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Donor derived HLA-G polymorphisms have a significant impact on acute rejection in kidney transplantation

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ABSTRACT

Human leucocyte antigen G (HLA-G) is a non-classical HLA-class I antigen that exerts immunoregulatory functions. The polymorphisms 14-base pair (bp) insertion/deletion (ins/del) (rs1704) and +3142C > G (rs1063320) could modify the expression level of HLA-G.

We genotyped 175 kidney recipients (41 with acute rejection and 134 without rejection) and additionally the corresponding donors for both polymorphisms in order to assess their impact on acute rejections one year after transplantation. In addition, we analyzed soluble HLA-G (sHLA-G) levels in sera of 32 living kidney donors and compared the sHLA-G levels in terms of the present genotype.

In kidney transplant recipients we did not observe an impact of the 14-bp ins/ins and the +3142GG genotypes on acute rejection. In contrast, we found a higher frequency of these genotypes in the donors of the no-rejection collective compared to the rejection collective (4.9% vs. 24.6%; $p = 0.010$; 9.8% vs. 31.3%; $p = 0.006$). Soluble HLA-G levels were highest in healthy kidney donors homozygous for the 14-bp insertion.

We conclude that the HLA-G polymorphisms of the donor are of importance for susceptibility of acute rejection in kidney transplantation. We suggest that the 14-bp ins/ins and the +3142GG genotypes are protective against kidney transplant rejection.

1. Introduction

The checkpoint molecule HLA-G exerts a key role in immunomodulation due to its tolerogenic features. In solid organ transplantation enhanced HLA-G expression has been widely associated with the prolongation of allograft survival and fewer episodes of acute rejections [1–9].

HLA-G differs from classical HLA-class I molecules by a limited number of allele variations and a limited tissue distribution. HLA-G is expressed in 7 different isoforms due to alternative splicing [10]. The membrane-bound as well as the soluble isoforms exert its immunomodulatory properties through binding four currently known receptors [11,12]: the leukocyte immunoglobulin-like receptors LILRB1/ILT-2/CD85j expressed on lymphoid as well as myeloid cells and LILRB2/ILT-4/CD85d expressed on myeloid cells [12], the killer immunoglobulin-like receptor KIR2DL4/CD158d expressed on NK-cells

Abbreviations: APC, antigen presenting cells; ASRA, allele-specific restriction analysis; bp, base pair(s); C, cytosine; cANCA, cytoplasmic anti-neutrophil cytoplasmic antibodies; CD, cluster of differentiation; CI, confidence interval; CNI, cyclosporine inhibitors; dATP, desoxyadenosine triphosphate; dCTP, desoxycytidine triphosphate; del, deletion; dGTP, desoxyguanosine triphosphate; dNTP, desoxynucleoside triphosphate; dTTP, desoxythymidine triphosphate; FOXP3, Forkhead-Box-Protein P3; G, guanine; HLA, human leucocyte antigen; IL, interleukin; ins, insertion; KIR, killer immunglobulin-like receptor; LILR, leukocyte immunglobuline-like receptor; M, mean; MMF, mycophenolate mofetil; mMol, millimolar; mTOR, mammalian target of rapamycin; ng, nanogramms; NK, natural killer; OR, odds ratio; pANCA, perinuclear anti-neutrophil cytoplasmic antibodies; PCR, polymerase chain reaction; pg, picogramms; pMol, picomolar; SD, standard deviation; sHLA-G, soluble HLA-G; SNP, single nucleotide polymorphisms; Treg, regulatory T cell; U, units; UTR, untranslated region

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and the receptor CD160 which occurs on endothelial cells. By binding these inhibitory receptors HLA-G downregulates the cytotoxic activity of NK-cells and of $CD8^+$ T-cells [13] and the alloproliferative responses of $CD4^+$ T-cells [14]. HLA-G is even capable to induce a new type of regulatory T-cells (Tregs) as well as the classical $CD25^+FOXp3^+$ Tregs [15,16]. Moreover, a new tolerable HLA-G^+ generation of dendritic cells producing high amounts of IL10 - the so called DC10 - have been described [17].

