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Genetics in Inflammatory Bowel Diseases

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ABSTRACT

Background: Genetic predisposition is the key factor for IBD. CARD15/NOD2 (nucleotide-binding oligomerization domain family, member 2) is responsible for recognizing of pathogen-associated molecular patterns. Activation of Janus kinase-2 (JAK2) transcription pathway leads to expression of pro-inflammatory cytokines and induce immune response. Genetic predisposition with polymorphism of NOD2/CARD15, JAK2 can play important role in pathogenesis of inflammatory bowel diseases (IBD).

Aim: to characterize genetic susceptibility to ulcerative colitis (UC), Crohn's disease (CD) in Caucasians.

Materials and methods: 115 IBD patients (58.3% UC and 41.7% CD) and 22 healthy controls were recruited. Single-nucleotide polymorphism (SNP) of NOD2/CARD15 (3020insC, Gly908Arg), JAK2 (Val617Phe) were determined by polymerase chain reaction (PCR) with electrophoretic detection in 3% agarose gel.

Results: 72 (62.6%) IBD patients had NOD2/CARD15 single-nucleotide polymorphism. An association with CD for 3020insC was detected more often – 58.3% cases (p=0,001; OR=10.0; 95% CI = 2,33 – 42,78), than with UC (37.3%, p=0,09). JAK2 mutation were revealed only in 11 (9.6%) patents with IBD and didn't show any positive interaction with IBD. The interaction with SNPs and severity of IBD was revealed for CARD15 3020 insC.

Conclusions: Important role of genetic predisposition in pathogenesis of IBD was revealed. Polymorphism of NOD2/CARD15 correlated with IBD. More significant association with CD comparing to UC was determined for CARD15 (Gly908Arg). Patients with IBD had combined mutations of NOD2/CARD15, JAK2. Significant positive interaction between risk genes and the severity of CD and UC was determined.

KEYWORDS inflammatory bowel diseases, NOD2/CARD15, JAK2.

Background

The etiology of inflammatory bowel diseases (IBD) involves both genetic and environmental components. Genetic predisposition is the key factor for IBD [1, 2, 3]. At the same time, approximately in 50% of cases of disease incidence environmental triggers such as diet regimen and food quality, emotional stress, episodes of intestinal infections and industrial burdens are also important [2, 4]. An influence of multiply combinations of environmental and non-environmental factors on health of population significantly increased in the last years. Changes of bacterial community are susceptible to different agents and can be caused by genetic and epigenetic factors. CARD15/NOD2 (nucleotide-binding oligomerization domain family, member 2) is responsible for recognizing of pathogen-associated molecular patterns play important role in innate immune host defense [2, 3, 5]. Activation of Janus kinase-2 (JAK2) transcription pathway leads to expression of pro-inflammatory cytokines and induce immune response. Genetic predisposition with polymorphism of NOD2/CARD15, JAK2 may have an influence on changes of gut microbiome, inducing of inflammatory process in the large and small intestine, and can play important role in pathogenesis of IBD.

Aim: to characterize genetic susceptibility to ulcerative colitis (UC), Crohn's disease (CD) in Caucasians.

Methods

Prospective study with 115 IBD patients (58.3% UC and 41.7% CD) has been conducted. 22 healthy controls were recruited. Investigated population were Caucasians (Ukraine). Average age was 38,3±9,2 years. Diagnosis of UC and CD was based on clinical symptoms, endoscopic, X-ray examination and histological findings. Patients with CD and UC were classified according to Montreal classification [4]. Clinical severity of UC was based on Mayo score assessment [3, 4, 5]. The activity of CD was measured by Crohn's disease activity index (CDAI) [5, 6, 7]. Endoscopic examinations were carried out with visual examination of the colon mucosa and assessment of endoscopical index (EI) [6, 7, 8, 9]. Bioptates of colon mucosa were stained by haematoxylin-eosin, alcian blue at pH 1.0 and 2.5 to determine of sulfated and non-sulfated glucosaminoglycans and glycoproteins, and goblet cells in the colon mucus layer. To characterize the mucus production the PAS-reaction was performed. The scoring of the staining was performed by an individual blinded assessment of morphologist.

