Review paper

Genetic polymorphisms in the pathogenesis and course of Crohn's disease

Polimorfizmy genetyczne w patogenezie i przebiegu choroby Leśniowskiego-Crohna

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Słowa kluczowe: polimorfizmy genetyczne, SNP, mutacje genetyczne, choroba Leśniowskiego-Crohna.

Abstract

Crohn's disease is a chronic inflammatory disease that affects the full length of the digestive tract. The precise aetiology is still unknown, but genetic, environmental, and immunologic factors are involved. The aim of this article is to summarize the current susceptibility genes and gene polymorphisms involved in the disease pathogenesis and clinical course. In recent studies an association was found between FUT2, ATG16L-1, IL-10, TLR, and NOD2 gene mutations and Crohn's disease. Each of them may affect the disease at a different stage of immune response, are involved in bacterial identification, or are related to bacterial dysbiosis. We present their effect of disease susceptibility, age of onset, severity, risk of surgery, and concomitant infections. Further research in this area may prove useful in early diagnosis, prognosis, and treatment of Crohn's disease.

Streszczenie

Choroba Leśniowskiego-Crohna jest przewlekłą chorobą zapalną obejmującą całą długość przewodu pokarmowego. Dokładna etiologia wciąż pozostaje nieznana. W jej patogenezie bierze się pod uwagę czynniki genetyczne, środowiskowe i immunologiczne. Celem artykułu jest podsumowanie aktualnych genów podatności i polimorfizmów genetycznych biorących udział w patogenezie i przebiegu klinicznym choroby. W ostatnich badaniach stwierdzono związek między mutacjami genów FUT2, ATG16L-1, IL-10, TLR, NOD2 i chorobą Leśniowskiego-Crohna. Każda z nich może wpływać na chorobę na innym etapie odpowiedzi immunologicznej, geny te są również zaangażowane w identyfikację bakterii lub są związane z dysbiozą bakteryjną. W artykule przedstawiono ich wpływ na podatność na zachorowanie, wiek zachorowania, ciężkość, ryzyko operacji i współistniejących infekcji. Dalsze działania w tym zakresie mogą okazać się przydatne we wczesnej diagnostyce, rokowaniu i leczeniu choroby Leśniowskiego-Crohna.

Introduction

Crohn's disease (CD) is one of two major types of inflammatory bowel disease (IBD). It is an idiopathic, chronic, incurable inflammatory pathology, which has the potential to affect all the gastrointestinal tract from the mouth to the anus. Most commonly it concerns the terminal ileum and colon. It appears in a non-continuous manner, asymmetrically, and represents segmental and transmural inflammation [1]. The aetiopathogenesis of CD is not entirely understood, and many aetiological factors are being considered. It is known that diet, cigarette smoking, and psychological and behavioural factors influence the regulation of gut microbiome and the host immune response [2]. It may be due to the breakdown in self-recognition of commensal bacteria together with mucosal barrier dysfunction in individuals with a given genetic background. CD is characterized by an exaggerated mucosal immune response to luminal gut contents in genetically susceptible individuals [3]. Based on the research conducted so far, it can be concluded that some people have a genetic predisposition to develop CD. It results in a chronic inflammatory disorder, in which the body's immune system defends the gastrointestinal tract, targeting microbial antigens [4]. While CD is an immune-related disease, it does not appear to be an autoimmune disease (in that the immune system is not being triggered by the body itself). The exact underlying immune problem is not clear. A study of families and twins has shown that the degree of inheritance in CD contributes significantly to the development of the disease. First-degree relatives (FDR) of IBD patients have an increased risk

