Black renal transplant recipients have poorer long-term graft survival than CYP3A5 expressers from other ethnic groups

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Abstract

Background. African American transplant recipients have poorer long-term outcomes than Caucasian Americans. This difference was not found in French patients, suggesting socialized medicine overcame this disparity. It has also been hypothesized that the difference relates to the higher prevalence of Black individuals who express the metabolic enzyme cytochrome P4503A5 (CYP3A5), with consequent altered handling of immunosuppressive drugs.

Methods. Records of 555 (50 Black; 505 non-Black) sequential renal transplant recipients from a single UK centre were analysed.

Results. Outcomes were significantly worse for Black patients: death-censored graft survival (5-year 66% versus 87%, \( P = 0.001 \)); halving of year one estimated glomerular filtration rate (mean 8.8 versus 10.8 years, \( P = 0.008 \)); first-year graft loss (12% versus 3.8%, \( P = 0.02 \)); and death-censored graft survival in patients surviving the first year with functioning grafts (5-year 77% versus 94%, \( P = 0.02 \)). Death-censored 5-year graft survival was poorer in Black CYP3A5 expressers than in non-Black CYP3A5 expressers (62% versus 93%, \( P = 0.002 \)). Following multivariate analysis, the Black group demonstrated poorer graft survival as compared to the non-Black group (hazard ratio 0.46, 95% CI 0.25–0.85, \( P = 0.002 \)). In a subgroup of genotyped transplant recipients, ethnicity (hazard ratio 0.31, 95% CI 0.15–0.64, \( P = 0.002 \)), and not CYP3A5 expresser status, persists as an independent risk factor for graft survival following multivariate analysis.

Conclusion. In this cohort of patients with socialized medicine, Black recipients had poorer long-term outcomes than individuals from other ethnic groups. This was independent of CYP3A5 expresser status.

Keywords: CYP3A5; ethnicity; pharmacogenetics; renal transplantation; tacrolimus

Introduction

African American renal transplant recipients, both adult [1–8] and paediatric [9–11], have been reported to have poorer long-term outcomes than Caucasian Americans. The following factors are suggested to underlie this discrepancy include: human leucocyte antigen (HLA) matching [12,13], cold ischaemia time [12], donor and recipient age [12], grafts from deceased or living donors [6], time on transplant waiting list [14] and comorbidities such as hypertension [15] and diabetes mellitus [16]. The majority of these factors have generally been accounted for in these observational studies by multivariate analyses. Acute rejection is more common in African Americans and more likely to be steroid resistant [17].

Recently, Pallet et al. reported no difference in renal transplant outcomes comparing African Europeans with Caucasian Europeans in a French cohort of 1092 patients. It was proposed that universal access to socialized medicine overcame the discrepancies noted in the American studies [18]. A similar lack of difference in outcomes for Black patients in Canada, based on analysis of their national renal transplant registry, was published earlier this year [19]. Access to healthcare in the United States may be restricted in the African American population.

Currently, the evidence for socioeconomic factors influencing graft survival is conflicting [20–22]. An attempt to address the impact of access to healthcare was made by Chakkera et al. who concluded that there was a similar increased risk ratio comparing African American and non-African American renal transplant graft survival in both those in the universal access to medical care environment of the Department of Veteran Affairs and those without universal access [23]. This suggested that the disparity in outcomes could not be fully overcome by universal healthcare. However, limitations already identified are that the population within the Department of Veteran Affairs may not reflect the general population and that the variation in care between Veteran Affairs hospitals is a potential confounding variable. Furthermore, there is an unexplained poorer
outcome in the patients treated under the Department of Veteran Affairs, independent of ethnicity.

Outside the United States, a Brazilian cohort demonstrated poorer graft survival in the Afro-Brazilian group [24], but this study only accounted for the source of graft and not other confounding factors. A recent study from UK Transplant based on a national transplant registry identified poorer 3-year graft survival (76% versus 82%) in the Black British population, but this was statistically significant only after correcting for recipient and donor age and HLA matching [25].

