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Allele *1 of HS1.2 Enhancer Associates with Selective IgA Deficiency and IgM Concentration1

Vincenzo Giambra,2* Rossella Cianci,2† Serena Lolli,‡ Claudia Mattioli,‡ Giacomo Tampella,§ Marco Catalini,¶ Sebnem S. Kilic,¶ Franco Pandolfo,† Alessandro Plebani,§ and Domenico Frezza3‡

Selective IgA deficiency (IGAD) is the most common primary immunodeficiency, yet its pathogenesis is elusive. The Ig (heavy) H chain human 3′ Regulatory Region harbors three enhancers and has an important role in Ig synthesis. HS1.2 is the only polymorphic enhancer of the 3′RRs. We therefore evaluated HS1.2 allelic frequencies in 88 IGAD patients and 101 controls. Our data show that IGAD patients have a highly significant increase of homozygosity of the allele *1 (39% in the IGAD patients and 15% in controls), with an increase of 2.6-fold.Allele *4 has a similar trend of allele *2, both showing a significant decrease of frequency in IGAD. No relationship was observed between allele *1 frequencies and serum levels of IgG. However, allele *1 was associated in IGAD patients with relatively low IgM levels (within the 30th lowest percentile of patients). The HS1.2 polymorphism influences Ig seric production, but not IgG switch, in fact 30th lowest or highest percentile of IgG in patients did not associate to different frequencies of HS1.2 alleles. The control on normal healthy subjects did not correlate high or low levels of IgM or IgG with HS1.2 allelic frequencies variation. Overall our candidate gene approach confirms that the study of polymorphisms in human diseases is a valid tool to investigate the function of these Regulatory Regions that confers multiple immune features. The Journal of Immunology, 2009, 183: 8280 – 8285.

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4 Abbreviations used in this paper: IGAD, Ig A deficiency; BAFF, B cell activating factor; BTK, Bruton tyrosine kinase.

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1 The human Ig H chain 3′ Regulatory Region harbors three enhancers and has an important role in Ig synthesis. HS1.2 is the only polymorphic enhancer of the 3′RRs. We therefore evaluated HS1.2 allelic frequencies in 88 IGAD patients and 101 controls. Our data show that IGAD patients have a highly significant increase of homozygosity of the allele *1 (39% in the IGAD patients and 15% in controls), with an increase of 2.6-fold. Allele *4 has a similar trend of allele *2, both showing a significant decrease of frequency in IGAD. No relationship was observed between allele *1 frequencies and serum levels of IgG. However, allele *1 was associated in IGAD patients with relatively low IgM levels (within the 30th lowest percentile of patients). The HS1.2 polymorphism influences Ig seric production, but not IgG switch, in fact 30th lowest or highest percentile of IgG in patients did not associate to different frequencies of HS1.2 alleles. The control on normal healthy subjects did not correlate high or low levels of IgM or IgG with HS1.2 allelic frequencies variation. Overall our candidate gene approach confirms that the study of polymorphisms in human diseases is a valid tool to investigate the function of these Regulatory Regions that confers multiple immune features.


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Because 3′RR in humans is duplicated it can be hypothesized 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 (24). When the switch involves the duplicated constant regions (γ2, γ4, e, α2) the 3′RR-1 will be deleted and the entire region could be regulated in the next steps of maturation or in memory cell and plasma cell by the presence of 3′RR-2 alone (22). In humans, only HS1.2 was found polymorphic, while HS3 and HS4 was not (25). The polymorphism consists of a 38 bp satellite repeated from one to four copies separated by different spacers of 17, 20, 16, 1 or 4 bp rich in GC. Giambra et al. (26) observed the change of the consensus for several transcription factors in the four alleles. The specific binding of SP1 for HS1.2 allele *1 and *2 and the exclusive binding of NF-κB for allele *2 which is absent in allele *1 was confirmed by competition EMSA. A different mechanism of activation can be hypothesized for the two alleles (see Fig. 1B) (27). These structural differences can give rise to a change in the function of the enhancer and consequently on the entire regulatory region, and therefore can influence the maturation and regulation of B cells. In vitro studies with B cell transfected with the different human alleles of HS1.2 occurring from one to four repeats showed an increasing effect on the reporter gene (28). The biological relevance and specific functions in humans of the 3′RRs in vivo can be studied only with correlative studies of polymorphisms with immuno-alterations or pathologies. Several case control studies show correlation of 3′RR1 HS1.2 alleles with immune diseases. These evidences indicate a specific role of this enhancer in B cell. Allelic frequency of 3′RR1 HS1.2 is highly variable and it is linked to different immunological diseases. In contrast, the frequency of 3′RR2 HS1.2 alleles is conserved among different populations (29). Taken together these data support that the two 3′RRs harbor different functions in the development of immune responses (30, 31).
Materials and Methods

