# Evaluation of Plasma Levels of Soluble HLA-G and HLA-G Genotypes in Kidney Transplant Recipients

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## ABSTRACT

In the field of transplantation, expression of HLA-G, a nonclassical HLA molecule with immunosuppressive functions and limited gene polymorphism, is considered beneficial for graft acceptance; various studies have aimed to demonstrate this role in transplantation. Recently, in other clinical conditions, it has been observed that insulin resistance was associated with HLA-G14bpins/del polymorphism, the most studied regulatory polymorphism of this molecule. In the present study, plasma levels of the soluble form of HLA-G (sHLA-G) were analyzed in kidney transplant recipients (n = 103) with different HLA-G14bpins/del genotypes. In a group of 26 recipients, sHLA-G was detected before and after transplantation (1 year) to evaluate early variations. In 77 recipients, sHLA-G was detected after transplantation (3-24 years) and correlated with occurrence of long-term post-transplant morbidity (diabetes mellitus, hyperlipidemia, hypertension, obesity, etc.).

Methods. Levels of sHLA-G were measured in plasma with an enzyme-linked immunosorbent assay; HLA-G14bpins/del and HLA-G+3142C>G genotypes were assessed using direct polymerase chain reaction.

Results. Plasma levels of sHLA-G significantly decreased during the first year after transplantation ($P = .019$); no significant correlations were found with genotypes or early post-transplant events. Lower levels of sHLA-G were found in recipients with post-transplant diabetes mellitus or obesity carrying the HLA-G14bpins/ins ($P = .006$ and $P = .003$, respectively) or HLA-G+3142G/G genotypes.

Conclusions. A complex modulation of HLA-G, which includes both immunologic and metabolic effects, could affect the risk for long-term post-transplant morbidity in kidney transplant recipients. Associations of HLA-G, diabetes, and obesity deserve to be investigated by deeply exploring HLA-G regulatory variants.

HLA-G is a nonclassical HLA molecule involved in immunosuppression and tolerance; its expression is genetically regulated. HLA-G polymorphism and post-transplant monitoring of soluble HLA-G molecules (sHLA-G) have a potential interest in kidney transplantation. HLA-G14bpins/del polymorphism was extensively studied in several diseases and transplantation; it is known to affect mRNA stability and protein expression. HLA-G+3142C>G is a target for microRNA (miRNA); the presence of a G, by increasing miRNA stability, was associated with a lower protein production [1]. In transplantation, sHLA-G has been primarily analyzed in relation to allograft rejection; a positive modulation in response to

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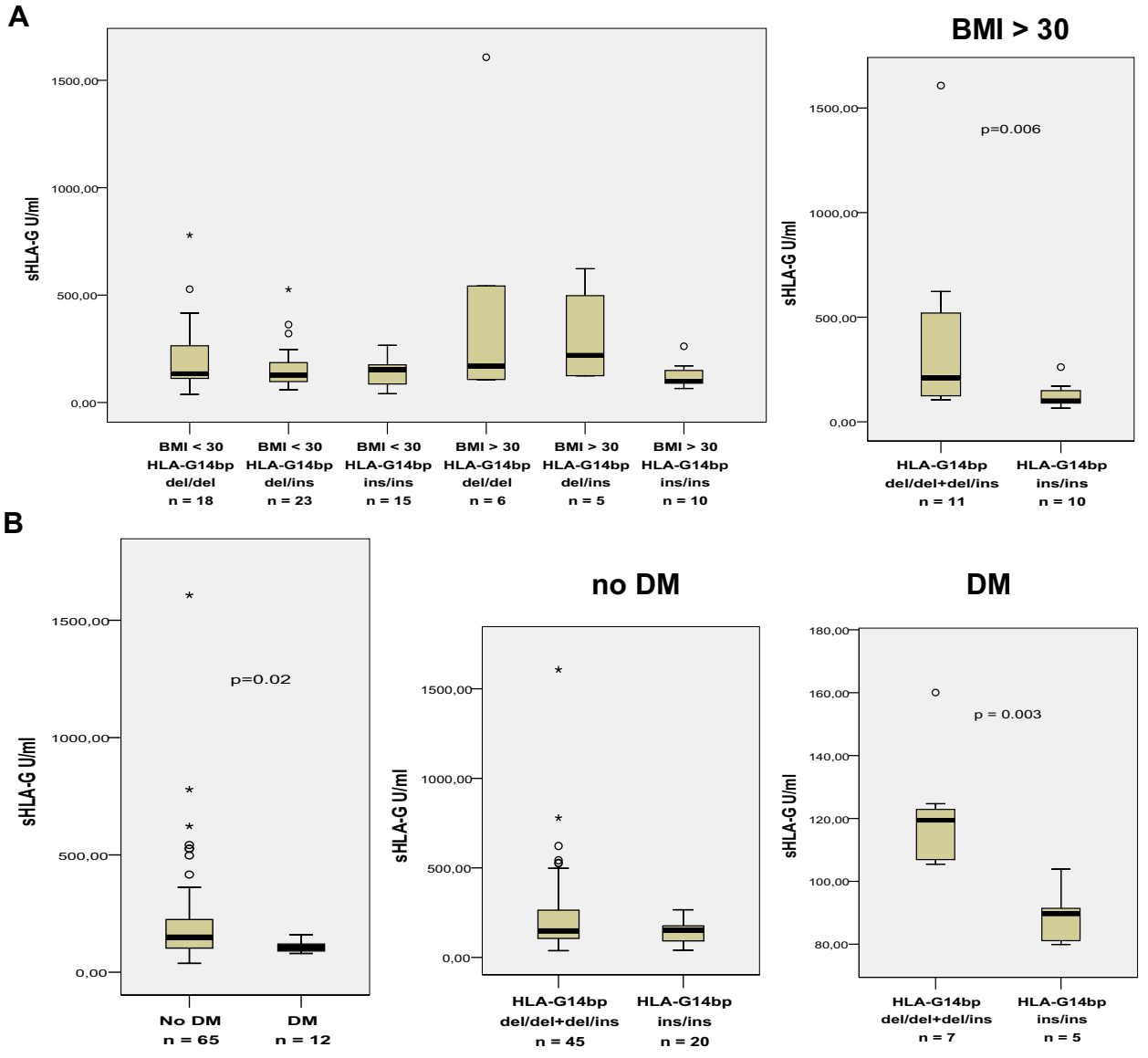


**Fig 1.** Box plot of post-transplant sHLA-G, HLA-G genotypes, and **(A)** obesity or **(B)** diabetes in kidney transplant recipients. Median levels are indicated by horizontal lines; quartiles and extreme values are shown. Significantly lower plasma sHLA-G levels were observed in **(A)** obese recipients (body mass index $> 30$), and in **(B)** recipients with DM (type 1 DM, n $= 3$, post-transplant DM, n $=$ 9) carrying the HLA-G14bp ins/ins ($P = .006$ and $P = .003$, respectively) or the $+3142$G/G genotype ($P = .030$ and $P = .041$, respectively, not shown). DM, diabetes mellitus.

inflammation in allograft acceptance was previously detected, while differential levels of sHLA-G among genotypes were not observed [2]. It is known that post-transplant diabetes, hypertension, obesity, and dyslipidemia are risk factors for long-term morbidity (mainly cardiovascular diseases and chronic allograft dysfunction [CAD]) in kidney transplantation. HLA-G expression has a complex regulation and seems to be involved in metabolic alterations [3,4]. In this study, sHLA-G in the plasma of kidney transplant recipients carrying different HLA-G genotypes was analyzed to evaluate potential associations with an increased susceptibility to post-transplant risk.

## METHODS

### Patients, sHLA-G Detection, and HLA-G Genotyping

Plasma levels of sHLA-G were analyzed in 103 kidney transplant recipients carrying different HLA-G14bp genotypes, recruited at the Transplant Unit of the S. Salvatore Hospital of L'Aquila, Italy. Of these, in a subset of 26 recipients (group A), sHLA-G was

assessed before and after transplantation (1 year) to evaluate early variations and post-transplant events; in the remaining 77 (group B), sHLA-G was measured after transplantation (8.96 ± 4.9 years, range 3-24) and associated with the onset of post-transplant diabetes mellitus (PTDM), obesity, hyperlipidemia, hypertension, and CAD (minimum monitoring time: 10 years). Immunosuppressive therapy consisted of cyclosporine or tacrolimus, steroids, and inhibitors of purine synthesis (86% of patients) or cyclosporine, steroids, and mammalian target of rapamycin inhibitors (14%). Written informed consent was obtained from all subjects; investigations were carried out by the principles of the Declaration of Helsinki, and the study was approved by the local ethics committee. Plasma levels of sHLA-G were measured using an enzyme-linked immunosorbent assay, which detects G1/G5 soluble isoforms (Bio-Vendor/Exbio, Czech Republic). HLA-G14bpins/del and HLA-G+3142C>G genotypes (3'UTR region) were assessed using direct polymerase chain reaction amplification of genomic DNA with specific primers, as previously described [5,6].

### Statistical Analysis

Correlations between sHLA-G and clinical variables were evaluated with Pearson's and Spearman's correlation coefficients, as appropriate. A nonparametric Mann-Whitney *U* test or Kruskal-Wallis test were employed to compare sHLA-G levels. Boxplots are used to display sHLA-G distribution in the different groups and genotypes (Fig 1).

## RESULTS

Group A (n = 26; 6 female, 23%; 20 male, 77%; mean age 50.4 ± 11.7 years) included 2 pretransplant obese patients and 2 recipients with type 1 diabetes; 6 recipients had acute rejection episodes, 1 developed PTDM, and 8 experienced obesity after transplantation. Significantly lower sHLA-G plasma levels were detected after transplantation compared with pretransplant levels (pretransplant: 216.93 ± 75.08 U/mL; after: 173.01 ± 69.21 U/mL; $P = .019$). No significant differences among genotypes were observed, except for a tendency toward reduced pretransplant levels (similar to that found after transplant) of sHLA-G in carriers of HLA-G+3142G/G (+3142C/C+C/G, 236.61 ± 67.25 U/mL vs +3142G/G, 179.74 ± 78.68 U/mL, $P = .075$). This observation of lower sHLA-G levels in HLA-G+3142G/G carriers was confirmed in the group B analysis ($P = .074$). In group B (n = 77; 28 female, 36%; 49 male, 64%; mean age 43.7 ± 10.6 years), posttransplant obesity affected 21 recipients (27%) (8 out of 21 were also obese pretransplant), diabetes mellitus (DM) 12 (16%, 3 type 1 diabetes and 9 PTDM), hypertension 64 (83%), and hyperlipidemia 55 (71%); 4 had cardiovascular complications, 9 (12%) CAD, and 13 (17%) organ loss. Posttransplant sHLA-G detection was characterized by a wide variability (Fig 1). Significant differences of sHLA-G between genotypes were observed in the presence of obesity or DM: lower sHLA-G plasma levels were observed in obese patients (body mass index > 30) and in recipients with DM carrying the HLA-G14bpins/ins genotype ($P = .006$ and $P = .003$, respectively) or the +3142G/G ($P = .03$ and $P = .041$, respectively), compared with other genotypes.

## DISCUSSION

In this study, differences in plasma levels of sHLA-G between HLA-G genotypes were observed in association with posttransplant DM or obesity in kidney transplant recipients. It was found that HLA-G14bpins/ins and +3142G/G genotypes are characterized by lower plasma levels of sHLA-G, compared with other genotypes (del/del+del/ins, +3142C/C+C/G), according to previous observations and that transplantation, in general, results in a decrease of sHLA-G in the early period compared with pretransplant plasma levels. In transplant recipients, interpretation of results of sHLA-G plasma levels could be rather difficult; in fact, pretransplant plasma detections are affected by dialysis, whereas after transplantation, the effects of immunosuppressive therapy, which affects both immunologic and metabolic pathways, and a wide variability of plasma levels should be considered. When considering HLA-G genotypes, a significant association between sHLA-G and obesity or DM was evidenced in this study, reinforcing the hypothesis of a potential functional role of HLA-G in these posttransplant conditions characterized by low-grade inflammation and insulin resistance. HLA-G has a complex genetic regulation that could have metabolic implications in subjects at risk, such as transplanted patients. Recently, in other clinical conditions, effects of HLA-G on insulin resistance were observed [3], and a putative link between HLA-G, diabetes and its cardiovascular complications has been highlighted, thus hypothesizing future innovative therapeutic perspectives that involve HLA-G [4]. Results indicate the opportunity for a deeper genetic and functional analysis aimed to identify transplant recipients more susceptible to the negative effects of immunosuppressive treatments and at a higher risk for long-term morbidity and transplant impairment.

## REFERENCES

[1] Tan Z, Randall G, Fan J, et al. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am J Hum Genet 2007;81:829–34.

[2] Hauer V, Risti M, Miranda BLM, et al. The association of HLA-G polymorphisms and the synergistic effect of sMICA and sHLA-G with chronic kidney disease and allograft acceptance. PLoS One 2019;14:e0212750.

[3] Marzuillo P, Bellini G, Punzo F, et al. Association between 14 bp insertion/deletion HLA-G functional polymorphism and insulin resistance in a cohort of Italian children with obesity. Pediatr Diabetes 2018;19:1357–61.

[4] Sommese L, Benincasa G, Schiano C, et al. Genetic and epigenetic-sensitive regulatory network in immune response: a putative link between HLA-G and diabetes. Expert Rev Endocrinol Metab 2019;14:233–41.

[5] Hviid TV, Hylenius S, Hoegh AM, Kruse C, Christiansen OB. HLA-G polymorphisms in couples with recurrent spontaneous abortions. Tissue Antigens 2002;60:122–32.

[6] Silva ID, Muniz YC, Sousa MC, et al. HLA-G 3'UTR polymorphisms in high grade and invasive cervico-vaginal cancer. Hum Immunol 2013;74:452–8.