Single-nucleotide polymorphism (SNP) of NOD2/CARD15 (3020insC, Gly908Arg), JAK2 (Val617Phe) were determined by polymerase chain reaction (PCR) with electrophoretic detection in 3 % agarose gel colored by ethidium bromide. Primary denaturation were applied at 93°C during 1 min with subsequent 35 cycles by 10 sec, annealing of primers at 64°C in 10 sec, elongation at 72°C during 20 sec. Gene Amp® PCR System 2400 (Applied Biosystems) was used for PCR analysis. Visualization of results conducted by «TFX-20.M» («Vilber Lourmat», France).

Statistics Individual data points were presented. The differences were considered as significant for $p \leq 0.05$, non-parametric Fisher's criteria was applied to ascertain differences. Odds ratios (ORs) and 95% confidence intervals (CI) were estimated using binary logistic regression. All statistics were done using MedStat and Statistica 6.1 (StatSoft).

Results

Crohn's disease

The most of patients with CD - 37 (77.1%) had established diagnosis between 17 and 40 years (A2), and only 11 (22.9%) patients had A3 (Table 1). Young patients with CD were not included in to the study. The inflammatory process characterized by involvement of different parts of the gastrointestinal tract. At the same time, patients with ileal or upper gastrointestinal involvement were not included in the study to receive homogeneous group for analysis. Colonic location (L2) of CD predominated – 23 (47.9%) patients, ileocolonic (L3) extension was observed in 12 (25.0%) patients. Terminal ileum was involved in 13 (27.1%) patients.

Non-stricturing, non-penetrating behavior of disease (B1) prevailed – 29 (60.4%) patients, strictures (B2) were found only in 5 (10.4%) patients. Penetrating course of disease (B3) occurred in 10 (20.8%) of CD patients. Perianal location was admitted only in 4 (8.4%) patients of the investigated group.

Minimal activity of CD was observed in 15 (31.2%) patients. Moderate activity was typical for the most of the group – 22 (45.8%) patients. Severe CD was in 11 (23.0%) patients. Total CDAI in CD group was equal 283.6$\pm$18.2 score points. EI was 1.9$\pm$0.7 in all patients with CD.

Extraintestinal manifestation were typical and occurred in 18 (37.5%) of total group of CD patients. It varied from reactive arthritis to arthralgia and from peripheral joints lesion to axial arthropathies, sacroiliitis and ankylosing spondylitis (25.0% patients). Affection of skin (eritema nodosum, pyodermia gangrenosum, psoriasis, vitiligo) also was found in 15 (31.3%) patients with CD. Systemic involvement of cardiovascular system (miocarditis), liver diseases (NASH, PBC, PSC) occurred rare (less than 5.0% of patients).

Predisposition to CD has been found in 7 (14.6%) patients, who had family history of disease.

Ulcerative colitis

The mean age of onset of disease in patients with UC was similar to patients with CD. At the same time, the most of the patients have suffered from disease at the middle age (Table 1). That's why distribution between the groups (before and after 40 years) was almost equal (41.8% and 58.2%). The location of UC characterized by the inflammation in the large intestine. Proctitis and proctosigmoiditis had 17 (25.4%) patients (E1). The majority of UC patients suffered from left-sided UC (E2) – 38 (56.7%) persons and only 12 (17.9%) patients had pancolitis (E3).

Patients with moderate severity of UC also predominated among all UC patients – 28 (4.1%) persons. Mild severity of disease occurred in 21 (31.3%) patients. Only 18 (26.9%) patients had severe UC. Index Mayo in all UC patients group consisted 2.6$\pm$0.9 score points. Severity of UC has correlated with extensive character of inflammation in the large intestine. As the majority of UC patients had moderate activity of disease, EI was equal to 2.1$\pm$0.5 in a whole group of patients with UC.

Extraintestinal manifestation in UC patients was similar to CD, but has been revealed relatively rare – in 15 (22.4%) of cases. Arthritis and arthralgia were found in 17.9% patients. The same features of skin affection: eritema nodosum, psoriasis and vitiligo except pyodermia gangrenosum, occur in 25.4% of patients with UC. Liver disorders (NASH) and cardiomyopathy were found with the same frequency as in CD patients.

Family history of UC had 5 (7.5%) patients, that was significantly rare, than in CD patients (p=0.05).

