Clinical applications of NOD2/CARD15 mutations in Crohn's disease.

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Summary

The recent identification of the CARD15/NOD2 gene as a susceptibility locus for Crohn's disease represents an important step in the immunopathogenesis of inflammatory bowel disease. The gene explains about 20% of the genetic susceptibility. CARD15 mutations are present in 30-50% of CD patients compared to 7-20% of healthy controls. The three risk alleles R702W, G908R and 1007fsInsC in NOD2 associated with susceptibility to Crohn's disease have demonstrated a remarkable amount of heterogeneity across ethnicities and populations, with regional variation across Europe. In non-Caucasian populations Crohn's disease continues to increase in incidence but this increase appears not to be a consequence of variation in NOD2. Genotype-phenotype analyses demonstrated an association of these mutations with ileum-specific disease and an increased incidence of the fibrostenotic phenotype. Although CARD15 variants do not predict response to the TNF alpha monoclonal antibodies, there are no data available on the possible influence of CARD15 mutations on response to other drugs. Screening for CARD15 mutations in order to identify high-risk individuals or to introduce an individualized disease management is therefore currently not recommended.

Key words: *CARD15/NOD2, Crohn´s disease, clinical application.*

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Aplicaciones clínicas de las mutaciones NOD2/CARD15 en la enfermedad de Crohn

Resumen

La reciente identificación del gen CARD15/NOD2 como locus de susceptibilidad para la enfermedad de Crohn representa un paso importante en la inmumopatogénesis de la enfermedad inflamatoria intestinal. El gen explica alrededor del 20% de la susceptibilidad genética. Las mutaciones en CARD15 aparecen en 30-50% de los pacientes con enfermedad de Crohn en comparación con 7-20% en los controles sanos. Los tres alelos de riesgo R702W, G908R y 100fsInsC del NOD2 asociados con susceptibilidad a la enfermedad de Crohn han demostrado una significativa heterogeneidad entre diferentes grupos étnicos y poblaciones, con variaciones regionales en toda Europa. En las poblaciones no caucásicas la enfermedad de Crohn aumenta en incidencia pero esta incidencia no parece ser una consecuencia de variación del NOD2. Los análisis de genotipo/fenotipo demostraron una asociación de estas mutaciones con enfermedad de localización ileal y una mayor incidencia del fenotipo fibroestenosante. Si bien las variantes del CARD15 no predicen una respuesta a los anticuerpos monoclonales TNF alfa, no hay datos disponibles sobre la posible influencia de las mutaciones del CARD15 como respuesta a otras drogas. Por consiguiente no podemos recomendar exámenes para las mutaciones del CARD15 con el fin de identificar individuos de alto riesgo ni para introducir un manejo individualizado de la enfermedad.

Crohn's disease (CD) aetiology is still unknown. An important role of genetic factors has been previously described by familial and epidemiologic studies. Familial aggregation of the disease and greater concordance in monozygotic than in dizygotic twins is well established. In last years a great number of DNA repositories were established, with DNA from thousands of individuals affected with CD. These repositories and the advances in genetic

technology have allowed the applications of techniques as genome-wide scans and candidate-gene approach to understand the genetics in CD. Although there is consensus that CD and ulcerative colitis are multifactorial and polygenic disease, the discovery in 2001 by two independent groups of the first CD susceptibility gene on chromosome 16q12, originally known as NOD2 and later renamed CARD15 by the International Nomenclature Committee[1,2] opened the possibility of using the presence of mutations of this gene in the diagnosis and evaluation of prognosis in clinical practice. In the present concise review we revise the current consensus of this finding for the clinician.

