World Journal of Gastrointestinal Surgery

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4240/wjgs.v8.i4.284

World J Gastrointest Surg 2016 April 27; 8(4): 284-293
ISSN 1948-9366 (online)

REVIEW

NOD2 mutations and colorectal cancer - Where do we stand?

Diogo Branquinho, Paulo Freire, Carlos Sofia

Diogo Branquinho, Paulo Freire, Carlos Sofia, Serviço de Gastrenterologia, Centro Hospitalar e Universitário de Coimbra, 3000-075 Coimbra, Portugal

Diogo Branquinho, Paulo Freire, Carlos Sofia, Faculdade de Medicina da Universidade de Coimbra, 3004-504 Coimbra, Portugal

Author contributions: Branquinho D performed the literature search and wrote the text; Freire P and Sofia C designed the text structure and made several critical corrections and revisions until the submitted version was achieved.

Conflict-of-interest statement: The above-mentioned authors of this manuscript hereby declare that they do not have any conflict-of-interest (including but not limited to commercial, personal, political, intellectual, or religious interests) related to the work submitted herein.

Correspondence to: Diogo Branquinho, MD, Serviço de Gastroenterologia, Centro Hospitalar e Universitário de Coimbra, Praceta Professor Mota Pinto, 3000-075 Coimbra, Portugal. diogofbranquinho@yahoo.com
Telephone: +351-914-251929
Fax: +351-239-701517

Received: July 28, 2015
Peer-review started: July 31, 2015
First decision: November 6, 2015
Revised: November 20, 2015
Accepted: February 14, 2016
Article in press: February 16, 2016
Published online: April 27, 2016

Abstract

Due to the overwhelming burden of colorectal cancer (CRC), great effort has been placed on identifying genetic mutations that contribute to disease development and progression. One of the most studied polymorphisms that could potentially increase susceptibility to CRC involves the nucleotide-binding and oligomerization-domain containing 2 (*NOD2*) gene. There is growing evidence that the biological activity of *NOD2* is far greater than previously thought and a link with intestinal microbiota and mucosal immunity is increasingly sought after. In fact, microbial composition may be an important contributor not only to inflammatory bowel diseases (IBD) but also to CRC. Recent studies have showed that deficient *NOD2* function confers a communicable risk of colitis and CRC. Despite the evidence from experimental models, population-based studies that tried to link certain *NOD2* polymorphisms and an increase in CRC risk have been described as conflicting. Significant geographic discrepancies in the frequency of such polymorphisms and different interpretations of the results may have limited the conclusions of those studies. Since being first associated to IBD and CRC, our understanding of the role of this gene has come a long way, and it is tempting to postulate that it may contribute to identify individuals with susceptible genetic background that may benefit from early CRC screening programs or in predicting response to current therapeutic tools. The aim of this review is to clarify the status quo of *NOD2* mutations as genetic risk factors to chronic inflammation and ultimately to CRC. The use of *NOD2* as a predictor of certain phenotypic characteristics of the disease will be analyzed as well.

Key words: Colorectal cancer; Fecal microbiota; Cancer susceptibility; Intestinal inflammation; Nucleotide-binding and oligomerization-domain containing 2 mutations

Core tip: Recently, data from animal models showed that nucleotide-binding and oligomerization-domain containing 2 (*NOD2*) deficiency leads to dysbiosis and to an increased risk of colitis and colitis-associated colorectal cancer (CRC). Furthermore, it is now known that this receptor has a much more expanded role than previously thought. Concerning population-based studies, and despite initial inconsistencies, recent data points to an important role for *NOD2* mutations in CRC susceptibility. Identifying carriers of such polymorphisms may allow them to be included in stricter CRC surveillance programs. A link between *NOD2* mutation carriage and response to different chemotherapy regimens is also a promising field of research.

Branquinho D, Freire P, Sofia C. *NOD2* mutations and colorectal cancer - Where do we stand? *World J Gastrointest Surg* 2016; 8(4): 284-293 Available from: URL: http://www.wjgnet.com/1948-9366/full/v8/i4/284.htm DOI: http://dx.doi.org/10.4240/wjgs.v8.i4.284

INTRODUCTION

Despite several advances in the diagnosis and treatment of colorectal cancer (CRC), it continues to be one of the most significant causes of morbidity and cancer-related deaths[1]. Apart from familial syndromes that account for 5%-10% of CRC cases[2], this disease is considered to have a multifactorial etiology and therefore predicting individual risk has been problematic, as there are many genetic polymorphisms with probably modest individual effect[3]. Due to its proven role as a genetic predisposing factor for chronic inflammation, most notably in Crohn's disease (CD)[4], nucleotide-binding and oligomerization-domain containing 2 (*NOD2*) mutations have been suggested to have a similar role in CRC. This assumption derives from the fact that several gastrointestinal cancers are strongly linked to chronic inflammatory conditions. The risk for malignancy may even increase according to the degree of underlying inflammation, as is the case for long-standing ulcerative colitis[5] and *Helicobacter pylori* (*H. pylori*)-induced chronic gastritis. One plausible hypothesis is that pro-inflammatory stimulus may lead to continuous cell proliferation, angiogenesis and eventually DNA damage[6]. If the association between chronic inflammation and cancer is based on solid experimental and epidemiological data, what remains to be fully understood is how do *NOD2* mutations lead to chronic inflammation?

The NOD2 protein plays an important role in innate immunity by recognizing bacterial lipopolysaccharides and activating the nuclear factor-kappaB. The mutant alleles for *NOD2* gene are thought to cause loss of function - deficient recognition, impaired clearance and proliferation of bacterial pathogens that lead to increased pro-inflammatory cytokines and subsequently to chronic inflammation[7]. Recently, the role of *NOD2* has been analyzed under a different light, as it seems to take a central place in the intricate balance between protection of the intestinal mucosa under physiological settings and the production of pro-inflammatory cytokines in chronic inflammatory conditions. Any shift, either gain or loss of function for *NOD2*, elicits a disturbance in the immune system that may lead to inflammation[8].

Another promising approach to understanding the pathogenesis of these diseases is the modulation of intestinal microbiota. Formerly considered a passive element in the homeostasis of the intestinal mucosa, the microbiota is nowadays considered as essential for epithelial differentiation and in maintaining a protective environment[9]. To that end, commensal bacteria and *NOD2* interact in a feedback-like mechanism - *NOD2* keeps bacterial proliferation in a steady, controlled state and the microbiota intervene in controlling *NOD2* expression[10]. Animal models with high risk genotype for colitis and CRC show less severe or even absent inflammation and fewer cases of adenomas if raised in selected bacterial or germ-free conditions[11], suggesting that changes in microbial species affect colitis and CRC development.

