SHORT COMMUNICATIONS

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Immunoglobulin Enhancer HS1,2 polymorphism: a new powerful anthropogenetic marker

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Summary

The human HS1,2 enhancer of the immunoglobulin (Ig) heavy chain 3′ enhancer complex plays a central role in the regulation of Ig maturation and production. Four common alleles HS1,2-A*1, *2, *3, *4 are directly implicated with the transcription level and at least one of them, HS1, 2-A*2, seems to be related to immune disorders, such as coeliac disease, herpetiform dermatitis and Berger syndrome. Given their clinical significance it is of interest to know the distribution of HS1,2-A variants in populations from different continents, as well as to determine whether the polymorphism is associated to specific evolutionary factors. In this paper we report the distribution of the HS1,2-A polymorphism in 1098 individuals from various African, Asian and European populations. HS1,2-A*3 and HS1,2-A*4 alleles are at their highest frequencies among Africans, and HS1,2-A*2 is significantly lower in Africans in comparison with both Europeans and, to a lesser extent, Asians. Analysis of molecular variance of the allele frequencies indicates that the HS1,2-A polymorphism can be considered as a reliable anthropogenetic marker.

Keywords: HS1,2 Ig enhancer, regulatory region, Immunoglobulines, Immune-pathologies, human populations, allelic frequencies

Introduction

An increasing number of syndromes have been correlated with the genetic background of ethnic groups (Yang *et al*. 2005). This could also be relevant for the incidence of pathologies ascribed to the immunoresponse (Nagel 2005).

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The immunoglobulin (Ig) heavy chain 3′ Enhancer Complex 1 (IgH3′EC-1) in humans includes three enhancers, the central of, which HS1,2-A, is polymorphic (Hu *et al*. 2000). No polymorphism has been described for the other two enhancers of the LCR complex (Guglielmi *et al*. 2004). The structure of HS1,2-A and HS1,2-B (Figure 1A) has been described by several authors (Denizot *et al*. 2001; Giambra *et al*. 2005). In apes and humans the Enhancer Complex is duplicated with a cluster of four constant heavy chain genes (Figure 1A) (Sepulveda *et al*. 2005). The function of the three enhancers of both 3′ complexes (Figure 1A)