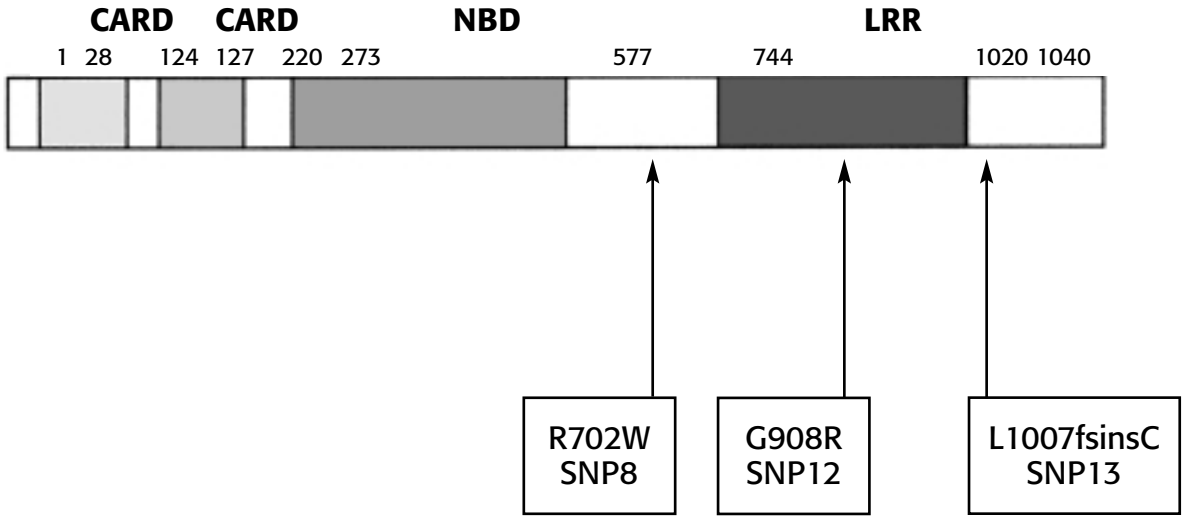
CARD15 is a cytosolic protein expressed primarily in monocytes, macrophages, dendritic cells and also detectable in some small intestinal Paneth cells.[3,4] Basic structure of the protein (1040 amino acides) is structured in three different regions: two N-terminal caspase recruitment domains (CARDs), these domains have been the reason for the name of the gene. A central nucleotide-binding site (NBS), and multiple carboxyl-terminal leucine-rich repeats (LRR), that is similar to the extracellular domains of toll-like receptors, implicated in pathogen resistance (figure 1).

CARD15 function consists on recognizing intracellular peptidoglycans from gram-negative and gram-positive bacteria through the detection of the muramyl dipeptide. The LRR region of CARD15 is essential in this peptidoglycan recognition. Binding of muramyl dipeptide to CARD15 leads to nuclear factor-kB (NF- kB) activation under normal circumstances. This has been relevant in CD pathogenesis, showing that NF- kB is a key intracellular signalling molecule, and has been shown elevated in CD tissues.[5]

Numerous CD-associated polymorphisms have been identified in CARD15 gene, but the most common are two missense mutations engendering arginine to tryptophan (R702W) or glycine to arginine (G908R) substitutions and a frameshifting cytosine insertion (L1007finscC) encoding truncated CARD15. The remaining variants or polymorphisms are unlikely to be of great significance because they are found in only a few individuals. In contrast, the three more common variants (R702W, G908R and 1007finscC), also called SNP8, SNP12 and SNP13 respectively, represent more than 80% of the mutated chromosomes. These three single nucleotide polymorphisms (SNP) in the NOD2/CARD15 gene have been associated with the incidence and the severity of acute graft-versus-host disease (GVHD) following allogeneic stem cell transplantation (SCT).[6,7]

After confirmation and validation in many populations that presence of CARD15 mutations increase susceptibility to CD, but not ulcerative colitis (UC), the global analyse of results confirms that CD is a complex genetic entity, and NOD2/CARD15 mutations are neither necessary nor sufficient for disease development. It was estimated that the three main

Figure 1. *Structure of NOD2/CARD15 gene with presence of CD-associated polymorphisms*



mutations of the gene do not explain more than 20% of the genetic predisposition to the disease.[8]

