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Predicting response to anti-tumor necrosis factor therapy in Crohn's disease via autophagy-related genes

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Abstract

As greater emphasis is currently being placed on the concept of 'personalized medicine', research with the objective of discovering a link between autophagy-related genes and treatment response of Crohn's disease (CD) patients to anti-tumor necrosis factor (anti-TNF) drugs is booming. The aims are to predict which CD patients are more prone to treatment failure and which ones will achieve better response to anti-TNF therapy; thus, providing more precise treatment. Below, we describe the three-key autophagy-related genes: nucleotide-binding oligomerization domain containing 2 (NOD2)/ caspase recruitment domain-containing protein 15 (CARD15), autophagy-related gene 16 like 1 (ATG16L1) and immunity-related guanosine triphosphatase M (IRGM). Up to now, investigations through clinical studies have established ATG16L1 as the most encouraging candidate for evaluation of response to anti-TNF agents in CD patients. However, further studies are warranted to assess the different genes and their impact on anti-TNF treatment.

Keywords: Autophagy-related gene, Crohn's disease, Atg16l1, Nod2/Card15.

INTRODUCTION

The classification of inflammatory bowel diseases (IBD) comprises: ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis [1]. Although still unclear, the pathogenesis of IBD is potentially influenced by several factors, including: genes, inflammation, infection, immune reactions and the

environment [2],[3]. The gut microbiota has also been reported to be less diverse in IBD, especially in CD patients [1]. Notably, genetic influences have a major impact on susceptibility to CD, especially in early-onset CD [3]. CD lesions can involve the entire gastrointestinal tract and its characteristic features are: transmural bowel inflammation, 'skip lesions' of inflammation, deep ulcers and fissures acquiring a 'cobblestone' appearance, perianal fistulas and various extra-intestinal complications [1]. Being a chronic, recurrent condition, CD eventually leads to bowel dysfunction [4]. Confirmatory diagnosis is made by visualization and biopsy during colonoscopy. Medical management of CD includes: 5-aminosalicyclic acid compounds, corticosteroids, immunomodulators (azathioprine, 6-mercaptopurine, methotrexate) and the revolutionary biologics [1]. Approved anti-TNF agents used for moderate to severe disease are infliximab, adalimumab and certolizumab [4]. Nonetheless, one-fifth of CD patients will be primary non-responders to anti-TNF therapy and a further one-third will experience secondary loss of response, necessitating alternative drugs or surgical management [4]. As precise medicine is more recognized, it is crucial to determine which group of patients will respond to or fail therapy with anti-TNF drugs. With the link between autophagy and CD unraveled, the aims of this review are to describe the key autophagy-related genes (ATGs) associated with CD susceptibility and discuss the evidence available surrounding their use to predict response to anti-TNF therapy.

AUTOPHAGY

Autophagy is a process of self-degradation, in which intracellular components of the cytoplasm are degraded or recycled [5]. It is a highly controlled cellular process, fundamentally regulated by the ATGs to maintain homeostasis [5],[6]. In response to a stimulus, the endoplasmic reticulum produces a phagophore, a double-membraned structure on which autophagy-related gene 16 like 1 (ATG16L1) combines with the ATG5-ATG12 conjugate. This complex generates a multimer and subsequently lipidates light chain 3-11 [7]. Concurrently, the phagophore engulfs the targeted cytoplasmic component to form an autophagosome, which subsequently fuses with a lysosome to form an autophagolysosome, in which enzymatic degradation occurs [7]. The two key signaling pathways regulating the activation and inhibition of autophagy are the beclin/B cell lymphoma-2 and the mechanistic target rapamycin complex-1 pathways, respectively [5]. Additional roles of autophagy are antigen presentation, infection control, involvement in cellular stress response during nutrient deprivation state and cancer [8]. Autophagy is not strictly non-specific as it can also be selective according to its target and comprises of mitophagy, xenophagy, aggrephagy, etc [2].

AUTOPHAGY AND IBD

Dysfunctions in autophagy have been associated with the development of several diseases, including IBD. The three major ATGs linked to IBD pathogenesis are nucleotide-binding oligomerization domain containing 2/ caspase recruitment domain-containing protein 15 (NOD2/CARD15), autophagy-related gene 16 like 1 (ATG16L1) and immunity-related guanosine triphosphatase M (IRGM).

