The Genetic Background of Crohn’s Disease
– Basic Genetic Definitions and the Gene CARD15

Genetyczne podłoże choroby Leśniowskiego-Crohna
– podstawowe pojęcia z zakresu genetyki i gen CARD15

Abstract
The complicated etiopathogenesis of Crohn’s disease includes a genetic background. Many epidemiological studies show geographical, racial, and ethnic differences in disease prevalence as well as familial occurrence. A genetic background is currently confirmed by molecular studies. The first gene identified as a “susceptibility” gene of Crohn’s disease was NOD2/CARD15. This paper explains the basic genetic definitions and the role of the CARD15 gene (Adv Clin Exp Med 2007, 16, 5, 689–694).

Key words: Crohn’s disease, CARD15.

Streszczenie

Słowa kluczowe: choroba Leśniowskiego-Crohna, CARD15.
determine the amino-acid sequence of the proteins, so any changes can lead to protein malfunction. The nucleotide changes in the DNA molecule can be grouped into three classes:

- base substitutions, which involve replacement of usually a single base, also called a single-nucleotide polymorphism (SNP),
- insertions, in which one or more nucleotides are inserted into a sequence,
- deletions, in which one or more nucleotides are eliminated from a sequence.

Sometimes DNA mutations do not result in a change of the amino-acid sequence of the protein, referred to as a silent mutations, whereas other DNA mutations leading to amino-acid changes are called missense mutations. When an amino-acid codon is replaced by a stop codon, the protein is truncated and often unfunctional, this type of mutation being defined as a nonsense mutation. A frameshift mutation can be produced by insertions, deletions, or splicing errors. Different nucleotide changes may undoubtedly affect the biological function of the protein, i.e. decreased activity or a loss or gain in function.

Genetic disorders can be autosomal dominant, autosomal recessive, or X-linked (the gene is located on the X chromosome). Autosomal recessive inheritance means that both alleles of the gene carry the mutation and only homozygotes manifest the disease symptom. Autosomal dominant inheritance means that the disease affects heterozygotes (only one allele is mutated). This type of inheritance makes it difficult to estimate genetic risk due to incomplete penetrance and expressivity. The penetrance is defined as the percentage of individuals with a given genotype who exhibit the phenotype associated with that genotype. A percentage of less than 100% shows incomplete penetrance, i.e. an individual may have a particular genotype but may not express the corresponding phenotype because of modifiers, i.e. epistatic genes, or because of a modifying effect of the environment. The other measure for describing the range of phenotypic expression is expressivity. Expressivity refers to the severity of the phenotype, i.e. the different degrees of expression in different individuals may be due to variations in the allelic constitution of the rest of the genome or to environmental factors.

Certain genetic diseases have a tendency to become more severe and appear at an earlier age as the disorder is passed from one generation to the next. This phenomenon is called anticipation. The conventional view is that human genetic diseases result from changes in the DNA sequence (i.e. mutations). However, identical twins having essentially the same DNA can develop different genetic defects. This phenomenon might be explained by heritable changes in the pattern of gene expression. Changes that are heritable but which do not depend on changes in the DNA sequence are called epigetetic.

**Crohn’s Disease**

Inflammatory bowel disease (IBD) encompasses two specific forms of the disease, known as ulcerative colitis (UC) and Crohn’s disease (CD). Approximately 5–10% of patients with inflammatory bowel disease have undetermined colitis. The etiopathogenesis of IBD is complex and still unclear, but it seems that genetic, environmental, and immunological factors play important roles in its etiology. Researchers are still looking for new markers to facilitate the diagnosis of IBD [1]. Ethnic, racial, and geographic variation, familial clustering, and twin studies provide strong evidence for genetic determinants in IBD [2]. Crohn’s disease and ulcerative colitis are more common in the developed world, in both the USA and Europe, and a north-south gradient has been observed (more common in the north than in the south). The incidence and prevalence of IBD is lower in Asia, South America, Africa, and the Pacific region. In Europe the incidence of ulcerative colitis is 10.4 cases per 100,000 persons per year and the incidence of Crohn’s disease 5.6 cases per 100,000 persons per year [3]. With regard to racial and ethnic variation, consistently increased incidence and prevalence of IBD in the Jewish population has been documented, the occurrence of disease being higher in Ashkenazi Jews than in Sephardic Jews. The prevalence of IBD is lower in the Afro-American than the Caucasian population. Environmental factors such as smoking may also play a role in causing Crohn’s disease; people who smoke or who have
smoked in the past have a higher risk than non-smokers. In addition, smokers with Crohn’s disease will face more severe forms of the disease with higher risk of flare-up and they will need surgery more often [4, 5].

