APOL1 genotyping by TaqMan Real-Time PCR for use in Black potential living kidney donors

Barts Health NHS Trust

Catherine Teenan[1], Eamonn Cudworth[1], Emma White[1], Delordson Kallon[1], Sarah Blow[1]

[1] Clinical Transplantation Laboratory, Barts Health NHS Trust, London, UK

APOL1 genotype testing

Mutations within apolipoprotein L1 (*APOL1*), have been identified as renal risk variants in African individuals. The reference allele is named *G0*. Allele *G1* describes two amino acid substitutions ((1024A>G) and (1152T>G)), whilst allele *G2* encodes a two amino acid deletion (TTATAA/-) in the APOL1 protein. Any combination of two copies of these renal risk variants, *G1* or *G2*, is described as "high-risk" as these genotypes have been found to be strongly associated with an increased risk of renal disease[1].

- **Kidney donors with high-risk genotypes, have been shown to experience increased rates of kidney failure compared to donors with 1 or 0 *APOL1* mutations**[2]
- **Recipients of a kidney from a high-risk genotype donor may have a shorter graft survival and require re-transplantation earlier**[3]

In light of mounting evidence, the British Transplantation Society (BTS) released guidelines in 2023, recommending *APOL1* genotyping for accurate risk stratification, in those with African heritage, undergoing assessment as a potential living kidney donor[4]. Here, we validate a TaqMan Real-Time PCR method for *APOL1* genotyping in the Clinical Transplantation Laboratory at Barts Health NHS Trust (BHNHST). We evaluate the genotype frequencies in our Black donor population and consider the impact of the new guidelines on renal donor selection at our centre.

APOL1 Genotype	Increased kidney disease risk	Genotype risk category
G0/G0	No	**Low Risk**
G0/G1,G0/G2	Undetermined	**Undetermined Risk**
G1/G1, G1/G2, G2/G2	Yes	**High Risk** If the donor <60 years old, advise against donation*

Table 1. *APOL1 Genotypes and renal disease risk. *Recommended by BTS. After this age, similar risk has been shown between white and black counterparts*[5]

Aims

1. Validate a TaqMan Real-Time PCR kit for the use of *APOL1* genotyping in kidney donors at BHNHST.
2. Investigate the impact of the new guidelines by observing the frequency of the risk alleles in our Black donor population.
3. Consider the appropriate test referral criteria by analysing samples from Black, Mixed, Other and Unknown ethnicity categories.
4. Consider the impact of potential guideline expansion by genotyping both living and deceased donor samples.

Methods

- DNA samples in storage were tested using a **Thermo Fisher Genotyping Master Mix Protocol** on an Applied Biosystems 7500 Fast Real-Time PCR System.
- The accuracy and reliability of the results were evaluated by re-testing 20 samples at a referral laboratory, using the same TaqMan technique and the gold standard, Sanger sequencing.

Ethnicity	Living Donor	Deceased Donor	Total
Black	34	17	51
Mixed	6	2	8
Other	5	1	6
Unknown	0	10	10
Total	45	30	**75**

Table 2. *Summary demographics of samples tested. White and Asian donors were not tested due to the strong evidence of risk variant absence in these populations.*

Assay performance

- Time required to perform testing and analysis was <3 hours, whilst also being simple to perform and interpret due to automated calling.
- Assay probe specificity allowed clear allelic discrimination for each sample.
- Genotyping results of the 20 samples sent for verification testing showed **100% concordance**.

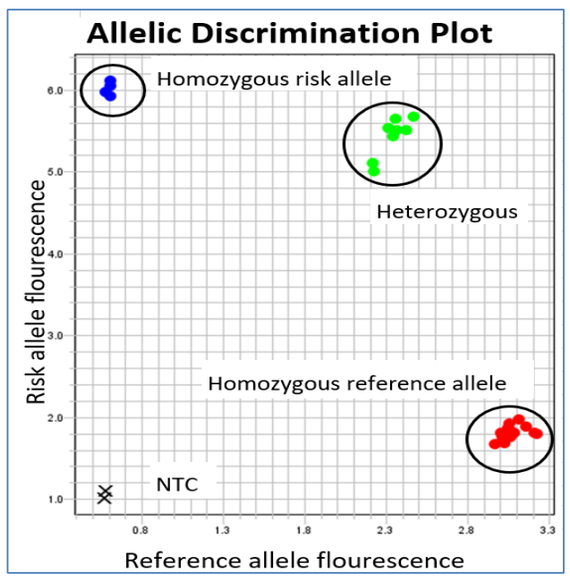

Figure 1. *An example allelic discrimination plot produced by TaqMan Genotyper Software. Automated calls were made by comparing the relative fluorescence of each competing probe. NTC = No template control.*