From 2004 to 2010, several population-based studies tried to find an increased risk for CRC in *NOD2* polymorphism carriers. The most studied mutations have been two missense mutations - R702W (rs2066844 C/T) and G908R (rs2066845 C/G), and a frameshift mutation - 3020insC (rs2066847 insC). The frequency of these polymorphisms differs greatly between populations, being much less common in Asian cohorts[12]. Even among Caucasian populations, there is significant genetic heterogeneity that may have limited the findings of such studies. Furthermore, the source of controls and the type of methodology used are also potential bias. Another relevant finding is that individual polymorphisms may increase the risk of CRC only in selected groups (*e.g*., German individuals under 45-year-old carrying R702W polymorphism[13]). These promising studies may ultimately lead to identifying individuals with susceptible genetic background, other than those with well-known familial syndromes, therefore benefitting from early diagnostic screening.

In order to find recent publications (2004 to present) on *NOD2* mutations and CRC susceptibility, an extensive literature search was performed using PubMed and MEDLINE. The key search terms used were *NOD2*/caspase recruitment domain 15 (CARD15) mutations, CRC genetics, microbiota, mucosal immunity and chronic inflammation, either alone or in combination. All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for further relevant papers.

THE EXPANDING *NOD2* ROLE

In order to protect the epithelium of the gastrointestinal tract, the largest surface in our body exposed to external environment, a number of obstacles within the mucosa are disposed to prevent the spread of pathogenic organisms. Besides mechanical barriers

such as the mucus layers, the innate immune system is probably the first mechanism to act against deleterious microorganisms, and once triggered it becomes activated within minutes[14]. This almost immediate response is due to pathogen recognition receptors (PRRs). One of the most prominent families of PRR includes Nod-like receptors (NLRs) that recognize bacterial wall component peptidoglycan. Certainly the most researched member of this family is *NOD2*, expressed in dendritic cells, leukocytes and epithelial cells of the gastrointestinal tract, especially after inflammatory stimuli[15]. Paneth cells are secretory epithelial cells found in small bowel crypts that express significant levels of *NOD2*. The structural features of NLRs include a central nucleotide-binding oligomerization domain, a variable N-terminal protein-protein interaction domain, defined by the CARD, and a C-terminal leucine-rich repeat that senses pathogen-associated molecular patterns[8]. After activation, recruitment of a serine threonine kinase called RIP2 occurs, which leads to the enrollment of the NF-κB signaling pathway, leading to the transcription of immune response genes[16].

Along with this traditional role, *NOD2* plays a part in the induction of autophagy as well. This process leads to the destruction of damaged proteins and organelles which has paramount importance not only in recycling biomolecules but also in eliciting anti-microbial properties[17]. Growing evidence has shown that *NOD2* may be also a relevant player in $CD4^+$ T cell function and in generating a Th1 response. This leads to the production of IFN-γ by T cells. In addition to detecting bacterial pathogens, *NOD2* has shown a different role in host defense. According to *in vitro* studies, *NOD2*-deficient animals showed an increased susceptibility to several viral infections[18]. The response to certain parasite infections may be compromised as well, due to a reduced production of IFN-γ[16,19]. In $NOD2^{-/-}$ animals, through stimulation of Th1-associated cytokines, an increase in mucosal permeability and low-grade chronic inflammation occurs[20].

The aforementioned evidence places *NOD2* in the center of the immune system, quite distant from its first described role as a "simple" pathogen sensor. But the essential question remains: How do *NOD2* mutations predispose to CD and do they have a role in cancer development?

Although our understanding of these complex interactions has improved in recent years, we still have no solid evidence to prove that polymorphisms in *NOD2* actually lead to the production of a stable protein. Currently, there are at least two possible ways in which *NOD2* mutations can lead to chronic inflammation and CD. The first is related with the basic function of *NOD2* as a positive regulator for innate response. We can assume that if there is no efficient *NOD2* activation during early phases of pathogen exposure, bacteria will proliferate and ultimately lead to chronic inflammation. An alternative explanation involves the deficient activation of *NOD2* as well, but in a later stage, during an ongoing inflammatory process. A continuous stimulus of pro-inflammatory pathways will take place, triggering toll-like receptors (TLR) (*NOD2* is a negative regulator of TLR signaling) and favoring Th1 response and the release of cytokines. Animal models have shown that *NOD2* has a paramount importance in the bactericidal activity of ileal crypts and the regulation of ileal microbiota. This allows us to hypothesize that ileal CD caused by *NOD2* mutations is due to the dysfunction of Paneth cells[8,10,21]. This diverse function of *NOD2* may explain the heterogeneity of CD[16].

In what concerns CRC, there are several factors that could explain its link to *NOD2* mutations. It is a well-known fact that the risk for CRC increases with duration and severity of the inflammatory process, while it decreases when anti-inflammatory drugs such as mesalazine and immunomodulators such as azathioprine are used in ulcerative colitis, consistent with a causative role for inflammation in colon carcinogenesis. The chemopreventive activity of aspirin and other nonsteroidal anti-inflammatory drugs in CRC supports this concept as well.

If these mutations play a consistent part in the pathogenesis of inflammatory bowel diseases (IBD), most notably in CD, then the hypothesis that such mutations are potential risk factors for cancer may have solid grounds. The study by Couturier-Maillard[9] shed some light in the role of *NOD2* in colitis-associated cancer: $NOD2^{-/-}$ mice showed increased tumor load in the distal colon than wild-type animals. Furthermore, this risk was shown to be transmittable if both animals were cohoused. An acceptable rationale to explain these findings may involve an unbalance between pro- and anti-inflammatory cytokines and lead to the loss of autophagy and apoptosis stimuli. This could eventually lead to increased risk of infection, chronic inflammation and cancer[22].

The most promising body of evidence that supports the role of *NOD2* in colorectal carcinogenesis involves its capacity to shape a protective assembly of gut bacterial communities. The deregulation of intestinal microbiota seems to be the essential element in the complex interaction between *NOD2* mutations and CRC.