An alternative hypothesis for this observed disparity in long-term outcomes is variations in drug metabolism. CYP3A5 expressers, more prevalent in Black renal transplant recipients than non-Blacks, on average require a 2-fold higher dose of tacrolimus to achieve target blood concentrations [26,27]. In the cohort of Black patients described in this study, 23 out of 26 (88%) transplant recipients who were genotyped were CYP3A5 expressers. This may result in sub-therapeutic dosing of immunosuppressants, especially in the initial stages or the potential for exposure to toxic metabolites. Studies have so far revealed mixed results for the impact of CYP3A5 expresser status on short-term outcomes of renal transplantation. CYP3A5 expressers had earlier onset of biopsy-proven acute rejection [26], but no difference in total incidence of acute rejection [26,28,29]. Biopsy-proven tacrolimus nephrotoxicity was found to be more common in CYP3A5 expressers, potentially related to an increased concentration of toxic metabolites [30].

We have addressed the issue of outcome in a cohort of Black patients with access to socialized healthcare in a single UK transplant centre, using tacrolimus as the primary immunosuppressive agent.

Subjects and methods

Patients and treatment

All 555 (50 Black; 505 non-Black) consecutive kidney-only renal transplants performed at St. George's Hospital, London, UK, between September 1995 and April 2006 were included in this study, including those who had returned to dialysis. Individuals genetically of sub-Saharan African origin were classified as Black (predominantly African Caribbean or West African). The follow-up period extended to April 2007. The care of patients referred to this tertiary renal transplantation centre was returned to the initial nephrologists after 3 months. Data were available for 395 patients with functioning renal transplants at least 1 year post-transplantation (135 patients transferred to referring renal centres and 25 graft loss events during the first post-transplantation year). The demographic data are shown in Table 1.

The standard immunosuppressive regimen throughout the study period was based on tacrolimus (98% of patients) and prednisolone, and further information on concomitant immunosuppression is shown in Table 1. From October 2001, all patients were treated with basiliximab. Immunosuppressant protocols, both initiation and maintenance, including target blood concentrations for tacrolimus did not differ between the ethnic groups but did evolve with time according to the latest evidence base. The one difference between ethnic groups was a change in the initial dose of tacrolimus in Black patients from 0.2 mg/kg daily to 0.4 mg/kg daily in July 2002 (135 patients transferred to referring renal centres and 25 graft loss events during the first post-transplantation year). The demographic data are shown in Table 1.

Genotyping

The study was approved by the Local Research Ethics Committee and subjects provided written informed consent. Genomic DNA was extracted from whole blood using a QIAGEN DNA Mini Kit (Quiagen, Crawley, UK). Genotyping for CYP3A5 was performed using a
LightCycler (Roche Diagnostics Ltd, Lewes, UK) with specific primer sequences (forward: 5′-ACTGCCCTTGACGACATTAG-3′; reverse: 5′-CATACCCCTAGTTGTACGAC-3′) for amplification [32]. Genotyping was performed for a subset of 224 (26 Black, 198 non-Black) transplant recipients. Individuals with at least one CYP3A5*1 allele, both heterozygotes and homozygotes, were classified as CYP3A5 expressers. Individuals homozygous for the CYP3A5*3 allele were classified as CYP3A5 non-expressers. The allelic distributions were in Hardy-Weinberg equilibrium.

A summary flow diagram of patients, genotyped subset and variations in initial tacrolimus dosing is shown in Figure 1.

**Patient outcomes**

Patient outcomes were obtained retrospectively from both electronic and paper records. The median follow-up period was 2.46 years (IQR 0.32–6.56).

Primary outcomes were time to graft loss (defined by death, return to dialysis or re-transplantation), patient survival and death-censored graft survival.

Secondary outcomes were estimated glomerular filtration rate (eGFR) at 3 months (n = 433) and 1 year (n = 306) post-transplantation, rate of progression of graft dysfunction and the time to halving of the Year 1 eGFR (abbreviated Modification of Diet in Renal Disease formula [33]).

eGFR at 1 year was defined as the nearest outpatient sample within a month of 1-year post-transplantation, explaining the smaller sample size. The rate of progression of graft dysfunction was calculated by linear regression analysis of the slope of outpatient eGFRs after the first post-transplantation year (minimum of 10 eGFR points to enter analysis = 287 patients; median 46 points per patient, IQR 25–68). The tacrolimus dose requirement, blood tacrolimus concentration and the calculated blood concentration per dose at 1-year post-transplantation were analysed.

Other potential risk factors considered for multivariate analysis were donor graft (donation after cardiac death versus others), donor age, recipient gender, combined number of HLA-A, HLA-B and HLA-DR mismatches (<3 versus ≥3), primary renal diagnosis recipient age at transplant, transplant number (first versus subsequent), era of immunosuppression; and in subgroup analyses, Year 1 eGFR or CYP3A5 expresser status. Grouping for categorical analysis for donor age and age of recipients at transplant was determined by the cut-off point of the median age (both at 47 years). To account for the potential bias in outcomes over time due to developments in immunosuppression, the cohort was split into two groups, the cut-off being October 2001, when routine use of basiliximab was introduced. Grouping of Year 1 eGFR was taken at the median (42.9 mL/min/1.73 m²).

**Statistics**

Differences between groups were compared using the Mann–Whitney U-test, the chi-squared test or Fisher’s exact test. Univariate analyses of differences in survival between groups were compared using Kaplan–Meier analysis. Multivariate Cox proportional hazards regression analysis was performed in a forward stepwise fashion with covariates with a P-value of <0.10 in univariate analyses. All analyses used SPSS version 14.0 (SPSS, Chicago, IL, USA). Data are presented as mean ± standard error, unless stated otherwise.

**Results**

**Demographics**

A significantly larger proportion of Black patients had hypertension and vasculitic disease as their primary diagnosis. The non-Black population had a larger proportion of patients with primary renal diagnoses related to structural abnormalities such as obstructive nephropathy or congenitally absent kidneys. The degree of HLA mismatch was significantly greater for the Black group, and a significantly larger proportion of grafts were donated after cardiac death. There were no significant differences between the two groups with regard to initial immunosuppression (Table 1).

**Outcomes**

Five-year patient survival was 96% for the Black and 90% for the non-Black groups (P = N.S.). Uncensored graft survival was significantly poorer for the Black cohort (5 year survival: 63% versus 80%, P = 0.03) as was death-censored graft survival (5 year survival: 66% versus 87%, P = 0.001; Figure 2). Black recipients received more kidneys from donors after cardiac death (36%) than non-Black recipients (12%). The recipients of renal grafts donated after cardiac death had significantly more combined graft failure and mortality events than other donor sources (17% versus 6.7%, P = 0.003).

A Cox proportional hazards analysis confirmed that our Black group had a poorer outcome independent of other potential confounding variables (hazard ratio = 0.46, P = 0.01; Table 2). There were significantly more graft failure events (12% versus 3.8%, P = 0.02), and combined graft failure and mortality events (16% versus 6.7%, P = 0.04) in the Black cohort in the first year.
Table 2. Cox proportional hazards analysis of the effect of ethnicity, graft type, donor age, gender, combined number of HLA-A, HLA-B and HLA-DR mismatches and primary renal disease on graft survival

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Hazard ratio for death-censored graft survival (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>0.46 (0.25–0.85)</td>
<td>0.01</td>
</tr>
<tr>
<td>Donation after cardiac death</td>
<td>0.43 (0.24–0.78)</td>
<td>0.005</td>
</tr>
<tr>
<td>Donor age (≥47)</td>
<td>0.50 (0.31–0.82)</td>
<td>0.006</td>
</tr>
<tr>
<td>Male</td>
<td>0.54 (0.31–0.94)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

545 patients available for analysis due to incomplete data set for 10 patients.

Covariates ‘Combined number of HLA-A, HLA-B and HLA-DR mismatches (3–6)’ and ‘Primary diagnoses more common in the non-Black group (ADPKD and structural)’ were removed from the forward conditional analysis, as they did not reach significance. ‘Primary diagnoses more common in the Black group (Hypertension and Vasculitis)’; ‘Primary diagnosis of diabetic renal disease’; ‘Recipient age at transplant (<47 versus ≥47 years)’, ‘Not first transplant’ and ‘Era of immunosuppression’ had P-values of >0.10 on univariate analysis and were not included in multivariate analysis.
Table 3. Mean survival (years ± SE) stratified by ethnicity and year 1 eGFR in transplant recipients surviving the first year with functioning grafts

<table>
<thead>
<tr>
<th>Year 1 eGFR</th>
<th>Black</th>
<th>Non-Black</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 42.9 mL/min/1.73 m²</td>
<td>7.1 ± 1.2 (n = 14)</td>
<td>10.1 ± 0.3 (n = 125)</td>
<td>0.02</td>
</tr>
<tr>
<td>≥ 42.9 mL/min/1.73 m²</td>
<td>9.9 ± 0.9 (n = 17)</td>
<td>11.9 ± 0.2 (n = 122)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Mean survival (years ± SE) comparing non-Black and Black groups for patients surviving first year post-transplantation with functioning graft within stratified Year 1 eGFR split at the median value.

Discussion

In a single Renal Transplant centre in the United Kingdom with universal access to healthcare, long-term graft survival in the Black population was poorer than that of non-Blacks. The impact of the greater degree of HLA-mismatch and higher proportion of grafts donated after cardiac death in the Black population did not fully account for this discrepancy as the independent effect of ethnicity persisted following multivariate analysis. The poorer graft survival was associated with a more rapid rate of deterioration in renal function when assessed by the time for eGFR to fall to 50% of the value at 1 year after transplantation.

The greater rate of first-year graft failure in the Black cohort may reflect the increased proportion of grafts donated after cardiac death and higher immunological risk. In order to minimize the influence of early events, an analysis was performed excluding patients who died or lost their grafts during the first year. This demonstrated poorer graft survival in Black patients. This difference in outcome was independent of Year 1 eGFR, which in itself is a strong predictor of long-term outcome [4]. There appeared to be an increase in the incidence of both early and late events in Black patients. However, early events may have increased the risk of chronic allograft damage. Of note, grafts donated after cardiac death and higher immunological risk are no longer a risk factor in the analysis of patients surviving the first year with a functioning graft, suggesting that they reflect risk for graft loss mainly in the first year.

One hypothesis for the discrepancy in outcomes between Black and non-Black individuals is the difference in pharmacokinetics of the immunosuppressive drugs due to the high prevalence of CYP3A5 expression in the Black patients. In a subgroup analysis of known CYP3A5 expressers, the poorer outcome in the Black population is still maintained with excellent outcome for non-Black CYP3A5 expressers. This finding persisted following multivariate analysis. With therapeutic drug monitoring, the genotyped subgroup has previously been reported to have trough blood tacrolimus concentrations within target therapeutic range, where the non-Black (previously split into White, Middle Eastern and South Asian) non-CYP3A5 expressers had significantly lower tacrolimus dose requirements and higher dose-normalized tacrolimus concentrations as compared to the non-Black CYP3A5 expressers [34]. Our genotyped subgroup did not appear to be a self-selected group, with no statistical differences in demographics and outcomes of the genotyped and non-genotyped subgroups (data not shown). Thus, CYP3A5 expresser status is unlikely to be a factor in the poorer graft survival, despite the well-characterized influence on tacrolimus dose requirement.

It has been hypothesized that the differences between the previous American [1–4,6–8,10,11,23] and French/Canadian [18,19] cohorts reflected socialized medicine levelling the outcomes between the two groups, excluding the influence of poverty as a confounding factor [20]. However, in our cohort, the difference in outcomes mirrors the American findings but in a socialized medical system similar to that in France [18]. Our findings confirm the results for the UK cohort reported by Rudge et al. [25], but with a longer follow-up time. Thus, access to treatment is unlikely to be the sole factor underlying the differences in long-term graft survival. However, universal access to treatment may not necessarily exclude poverty per se as a socioeconomic risk factor for allograft survival, as it may still impact on environmental factors affecting healthcare.