Donor *APOL1* genotype analysis

- Risk alleles G1 and G2 were identified only within Black and Mixed individuals tested, however the sample sizes were too small to determine the statistical significance of this.
- 40% of individuals tested possessed one risk allele whilst 11% possessed two.
- Five of the six possible genotype combinations were identified, excluding the rare G2/G2.

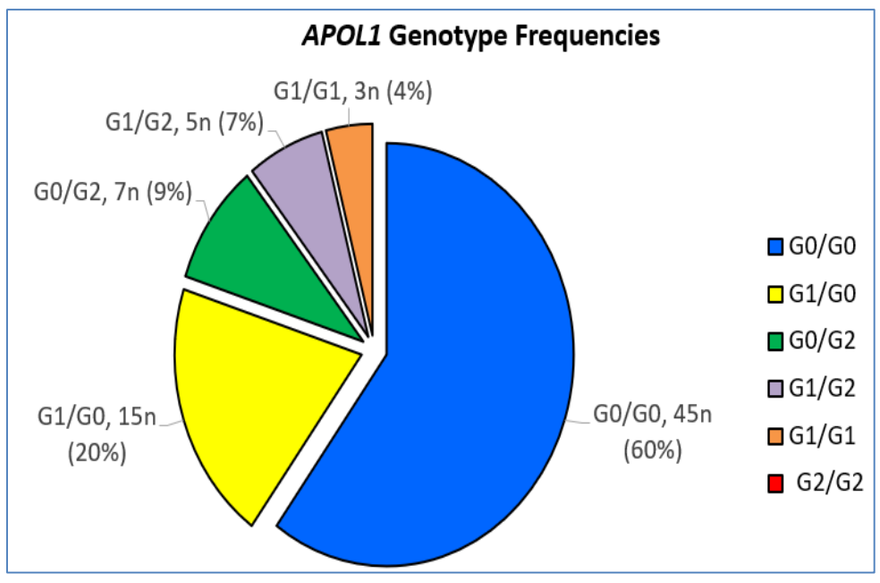

Figure 2. *The frequency of each APOL1 genotype in the total population tested.*

Assessment of impact on donor selection

- 20% of Black or Mixed living donors tested, possessed high-risk genotypes.
- All of these individuals were <60 years of age at the time of donation, meaning they would be counselled against donation under the new guidance today.
- High risk genotypes were only identified within the Black living donors tested.
- Risk alleles were present in the Black and Mixed deceased donors, although no high risk genotypes were identified.

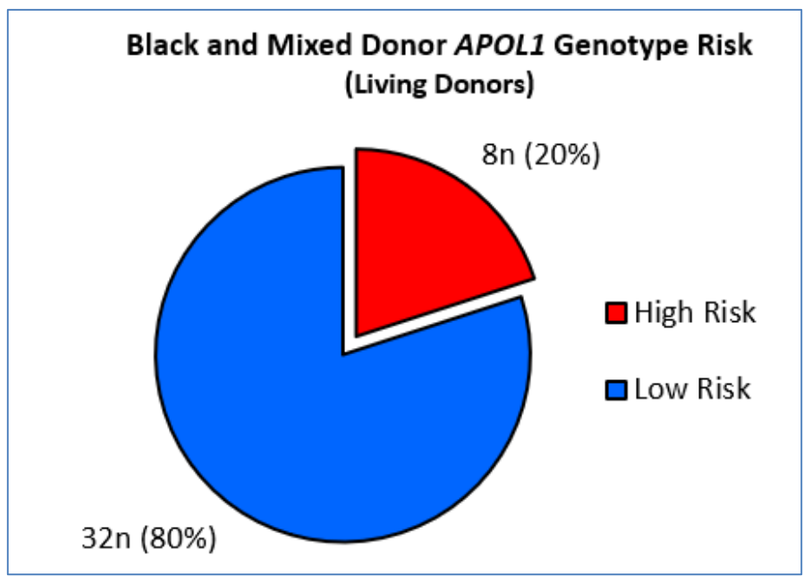

Figure 3. *The percentage of Black and Mixed living doors tested with high-risk APOL1 genotypes.*

