
Folwaczny C (69)	Germany	Carriage 1007fs					X					*CARD15* variant increases risk for B2, ileocaecal resection and fibrostenosis
Abreu M (71)	Caucasian/ Jews and non-Jews (two cohorts)	Carriage *CARD15* variants*					X					*CARD15* variants are associated with fibrostenosing disease
Vermiere S (73)	Canada/ Belgium?	Carriage *CARD15* variants*					X					*CARD15* variant associated with ASCA+ and risk for L1
Crichton D (72)	Scotland	Carriage 1007fs						X				*CARD15* variant increases risk for B2

*Leu1007fs, Gly908Arg, Arg702Trp. †Disease-causing mutation. A1: Age of onset <40 years. A2: Age of onset >40 years. B1: Inflammatory. B2: Stricturing. B3: Penetrating. L1: terminal Ileum. L2: Colon. L3: Ileo-colon. L4: Upper gastrointestinal tract.

response allowing changes in the pathogenic mechanisms of responses to the ubiquitous bacteria of these populations.

The evidence of ethnic genetic variation that predisposes to Crohn disease suggests that other mutations might play a role in the increased susceptibility to Crohn disease, at least in Jewish and Japanese populations. This confirms the hypothesis that Crohn disease is a polygenic disease. The likely different pathogenic mechanisms involved in Crohn disease will guide future research in identifying additional susceptibility genes and ultimately could be helpful for developing therapeutic measures, which could differ for different ethnic populations.

Crohn disease and ulcerative colitis are disorders with overlapping phenotypes (1). The frequencies of the *CARD15* gene variants in ulcerative colitis are similar to those in controls, at least in Western populations. This could help identify potential differences in pathogenic mechanisms for ulcerative colitis and Crohn disease and might facilitate differential diagnostic of these diseases.

CARD15 mutations and the entity concept

The strong association of *CARD15* mutations with only a group of patients with Crohn disease and only in specific ethnic backgrounds further strengthens the hypothesis proposed some years ago by Walvoort & Peña (58). The evidence to suggest that Crohn disease was a defined disease was challenged and it was concluded that Crohn disease was a heterogeneous disorder composed of groups of patients with different pathogenesis and prognosis. C. Marina Fiol previously discussed this concept in 1960: 'In my view terminal ileitis can constitute a histological picture of different aetiologies. Consequently, it is a syndrome of different aetiologies, which lead to the same histological lesions' (59, 60). Crohn disease syndrome reflects a basic pattern of the gastrointestinal tract to more than one eliciting agent and the histopathological presentation of the disease seems to be mediated through specific dysregulated immunopathologic processes which may be triggered by non-specific agents. Identification of the intracellular lipopolysaccharide sensing NOD2 receptor seems to be the previous missing link for some of the Crohn patients.

Conclusions and future directions

The *CARD15* gene seems to play an important role in Crohn disease susceptibility in Western populations. These mutations link to early onset ileal and fibrostenotic disease corresponding to the A1/L1/B2 subgroup of the Vienna classification (61). Further studies should be designed to investigate the gene–environment interaction in the pathogenesis of Crohn disease and to determine the role of the leucine-rich repeats region in stimulating (or inhibiting) NFκB signalling and apoptosis activity. The mechanisms by which the genetic variations of the *CARD15* gene increase the risk of Crohn disease and determine clinical manifestations remain to be elucidated. A better understanding of the pathogenic mechanisms and phenotypic classification could provide a new focus for better and more specific therapeutic treatments with the promise of a better quality of life for those suffering from this debilitating disease.

However, the *CARD15* gene is not sufficient to explain the overall disease susceptibility. Because the genetic susceptibility to Crohn disease is not limited to chromosome 16, at least five other loci have been implicated and await further elucidation. Moreover, the *CARD15* gene does not account for all the linkage demonstrated at 16q12. This suggests that other *CARD15* gene variants or even additional genes on chromosome 16 may predispose to Crohn disease. There are other Crohn disease susceptibility genes that are located on chromosomes 6 (IBD3), 12 (IBD2) and 14 (IBD4) and it is possible that these loci also contain polymorphisms involved in regulation of the immune system pathways of NOD2. Future studies will provide the final answer to the challenge that is still present, namely: Is Crohn disease an entity or is it a combination of different diseases with different pathogenesis and prognosis?

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