The 14-base pair (bp) insertion/deletion (ins/del) polymorphism (rs1704) and the +3142C > G single nucleotide polymorphism (SNP) (rs1063320), both located in the 3′ untranslated region (UTR), seem to be of crucial importance for HLA-G expression. Data obtained in 2003 reveal that the 14-bp insertion leads due to a splice out of the first 92 bp at mRNA-level to a more stable mRNA transcript [18]. Subsequently it has been shown, that due to the presence of the insertion more HLA-G1 is expressed in vitro [19]. However, most studies reported lower soluble HLA-G (sHLA-G) levels for individuals expressing the insertion allele [20–23]. The 3142C > G SNP, also located in the 3′UTR reveals a potential binding site for microRNAs modifying the expression level of HLA-G [24].

One study assessing the relevance of the +3142C > G SNP in kidney transplant patients could not find an association with the occurrence of acute rejections [25]. Another recently published study could not reveal an influence of the +3142C > G SNP on the occurrence of acute rejections as well - neither of the recipients nor of the donors genotype but showed an association of the patients CC genotype with a reduced allograft survival and a susceptibility to CMV-infection [26]. Results concerning the 14-bp ins/del polymorphism are heterogeneous [9,27–31].

Since HLA-G is expressed on the engrafted kidney [32–36], it is conceivable that the polymorphic sites of the donor could exert an influence on the occurrence or the extent of rejection episodes. The present study aimed at assessing the association between the 14-bp ins/del polymorphism and the +3142C > G SNP and the occurrence of rejection episodes after kidney transplantation.

2. Subjects and methods

2.1. Subjects

The present study includes 175 adult patients who underwent allograft kidney transplantation. Furthermore, we investigated the corresponding 175 kidney organ donors. Patients under the age of 18 years were excluded. Individuals who were of AB0-incompatibility regarding their organ donation were excluded as well. The patients received either a living or a deceased organ donation. All patients were transplanted between January 2009 and December 2013 at the Department of Hepatobiliary and Transplant Surgery of the University Medical Center Hamburg-Eppendorf. The included patients were predominantly Caucasian (> 90%). All subjects gave informed consent for study participation. The study was approved by the local ethics committee (PV3248).

Demographic and relevant clinical data are presented in Table 1. All patients received standard triple drug immunosuppression consisting of calcineurin inhibitors (CNI), an antiproliferative drug [mycophenolate mofetil (MMF) or an mTOR inhibitor] and steroides. Standard induction therapy was conducted with basiliximab. Patients with a higher immunological risk only received antithymocyte globulin.

According to the development of rejection episodes kidney transplant patients were divided into two groups. The first group consisted of 41 patients (23%) who had experienced an acute rejection (rejectors) within the first year post transplantation, while the remaining 134 kidney transplant patients (77%) did not experience any episode of rejection within at least one year after organ transplantation (no-rejectors). Group allocation of the corresponding kidney donors was also based on the rejection status of the organ recipients.

Table 1
Demographic and clinical characteristics of kidney transplant recipients.

	Acute rejection (n = 41)		No rejection (n = 134)		
	M/n	*SD/(%)*	*M/n*	*SD/(%)*	*p-Value*
Mean age (years)	53.10	± 15.274	53.27	± 16.062	0.953
PRA max	11.00	± 23.901	4.93	± 16.435	0.134
PRA at time of transplantation	6.56	± 21.369	1.20	± 9.584	0.126
Gender					
male/female	29/12	(70.7/29.3)	90/44	(67.2/32.8)	0.112
HLA-mismatches					0.114
HLA-A 0/1/2	12/21/7	(30.0/52.5/17.5)	31/68/31	(23.8/52.3/23.8)	0.533
HLA-B 0/1/2	2/23/15	(5.0/57.5/37.5)	24/69/37	(18.5/53.0/28.5)	0.044
HLA-DR 0/1/2	6/21/13	(15.0/52.5/32.5)	41/66/23	(31.5/50.8/17.7)	0.045
Type of transplantation					
Living/deceased organ donation	21/20	(51.2/48.8)	49/85	(36.6/63.4)	0.201

M, mean; SD, standard deviation; MHC, major histocompatibility complex; PRA, panel reactive antibodies.

Biopsies were performed based on clinical indication (indication biopsy), no protocol biopsies were done. All rejections were biopsy proven and treated. All suspected rejections were retrospectively reviewed by one of the authors (MK) for conclusive response to anti-rejection treatment. Cellular, vascular and humoral rejections were included and not differentiated due to the low numbers. Patients with unclear histological results (e.g. borderline rejection) were not included in the study.