of developing the disease; moreover, this risk in CD is higher than in ulcerative colitis (UC) [5]. Moller *et al.*, in their study encompassed the entire Danish population during the period 1977–2011, showed that the risk of CD in FDR was significantly increased (IRR = 7.77; 95% confidence interval (CI): 7.05–8.56), in second-degree (IRR = 2.44; 95% CI: 2.01–2.96), and third-degree relatives (IRR = 1.88; 95% CI: 1.30–2.71) of patients with CD, and was less pronounced in relatives of UC cases [6]. Research in families and twins with IBD also shows that there is a genetic predisposition that contributes to the occurrence of CD. One study based on a total group of 189 pairs of twins in which at least one member had IBD (68 monozygotic and 121 dizygotic pairs) showed that the incidence of CD in both monozygotic twins is 30–35% [7]. Recently, it was observed that the incidence of CD in Europe ranged between 0.4 and 22.8 per 100,000 person-years, and the highest incidence of CD was reported in the Netherlands (22.8 per 100,000 person-years) and the lowest in Moldova (0.4 per 100,000 person-years) [8]. Genetic polymorphism is defined as the inheritance of the trait controlled by a single genetic locus with 2 alleles, in which the least common allele has a frequency in the population of about 1% or greater. Genetic polymorphism is a place in the DNA sequence in which there is variation among individuals, groups, or populations [9]. In this review, we summarize the available evidence on the gene mutation and gene polymorphism in CD. We searched the PubMed database mainly with keywords such as CD, IBD, SNP, single nucleotide polymorphism, mutations, and genetic impact. Our work focuses on the review of meta-analyses and research up to March 2023. We evaluated associations between many polymorphisms and the correlation with CD onset and clinical course.

We selected these mutations on account of the most common occurrence, and our objective was to collect the most up-to-date information. We would like to point out the importance of future work to find and clarify deeply the pathogenesis of CD.

FUT-2

The fucosyltransferase 2 (FUT2) gene located on chromosome 19q13.33 encodes for a critical α-(1, 2)-fucosyltransferase enzyme responsible for blood group secretor status and mucosal protective functions [10]. The mechanism by which the FUT2 gene is associated with CD is still unclear. It is hypothesized that changes in the gut microbiota associated with the FUT2 mutation may play a role in the pathogenesis of CD [11]. About 20% of the population who have both FUT2 alleles inactive are "non-secreting". In this situation they do not express ABO antigens both in the gastrointestinal tract and in the body secretions. The dominant polymorphism of the FUT2 gene in Caucasians is rs601338, while rs1047781 is the most common variant in Asians; both are loss-of-function alleles. Genome-wide association studies (GWAS) in European populations have shown that the nonsense allele in FUT2 (non-secretory) is one variant for CD susceptibility [12]. A GWAS, involving 1735 CD patients and 8074 healthy subjects (Korean population), showed the rs1047781 polymorphism in 883 CD patients and the significant association with CD with genome-wide significance (OR = 1.39, 95% confidence interval (CI): 1.25–1.53, p-value = 6.99×10^{-10}) [13]. O'Brien *et al.* in the study compared 189 patients (of whom 27 had afferent limb stenosis, which is an infrequent complication following ileal pouch-anal anastomosis (IPAA) suggesting underlying CD), 108 patients with CD narrowing phenotype, and 54 patients with CD non-narrowing phenotype. In this study, the authors showed that FUT2 AG and GG (rs601338) genotypes were more strongly associated with both stricturing CD (OR = 1.99, 95% CI: 0.93–4.25, p-value = 0.07 and OR = 3.03, 95% CI: 0.98–9.37, p-value = 0.05, respectively) and afferent limb stenosis (OR = 4.81, 95% CI: 1.36–17.05, p-value = 0.02 and OR = 6.75, 95% CI: 1.45–31.37, p-value = 0.01, respectively), compared to non-stricturing CD [11]. One study involving 671 IBD patients (396 UC, 275 CD) found that the frequency of the FUT2 A385T allele and genotype was significantly increased in patients with CD, as opposed to UC (49.27% versus 43.33%, p-value = 0.024, OR = 1.271, and 95% CI: 1.031–1.565; 27.64% versus 16.53%, p-value ≤ 0.001, OR = 1.927, and 95% CI: 1.353–2.747, respectively). The same results were obtained for the G428A polymorphism (1.64% versus 0.50%, p-value = 0.023, OR = 3.324, and 95% CI: 1.108–9.968; 3.27% versus 1.00%, p-value = 0.044, OR = 1.116–10.137, and 95% CI: 1.116–10.137, respectively). It was also shown that, compared to the control sample, the incidence of TT haplotype formed by C357T and A385T was significantly increased in patients with CD (OR = 1.277 and 95%, p-value = 0.020, 95% CI: 1.036–1.573). This shows that mutations of the FUT2 allele and genotype (A385T and G428A) and the mutant of the TT haplotype were more common in CD patients compared to the controls, suggesting that loss of FUT2 function of the gene may increase susceptibility to CD [14]. The presented studies show that the loss of the function of the FUT2 gene in the form of the rs601338 and rs1047781 polymorphisms may affect the dysbiosis of the intestinal microbiota and contribute to the occurrence of CD. One recent study in FUT2 knock-out mice showed that an epithelial-specific FUT2 deficiency increases IBD susceptibility by modulating the gut microflora and producing lysophosphatidylcholine (LPC). Moreover, a positive correlation has been demonstrated between the production of LPC and the increase in the production of pro-inflammatory cytokines and damage to the epithelial barrier *in vivo* and *in vitro*. In summary,

Table 1. FUT-2 polymorphisms in Crohn's disease

Author	Year	Type of study	FUT-2 Gene mutation	Clinical features
O'Brien SJ [11]	2021	Clinical study	Single-nucleotide polymorphism (SNP) **rs601338**	Describes the genotypic and phenotypic characteristics of afferent limb stenosis patients as compared to those with stricturing CD and non-stricturing CD
McGovern DPB [12]	2010	Meta-analysis	Single-nucleotide polymorphism (SNP) **rs601338** **rs1047781**	Explores clinical features of FUT-2 gene mutations in CD susceptibility and highlights the role of the mucus layer in the development of CD
Ye BD [13]	2020	Clinical study	Single-nucleotide polymorphism (SNP) **rs1047781**	Explores the clinical features of the O blood group and FUT2 secretor status as protective factors against CD in Asians
Wu H [14]	2017	Clinical study	Gene mutation **A385T** **G428A** **C357T**	Found that FUT2 gene polymorphisms and haplotypes were associated with susceptibility to CD but not UC
Tang X [15]	2021	Clinical study	Single-nucleotide polymorphism (SNP) **rs601338** **rs1047781**	Explores clinical features of intestinal epithelium-specific Fut2 deficiency, which increase susceptibility to CD and UC through modulation of gut microbiota and generation of LPC

the role of FUT2 in IBD susceptibility here was based on the structural and functional modulation of the intestinal microflora, which increased the production of LPC, which exacerbates colitis [15]. The studies presented in this section are summarized in Table 1.