Non-compliance, which has been associated with increased renal graft failure, has also been proposed to contribute to the poorer outcomes in the African American population [35]. However, the influence of ethnic group on compliance is not universally supported by the published literature [36] and is unlikely to be a major factor in the discrepancy in outcomes. A potential underlying factor for the difference between our findings and those of Pallet [18] is the relatively low usage of anti-proliferative agents in our population. However, this could not account for the differences noted in the United States, where the majority of
patients in the published series were given antiproliferative agents.

Other explanations for this observed difference are the differences in comorbidities, genetic or socioenvironmental factors. Although this study accounted for hypertension and diabetes as the primary renal diseases, we were unable to assess the contribution of these conditions as comorbidities if not documented as the primary cause of renal disease due to the retrospective nature of the study. This could be important as diabetes and hypertension as comorbidities have been demonstrated to have an impact on long-term renal transplant outcomes [15,16,37]. In particular, NODAT (new-onset diabetes after transplantation) is more prevalent in the Black population [16,38]. Furthermore, Black patients in the UK awaiting kidney-only transplants are known to have a longer time on the waiting list [24], a known risk factor for poorer graft survival [14] and a higher level of panel reactive antibodies—another risk factor [39].

A range of potential genetic polymorphisms have been proposed as potential contributors to the observed poorer outcome [40–42], predominantly related to increased immune responsiveness in the Black population. One other previously hypothesized genetic contributor is the single nucleotide polymorphisms of the ABCB1 gene, encoding P-glycoprotein. In this cohort, 208 (183 non-Black, 25 Black) renal transplant recipients with ABCB1 genotype data, there was no influence of the g.1236C > T, g.2677G > [T,A] and g.3435C > T single-nucleotide polymorphisms individually or in combination on primary or secondary outcomes (data not shown).

In this retrospective study, it was not possible to determine the potential contributions of social or environmental factors. Our study did not differentiate between more specific ethnic groups that are represented by our broad definition of the Black population, and may potentially explain the differences in the observations compared to the French cohort with differences in traditional migration patterns [18]. As causes of graft failure or decline in renal function were not identified, they could not be directly attributed to immune-mediated graft loss and cannot be regarded as direct evidence to support ethnicity as a factor in selection of the immunosuppressive regimen. In the long-term, ethnicity is unlikely to be easily defined due to an increasing degree of ethnic admixture, but ethnic group remains a surrogate marker for environmental and genetic risk factors.

Our data suggest the need to investigate the potential biological causes underlying poor transplant outcomes in Black patients.

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References


Use of isoniazid chemoprophylaxis in renal transplant recipients

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Abstract

**Background.** The use of isoniazid (INH) as chemoprophylaxis for tuberculosis (TB) in renal transplant recipients has not been widely studied or reported from a country where TB is endemic. We are reporting here the results of the largest ever-reported randomized, prospective study of the use of INH in renal transplant recipients.

**Methods.** Four hundred consecutive live related renal transplant recipients between April 2001 and September 2004, from this single center, were randomized to receive or not receive INH for 1 year after transplantation.

**Results.** There were 12 dropouts. Of the remaining 388, 181 recipients received INH for 1 year post-transplant and 207 did not. The primary disease, comorbidities, HLA (human leucocyte antigen) match, immunosuppression, episodes of rejection, the use of anti-rejection agents, a past history of TB in the donor, the recipients and in family members living in same house and a history of TB in the family were factors compared in the two groups. The only significant difference between the two groups was that there was an increased family history of TB in recipients who received INH ($P = 0.01$). One recipient from the INH group and 16 recipients from the non-INH group developed TB ($P = 0.0003$). Discontinuation of INH for hepatotoxicity was not required in any patient.

**Conclusion.** These results provide evidence that the use of INH following renal transplantation should be considered mandatory in geographical areas where the prevalence

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