2.2. Genotyping of the HLA-G 14-bp ins/del polymorphism and the +3142C > G SNP

We genotyped all 175 kidney recipients as well as the organ donors for the mentionend HLA-G gene polymorphisms. DNA was extracted from peripheral blood samples using the innuPREP Blood DNA mini Kit (Analytik Jena Biometra, Jena, Germany) according to the instruction manual. The HLA-G 14-bp ins/del polymorphism was genotyped by polymerase chain reaction (PCR) performed by a Thermocycler (Biometra GmbH, Göttingen, Germany) as previously described [9].

The +3142C > G SNP was genotyped as previously described [9,37]. The selected Real-Time Endpoint genotyping analyses were performed using the Lightcycler 480 System (Roche, Mannheim, Germany). All samples were run in duplicates.

2.3. Detection of sHLA-G

Of our 175 kidney donors we selected 32 healthy living donors to determine the serum level of sHLA-G and compared them with the donor's genotype (ins/ins = 5; ins/del = 11; del/del = 16). Sera were obtained from peripheral blood vessels before donation and stored until usage at −80 °C. The levels of soluble HLA-G5 and shedded HLA-G1 were determined using a commercial sHLA-G enzyme-linked immunoabsorbant assay kit (Exbio, Praha, Czech Republic) according to the instruction manual. Each sample was analyzed in duplicates. Absorbance was measured at a wavelength of 450 nm with a reference wavelength of 630 nm. A standard curve was constructed by plotting the measured absorbance against the concentration of calibrators prepared on the basis of a dilution series. According to this calibration curve, sHLA-G levels were defined. The minimum possible detection level was 1.3 ng/ml.

2.4. Statistical analysis

Compliance of the observed genotype frequencies to the expected genotype proportions according to the Hardy-Weinberg-Equilibrium was checked by Chi-square test for goodness of fit, using HAPLOVIEW v4.1 (Broad Institute of MIT and Harvard, Boston, USA).

Differences in age and all other demographic and clinical variables were compared between the two groups. Comparisons of dichotomous variables such as genotype, gender, underlying HLA-mismatches were performed using two-sided Fisher's exact test, while age and sera concentration as continuos variables were analyzed using Students' *t*-test.

The recipients' and the donors' genotypes were compared between the acute rejection group and the no-rejection group concerning the HLA-G 14-bp ins/del and the +3142C > G polymorphisms by a binary logistic regression. Probabilities of graft rejection within one year and associations of the polymorphisms and allograft loss are calculated with log rank tests and results are delineated in kaplan meier survival functions.

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Chicago, IL, USA), release 24.0 for Macintosh. Haplotype analyses were performed using the program unphased [38]. The power calculation was conducted by G*Power 3.1 (Institute for Experimental Psychology, Heinrich Heine University, Düsseldorf, Germany) according to the programmer's instruction manual. We corrected for multiple testing by applying the procedure of the False Discovery Rate according to Benjamini and Hochberg [39].

3. Results

3.1. Results of genotyping for HLA-G polymorphisms

In the present study we genotyped kidney transplant recipients ($n = 175$) and the related donors for the 14 bp ins/del polymorphism and the +3142C > G SNP. Genotype frequencies of donors and recipients complied with the expectations of Hardy-Weinberg equilibrium.

No significant differences between the acute rejection group and the group of no-rejectors were found concerning gender, age, or type of organ donation (Table 1). Nevertheless, significant differences were revealed concerning HLA-B as well as HLA-DR mismatches. Mismatches in HLA loci are a well known risk factor for acute rejection. In order to control for possible influences we performed further analyzes concerning genotype distributions by a binary logistic regression. 70.7% of the probands of the acute rejection collective underwent the first rejection episode within the first 3 months after solid organ transplantation, whereas the remaining 29.3% of the rejection group developed the first acute rejection after 3 months but within the first year post transplant. 80.5% of the probands of the acute rejection collective underwent only one acute rejection episode, whereas the remaining 19.5% experienced multiple episodes.

Recipients' and donors' genotype and allele distributions in the rejection as well as in the no-rejection group concerning the 14-bp ins/del polymorphism and the +3142C > G SNP are shown in Table 2. In organ donors the 14-bp ins/ins genotype occurs significantly more often within the no-rejection group than within the rejection group (OR = 0.142; CI95% = 0.032–0.627; $p = 0.010$). This result reached a power of 91.7%. The del/del genotype did not show any significant differences between the rejection and the non-rejection group. Also, no differences were found concerning the distribution of the alleles in the 14-bp ins/del polymorphism between the two groups. Probability of acute rejection was significantly lower for kidney transplants with the ins/ins genotype compared to either the donor's del/del or the ins/del genotype as presented in a kaplan meier curve (Fig. 1, log rank test: $p = 0.010$).

