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Predisposition to idiopathic thrombocytopenic purpura maps close to the major histocompatibility complex class I chain-related gene A

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ABSTRACT

Idiopathic thrombocytopenic purpura (ITP) is an autoimmune condition with poorly known etiology, characterized by platelet destruction. Genetic association studies of this disease are scarce, discrepant, and restricted to major histocompatibility complex (MHC) polymorphisms. Hence, a case–control study was conducted with an aim to map the MHC to IPT susceptibility using *HLA-B* and nine microsatellite loci encompassing MHC class I, II, and III regions. We compared the allelic frequencies in samples of unrelated healthy controls and ITP patients. After correction for multiple tests, only allele MICA*183, also known as A5.1, demonstrated an association, resulting in the identification of a major predisposing region close to *STR-MICA*. This result may highlight the putative functional role of MICA in the immune response to ITP.

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1. Introduction

Autoimmune thrombocytopenia arises from the early destruction of platelets by antiplatelet autoantibodies in the activated endothelial reticulum system [1,2]. The resulting disease is characterized by a severe decrease in platelet numbers in the peripheral blood, followed by symptoms such as petechiae, purpura, conjunctional hemorrhage, or other types of mucocutaneous bleeding [3]. Autoantibodies found in autoimmune thrombocytopenia may have distinct origins, differentiating the condition into four broad groups: idiopathic thrombocytopenic purpura (ITP), secondary immune thrombocytopenia (*e.g.*, secondary to systemic lupus erythematosus), drug-induced immune thrombocytopenia, and viral infection-related thrombocytopenia (*e.g.*, HIV) [1]. Among those groups, ITP has a significant incidence of around 6:100,000 inhabitants per year [4], although the fundamental disturbances that lead to its autoimmune response are unknown. Genetic factors can influence the development of autoimmune diseases, especially immune-response genes, including the human leukocyte antigen (HLA) genes. Indeed, some association studies have indicated genetic polymorphisms on HLA genes as predisposing factors to ITP [5–10]. However, the associations reported were generally weak and varied among the studies. Possible reasons for the lack of reproducibility in previous studies include different diagnostic criteria for ITP, ethnic variability in the HLA allele distribution, and the use of serological typing methods, which are unable to identify HLA alleles at the amino acid level. In addition, HLA analysis may be further complicated by linkage disequilibrium with other genes of the major histocompatibility complex (MHC).

The first stage of the present study aimed to map the MHC using microsatellite markers encompassing the most relevant class I, II, and III recombination blocks [11] to identify the primary ITP predisposing/protective regions. Because the region around major histocompatibility complex class I chain-related gene A (MICA), represented by the *STR-MICA*, exhibited the strongest association signal in our study and the HLA-B locus was reported to be associated with ITP in some studies [8,10], this locus was genotyped and analyzed to certify that the possible association of ITP with *MICA* polymorphisms was not secondary based on linkage disequilibrium with this locus.

2. Subjects and methods

Of about 400 patients registered at the Foundation Center of Hemotherapy and Hematology of Pará (a reference state hematology center in northern Brazil) as autoimmune thrombocytopenia carriers, only 51 (11 men and 40 women, mean age of 35 years old at examination) could be diagnosed as adult ITP carriers using the absence of background diseases and the presence of GPIIb/IIIa and/or GPIb/IX serum autoantibodies as criteria (PakAuto Kit, GTI,

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Brookfield, WI, USA). The control sample was composed of 145 unrelated individuals (65 men and 80 women, mean age of 31 years old) representative of the same population from which the ITP carriers were obtained. Men and women in control sample underwent allelic frequency comparison to determine whether their lower female/male ratio could bias the results. DNA samples were isolated according to Sambrook *et al.* [12].

The nine microsatellites used for genetic mapping (Table 1) were located in the following MHC recombination blocks: ε (*D6S2749* locus) and δ (*G51152* and *D6S2883*) blocks in the class II region; in-between γ and δ blocks (*D6S273* and *BAT2GT*) and β block (*TNFd*) in the class III region; and β (*STR-MICA* and *D6S2811*) and α (*D6S510*) blocks in the class I region (Figure 1) [11,13,14].

Polymerase chain reaction (PCR) conditions for each loci were 94°C for 10 minutes, followed by 30 cycles at 94°C for 30 seconds, primer-specific annealing temperature for 30 seconds, 72°C for 1 minute, and final extension at 72°C for 1 hour. *BAT2GT* and *D6S2883* were multiplexed at an annealing temperature of 60°C, whereas *D6S2749*, *G51152*, *D6S273,* and *D6S2811* were multiplexed at an annealing temperature of 57°C. Annealing temperatures for *TNFd*, *STR-MICA,* and *D6S510*, loci that could not be multiplexed, were 64, 57, and 65°C, respectively. The final volume of each reaction was 15 μL, composed of reaction buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), 3.3 mM $MgCl_2$, 0.75 μL DMSO, 0.08 mM dNTP, 1 U Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), and 50 ng of genomic DNA. Primer concentrations were 0.25 mM for *D6S510*, *TNFd*, *G51152,* and *MICA*, 0.35 mM for *D6S2883*, 0.15 mM for *BAT2GT*, 0.1 mM *D6S2749*, 0.2 mM for *D6S273,* and 0.3 mM for *D6S2811*. Forward primers (Table 1) were fluorescently tagged at the 5′ ends, allowing genotyping using an ABI 377 automated sequence analyzer (Applied Biosystems, Foster City, CA, USA).

