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SHORT COMMUNICATION

The role of the NOD2/CARD15 gene in surgical treatment prediction in patients with Crohn's disease

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Abstract

Purpose Crohn's disease (CD) belongs to chronic disorders with unpredictable disease course. The aim of this study was to identify how genetic testing (NOD2/CARD15) can be used in patients with CD to predict the need for surgical treatment (to define an aggressive type of disease where the patient can profit from early surgery).

Methods The patients who were tested genetically had undergone a surgery due to CD at the Department of Surgery University Hospital Brno Bohunice between 2010 and 2016. The control group consisted of patients with CD who had been diagnosed with CD at least 5 years prior to the testing and had not required any surgical intervention. The second control group was healthy subjects.

Results In total, there were 117 operated patients for CD, 77 patients with CD that had not undergone surgery for CD and 30 healthy subjects. For patients with at least one genetic mutation, the risk of the necessity of surgical treatment of CD is 1.96 times higher than for patients with no mutation. Patients with two or more mutations were generally operated on at a younger age, in a shorter time after being diagnosed and each patient had a partial resection of the ileum.

Conclusion The group of operated patients with CD had a significantly higher distribution of at least one genetic mutation as opposed to the non-operated group. In patients with two or more mutations, the disease course was more aggressive. This group of patients might profit from the conservative top-down or early surgical therapy.

Keywords Crohn's disease · Inflammatory bowel disease · Disease course · Genetics · NOD2/CARD15 · Surgery · Intestinal resection · Czech cohort

Introduction

Crohn's disease (CD) is a chronic bowel inflammatory disease with an increasing incidence, affecting mostly patients at an early age. It is a disease of unknown etiology, unpredictable development, and in addition most patients need to undergo surgical treatment of CD during their life [1].

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A possible relation between CD and a mutation of the NOD2/CARD15 gene was first published by Ogura et al. [2] in 2001. Several independent studies describe three mutations of this gene in the western (Caucasian) population that are related to CD (R702W, G908R, and L1007fs, sometimes also referred as 3020insC). A carrier of a mutant allele (heterozygote) in the NOD2/CARD15 gene is 2–4 times more likely to develop CD and a carrier of two mutant alleles (homozygote or a mixed heterozygote) is 20–40 times more likely to develop CD [3].

According to the ECCO (European Crohn's and Colitis Organisation) guidelines, the general factors of an aggressive disease course and factors increasing the risk of surgical treatment include the following: cigarette smoking, a stricturing or penetrating form of the disease, an early necessity to prescribe corticosteroids in medication, location of the disease in the ileum, or an early age of the patient at the time of diagnosis [4].

Some studies have proved a connection between the NOD2/CARD15 gene mutations and a complicated disease course [5]. The relation between an aggressive form of CD

and a NOD2/CARD15 gene mutation is especially noticeable in cases of patients diagnosed at an early age, dominant ileum location or a stricturing form of the disease [6, 7].

The aim of the analysis was to find a clinical relation between the mutations in the NOD2/CARD15 gene and the CD phenotype, relation to the operative and postoperative course of the disease and try to determine CD patients with a fast-progressing form of the disease who would profit from an early surgical solution.

Material and methods

Three groups of patients underwent genetic testing. The main group consisted of CD patients who had undergone surgical treatment during 2010–2016 for CD. The first control group was CD patients who had been diagnosed with CD for at least 5 years and had not undergone any surgical treatment for this disease. This group consisted of patients with a less aggressive form of CD, in which we supposed a lower occurrence of NOD2/CARD15 gene mutations. The second control group was healthy individuals without any disease or internal diagnosis. All the patients including the control groups were tested for a genetic mutation in the NOD2/CARD15 gene.

The study was prospective, and the patients were monitored for these basic parameters—age, gender, Montreal classification of CD, medication, cigarette smoking, number of previous CD surgeries, history of CD in the family, and genetic analysis of NOD2/CARD15.

Furthermore, other data related to the operation and postoperative development were recorded—the time interval between being diagnosed with CD and surgery, type of surgical treatment, and the extent of bowel resection, type of anastomosis, operative approach, and postoperative complications.