ATG16L-1

Autophagy is the major intracellular degradation system delivering cytoplasmic components to lysosomes, and it accounts for degradation of most long-lived proteins, pathogens, and organelles. Autophagy is a relevant element in maintaining immune homeostasis in the intestines and therefore influences the innate and adaptive response [16]. Autophagy contributes to the maintenance of the antimicrobial defence, the integrity of the epithelial barrier, and the immune response of the mucosa. When the autophagy process is disrupted, the intestinal epithelial function and the immune system may be impaired, which may lead to an abnormal immune response and inflammation [17]. Autophagy-related 16 like 1 (ATG-16L1) is a protein encoded by the ATG-16L1 gene located on chromosome 2q37.1 [16]. ATG-16L1 protein is expressed in the colon, small bowel, intestinal epithelial cells, leukocytes, and spleen and constitutes an essential component of the autophagic pathway involved in autophagosome formation [18, 19]. Single nucleotide polymorphisms (SNPs) in the gene encoding the ATG1 6L1 protein have been associated with an increased risk of developing CD (especially CD of the ileum) in many populations. The rs2241880 (T300A) ATG16L1 CD-associated gene variant is common in the population (33.2% of people of Western European descent are homozygous for the risk variant). Subjects with one copy of the ATG16L1 CD-related risk variant have a 1.86–3-fold increased risk (95% CI: 1.09–3.24), and with 2 copies, a 2.38-fold higher risk of developing CD (95% CI: 1.40–4.04). This shows that the effect of this risk allele is gene dose dependent [20]. The ATG16L1 risk variant rs2241880 (T300A) in humans contribute to increased production of cytokines such as IL-1β and IL-18, and to the impairment of cellular autophagy and bacterial degradation [20, 21]. One study involving 236 Danish CD patients found that the presence of a variant in T300A (homozygous variant AA, heterozygous variant AG, respectively) is associated with an increased risk of complicated fistula disease and postsurgical infections. In addition, studies suggest that the ATG-16L1 gene polymorphisms are associated with the increased production of TNF-α and IL-1β (related to the response to pathogenic bacteria) and contribute to the weak innate immune response to pathogens [22]. Rs2241879 C/T (single nucleotide variation) is another SNP associated with CD. Baradaran Ghavami *et al.* in their case control study on a total of 101 Iranian IBD patients (75 UC and 26 CD and 99 healthy controls) showed that CC genotype has a protective effect against IBD (p-value = 0.01; adjusted OR = 1.68; 95% CI: 1.135–2.506) and patients with Allele A of rs2241879 show a significant relationship with 1.687-fold increased risk of IBD, compared to the control (p-value < 0.01; adjusted OR = 1.68; 95% CI: 1.13–2.50) [23]. In addition, it was also shown that the C allele may increase the risk of disease recurrence, and patients with genotype AA were more often in remission, which demonstrates the protective role of the A allele [23]. The studies presented in this section are summarized in Table 2.

Table 2. ATG16-L1 polymorphisms in Crohn's disease

Author	Year	Type of study	ATG16-L1 gene mutation	Clinical features
Ngoh E [20]	2015	Clinical study	Single-nucleotide polymorphism (SNP) **rs2241880**	Explores a novel mechanism by which the ATG16L1 CD-associated gene variant may predispose people to develop intestinal inflammation
Kim S [21]	2019	Systematic review	Single-nucleotide polymorphism (SNP) **rs2241880**	The data in the review suggest that the functional defect in ATG16L1 is involved in the dysregulation of intestinal homeostasis and CD pathogenesis
Larabi A [22]	2020	Systematic review	Single-nucleotide polymorphism (SNP) **rs2241880**	The review suggests that the defective autophagy may have a strong impact on the course of IBD via disruption of intestinal homeostasis
Baradaran Ghavami S	2019	Case control study	Single-nucleotide polymorphism (SNP) **rs2241879**	Shows that the ATG16L1 gene polymorphism has a significant relationship with increased risk of IBD in an Iranian population

In summary, the ATG-16L1 protein plays a role in maintaining intestinal homeostasis, and mutations related to its gene influence the development of CD. In addition, the latest research provides new information on CD immunopathogenesis induced by impaired induction of autophagy.

IL-10

Interleukin-10 (IL-10) is a multifunctional cytokine, secreted by a wide variety of cells. It is a crucial anti-inflammatory mediator, which limits excessive immune responses [24]. Significantly, IL-10 plays a crucial role in maintaining intestinal homeostasis. IL-10 regulates the anti-microbial immune responses within the intestine [25]. IL-10 signals through an interleukin-10 receptor (IL-10R) complex consisting of 2 subunits: IL-10Rα and IL-10Rβ [26].

The role of IL-10 in the pathogenesis of IBD is widely evaluated. Defects in IL-10 and IL-10R genes are closely related to early onset IBD. Glocker *et al.* (2009) found loss-of-function mutations in Il-10R subunits in patients with very early onset of CD, involving hyperinflammatory immune responses in the intestine due to a lack of negative-feedback signalling mediated by IL-10 [27–32]. Several studies continuously discovered the loss of function mutations encoding IL-10 and IL-10R in patients with early onset of IBD [33]. Beser *et al.* described a mutation in Il-10Rβ: c.G477A, p.Trp159 and 2 mutations in the IL-10Rβ: c.T192G, p.Tyr64 and c.T133G, p.Trp45Gly genes. It was demonstrated that the patients bearing mutations in the IL-10 receptor genes have the severe form of the disease and respond poorly to the treatment. Furthermore, a Gly15Arg mutation in the leader sequence of Il-10 was found in multiple CD-affected families. This altered leader sequence decreases IL-10 secretion, thereby reducing the anti-inflammatory effect [34].

Concurrently, the results of a systematic review performed by Sharifi Nejad *et al.* investigated the features of patients with IL-10/IL-10R deficiency. In this analysis, which included 110 studies and 286 patients, the authors demonstrated 91 unique mutations. The most frequent mutations among patients with Il-10Rα were c.301C>T (exon 3, p.R101W) and c.537G>A (exon 4, p.T179T-splicing mutation), detected in a total of 48% of families. The predominant mutations in Il-10Rβ deficiency were c.139A>G (exon 2, p.K47E), c.477G>A (exon 4, p.W159X), and c.611G>A (exon 5, p.W204X), identified in 34% of families. Moreover, large deletion mutations were reported in 21 families consisting of deletion containing exon 1, exon 1–3, and exon 2–4 (p. V23fsX31) of the Il-10Rα gene, and deletion in exon 2 and exon 3 (g.34647434_34650412delinsCT), duplication of exon 6, and deletion of exon 3–7, exon 4, and part of exon 5 (g.11930_17413del), and exon 4 and exon 5 (g.17030_22177del) of the IL-10Rβ gene [35].

CD with an early onset differs phenotypically and genetically from IBD with the onset of older age. IL-10Rα and IL-10Rβ mutations are common in children with very early onset of IBD and particularly in cases that are associated with perianal fistulae and severe colitis. Children with very early onset of CD, who had IL10R mutations, were resistant to the conventional therapies used for IBD. Moreover, they often require early surgical interventions [32, 36, 37]. The studies presented in this section are summarized in Table 3. As discussed above, such information could potentially be used in the future to guide the screening of VEO-IBD patients with IL-10 and/or IL10-R mutations. Nevertheless, the proper mainstay of management still requires careful attention and additional studies to establish broader screening in the child population.

TLR

Toll-like receptors (TLRs) are transmembrane proteins expressed in the membrane of various cells [38]. TLRs play a significant role in immune responses, especially pathogen recognition by the extracellular

Table 3. IL-10 gene mutations in Crohn's disease

Author	Year	Type of study	IL-10 gene mutation	Clinical features
Shim *et al*. [28]	2013	Clinical study	IL-10 receptor gene mutations	Investigates IL-10 receptor mutations in children with neonatal-onset Crohn's disease and intractable ulcerative enterocolitis
Beser *et al*. [29]	2015	Clinical study	IL-10 receptor gene mutations	Explores the clinical features of IL-10 receptor gene mutations in children with very early onset inflammatory bowel disease
Huang *et al*. [30]	2017	Clinical study	Comprehensive mutation screening	Explores mutations in IL-10 receptor and clinical phenotypes in Chinese patients with VEO-IBD
Xiao *et al*. [32]	2016	Clinical study	Comprehensive mutation screening	Conducts comprehensive mutation screening for 10 genes, including IL-10 receptor, in Chinese patients with VEO-IBD
Kotlarz *et al*. [33]	2012	Clinical study	Loss of IL-10 signalling	Explores loss of IL-10 signalling in infantile inflammatory bowel disease, impacting diagnosis and therapy
van der Linde *et al*. [34]	2003	Clinical study	Gly15Arg mutation	Identifies a Gly15Arg mutation in the IL-10 gene associated with reduced IL-10 secretion in Crohn's disease
Sharifinejad *et al*. [35]	2022	Systematic review	IL-10/IL-10R deficiency	Conducts a systematic review on clinical, molecular, and therapeutic features of patients with IL10/IL10R deficiency
Khoshnevisan *et al*. [36]	2019	Clinical study	IL-10 receptor gene mutations	Analyses IL-10 receptor mutations in Iranian IBD cohort
Shim *et al*. [37]	2014	Clinical study	IL-10 receptor gene mutations	Highlights IL-10 receptor mutations as a distinct disease entity in very early onset IBD

