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Genetic variants in autophagy-related genes and granuloma formation in a cohort of surgically treated Crohn's disease patients

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Abstract

Background and aims: Granulomas are a characteristic microscopic finding in Crohn's disease. Their clinical significance is controversial and their pathogenesis is unknown, but impaired processing of bacterial components has been suggested. Autophagy is a fundamental process involved in the elimination of intracellular bacteria. Genetic variants in autophagy genes *IRGM* and *ATG16L1* have been associated with susceptibility to Crohn's disease. We therefore investigated whether variants in autophagy genes contribute to granuloma formation.
Methods: Surgical specimens from 464 clinically well-documented Crohn's patients were reviewed and scored for the presence and distribution of granulomas. All patients were genotyped for the CD-associated SNPs in *ATG16L1* and *IRGM* as well as for 77 haplotype tagging SNPs in 13 additional autophagy genes.
Results: Granulomas were found in 75% of the patients. Their frequency increased with more distal involvement of the GI tract. Granuloma positive patients were significantly younger at the time of diagnosis and surgery, and were more likely to smoke. We identified associations between granulomas and autophagy gene variants *ATG4A* (rs5973822), *FNBP1L* (rs17109951) and *ATG4D* (rs7248026; rs2304165; rs10439163).
Conclusion: These findings suggest that granuloma formation is a marker of a more aggressive disease course, and that variants in autophagy genes *ATG4A*, *ATG2A*, *FNBP1L* and *ATG4D*, may contribute to granuloma formation.

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1. Introduction

Crohn's disease (CD) is characterized by discontinuous chronic inflammation of the gut that can involve any segment of the gastrointestinal tract from the mouth to the anus. CD is presumed to result from a dysregulated immune response to the host's microbiota in a genetically susceptible individual. The inflammatory process in CD is transmural involving all layers of the bowel wall with epithelioid granuloma as a characteristic histopathological finding. Although granulomas are considered an important feature in CD, they are found in only 45–70% of surgical samples of CD patients. Thus, a significant proportion of CD patients do not develop granulomas.[1–3] Frequency of granulomas is not only variable between patients, but it also varies in a single patient with a higher frequency reported in the distal part of the colon.[1,4] The absence of granulomas in a proportion of CD patients raised the possibility that these structures are markers of a particular clinical subphenotype. Indeed, some reports indicate that granulomas are more prevalent in young patients with a short disease duration. They have also been associated with a more aggressive disease course and a higher rate of postoperative recurrence.[2,5–7] However, these findings are controversial, with opposite conclusions being reached in other studies.[3,4,8] Furthermore, although granuloma formation is a characteristic finding in Crohn's disease, the reason for granuloma formation is still unknown. DNA from various microorganisms, such as *Mycobacterium avium* subspecies *paratuberculosis* and *Escherichia coli* has been detected in granulomas of CD patients, but these findings were not conclusively replicated and may represent nothing more than a bystander phenomenon.[9–11]

In the last decade, numerous genetic studies have highlighted the role of innate immunity in CD pathogenesis. The disease is strongly associated with particular variants of the *NOD2/CARD15* gene in western populations.[12,13] The protein product of this gene functions as a pattern recognition receptor binding the bacterial wall component muramyl-dipeptide, which leads to nuclear factor kB pathway activation. It has been conceived that impaired recognition of bacterial components by the innate immune system could result in granuloma formation. However, no significant association between variants of *NOD2/CARD15* or *TLR4* and granuloma formation was found.[1,14] Also in recent years, variants in the autophagy genes *ATG16L1* and *IRGM* have been linked to CD by genome-wide association scans (GWAS).[15–18] Autophagy is a fundamental homeostatic cellular process found in all eukaryotic cells. It involves delivery of cytoplasmic components to the lysosome for the degradation within a specific double membrane bound vesicle called the autophagosome.[19] Autophagy can also eliminate intracellular pathogens including viruses, parasites and bacteria and thus plays a role in innate and adaptive immunity, making it an intrinsic part of our immune system.[19–25] For example, *IRGM* is necessary for autophagy in human macrophages and has a documented role in the clearance of intracellular Mycobacteria.[21] Also, carriers of a particular single-nucleotide polymorphism in *ATG16L1* have an impaired autophagic clearance of *Salmonella*, while the other functions of autophagy remain intact.[26]

Considering the controversial clinical significance of granulomas, we investigated whether their presence, number or distribution in the bowel wall is related to particular phenotypical characteristics of CD patients. We also hypothesized that genetic variants in autophagy genes may lead to impaired processing of intracellular bacterial components, which in turn could lead to granuloma formation in CD patients. We therefore studied variants in autophagy genes for the association with granuloma formation in CD patients who had undergone bowel resection.

2. Materials and methods

2.1. Patients

All CD patients undergoing surgical resections in the University Hospital Gasthuisberg, Leuven, Belgium, in the period between January 1st, 1991 and December 31th, 2007 were included, except if they were operated for perianal disease only. A total of 464 patients were thus selected. All clinical records were reviewed for gender, age at diagnosis, smoking status, localization and behavior of disease, disease duration prior to surgery, indication for surgery, age at operation, and number of surgical interventions (Table 1). Localization and behavior of disease at the time of surgery were defined according to Montreal classification.[27] For all included patients, DNA as well as tissue blocks from the resection specimens were available. Informed consent was obtained from all patients and the study was approved by the Ethics Committee of the University hospitals Leuven.

Table 1 Clinical characteristics of CD patients.

Male/female (%)	213/251 (45.9/54.1)
Median (IQR) age at diagnosis (years)	23.8 (18–31.1)
Median (IQR) age at first surgery (years)	36.4 (27.4–45.7)
Median (IQR) disease duration to surgery (years)	10.2 (4.0–16.3)
Location of disease (%)	
L1 ± L4	208 (44.8)
L2 ± L4	29 (6.2)
L3 ± L4	226 (48.7)
L4	1 (0.2)
Disease behaviour (%)	
B1 ± p	29 (6.3)
B2 ± p	259 (55.8)
B3 ± p	176 (37.9)
Perianal	159 (34.2)
Smoking at surgery (%) (n = 409)	189 (46.2)
Granuloma positive (%)	348 (75.0)
Indication for surgery (%)	
Intractable disease	32 (6.9)
Obstruction	263 (56.7)
Fistula	146 (31.4)
Perforation	18 (3.9)
Malignancy	5 (1.1)