AUTOPHAGY-RELATED GENES:

NOD2/CARD15

The first CD-susceptibility gene discovered in 2001 is the NOD2/CARD15 gene which is located on chromosome 16q12[3],[5]. NOD2/CARD15 gene encodes an intracellular pattern recognition receptor expressed in monocytes, macrophages and dendritic cells, especially in the Paneth cells of the small intestine. Also, it has a role in innate immunity and defense against pathogenic bodies [8]. Under normal conditions, the gene has a low level of expression while it is enhanced under inflammatory conditions [9]. Moreover, recruitment of ATG16L1 protein for autophagosome formation is also mediated by NOD2/CARD15, thus illustrating its role in autophagy [10]. Mutations of the NOD2/CARD15 gene leads to alteration of the inflammatory immune response and it has been reported that harboring homozygous mutation of the NOD2/CARD15 gene augments the risk of developing CD by 20- to 40-fold [5],[11]. Westerners, unlike Asians, are the main carriers of aberrant NOD2/CARD15 genetic variants [8]. The three leading NOD2/CARD15 mutations associated with CD are R702W, G908R, and 3020insC. Mutations in NOD2/CARD15 correlate with early disease onset, primary involvement of the terminal ileum, complications such as perianal disease, stricture and fistula formation as well as high probability of surgical intervention [3],[11],[12]. Gazouli *et al*. found NOD2/CARD15 3020insC polymorphism to be considerably higher in patients with CD onset in childhood [13].

ATG16L1

The association of ATG16L1 with increased CD susceptibility was initially described in 2007 by Hampe *et al* [14]. ATG16L1 is involved in the autophagic process as it is necessary for autophagosome formation, bacterial clearance, T-cell responses and it also has a role in the exocytotic pathway of Paneth cells [8],[15]. Subsequently, studies have demonstrated reduced autophagy levels and Paneth cell dysfunction in certain cell types with NOD2/CARD15 and ATG16L1 T300A mutations obtained from CD patients [5]. ATG16L1 dysfunction can also lead to disruption of the intestinal microbiota, thus enhancing the severity of CD flares [15]. The most investigated variant of ATG16L1 is rs2241880, which encodes a missense variant leading to a substitution of threonine-to-alanine amino acid at position 300 (T300A) [15]. It was found that people harboring the T300A variant had double the risk of developing CD [7]. CD patients homozygous for T300A are more prone to develop structuring complications and experience recurrent relapses; consequently, this group is found to be treated with immunomodulators earlier in the disease course [15]. Knowing the genotype-phenotype relationship can support more assertive therapy in special high-risk patients to minimize the risk of complications and relapses.

IRGM

Located on chromosome 5q33.1, IRGM is another autophagy-related gene implicated in CD susceptibility [16]. Its role in autophagy is via its encoded GTP-binding protein which has the

ability to restrict replication of intracellular bacteria; thus, lack of normal IRGM gene activity may eventually lead to chronic intestinal inflammation [17]. A meta-analysis including over 75,000 IBD and control subjects, found a robust association between IRGM and CD [18]. Moreover, in a large case-control study, Runifi *et al* analyzed the association of IRGM with CD and found two variants of IRGM, rs13361189 and rs4958847, to influence the phenotype of CD [19]. The polymorphism rs13361189 was noted to augment the risk of strictures, ileal and perianal disease, as well as, surgery in CD patients. In a meta-analysis of case-control studies, the latter IRGM polymorphism showed a significant association with CD, thus, discovery of these potential markers could have a significant impact on the future of CD treatment strategies [16]. In their study involving the German population, Glas *et al* also found IRGM to have a significant association with CD susceptibility, thereby supporting its potential involvement in CD pathogenesis, although evidence for the role of ATG16L1 in CD pathogenesis is more substantial [17]. However, since most studies have focused on NOD2/CARD15 and ATG16L1, the impact of altered IRGM on CD phenotype remains uncertain.

RESPONSE TO BIOLOGICS

Regarding the role of autophagy-related genes for the prediction of response to biologic therapy, NOD2/CARD15 and ATG16L1 are the two most investigated genes.

NOD2/CARD15

Interest in the NOD2/CARD15 gene has fuelled the existence of various studies and researches to assess its role in anti-TNF therapy for CD. A recent genotype-phenotype retrospective analysis with 161 CD patients conducted by Schaffler *et al* found that 34.2% of the study sample harbored NOD2/CARD15 genetic mutations [12]. In addition to the above-mentioned clinical picture seen with NOD2/CARD15 mutations, they commonly found anti-TNF levels in a sub-therapeutic range during therapeutic drug monitoring and reduced anti-TNF trough levels compared to NOD2/CARD15 wild-type. Consequently, it was deduced that higher doses of anti-TNF drugs would be more beneficial to CD patients with NOD2/CARD15 mutations. Despite the above-mentioned findings, Wang *et al* performed a meta-analysis, involving 4 studies and a total of 355 CD patients treated with adalimumab and/or infliximab and the study demonstrated no significant association of NOD2/CARD15 polymorphisms in the prediction of treatment response; however, the studied sample size was small [11]. Moreover, in 2010, another study reported no apparent association between NOD2/CARD15, TLR4 and CD14 gene polymorphisms and response to adalimumab therapy [20]. In a large study, Mascheretti *et al*. [5] found no positive correlation between therapeutic response to infliximab and NOD2/CARD15 mutations [21]. Similarly, Vermeire *et al* found no association between response to infliximab and NOD2/CARD15 mutations; however, in their study, 32.6% of CD patients compared to 15% of healthy participants harbored NOD2/CARD15 mutations [22]. Further clinical studies are warranted to evaluate the association of NOD2/CARD15 gene with biologic therapy to choose the most efficient therapy for different CD genotypes.

ATG16L1

In the search for a superior and more personalized management strategy for IBD, advanced research to find a relation between autophagy-related genes and response to anti-TNF agents is ongoing. Currently, the gene displaying the most potential is ATG16L1[23]. A recent study found that CD patients carrying mutant ATG16L1 are more frequently treated with adalimumab; thus, hinting that disease genotype may influence drug choice for therapy [23]. In another study, Dezelak *et al* considered the role of autophagy-related genes: ATG12 and ATG5, which interact with ATG16L1 in the autophagy process, for the prediction of adalimumab treatment response in CD patients [24]. Using inflammatory bowel disease questionnaire (IBDQ) scores, they found that polymorphisms rs9373839 and rs510432 of ATG5 are promising positive predictors of adalimumab response. The study by Koder *et al* enrolled 102 Slovenian CD patients and similarly used IBDQ index and C-reactive protein (CRP) levels to evaluate the role of single nucleotide polymorphisms (SNPs) in predicting adalimumab treatment response [25]. The results showed ATG16L1 SNP rs10210302 to have a robust association with adalimumab response. A prospective cohort study by Netz *et al* identified Fas Ligand SNP (rs763110) and TNF gene-308 (rs1800629), to be related with non-response of CD patients to anti-TNF treatment [26]. On the other hand, no association was demonstrated with ATG16L1 SNPs. This study emphasizes the importance of detecting non-responders to anti-TNF agents via genetic screening to reduce the burden of expenses for patients who might not respond to such therapies and to avoid subjecting such patients to the risks of anti-TNF drugs.

CONCLUSION

With advanced genetic research, more and more genetic components linked to chronic conditions are being uncovered. As the relation between CD and autophagy-related genes has been established, it is further being investigated as a tool to predict the success or failure of anti-TNF therapy. So far, the most promising gene is ATG16L1, which has shown potential to predict response to adalimumab. However, for a more extensive appreciation of this association, further and larger sample sized studies are necessary. Conversely, in numerous studies, NOD2/CARD15 polymorphisms have shown no significant associations with treatment response to anti-TNF agents. As precision medicine is still an evolving field, further research will benefit the management of CD patients by classifying them as suitable or unsuitable for anti-TNF therapy. This would lead to prompt and a more cost-effective management of CD and also avoid subjecting unsuitable patients to unnecessary risks linked to biologics.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' contributions

DLP designed and drafted the manuscript. TGW and NET

critically revised the work. HG and XC conceptualized, designed and critically revised the manuscript. DLP, XC and HG gave final approval of the version to be published. HG and XC contributed equally to the manuscript.

