

Article type : O - Original Article

Long and short term outcomes in renal cadaveric allografts: a large recipient and donor genome-wide association study

Authors: UKIRTC, WTCCC3

Maria P Hernandez-Fuentes^{1*†}, Christopher Franklin^{2*}, Irene Rebollo-Mesa^{1*†}, Jennifer Mollon^{1,34}, Florence Delaney^{1,3}, Esperanza Perucha¹, Caragh Stapleton⁶; Richard Borrows⁴, Catherine Byrne⁵, