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The involvement of IgH enhancer HS1.2 in the pathogenesis of Crohn's disease: how the immune system can influence a multifactorial disease

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Abstract. – OBJECTIVE: To study the 3′ immunoglobulin heavy-chain regulatory region (3′RR) enhancer complex, active in class switching recombination and in B-cells, in Crohn's disease.

PATIENTS AND METHODS: A total of 167 patients [79 females (47.3%) and 88 males (52.7%)] affected by Crohn's disease were enrolled in the study. As a control, we included 64 healthy subjects, age and sex matched, from the same geographical area. Blood tests were performed on all subjects to determine their antibody levels and to detect the presence of any possible infections. We conducted a selective PCR, which amplified the hs1.2-A region. The nested second PCR to amplify the polymorphic core of the enhancer was performed.

RESULTS: No differences between cases and controls were observed with respect to sex distribution (43.8% females among controls and 49.5% among cases), age, tTG IgA, RF, serum or secretory IgA, IgG1, IgG2 and IgG3. No correlation was found between both seric and secretory immunoglobulins levels, with except of statistically significant differences between cases and controls with respect to IgA and IgG ASCA positivity ($p<0.001$), serum IgG4 ($p<0.001$) and IgD ($p=0.001$).

CONCLUSIONS: We have demonstrated that in Crohn's disease, the HS1,2 immunoglobulins enhancer is not implicated in the disease pathogenesis. Moreover, we have found that IgG4 levels are lower in Crohn's disease patients than in controls; these data may be related to an impairment of number and function of Tregs, further linked to the presence of tissue inflammation. Crohn's disease is a complex multifactorial disease. The pathogenesis of Crohn's disease is incompletely understood although it is clear that the disease involves multiple interacting agents.

Key Words
Crohn's disease, IgH Enhancer hs1.2, Immunoglobulins, Anti-Saccharomyces cerevisiae antibodies (ASCA), Dysbiosis.

Abbreviations

Anti-Saccharomyces Cerevisiae Antibodies (ASCA); inflammatory bowel disease (IBD); immunoglobulins (Igs); heavy (H); light (L); variable (V); constant (C); 3′ Immunoglobulins' heavy-chain regulatory region (3′RR); DNase-hypersensitive region 3 (HS3); DNase-hypersensitive A region 1,2 (hs1.2-A); Toll like receptors (TLRs); Nod like receptors (NLRs); Single Nucleotides Polymorphisms (SNPs); tissue transglutaminase (anti-tTG); Rheumatoid Factor (RF); T regulatory cells (Tregs).

Introduction

Crohn's disease is a complex multifactorial disease. Although the precise etiology of this inflammatory bowel disease (IBD) remains obscure, several reports have indicated that an altered function of the mucosal immune system plays an important role in its pathogenesis[1]. The gastrointestinal tract is continuously exposed to a variety of antigens, including ones from enteric bacteria and foods. Homeostasis of the gut is maintained in the normal state by suppressing excessive immune responses to non-self-antigens. Disfunction of regulatory mechanisms may lead to abnormal immune responses to enteric antigens and cause chronic intestinal inflammation, leading to mucosal damage[2]. The pathogenesis of Crohn's disease remains

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incompletely understood; multiple, complex interacting agents are involved. There is a genetic component, but this is of minor importance. The complex interaction among environmental factors, gut microbiota and mucosal immunity appear to be much more important in determining the severity of the disease. Thus, for a better understanding of the pathogenesis of Crohn's disease, we have to consider and study these factors.

It is well established that host genetic susceptibility plays a key role in the risk of development of IBD. The risk in homozygous twins is higher than in the general population[3]. Many IBD susceptibility genes have been identified, and some of these are associated with host immune function, including epithelial barrier functions, host defense mechanisms in response to pathogens and host immune response[4].

The rapid increase in the incidence of IBD in the last decade cannot be fully explained by genetic drift. IBD may be caused by exposure to other factors, such as diet and life style, in genetically predisposed individuals, leading to the abnormal host immune response.

