Kidney transplantation beyond HLA
APOL1 testing by qPCR

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INTRODUCTION

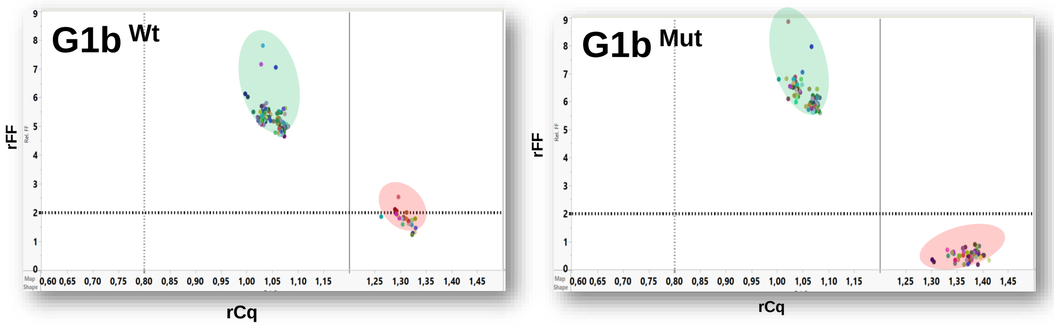
Human leukocyte antigen (HLA) matching is of vital importance to the success of solid organ transplants and remains a stable predictor for short- and long-term allograft survival. There are however other, non-HLA genetic factors, that contribute to poor transplant outcomes. Variants of APOL1 (22q12.3), namely G1a, G1b (referred to simply as G1) and G2 have been identified as associated with an increased risk of developing of chronic kidney disease (CKD) [1-2]. G1 and G2 variants evolved in Western Africa to protect from sleeping sickness and are estimated to exist in African American (AA) population at frequency of 0,22 (for G1) and 0,13 (for G2) [3]. The risk of CKD, and later progression to end-stage renal disease (ESRD), is increased when two risk variants are present, and is exacerbated by co-existing conditions, like hypertension or HIV infection [4]. A recent study in France revealed high occurrence of G1/G2 variants among focal segmental glomerulosclerosis (FSGS) patients from French West Indies and confirmed the need for broad APOL1 testing among European populations [5]. Transplanting a kidney with the high risk APOL1 variants is correlated with shorter allograft lifespan and potentially worsening the life quality for the donor [6].

Here we present a qPCR-based test for rapid APOL1 genotyping, with zygosity determination for the high-risk variants. We used the APOL1 assay to determine the frequency of the APOL1 risk variants among AA kidney transplant recipients (KTR) recruited within the AlloSure® Circulating Donor-Derived Cell-Free DNA in Blood for Diagnosing Acute Rejection in Kidney Transplant Recipients (DART) study [7].

METHODS

APOL1 assay. Six qPCR tests were developed to report the presence of the common (wild-type; Wt) or the risk variant (Mut) in each of the polymorphic sites: G1a, G1b and G2 (Table 1). Each well of the APOL1 assay contains dried primer pairs and a reporter TaqMan® probe designed to specifically bind the APOL1 variants and, additionally, an internal control assay (Figure 1.). The APOL1 assay is compatible with QTYPE® 11 HLA-typing kit. It can work as a stand-alone test or an integral part of QTYPE 11, utilizing the same interpretation software, Score™ 6. As a standalone test, APOL1 typing plate allows for screening of up to 48 samples and utilizes 70ng of DNA . **Cell line DNA typing.** A random selection of 112 cell line DNA samples from the IHW collection was typed using the APOL1 plate. Accuracy of the qPCR-based APOL1 typing was verified on 13 samples by Sanger sequencing, showing 100% concordance. **AA KTR sample typing.** Genomic DNA was extracted using FlexiGene DNA Kit (Qiagen) from buffy coat fractions collected from 124 African American kidney transplant recipients (KTR). The samples were typed on a stand-alone APOL1 typing plate. Accuracy of the qPCR-based APOL1 typing was verified on a subset of 25 samples by Sanger sequencing, showing 100% concordance.

Figure 1. Positive and negative signals for the two mixes targeting G1b polymorphic site



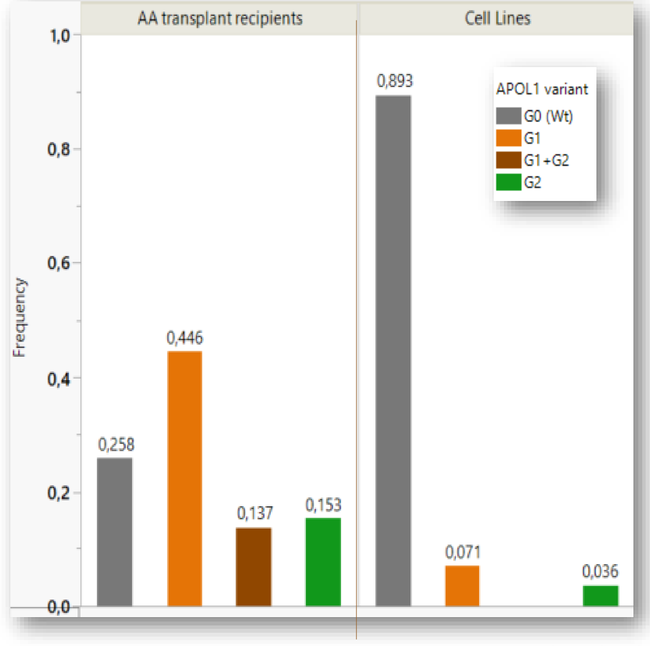
The graphs show positive and negative signals for two selected reactions in the assay (G1b ^{Wt} and G1b ^{Mut}). The results are reported as a function of final fluorescence (FF) and Cq obtained for APOL1 variant, relative to the FF and rCq for the internal control (rFF and rCq, respectively). The assay allows for a good resolution between positive (green) and negative (red) signals. rFF thresholds and rCq cut-offs are set for each reaction separately.

Table1. APOL1 polymorphic sites detected and their annotations

Polymorphic site	Variant	Variant	Genomic annotation
G1a	$G1a^{A}$	$G1a^{Wt}$	NM_003661.3:c.1024[=]
G1a	$G1a^{G}$	$G1a^{Mut}$	NM_003661.3:c.1024A>G
G1b	$G1b^{T}$	$G1b^{Wt}$	NM_003661.3:c.1152[=]
G1b	$G1b^{G}$	$G1b^{Mut}$	NM_003661.3:c.1152T>G
G2	$G2^{TAATTA}$	$G2^{Wt}$	NM_003661.3:c.1164_1169[=]
G2	$G2^{\Delta TAATTA}$	$G2^{Mut}$	NM_003661.3:c.1164_1169del TTATAA

The non-synonymous substitution (G1a and G1b) and the 6-nucleotide deletion (G2), which constitute the APOL1 risk variants, cluster near the 3' end of the APOL1 coding region.

Figure 2. Frequency of G0 (Wt), G1 and G2 alleles in AA kidney transplant recipients' samples and the cell line samples



The graph shows frequency of the wild-type only (G0 (Wt); grey bar), G1- (G1; orange bar), G1 and G2- (G1+G2; brown bar) and G2- (G2; green bar) harboring genotypes in the analysis of a random cell line panel, AA KTR samples and in the reference cohort of African Americans, as reported by Limou et al. [3].

RESULTS

Typing confirms high frequency of the risk alleles among AA kidney transplant recipients.
124 kidney transplant recipients of an African ancestry were typed for the presence of the APOL1 risk variants. The results are shown in Figure 2, alongside results obtained for a random panel of 112 cell lines from the IHW collection. The AA KTR showed much higher frequencies of G1 risk variants (0,446), compared to the random panel (0,07). The G2 risk variant frequency was slightly higher in the AA KTR cohort (0,153), compared to the random cell line panel (0,036). Additionally, we detected presence of both G1 and G2 risk variants in 19 AA KTR samples (frequency of 0,137). Overall, occurrence of at least one risk variant was much higher in the AA KTR, reaching frequency of 0,742, compared to 0,11 for the random cell line panel.

The test allows for identification of homozygous samples.
The test allows for detection of both wild-type and the risk alleles, allowing therefore for the zygosity determination. Among the AA KTR samples with at least one of the risk variants we identified 25 homozygous for G1 variant and 3 homozygous for G2 variants (frequency of 0,2 and 0,024; respectively). We also identified 1 individual homozygous for G1a and heterozygous for G1b (Table 2.). Typing for this particular sample and for a random selection of homozygous samples was confirmed by sequencing.

CONCLUSIONS

Our studies show high frequencies of APOL1 risk variants, namely G1 and G2 among kidney transplant recipients of an African ancestry, compared to frequencies for these alleles reported previously by Limou et al. (2014) for the general AA population [3]. We identified high number of individuals with high risk genotypes, i.e. either homozygous for G1 or G2 (28 out of 124 analyzed) or compound heterozygotes (i.e. carrying G1 and G2; 19/124). These numbers are also higher, compared to 14% of individuals carrying 2 risk alleles reported previously for the general AA population [3]. Our results are in agreement with previously reported studies, showing frequent occurrence of the high risk APOL1 genotypes among the individuals suffering from kidney injury [1,2].

The very low frequencies of the risk alleles we observed in the random cell panel can be explain by the fact that only 12 of these lines were obtained from individuals reporting African ancestry. We have not detected any sample carrying three risk variant, which is consistent with what is known about APOL1 G1/G2 evolution and segregation. G1 and G2 originated independently on separate chromosomes. Due to their physical proximity (G1b and G2 sites are only 9 base pairs apart), the recombination event that would bring them together on the same haplotype is impossible [3]. One of the samples was typed as G1a homozygous and G1b heterozygous. This is an unusual result, but G1a has been infrequently observed in the absence of G1b [3].

Table 2. High risk genotypes detected in the AA KTR cohort

Zygocity within AA kidney transplant recipients	No. of samples	Frequency (n=124)
G1 homozygous	25	0,20
G2 homozygous	3	0,024
G1 heterozygous and G2 heterozygous (compound heterozygote)	19	0,137
G1a homozygous and G1b heterozygous	1	0,001
Total	**48**	

The frequencies are shown in relation to all AA KTR samples analyzed.

We have developed a rapid qPCR-based test for APOL1 renal risk variant typing. Accuracy of the test was confirmed by sequencing. The assay allows for detection of all 3 risk variants and zygosity reporting. Overall, our observations of the high frequency of 2 risk variants among kidney transplant recipients confirm previously reported association between the high risk APOL1 genotypes and kidney injury. The results obtained in this study provide functional validity for our APOL1 typing assay. Moving forward, future studies should address potential association of the APOL1 risk variants with transplant rejection within the AA KTR cohort.

MAJOR TAKEAWAYS

- We developed qPCR- base test for a rapid detection of the APOL1 risk variants.
- Following additional validations and approval, the test can be combined with our QTYPE 11 HLA-typing kit and offers a more comprehensive pre-transplant matching beyond classical HLA typing.
- The functionality of the test was confirmed on a cohort of AA kidney transplant recipients.

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