Concerning the +3142C > G SNP, patients of whom the donors' genotype was homozygous for the G belonged more often to the collective of non-rejectors (OR = 0.210; CI95% = 0.069–0.635; $p = 0.006$). This result reached a power of 88.6%. Moreover, probability of acute rejection was significantly lower for the donor's genotype +3142GG compared to either the CC or the CG genotype as presented in a kaplan meier curve (Fig. 2, log rank test: $p = 0.012$).

Regarding the allele frequencies, the C allele was found more often within the collective of rejectors (OR = 2.001; CI95% = 1.181–3.391; $p = 0.010$), this difference was even significant after correcting for multiple testing.

HLA-DR but not HLA-B mismatches became significant as a risk factor for rejection though not as significant as genotype distributions between the rejection and the no rejection group of both investigated polymorphisms ($p = 0.030$).

The results of our haplotype analyses partly support our genotyping results. Estimated haplotype frequencies and *p*-values are shown in Table 3. The haplotype composing of the 14-bp del allele combined with the +3142C allele was overrepresented in the rejection group ($p = 0.009$). Indeed, combination of both genotypes - the 14-bp del and the +3142C – indicates a higher risk of an acute rejection than both genotypes alone do ($p = 0.051$; $p = 0.010$). The opposite haplotype consisting of 14-bp ins allele and the +3142G allele was more frequent in the no-rejection collective if compared to the rejection group even though this comparison did not become significant ($p = 0.091$).

In contrast to the differences described concerning kidney donors, no significant differences were observed in the recipients' genotypes or the allele frequencies between the rejection and no-rejection group.

None of both donor polymorphisms had an influence on time of graft loss. Nevertheless, as shown in Fig. 3 there seems to be a slight tendency for the 14 bp ins/ins leading to better graft survival without reaching statistical significance.

Table 2
The genotype and allele frequencies of the 14 bp Ins/Del (rs1704) and +3142C > G (rs1063320) polymorphisms in kidney transplant recipients and in the concerning donors.

	Rejection ($n = 41$) *n (%)*	No Rejection ($n = 134$) *n (%)*	*p*	*OR*	*CI (95%)*
Donors					
14 bp Ins/Del (rs 1704)					
Del/Del	14 (34.1)	40 (29.9)	0.791	1.116	0.496–2.508
Ins/Ins	2 (4.9)	33 (24.6)	0.010	0.142	0.032–0.627
Ins/Del	25 (61.0)	61 (45.5)	0.130	1.756	0.847–3.642
Del	53 (64.6)	141 (52.6)	0.051	1.701	0.997–2.903
Ins	29 (35.4)	127 (47.4)			
+3142C > G (rs 1063320)					
C/C	13 (31.7)	30 (22.4)	0.107	1.935	0.867–4.321
G/G	4 (9.8)	42 (31.3)	0.006	0.210	0.069–0.635
C/G	24 (58.5)	62 (46.3)	0.205	1.599	0.774–3.300
C	50 (61.0)	122 (45.5)	0.010	2.001	1.181–3.391
G	32 (39.0)	146 (54.5)			
Recipients					
14 bp Ins/Del (rs 1704)					
Del/Del	13 (31.7)	47 (35.1)	0.932	0.966	0.440–2.120
Ins/Ins	6 (14.6)	28 (20.9)	0.511	0.720	0.270–1.917
Ins/Del	22 (53.7)	59 (44.0)	0.558	1.248	0.594–2.620
Del	48 (58.5)	153 (57.1)	0.924	1.062	0.608–1.730
Ins	34 (41.5)	115 (42.9)			
+3142C > G (rs 1063320)					
C/C	8 (19.5)	38 (28.4)	0.471	0.719	0.293–1.762
G/G	7 (17.1)	40 (29.9)	0.126	0.465	0.175–1.241
C/G	26 (63.4)	56 (41.8)	0.055	2.120	0.984–4.567
C	42 (51.2)	132 (49.3)	0.633	1.134	0.677–1.898
G	40 (48.8)	136 (50.7)			

After correcting for multiple testing using the Benjamini and Hochberg approach *p*-values below 0.019 were considered as significant.
OR, odds ratio; CI, confidence interval; bp, base pairs; Ins, insertion; Del, deletion; C, cytosine; G, guanine.