LINKING MICROBIOTA TO MUCOSAL IMMUNITY AND CRC

Recently, a significant effort has been made to identify the components and the role of intestinal microbiota in colonic health and homeostasis. The microbiota has been increasingly recognized as a major player in normal metabolic and physiologic processes. This community of 10^{13}-10^{14} microorganisms represents a perfect example of symbiotic relationship. It exerts several essential functions like the synthesis of essential compounds for the normal growth of colonic mucosa, regulation of its lymphoid tissue and synthesis of amino acids that inhibit the growth of pathogenic microorganisms. The latter is

of paramount importance, as a delicate equilibrium has to be achieved between commensal bacterial load and the innate immune system. The commensal bacteria must be contained in their capacity to proliferate and should be maintained in adequate amounts. Conversely, any change in the composition of luminal bacterial flora may favor the production of toxins and metabolites associated with carcinogenesis and induce dysregulation of the immune response, allowing pathogenic agents to replicate, therefore promoting and sustaining inflammation and carcinogenesis[23]. A well balanced gut microbiota leads to healthy colocytes through the production of important compounds and the correct modulation of the immune system. It is now believed that *NOD2* mutations may result in altered host-microbial interactions in the intestinal mucosa.

COMPOSITION OF THE MICROBIOTA

It is known that the human intestine is sterile in utero, but progressively over 36000 bacterial species colonize the gastrointestinal tract and develop a symbiotic relation with their host. The human distal intestinal microbiota includes two predominant phyla, the Firmicutes and Bacteroidetes, with less significant contributions from Proteobacteria and Actinobacteria, and minor contributions from Fusobacteria, Verrucomicrobia, and Cyanobacteria[24]. The production of propionate and butyrate from the degradation of indigestible polysaccharides is one of its main roles. Despite being outnumbered by other species, Actinobacteria, Proteobacteria (including *Escherichia coli*) and Verrucomicrobia, showed potential to influence health outcomes[25]. A significant effort is being made to characterize the diverse genetic material of these numerous microorganisms (also known as microbiome). Ultimately, the goal is to understand the link between variations in the composition of these communities and common diseases, such as IBD and CRC. The hypothesis formulated by several investigators lies on the ability of these variations of the microbiota to cause a breakdown of the balance between bacterial communities and the epithelial barrier may lead to chronic inflammation. It is thought that some species may not be able to maintain a quiet state of protective immunity in dysbiotic conditions. For example, *Bacteroides thetaiotaomicron* was found to be not only a commensal but also an opportunistic microorganism in predisposed individuals[26].

A special attention has been devoted to factors that may influence the composition of the microbiota. Aging, place of birth and mode of delivery are quite relevant determinants. Antibiotics are also important and modifiable factors, especially with larger antimicrobial spectrums, as their effect on the microbiota varies from drastic to only temporary. After the end of the antibiotic treatment, certain species may recover in about a month while others may not recover at all, most often in children[27]. Besides antibiotics, different dietary habits may modify significantly the microbiota as well. Quite interestingly, even a short-term increase in fat and carbohydrate consumption may influence not only the relative abundance of each bacterial species, but also the functionality of the microbiota[28]. Obesity has also been proposed as a major factor in this equation, as it may alter the composition of the microbiota and increase its metabolic potential to harvest energy from the host diet. The capacity of the microbiota to confer host traits was revealed by studies where fecal content was transplanted from obese mice into lean germ-free recipients. The recipients showed significant weight gain and increased adiposity[29]. This surprising discovery opened the door for further investigation on genetic and microbiota manipulation and its ability to cause disease in animal models. Another interesting concept was revealed by Couturier-Maillard *et al*[9]. The absence of *NOD2* confers a transmissible risk for colitis and CRC, even to immunocompetent hosts. In other words, after sharing the same environment with *NOD2*$^{-/-}$ mice, wild-type animals treated with dextran sodium sulfate revealed an increased risk for colitis, probably due to an altered commensal flora acquired from knockout mice. This dysbiotic microbiota is then passed to the next generation. Growing evidence now shows us that the risk for colitis and CRC is influenced by specific members of the commensal microbiota. In fact, in *IL10*-deficient mice treated with colon-specific carcinogen azoxymethane (AOM) showed high levels of mucosal inflammation and adenoma development when raised in conventional conditions, but when *Bacteroides vulgatus* is the only commensal, the carcinogenicity of AOM is somewhat attenuated. Even more surprising is the fact that these mice show almost no inflammation or adenomas if created in a germ-free environment[11]. This is consistent with the notion that treating *NOD2*-deficient mice with broad spectrum antibiotics may mitigate its disease risk[9]. In the opposite direction, a recent study suggests that antibiotics promote inflammation through translocation of commensal colonic bacteria and it is suggested that this may explain the association between increasing antibiotic use and the growing incidence of inflammatory disorders[30].

The importance of *NOD2* as a regulator of microbiota and consequently as a risk factor for injury of the colonic mucosa was reinforced by studies using reciprocal fecal microbiota transplantation. Such interventions led to profound changes in the microbiota. The end result was that *NOD2*-deficient mice that received fecal transplantation from wild-type animals showed decreased mucosal injury and inflammation. On the contrary, there was an increased risk of colonic disease in wild-type hosts that received dysbiotic fecal microbiota from *NOD2*-deficient mice[9]. A crucial role in this equilibrium is played by Paneth cells, specialized secretory epithelial cells of the small intestinal crypts that express *NOD2* at high levels. Recent studies demonstrated that *NOD2*-deficient mice had a significant rise in the amount of commensal bacteria in the terminal ileum, probably due to impaired cryptal activity[31]. This is a potential mechanism by which *NOD2* mutations may disturb intestinal homeostasis and

lead to CD and colitis-associated CRC. But the intestinal microbiota role in this equilibrium is not passive at all. On the contrary, it is now known that it plays a part in controlling the expression of *NOD2* as well.

The influence of *NOD2* in microbial communities and its consequences on disease risk have solid basis. But how exactly does an altered microbiota lead to colonic inflammation? One possible explanation is the production of bioproducts with anticancer properties by the metabolic machinery of the microbiota. Butyrate and other small-chain fatty acids are nutrients formed by the fermentation of indigestible carbohydrate and are known to have an interesting paradoxal activity. In colon carcinoma cells, it leads to apoptosis, inhibits cell proliferation and angiogenesis, therefore showing a protective effect. On the other hand, in normal colonic cells it shows opposite effects as it prevents apoptosis[32]. Another potential role for the microbiota and especially for butyrate is maintaining inhibition of the histone deacetylase, therefore maintaining histones in an acetylated state, thus facilitating the transcription of anti-oncogenes[33]. This is an objective of anticancer drugs such as Vorinostat, an approved agent for the treatment of cutaneous T cell lymphoma. Detrimental influences are provided by the accumulation of toxic compounds in the gut that can exert a mutagenic action. Poli-heterocyclic amines, deoxycholic acid and calibactin are examples of compounds directly or indirectly produced by commensal bacteria that harbor potential to damage colonocytes' DNA[34]. This conflicting evidence supports the idea that the role of the microbiota shouldn't be considered univocal, but rather be regarded as a complex set of influences that may have a protective or deleterious effect on mucosal immunity, depending on its specific components. It is reasonable to postulate that the same bacterial agents will elicit different effects according to each individual genetic background and environmental exposure.

