Genetic risk factors in solid organ transplantation: beyond classical human leukocyte antigen

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Introduction

HLA matching is crucial for solid organ transplant survival, but it is not the only important factor in determining the successful outcome of a transplant. Several independent studies identified genetic variants of Apolipoprotein L1 (*APOL1*, 22q12.3), LIM zinc finger domain containing 1 (*LIMS1*, 2q12.3) and 3'-UTR low expression variant of *HLA-G* (G+3196, 6p22.1) as either contributing to allograft failure or correlated with the risk of allograft rejection, when present as homozygous.

Out of these, APOL1 has been studied most extensively. *APOL1* G1 and G2 variants (G1a-rs73885319, G1b-rs60910145 and G2-rs71785313) are associated with an increased risk of developing chronic kidney disease [1-3] and commonly referred to as renal risk variants (RRV). Additionally, transplanting kidneys carrying two RRVs was associated with a higher risk of an earlier allograft failure. In three studies of deceased donor transplants, the risk of graft failure ranged from 2,05 to 3,84 at 24- or 36-month post transplantation follow-up [4-6]. Because of the potential risk of kidney disease associated with RRVs, decision of kidney donation might potentially also carry risk for living donors [7]. Broad studies evaluating risk of living kidney donations for donors carrying two RRVs are underway [8].

Transplanting kidney to a recipient homozygous for *LIMS1* mismatch at rs893403 might result in genomic collision and production of anti-LIMS1 antibodies, leading to a chronic rejection. The genomic collision at chromosome 2q12.3, leads to a risk of rejection that is nearly 60% higher than the risk among donor–recipient pairs with non-collision genotypes [9]. *LIMS1* genomic collision would be expected to occur in approximately 12-15% of transplants from unrelated donors among individuals of European and African ancestry. Increased risk for chronic T-cell mediated rejection in *LIMS1* collision background was later reported in another study by Caliskan et al. [10].

HLA-G is a well-established modulator of the immune response and increased expression of *HLA-G* was shown to increase allograft acceptance [11]. High-expression variant of *HLA-G* reduces the risk of rejection by 66,9%, compared to the low-expression variant (rs1610696 C>G), carrying single nucleotide change in 3'-UTR in position +3196 [12].

The relevance of the high-risk variants is supported by their strong penetration across different populations. These variants are present at ca. 40% among individuals of European and African ancestry (*LIMS1*), 35% among African Americans (*APOL1*) and 20-30% among individuals of European, African and Asian ancestry (G+3196).

Methods and Materials

The assay. We developed qPCR-based tests utilizing TaqMan® probes and targeting high risk variants of *APOL1*: G1a (rs73885319), G1b (rs60910145) and G2 (rs71785313); *LIMS1* (rs893403), and G+3196 (rs1610696), as well as their common reference variants (ten reactions in total and one no-template control; Table 1). Each reaction also includes an internal control assay. The reactions were dispensed and dried in a 384-well plate format.

APOL1 Polymorphic site	Allele	Variant	Genomic annotation
rs73885319 (G1a)	A	G1a^{Wt}	NM_003661.4:c.1024[=]
	G	G1a^{HR}	NM_003661.4:c.1024A>G
rs60910145 (G1b)	T	G1b^{Wt}	NM_003661.4:c.1152[=]
	G	G1b^{HR}	NM_003661.4:c.1152T>G
rs71785313 (G2)	TTATAA	G2^{Wt}	NM_003661.4:c.1164_1169[=]
	-	G2^{HR}	NM_003661.4:c.1164_1169del
***LIMS1* Polymorphic site**	**Allele**	**Variant**	**Genomic annotation**
rs893403	A	*LIMS1*^{Wt}	NC_000002.12:g.108606280[=]
	G	*LIMS1*^{HR}	NC_000002.12:g.108606280G>A
G+3169 Polymorphic site	**Allele**	**Variant**	**Genomic annotation**
rs1610696	C	G+3196^{Wt}	NC_000006.11:g.29798803[=]
	G	G+3196^{HR}	NC_000006.11:g.29798803C>G

***Table 1. Variants detected**. The test detects high-risk (HR) and common (Wt) variants of APOL1, LIMS1 and G+3196.*

Cell line DNA typing. 110 cell line DNA samples from the IHW collection were typed using the test plate. Accuracy of the qPCR-based typing was verified by AlloSeq® hybrid-capture assay (*APOL1*, G+3196; CareDx Pty Ltd, Australia) and Sanger sequencing (*LIMS1*; Eurofins Genomics, Germany).