Population studies

Most of studies have been carried out in Caucasian populations. The results of these multicentric and heterogeneous studies shows that 30 to 40% of CD patients are heterozygotes for one of these three mutations,[9] whereas 3%-15% of patients are either homozygotes (same mutation on both chromosomes 16) or compound heterozygotes (2 different mutations on both chromosomes 16). By comparison, 7%-12% and 0%-1% of healthy control subjects are heterozygotes and homozygotes, respectively. A dose effect of the CD-associated CARD15/NOD2 risk alleles was observed, so the relative risk developing CD when carrying one mutation is 2-3, whereas homozygotes and compound heterozygotes have a 20-40 fold risk. Assuming a prevalence of 1-2/1000 for CD in Western countries, from the previously related relative risks can be deduced that the probability of developing the disease is 4-6% in the group of two mutations. With these results we assume that less than 10% of all persons carrying two CARD15 risk alleles will develop CD.[10,11]

A first meta-analysis of NOD2 variants on CD in diverse populations[12] contributed to map the frequency and geographical differences of the mutations. This study showed the results from 42 previously published populations (nowadays there are approximately 70), most of them Caucasian and with European origin. In this meta-analysis people carrying only one high-risk allele had 2.39-fold (95%CI: 2.00-2.86) increased odds of CD, and those carrying two or more of the risk alleles had 17.1-fold (95%CI: 10.7-27.2) higher odds of CD compared to people without any high-risk alleles. Analyzing separately the three main mutations, they observed a greater risk for CD in carriers of the frameshifting mutation (SNP13).

CARD 15 and geographic variations

Prevalence studies have shown important geographically variations in IBD rates, with a north-south gradient in risk. After analysing globally results, we can conclude that important geographical differences have been described in population studies of CARD15 mutations. It is notably that in Asiatic[13-16] and African[17] populations there has not been significant differences in carriers of CARD15 mutations between CD patients and controls (table 1). Most of these studies showed a total absence of carriers of any of the three major risk alleles. Recently a multicenter study showed that CARD15 mutational frequencies among African American and Hispanic children are lower compared with white children[18]. Nevertheless, important associations between CARD15 and CD have been described in other very distant populations from Europe like Australia,[19] Canada[20] and United States of America.[21] The explications for these results, very similar to most of European studies, should be the Caucasian origin of the subjects of those studies.

In European populations around 35-40% of CD patients carry at least one mutation in the

Table 1. *Populations without presence of CARD15 mutations or without significant differences with healthy controls*

AUTHOR	(Ref)	Year	POPULATION	CD (n)	UC (n)	C (n)
Inoue et al	(13)	2002	Japanese (3 hospitals in Tokyo) No mutations	350	272	292
Sugimura et al	(14)	2003	Japanese Tokyo No mutations	188	-	192
Croucher et al	(15)	2003	Korean No mutations	126	-	116
Leong et al	(16)	2003	Chinese No mutations	65	63	`70
Zouiten-Mekki et al	(17)	2005	Tunisian Significant less than French population	130	-	90

NOD2/CARD15 gene,[9,22-24] but a lower prevalence has been reported in some geographically peripheral and homogeneous populations as Finnish,[25] Scottish,[26] Irish[27] and Galician.[28] It is especially interesting that there was not significance of R702W mutation in the last three populations described; the explication may be the historically accepted Celtic origin of them.

CARD15 and relationship to the Vienna Classification

CD is a clinically heterogeneous disease, with multiple and timely variable phenotypes. There is no a universally accepted classification of CD phenotypes, but in last years, the most frequently applied is the Vienna Classification; this classification is based on parameters such as disease location, behaviour and age of diagnosis.[29] Some authors had hypothesized that phenotypic differences could be explained by genetics.

An important British study[30] was the first that showed that CARD15 mutations were significantly associated with ileal disease, with a relative risk increased from 4, in patients with possession of one variant allele, to more than 30, in patients with more than one mutation. These results have been corroborated in most of later published studies and, also in Economou´s meta-analysis,[12] which showed a predisposition to small bowel involvement [OR 2.53 (95% CI: 2.01-3.16)]. The association with ileal disease also appears to be present in patients with ileocolonic disease (L3 in the Vienna Classification); an explication could be the prominent expression of CARD15 in Paneth cells typically found in the terminal ileum.[4,31] As well as an association with ileal disease, other studies have shown that CARD15 variants were inversely correlated with colonic disease;[20,22] another potential hypothesis is the difference between the immune tolerance mechanisms of the ileum and of the colon; based in that the colon is exposed to much higher bacterial concentrations than the ileum, and it may utilize immune mechanisms that do not depend on intact CARD15 function.[11,32]