CASE-CONTROL STUDIES ON *NOD2* POLYMORPHISMS

Conflicting

This is a common word found in the introduction of most studies on *NOD2* mutations as a risk factor for CRC. However, for an adequate analysis of the published results, several factors should be taken into consideration.

First, the most studied polymorphisms of *NOD2* have significantly different prevalence in different populations. Three of the most common polymorphisms were not found in 342 patients included in a Malaysian study[12]. On the contrary, in a Danish study that included more than 40000 individuals, about 13% were carriers of at least one of the polymorphisms[35]. An obvious conclusion is that we should analyze these results according to the geographic region where they were conducted. Even in European studies, where these polymorphisms are thought to be more common, the number of carriers is often low. For example, in a study from Finland, there was only one homozygote for the R702W mutation in a universe of 1400 subjects[36]. Achieving solid conclusions with such low numbers is extremely difficult. Another limiting aspect that may hinder the conclusions of such studies is the source of controls. The results differ according to the source of controls. According to a meta-analysis that included 30 case-control studies about *NOD2* polymorphisms and cancer risk[37], there was only an increased risk in the subgroup with hospital-based controls, while no significant risk was observed in population-based studies. A factor worth looking at as well is that the great majority of studies about *NOD2* polymorphisms described so far analyzed only DNA extracted from nonneoplastic tissue (searching only germline mutations). For a complete understanding of the role of these mutations in the pathogenesis of CRC, an investigation of the neoplastic tissue should be undertaken as well (somatic mutations). To our knowledge, there is only one study that tried to determine if these mutations were of germline or somatic nature[38]. A total genotypic agreement between blood and neoplastic samples was observed, therefore suggesting that CRC susceptibility associated with these variants is linked to germline mutations, apparently without the participation of somatic mutations.

Most studies addressing *NOD2* polymorphisms and CRC are essentially linkage studies concerning a specific country or region. Nowadays, genome-wide association studies (GWAS) have a significant impact on medical research. As they search for differences in allele frequencies or genotypes in a large number of patients, through the identification of thousands of single-nucleotide polymorphisms, GWAS have stronger statistical power than linkage studies. However, as it was already mentioned, the frequency of *NOD2* polymorphisms shows a significant geographic variability. As GWAS often recur to samples from a quite diverse set of countries, the effect of these polymorphisms in a certain population may go unnoticed.

When analyzing the results from these studies, it is important to consider each polymorphism separately as well. It seems plausible to admit that each polymorphism will have different effects on *NOD2* function and therefore its effect on cancer risk will probably be different as well. The most studied polymorphisms are two missense mutations - rs2066845 C/G (G908R) and rs2066844 C/T (R702W), and one frameshift mutation - rs2066847 insC (3020insC). For the first missense mutation, G908R, results are equivocal (Table 1). Case-control studies failed to identify an increased susceptibility to CRC for G908R mutation carriers in German, Portuguese and Hungarian populations[13,38,39]. On the other hand, a Greek study was able to find an association between this mutation and CRC susceptibility[40]. In what concerns meta-analysis, the results are conflicting. In the meta-analysis by Tian *et al*[41], there is evidence for an increased risk, but more recently, in 2014, a new meta-analysis showed no association between CRC and the G908R mutation[37]. For the last of the missense mutations,

Table 1 Genotype frequencies of the nucleotide-binding and oligomerization-domain containing 2 polymorphisms

	Country	Finland[36]	Greece[40]	Hungary[39]	New Zealand[43]	Portugal[38]
	CRC (*n*)	953/960/926[1]	104	194	133	112
	Controls (*n*)	508/508/348[1]	100	200	201	152
R702W						
CRC	% Allele frequency	2.2	4.8	1.8	7.1	12.5
Control	% Allele frequency	2.1	1.0	1.5	3.0	5.3
	P value	0.88	0.02	0.78	0.03	0.03
G908R						
CRC	% Allele frequency	0.3	8.65	1.8	2.2	2.73
Control	% Allele frequency	0.2	3.5	1.8	0.8	3.29
	P value	0.57	0.025	0.95	0.09	0.77
3020insC						
CRC	% Allele frequency	1.9	12.5	3.6	2.2	0.89
Control	% Allele frequency	1.9	6	2.5	1.0	1.3
	P value	0.96	0.017	0.40	0.19	0.75
At least one mutation						
CRC	% Genotype frequency	-	51.9	14.4	21.8	16.1
Control	% Genotype frequency	-	21.0	11.5	8.9	9.9
	P value	-	< 0.0001	0.45	0.001	0.132

[1]CRC cases and controls for each mutation, respectively (R702W, G908R, 3020insC). CRC: Colorectal cancer.

R702W, there is strong evidence for an important role in CRC susceptibility. Several population-based studies and two meta-analyses revealed a significantly increased prevalence of this mutation in CRC patients (Table 1). For the frameshift mutation 3020insC, most studies support a relevant role for an increased risk of disease (Table 1)[40,42]. Both meta-analysis published on this subject revealed that carrying the 3020insC mutation was associated with a higher risk for CRC development[37,41].

If our objective is to assess the risk for CRC in *NOD2* polymorphism carriers, we should analyze the combined effect of the three main mutations. Several studies tried to determine if there was an increased risk for an individual carrying at least one of these polymorphisms. According to at least three population-based studies[13,38,40], there was evidence of an increased CRC risk if one or more of the described polymorphisms were identified.

Besides searching for a potential role for *NOD2* mutations and a hypothetic increased susceptibility for CRC, several groups searched for genotype-phenotype correlations in these patients. One of the main concerns for investigators and clinicians working in the field of CRC are young patients suffering from this disease. Finding a marker of increased risk that could identify patients under 50 that should enter an early surveillance program is obviously a sought-after goal. In a German cohort of patients under 50, a significant association between *NOD2* mutations and CRC susceptibility was described[13]. In a Portuguese study[38], the R702W variant was associated with an increased risk for CRC only in female patients under 60. In the opposite direction, the groundbreaking work by Kurzawski *et al*[42], the first group that tried to find a correlation between these polymorphisms and CRC, revealed a bigger propensity of 3020insC mutation carriers to develop CRC at a later age. An association with certain phenotypic characteristics was researched as well. Tumor location and size, vascular or lymphatic invasion, differentiation and distance to margins in resected specimens showed no relation with the presence of *NOD2* polymorphisms in most studies[38,39,43]. This lack of genotype-phenotype agreement may be due to the genetic heterogeneity of the disease or it can be explained by the small number of mutation carriers diagnosed with CRC, therefore limiting the ability to reach such a conclusion. Only a Greek study was able to show a relevant association between tumor stage (TNM classification) and the occurrence of these mutations[40].