SNP's analysis

72 (62.6%) IBD patients had NOD2/CARD15 single-nucleotide polymorphism. An association with CD for 3020insC was detected more often – 58.3% cases (p=0,001; OR=10.0; 95% CI = 2,33 – 42,78), than with UC (37.3%, p=0,09) (Table 2). Strong positive interaction was found also for Gly908Arg and CD, in spite of occurrence of this polymorphism was relatively rare – 32.0% cases (p=0,015; OR=10,353; 95% CI = 1,178 – 90,956). Only 11 (16.4%) patients with UC had Gly908Arg SNP (p=0,053) (Table 3). Nevertheless, it was also considered as a risk factor of colitis in comparison with control group (OR=1,872; 95% CI = 0,198 – 17,748). 22.9% of patients with CD had combined 3020insC and Gly908Arg SNPs (p=0,01). At the same time only 8.9% UC patients had the same SNPs combination. This allow to suppose that predisposition to CD more often associated with combined mutation of CARD15, than UC.

JAK2 mutation were revealed only in 11 (9.6%) patents with IBD (Table 4) and didn't show any positive interaction with IBD in the investigated group (OR= 0,31; 95% CI = 0,163 - 4,082). At the same time, frequency of SNPs for JAK2 was higher in patients with CD (63.6% of all cases), than with UC. That can be caused by peculiarities of the region as well as small number of the examined cohort.

The interaction with SNPs and severity of IBD was revealed for CARD15 3020 insC (Table 5, 6). This SNP was observed more often in patients with high severity of CD (OR=3,61; 95% CI = 0.35-1.07, p=0,05) and moderate severity of UC (OR=2,66; 95% CI = 0,31-1,04, p=0,03). Thus, 3020 insC can be assumed as one of key target in control of IBD incidence and severity. SNPs of CARD15 (Gly908Arg) and JAK2 (Val617Phe) didn't show positive interaction with the course and severity of IBD.

Discussion

IBD is one of the major question in modern genetical investigation. CARD/NOD2 was the first gene associated with IBD. CARD/NOD2 recognizes peptidoglycans and modulates both innate and adaptive immune responses [10, 11]. By stimulation of autophagy, bacterial replication and antigen presentation, it influences on dendritic cells together with toll-like receptors, promoting T-cells differentiation and immune tolerance [11, 12]. This pathway is damaged in patients with Crohn's disease-associated mutation 3020insC [13]. At the same time, CARD/NOD2 takes part in muramyl dipeptide-independent pathways, such as the control of T-cell response and interferon sintesys to microbial single-stranded RNA stimulation, showing immunomodulatory properties [11, 13].

JAK family enzymes (JAK1, JAK2, JAK3) and tyrosine kinase 2 (TYK2) play role in cytokine-induced cell signaling and implicated in cell signaling processes important in cancer and immune-inflammatory diseases. Progression in the field has taken a recent step forward with a selective inhibitor of JAK1/2 (ruxolitinib) and tofacitinib - a pan-JAK inhibitor [14]. Investigators have an optimistic view on combinations of JAK inhibitors with other targeted agents able to control IBD.

At the same time, gender, environment and individual bad habbits (smoking cessation, alcogol abuse, "junk food") may have a significant influence on epigenetic alterations in IBD. It is also confirms by the high discordance rate between CD (68%) and UC (85%) in monozygotic twins, who has identical genomes [10].

In our study we examined Caucasian patients with IBD, who live generaly in same environmental conditions – industrial Donetsk region. Gender and age differences were also not significant between UC and CD groups of patients. Behaviour

of inflammatory diseases and extraintestinal manifestations were typical for both pathlogies. Genetic predisposition to IBD was revealed in investigated group and confirmed not only by family history of disease, but also by evidence of SNPs in the most of patients with UC and CD. More over, genetic susceptibility was higher in CD, than in UC. CARD15 (3020insC) polymorphism showed significant interaction with IBD, which was greater for CD, and can be a risk factor of severe course of disease. SNPs of CARD15 (Gly908Arg) also occur more often in patients with CD. Thence, combined mutation of CARD15 (3020insC) and (Gly908Arg) may leads to more aggressive course of disease, can be predictor of early onset of CD. Chronic inflammation caused by bacterial infections (haemolysing and enteropathgenic E.coli, Salmonella enteritidis, Clostridium difficile ect.), more typical for CD than for UC. It can be induced by binding of specific pathogen-associated molecular patterns receptors (NOD2/CARD15) that protect mucosa from microbial and parasites invasion. Thus, SNPs of CARD15 can lead to abnormal responce to colon microbiota or bacterial pathogenes in the large intestine. Mutation of NOD2/CARD15 more often occurred in patients with CD and confirm the hypothesis about the role of infections in affected innate immune response as features in pathogenesis of CD. That can be one of the multiply risk factors also for UC, but evidently not as a single mutation, but probably in combination with other genetic or epigenetic factors.