The production of circulatory and secretory immunoglobulins has a pivotal role in the mucosal protection against intestinal pathogens. Soluble immunoglobulins (Igs) represent the 20% of total serum proteins and are produced by plasma cells by a tightly regulated process. The Igs are composed of two heavy (H) and two light (L) chains. Both the light chains and the heavy chains have a variable (V) and a constant (C) region that are held together by disulfide bonds. The variable regions of both the heavy and light chains form the antigen binding site determining the antibody's specificity. There are five different isotypes for the constant region (Alpha, Delta, Epsilon, Gamma and Mu); each isotype is encoded from a different DNA segment and defines the specific class of the antibodies (IgA; IgD; IgE; IgG and IgM).

The 3′ Immunoglobulin heavy-chain regulatory region (3′RR) enhancer complex[5-7] is active in murine class switching recombination and B-cell plasma cells[8,9]. The 3′RR enhancers function as a regulatory complex and play a role in germline transcription[10-12].

The 3'RR is required for class switching recombination during which it creates an architectural scaffolding through physical interactions with the H chain variable region[13,14]. In addition to its activity in Ig production, the enhancer complex is thought to be responsible for a wider range of regulatory actions under B cell control[15,16]. The complex interactions during B cell development also involve the function of these regulatory regions activated in a time-dependent manner[17].

In humans, the 3′RR is duplicated; a copy lies downstream of the constant Alpha-1 and the constant Alpha-2 genes[6,7,18]. Because 3′RR in humans is duplicated, it can be hypothesized that there is a coordinated activity for the two 3′RRs during Ig maturation. In addition, they are considered relevant for germline transcription of constant regions during B cell maturation[19]. When class switching involves the duplicated constant regions (Gamma-2, Gamma-4, Epsilon, Alpha-2) the 3′RR-1 gets deleted and the entire region could be regulated in the next steps of maturation or memory cell and plasma cell by the presence of the 3′RR-2 alone[20].

Human 3′RRs contain three enhancers: DNase-hypersensitive region 3 (HS3), hs1.2, and HS4. The central hs1.2 enhancers are polymorphic[7,21,22], consisting of one to four copies of a 40 bp satellite separated by GC rich spacers of 17, 20, 16, 1 or 4 bp. Giambra et al[21] observed a change in the consensus for several transcription factors in the four alleles. The specific binding of Sp-1 for hs1.2 allele *1 and *2 and the exclusive binding of NF-κB for allele *2 but not allele *1 (Figure 1).

A significant correlation of the *2 allele of DNase-hypersensitive A region 1,2 (hs1.2-A), downstream of Alpha-1 gene, was already shown in patients with autoimmune disorders: celiac disease, systemic sclerosis, rheumatoid arthritis, psoriatic arthritis, plaque psoriasis and systemic lupus erythematosus[23-27].

Abnormal immune responses to commensal bacteria and/or food antigens may be a central part of IBD pathogenesis. Immunoglobulins have a pivotal role in response to antigens.

In the gut lumen, it is possible to have interaction among food antigens, microbiota and components of the immune system. In particular, there are some receptors that may mediate these interactions between antigens, bacteria and immune cells (Figure 2). Some of these are the Toll-like receptors (TLRs) and Nod-like receptors (NLRs). The NLR family members comprise NOD1 and NOD2. These receptors are stimulated by different components of bacterial peptidoglycan and result in NF-κB activation (Figure 3). Other NLRs contribute to the formation of the inflammasome that leads to caspase activation and IL-1 production and secretion by immune cells[7,21]. NOD2 was the first gene to be identified to confer increased risk to Crohn's disease[28].