Currently, new therapies for cancer are designed for a specific set of patients that are expected to respond, according to certain clinical and biological features. For example, Cetuximab is only prescribed to patients with advanced CRC and no mutation in the *KRAS* gene (wild type at codons 12 and 13 of *KRAS*). For *NOD2* mutations, a potential role for predicting response to treatment was researched as well. In 2014, Omrane *et al*[44] described an association between CRC patients carrying 3020insC polymorphism and the need for neoadjuvant chemotherapy. The presence of this polymorphism was able to predict failure of neoadjuvant chemotherapy. Conversely, the presence of 3020insC mutation was predictive of successful adjuvant chemotherapy[44]. Despite the relatively small size of the sample, this may be a promising role for *NOD2* mutations, needing confirmation by large-scale studies.

Routine detection of *NOD2* mutations is still not being offered in the management of CRC patients. However, there is a new simple and cost-effective tool for the genetic screening of CRC[45]. Besides well-known mutations in *MLH1*, *MSH2* and *MSH6* genes, the DNA microarray assay also searches the 3020insC polymorphisms in the *NOD2* gene. This may be a useful tool in clinical practice in CRC screening programs.

Due to the already described conflicting nature of the results from studies on *NOD2* mutations and CRC risk,

Table 2 Nucleotide-binding and oligomerization-domain containing 2 polymorphisms and cancer risk: Results of meta-analysis

Meta-analysis		Liu *et al*[37] (2014)	Tian *et al*[41] (2010)
R702W			
Variant genotypes *vs* homozygous wild-type	OR	1.32	1.59
	95%CI	1.01-1.72	1.09-2.32
	P value	0.04	0.02
G908R			
Variant genotypes *vs* homozygous wild-type	OR	1.32	1.98
	95%CI	1.01-1.72	1.14-3.44
	P value	0.04	0.01
3020insC			
Variant genotypes *vs* homozygous wild-type	OR	1.23	1.44
	95%CI	1.10-1.38	1.13-1.84
	P value	< 0.001	0.003
R702W/G908R/3020insC			
Variant genotypes *vs* homozygous wild-type	OR	-	1.58
	95%CI	-	1.03-2.42
	P value	-	0.03

OR: Odds ratio.

two meta-analyses on this subject were conducted (Table 2)[37,41]. The first was published in 2010 and remains, to our knowledge, the only meta-analysis that studied *NOD2* mutations and CRC exclusively. A total of 85 papers were screened, but only 8 were considered appropriate to be included (all from Caucasian populations), totaling 3524 CRC cases and 2364 controls (Table 2). The most significant risk for CRC was found in patients carrying the G908R mutation (5 studies; OR = 1.98; 95%CI: 1.14-3.44). Increased risk was also found for the R702W mutation (5 studies; OR = 1.59; 95%CI: 1.09-2.32) and the missense mutation 3020insC (7 studies; OR = 1.44; 95%CI: 1.13-1.84). For an individual carrying at least one of these high-risk alleles, there is also an increased probability of developing CRC (5 studies; OR = 1.58; 95%CI: 1.03-2.42). The most recent meta-analysis on this subject studied the effect of *NOD2* mutations in the susceptibility for several types of cancer (melanoma, breast cancer, non-Hodgkin lymphoma and different gastrointestinal tumors). A total of 30 articles were included, 11 of which exclusively with CRC patients, published from 2004 to 2010. The results were similar to the ones described by Tian *et al*[41], as it was demonstrated that both R702W and 3020insC were risk factors for CRC (Table 2). The only discrepancy was the effect of carrying G908R polymorphism. This allele was found to contribute to the overall risk of cancer, but not specifically to CRC. The studies included in this meta-analysis showed no obvious heterogeneity (I^2 < 50%). Excluding any of the included studies would not change significantly the outcome, therefore suggesting that these results are statistically robust[41].

NOD2 MUTATIONS AND OTHER MALIGNANT AND NON-MALIGNANT CONDITIONS

Since the publication in 2001 by Ogura *et al*[4] of the first study about *NOD2* mutations and increased susceptibility to CD, several investigators tried to understand the intricate mechanism behind this association and to find a link between these polymorphisms and other inflammatory and malignant conditions.

In fact, a role for *NOD2* mutations has been postulated for several malignant diseases other than CRC. In common digestive tract tumors, a significant number of published studies have addressed this putative relation. The 3020insC missense mutation was shown to be a risk factor for intestinal type gastric cancer in a Portuguese population[46]. The same was demonstrated by an Italian study for the R702W and 1007fs polymorphisms[47]. According to the meta-analysis by Liu *et al*[37], there was an increased risk for gastric cancer for carriers of the G908R and 3020insC mutations, but the same was not observed for the R702W polymorphism. In a recent population-based Chinese study, an increased risk for gastric cancer was found for individuals carrying the rs718226 AG or GG genotype. Interestingly, this single-nucleotide polymorphism revealed significant joint effects with *H. pylori* in dysplasia and gastric cancer risk. On the contrary, both the rs2111235 C allele and the rs7205423 G allele showed a protective effect, as they were associated with a decreased risk of progression to dysplasia and gastric cancer in *H. pylori*-infected subjects[48].

In what concerns pancreatic cancer, there was no evidence of increased risk neither in the familial nor the sporadic form of the disease[37,49]. The same meta-analysis revealed an increased risk for MALT lymphoma, breast, lung and laryngeal cancer for the carriers of the 3020insC mutation. On the other hand, none of the *NOD2* mutations were found to be risk factors for melanoma or non-Hodgkin lymphoma[37].

These discrepancies found between different studies may be attributed to a variety of factors, especially those that influence the expression of these polymorphisms, as well as differences in sample size, geographic variation or genotyping methods[22].

