REVIEW

Role of Mutations in NOD2/CARD15, ATG16L1, and IRGM in the Pathogenesis of Crohn's Disease

Igor’ V. Maev, PhD, ScD; Dmitry N. Andreev*

Moscow State University of Medicine and Dentistry named after A.I. Evdokimov
Moscow, Russian Federation

Abstract

This review article summarizes the issues concerning the pathogenesis of Crohn’s disease (CD) based on the results of large-scale genome-wide association studies. The role of defects in innate immunity associated with mutations in specific genes such as those regulating bacterial pattern recognition (NOD2/CARD15) and autophagy/xenophagy (ATG16L1 and IRGM) in CD is also discussed. Basic pathogenetic hypotheses that aim to interpret the association between specific gene mutations and CD development are presented.

Keywords: Crohn’s disease, innate immunity, autophagy, xenophagy, microbiota, NOD2/CARD15, ATG16L1, IRGM

Introduction

Crohn’s disease (CD) is a multisystemic disease with unknown etiology and is characterized by nonspecific granulomatous transmural inflammation with segmented injuries in any part of the gastrointestinal tract (GIT) [1, 2]. Epidemiological data shows that the incidence and prevalence of CD in northern Europe and North America have increased, which also has major social impact [3, 4]. In last decades, the significant increase in understanding of molecular genetics of CD, allowed the identification of genetic determinants associated with CD pathology [2, 5].

The results of various epidemiological studies have identified the role of genetic factors in the pathogenesis of CD. In particular, several observations provide evidence that this specific pathology commonly occurs in families of patients with CD [1, 2, 6, 7]. Approximately one of every five patients with CD has at least one relative with the same pathology [8]. However, in studies involving monozygotic twins, a 67% concordance (comorbidity in both twins) has been demonstrated for CD [2]. In addition, the ethnic variations in CD incidence rate are in favor of a genetic component in its pathogenesis. For example, the CD incidence rate is 2–4 times higher in Eastern Europe, with the exception of the Ashkenazi Jews and other ethnic groups [9]. Therefore, the enumerated factors serve as prerequisites for the identification of CD susceptible loci.

The evolution of molecular genetic technologies has resulted in a new epoch of genome-wide association studies (GWAS), which facilitates in the identification of the new candidate genes for CD based on their genomic localization, without reference to its biological effect or mode of inheritance [10]. GWAS have become the all-powerful catalyst in understanding the pathogenesis of CD, resulting in the identification of approximately more than 30 loci that confer a predisposition to CD [11, 12]. The most associations between CD and specific mutations include those regulating the bacterial pattern recognition and autophagy [2, 6, 13].

NOD2/CARD15. A first susceptibility locus identified for CD is NOD2, also known as CARD15; this gene was identified by two independent research groups in early 2001 [14,15]. This particular investigation could be considered as the most significant achievement in genetic studies of inflammatory bowel diseases [1,5].

NOD2/CARD15 encodes for the cytosolic protein, NOD2, which is an intracellular pattern-recognizing receptor binding to muramyl dipeptide (MDP) [16,17]. The last one is the component of bacterial peptidoglycan from walls of Gram-positive and Gram-negative bacteria [17]. NOD2 is preferentially expressed in immunocompetent cells such as macrophages, dendritic cells, and Paneth cells [18]. Paneth
cells are specialized cells of the small intestines that produce antibacterial substances called α-defensins [19].

NOD2 consists of two CARD domains, one nucleotide binding domain (NBD), and a leucine-rich repeat region (LRR), which serves as the domain for MDP recognition [20]. Pattern recognition by NOD2 is associated with activation of signal transduction, which results in the induction of the transcription factor, NF-kB. Its activation results in the transcription of several pro-inflammatory genes [17, 21, 22]. NOD2 is the most important component of innate immunity, which subsequently confirms the hypothesis that an alteration in the immune response to gastrointestinal microbiota influences the development of CD [2].

At least 30 alleles of NOD2/CARD15 have been identified [23]. However, the most frequent allele variants in European and American populations that are associated with CD include two missense mutations, Arg702Trp, Gly908Arg, and a frameshift mutation Leu1007fsinsC [2, 6, 13-15]. These particular mutations represent 82% of all NOD2/CARD15 gene variations that have been associated with CD [24].