Data analysis. The Cq and final fluorescence were calculated from qPCR amplification curve, for both, the target gene and the internal control. Relative Cq (rCq) and relative fluorescence (rFF) were calculated by normalizing values for the gene variant to its internal control. The combination of rCq and rFF was used to determine whether the sample was positive or negative in each reaction.

Results

In this study, five pairs of qPCR reactions were developed to identify the high-risk (HR) genetic variants of *APOL1*, *LIMS1*, and G+3196. The assay delivers a good resolution in distinguishing HR variants from common (Wt) variants (Figure 1.) The test allows for detection of all the variants and zygosity determination at each of the polymorphic sites. The accuracy of the assay was confirmed with 100% concordance for all the samples (Table 2).

APOL1 Polymorphic site	Numbers of samples tested	Concordance
APOL1: rs73885319 (G1a)	110	100 %
APOL1: rs60910145 (G1b)	110	100%
APOL1: rs71785313 (G2)	110	100%
***LIMS1* Polymorphic site**	**Numbers of samples tested**	**Concordance**
LIMS1: rs893403	110	100%
***HLA-G* Polymorphic site**	**Numbers of samples tested**	**Concordance**
G+3196 rs1610696	110	100%

***Table 2. Test accuracy.** Accuracy of the test was confirmed for all the samples tested with 100% concordance.*

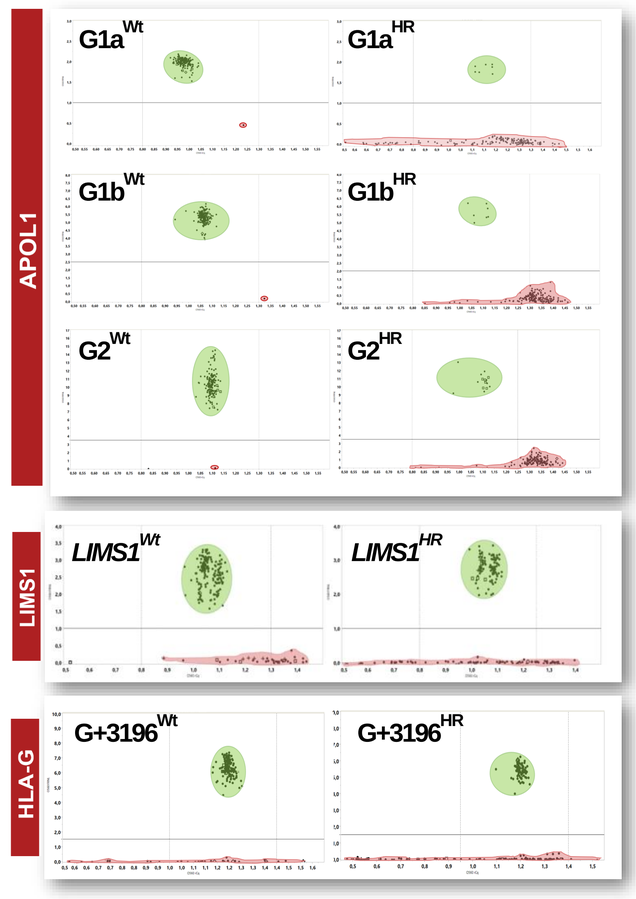

***Figure 1.** 110 cell line testing for common (Wt) and high-risk variant (HR) of APOL1 (G1a, G1b, G2), LIMS1, and HLA-G+3196. All samples were plotted with relative fluorescence intensity (y-axis) and relative Cq (x-axis) obtained for each reaction and its internal control. Colored shading highlight the positive (green) and negative (red) result of respective reaction.*

Conclusions and major takeaways

- We developed a rapid test for detection of high-risk variants of *APOL1* (G1 and G2), *LIMS1* (rs1610696 C>G) and G+3196, and confirmed its accuracy on 110 samples.
- Growing evidence suggests that additional factors, other than classical HLA genes, such as *APOL1*, *LIMS*, and *HLA-G* expression variant (G+3196) could have an effect on allograft failure or rejection. The relevance of these factors is supported by the high frequencies of their risk variants across different populations.
- Inclusion of these risk variants, in addition to HLA typing, provides a more comprehensive transplant care, from better pre-transplant matching to post-transplant surveillance and guidance in administration of immunosuppressives.
- All the above make APOL1, LIMS1 and G+3196 valuable targets for personalized medicine.

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