The effect of *NOD2* as a risk factor for disease is best established in CD. After an etiologic role was consolidated for these mutations in CD, further investigation was undertaken to find out if these mutations influenced the behavior, prognosis and response to treatment as well. The presence of one mutation increased the risk for structuring or penetrating disease by 8% and this effect was largely increased if two *NOD2* mutations were present (41% risk increase)[38]. An Australian study revealed as well that carriers have a more aggressive disease, needing more frequent and more precocious surgery[50]. A recent European multicenter cohort study recently revealed that *NOD2* mutations and early use of immunomodulatory drugs are the most relevant predictors of the course of disease[51].

It was speculated as well that certain disease phenotypes and their response to treatment could be influenced by *NOD2* mutations. The development of perianal fistulas is thought to depend on the proliferation of luminal bacteria. As such a possible connection between *NOD2*, a regulator of host response to microbial agents, and perianal fistulas was evaluated in recent literature. These fistulas showed significantly worse response to antibiotics in *NOD2* mutation carriers[52], probably due to impaired recognition of intestinal bacteria and a decreased ability to mount an effective innate immune response. This kind of studies emphasizes the importance of gene mapping and corresponding phenotypic correlations in order to predict disease severity and optimize treatment strategies.

CONCLUSION

In the last fifteen years, the proposed role of *NOD2* and its mutations in disease has grown significantly. From only a susceptibility gene to an important predictor of prognosis and response to treatment in CD, these mutations have been postulated as a risk factor in several conditions such as mycobacterial infections, common gastroenterological disorders and malignant diseases like gastric and colorectal cancer. The expanding role of this receptor as a major coordinator of several inflammatory pathways and a modulator of microbiota is increasingly accepted, mainly due to evidence arising from *NOD2*-deficient animal models. In fact, it was shown that losing *NOD2* activity leads to more severe colitis and higher propensity to adenomas and CRC. It seems likely that *NOD2* may be the key element of the intricate puzzle that links the disturbance of mucosal immune defense, dysbiotic bacterial communities and conditions such as CD and colitis-associated CRC.

Furthermore, *NOD2* polymorphisms such as 3020insC and R702W seem to increase susceptibility to CRC. The search of these mutations is still not offered routinely in clinical practice. However, the identification of its carriers would allow such patients to be included in a more intense CRC surveillance program, contributing to early diagnosis of a disease that carries such a heavy burden. Predicting response to different chemotherapy regimens according to the presence of *NOD2* polymorphisms could become a useful tool for clinicians. More large-scale studies should be conducted to confirm this association. The development of new therapeutic targets based on research about NOD2 protein function and interactions could ultimately lead to a tailored approach to the treatment of CRC.

REFERENCES

1 **Siegel R**, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014; **64**: 104-117 [PMID: 24639052 DOI: 10.3322/caac.21220]

2 **Tejpar S**, Van Cutsem E. Molecular and genetic defects in colorectal tumorigenesis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 171-185 [PMID: 11969232 DOI: 10.1053/bega.2001.0279]

3 **Boland CR**. Chronic inflammation, colorectal cancer and gene polymorphisms. *Dig Dis* 2010; **28**: 590-595 [PMID: 21088407 DOI: 10.1159/000320053]

4 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606 [PMID: 11385577 DOI: 10.1038/35079114]

5 **Rubin DT**, Huo D, Kinnucan JA, Sedrak MS, McCullom NE, Bunnag AP, Raun-Royer EP, Cohen RD, Hanauer SB, Hart J, Turner JR. Inflammation is an independent risk factor for colonic neoplasia in patients with ulcerative colitis: a case-control study. *Clin Gastroenterol Hepatol* 2013; **11**: 1601-1608.e1-e4 [PMID: 23872237 DOI: 10.1016/j.cgh.2013.06.023]

6 **Sipos F**, Molnár B, Zágoni T, Berczi L, Tulassay Z. Growth in epithelial cell proliferation and apoptosis correlates specifically to the inflammation activity of inflammatory bowel diseases: ulcerative colitis shows specific p53- and EGFR expression alterations. *Dis Colon Rectum* 2005; **48**: 775-786 [PMID: 15747078 DOI: 10.1007/s10350-004-0831-5]

7 **Philpott DJ**, Sorbara MT, Robertson SJ, Croitoru K, Girardin SE. NOD proteins: regulators of inflammation in health and disease. *Nat Rev Immunol* 2014; **14**: 9-23 [PMID: 24336102 DOI: 10.1038/nri3565]

8 **Corridoni D**, Arseneau KO, Cifone MG, Cominelli F. The dual role of nod-like receptors in mucosal innate immunity and chronic intestinal inflammation. *Front Immunol* 2014; **5**: 317 [PMID: 25071778 DOI: 10.3389/fimmu.2014.00317]

9 **Couturier-Maillard A**, Secher T, Rehman A, Normand S, De Arcangelis A, Haesler R, Huot L, Grandjean T, Bressenot A, Delanoye-Crespin A, Gaillot O, Schreiber S, Lemoine Y, Ryffel B, Hot D, Nùñez G, Chen G, Rosenstiel P, Chamaillard M. NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J Clin Invest* 2013; **123**: 700-711 [PMID: 23281400 DOI: 10.1172/JCI62236]

10 **Biswas A**, Petnicki-Ocwieja T, Kobayashi KS. Nod2: a key regulator linking microbiota to intestinal mucosal immunity. *J Mol Med* (Berl) 2012; **90**: 15-24 [PMID: 21861185 DOI: 10.1007/s00109-011-0802-y]

11 **Uronis JM**, Mühlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS One* 2009; **4**: e6026 [PMID: 19551144 DOI: 10.1371/journal.pone.0006026]

12 **Lau TP**, Roslani AC, Lian LH, Lee PC, Hilmi I, Goh KL, Chua KH. NOD2/CARD15 variants in Malaysian patients with sporadic colorectal cancer. *Genet Mol Res* 2014; **13**: 7079-7085 [PMID: 24682985 DOI: 10.4238/2014.March.19.3]

