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Maternal *HLA-G*01:01:01:04* protects from anti-HLA-class II immunization in pregnant women

Mohamed Slimane[a], Christophe Picard[b,c], Jacques Chiaroni[b,c], Anderson Loundou[d], Julien Paganini[e], Pierre Tiberghien[a,f], Jean-Michel Rebibou[a,g], Julie Di Cristofaro[b,c,*]

[a] UMR 1098, Inserm, Université de Franche-Comté, Etablissement Français du Sang BFC, IFR 133, Besançon, France
[b] Aix Marseille Univ, CNRS, EFS, ADES, "Biologie des Groupes Sanguins", Marseille, France
[c] Etablissement Français du Sang PACA Corse, Marseille, France
[d] Department of Public Health -EA 3279 Research Unit, University Hospital Marseille, Aix-Marseille University, Marseille, France
[e] Xegen, Gemenos, France
[f] Laboratoire d'Immunogénétique, Établissement Français du Sang BFC, Besançon, France
[g] Service de Néphrologie, Centre Hospitalier Universitaire, Dijon, France

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ABSTRACT

Factors determining anti-HLA immunization are poorly understood, although anti-HLA immunization following pregnancy is well described.

The HLA-G molecule has been extensively described for its implication in immunological tolerance, especially during pregnancy. Transplant studies show an association between *HLA-G* haplotypes and alloimmunization.

Our aim was to investigate the association of *HLA-G* haplotypes with anti-HLA class I and II immunization in a cohort of women having experienced one or more pregnancies and with no transfusion history.

Maternal blood samples (n = 270) collected at delivery and formerly screened for anti-HLA antibodies, HLA-A and HLA-B antigens, were screened by NGS for *HLA-G* gene polymorphism.

Univariate analysis further confirmed that the number of pregnancies was significantly associated with anti-HLA class I immunization, whereas no other variable remained significant after Bonferroni correction. Our results showed however that anti-HLA class II immunization was associated with the number of children whereas the *HLA-G*01:01:01:04* allele was protective against this immunization.

1. Introduction

The *HLA-G* molecule has been extensively described for its implication in immunological tolerance, especially during pregnancy. Although it displays a restricted number of alleles, many studies associated *HLA-G* alleles with differential level of tolerance in achallenging immune situations such as pregnancies and transplantation outcome. Indeed *HLA-G* displays few haplotypes (including 5'URR, coding and 3'UTR regions) associated with differential protein expression patterns [1–5] and differential clinical outcome of immunosensitive events. *HLA-G* alleles were associated with pregnancy complications [6,7] and recurrent miscarriages [8,9] as well as with immunization and poor prognosis in lung transplant patients [10].

Alloimmunization, especially against HLA molecules, is also responsible for adverse effects and acute rejection in blood transfusion and organ transplantation; including febrile non-haemolytic transfusion reactions, immunological platelet refractoriness or transfusion-related acute lung injury. The main cause of naturally occurring anti-HLA immunization is pregnancy, with an extensively confirmed increased HLA antibody prevalence with a greater number of pregnancies [11–13]. Anti-HLA antibodies are the most studied antibodies, but little is known about non-HLA antibodies mainly directed against endothelial cells, and although this field has been intensively studied, their prevalence and implication in physio-pathological process remain unclear [14].

Biological and genetic factors determining anti-HLA immunization remain poorly understood with the exception of HLA-A, B or DR antigens or eplets [11,12,15–19]. HLA immunogenicity during pregnancy was found to be lower in women who experienced a prior miscarriage compared to women who had a prior successful pregnancy [17]. Masson et al. analyzed genetic polymorphisms implicated in humoral

Abbreviations: HLA, Human Leukocyte Antigen; CTL, Cytotoxic T-Lymphocyte; NK, Natural Killer cells; ILT, Immunoglobulin-Like Transcript; KIR, Killer-cell Immunoglobulin-like Receptor; sHLA-G, soluble HLA-G; UTR, UnTranslated Region; URR, Upstream Regulatory Region; gDNA, genomic DNA

* Corresponding author.
E-mail address: julie.dicristofaro@efs.sante.fr (J. Di Cristofaro).

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immunization, and none of the following parameters influenced immunization rate: *HLA*-typing, *TLR4* gene polymorphisms D299G, T399I; *IL-6* promoter polymorphism -174 C/G and *BAFF* intron G/T and promoter polymorphism -871 C/T [20]. In women having had only one successful pregnancy, there was a significant association between *IL-6* promoter -174G/C and immunization [20]. HLA-G expression was found to be lower in women with antibodies detectable both by CDC and Luminex than in women with antibodies detected only by Luminex [20]. Such serological testing is however poorly reproducible [1], whereas most HLA laboratories nowadays have DNA analysis facilities making *HLA-G* studies a feasible option. Thus, *HLA-G* molecule may represent a good complementary genetic predictive factor for anti-HLA immunization.

To get further insight into the association of *HLA-G* haplotypes with immunization, we recently investigated *HLA-G* phylogeny with regards to inflammatory response [21]. Our results split *HLA-G* haplotypes phylogenetically into four main clades respectively containing (1) *HLA-G*01:01:02:01*, *G*01:01:02:02*, *G*01:05N* and *G*01:06* (associated with haplotype *H10*); (2) *G*01:04:01* and *G*01:04:04* (*H23*); (3) *G*01:03:01:02* (*H21, H20* and *H19*) and (4) *G*01:01:01:01* and *G*01:01:01:05* (*H01, H02, H03, H04, H05, H46, H47, H49, H54*). Whereas *HLA-G* alleles associated with poor prognosis in transplantation, pregnancy or inflammatory disease were grouped in clades 1, 2 and 3, some of the *HLA-G* alleles in the fourth clade were associated with a protective effect against inflammation [1,10,21].

The main goal of this study was to confirm the association of *HLA-G* phylogeny with differential immunological tolerance induction. We thus investigated *HLA-G* haplotype association with the presence of anti-HLA antibodies in women who had experienced one or more pregnancies but who had no transfusion history.

2. Material and methods

2.1. Population and sample collection

Two hundred and seventy women having had one or more pregnancies were included in the study. None of these women had any history of transfusion. Maternal blood samples and umbilical cord samples were collected at delivery [20]. Patient data (age, number of children, pregnancies and miscarriages) are given in Table 1. The study was designed in collaboration with the Besançon Cord Blood Bank (Etablissement Français du Sang) and carried out in accordance with the recommendations of Besançon Hospital's Ethics Committee with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Besançon Hospital's Ethics Committee.

2.2. HLA-G analysis and HLA typing

Maternal DNA was sequenced for *HLA-G* gene from position -1983 to +3447 by Next Generation Sequencing (NGS). PCR fragments were sequenced using an NGS platform (MiSeq, Illumina, The Netherlands) [21].

HLA-G NGS data were analyzed using PolyPheMe (Xegen, France) [22]. *HLA-G* allelic assignment at 8 digits was based on the HLA sequences listed in the official IMGT/HLA database 3.28.0 [23]. All polymorphic variations from position -1983 to -1 in 5'URR and from +2540 to +3447 in 3'UTR, *i.e.* 37 SNPs, 1 base deletion, 1 base insertion and a 14bp insertion were used for haplotype estimation using PHASE. *HLA-G* haplotype identification numbers were coded according to [24]. Allelic and haplotype frequencies were estimated using an EM algorithm implemented in the Gene [Rate] computer tools [25].

Both maternal and child DNA were typed for HLA-A and HLA-B at a low resolution level by Luminex™ technology (One Lambda LABType® SSO, InGen, France), complete procedure is described in [20]. HLA-C, DR or DQ were not investigated.

Latest child/paternal HLA-A and HLA-B mismatch were deduced from mother and latest child HLA-A and HLA-B typing results. Maternal *IL-6* -174 C/G genotype results obtained by Snap Shot were included in this study, complete procedure is described in [20].

2.3. HLA antibody testing

Each serum was screened for anti-HLA antibodies using two techniques: the standard CDC method (for anti-class I antibodies) and the Luminex screening kits (for anti-class I and class II antibodies), complete procedure is described in [20].

2.4. Statistical analysis

Missing data led to the exclusion of the concerned sample from further analyses. No multiple imputation was used. Analysis were performed using GRAPH PAD Prism 5 software (CA, www.graphpad.com), SPSS 24.0 software (SPSS Inc., Chicago) and SAS 9.4 (SAS Institute Inc. Cary, USA).

Biological and genetic data frequencies between anti-HLA class I immunized women, anti-HLA class II immunized women and non-immunized women were compared with Chi-square tests. The presence of anti-HLA class I antibodies and anti-HLA class II antibodies were independently tested in univariate analyses according to biological data (women's age, number of pregnancies, number of children delivered, number of miscarriages, sex of the latest child) and according to genetic data (*HLA-G* alleles and *HLA-G* haplotypes, *IL-6* -174 C/G genotype, HLA-A and HLA-B antigen, latest child/paternal HLA-A and HLA-B antigen mismatch). HLA-A and HLA-B antigens and latest child/paternal HLA-A and HLA-B mismatches were included for their association with allo-immunization because of the Linkage Disequilibrium between *HLA-G* and *HLA-A* loci and *HLA-A* and *HLA-B* [26]. *IL-6* -174 C/G genotype was included in the study since it was described as being significantly associated with immunization in women having had only one successful pregnancy [20].

Bonferroni correction for multiplicity testing procedure was applied and variables with p-values below 0.001 in univariate analyses were further investigated in multivariate regression analysis. The hazard ratios were estimated by using Firth's penalized partial likelihood approach. Association results are expressed as odds ratio (OR) and confidence intervals of 95% (CI 95%).

3. Results

Anti-HLA class I and class II immunization data according to biological characteristics are shown in Table 1. Univariate analyses showed no difference between anti-HLA-I immunized and anti-HLA-I non-immunized women, or between anti-HLA-II immunized and anti-HLA-II non-immunized women according to age or gender of latest child. Anti-HLA-I and anti-HLA-II immunization were associated both with the number of pregnancies and the number of children (both $p < 0.0001$) but not with miscarriages; the association of miscarriages with anti-HLA-II immunization did not reach significance after Bonferroni correction ($p = 0.007$).

HLA-G allelic frequency and characteristics of estimated *HLA-G* haplotypes with a frequency above 1% are shown in Table 2. Most *HLA-G* haplotypes with a frequency above 1% display an exclusive association with an *HLA-G* allele defined at high resolution, i.e. 8 digits.

The characteristics of anti-HLA class I and class II antibody groups according to HLA-G alleles and haplotypes are shown in Table 3.

Univariate analyses showed no significant difference between anti-HLA-I immunized and anti-HLA-I non-immunized women according to HLA-A and HLA-B antigens (data not shown), *IL-6* G/C genotype (data not shown), *HLA-G* allele or haplotype, and latest child/paternal HLA-A and HLA-B mismatch (data not shown) after Bonferroni correction.

None of these variables displayed any significant differences either

Table 1
Women's data under study. Age, sex of latest child, number of pregnancies, children and miscarriages are given for all women, anti-HLA class I immunized, anti-HLA class I non-immunized, anti-HLA class II immunized and anti-HLA class II non-immunized women; p-values under 0.001 are in bold.

	All	Anti-HLA class I immunized	Anti-HLA class I nonimmunized	p-value	Anti-HLA class II immunized	Anti-HLA class II non-immunized	p-value
Number of patients (%)	270	100 (37.1)	170 (62.9)		70 (26.0)	200 (74.0)	
Age (yrs) (median and range)	29.3 [18-42]	29.7 [19-40]	29.0 [18-42]	0.176	29.9 [19-41]	29.0 [18-42]	0.209
Sex of latest child sex (Male %)	51.5	55	49.4	0.375	59.4	48.8	0.126
Pregnancies (median and range)	2.1 [1–6]	2.4 [1–6]	1.9 [1–6]	< **0.001**	2.6 [1–6]	1.9 [1–6]	< **0.001**
1 (%)	35.2	20.0	44.1	< **0.001**	14.5	42.3	< **0.001**
2 (%)	36.3	45.0	31.2	0.022	39.1	35.3	0.570
3 (%)	15.2	16.0	14.7	0.774	23.2	12.4	0.032
4 (%)	10.0	14.0	7.6	0.092	17.4	7.5	0.018
5 (%)	2.6	4.0	1.8	0.264	4.3	2.0	0.288
6 (%)	0.7	1.0	0.6	0.703	1.4	0.5	0.426
Children (median and range)	1.6 [1–5]	1.96 [1–4]	1.5 [1–5]	< **0.001**	2.0 [1–4]	1.58 [1–5]	< **0.001**
1 (%)	48.1	31.0	58.2	< **0.001**	26.1	55.7	< **0.001**
2 (%)	38.1	49.0	31.8	0.005	50.7	33.8	0.013
3 (%)	10.0	13.0	8.2	0.208	17.4	7.5	0.018
4 (%)	3.3	7.0	1.2	0.010	5.8	2.5	0.186
5 (%)	0.4	0.0	0.6	0.442	0.0	0.5	0.557
Miscarriages (median and range)	0.41 [0-3]	0.44 [0-2]	0.39 [0-3]	0.437	0.59 [0-2]	0.35 [0-5]	0.007
0 (%)	67.0	64.0	68.8	0.416	55.1	71.1	0.014
1 (%)	25.6	28.0	24.1	0.480	30.4	23.9	0.281
2 (%)	6.7	8.0	5.9	0.501	14.5	4.0	0.003
3 (%)	0.7	0.0	1.2	0.276	0.0	1.0	0.406

between women with anti-HLA-II antibodies and anti-HLA-II non-immunized women (data not shown), except the *HLA-G* allele *G*01:01:01:04* and haplotype *H03*. This allele was never observed in anti-HLA-II immunized women whereas it were present in 11.5% of anti-HLA-II non-immunized women.

Of note, variables that did not reach significance after Bonferroni correction and were thus not included in multivariate analyses were the following for anti-HLA class I antibody presence: *H03-G*01:01:01:04*, B*13 and B38, and for anti-HLA class II presence: *H19-G*01:03:01:02*, A*29, A*31, B*18, B*57, B*60, paternal mismatch B*51 and paternal mismatch B*62 (Table 2). It should be noted that *HLA-G*01:03:01:02* was the only allele observed for *HLA-G*01:03*.

Multivariate analysis was performed by logistic regression hazard modeling using Firth's penalized partial likelihood approach with variables showing significant p-values after Bonferroni correction. One variable of those displaying high correlation was chosen (either number of pregnancies or number of children for biological variables, and either *H03* or *G*01:01:01:04* for genetic variables). Anti-HLA class II immunization displayed significant association with number of children ($p < 0.0001$, hazard ratio: 1.725; CI 95%: 1.1339-2.223) whereas *G*01:01:01:04* allele was significantly protective against anti-HLA class II immunization ($p = 0.0422$, hazard ratio: 0.0422; CI 95%: 0.003-0.0899) (Table 4).

4. Discussion

Anti-HLA immunization is a main issue in blood transfusion and organ transplantation. Pregnancy is the main source of HLA sensitizing

Table 2
Characteristics of *HLA-G* haplotypes estimated with a frequency above 1% (ID: identification number according to [24], N: Number, Fq: Frequency).

Haplotype ID	-1898 T>C	-1746 C>A	-1573 T>C	-1305 G>A	-1179 A>G	-1155 G>A	-1140 A>T	-1138 A>G	-1121 C>T	-964 A>G	-762 T>C	-725 C>G>T	-716 G>T	-689 G>A	-666 T>G	-646 A>G	-633 A>G	-546 *>G	-540 A>*	-509 C>G	-486 C>A	-483 A>G	-477 G>C	-400 G>A	-391 G>A	-369 A>C	-201 A>G	-56 C>T	2798 G>A	2804 G>T	2838 C>G	ex8 D>I	3003 T>C	3010 C>G	3027 C>T	3035 C>T	3142 C>G	3187 A>G	3196 C>G	3227 G>A	*Associated allele*	*GenBank accession number*	*N*	*Fq*
H01	T	C	T	G	A	G	A	A	C	G	C	C	T	A	G	A	G	*	A	C	A	A	C	G	G	C	G	C	G	G	C	D	T	G	C	C	C	G	C	G	*G*01:01:01:01*	MG825364	145	26.9
H10	C	A	C	A	G	G	T	A	C	A	T	C	G	G	T	A	A	*	A	C	C	A	G	G	G	A	A	C	A	T	G	I	T	C	C	C	G	A	G	G	*G*01:01:02:01*	MG825359	112	20.7
																																									*G*01:06*	MG825360	28	5.2
																																									*G*01:05N*	MG825361	12	2.2
																																									*G*01:01:02:02*	MG825362	3	0.6
H04	T	C	T	G	A	G	A	A	T	G	C	G	T	A	G	A	G	*	A	C	A	A	C	G	G	C	G	C	G	G	C	D	C	G	C	C	C	A	C	G	*G*01:01:01:05*	MG825356	46	8.5
H02	T	C	T	G	A	G	A	A	C	G	C	G	T	A	G	A	G	*	A	C	A	A	C	G	G	C	G	C	G	G	C	D	C	G	C	C	C	A	C	G		MG825358	38	7.0
H23	T	C	C	A	G	A	A	A	C	A	T	C	G	G	T	A	A	*	A	C	C	A	G	G	G	A	A	C	G	T	G	D	T	C	C	C	G	A	C	G	*G*01:04:01*	MG825349	37	6.9
																																									*G*01:04:04*	MG825348	7	1.3
H16	C	A	C	A	G	G	T	A	C	A	T	C	G	G	T	A	A	*	A	C	C	A	G	G	G	A	A	C	G	T	G	I	T	C	A	T	G	A	C	G	*G*01:01:03:03*	MG825347	22	4.1
H03	T	C	T	G	A	G	A	A	C	G	C	C	T	A	G	A	G	*	*	C	A	A	C	G	G	C	G	C	G	G	C	D	T	G	C	C	C	A	C	A	*G*01:01:01:04*	MG825363	22	4.1
H21	T	C	C	G	G	G	A	G	C	G	C	T	T	A	G	G	G	*	A	G	A	A	G	A	A	A	G	T	G	T	G	I	T	C	C	T	G	A	C	G	*G*01:03:01:02*	MG825350	8	1.5
H20	T	C	C	G	G	G	A	G	C	G	C	T	T	A	G	A	G	G	A	G	A	A	G	A	A	A	G	T	G	T	G	I	T	C	C	T	G	A	C	G		MG825354	8	1.5
H19	T	C	C	G	G	G	A	G	C	G	C	T	T	A	G	A	G	G	A	C	A	A	G	A	A	A	G	T	G	T	G	I	T	C	C	T	G	A	C	G		MG825357	7	1.3

Table 3
Characteristics of immunized and non-immunized anti-HLA class I and anti-HLA class II groups for *HLA-G* allele and haplotype frequencies; p-values under 0.001 are in bold.

