Interleukin-1A and interleukin-1B gene polymorphisms in gastroesophageal reflux disease

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Abstract. Inflammation may play contradictory roles in the pathogenesis of gastroesophageal reflux disease (GERD): gastritis decreases gastric output and reduces the risk of esophagitis, while interleukins may favor mucosal inflammation. The inflammation may cause esogastric motility changes and thus increase the risk of esophagitis. Considering the genetic influence of inflammatory response, we looked for the genetic polymorphisms of IL-1 in GERD manifested as reflux esophagitis. This is a prospective study carried out in GERD and healthy controls. We assessed in these groups the following single nucleotide polymorphisms (SNPs): IL-1A (rs1800587), IL-1B (rs16944), IL-1B (rs1143634) and the VNTR for IL-1RN. Both groups were similar according to biographical data. Reflux esophagitis was confirmed by endoscopy and where necessary by pH-impedance monitoring. Reflux esophagitis was associated only with the polymorphism rs16944. No other correlations with the other three genetic polymorphisms were detected. These data suggest that the diverging effects of proinflammatory factors on the upper digestive tract may have deleterious effect on GERD. The IL-1B (rs16944) SNP correlates with reflux esophagitis.

Introduction

Gastroesophageal reflux disease (GERD) is a common pathological condition, including extraesophageal clinical manifestations (1). In the pathogenesis of GERD the main role is played by the gastric acid (2). Among the factors contributing to gastric acid secretion, the inflammation of gastric mucosa is very important (3). It is expected that inflammation of gastric mucosa is associated with hypochlorhydria and thus has a protective effect on esophageal mucosa, preventing reflux esophagitis. Genetic factors are involved, beside lifestyle factors, in the occurrence of GERD. Proof of the genetic role in esophagitis have been gathered from first twin studies (4) and from more recent investigations on genetic polymorphisms (5). Few studies are dedicated to inflammatory biomarkers in GERD (6,7).

Some polymorphisms protect against GERD, i.e. reflux esophagitis. Interleukin-1α (IL-1α) is a cytokine encoded by the IL-1A gene; IL-1B is another cytokine from the same IL-1 family and is encoded by the IL-1B gene (8). Both IL-1A and IL-1B are proinflammatory cytokines. The IL-1 receptor antagonist IL-1RA has anti-inflammatory effects and is encoded by the gene IL1RN (9,10).

The aim of our study was to look for gene polymorphisms of IL-1A and IL-1B in GERD, in order to establish whether they have a protective or harmful effect on the pathogenesis of reflux esophagitis.

Patients and methods

Protocol. This is a single center prospective controlled study looking to the gene polymorphisms of ILA and ILB in GERD versus healthy controls (all Caucasians living in Romania).

Subjects. The GERD subjects were recruited from a specialized tertiary center where they were referred for heartburn and for investigations including upper digestive endoscopy and pH-monitoring. Inclusiong criteria: age 18-70 years, symptoms of GERD and at least Los Angeles class B esophagitis at endoscopy. Los Angeles class A esophagitis was discarded since this class is not specific for symptomatic esophagitis. If symptoms were persistent, pH-monitoring with impedance was also carried out as usual workup. This investigation was peformed after the discontinuation of proton pump inhibitors for at least two weeks. Exclusion criteria: Refusal to

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participate, coexistence of inflammatory gastrointestinal or systemic diseases, or immune deficits. *Helicobacter pylori* status was not investigated.

The control group consisted of age and sex matched apparently healthy subjects. None had heartburn or other symptoms suggestive of GERD and no comorbidities or medications able to bias the study.

All participants were recruited in consecutive order. They filled a questionnaire on esophageal symptoms, medication, and lifestyle.

Polymorphism assessment. Samples of 2 ml of blood in EDTA were collected from each subject. The samples were stored in a freezer before being processed.

The DNA was extracted from leukocytes from peripheral venous blood. The DNA concentration and purity were subsequently assessed by measuring its absorbance at 230, 260 and 280 nm, respectively, using a nano-spectrophotometer. A method of separating and UV visualizing PCR products or digestion fragments (obtained after the use of restriction enzymes) was used, allowing the control of PCR amplification and/or restriction analysis by horizontal electrophoresis in agarose gel. All the electrophoretic pattern images were recorded digitally using a gel documentation system and two researchers independently performed the interpretation of PCR-RFLP (restriction fragment length polymorphism) markers; no differences were identified.