13 **Möckelmann N**, von Schönfels W, Buch S, von Kampen O, Sipos B, Egberts JH, Rosenstiel P, Franke A, Brosch M, Hinz S, Röder C, Kalthoff H, Fölsch UR, Krawczak M, Schreiber S, Bröring CD, Tepel J, Schafmayer C, Hampe J. Investigation of innate immunity

genes CARD4, CARD8 and CARD15 as germline susceptibility factors for colorectal cancer. *BMC Gastroenterol* 2009; **9**: 79 [PMID: 19843337 DOI: 10.1186/1471-230X-9-79]
14 **Werts C**, Rubino S, Ling A, Girardin SE, Philpott DJ. Nod-like receptors in intestinal homeostasis, inflammation, and cancer. *J Leukoc Biol* 2011; **90**: 471-482 [PMID: 21653239 DOI: 10.1189/jlb.0411183]
15 **Antosz H**, Osiak M. NOD1 and NOD2 receptors: integral members of the innate and adaptive immunity system. *Acta Biochim Pol* 2013; **60**: 351-360 [PMID: 23901396]
16 **Shaw MH**, Kamada N, Warner N, Kim YG, Nuñez G. The ever-expanding function of NOD2: autophagy, viral recognition, and T cell activation. *Trends Immunol* 2011; **32**: 73-79 [PMID: 21251876 DOI: 10.1016/j.it.2010.12.007]
17 **Boyle JP**, Parkhouse R, Monie TP. Insights into the molecular basis of the NOD2 signalling pathway. *Open Biol* 2014; **4**: pii: 140178 [PMID: 25520185 DOI: 10.1098/rsob.140178]
18 **Sabbah A**, Chang TH, Harnack R, Frohlich V, Tominaga K, Dube PH, Xiang Y, Bose S. Activation of innate immune antiviral responses by Nod2. *Nat Immunol* 2009; **10**: 1073-1080 [PMID: 19701189 DOI: 10.1038/ni.1782]
19 **Shaw MH**, Reimer T, Sánchez-Valdepeñas C, Warner N, Kim YG, Fresno M, Nuñez G. T cell-intrinsic role of Nod2 in promoting type 1 immunity to Toxoplasma gondii. *Nat Immunol* 2009; **10**: 1267-1274 [PMID: 19881508 DOI: 10.1038/ni.1816]
20 **Barreau F**, Madre C, Meinzer U, Berrebi D, Dussaillant M, Merlin F, Eckmann L, Karin M, Sterkers G, Bonacorsi S, Lesuffleur T, Hugot JP. Nod2 regulates the host response towards microflora by modulating T cell function and epithelial permeability in mouse Peyer's patches. *Gut* 2010; **59**: 207-217 [PMID: 19837677 DOI: 10.1136/gut.2008.171546]
21 **Watanabe T**, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. *Nat Immunol* 2004; **5**: 800-808 [PMID: 15220916]
22 **Kutikhin AG**. Role of NOD1/CARD4 and NOD2/CARD15 gene polymorphisms in cancer etiology. *Hum Immunol* 2011; **72**: 955-968 [PMID: 21745515 DOI: 10.1016/j.humimm.2011.06.003]
23 **Tomasello G**, Tralongo P, Damiani P, Sinagra E, Di Trapani B, Zeenny MN, Hussein IH, Jurjus A, Leone A. Dismicrobism in inflammatory bowel disease and colorectal cancer: changes in response of colocytes. *World J Gastroenterol* 2014; **20**: 18121-18130 [PMID: 25561781 DOI: 10.3748/wjg.v20.i48.18121]
24 **Arumugam M**, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A, Delorme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F, Huber W, van Hylckama-Vlieg J, Jamet A, Juste C, Kaci G, Knol J, Lakhdari O, Layec S, Le Roux K, Maguin E, Mérieux A, Melo Minardi R, M'rini C, Muller J, Oozeer R, Parkhill J, Renault P, Rescigno M, Sanchez N, Sunagawa S, Torrejon A, Turner K, Vandemeulebrouck G, Varela E, Winogradsky Y, Zeller G, Weissenbach J, Ehrlich SD, Bork P. Enterotypes of the human gut microbiome. *Nature* 2011; **473**: 174-180 [PMID: 21508958 DOI: 10.1038/nature09944]
25 **Lagier JC**, Armougom F, Million M, Hugon P, Pagnier I, Robert C, Bittar F, Fournous G, Gimenez G, Maraninchi M, Trape JF, Koonin EV, La Scola B, Raoult D. Microbial culturomics: paradigm shift in the human gut microbiome study. *Clin Microbiol Infect* 2012; **18**: 1185-1193 [PMID: 23033984 DOI: 10.1111/1469-0691.12023]
26 **Bloom SM**, Bijanki VN, Nava GM, Sun L, Malvin NP, Donermeyer DL, Dunne WM, Allen PM, Stappenbeck TS. Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. *Cell Host Microbe* 2011; **9**: 390-403 [PMID: 21575910 DOI: 10.1016/j.chom.2011.04.009]
27 **Jakobsson HE**, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One* 2010; **5**: e9836 [PMID: 20352091 DOI: 10.1371/journal.pone.0009836]
28 **Graf D**, Di Cagno R, Fåk F, Flint HJ, Nyman M, Saarela M, Watzl B. Contribution of diet to the composition of the human gut microbiota. *Microb Ecol Health Dis* 2015; **26**: 26164 [PMID: 25656825 DOI: 10.3402/mehd.v26.26164]
29 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540]
30 **Knoop KA**, McDonald KG, Kulkarni DH, Newberry RD. Antibiotics promote inflammation through the translocation of native commensal colonic bacteria. *Gut* 2015; pii: gutjnl-2014-309059 [PMID: 26045138 DOI: 10.1136/gutjnl-2014-309059]
31 **Rehman A**, Sina C, Gavrilova O, Häsler R, Ott S, Baines JF, Schreiber S, Rosenstiel P. Nod2 is essential for temporal development of intestinal microbial communities. *Gut* 2011; **60**: 1354-1362 [PMID: 21421666 DOI: 10.1136/gut.2010.216259]
32 **Tralongo P**, Tomasello G, Sinagra E, Damiani P, Leone A, Palumbo VD, Giammanco M, Di Majo D, Damiani F, Abruzzo A, Bruno A, Cassata G, Cicero L, Noto M, Tomasello R, Lo Monte A. The role of butyric acid as a protective agent against Inflammatory Bowel Diseases. *Euro Biomed J* 2014; **9**: 24-35 [DOI: 10.3269/1970-5492.2014.9.4]
33 **Shenderov BA**. Gut indigenous microbiota and epigenetics. *Microb Ecol Health Dis* 2012; **23**: 17195 [PMID: 23990811 DOI: 10.3402/mehd.v23i0.17195]
34 **Arthur JC**, Jobin C. The struggle within: microbial influences on colorectal cancer. *Inflamm Bowel Dis* 2011; **17**: 396-409 [PMID: 20848537 DOI: 10.1002/ibd.21354]
35 **Yazdanyar S**, Nordestgaard BG. NOD2/CARD15 genotype and common gastrointestinal diseases in 43,600 individuals. *J Intern Med* 2010; **267**: 228-236 [PMID: 19570052 DOI: 10.1111/j.1365-2796.2009.02137.x]
36 **Tuupanen S**, Alhopuro P, Mecklin JP, Järvinen H, Aaltonen LA. No evidence for association of NOD2 R702W and G908R with colorectal cancer. *Int J Cancer* 2007; **121**: 76-79 [PMID: 17351900]
37 **Liu J**, He C, Xu Q, Xing C, Yuan Y. NOD2 polymorphisms associated with cancer risk: a meta-analysis. *PLoS One* 2014; **9**: e89340 [PMID: 24586700 DOI: 10.1371/journal.pone.0089340]
38 **Freire P**, Portela F, Donato MM, Figueiredo P, Ferreira M, Amaro P, Sá A, Andrade P, Gouveia H, Sofia C. CARD15 mutations and colorectal cancer in a South European country. *Int J Colorectal Dis* 2010; **25**: 1211-1219 [PMID: 20676658 DOI: 10.1007/s00384-010-1028-0]
39 **Lakatos PL**, Hitre E, Szalay F, Zinober K, Fuszek P, Lakatos L, Fischer S, Osztovits J, Gemela O, Veres G, Papp J, Ferenci P. Common NOD2/CARD15 variants are not associated with susceptibility or the clinicopathologic characteristics of sporadic colorectal cancer in Hungarian patients. *BMC Cancer* 2007; **7**: 54 [PMID: 17389035 DOI: 10.1186/1471-2407-7-54]
40 **Papaconstantinou I**, Theodoropoulos G, Gazouli M, Panoussopoulos D, Mantzaris GJ, Felekouras E, Bramis J. Association between mutations in the CARD15/NOD2 gene and colorectal cancer in a Greek population. *Int J Cancer* 2005; **114**: 433-435 [PMID: 15578724 DOI: 10.1002/ijc.20747]
41 **Tian Y**, Li Y, Hu Z, Wang D, Sun X, Ren C. Differential effects of NOD2 polymorphisms on colorectal cancer risk: a meta-analysis. *Int J Colorectal Dis* 2010; **25**: 161-168 [PMID: 19787357 DOI: 10.1007/s00384-009-0809-9]
42 **Kurzawski G**, Suchy J, Kładny J, Grabowska E, Mierzejewski M, Jakubowska A, Debniak T, Cybulski C, Kowalska E, Szych Z, Domagała W, Scott RJ, Lubiński J. The NOD2 3020insC mutation and the risk of colorectal cancer. *Cancer Res* 2004; **64**: 1604-1606 [PMID: 14996717 DOI: 10.1158/0008-5472.CAN-03-3791]
43 **Roberts RL**, Gearry RB, Allington MD, Morrin HR, Robinson