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REFERENCES

1. Gajendran M, Loganathan P, Catinella AP, Hashash JG. A comprehensive review and update on Crohn's disease. Dis Mon. 2018;64(2):20-57.
2. Ardizzone S, Bianchi Porro G. Biologic therapy for inflammatory bowel disease. Drugs. 2005;65(16):2253-86.
3. Roesler J, Thurigen A, Sun L, Koch R, Winkler U, Laass MW, *et al*. Influence of CARD15 mutations on disease activity and response to therapy in 65 pediatric Crohn patients from Saxony, Germany. J Pediatr Gastroenterol Nutr. 2005;41(1):27-32.
4. Barber GE, Yajnik V, Khalili H, Giallourakis C, Garber J, Xavier R, *et al*. Genetic Markers Predict Primary Non-Response and Durable Response To Anti-TNF Biologic Therapies in Crohn's Disease. Am J Gastroenterol. 2016;111(12):1816-22.
5. Hooper KM, Barlow PG, Stevens C, Henderson P. Inflammatory Bowel Disease Drugs: A Focus on Autophagy. Journal of Crohn's & colitis. 2017;11(1):118-27.
6. Kim S, Eun HS, Jo E-K. Roles of Autophagy-Related Genes in the Pathogenesis of Inflammatory Bowel Disease. Cells. 2019;8(1):77.
7. Iida T, Onodera K, Nakase H. Role of autophagy in the pathogenesis of inflammatory bowel disease. World J Gastroenterol. 2017;23(11):1944-53.
8. Iida T, Yokoyama Y, Wagatsuma K, Hirayama D, Nakase H. Impact of Autophagy of Innate Immune Cells on Inflammatory Bowel Disease. Cells. 2018;8(1):7.
9. Poniewierka E, Neubauer K, Kempinski R, Sadakierska-Chudy A. Disease duration and age influence CARD15 expression in Crohn's disease. Postepy Hig Med Dosw (Online). 2016;70:10-3.
10. Travassos LH, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhaes JG, *et al*. Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol. 2010;11(1):55-62.
11. Wang X, Qin L, Cao J, Zhao J. Impact of NOD2/CARD15 polymorphisms on response to monoclonal antibody therapy in Crohn's disease: a systematic review and meta-analysis. Curr Med Res Opin. 2016;32(12):2007-12.
12. Schaffler H, Geiss D, Gittel N, Rohde S, Huth A, Glass A, *et al*. Mutations in the NOD2 gene are associated with a specific phenotype and lower anti-tumor necrosis factor trough levels in Crohn's disease. J Dig Dis. 2018;19(11):678-84.
13. Gazouli M, Pachoula I, Panayotou I, Mantzaris G, Chrousos G, Anagnou NP, *et al*. NOD2/CARD15, ATG16L1 and IL23R gene polymorphisms and childhood-onset of Crohn's disease. World J Gastroenterol. 2010;16(14):1753-8.
14. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, *et al*. A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet. 2007;39(2):207-11.
15. Salem M, Ammitzboell M, Nys K, Seidelin JB, Nielsen OH. ATG16L1: A multifunctional susceptibility factor in Crohn disease. Autophagy. 2015;11(4):585-94.
16. Li Y, Feng ST, Yao Y, Yang L, Xing Y, Wang Y, *et al*. Correlation between IRGM genetic polymorphisms and Crohn's disease risk: a meta-analysis of case-control studies. Genet Mol Res. 2014;13(4):10741-53.
17. Glas J, Seiderer J, Bues S, Stallhofer J, Fries C, Olszak T, *et al*. IRGM variants and susceptibility to inflammatory bowel disease in the German population. PloS one. 2013;8(1):e54338.
18. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, *et al*. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012;491(7422):119-24.
19. Rufini S, Ciccacci C, Di Fusco D, Ruffa A, Pallone F, Novelli G, *et al*. Autophagy and inflammatory bowel disease: Association between variants of the autophagy-related IRGM gene and susceptibility to Crohn's disease. Digestive and Liver Disease. 2015;47(9):744-50.
20. Barreiro-de Acosta M, Ouburg S, Morre SA, Crusius JB, Lorenzo A, Potel J, *et al*. NOD2, CD14 and TLR4 mutations do not influence response to adalimumab in patients with Crohn's disease: a preliminary report. Rev Esp Enferm Dig. 2010;102(10):591-5.
21. Mascheretti S, Hampe J, Croucher PJ, Nikolaus S, Andus T, Schubert S, *et al*. Response to infliximab treatment in Crohn's disease is not associated with mutations in the CARD15 (NOD2) gene: an analysis in 534 patients from two multicenter, prospective GCP-level trials. Pharmacogenetics. 2002;12(7):509-15.
22. Vermeire S, Louis E, Rutgeerts P, De Vos M, Van Gossum A, Belaiche J, *et al*. NOD2/CARD15 does not influence response to infliximab in Crohn's disease. Gastroenterology. 2002;123(1):106-11.
23. Nuij V, Peppelenbosch MP, van der Woude CJ, Fuhler GM. Genetic polymorphism in ATG16L1 gene is associated with adalimumab use in inflammatory bowel disease. J Transl Med. 2017;15(1):248.
24. Dezelak M, Repnik K, Koder S, Ferkolj I, Potocnik U. A Prospective Pharmacogenomic Study of Crohn's Disease Patients during Routine Therapy with Anti-TNF-alpha Drug Adalimumab: Contribution of ATG5, NFKB1, and CRP Genes to Pharmacodynamic Variability. Omics. 2016;20(5):296-309.
25. Koder S, Repnik K, Ferkolj I, Pernat C, Skok P, Weersma RK, *et al*. Genetic polymorphism in ATG16L1 gene influences the response to adalimumab in Crohn's disease patients. Pharmacogenomics. 2015;16(3):191-204.
26. Netz U, Carter JV, Eichenberger MR, Dryden GW, Pan J, Rai SN, *et al*. Genetic polymorphisms predict response to anti-tumor necrosis factor treatment in Crohn's disease. World J Gastroenterol. 2017;23(27):4958-67.