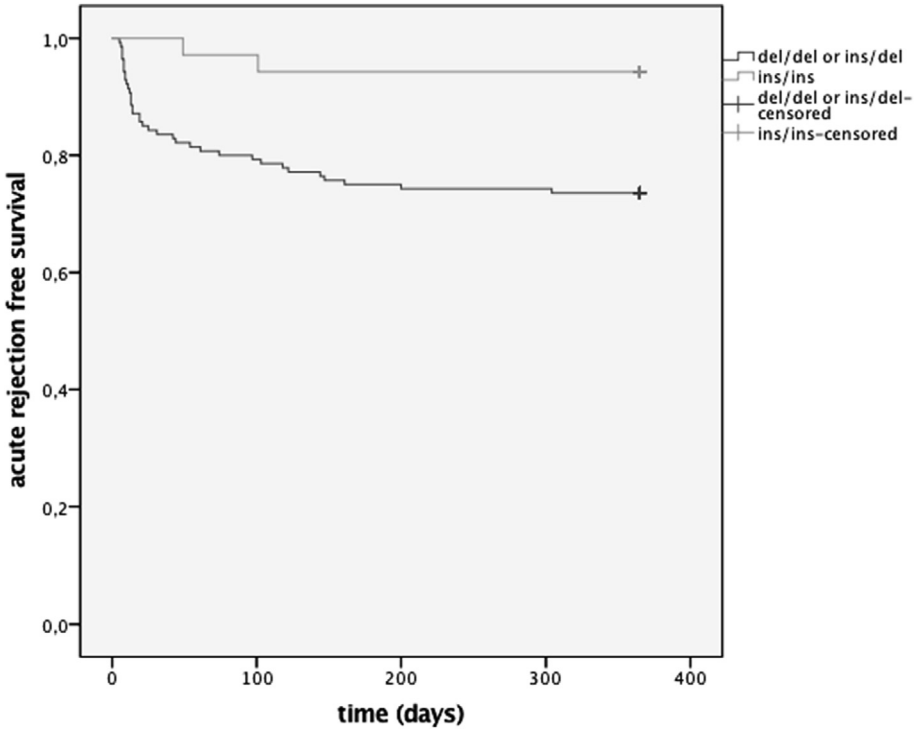

Fig. 1. Kaplan Meier curve analysis and corresponding log rank test for the probability of graft rejection within one year for the 14-bp polymorphism of the donors ($p = 0.010$).

3.2. Results of sHLA-G sera levels

Fig. 4 displays our results regarding sHLA-G levels in living kidney donors in correlation to HLA-G 14-bp ins/del genotypes. Mean serum concentrations of living donors with the 14-bp ins/ins genotype were significantly higher (mean: 43.65 ng/ml; SD: 42.87 ng/ml) than those of living donors with the del/del genotype (mean: 10.28 ng/ml; SD: 5.16 ng/ml; $p = 0.004$) or ins/del genotype (mean: 12.99 ng/ml; SD: 15.86 ng/ml; $p = 0.05$).

4. Discussion

The present study shows a significant association between the 14-bp ins/ins genotype and the +3142GG genotype of the organ donor and