Results

The group of patients operated for CD included 117 patients; 53 of them were men (45.3%) and 64 women (54.7%). The most frequent location of the disease was the ileum and the colon (L3) in 55 of the cases (47.0%), closely followed by the ileum (L1) in 51 of the cases (43.6%) and colon (L2) in 11 patients (9.4%). Twenty-one (17.9%) of the patients suffered from an inflammatory type (B1) of the disease, 56 (47.9%) patients had a stricturing type (B2), and 40 (34.2%) patients had a penetrating type (B3). The difference in the number of patients with a stricturing and penetrating type was significantly higher than in the group of patients without surgery. Eighty-three patients (70.9%) underwent surgery for CD for the first time, 27 patients (23.1%) had already undergone one CD-related surgery in the past, 3 patients (2.6%) had undergone 2 surgeries in the past, and 4 patients (3.4%) even 3 CD-related surgeries.

The first control group consisted of 77 CD patients who had not required any surgery for this diagnosis during their life. Female gender prevailed—there were 50 (64.9%) women as opposed to 27 (35.1%) men. CD duration median was 13 years (9–18 years IQR). In this group, the most frequent location was the ileum (L1) in 36 patients (46.8%), followed by the ileum and colon (L3) in 28 patients (36.4%). The least frequent location was the colon (L2) in 13 of the cases (16.9%). The prevailing type of the disease was the inflammatory form (B1), specifically in 68 patients (88.3%).

The second control group of persons without CD or any other internal diagnosis who underwent genetic testing consisted of 30 healthy individuals (16 of whom were men and 14 women).

Out of a total of 117 surgical procedures, the most common one was ileocecal resection ($n = 72$), followed by left hemicolectomy or rectal resection ($n = 21$), then resection of anastomosis in 13 patients and right hemicolectomy in 11 patients. The laparoscopic approach was chosen for 28 patients (24%) and open surgery for 89 patients (64%). Regarding the construction of anastomosis, the most frequent one was side-to-side (STS) anastomosis ($n = 83$) in comparison with end-to-end (ETE) anastomosis ($n = 18$) and 14 patients had terminal ileostomy.

Afterwards, a statistical analysis of the high-risk mutations in the NOD2/CARD15 gene was performed in all three studied groups. The distribution according to the number of mutations is shown in Table 1 and the particular alleles of the gene summarizes Table 2. The associations between a specific number of mutations and individual characteristics of operated CD patients can be seen in Table 3. Operated patients with two or more mutations had their first CD-related surgery performed at a considerably lower age than patients with one or no mutation. In particular, the median age at the time of their first CD-related surgery was 24 years, as opposed to 31 or 32 years, respectively. Another important finding was that patients with two or more mutations had an operation much earlier after being diagnosed than patients with one mutation. Operated CD patients with two or more mutations were therefore generally operated on at an earlier age and needed surgery earlier after being diagnosed. Furthermore, in the group of patients with two or more mutations, each patient had a resection of the ileum (100%) as opposed to patients with one or no mutation, which means that this information presents a high specificity.

The relation between a mutation in the gene and the time interval between diagnosis and the first CD-related surgery has also been measured. Patients with two or more mutations underwent surgery earlier in comparison with the patients with one mutation. The median interval between diagnosis and surgery was only 1 year compared to 5 years.

Another statistically significant correlation was found regarding the form of the disease. Patients with two or more

Table 1 Number of mutations in NOD2/CARD15 gene

	No mutations	1 mutation	≥ 2 mutations	1–3 mutations
Operated CD patients ($N = 117$)	52 (44.4%)	43 (36.8%)	22 (18.8%)	65 (55.5%)
Non-operated CD patients ($N = 77$)	47 (61.1%)	17 (22.1%)	13 (16.8%)	30 (38.9%)
Controls ($N = 30$)	29 (96.7%)	1 (3.3%)	0 (0.0%)	1 (3.3%)
P^1	*0.028*	*0.039*	0.849	*0.028*
P^2	*< 0.001*	*< 0.001*	*0.008*	*< 0.001*
P^3	*< 0.001*	*0.021*	*0.018*	*< 0.001*

P value of Fisher's exact test compares relative frequencies of genotypes between operated CD patients and non-operated CD patients[1] , operated CD patients and controls[2] , and non-operated CD patients and controls[3]

mutations were operated on significantly earlier after being diagnosed as opposed to patients with one mutation if the patient had a stricturing or penetrating form of the disease (B2 and B3 were counted together, $P = 0.016$).

No relation between the genetic mutation and a higher number of reoperations for CD (resection of anastomosis in stenosis, etc.) was found and there was not observed any association between the genetic mutation and the extent of intestine resection.