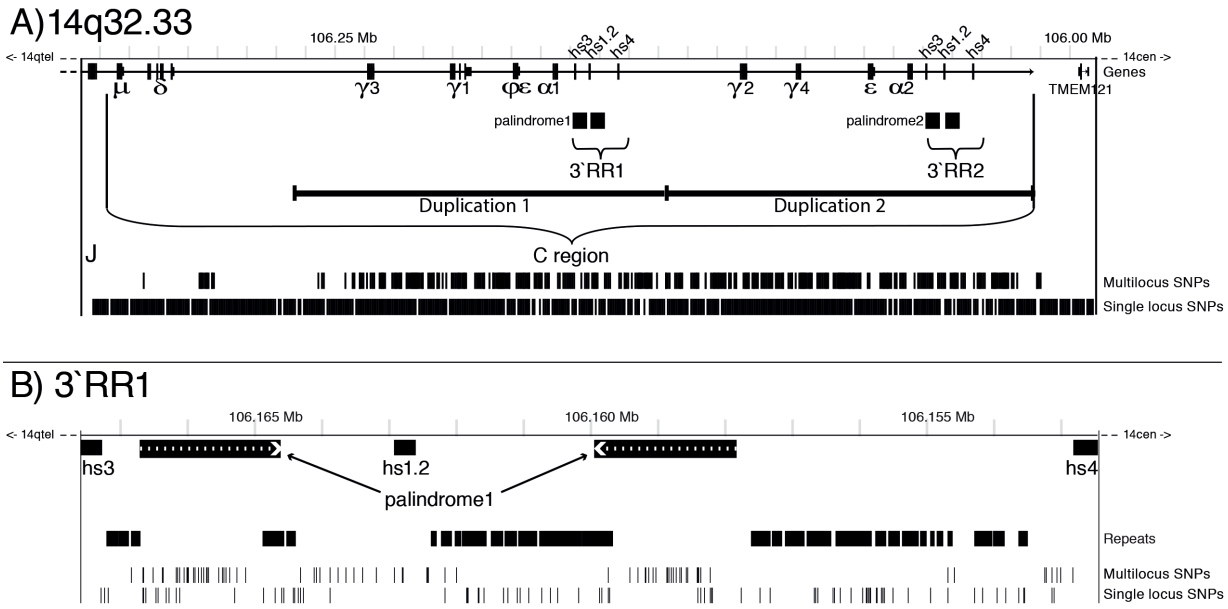

Figure 1. The 3' Immunoglobulins' heavy-chain (IgH) regulatory region (3'RR) enhancer complex. **A**, Map of the human genomic region 14q32 containing the constant genes of the Ig heavy chain generated by the duplication including the two 3' Regulatory Regions with the three enhancers determined by DNase-I hypersensitive test. The lower part shows the multilocus SNPs and the single locus SNPs. **B**, Enlargement of the 3'RR-1 with the three enhancers and the internal palindromic region conserved for the structure and not for the nucleotides. The lower part shows the sequences with repeated motif, single copy and the multilocus SNPs.