Subjects with IGAD and control of local population

Eighty-eight patients with a median age of 11.75 years (ranges: from 23.3 to 31.1 years), 49 males and 39 females with a diagnosis of selective IgA deficiency based on IgA serum levels below 5 mg/dl, without impairment of IgG and IgM, were included in the present study. None of them had defect of T cell compartment as evaluated by T cell subsets evaluation. A group of 101 subjects, age and sex matched from the same geographical region, was included as control. The Ig levels of a normal group was analyzed in 114 subjects of same geographical area and HS1.2 allelic frequencies determined.

Lymphocyte cells and immunoglobulin serum determination

IgA, IgM, and IgG serum levels were evaluated with standard techniques by the service of clinical analysis of the Pediatric Clinic of the University of Brescia. The IGAD subjects and Control were stratified for two groups of 30% each of the total with relatively low and high IgM or IgG levels.

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 (26). Genomic DNA was extracted from peripheral blood nucleated cells or from buccal mucosal swabs and was amplified with the primers described previously (26). The cycle conditions were 94°C 2 cycles and 94°C 30 s, 61°C 30 s, 68°C 5 s for a first step, followed by 94°C 30 s, 61°C 30 s, 68°C 5 s for 10 cycles and 94°C 30 s, 59°C 30 s, 68°C 5 s for 20 cycles, ending with 72°C 10 min. 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), 15 pmols of each primer, 1.5 mM MgSO4, 50 microM each dNTP, and 1X buffer High Fidelity (600 mM Tris-SO4 (pH 8.9), 180 mM (NH4)2SO4) (Invitrogen), by using GeneAmp PCR System 9700 (Applied Biosystems). 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). 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. (26). 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). PCR products were analyzed on a 3.0% agarose gel stained with SYBR Safe DNA gel stain (Invitrogen).

Statistical analysis

The frequencies of the variables considered are described in percentages. Comparisons between frequencies of categorical variables in different groups were performed by chi-square test. The p value was set at 0.05. Smith’s Statistical Package, version 2.80 (Pomona College, GraphPad Instat, and Stata 6.0 TM software were used for statistical analysis.

Results

The frequency of the HS1.2 3′RR1 alleles and IGAD patients

The analysis of 3′RR1 HS1.2 allelic frequencies of 88 IGAD patients and 101 healthy subjects of the same geographical area is reported in Table I. Comparison of the results shows a significant difference: homozygosity of the allele *1 that has a frequency of 39% in the IGAD patients and 15% in the control, with an increase of 2.6-fold; the heterozygous subjects with genotype *1/*2 also have a marked shift from 17% in IGAD to 33% in the control population. The two cohorts are in the conditions expected for the Hardy Weinberg equilibrium. The differences of the allelic frequencies among the two groups resulted highly significant (p = 0.001). The histogram in Fig. 2 shows the inversion of the frequencies among allele *1 and *2 in the two groups, where the relative ratio shifted from 1.24 in the patients with IGAD to 0.68 in the control. Allele *4 has a similar trend of allele *2 with a ratio of control vs patients with a significant decrease. The frequency of allele *3 is too low from 1.24 in the patients with IGAD to 0.68 in the control. IgA defect Genotypes Observed % Expected Controls IgA defect Observed % Expected p value

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Observed</th>
<th>%</th>
<th>Expected</th>
<th>Observed</th>
<th>%</th>
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<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</table>

*NS, not significant.
HS1.2 allelic frequency in IGAD patients and control related to IgG and IgM serum concentration