There was significantly higher occurrence of mutations in the operated group than in the group of non-operated CD patients (55% of the operated patients had a mutation in the gene, compared with 39% of the non-operated patients with a mutation in the gene, $P = 0.028$). Patients with a mutation in the gene are 1.96 times more likely to have another CD-related surgery compared to patients without a mutation.

Table 2 Distribution of NOD2/CARD15 risk alleles (R702W, G908R and 3020insC)

	R702W	G908R	3020insC
Operated CD patients ($N = 117$)			
Homozygous wild-type (−/−)	96 (82.1%)	103 (88.0%)	74 (63.2%)
Heterozygous mutation (±)	21 (17.9%)	14 (12.0%)	33 (28.2%)
Homozygous mutation (+/+)	0 (0.0%)	0 (0.0%)	10 (8.5%)
Non-operated CD patients ($N = 77$)			
Homozygous wild-type (−/−)	70 (90.9%)	68 (88.3%)	57 (74.0%)
Heterozygous mutation (±)	7 (9.1%)	7 (9.1%)	15 (19.5%)
Homozygous mutation (+/+)	0 (0.0%)	2 (2.6%)	5 (6.5%)
Controls ($N = 30$)			
Homozygous wild-type (−/−)	30 (100.0%)	30 (100.0%)	29 (96.7%)
Heterozygous mutation (+/−)	0 (0.0%)	0 (0.0%)	1 (3.3%)
Homozygous mutation (+/+)	0 (0.0%)	0 (0.0%)	0 (0.0%)
P^1	0.098	0.205	0.330
P^2	*0.008*	0.074	*< 0.001*
P^3	0.187	0.249	*0.027*

P value of Fisher's exact test compares relative frequencies of genotypes between CD patients with surgery and without surgery[1] , CD patients with surgery and controls[2] , and CD patients without surgery and controls[3]

Discussion

In 2005, Annese et al. [5] published a study of a group of 316 CD patients which proved that the carriers of at least one mutant allele in the NOD2/CARD15 gene had a tendency toward a more aggressive disease course. These conclusions correspond to ours, where the number of patients with at least one mutation was significantly higher in the operated group compared to the control non-operated group of CD patients ($P = 0.028$). After a subdivision into individual alleles, the operated group of CD patients was found to have more mutations in the R702W and 3020insC allele compared to a healthy population. In the group of non-operated CD patients compared to the healthy group, only the 3020insC allele had a higher representation. In 2006, Seiderer et al. [8] even described that a mutation in this particular allele (3020insC) significantly increases the risk of intestinal stenosis and resection in CD patients.

Some studies correlate the NOD2/CARD15 gene mutation with a greater risk of an earlier intestinal resection after being diagnosed with CD [7, 9] or a generally greater risk of a CD-related surgery [8]. A greater risk for CD patients with a mutation of the NOD2/CARD15 gene is, according to previous studies, mainly connected to a disease of the ileum, the stricturing and penetrating form and being diagnosed with CD at an early age (unfavorable factors causing a more aggressive disease course) [8, 10].

In our group, patients with two or more mutations (homozygotes or mixed heterozygotes) formed a risk group with a more aggressive disease course. Specifically, patients with two or more mutations were operated on for CD at a significantly lower age and also significantly earlier after being diagnosed. We have also demonstrated the relation of an earlier operation after diagnosis in patients with two or more mutations and a stricturing or penetrating form of the disease. Another fact that makes this group of patients with two or more mutations high-risk is that 100% of them had to undergo a small bowel resection. This corresponds with many studies that have demonstrated the relationship between the ileum (L1) and mutations in the NOD2/CARD15 gene [7, 9, 10].

Table 3 Associations between mutations in NOD2/CARD15 and characteristics of operated CD patients