The specific mutations in NOD2/CARD15 affect the LRR–MDP recognition region and disrupt the binding of MDP to the NOD2 protein [25]. However, no unifying model currently explains the association between NOD2/CARD15 mutations and CD pathogenesis. Following the one of hypothesis the proinflammatory mediators’ transcription and inflammation induction even under minimal bacterial invasion is explained by that the NOD2/CARD15 gene mutations leads to hyperamplification in response to MDP stimulation in signal transduction through NF-κB [26]. On the over side that is out of this model frames it has been demonstrated for today that as minimum the NOD2/CARD15 gene missense mutations functionally inactivate the protein [27].

Another theory on CD pathogenesis is that NOD2 inhibits TLR2-associated signal pathways, thereby inducing the synthesis of proinflammatory cytokines such as IL-12 [28]. Mutations in NOD2/CARD15 result in the upregulation of IL-12, followed by Th1-type adaptive immunity polarization [29].

Another hypothesis is that NOD2/CARD15 mutations lead to not only changes in MDP recognition but also to a downregulation of α-defensin production by Paneth cells (Fig. 1) [18]. However, patients with CD and NOD2/CARD15 mutations show a 50% decrease in α-defensin HD5 expression [30, 31]. These changes disrupt the homeostasis between the macro-organism and bowel microbiota [32]. The decrease in the protective potential results in quantitative and qualitative alterations in the microbiotic content, with an expansion of pathogenic and commensal bacteria, which mediates their translocation into the mucosal layer followed by the activation of adaptive immunity that primarily involves antigen-presenting cells (APC) and T-lymphocytes [32, 33].

Current clinical trials have demonstrated that NOD2/CARD15 gene polymorphisms are associated with early CD onset with inflammation, particularly involving the ileum (ileitis), as well as the early formation of strictures [34-36].

A previous report has shown that 20%–30% of patients with CD harbor NOD2/CARD15 mutations [2]. However, the penetrance of these variants is less than 1%; thus, these variations can occur even in individuals without CD [37]. Therefore, NOD2/CARD15 mutations are not exclusively responsible for CD development.

Figure 1. Intracellular signal transduction processes associated with α-defensin production in Paneth cells

**Autophagy genes (ATG16L1 and IGRM)**

Subsequent investigations in CD genetics have implicated autophagy elements [2, 6, 13, 38]. Autophagy is a cell mechanism involving excessive or damaged proteins, protein complexes, and performing cell organelles utilization by cell own lysosomes [39, 40]. Autophagy plays an important role in immune protection against viral, bacterial, and parasitic infections by selective microorganisms’ utilization in lysosomes, called xenophagy [41]. Autophagy also serves as an integral link between innate and adaptive immunity, providing antigens with major histocompatibility complexes class II for the presentation (MHC-II) [42].

According to results of some GWAS, single nucleotide polymorphisms in ATG16L1 (T300A) are associated with high-risk CD development [11, 43, 44]. The ATG16L1 product is an autophagy modulating protein, ATG16L1, which forms a complex with ATG5-ATG12 and is responsible for autophagosome formation (Fig. 2) [45]. The role of NOD2 in autophagy induction in association with ATG16L1 has been established, although there is still a need to establish the significance between NOD2/CARD15 gene mutations and CD pathogenesis [46, 47].
In general, ATG16L1 mutations are believed to be associated with alterations in xenophagy activation based on the recognition of intracellular bacteria patterns by NOD2 [48]. In addition to the ATG16L1 defects that lead to bowel deregulation, Paneth α-defensin exocytosis may also play a direct role in CD development [49].

GWAS have identified another autophagy gene, IRGM, which has been associated with CD [11, 50]. This gene encodes the immunity-related GTPase family M protein (IRGM) [51]. The actual function and role of this protein is unknown. It has been suggested that IRGM plays a role in protecting the cell against intracellular bacteria by inducing autolysosome substrate degradation [52]. Defects in autophagy play a major role in CD pathogenesis. These defects result in an insufficiency in the clearance of pathogens in the GIT and interruptions in the activation of adaptive immunity involving MHC-II molecule antigen presentation [38].

**Conclusion**

The last few decades have been marked by major breakthroughs in understanding the molecular genetic basis of CD. Results of large-scale GWAS have substantially changed our view on the pathogenesis of CD. Alterations in innate immunity, which influence autophagy and the recognition of microbiota structures, appear to be the most important stage of CD pathogenesis, followed by the irrational activation of adaptive immunity components.

**Acknowledgements**

The authors would like to thank Enago (www.enago.com) for the English language review.

**Competing interests**

The authors declare that they have no competing interests.

**References**

19. Ayabe T, Satchell DP, Wilson CL, Parks WC, Selsted ME,