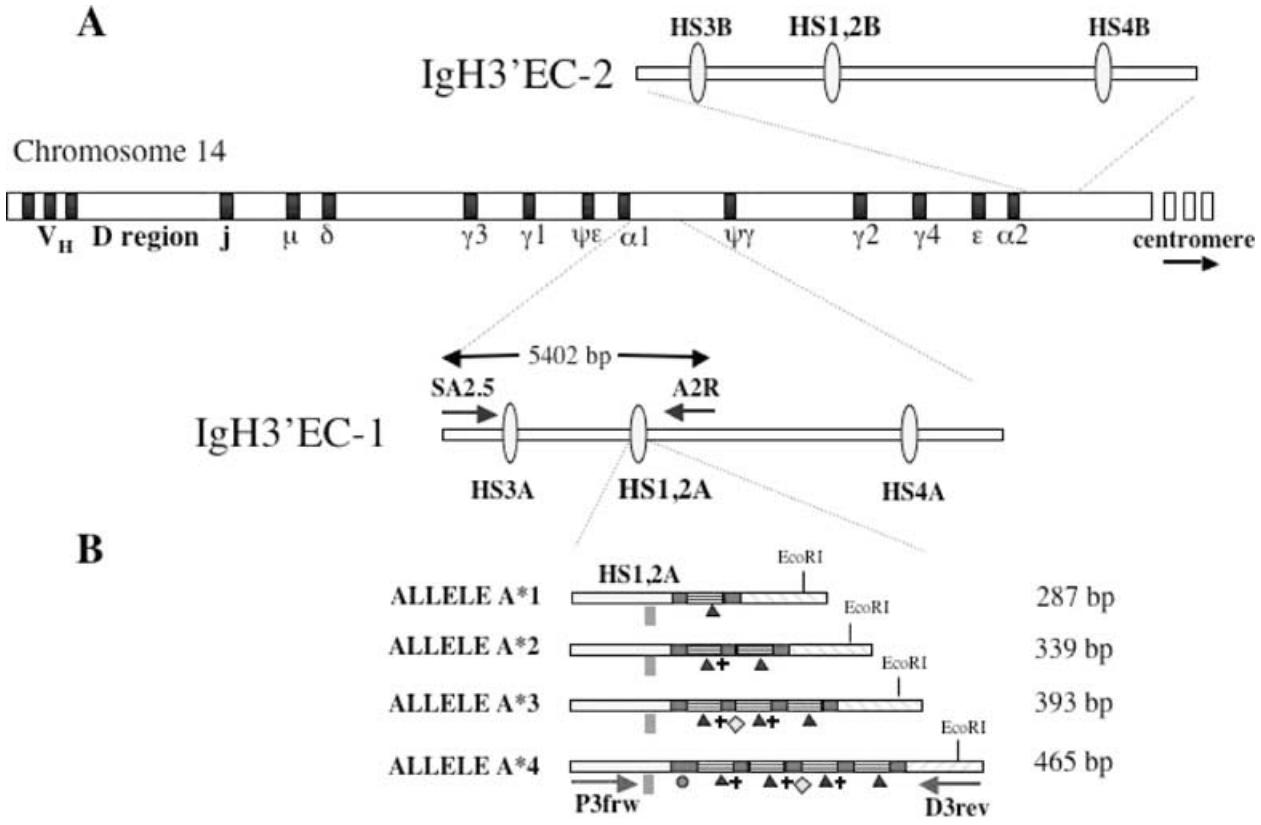

Figure 1 A. Genomic organization of the Ig constant heavy chain genes in the chromosome 14q32 region: enlargement of the 3′ Cα-1 EC-1 and 3′ Cα-2 EC-2. The elliptic inserts represent the three enhancers of both Enhancer Complexes. The arrows indicate the position and direction of the primer sequences for selective (Sa2.5/A2R) and nested (P3Frw/D3Rev) amplifications of the HS1,2 A enhancer. **B.** Schematic representation of the 4 alleles aligned to show the conserved and duplicated elements. The structure of the polymorphism consists of repeated elements of 38 bp separated by spacers containing stretches of C. The 38 bp repeated element, the internal spacers and the borders present transcription factor consensus sites, as well as the core of the enhancer. The names of the transcription factors, the functions of which are not B cell-specific except when combined in the transriptome complex, are reported with the corresponding symbols mapped on the alleles. (■) for CEBP, CMYB, HSF, MEF2, OCT1/HOXC4, SR-Y; STAT, TH1E47 (plus strand), CETS1P54, YY1 (minus strand); (▲) for IK2 and Nf-kB (p50); (+) for Nf-kB; (◇) for c-MYB; (●) for AP4, E47, MYOD, μE5.

is essential for the isotypic switch and for the control of Ig production in plasma cells (Laurencikiene *et al*. 2001). The modulation of these events could also be ascribed to the polymorphic structure of the HS1,2-A and HS1,2-B enhancers, since the polymorphism involves a different number of consensus sites for transcription factors, including NF-kb (Figure 1B) (Giambra *et al*. 2005).

The aim of this study is to gather knowledge on the distribution of the Immunoglobulin Enhancer HS1,2 polymorphisms in various African, Asian and European human populations. This is relevant to establishing their genetic background for further epidemiological studies on immune-disorders, as suggested by the correlation of the HS1,2 polymorphisms to coeliac disease, herpetiform dermatitis (Frezza *et al*. 2004) and Berger disease (Aupetit *et al*. 2000).

Material and Methods

One thousand and ninety-eight individuals belonging to various African, Asian and European populations were analyzed for the Immunoglobulin Enhancer HS1,2-A polymorphism. Each sample comprises autochthonous, unrelated and healthy donors of both sexes, who gave their informed consent. Each donor was asked to supply their name, birthplace, language and ethnicity for three generations in order to exclude recent

Table 1 Sample size and frequencies of HS1,2-A alleles

Populations	Total	Alleles			
		∗1	∗2	∗3	∗4
Benin	100	0.590 ± 0.035	0.000 – 0.01∗	0.245 ± 0.030	0.165 ± 0.026
Cameroun	86	0.442 ± 0.038	0.087 ± 0.021	0.250 ± 0.033	0.221 ± 0.032
Burkina	101	0.322 ± 0.033	0.049 ± 0.015	0.416 ± 0.035	0.213 ± 0.029
Mongolia	95	0.658 ± 0.034	0.263 ± 0.032	0.016 ± 0.009	0.063 ± 0.018
Siberia	70	0.650 ± 0.040	0.279 ± 0.038	0.014 ± 0.010	0.057 ± 0.020
Iceland	96	0.536 ± 0.036	0.307 ± 0.033	0.036 ± 0.013	0.120 ± 0.023
Bulgaria	95	0.363 ± 0.035	0.558 ± 0.036	0.016 ± 0.009	0.063 ± 0.018
Turkey	103	0.563 ± 0.035	0.383 ± 0.034	0.024 ± 0.011	0.029 ± 0.012
Spain	104	0.457 ± 0.034	0.346 ± 0.033	0.058 ± 0.016	0.139 ± 0.024
Italy	148	0.399 ± 0.028	0.416 ± 0.029	0.054 ± 0.013	0.132 ± 0.020
Sicily	100	0.500 ± 0.035	0.365 ± 0.034	0.070 ± 0.018	0.065 ± 0.017

∗S.D. calculated according to Morpurgo *et al*. 1983

admixture. Genomic DNA was isolated from whole blood by standard methods, or from mouthswab (Budowle *et al*. 2000).

The PCR assay of the HS1,2-A region was performed on genomic DNA (50 ng) with primers and conditions as described in Giambra *et al*. (2005).

Allele frequencies were calculated by direct count and using Arlequin package software (Schneider *et al*. 2000). Bayesian 95% credible regions (CRs) for allele frequencies were calculated with the computer program Sampling (kindly provided by V. Macaulay, Department of Statistics, University of Glasgow). Departures from Hardy-Weinberg equilibrium were evaluated.

Population genetic structure was tested through analysis of molecular variance by comparing allele frequencies (AMOVA) (Excoffier *et al*. 1992). The statistical significance of F- values was estimated by permutation analysis using 100,000 random permutations (Arlequin v2.0, Schneider *et al*. 2000).

Results and Discussion

The alleles of HS1,2-A were determined by nested PCR analysis; the four alleles differ for 1–4 copies of a tandem repeat separated by stretches of poly-C. Allele *1 is 72 bp long, allele *2 is 124 bp long, allele *3 178 bp and 250 bp allele *4, as shown in Figure 1B (GenBank accessions AJ544218, AJ544219, AJ544220, AJ544221). The HS1,2-B locus was analyzed at first only in Italian and Cameroon populations, in order to ascertain the discriminating power at population level of this locus. Since no significant variation was detected between south-Saharan and European populations when 95% credible regions were calculated, no further investigations on the other collected samples were performed.

The HS1,2-A allele frequencies of the eleven populations are reported in Table 1. In all groups the observed genotypes are in agreement with those expected under Hardy-Weinberg equilibrium. The HS1,2-A*3 allele shows the highest frequencies among African populations, and HS1,2-A*2 is significantly lower in Africans in comparison with both European and, to a lesser extent, Asian populations ($P > 0.001$). The HS1,2-A*4 allele is also higher in Africans while allele HS1,2-A*1 does not seem to differentiate the major human population groups.

The frequency distribution of HS1,2-A alleles may explain the characteristic geographic distribution of IgA nephropathy: its low incidence among Africans and African ancestry populations, and the fact that European, Asian and Asian derived populations are more prone to Berger disease (Levy & Berger, 1988; Kobet *et al*. 1996; Hall *et al*. 2004).

In order to provide a quantitative estimation of the reliability of the HS1,2-A polymorphism as useful anthropological marker, an analysis of molecular variance (AMOVA) was performed on the studied population samples. As a rule in human populations most of the variance observed was due to the intra-population variability. At the geographical level, considering the populations subdivided according to the different continents and, therefore, including the Turkish sample from Bursa within Asian populations, it can be seen that the among groups variation is relatively higher (9.8%, $P < 0.001$)

compared to the among populations within groups variance (1.6%, $P < 0.001$). This analysis highlights the great variation in allele frequency distributions among the major human population groups, while the level of differentiation of the HS1,2-A polymorphism within each continent is quite low. This is particularly remarkable considering the high cultural and ethnic heterogeneity of the groups sampled within each main geographic area.

However, if we consider the distribution of the variation within each continental group, it is evident that Africa peoples display the highest heterogeneity, with a percentage of variation among populations of 5.7 ($P < 0.001$), while Europeans show a fairly low value of differentiation (1.5%, $P = 0.008$), followed by Asians who appear to be the most homogeneous, although not significantly (0.3%). These results do not change if we consider the Turkish as part of a "Mediterranean group", which includes the samples from Bulgaria, continental Italy, Sicily and Spain, or as part of the European group (in both cases the percentage of variation is equal to 1.65, $P < 0.001$). On the contrary, excluding the Turkish from the Asian group, the degree of homogeneity within this continent increases from 0.3% of variance (Turkish included) to -1.2%. This result can be explained by the high genetic similarity between Mongolian and Siberian populations, as pointed out by several studies based on classical, mitochondrial and autosomal DNA markers (see Cavalli-Sforza *et al*. 1994). Also the remarkable heterogeneity found among the African populations is in line with other genetic studies which indicate that Africa presents the most complex genetic picture of any continent, and therefore that modern humans originated in Africa during the Middle Stone Age, about 150,000 years ago or somewhat earlier (see Cavalli-Sforza *et al*. 1994).

In conclusion, the present work for the first time provides information on the world distribution of the HS1,2-A polymorphism, highlighting the fact that its frequencies are differentially distributed in different geographic groups. This enhances the importance of this polymorphism in epidemiological studies related to immune-disorders (such as Berger disease, the pathogenesis of which is still unknown), especially in view of the current environmental modifications, such as changes in diet and exposure to pesticides, poisons and particular drugs, to which different populations are exposed.

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