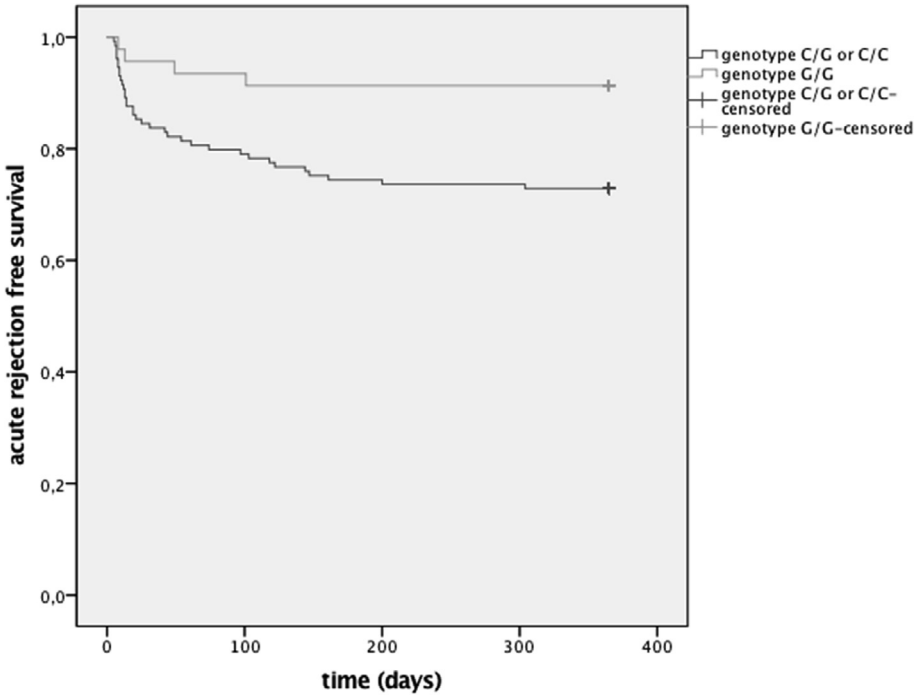

Fig. 2. Kaplan Meier curve analysis and corresponding log rank test for the probability of graft rejection within one year for the +3142C > G SNP of the donors ($p = 0.012$).

Table 3
Results of haplotype analysis in donors.

HLA-G haplotype	Rejection (n = 82) *n (%)*	No Rejection (n = 268) n (%)	*p*	*OR*	*CI (95%)*
Del-C	50.0 (61.0)	118.7 (44.3)	0.009	1	1–1
Del-G	3.0 (3.7)	23.25 (8.7)	0.139	0.3064	0.08795–1.067
Ins-G	29.0 (35.4)	122.7 (45.8)	0.091	0.5611	0.3328–0.9461

Haplotype HLA-G polymorphism order: *rs1704 - rs1063320*. Haplotypes with overall frequency beneath 5% are not shown. After correcting for multiple testing using the Bonferroni approach *p*-values below 0.02 were considered as significant. OR, odds ratio; CI, confidence interval; Ins, insertion; Del, deletion; C, cytosine; G, guanine.

the absence of acute rejections in kidney transplantation. However, we found no association between the genotypes of the recipients and the occurrence of acute rejections.

A beneficial role on prolonged graft survival has been reported for HLA-G expression [1–9]. In this context it should be mentioned that expression of HLA-G on kidney epithelial cells has also been widely described [32–36]. The up-regulation of HLA-G on the surface of the kidney tubular epithelial cells due to the presence of the 14-bp ins/ins genotype could provide a mechanism of immunologic protection, since cytotoxic $CD8^+$ T-cells express the immunosuppressive antigen CD94 NKG2A and CD85j as ligands [40]. Due to an up-regulated HLA-G expression, the interaction with CD85j and probably CD94 NKG2A is higher, which in turn leads to a greater suppression of alloreactive cytotoxic $CD8^+$ T-cells [41,42] and thus to a reduction of acute rejections. NK-cells express CD94 NKG2A as well [40]. It has been observed in NK cytotoxicity assays that the cell specific lysis mediated through NK-cells was significantly lower if the cells were of the 14 bp ins/ins genotype [19]. Unfortunately we couldn't find an association of any of the investigated genotypes on graft survival what may be due to the relatively small collectives (Fig. 3). Nevertheless, in context of acute allograft rejection we could reveal an association between the 14-bp ins/ins genotype and the +3142 GG genotype and the absence of acute rejections.

Our results concerning the sHLA-G sera levels underline possible associations of the 14-bp ins/ins genotype and higher HLA-G or sHLA-G levels depending on the context. In healthy probands this genotype is associated with highest sHLA-G sera concentrations. These findings are in contrast to other studies describing either no association or an association between higher levels of sHLA-G and the 14-bp del/del genotype [20–23,37,43–51]. In terms of solid organ transplantation this discrepancy might be explained by the fact that most studies determined sHLA-G levels in patients suffering from autoimmune diseases or post-transplantation. The underlying disease or the immunosuppressive treatment is likely to influence sHLA-G levels. Nevertheless, Svendson et al. showed in vitro an increased surface expression of HLA-G1 for mRNA from the 14-bp insertion but also that the ratio of released sHLA-G to surface expressed HLA-G1 is lower [19]. This is in line with almost all data obtained in the field of feto-maternal medicine. Low levels of sHLA-G have been widely associated with the ins/ins genotype in pregnant women [44–47]. A recent study described high levels of sHLA-G, partly released in exosomes, in 14-bp deletion variants [48]. Rizzo et al. found the highest sHLA-G production in patients with multiple sclerosis that showed the 14-bp del/del genotype as well as the +3142 CC genotype. Lowest amounts were on the other hand found in patients with the ins/ins and the GG genotypes [37]. Furthermore, for other autoimmune diseases such as Crohn's disease the patients with del/del genotype have been reported to be high sHLA-G producers [49]. Moreover, in healthy individuals highest production of soluble HLA-G have been described for the del/del genotype [50].