Genotyping features of IL-1A and IL-1B variants (four polymorphisms). i) The single nucleotide polymorphisms (SNP) in IL-1A-889C/T (rs1800587) was identified by the PCR-RFLP technique (11,12). ii) For the identification of SNP in the IL-1B gene at -511C/T (rs16944), we optimized the original method of Di Giovine *et al* (13). The gene variants were: CC genotype (115+190 bp), CT genotype (115+190+305 bp) and TT genotype (305 bp, fragments not-digested). iii) For the detection of SNPs in the IL-1B gene at + 3953C/T (rs1143634), the original method according to Kornman *et al* was optimized (14). iv) The study of polymorphism type variable number of tandem repeats (VNTR) in IL-1RN gene was based on the PCR method for the detection of a 86 bp sequence in the second intron of the gene, according to previous literature.

Statistical analysis. The data obtained were analyzed using the SPSS statistical software. Descriptive statistics were performed. For comparison of continuous variables the Student's two-tailed test and for nonparametric variables the Chi2 test were used. Correlation was assessed by Pearson's test and by multiple regression. Significance level was set at 0.05. The distribution of alleles identified for a particular locus was calculated for the four SNPs analyzed in our study, using the Hardy-Weinberg Equilibrium.

Ethical issues. The study was approved by the Institutional Review Board of the 'Iuliu Haţieganu' University of Medicine and Pharmacy (Cluj-Napoca, Romania) and was conducted according to the Declaration of Helsinki on Human and Animal Studies. Written informed consent was obtained from each subject.

Results

The GERD group included 27 subjects (24-67 years, mean ± SD: 49±12 years; 18 males/9 females). The control group included 26 age and sex matched controls (22-65 years, mean ± SD: 46±12 years; 17 males/8 females). The BMI of the subjects were comparable: 31±8 vs. 33±10, 14/27 had hiatal hernia, but all were small.

The genotypes of the four polymorphisms are shown in Table I.

There was no difference between GERD versus controls and no correlation with genotype IL-1A SNP rs1800587. However, we detected a significant correlation with the genotype IL-1B SNP rs16944 (Pearson -.329). There was no correlation with the other polymorphisms investigated: IL-1A (rs1800587), IL-1B (rs1143634) and IL-1RN (χ^2>0.05).

The multiple regression confirmed that GERD depends on polymorphism rs16944 in IL-1B gene.

Thus, the single polymorphism of IL-1 associated with GERD in the present study was rs16944 in IL-1B gene.

Discussion

This original study looked for the correlations between IL-1A and IL-1B genotypes (rs16944 and rs1143634) and IL-1RN, respectively, in patients with GERD versus controls.

The hypothesis of this study was the following: GERD and mainly esophageal mucosa erosions are due to too much refluxed gastric acid. Beside anatomical and lifestyle factors, gastric acid output is considered to play a pathogenic role. Inflammation of gastric mucosa leading to chronic gastritis reduces gastric acid secretion and thus is suspected to reduce the risk of esophagitis (apart of the risk of gastric cancer). On the other hand, the epigastric pain caused by gastric acid in acid related dyspepsia is frequently associated with heartburn. Therefore, it is important to establish whether inflammatory interleukins may cause or protect against GERD. Data are controversial (15).

We studied four SNP in the genes of IL-1A-889C/T (rs1800587), IL-1B -511C/T (rs16944) 3953C/T (rs1143634) and IL-1RN, respectively (the more frequently identified variants are marked 1/1, 1/2 or 2/2). The association of GERD with the polymorphisms evidenced was statistically analyzed.

A correlation between GERD and IL-1B (rs16944) was detected, not with the three other polymorphisms.

Our data show that the proinflammatory effect of IL-1B (rs16944) is not correlated with severity of GERD symptoms, but rather seem to influence the esophageal mucosa and favors esophagitis symptoms (heartburn, pain). The data contradict previous studies (16,17). It is assumed that differences can be explained by sample size and other genetic differences. Cooperative studies are necessary to shed light on this.

It also indicates that not only genetic, but also extragenetic factors play a role in GERD onset and evolution: i.e., lifestyle and anatomical particularities (18). The complex mechanism of the symptom production in reflux esophagitis is thus involved also in the mucosal inflammation of the esophagus.

It should be considered that the proinflammatory factors intervene in GERD pathogenesis not only by reducing gastric

Table I. Genotypes of the four polymorphisms investigated.