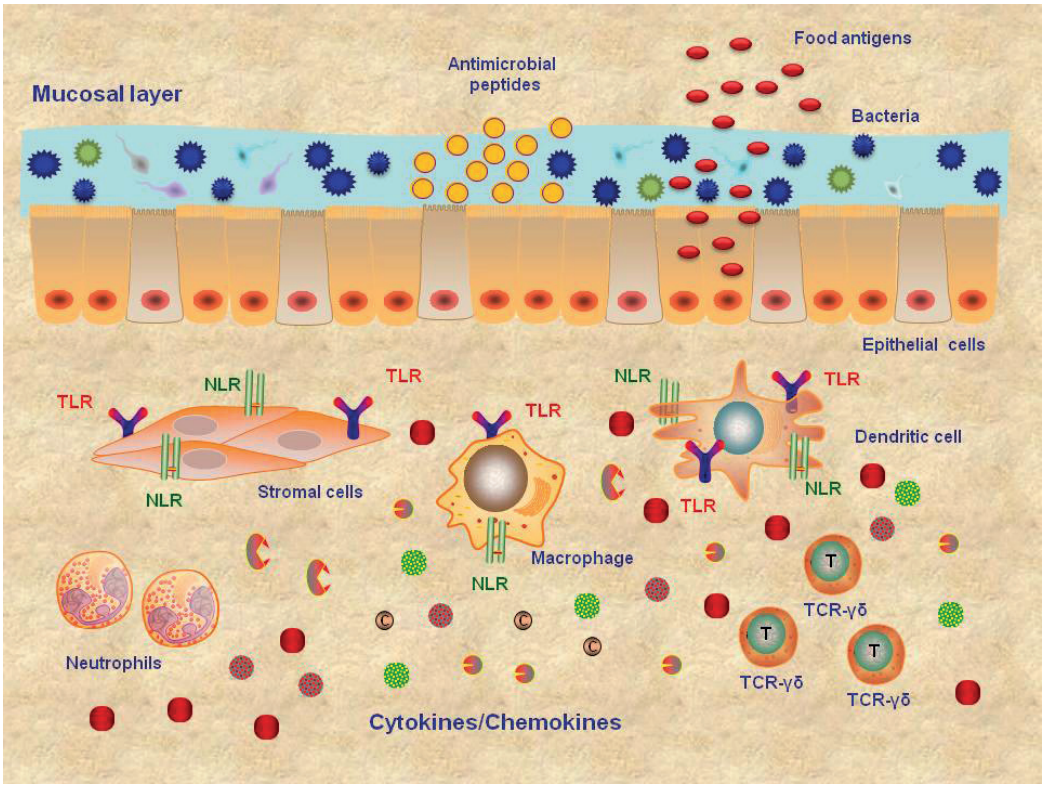

Figure 2. The gut barrier. The gut barrier is primarily composed of the mucosal layer that represents the mechanical defense from pathogens. In the gut lumen, it is possible to have interaction among food antigens, microbiota and components of the immune system, both innate and adaptive immune cells. In particular, there are some receptors that may mediate these interactions between antigens, bacteria and immune cells.

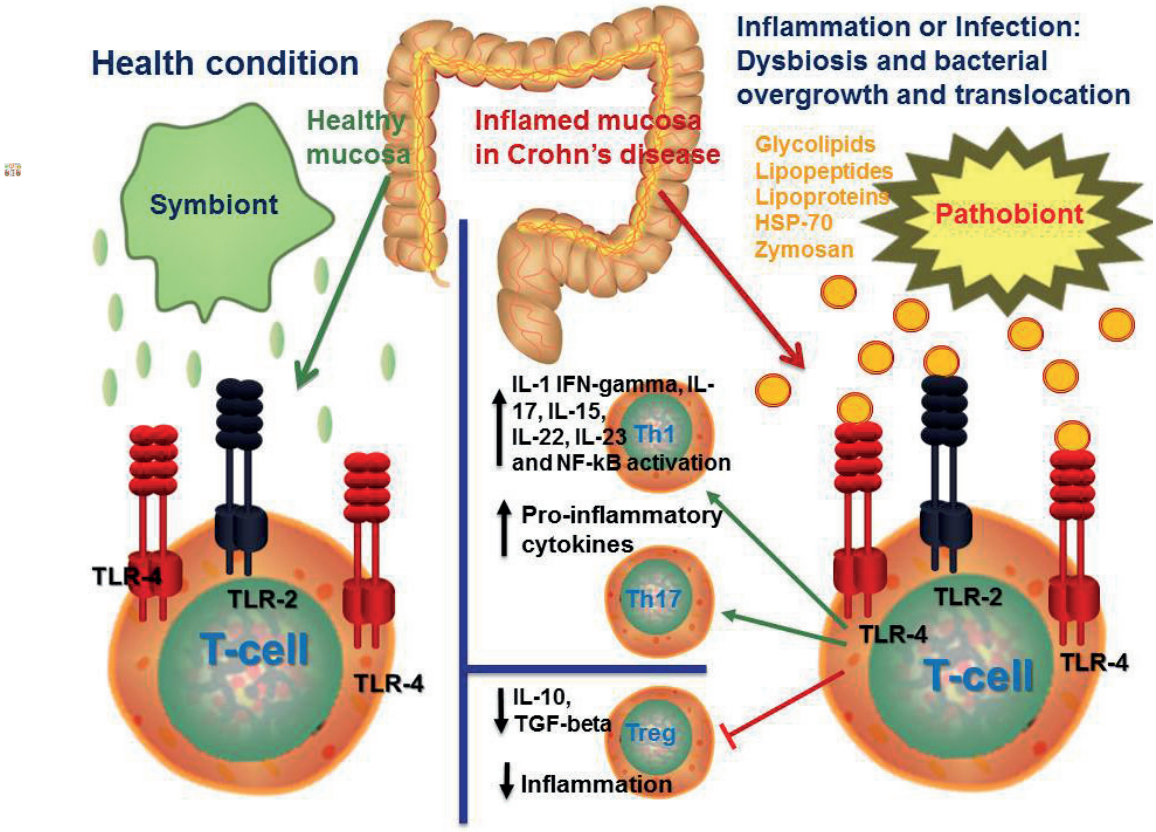

Figure 3. The role of TLRs in Crohn's disease and human pathology. In healthy human gut mucosa, there is an equilibrium between the host immune system and the gut microbiota, and consequently, the homeostasis is maintained. In inflamed gut mucosa of Crohn's patients, this perfect homeostasis is broken due to bacterial overgrowth and dysbiosis, and TLRs are activated, further leading to the activation of proinflammatory T-cells subpopulations (Th1 and Th17 cells) and the inhibition of the anti-inflammatory T-regulatory cells (Tregs). Thus, TLRs activation contributes to the maintenance of acute and chronic gut inflammation of Crohn's disease.

Three uncommon Single Nucleotides Polymorphisms (SNPs) in NOD2 have been associated with susceptibility to ileal Crohn's disease with an odds ratio of 2.4 in heterozygote individuals and 17.1 in homozygotes or compound heterozygotes, representing the strongest association with IBD to date[28].

The aim of our study was to investigate whether the IgH hs1.2 alleles are involved in the pathogenesis of Crohn's disease.

Patients and Methods

Patients with Crohn's Disease and Control of Local Population

A total of 167 patients [79 females (47.3%) and 88 males (52.7%)] affected by Crohn's disease were enrolled in the study. As a control, we included 64 healthy subjects, age and sex matched, from the same geographical area. The mean age of patients was 37 years (SD 12.7 years); the mean age of controls was 38 years (SD 10.0 years). None of the patients nor the controls had defects of the T cell compartment as evaluated by a T cell subsets cytofluorimetric count. Diagnosis of Crohn's disease was based on standard clinical, endoscopic, histological and laboratory *criteria*. For all patients, we have recorded steroid use, immunosuppressive and anti-TNF therapy. Furthermore, we have reported the clinical activity and the pattern of disease (inflammatory, stenosing, and fistulizing). To exclude association with Celiac disease, all patients and controls were assessed for antibodies to tissue transglutaminase (anti-tTG) by immunoenzymatic assay (Sigma-Aldrich, Saint Louis, MO, USA); moreover, to exclude association with other autoimmune diseases all patients and controls were assessed for Rheumatoid Factor (RF) by the immunoturbidimetric assay (Human Diagnostic, Wiesbaden, Germany) and Anti-Saccharomyces

Cerevisiae Antibodies (ASCA) (Diamedix Corporation, Miami, FL, USA). In patients and controls in which we found abnormal IgA and IgG counts, we performed serological tests, according to standard procedures, to detect *Adenovirus*, *Yersinia enterocolitica* and *Influenza* virus type A and B. These tests were performed at Central Laboratory, I.R.C.C.S. G. Gaslini, Genoa, Italy. The presence or absence of NOD in all patients was determined.

Immunoglobulins Determination

Seric IgA, IgM, IgE, IgD, IgG and its subgroups (IgG1, IgG2, IgG3, IgG4), were evaluated by radial immunodiffusion (Dade Behring, Marburg, Germany) at Central Laboratory, I.R.C.C.S. G. Gaslini, Genoa, Italy.

Secretory IgA levels were evaluated by radial immunodiffusion (plates R.I.D. and MONORID, Astra s.r.l., Milan, Italy) at Central Laboratory, I.R.C.C.S. G. Gaslini, Genoa, Italy.

PCR Assay

To estimate the frequencies of the four alleles of hs1.2-A respectively (Gene Bank acc. num. AJ544218, AJ544219, AJ544220, AJ544221), we conducted a selective PCR, which amplified the hs1.2-A region, but not the identical inverted hs1.2-B region[21]. Genomic DNA was extracted from peripheral blood nucleated cells (PBMCs) or buccal mucosal swabs and amplified with the primers described previously[21]. The cycle conditions were 94°C 2′ for a first step, followed by 94°C 30″, 61°C 30″, 68°C 5′ for 10 cycles and 94°C 30″, 59°C 30″, 68°C 5′ for 20 cycles, ending with 72°C 10′. PCRs were conducted in 50 microliters of reaction volume containing: 2 microliters of extracted DNA (10 ng), 1.5 U Platinum TaqDNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA, USA), 15 pmols of each primer, 1.5 mM $MgSO_4$, 50 microM each dNTP, and 1× buffer High Fidelity (600 mM Tris-SO_4 (pH 8.9), 180 mM $((NH_4)_2SO_4)$ (Invitrogen, Waltham, MA, USA), by using GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). To prevent carryover contamination, pre-PCR procedures were performed with dedicated equipment in a laminar flow hood, using aerosol-resistant plugged pipette tips (ART, Molecular Bio-Product, Toronto, Canada). Permanent devices were sterilized by UV irradiation between uses. Negative and positive controls, without DNA template or with a control DNA of a heterozygote, were always included. The nested second PCR to amplify the polymorphic core of the enhancer hs1.2-A was performed with 1/50 of the volume of the first PCR, avoiding the carryover of the genomic DNA of the first reaction. Control reactions were performed with 1 and 5 ng of total genomic DNA and resulted in no visible amplification in those conditions on gel agarose electrophoresis. The primers for the PCRs are reported in Giambra et al[21].