In contrast to the results of the present study, we could not observe a significant association between the genotype of the donor and acute rejections in the setting of liver transplantation in our previously published study [9]. Differences between liver and kidney transplant patients concerning the associations described above may lay in different expression patterns of HLA-G described for both organs. With particular regard to kidney transplantation, expression was detected in tubular epithelial cells after kidney and combined liver-kidney transplantations [1–3,32–36]. According to Creput et al. 55.5% of the tubular epithelial

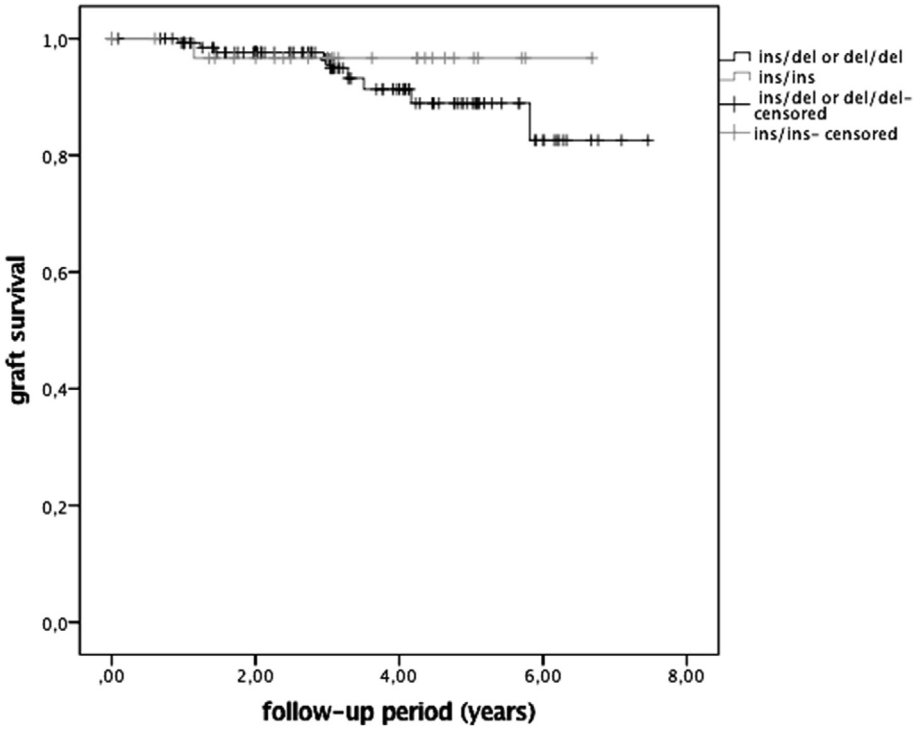

Fig. 3. Survival function of graft loss and 14-bp polymorphism of the donors (p = 0.497).