There is a great variation between genetic and epigenetic alterations and need for further studies to reveal the effect of disease-associated alleles to explain the precise influence on incidence, behaviour and severity of IBD.

Conclusion

Thus, important role of genetic predisposition in pathogenesis of IBD was revealed. Polymorphism of NOD2/CARD15 correlated with IBD. More significant association with CD comparing to UC was determined for CARD15 (Gly908Arg). Patients with IBD had combined mutations of NOD2/CARD15, JAK2. Significant positive interaction between of at least two risk genes and the severity of CD and UC was determined.

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Author Contributions

Design of the experiment and analytic strategy: A.E. Dorofeyev, O.A. Rassokhina. Performed the experiment: O.A. Rassokhina, M.S. Kishenya. Analyzed the data: A.E. Dorofeyev, O.A. Rassokhina, M.S. Kishenya, E.A. Kiriyan, E.E. Sabodash, I.A. Derkach. Wrote the manuscript: A.E. Dorofeyev, O.A. Rassokhina. Literature review and technical support: O.A. Rassokhina, E.A. Kiriyan, E.E. Sabodash, I.A. Derkach.

Competing interests

Authors declare no competing interests.

Table 1. Clinical characteristics of IBD patients

Disease	UC	CD
Total number	67	48
Male/Female	1.3:1	1:1.5
Age of onset Mean (years) Below 16 (A1) 17- 40 (A2) Above 40 (A3)	 38.3±9.2 - 28 (41.8%) 39 (58.2%)	 32.5±11.8 - 37 (77.1%) 11 (22.9%)
Location Terminal ileum (L1) Colon (L2) Distal Left-sided Total Ileocolon (L3) Ileocolon+upper GI (L4)	 - 67 (100%) 17 (25.4%) 38 (56.7%) 12 (17.9%)	 13 (27.1%) 23 (47.9%) 12 (25.0%) -
Behavior Non-stricturing, non-penetrating (B1) Stricturing (B2) Penetrating (B3) Perianal disease (P)	 - - - -	 29 (60.4%) 5 10.4% 10 20.8% 4 8.4%
Severity (index) Total Mild Moderate Severe	Mayo 2.6±0.9 21 (31.3%) 28 (41.8%) 18 (26.9%)	CDAI 283.6±18.2 15 (31.2%) 22 (45.8%) 11 (23.0%)
Extraintestinal manifestation	15 (22.4%)	18 (37.5%)
Family history of IBD	5 (7.5%)	7 (14.6%)

Table 2. CARD15 (3020 ins C) SNP in patients with CD and UC

3020 insC	CD (N=48)		control (N=22)		p (F)	OR	95% CI	χ^2
	n	%	n	%				
normal	10	0,21	19	0,820	0,001	0,118	0,031 - 0,458	11,508
SNP	28	0,583	3	0,130	0,001	10,000	2,337 - 42,784	

3020 insC	UC (N=67)		control (N=22)		p (F)	OR	95% CI	χ^2
	n	%	n	%				
normal	42	0,626	19	0,820	0,076	0,500	0,187 - 1,338	2,027
SNP	25	0,373	3	0,130	0,093	1,826	0,691 - 4,826	

Table 3. CARD15 (Gly908Arg) SNP in patients with CD and UC

Gly908Arg	CD (N=48)		control (N=22)		p (F)	OR	95% CI	χ^2
	n	%	n	%				
normal	29	0,64	21	0,913	0,023	0,169	0,032 - 0,895	6,047
SNP	19	0,32	1	0,043	0,015	10,353	1,178 - 90,956	