Evolution of human IgH3′EC duplicated structures: both enhancers hs1.2 are polymorphic with a variation of transcription factor's consensus sites. The second PCR was conducted with the same volumes and concentrations used in the first PCR, except for the use of 1 U of Platinum TaqDNA polymerase (Invitrogen, Carlsbad, CA, USA). PCR products were analyzed on a 3.0% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen).

Statistical Analysis

The statistical review of the study was performed by a biomedical statistician. The Hardy-Weinberg *equilibrium* was used in order to calculate genomes expected frequencies and allele frequencies.

The parameters investigated were analyzed with descriptive statistics (mean, standard deviation [SD], median (minimum-maximum) and interquartile range [IQR], proportion, and 95% confidence interval [CI]), as appropriate. Comparison of continuous variables was performed with 1- or 2 analysis of variance, as appropriate. The differences between proportions were compared using the chi-square, Fisher exact test or Odds Ratio, as appropriate. All statistical analyses were performed using the STATA software, version 11.1 (StataCorp Lp, College Station, TX, USA). A p-value <0.05 was considered statistically significant.

Results

Tables I and II show genotypes and alleles frequencies of the enhancer hs1.2-A in Crohn's patients (cases) and healthy subjects (controls). Hardy-Weinberg *equilibrium* was proven for both cases and controls. Results do not show any significant differences with respect of genome and allele frequencies.

No differences between cases and controls were observed with respect to sex distribution (43.8% females among controls and 49.5% among cases), age, tTG IgA, RF, serum or secretory IgA, IgG1, IgG2 and IgG3 (Table III).

Table I. Frequency of hs1.2-A genotypes in patients with Crohn's disease and healthy controls.

	Controls (48)			Cases (85)			*p*
	Observed	%	Expected	Observed	%	Expected	
1/1	4	8.3%	3.5	10	11.8%	7.1	n.s.
1/2	14	29.2%	14.6	23	27.1%	26.2	n.s.
1/3	-	-	-	-	-	0.3	
1/4	4	8.3%	4.3	6	7.1%	8.4	n.s.
2/2	14	29.2%	15.2	22	25.9%	24.4	n.s.
2/3	-	-	-	1	1.2%	0.5	n.s.
2/4	12	25.0%	9	23	27.1%	15.5	n.s.
3/3	-	-	-	-	-	0.0	
3/4	-	-	0	-	-	0.2	
4/4	-	-	1.3	-	-	2.5	

No correlation was found between both seric and secretory immunoglobulins levels, with except of ASCA IgA and IgG, IgG4 and IgD. Statistically significant differences were found between cases and controls with respect to IgA and IgG ASCA positivity ($p<0.001$), serum IgG4 ($p<0.001$) and IgD ($p=0.001$) (Table III).

No differences were found in the association between different genotypes and immunoglobulin levels, in patients and controls, with the exception for IgG4 in patients affect by Crohn's disease (Table IV). No association exists in Crohn's patients among the presence or absence of NOD and genotypes and alleles frequencies of the enhancer hs1.2-A, clinical activity, sex, steroid and immunosuppressive therapy.

Discussion

In the previous reports[24,26,27,29], it has been shown that the hs1.2-A enhancer *2 allele's frequency is increased in several autoimmune diseases, such as Celiac disease, Systemic Sclerosis, Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis, Dermatitis Herpetiformis, Plaque and Arthritic Psoriasis. Susceptibility to immune diseases is considered a multigenic phenotype affected by a variety of genetic and environmental or stochastic factors[29].