IL-10 – interleukin-10, IBD – inflammatory bowel disease, VEO-IBD – very early onset inflammatory bowel disease.

matrix [39]. In the digestive system intestinal epithelial cells normally express TLR3 and TLR5, while TLR2 and TLR4 are barely observable. However, in acute intestinal injury, TLR4 expression is increased [40]. The interaction of TLR4 with its ligand-lipopolysaccharides (LPS) triggers inflammation within the intestine. LPS may hyperactivate the TLR4 and lead to sustained response and thus to chronic inflammation, at least in susceptible patients [41, 42]. Several studies have examined TLR polymorphisms as candidate genes in IBD. The meta-analysis by Wang *et al*. evaluated the associations between TLR2 and TLR4 polymorphisms and IBD, and they showed that the TLR2 Arg753Gln polymorphism does not significantly increase CD susceptibility. Nevertheless, the TLR4 Asp299Gly polymorphism was significantly associated with an increased risk of CD. In this study the higher risk of the CD refers only to the Caucasian race. The TLR4 Thr399Ile polymorphism was not connected with the risk of CD in any genetic model: dominant, additive, recessive, and allele models. Among all 4 genetic models, this polymorphism did not correlate with CD susceptibility in Caucasian or Asian ethnic groups [43]. Thus, the novel association with the TLR4 Asp299Gly polymorphism strongly suggests a genetic influence in predisposition to CD [44]. The studies presented in this section are summarized in Table 4.

NOD2

NOD2 mutations are the most common in Crohn's disease. NOD2 (nucleotide-binding oligomerization domain 2), also known as CARD15 (caspase recruitment domain family, member 15), is a protein encoded by the gene with the same name located on chromosome 16 (16q12.1) engaged in immune reaction [45]. The protein is an intracellular pattern recognition receptor (PRR) and plays a vital role in regulating intestinal homeostasis. When the metabolic equilibrium is maintained, the NOD2 responds to the intracellular bacterial lipopolysaccharides (LPS) by recognizing their muramyl dipeptide (MDP) and activating the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) protein. Then, antimicrobial peptides (AMPs) are produced by Paneth cells located in the crypts of the small intestine [46]. Eventually, AMPs are produced. It was found that each of the AMP peptides act as a mitogen and protective agent for intestinal epithelial cells. Increasing accumulation of (these) proteins, preventing their loss during injury, and facilitating assembly of new tight junc-

Table 4. TLR polymorphisms in Crohn's disease

Author	Year	Type of study	Findings
Takeuchi *et al.* [41]	2010	Review	Discusses pattern recognition receptors (PRRs) and their role in inflammation. It covers Toll-like receptors (TLRs) and other PRRs, highlighting their importance in recognizing pathogens and initiating immune responses
Candelli *et al.* [42]	2021	Clinical study	Explores the interaction between lipopolysaccharide (LPS) and gut microbiota in inflammatory bowel diseases (IBD). The study suggests that LPS from gut bacteria might contribute to the inflammatory process in IBD
Wang *et al.* [43]	2019	Meta-analysis	Conducts a meta-analysis of TLR polymorphisms and their association with inflammatory bowel disease (IBD). Finds correlations between TLR polymorphisms and increased susceptibility to IBD
Cheng *et al.* [44]	2015	Meta-analysis	Performs a meta-analysis on TLR2 and TLR4 gene polymorphisms and their relationship with the susceptibility to inflammatory bowel disease (IBD). Indicates a potential association between TLR2 polymorphisms and IBD susceptibility

PRRs – pattern recognition receptors, TLRs – Toll-like receptors, LPS – lipopolysaccharide, IBD – inflammatory bowel disease.