Because HS1.2 is involved in a coordinated manner in Ig maturation, indirectly it could be involved in B cell maturation and survival (13, 17). Therefore, we investigated whether in our patients beside the IgA defect 3/11032 RR-1 HS1.2 could be involved in differences in plasma IgM concentration or IgG. We evaluated serum levels of IgM and IgG to determine a possible correlation in the production of circulating Ig with the polymorphisms before and after isotype switch. The subjects with the values of IgM or IgG corresponding to the 30th percentile with low serum levels vs 30th percentile with high serum levels were compared for the frequencies of HS1.2 alleles in IGAD patients and controls. The results reported in Table II indicate that there is a remarkable difference of the HS1.2 allelic frequencies as far as concerns the IgM values in IGAD subjects with relatively low or high levels in the serum. No differences were observed for IgG concentrations and HS1.2 frequencies. The frequency of the 1/1 homozygous individuals with low or high levels of IgM from 46% changes to 25% with a decrease of 1.84-fold and with an increase of 1.85-fold, respectively, for the 1/2 genomes, respectively. However for the IgG high and low values a change of frequency is observed only for the 1/2 genotypes, but on the whole does not influence the frequency of the four alleles among the two groups that show almost identical values (Table II). The variation of the four allele frequency is statistically significant for the comparison of patients with low or high IgM values with a p value of 0.0391 (O.R. = 2.023) (Table II B). The analysis in the Control group stratified for the 30% of subjects with normal high or low levels of the IgM and IgG in the serum showed no significant difference for the genotype frequencies of the HS1.2 alleles as reported in Table III.

Discussion

In this study, we first report a significant increase of HS1.2 allele *1 in the cohort of patients with IGAD, suggesting a possible role of this allele in the pathogenesis of the disease. The precise role of HS1.2 in the pathogenesis of IGAD is not known, but its strong association

![FIGURE 2. The histogram represents the comparison of HS1.2 allelic frequencies in IGAD patients and a control population of same geographical area. Inversion of frequency is observed for allele *1 in respect to alleles *2 and *4. The frequency of allele *3 is statistically not relevant. The $\chi^2$ analysis on the allelic frequencies shows a difference with a p value of 0.001 highly significant. Values are indicated with the SEM. Total number of subjects is 88 for IGAD and 101 for the control. The single genotypes are reported in Table I.](http://www.jimmunol.org/)

Table II. HS1.2 frequencies in IGAD patients of the 30th percentile with low or high values of IgM and IgG in the serum

<table>
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<th>Genotypes</th>
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<th>IgG IGAD</th>
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<td>High values</td>
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<tr>
<td></td>
<td>Obs % Exp</td>
<td>Obs % Exp</td>
</tr>
<tr>
<td>1/1</td>
<td>27 45.7 18.4</td>
<td>7 25 4</td>
</tr>
<tr>
<td>2/2</td>
<td>15 25.4 7.8</td>
<td>9 32.1 7</td>
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<td>3/3</td>
<td>- - - -</td>
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<tr>
<td>4/4</td>
<td>- - - -</td>
<td>- - - -</td>
</tr>
<tr>
<td>1/2</td>
<td>8 13.5 24</td>
<td>7 25 10.6</td>
</tr>
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<td>1 1.7 1</td>
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<td>2/4</td>
<td>4 6.7 2</td>
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<table>
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<th>Alleles</th>
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<tbody>
<tr>
<td>*1</td>
<td>0.559 ± 0.045</td>
<td>66</td>
<td>0.392 ± 0.063</td>
<td>22</td>
<td>0.517 ± 0.065</td>
<td>30</td>
<td>0.569 ± 0.065</td>
<td>33</td>
</tr>
<tr>
<td>*2</td>
<td>0.364 ± 0.044</td>
<td>43</td>
<td>0.518 ± 0.065</td>
<td>29</td>
<td>0.397 ± 0.064</td>
<td>23</td>
<td>0.379 ± 0.063</td>
<td>22</td>
</tr>
<tr>
<td>*3</td>
<td>0.026 ± 0.014</td>
<td>3</td>
<td>0.001 ± 0.001</td>
<td>0</td>
<td>0.017 ± 0.016</td>
<td>1</td>
<td>0.017 ± 0.016</td>
<td>1</td>
</tr>
<tr>
<td>*4</td>
<td>0.051 ± 0.020</td>
<td>6</td>
<td>0.089 ± 0.033</td>
<td>5</td>
<td>0.069 ± 0.033</td>
<td>4</td>
<td>0.035 ± 0.023</td>
<td>2</td>
</tr>
</tbody>
</table>

* IgM: $\chi^2 = 4.257; p$ value (two sided) = 0.0391; OR (95% CI) = 2.023 (1.031–3.971).