Parameters		No mutations $N = 52$	1 mutation $N = 43$	≥ 2 mutations $N = 22$	P^1
Clinical characteristics					
Sex	Male	24 (46.2%)	19 (44.2%)	10 (45.5%)	0.999
	Female	28 (53.8%)	24 (55.8%)	12 (54.5%)	
Age at diagnosis	Years	26 (22; 37)	26 (22; 32)	22 (17; 29)	0.061
	< 17 years	2 (3.8%)	5 (11.6%)	5 (22.7%)	0.089
	17–40 years	41 (78.8%)	35 (81.4%)	15 (68.2%)	
	> 40 years	9 (17.3%)	3 (7.0%)	2 (9.1%)	
Location of disease	L1—ileum	27 (51.9%)	17 (39.5%)	7 (31.8%)	*0.031*
	L2—colon	8 (15.4%)	3 (7.0%)	0 (0.0%)	
	L3—ileum + colon[#]	17 (32.7%)[a]	23 (53.5%)[ab]	15 (68.2%)[b]	
	Not L4	51 (98.1%)	40 (93.0%)	20 (90.9%)	0.286
	L4—upper GIT (concomitant)	1 (1.9%)	3 (7.0%)	2 (9.1%)	
Behavior of disease	B1—inflammatory	12 (23.1%)	7 (16.3%)	2 (9.1%)	0.638
	B2—stricturing	25 (48.1%)	20 (46.5%)	11 (50.0%)	
	B3—penetrating	15 (28.8%)	16 (37.2%)	9 (40.9%)	
Perianal disease	No	36 (69.2%)	28 (65.1%)	14 (63.6%)	0.902
	Yes	16 (30.8%)	15 (34.9%)	8 (36.4%)	
Surgical characteristics					
Age at first surgery	Years	32 (25; 42)[a]	31 (27; 37)[a]	24 (20; 34)	*0.005*
Time from diagnosis to first surgery	Years	3 (1; 8)[ab]	5 (2; 9)[a]	1 (1; 7)[b]	*0.024*
Surgical procedure	IC resection	32 (61.5%)	25 (58.1%)	15 (68.2%)	0.093
	Right-sided hemicolectomy	4 (7.7%)	4 (9.3%)	3 (13.6%)	
	Resection of anastomosis	3 (5.8%)	6 (14.0%)	4 (18.2%)	
	Resection of left colon or rectum	13 (25.0%)	8 (18.6%)	0 (0.0%)	
Ileum resection	No	13 (25.0%)	10 (23.3%)	0 (0.0%)	*0.016*
	Yes	39 (75.0%)[a]	33 (76.7%)[a]	22 (100.0%)	
	(cm; if yes)	25 (15; 30)	25 (20; 30)	25 (15; 30)	0.977
Anastomosis	STS	34 (66.7%)	31 (73.8%)	18 (81.8%)	0.602
	ETE	8 (15.7%)	7 (16.7%)	3 (13.6%)	
	Terminal ileostomy	9 (17.6%)	4 (9.5%)	1 (4.5%)	
Surgical approach	Open	40 (76.9%)	30 (69.8%)	19 (86.4%)	0.379
	Laparoscopic	12 (23.1%)	13 (30.2%)	3 (13.6%)	
Complications	No	34 (65.4%)	30 (69.8%)	13 (59.1%)	0.667
	Yes	18 (34.6%)	13 (30.2%)	9 (40.9%)	

Continuous variables are described by median (IQR); categorical variables are characterized by absolute and relative frequencies. [a, b] Same letters denote groups of patients which do not significantly differ (post hoc analysis with Bonferroni correction applied)

[1] *P* value of Fisher's exact test for categorical variables or *P* value of Kruskal-Wallis test for continuous variables

[#] Significant difference between groups of patients (post hoc analysis with Bonferroni correction applied)

The work of Jürgens et al. [11] from 2010 also points to patients with a homozygous mutation of the 3020insC allele as being at a high risk for stenosis and intestinal resection. Another important work by Adler et al. [7] from 2011 also finds out that patients with two mutations are at a higher risk of an aggressive and complicated course of CD.

In 2009, Maconi et al. [12] did not show any correlation between the mutation in the gene and higher frequency of reoperations (stenosis in anastomosis, involvement of another part of the intestine, etc.). In our analysis, we also did not detect any relation between a mutation in the gene and more frequent reoperation in CD patients.

Conclusion

An essential finding was that patients with two or more mutations were generally operated on at a younger age and also earlier after being diagnosed with CD. Moreover, each patient with two or more mutations had a resection of a part of the

ileum. This group of patients could therefore be considered as a group with a more aggressive type of CD and a higher probability of a resection of the small intestine, and would benefit from conservative top-down therapy or early surgical treatment.

In the operated group, there was at least one mutation in the gene found significantly more often than in the non-operated group. Patients with a mutation in the NOD2/CARD15 gene are at a 1.96 times greater risk of CD-related surgery than patients without a mutation.

Compliance with ethical standards

Ethical standard statement All procedures which followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Informed consent was obtained from all patients for being included in the study.

Conflict of interest The authors declare that they have no conflict of interest.

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