tions (TJs) following barrier disruption, were found to be the mechanisms by which AMP peptides provide barrier protection and surveillance. Additionally, in response to bacterial and cholinergic stimuli, the Paneth cells secrete the human α-defensins (HD-5 and HD-6), through the NF-κB pathway and with T-cell factor 4 (TCF-4) [47]. α-defensins are broad-spectrum microbicides that kill commensal or pathogenic bacteria. They function by binding to the microbial cell membrane and forming pore-like membrane defects that allow efflux of ions and nutrients [48]. Furthermore, NOD2 interaction with the ATG16L1 promotes the formation of autophagosome in intestinal epithelial cells (IECs), and ATG16L1 maintains autophagy by providing intraepithelial bacterial clearance [49]. In Crohn's disease (CD) NOD2 is often mutated. At least one NOD2 mutation is detected in 30–40% of CD cases, compared to 6–7% present in healthy subjects [50]. It is suggested that mutations impair bacterial recognition and clearance, leading to autoimmune, chronic inflammation [51]. Induction of inflammatory cytokines after MDP stimulation is impaired. In turn, Paneth cells do not secrete AMPs and α-defensins. Furthermore, impaired autophagy is also observed, accompanied by failure to kill intracellular bacteria due to impaired secretion of defensins. Consequently, there is defected barrier function and bacterial clearance. These alterations can lead to enhanced mucosal adherence with the development of dysbiosis [49, 51]. The NOD2 mutations and inflammation process are associated with ileal and not colonic disease involvement, which correlates with the ileal location of Paneth cells. The NOD2 mutations also correlate with fibrostenotic disease, earlier age of onset, and a family history of CD [52]. There are 3 common variants of mutations in CD: rs2066844(R702W) and rs2066845 (G908R) due to a single nucleotide substitution, and one resulting from a frameshift mutation: rs41450053(L1007fs). Overexpression of the last one is the most frequent NOD2 variant [53]. The presence of one risk allele suggests a 2–4-fold increased risk for CD occurrence, whereas for the presence of 2 risk alleles the risk increases 20–40-fold [54]. Furthermore, the presence of a single NOD2 mutation predicted an 8% risk increase for complicated disease, and 2 mutations predicted a 41% risk increase [55]. Additionally, the presence of NOD2 mutations correlated with increased risk of surgical interventions and reduced incidence of perianal disease [56]. In addition, there is a SNP in a region of TCF-4, rs3814570, which is associated with ileal CD phenotypes, decreased expression of TCF-4, and as a result with differentiated PC secretion of α-defensins. Patients who had these rs3814570 alleles showed higher risk of stricturing ileal CD and upper GIT involvement [47]. When it comes to treatment, defensin-like drugs have been developed as antibacterial treatments. They are proteolysis resistant and have low molecular weight and strong antimicrobial activity. Nevertheless, their costs of production, potential toxicity and low stability *in vivo* make them unattractive as a potential CD therapeutic [50]. NOD2 protein mutations provide convincing evidence in the pathogenesis of Crohn's disease, but there are still a lot of unanswered questions. Based on recently published evidence, NOD2 sequencing could help in a more precise diagnosis among CD patients with a substantial risk of surgery. This could be clarified through segregation analysis of the mother and father sequences, which will allow us to ascertain if the variants are inherited on the same chromosome or separately on a maternal or paternal chromosome. The concept came out after detection of the mechanism that variants inherited on different chromosomes would lead to 2 dysfunctional copies of the gene, when the same variants inherited on the same chromosome would result in a preserved

Table 5. NOD2 polymorphisms in Crohn's disease

Author	Year	Type of study	NOD2 gene mutation	Clinical features
Frade-Proud'Hon-Clerc S [53]	2019	Clinical study	rs2066844(R702W) rs2066845 (G908R) rs41450053(L1007fs)	Identifies new coding variations related to CD and performs whole exome sequencing (WES)
Yang E [47]	2021	Review	rs3814570	Mutation is associated with ileal CD phenotypes, decreased expression of TCF-4, and as a result with differentiated PC secretion of α-defensins. Higher risk of stricturing ileal CD and upper GIT involvement

functional version of NOD2 (because there is still a functional copy of the NOD2 gene present). Nonetheless, it will require precise sequencing, phased data to clarify recessive inheritance patterns in adults and children, systematic interpretation of variants, and longitudinal phenotyping in the context of specific genotypes [51]. Being able to predict the course of the disease in high-risk patients can prove useful in not only preventing complications but also in modifying the natural history of the disease. The studies presented in this section are summarized in Table 5.