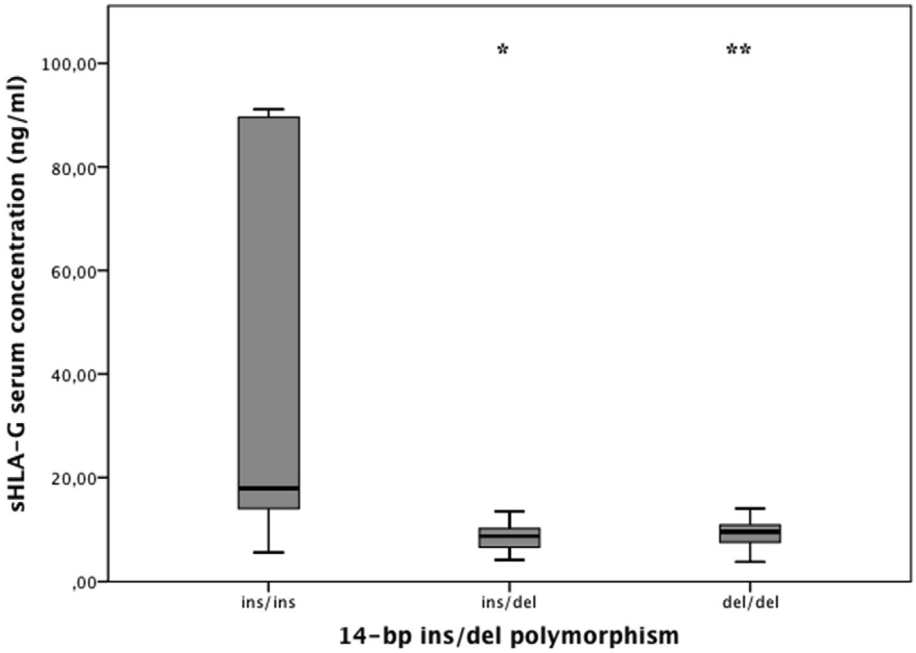

Fig. 4. Box-plot serum concentrations of sHLA-G (HLA-G1 and HLA-G5) in living kidney donors pre-transplantation. Median values are indicated by horizontal lines. *ins/ins vs. ins/del, $p = 0.050$; ** ins/ins vs. del/del, $p = 0.004$.

cells of the transplanted kidney express HLA-G [2]. In contrast to kidney tissue a study of Le Rond et al. [43] described no detection of HLA-G expression on liver cells one year after liver transplantation. According to our results we suppose that the engrafted kidney may also be able to produce higher amounts of membrane bound HLA-G.

Our observations in the setting of kidney transplantation are in line with the results of the studies performed by Alves, Aghdaie and Piancatelli [25,30,51]. Recently published data by the Rebmann group go in the same direction as our findings as they found an association of the CC genotype of the recipients and an increased risk of five-year allograft loss [26]. Nevertheless, they did not reveal an association of the donor's genotype neither with rejection episodes nor CMV re-activations even though there was borderline significance concerning the risk of allograft loss. Our results differ from those reported by Misra and Jin [28,29]. However, Misra and Jin included exclusively living organ donors - a fact in which their collective differs from ours since we included living as well as deceased organ donors. Moreover, the ethnic origin of the patients included by Misra and Jin differ from the ethnic origin of our cohort, which could have possibly influenced the findings as well. In this context, it should be mentioned that for the +3142C > G SNP the C in the European population has a frequency of 46%, whereas in the Japanese population its frequency is about 73% [52].

None of the currently existing studies conducted a power analysis. Therefore, the validity of previously published results is difficult to evaluate. We constantly presented a statistically significant power level above 80% indicating a high validity level. Nevertheless, we cannot exclude that we missed clinically relevant results due to a low number of patients, e.g., for the kidney donors. The small sample size is the most important limitation of our study. This is most obvious in our collective of healthy kidney donors. Due to the fact that data according to the clinical relevance of HLA-G is still controversial, studies capable of including more probands are needed.

HLA-DR mismatches became significant according to the experience of at least one acute rejection episode. Mismatches are a well known risk factor in solid organ transplantation for acute rejections. Nevertheless, in multivariate analyses the investigated polymorphisms seem to have a stronger impact on acute rejections than mismatches in HLA-DR.

In our study we included living as well as deceased organ donors. It has to be noted that in the no-rejection collective the deceased donations are overrepresented. Nevertheless, no significant difference could be observed concerning the probability of acute graft rejections between both groups.

We found exclusively a combined association for the 14-bp ins/ins genotype and the +3142GG genotype that displays a linkage disequilibrium, which was previously described for the 14-bp ins/ins genotype and the +3142GG genotype [53].

In summary, our results show that the 14-bp ins/ins genotype is associated with higher levels of sHLA-G in living kidney donors and that the +3241GG genotype and the 14-bp ins/ins genotype of the HLA-G gene are associated with the absence of acute rejection in the setting of kidney transplantation, even though we could not reveal an association of any of the investigated genotypes on graft survival. Thus, both investigated polymorphisms seem to be of crucial importance for the prevention of acute rejection following kidney transplantation.

Conflict of interest

The authors declare no conflicts of interest.

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