Gly908Arg	UC (N=67)		control (N=22)		p (F)	OR	95% CI	χ^2
	n	%	n	%				
normal	56	0,836	21	0,913	0,330	0,876	0,157 - 4,889	0,623
SNP	11	0,164	1	0,043	0,36	1,872	0,198 - 17,748	

Table 4. JAK2 (Val617Phe) SNP in IBD

Val-617Phe	UC & CD (N=115)		control (N=22)		p (F)	OR	95% CI	χ^2
	n	%	n	%				
normal	104	0,904	20	0,636	0,038	0,73	0,119 - 4,843	0,238
SNP	11	0,096	2	0,09	0,06	0,31	0,163 - 4,082	

Table 5. SNPs in severity of CD

SNPs	I		II		III		Control	
	N 15	%	N 22	%	N 11	%	n 22	%
CARD15 3020 insC	7	46,7	9	40,9	8	72,7*	2	9,1
CARD15 Gly908Arg	8	53,3*	5	22,7	6	54,5*	1	4,5
JAK2 Val-617Phe	0	-	4	18,2	3	20	1	4,5

*p=0.05

Table 6. SNPs in severity of UC

SNPs	I		II		III		Control	
	N 21	%	N 28	%	N 18	%	n 22	%
CARD15 3020 insC	3	14,3	15	53,7*	7	38,9*	1	4,5
CARD15 Gly908Arg	4	19	4	14,3	3	16,7	1	4,5
JAK2 Val617Phe	0	-	2	7,1	2	11,1	1	4,5

*p=0.05

REFERENCES

1. Silverberg M., Satsangi J., Ahmad T., Arnott I., Bernstein C., et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol. 2005;19(Suppl A):5–36. | 2. Cho J.H. The genetics and immunopathogenesis of inflammatory bowel disease. Nat Rev Immunol. 2008 Jun; 8(6): 458-466. Abraham C., Cho J.H. Inflammatory bowel disease. N Engl J Med. 2009; 361: 2066–2078. | 3. Satsangi J., Silverberg M.S., Vermeire S., Colombel J-F. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut. 2006; 55:.749-753 | 4. Sartor R.B., Sandborn W.J. Kirsner's. Inflammatory Bowel Disease.-London: Saundrs, 2004.-6th ed.-754 p. | 5. Dignass A., Van A.G., Lindsay J.O., et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease. J. Crohn's &Colitis. 2010; 4/1: 29-62. | 6. Andrews J.M. Australian Guidelines for General Practitioners and Physicians. Inflammatory Bowel Disease. Third edition 2013. P.31 | 7. Mowat C., Cole A., Windsor A. et al. Guidelines for the management of inflammatory bowel disease in adults. Gut 2011;60(5). P.571- 607. | 8. Dignass A. et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis Part 1: Definitions and diagnosis // JCC Dec 2012; 6(10). P.965-990. | 9. Dignass A. et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis Part 2: Current management // JCC Dec 2012; 6(10). P.991-1030. | 10. Spehlmann M E, Begun AZ, Burghardt J, Lepage P, Raedler A, Schreiber | S. Epidemiology of inflammatory bowel disease in a German twin cohort: results of a nationwide study. Inflamm Bowel Dis 2008; 14:968-976. | 11. Cooke J, Zhang H, Greger L, Silva A, Massey D, Dawson C et al. Mucosal | genome-wide methylation changes in inflammatory bowel disease. Inflamm | Bowel Dis 2012; 18:2128-2137. | 12. Rubino S J, Selvanantham T, Girardin SE, Philpott DJ. Nod-like receptors | in the control of intestinal inflammation. Curr Opin Immunol 2012; 24:398-404. | 13. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory | bowel disease. Nature 2011; 474:307-317. | 14. Dymock BW, See CS. Inhibitors of JAK2 and JAK3: an update on the patent literature 2010 – 2012 . Expert opinion on therapeutic patients. April 2013, Vol. 23, No. 4 , Pages 449-501. | 15. Barnett M, Bermingham E, McNabb W, Bassett S, Armstrong K, Rounce J et al. Investigating micronutrients and epigenetic mechanisms in relation to inflammatory bowel disease. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 2010; 690:71-80. | 16. Quigley E M. Epigenetics: filling in the 'heritability gap' and identifying | gene-environment interactions in ulcerative colitis. Genome medicine 2012; 4:1-3. |