Familial aggregation of inflammatory bowel disease was recognized many years ago; the relatives of patients with IBD have a higher risk of illness than the general population. The prevalence in first-degree relatives of patients with CD is 2.2–16.2% and with UC 5.7–15.5% [6].

**Molecular Genetics of Crohn’s Disease**

In recent years, genetic determinants in IBD have been confirmed by molecular studies. Attempts to localize genes involved in inflammatory bowel disease have identified putative loci on several chromosomes by genome-wide linkage studies (Table 1).

Many IBD-associated loci with confirmed linkage have been identified in chromosomal areas (Fig. 2).

Crohn’s disease is the result of an abnormal immune response of the gut mucosa to microorganisms or antigens in genetically susceptible people. Csillag et al. studied the gene expression profiles of non-inflamed colonic mucosal cells from patients with Crohn’s disease and from controls. They found that, compared with controls, CD patients had two up-regulated genes related to the innate immune system. It is known that the expression of these genes can be induced by microorganisms, which suggests either increased microfloral antigenicity or an altered function in the mucosal defense barrier [7]. Devlin et al. studied 732 patients with CD, 220 unaffected relatives, and 200 healthy controls. The results showed that the patients with Crohn’s disease and unaffected relatives carrying variants of the NOD2 gene had increased adaptive immune responses to microbial antigens. Ott et al. demonstrated that mucosal inflammation in inflammatory bowel disease is associated with loss of normal luminal anaerobic bacteria. These authors did not find an association between CARD15 mutations and bacterial diversity in CD patients [9].

**Gene CARD15**

Hugot et al. mapped the first susceptibility locus for Crohn’s disease on chromosome 16q12.1, called IBD1 [10]. In 2001, three independent studies reported the identification of the gene NOD2 within the IBD1 locus, subsequently renamed CARD15 by the International Nomenclature Committee. Three common mutations in the CARD15 gene are known to be associated with susceptibility to Crohn’s disease [11–13]:

- 1007fsinsC, a frameshift mutation which leads to truncation of CARD15 protein,
- R702W and G908R, missense mutations that result in amino-acid changes.

The relative risk of Crohn’s disease is increased 2-to 4-fold in heterozygotes and 20- to 40-fold in homozygotes or combined heterozygotes [10]. However, these three variants of CARD15 gene do not account for all the disease-causing mutations, which is why further investigations are being performed to search for rare mutations. In the European population, 27 rare mutations have been identified representing 19% of disease-causing mutations in total, whereas the

---

**Table 1. Susceptibility loci for Crohn’s disease**

<table>
<thead>
<tr>
<th>Locus (Locus)</th>
<th>Gene name (Nazwa genu)</th>
<th>Chromosome (Chromosom)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD1</td>
<td>CARD15</td>
<td>16q12</td>
</tr>
<tr>
<td>IBD2</td>
<td>unidentifed (niezidentyfikowany)</td>
<td>12p13.2−q24.1</td>
</tr>
<tr>
<td>IBD3</td>
<td>TNF-α</td>
<td>6p</td>
</tr>
<tr>
<td>IBD4</td>
<td>unidentifed (niezidentyfikowany)</td>
<td>14q11-12</td>
</tr>
<tr>
<td>IBD5</td>
<td>SLC22A4, SLC22A5</td>
<td>5q31</td>
</tr>
<tr>
<td>IBD6</td>
<td>ICAM-1</td>
<td>19p13</td>
</tr>
<tr>
<td>IBD7</td>
<td>IL23R</td>
<td>1p36</td>
</tr>
<tr>
<td>IBD8</td>
<td>unidentifed (niezidentyfikowany)</td>
<td>16 q/p</td>
</tr>
<tr>
<td>IBD9</td>
<td>CCR2, CCR5</td>
<td>3p</td>
</tr>
<tr>
<td>IBD10</td>
<td>ATG16LI</td>
<td>2q37.1</td>
</tr>
</tbody>
</table>