Crt. no.	Subject	Genotype IL-1A (rs1800587)	Genotype IL-1B (rs16944)	Genotype IL-1B (rs1143634)	Genotype IL-1RN	GERD/Control
1	B.V.	C/T	T/T	C/T	2/2	GERD
2	R.A.	C/T	C/T	C/C	1/1	GERD
3	C.D.	T/T	C/T	C/T	1/1	GERD
4	P.M.N	C/T	C/C	C/T	1/1	GERD
5	B.G	C/T	C/T	C/C	1/2	GERD
6	S.L.	C/T	C/T	C/C	1/1	GERD
7	D.N.	C/T	C/T	C/C	1/1	GERD
8	T.D	C/T	C/C	C/C	1/1	GERD
9	F.R.	C/T	C/T	C/C	1/2	GERD
10	P.I.	C/T	T/T	C/C	2/2	GERD
11	M.N	C/T	C/T	C/C	1/1	GERD
12	K.E.	C/T	T/T	C/C	2/2	GERD
13	B.O.	C/T	C/T	C/T	1/2	GERD
14	B.V	C/T	C/C	C/C	1/1	Control
15	C.M	C/T	C/C	C/C	1/1	GERD
16	B.S.	C/T	C/T	C/C	1/2	Control
17	R.I.	C/T	C/T	C/C	1/2	GERD
18	T.S.	C/T	C/T	C/C	1/2	Control
19	M.L	C/T	C/T	C/C	1/2	Control
20	R.M.	C/T	C/C	C/C	1/2	Control
21	E.V.	C/T	C/T	C/T	1/2	Control
22	C.C.	C/T	C/T	C/C	1/2	GERD
23	O.V.	C/T	C/C	C/T	1/1	Control
24	T.H.	C/T	C/C	C/C	1/1	Control
25	P.A.	C/T	C/T	C/C	2/2	Control
26	S.V.	C/T	C/T	C/C	1/1	Control
27	C.D.	C/T	C/C	C/T	1/1	GERD
28	V.L.	C/T	C/T	C/C	1/1	GERD
29	D.L.	C/T	C/T	C/T	1/1	GERD
30	A.M.	C/T	C/T	C/T	1/2	Control
31	P.V.	C/T	C/T	C/C	1/2	Control
32	S.G.	T/T	C/C	C/T	1/2	Control
33	P.M.	C/C	C/C	C/C	1/1	Control
34	P.O.	C/C	C/T	C/C	2/2	Control
35	M.O.	T/T	C/C	C/T	1/1	Control
36	I.M.	C/C	C/T	C/C	1/2	Control
37	B.C.	T/T	C/C	C/T	1/2	Control
38	H.M.	C/C	C/T	C/C	1/2	Control
39	G.V.	C/C	C/C	C/C	1/1	Control
40	N.R.	C/T	C/C	C/C	1/2	Control
41	P.E.A.	C/C	C/C	C/C	1/1	Control
42	B.I.	C/C	C/T	C/C	1/1	Control
43	P.D.M.	C/C	T/T	C/C	1/1	Control
44	C.G.	C/T	T/T	C/T	2/2	Control
45	P.D.	C/C	C/T	C/C	1/2	GERD
46	B.I.	C/T	C/T	C/C	1/1	GERD
47	R.I.	C/T	C/T	C/T	1/1	GERD
48	O.I.	C/T	C/T	C/C	1/2	GERD
49	F.B.	C/C	C/T	C/C	1/2	GERD
50	V.I.	C/C	T/T	C/C	1/2	GERD
51	M.M.	C/T	C/C	C/C	1/3	GERD
52	M.B.	C/T	T/T	C/T	1/2	GERD
53	I.R.	T/T	C/C	C/T	1/1	Control

secretion, but also by influencing the esogastric motility, another pathogenic factor in GERD (19,20).

The main shortcoming of this study is the small size of patient samples. However, we consider it enough to allow pertinent conclusions.

Therefore, we consider that in GERD patients with recurrent and/or severe symptoms, the evaluation of the proinflammatory IL-1B SNPs is useful in order to identify those patients who run the risk to develop Barrett esophagus and esophageal cancer. Investigation of the correlation of heartburn and esophageal chest pain with other polymorphisms is recommended.

In conclusion, this study detected a significant correlation between GERD and IL-1B (rs16944). This finding represents a proof that nongenetic factors such as lifestyle or anatomical particularities are important in the pathogenesis of GERD. The reduction of gastric acid output by interleukin triggered gastritis did not lead to a decrease in GERD symptoms in this study.

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Availability of data and materials

All data analyzed are included in the present manuscript and the data and materials are available at 'Iuliu Haţieganu' University of Medicine and Pharmacy.

Authors' contributions

AndreiP contributed to the design of the study and the acquisition of the data, and significantly contributed to the writing of the manuscript. AlinaP designed the study, contributed to the data analyses, the literature research and the writing of the manuscript. RV and AC undertook the genetic analysis and suggested references, and also contributed to the drafting of the manuscript. AndraP contributed to the acquisition of the data and the creation of the database. IC performed the statistical analysis and offered advice. NP contributed to the acquisition of the data and the creation of the database. DLD conceived the study, offered advice and suggestions, contributed to the acquisition of the data and revised critically the manuscript. All authors had intellectual contribution to be granted as authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of the 'Iuliu Haţieganu' University of Medicine and Pharmacy (Cluj-Napoca, Romania) and was conducted according to the Declaration of Helsinki on Human and Animal Studies. Written informed consent was obtained from each subject.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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