Similarly, Crohn's disease is thought to be a multifactorial condition. This means that it is likely associated with the effects of multiple genes, in combination with lifestyle and environmental factors. In fact, several *loci* have been implicated in Crohn's pathogenesis[30]. Many Crohn's disease *loci* are also implicated in other immune-mediated disorders[31].

Table II. Relative and absolute frequency of hs1.2-A alleles in patients with Crohn's disease and healthy controls.

	Controls (48)	Cases (85)	*p*
Allele 1	0.271 (26)	0.288 (49)	n.s.*
Allele 2	0.563 (54)	0.535 (91)	
Allele 3	0.000 (0)	0.006 (1)	
Allele 4	0.167 (16)	0.171 (29)	
Hardy-Weinberg (χ^2)	<0.001	9.453	
p	n.s	n.s	

*χ2: 0.719; 3 degree of freedom

Table III. Cases and controls parameters [median (minimum-maximum)].

	Controls	Cases	*p*
Age (166*)	37 (20-73)	34 (15-69)	n.s.
tTG IgA (104°)	1 (1-12)	1 (1-163)	n.s.
RF (104°)	6.5 (4.7-10.9)	6.9 (1.6-33.8)	n.s.
ASCA IgA (167)	5 (0.9-19.8)	10.3 (1.1-154)	<0.001
ASCA IgG (167)	1.3 (0-78.4)	11.9 (0.1-128)	<0.001
Serum IgA (166*)	274 (22-1030)	288 (77-1590)	n.s.
Secretory IgA (99^)	43.8 (10.7-92)	41.6 (2-141.8)	n.s.
IgD (104°)	0 (0-211)	26 (0-123)	0.001
IgM (166*)	140 (15-975)	154 (25-609)	n.s.
IgG (165**)	1374 (15-975)	1290.5 (479-6350)	n.s.
IgG1 (104°)	584 (277-854)	545 (242-1310)	n.s.
IgG2 (104°)	327 (201-597)	348 (152-1110)	n.s.
IgG3 (104°)	35.1 (10.4-110)	38.1 (7.1-153)	n.s.
IgG4 (104°)	59.3 (8-168)	26.5 (1-315)	<0.001
IgE (104°)	116.55 (2.9-652)	128.24 (1-1659)	n.s.

Table IV. Association between different genotypes and Immunoglobulins levels, in patients and controls.

	Controls					
	1;1	**1;2**	**1;4**	**2;2**	**2;4**	***p***
IgD	0 (0-12) [3]	26.5 (0-211) [10]	0 (0-0) [4]	0 (0-70) [11]	0 (0-67) [7]	0.109
IgM	1102.5 (876-1700) [4]	1200 (853-5000) [13]	966 (829-1188) [4]	1254.5 (761-2970) [14]	1696.5 (921-4660) [12]	0.165
IgG	148 (92-332) [4]	95 (15-975) [13]	114 (54-198) [4]	131 (17-296) [14]	244.5 (20-690) [12]	0.578
IgG4	24.8 (23-108) [3]	35.9 (8-133) [10]	49.6 (23-123) [4]	61.6 (19-134) [11]	77.1 (39-113) [7]	0.685
	Cases					
	1;1	**1;2**	**1;4**	**2;2**	**2;4**	***p***
IgD	99 (0-99) [6]	36 (0-99) [11]	14 (0-123) [5]	11.5 (0-99) [14]	32 (0-99) [19]	0.251
IgM	1538.5 (791-6350) [10]	1644 (706-5180) [23]	1244 (668-4480) [6]	1387 (881-4170) [21]	1104 (479-4690) [23]	0.244
IgG	231 (57-554) [10]	155 (29-534) [23]	131 (53-609) [6]	161 (48-335) [22]	116 (25-390) [23]	0.130
IgG4	23.25 (5-100) [6]	20.8 (4-117) [11]	13.2 (1-27) [5]	44.75 (13-315) [14]	20.2 (5-123) [19]	0.025

On the other hand, the presence of large number of *loci* implicated in Crohn's pathogenesis is not sufficient to completely explain the risk of occurrence of disease, suggesting that other factors, such as environment, gut microbiota dysbiosis and infections may influence the development of the disease.