BA, Frizelle FA. Caspase recruitment domain-containing protein 15 mutations in patients with colorectal cancer. *Cancer Res* 2006; **66**: 2532-2535 [PMID: 16510569]

44 **Omrane I**, Mezlini A, Baroudi O, Stambouli N, Bougatef K, Ayari H, Medimegh I, Bouzaienne H, Uhrhammer N, Bignon YJ, Benammar-Elgaaied A, Marrakchi R. 3020insC NOD2/CARD15 polymorphism associated with treatment of colorectal cancer. *Med Oncol* 2014; **31**: 954 [PMID: 24719038 DOI: 10.1007/s12032-014-0954-z]

45 **Stojcev Z**, Banasiewicz T, Kaszuba M, Sikorski A, Szczepkowski M, Bobkiewicz A, Paszkowski J, Krokowicz Ł, Biczysko M, Szmeja J, Jurkowska M, Majewski P, Mackiewicz A, Lamperska K, Drews M, Wojciechowicz J. Development of a new, simple and cost-effective diagnostic tool for genetic screening of hereditary colorectal cancer--the DNA microarray assay. *Acta Biochim Pol* 2013; **60**: 195-198 [PMID: 23741719]

46 **Freire P**, Figueiredo P, Cardoso R, Donato MM, Sá A, Portela F, Romãozinho JM, Sofia C. Card15 mutations and gastric cancer in a Portuguese population. *Scand J Gastroenterol* 2013; **48**: 1188-1197 [PMID: 24047397 DOI: 10.3109/00365521.2013.832370]

47 **Angeletti S**, Galluzzo S, Santini D, Ruzzo A, Vincenzi B, Ferraro E, Spoto C, Lorino G, Graziano N, Calvieri A, Magnani M, Graziano F, Pantano F, Tonini G, Dicuonzo G. NOD2/CARD15 polymorphisms impair innate immunity and increase susceptibility to gastric cancer in an Italian population. *Hum Immunol* 2009; **70**: 729-732 [PMID: 19397946 DOI: 10.1016/j.humimm.2009.04.026]

48 **Li ZX**, Wang YM, Tang FB, Zhang L, Zhang Y, Ma JL, Zhou T, You WC, Pan KF. NOD1 and NOD2 Genetic Variants in Association with Risk of Gastric Cancer and Its Precursors in a Chinese Population. *PLoS One* 2015; **10**: e0124949 [PMID: 25933107 DOI: 10.1371/journal.pone.0124949]

49 **Nej K**, Bartsch DK, Sina-Frey M, Rieder H, Hahn SA, Lubiński J. The NOD2 3020insC Mutation and The Risk of Familial Pancreatic Cancer? *Hered Cancer Clin Pract* 2004; **2**: 149-150 [PMID: 20233470 DOI: 10.1186/1897-4287-2-3-149]

50 **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* 2014; **20**: 5008-5016 [PMID: 24803813 DOI: 10.3748/wjg.v20.i17.5008]

51 **Cleynen I**, González JR, Figueroa C, Franke A, McGovern D, Bortlík M, Crusius BJ, Vecchi M, Artieda M, Szczypiorska M, Bethge J, Arteta D, Ayala E, Danese S, van Hogezand RA, Panés J, Peña SA, Lukas M, Jewell DP, Schreiber S, Vermeire S, Sans M. Genetic factors conferring an increased susceptibility to develop Crohn's disease also influence disease phenotype: results from the IBDchip European Project. *Gut* 2013; **62**: 1556-1565 [PMID: 23263249 DOI: 10.1136/gutjnl-2011-300777]

52 **Freire P**, Portela F, Donato MM, Ferreira M, Andrade P, Sofia C. CARD15 mutations and perianal fistulating Crohn's disease: correlation and predictive value of antibiotic response. *Dig Dis Sci* 2011; **56**: 853-859 [PMID: 20632099 DOI: 10.1007/s10620-010-1331-1]

P- Reviewer: Hammerman A, Noshiro H, Uppara M
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com