Summary and conclusion

This review provides new insight into genetic polymorphisms in the pathogenesis and course of CD. Despite the large number of data on the genetic and epigenetic background of the disease, the genetic polymorphisms discovered so far explain only a small part of disease variance. CD is a complex disease the incidence of which is increasing and the course is heterogeneous, which poses a great medical challenge. Considering this aspect as well as natural history and therapeutic response, CD seems to be an ideal model for individual or targeted therapies. A better understanding of the genetic background can help predict the disease occurrence and the therapy stratification. However, it is also evident that the gene-environment interaction is important and the intestinal microbiome and inflammatory factors have an influence on the development and clinical course of CD.

Disturbances in the intestinal microbiota and impaired immune response are associated with the development of CD. There is increasing evidence in the literature that FUT2 is involved in the pathogenesis of many human diseases, among them CD. particular FUT2 gene SNPs are a risk factor for the development of CD. Although the mechanism of the impact of the FUT2 gene mutation is still unknown, the studies presented in this review shows that there is a relationship between the occurrence of the FUT2 gene mutation and bacterial dysbiosis. This fact shows that FUT2 gene products affect the formation of the gut bacterial community, its composition, and diversity in CD patients and are related to the dysregulation of the immune system, which contributes to the development of the disease. In summary, further studies linking FUT2 mutations, gut microbiota, and CD are needed. This knowledge may influence the discovery of new, targeted therapies in people with mutations in the FUT2 gene, which may contribute to faster remission in these patients. Dysregulated immunological responses cause the development of CD. IL-10 controls the immunological microbial responses in the gut. Early-onset IBD and defects in IL-10 and IL-10R are strongly connected. Recent advances in genetic testing showed that loss-of-function mutations in IL-10R subunits are associated with patients with a very early onset of CD. Importantly, traditional IBD treatments were ineffective in infants with IL10R mutations. Therefore, gene therapy may in future prove useful in the management of the early onset of CD. The ATG16-L1 gene encodes the protein that plays a role in maintaining intestinal homeostasis, and mutations related to this gene influence the development of CD. Increased secretion of cytokines such as IL-1β and IL-18 impair cellular autophagy and bacterial degradation in patients with the most common rs2241880 (T300A) risk variant, which is associated with the development of CD. Recent studies also show that this variant also has an increased risk of fistulas and postoperative infections. Understanding the impact of autophagy and disorders in its process through mutations in the ATG16L-1 gene may help in diagnosis and treatment planning. TLR4 is involved in bacterial identification. A correlation between CD and the TLR4 Asp299Gly polymorphism has been established. Thus, the genetic link between CD and TLR4 Asp299Gly strongly implies that abnormal interactions with both gram-positive and gram-negative bacteria contributed to CD triggering. On the contrary, there is no significant association between TLR2 Arg-753Gln and TLR4 Thr399Ile gene polymorphisms and the development of CD. These findings demonstrate the importance of various genetic profiles linked to CD. The NOD2 mutations are strongly connected to the CD susceptibility. They contribute to abnormal recognition, sensing, and reduced clearance of invading microbes. They correlate with ileal involvement,

fibro-stenotic disease, earlier age of onset, and a family history of CD. The presence of a double NOD2 mutation predicts a 41% increased risk of the complicated disease. A recent study considered defensin-like drugs as possible new antibacterial agents, but potential toxicity and low stability *in vivo* make them unattractive in the near future. Sequencing of NOD2 could help in precise diagnosis and treatment planning in CD.

More advanced research analysing the genomic, proteomic, metabolomic, transcriptomic, and microbiomic signatures is needed for better patient stratification. In addition, the use of new technologies such as DNA and RNA sequencing will allow the study and understanding of the impact of genetic polymorphisms in various cell populations and biopsy samples.

Conflict of interest

The authors declare no conflict of interest.

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