A significant correlation of hs1.2 allele 2 was shown to be associated with several autoimmune disorders such as Celiac Disease, Systemic Sclerosis, Rheumatoid Arthritis, Psoriatic Arthritis, Plaque Psoriasis and SLE. However, our study shows no correlation between Crohn's disease and hs1.2 allele 2. Thus, we suppose that the genetic burden of immunoglobulin alleles in Crohn's disease is less important than in the other autoimmune diseases mentioned above. Our study demonstrates that in Crohn's disease the hs1.2 immunoglobulins enhancer is not implicated in the disease pathogenesis. However, we have observed an increase in both IgA and IgG Anti-Saccharomyces Cerevisiae Antibodies (ASCA). These data lead to the hypothesis that genetic factors have a differential influence on disease pathogenesis. The most important role in the pathogenesis of Crohn's disease is played by the interaction between external environment, mediated by dietary food, and bacterial overgrowth or bacterial translocation subsequent to dysbiosis present in the gut of affected patients. This hypothesized mechanism explains the pathognomonic increase in ASCA that is an *epiphenomenon* in Crohn's disease; in fact, ASCA are well known serologic markers of Crohn's disease. It is easily hypothesized that in Crohn's disease patients the level of both ASCA IgA and IgG is higher than in controls because these antibodies normally correlate with the bacterial burden.

Furthermore, in our study, we found that IgD levels are higher in Crohn's disease patients than in controls. IgDs are implicated in the maturation of B cell precursors; IgDs are not present in soluble serum form, but function as surface cellular membrane antibodies which are eliminated from B-cell precursors' surface during their maturation process after antigen presentation. Normally, IgDs are not present in the serum of healthy subjects, as showed in our control group. Instead, the increase of IgD levels in Crohn's patients observed in our study may be explained by selective pressure caused by the bacterial burden and dysbiosis on the mucosal immune system.

As explained above, both ASCA and IgD levels are directly linked to the bacterial burden and dysbiosis common in Crohn's disease patients. Both of these antibodies are increased in Crohn's disease, and this fact may simply be an *epiphenomenon* of the disease, directly connected to the bacterial burden and resulting from tissue inflammation. Alternatively, the observed increase in these antibodies may have a pathogenic effect by influencing the mucosal immunopathogenetic processes.

In our study, we have found that IgG4 levels are lower in Crohn's disease patients than in controls. These data might be connected to a related observed increasing IgE trend in Crohn's patients *vs.* control subjects. In fact, it has been demonstrated that a link between IgG4 and IgE exists[32]. In particular, these antibody subtypes are linked to parasite and allergen stimulation and to an immune response connected to activation of mast cells, basophils, and eosinophils during allergic disorders. However, IgG4 and IgE may have opposite effects; in fact, *in vitro* data in which human lymphocytes were stimulated with a Th2 cytokine pattern in addition to the anti-inflammatory IL-10 resulted in a preferential production of IgG4 over IgE. IL-10 is an anti-inflammatory cytokine specifically produced by T regulatory cells (Tregs); so the presence of Tregs may increase levels of IgG4[32]. It is well known that in Crohn's disease there is a significant impairment in the number and function of Tregs[28]. Moreover, in the inflamed mucosa of Crohn's patients, there is a prevalent pro-inflammatory cytokine pattern with consequent lower levels of IL-10 and TGF-beta[28,33]. Thus, our data describing an impairment of IgG4 in Crohn's patients may be linked to the presence of an impairment of T regulatory function that is characteristic in affected Crohn's mucosa.

These data need to be extended with a focus on the mucosal micro-environment in order to study the cytokine pattern involved in tissue inflammation and damage. Considering the evidence of decreased IgG4 levels in Crohn's patients reported in our study, we have hypothesized an impairment in Treg number and function in Crohn's disease. This has been already confirmed in several experimental works performed both in peripheral blood and tissue level[34].

However, more specific studies are needed to define better the pathogenetic mechanisms of disease that occur at the tissue level. In our previous works[35-37], we defined and proposed the model of 'immunological niche' to explain the pathogenesis of immune-mediated tissue damage in other gastrointestinal diseases.

Conclusions

This study allows us to confirm that genetic burden is not predominant in the pathogenesis of Crohn's disease, thus, major attention may be direct to the interaction between the environmental factors, such as food antigens, and local mucosal immune system.

Institutional review board statement

All blood and salivary samples from patients were taken after informed consent and Ethical Permission was obtained for participation in the study.

Conflict of interest

All authors declare that they have no conflict of interest.

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