---

**Fig. 2. Susceptibility and suggested linkages in inflammatory bowel disease**

**Ryc. 2. Chromosomowe umiejscowienie genów związanych z nieswoistym zapaleniem jelit**
three main polymorphisms R702W, G908R, and 1007fsinsC represent 32%, 18%, and 31% of disease-causing mutations, respectively [14]. It turned out that 93% of all the mutations were located in the leucine-rich region (LRR) of the gene. Many studies of allele frequencies of the CARD15 mutations revealed ethnic and geographic variation in selected CD populations throughout the world [Table 2].

Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD) indicated that CARD15 mutations were less present in the Scandinavian countries (12.1%) than in the rest of Europe (32.8%) and did not correlate with the incidence of CD [15]. Popular mutations in the CARD15 and TLR4 genes that are common in the West are not associated with CD in Taiwanese children [16]. The CARD15 gene encodes a 1040-amino-acid protein, a member of the cytosolic protein family involved in apoptosis (programmed cell death) and intracellular regulation of nuclear factor-κB (NFκB) activity [17]. Despite sequence similarities with other family members, particularly NOD1/CARD4, mutations in this gene are not involved in susceptibility to CD [18]. The CARD15 protein contains two N-terminal caspase activation and recruitment domains (CARDs), a centrally located nucleotide-binding oligomerization domain (NOD), and leucine-rich repeats (LRRs) at the C-terminus (Fig. 3).

The LRR domains are important for bacterial binding and recognize muramyl dipeptide (MDP) derived from bacterial peptidoglycan (PGN), a major cell-wall component of most Gram-positive and Gram-negative bacteria. Binding of MDP to the LRR domains leads to NFκB activation and induces NFκB-dependent immune response gene expression (Fig. 4) [19, 20].
The identified mutations modify the structure of the LRR domains of CARD15 protein or adjacent ones, cause an altered immune response [12]. The expression of \textit{CARD15} gene was noted in monocytes, macrophages, B cells, and intestinal epithelial cells, an especially high expression level being observed in Paneth cells. Paneth cells are specialized epithelial cells located in the crypts of the small intestine and they secrete a number of anti-bacterial substances in response to MDP and bacterial products [14]. The antibacterial substances are located in granules within the cytosol; close proximity of \textit{CARD15} protein and secretory granules has recently been demonstrated. It is speculated that \textit{CARD15} may be involved in degradation and release of mediators [17].

The authors conclude that the discovery of the first susceptibility gene for Crohn’s disease was very important for further genetic studies. Using a novel approach, researchers identified other loci associated with Crohn’s disease, for example IBD2, IBD3, IBD4, IBD5, and IBD6, and putative susceptibility genes (\textit{DLG5}, \textit{ATG16L1}, \textit{SLC22A4}). Dutch scientists have shown that in Crohn’s disease, carriage of at least one \textit{CARD15} mutation is associated with a more complicated disease behavior [21]. In the Finnish population a carrier status for \textit{CARD15} mutations predicted an ileal localization of CD as well as a liability to complications of CD, such as strictures and fistulation [22]. A Polish study revealed that \textit{CARD15} mutations can induce an early onset of Crohn’s disease, joint diseases, iridoc choroiditis, and erythema nodosum [23]. Recent data suggest that polymorphisms in other genes can also cause Crohn’s disease. Noble et al. discovered that the IBD5 locus contains two organic cation transporter genes: OCTN1 (\textit{SLC22A4}) and OCTN2 (\textit{SLC22A5}) influence susceptibility, progression, and the need for surgery in CD patients [24].

**References**


Address for correspondence:

Katarzyna Neubauer
Gastroenterology and Hepatology Department
Silesian Piasts University of Medicine
Browarska 213
50-556 Wrocław
Poland
Tel.: +48 71 733 21 22
E-mail: gastro@gastro.am.wroc.pl

Conflict of interest: None declared

Received: 1.08.2007
Revised: 11.09.2007
Accepted: 30.10.2007