	All	Anti-HLA class I non-immunized	Anti-HLA class I immunized	p-value	Anti-HLA class II non-immunized	Anti-HLA class II immunized	p-value
***HLA-G* allele carrier**							
G*01:01:01:01	49.3	51.8	45.0	0.172	52.0	41.4	0.105
G*01:01:01:02	0.4	0.0	1.0	0.370	0.0	1.4	0.256
G*01:01:01:03	0.7	1.2	0.0	0.396	1.0	0.0	0.553
G*01:01:01:04	8.5	11.2	4.0	0.031	11.5	0.0	**0.001**
G*01:01:01:05	28.5	26.5	32.0	0.202	28.0	30.0	0.396
G*01:01:01:06	0.4	0.0	1.0	0.370	0.0	1.4	0.256
G*01:01:02:01	38.1	38.2	38.0	0.537	38.0	38.6	0.477
G*01:01:02:02	1.1	1.8	0.0	0.248	1.5	0.0	0.411
G*01:01:03:03	8.5	8.8	8.0	0.503	9.5	5.7	0.252
G*01:01:05	0.4	0.6	0.0	0.630	0.5	0.0	0.744
G*01:01:12	0.4	0.6	0.0	0.630	0.5	0.0	0.744
G*01:01:15	0.4	0.6	0.0	0.630	0.5	0.0	0.744
G*01:03:01:02	8.1	8.2	8.0	0.571	6.5	12.9	0.075
G*01:04:01	16.3	15.3	18.0	0.338	16.0	17.1	0.453
G*01:04:04	3.0	2.4	4.0	0.337	2.0	5.7	0.118
G*01:05N	4.4	5.9	2.0	0.114	5.5	1.4	0.142
G*01:06	10.7	8.8	14.0	0.131	11.0	10.0	0.528
G*01:10	0.7	1.2	0.0	0.396	1.0	0.0	0.553
***HLA-G* haplotype carrier**							
H01	46.7	49.4	42.0	0.146	48.0	42.9	0.318
H02	14.1	12.4	17.0	0.189	13.0	17.1	0.233
H03	8.1	10.6	4.0	0.042	11.0	0.0	**0.001**
H04	16.7	15.9	18.0	0.386	16.5	17.1	0.492
H05	1.9	1.8	2.0	0.612	2.0	1.4	0.621
H06	0.4	0.0	1.0	0.370	0.0	1.4	0.256
H08	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H09	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H10	50.0	48.8	52.0	0.353	50.5	48.6	0.500
H11	0.4	0.0	1.0	0.370	0.0	1.4	0.256
H13	1.1	1.8	0.0	0.248	1.5	0.0	0.411
H16	8.1	8.2	8.0	0.571	9.0	5.7	0.293
H19	2.2	1.2	4.0	0.138	0.5	7.1	0.005
H20	3.3	4.7	1.0	0.094	3.5	2.9	0.585
H21	3.0	2.9	3.0	0.622	2.5	4.3	0.334
H23	16.3	15.3	18.0	0.338	15.5	18.6	0.312
H26	0.4	0.0	1.0	0.370	0.5	0.0	0.744
H28	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H31	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H32	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H36	0.7	0.6	1.0	0.604	0.5	1.4	0.447
H38	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H44	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H46	1.1	0.6	2.0	0.309	1.5	0.0	0.411
H48	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H49	0.4	0.0	1.0	0.370	0.0	1.4	0.256
H52	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H53	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H54	0.7	0.0	2.0	0.136	1.0	0.0	0.553
H56	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H58	0.4	0.0	1.0	0.370	0.0	1.4	0.256
H61	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H63	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H71	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H72	0.4	0.6	0.0	0.630	0.5	0.0	0.744
H73	0.4	0.0	1.0	0.370	0.0	1.4	0.256

Table 4
Associated factors for anti-HLA class II immunization according to logistic regression on number of pregnancies and HLA-*G*01:01:01:04* (HR, hazard ratio; CI, confidence interval).

	Estimated HR (95% CI)	p-value
Pregnancies	1.725 (1.339-2.223)	0.0001
HLA-G*01:01:01:04	0.049 (0.003-0.899)	0.0422

events: half to two thirds of pregnant women seem to develop anti-HLA antibodies [11,13].

HLA-G is extensively reported to play a pivotal role in fetomaternal tolerance and transplantation outcome [1]. We previously showed that *HLA-G* haplotypes, i.e. SNPs associated in Linkage Disequilibrium, (1) remain preserved between populations with different geographical origins, (2) can reliably be used to predict sHLA-G levels, (3) are associated with alloimmunization and inflammation in transplantation and inflammatory disease and (4) are grouped into four main clades, three of which contain alleles associated with lower immunotolerance whereas the fourth seems to be associated with higher immunotolerance [4,5,10,21].