* IgG: $\chi^2$ test is not significant.
with the disease and the complex effects of this enhancer on the immune systems prompted us to make some speculations. HS1.2 might be directly associated to IGAD pathogenesis. Alternatively, and more likely, the presence of a high frequency of allele *1 and low frequency of allele *2, may be associated to a given pattern of immune response characterized by several features: both IGA and IGM production. It has been shown that specular (high allele *2, low allele *1) pattern of HS1.2 allelic distribution is present in diseases with increased or abnormal IgA production such as IgA nephropathy and Celiac disease (30, 34). These results expand our knowledge of the possible role of HS1.2 allelic distribution in diseases with increased or abnormal IgA production; in light with this data in the present study, we report in IGAD patients HS1.2 enhancer alleles association with IgM seric levels. These findings are elusive, but a clue for their interpretation can be obtained by recent results by Vincent-Fabers et al. (17) who described a mouse KO for HS4. This mouse has a reduced number of spleen B cells. The two findings, KO for mouse HS4 and polymorphism of Human HS1.2, may appear contradictory, however the enhancers of 3’RR act synergistically and the KO of HS4 in mouse or polymorphisms of HS1.2 in IGAD patients both can alter the 3’RR activity and consequently B cell circulating number and Ig production.

The association between allelic variability in 3’RR-1 and immune diseases is still unclear, but some speculation can be made. HS1.2 allele *2 has a binding site for NF-κB, while allele *1 does not. It has been shown that BAFF, proliferation-inducing ligand (APRIL), and BTK, all regulate B cell survival via NF-κB. It can be hypothesized that HS1.2 enhancer of 3’RR1 HS1.2 allele frequencies, this suggests that the alleles cooperate differently before and after the isotype switch at least for the haplotypes associated in IGAD patients, though HS1.2 alleles interfere positively and negatively with Ig and also affect IgM production. HS1.2 allele *2 has a binding site for NF-κB (3, 8), and therefore in subjects with allele *1 homozygosis, the lacked binding sites for NF-κB may interfere with this pivotal transcription factor leading to Ig maturation and production thus affecting also survival of B cells. The penetrance of the allele *1 for the absence of circulating IgA is partial but can coregulate also IgM production. We do not exclude that the presence of specific haplotypes associated to IGAD and determined by polymorphisms distributed on the entire 3’RRs can cause conformational changes with different structural variation of the Regulatory Regions relevant for methylation changes and other epigenetic events. Interestingly, in mouse where polymorphisms are not studied was observed the relevance of the 3’RR for the epigenetic regulation (35). However further studies are needed to clarify the role, if any, of HS1.2 allele *1 in the pathogenesis of IGAD and to confirm a pattern of allelic frequencies associated to increased (allele *2 high; allele *1 low) or decreased (allele *1 high; allele *2 low) IgA production.
production. It is also worth noting that IGAD increases susceptibility to celiac disease (34, 36).

One additional point that can be made based on the results presented herein and those already available of a correlation between allelic frequency and immune mediated diseases is related to the functions of 3’RR-1 and 3’RR-2. In humans it is present an allelic hypervariability in 3’RR1, while alleles in 3’RR-2 have constant frequency. The variability in 3’RR-1 is associated with different diseases as we have discussed above. These findings suggest independent roles of the two 3’RRs in the development of the immune function as well as in susceptibility to immune-mediated diseases. Thus we suggest that the human 3’RRs can play different roles for Ig maturation and B cell control. They could act differently either for different polymorphisms that can bear, either for different time activation caused by the position that have in the cluster (Fig. 1).

Only further studies in different diseases will confirm whether that pattern of IgM is specific for IgAD or is related to the activity of a given set of alleles present not only in IgAD but synergistic with other cofactors leading in each case to different pathologies. Only the whole pattern of polymorphisms of both 3’RRs will show whether the presence of specific haplotypes may condition the regulatory activity in linkage to polymorphic enhancer HS1,2.

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Disclosures

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References