HLA-B genotyping was performed using the INNO-LiPA HLA-B Update Plus probe assay (Innogenetics, Gent, Belgium) according to the manufacturer's instructions.

3. Results

Hardy–Weinberg genotypic deviations were calculated using GENEPOP software [16]. Genetic diversities (*H*) for each locus were estimated according to Nei [17] and compared using Wilcoxon's test. The χ^2 test was applied for each allele and the *p* value was accessed by Monte Carlo simulations, using the CLUMP software [18]. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for alleles that significantly differed in their frequencies between patients and controls. Multiple test correction was applied by multiplying allelic *p* values by the number of alleles at each locus according to Bonferroni's correction linkage disequilibrium and haplotypes were inferred using Pypop software [19].

All loci were in Hardy–Weinberg equilibrium, and male and female control subsamples did not differ in terms of their allelic frequencies ($\chi^2 = 26.618$; $p = 0.1464$). Microsatellite genetic diversity (*H*) and number of alleles (*k*) did not differ statistically between patients and controls (Wilcoxon's test: $Z = 1.481$, $p = 0.139$, for *H* comparison; $Z = 1.782$, $p = 0.075$, for *k*; Table 2); in addition, the

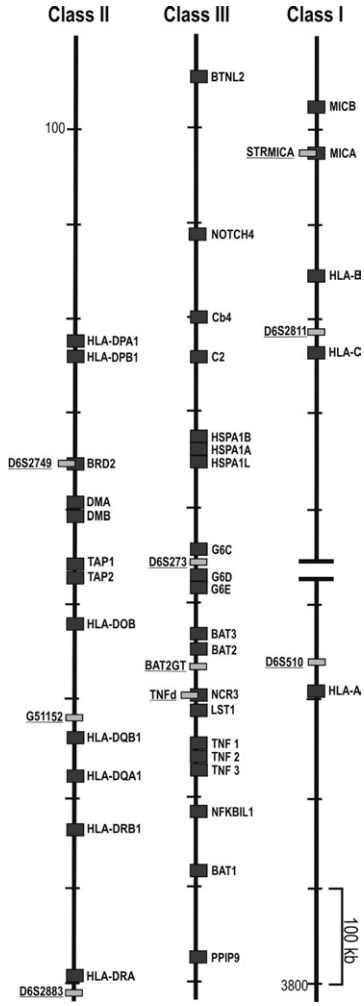

Fig. 1. Localization map of the microsatellites in the MHC. The nine microsatellites studied (gray boxes) are shown in their relative position to relevant MHC genes (black boxes).

Table 1
Characteristics of the nine selected MHC microsatellites

Loci	Primer 1 Primer 2	Core motif	References
D6S2749	GAGGTAATGTCACAGGATGGG TGCTTATAGGGAGACTACCG	GT	13
G51152	GGTAAAATTCCTGACTGGCC GACAGCTCTTCTTAACCTGC	GT	13
D6S2883	ACATTTGTATGCTTCAGATG TGGAATCTCATCAAGGTCAG	AC	13
D6S273	ACCAAACTTCAAATTTTCGGC GGAGAAGTTGAGTATTTCTG	GT	13
BAT2GT	CTCCAGCCTGGATAACAG ACAAGGGCTTTAGGAGGTCT	GT	13;14
TNFd	CATAGTGGGACTCTGTCTCCAAAG AGATCCTTCCCTGTGAGTTCTGCT	AG	13
STR-MICA	CCTTTTTTTCAGGGAAAGTGC CCTTACCATCTCCAGAAACTGC	GCT	13;15
D6S2811	TGCCATTTGGCCCTAAATGC TGGGCAATGAGTCCTATGAC	GT	13
D6S510	AATGGGCTACTACTTCACACC CAACACACTGATTTCCATAGC	AC	13

Table 2
Genetic variability estimates for each locus in controls and patients samples

Loci	Control ($2n = 288$)		Patients ($2n = 102$)	
	H	*k*	*H*	*k*
D6S2749	0.744	08	0.760	06
G51152	0.707	10	0.759	09
D6S2883	0.850	10	0.815	10
D6S273	0.779	08	0.780	08
BAT2GT	0.789	13	0.822	14
TNFd	0.666	08	0.726	06
STR-MICA	0.772	05	0.774	05
D6S2811	0.930	21	0.940	21
D6S510	0.846	13	0.837	09
HLA-B	0.931	27	0.944	26
Average	0.800	12.3	0.816	11.4

H = expected heterozygosity; *k* = number of alleles; $2n$ = number of chromosomes sampled.

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