In this study, we aimed to confirm whether *HLA-G* haplotypes could be a predictive genetic risk or protective factor for anti-HLA immunization and may help clinicians to estimate the risk of rejection in

each single case, while considering the consequences of performing the graft or not.

We thus investigated *HLA-G* haplotype association with anti-HLA immunization in women having experienced pregnancy but with no transfusion or transplant history.

We performed *HLA-G* genetic analysis on 270 maternal blood samples collected at delivery and tested for anti-HLA antibodies. Statistical analysis included variables reported to be associated with anti-HLA immunization such as the number of pregnancies, number of children, number of miscarriages, HLA-A and HLA-B antigen typing and paternal HLA-A and HLA-B mismatch.

We found that H03-HLA-G*01:01:01:04 was the only genetic variable to remain significantly associated with anti-HLA class II immunization after Bonferroni correction ($p < 0.001$). Its protective value against anti-HLA class II immunization remained significant in multivariate analysis including the number of pregnancies ($p = 0.0422$). However, it should be noted that since only 22 women presented *HLA-G*01:01:01:04*, an accidental better HLA class II matching of mothers and children in this group is not excluded. Furthermore, when anti-HLA class I immunization is considered, for which latest child/paternal mismatch displayed no significant results, this same HLA-G allele was found to be protective ($p = 0.031$) but it was not included in multivariate analysis because of Bonferroni correction.

Although this study was not designed to decipher the biological mechanism leading to our results, it seems to support our previous work on phylogenetic analysis of *HLA-G* alleles defined at high resolution. Phylogenetic structure of *HLA-G* sequences consisted of four main clades which may reflect HLA-G tolerogenic properties; indeed each clade displayed specific transcription factor sites and coding sequence variations [21]. The conservation of *HLA-G* sequences worldwide suggests that those which lowered immunotolerance might provide an advantage in specific contexts [27]. Tolerogenicity would come from HLA-G binding with the ILT-2 receptor on B cells and thus limiting their proliferation, differentiation and immunoglobulin secretion [28].

In pregnancy, reduced expression of HLA-G seemed to be associated with complications, such as miscarriage or preeclampsia. Interestingly, women with preeclampsia harbor higher concentrations of cellular fetal microchimerism and anti-paternal allo-antibodies [29,30].

Masson *et al.* showed, in the same cohort as this study, that anti-HLA immunization increased with the number of pregnancies and of children delivered but they could not find any association with HLA-A and HLA-B antigens. Only the -174 G/C polymorphism in the *IL-6* promoter (located on chromosome 7) was associated with a lower risk of anti-HLA antibody development among primiparous pregnant women with no history of miscarriage [20]. Our analysis further confirmed their main results. We did not find any association with HLA-A, HLA-B or their paternal mismatch, on the contrary to Piacasdia *et al.* who showed that HLA-B*14 and HLA-B*51 were associated with a lower risk of anti-HLA antibody development after pregnancy, while having the A*11 allele seemed to represent a higher risk in a cohort of 161 pregnancy-only sensitizing events [18].

This preliminary study seems to confirm the role of HLA-G in inflammation and immune control; it needs however to be completed by a larger number of patients to get a better insight into the nature of the association with each individual HLA locus. We previously reported a strong linkage disequilibrium of both *HLA-A* and *HLA-F* with *HLA-G* [26]; such extended HLA class I haplotypes may represent even more pertinent predictive values, notably HLA-F which is shown to play an important part in immune regulation [31]. This descriptive study would also benefit from investigating into biological mechanisms leading the association of *HLA-G* phylogeny with differential immunological tolerance induction.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

Conception or design of the work: MS, CP, JC, PT, JMR, JDC

Acquisition, analysis and interpretation of data: MS, CP, JC, AD, JP, PT, JMR, JDC

Drafting the work or revising it critically for important intellectual content: MS, CP, JC, AD, JP, PT, JMR, JDC

Final approval of the version to be published MS, CP, JC, AD, JP, PT, JMR, JDC

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: MS, CP, JC, AD, JP, PT, JMR, JDC

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