Discussion

- Validation has been successful. Complete concordance with the referral laboratory shows the test is accurate and reliable compared to the gold standard method, whilst being quicker and easier to perform. There can be more time for counselling of patients and donors on their individual risks. The presence of other non-target polymorphisms within the binding region remains an interference risk and there is additionally a very small risk that individuals can be *APOL1* null. In the case of any uncertainty or doubt, samples can be sequenced.
- The prevalence of high-risk genotypes in our Black and Mixed living donor population highlights that the new BTS guidelines will have significant repercussions on patients and donors at BHNHST. A number of renal transplant patients will need to explore options for alternative living donors or listing on the deceased donor waiting list. There is a risk that this may exacerbate already existing disparities in access to suitable donors in Black communities.
- Introduction of *APOL1* genotyping will help to provide recipients and donors with a transparent review of the risks involved with transplantation, as well as reassuring anxieties in those who do not carry the additional risk alleles. There remains an unmet need for targeted treatment and management protocols for people in whom high-risk genotypes are identified. Not all individuals with high-risk genotypes will go on to develop kidney disease and it is unknown what the determining factors are.
- It should be noted that a large percentage of our living donors were related to their recipients. Their shared genetic ancestry with a family member who is of African descent and affected by kidney disease, the cause of which is unknown to us, may increase the likelihood that they carry the risk alleles within their family. This accurately reflects the clinical setting in which living donors are often first-degree relatives of the patient.
- The BTS guidelines state that clinicians should approach donor assessments on an individual basis. This leaves them open to interpretation, which whilst allowing flexibility, provides a lack of clarity on how to approach difficult questions. The BTS guidelines do not propose that high-risk *APOL1* genotypes constitute an absolute contraindication to donation. Instead, policies should allow assessment on a case-by-case basis with patient and donor involvement in the discussion.
- This study highlighted the need for improved ethnicity recording processes. We encountered difficulties with incomplete ethnicity reporting, use of non-standardised categories as well as inconsistencies in data transfer between databases. Ethnicity is not a required parameter in our database and is not provided to the recipient centre during deceased donation. It is important to address this before the test is brought into clinical practice.

Conclusion

The chosen TaqMan method is suitable for detecting *APOL1* risk alleles. Identifying high-risk *APOL1* genotypes within our donor cohort provides evidence that the test has clinical utility at our centre. Implementation of this test will likely have a significant impact on our donor selection process. The results reported here will allow timely implementation of *APOL1* genotyping into clinical practice at BHNHST, allowing us to provide the most accurate risk stratification to patients and donors under the renal transplantation service. This aligns with our values as a trust and the wider values of the NHS, as we work towards improving care by implementing cutting edge genomic science to evolve our service in line with research and innovation.

References

1. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, et al. Association of Trypanolytic ApoL1 Variants with Kidney Disease in African Americans. Science. 2010;329(5993):841-5.
2. Doshi MD, Ortigosa-Goggins M, Garg AX, Li L, Poggio ED, Winkler CA, et al. APOL1 genotype and renal function of black living donors. Journal of the American Society of Nephrology. 2018;29(4):1309-16
3. Guidelines for APOL1 testing in potential living kidney donors. British Transplantation Society; 2023
4. Friedman DJ. COVID-19 and APOL1: Understanding Disease Mechanisms through Clinical Observation. Journal of the American Society of Nephrology. 2021;32(1):1-2.
5. Wainright JL, Robinson AM, Wilk AR, Klassen DK, Cherikh WS, Stewart DE. Risk of ESRD in prior living kidney donors. American Journal of Transplantation. 2018;18(5):1129-39.