The results on disease behaviour are less consistent; the previously cited meta-analysis[12] showed that the three studied alleles were over-represented among the CD patients with stricturing disease behaviour [OR 1.94 (95% CI 1.61-2.34)]. After analyzing separately the main published studies we can conclude that most of patients with a fibrostenotic pattern (B2 in Vienna Classification) presented more allele mutations than the other behaviour groups;[20,22,30] being more consistent the association in those patients that showed the frameshifting mutation (1007finscC).[33] However, the correct interpretation of these associations is complicated, first of all because of the changing nature of behaviour of this disease; and, especially because we do not know if the association between CARD15 mutations and stricturing disease is a primary association since it is well known that ileal disease and stricture formation are not independent variables.[30,34]

More controversy exists in the relationship between CARD15 mutations and age of diagnosis of CD; mainly because of the age values of Vienna Classification (<40 and >40) are very lax. Another problem is that age of diagnosis is referred in very few published studies. Nevertheless two independent groups have observed an earlier age of presentation of the disease in those subjects who presented at least two mutations.[22,30]

CARD15 and Extraintestinal manifestations

No association between CARD15 mutations and extraintestinal manifestations (EIMs) of CD have been observed in the scarce number of studies that had included them.[20,22] No association was found after analyzing separately the most important EIMs and the three main mutations of CARD15.[35]

CARD15 and Enviromental factors

Smoking habits were similar between mutation carriers and noncarriers; this data suggest that other independent factors could interact with this well-characterized environmental factor.[22,28]

In relation to familial disease despite that most of the previous published studies had not found differences in the frequency of CARD15 variants in familial and non familial cases;[20,22,28] the meta-analysis[12] showed that the presence of any of the mutations increased the risk of familial CD [OR 1.41 (95% CI: 1.17-1.69)].

CARD15 and risk for surgery

Some studies had found a positive association between the presence of mutations, especially 1007finscC, and previously surgical procedure related to CD, mainly ileal resections.[33,36] A recently published German study observed that NOD2/CARD15 mutations are associated with ileocecal

resections and a high risk of postoperative relapse and reoperation.[37]

Medical treatment

There are not data available on the possible influence of CARD15 mutations on response to aminosalicylates, steroids, antibiotics or immunosuppressors. It has been hypothesized that mutations in CARD15 might be involved in response to infliximab®, based on the altered activation of NF-kβ and subsequently production of tumor necrosis factor-α, but first published studies did not show relationship between the CARD15 mutations and this treatment, neither induction nor manteinance.[38,39]

Screening

The previously reviewed shows that the clinical application of CARD15 mutations is still controversial. However, more data is needed, in particular data obtained in prospective studies on evolution of the disease and response to treatment. The first published studies showing an association between CARD15 mutations and CD, but not in UC, it was thought that the presence of mutations could help in the diagnosis of indeterminate colitis, which represent about 5-10% of IBD patients. Nevertheless this idea was rejected after knowing that CARD15 mutations are fundamentally associated with ileal CD.

It has been ruled out the use of CARD15 mutations as screening test is symptomatic patients due to the low sensitivity of the test; the absence of a mutation in an unaffected individual would not exclude the development of CD, nor would the presence of a mutation necessarily lead to clinical symptoms.[10,40] IBD relatives who are at higher risk for developing disease, sometimes, the patients for their descendants, frequently request presymptomatic screening.[41] But CARD15 mutations could not predict the development of CD on its own and furthermore there are no preventive strategies to avoid the development of the disease. At this stage, the value of a positive CARD15 mutation in relatives or in healthy controls (about 15%) would be to strengthen the advice to avoid cigarette smoking.

Conclusion

The major finding of the CARD15 gene in the susceptibility of Crohn's disease in Caucasians changed the focus of the investigation from the acquired to the innate immunity and no doubt will contribute to understand the physiopathology and immunology of a subgroup of patients. For the clinician in medical practice the significance of this finding is for the time being modest as can be concluded based on the evidence reviewed.

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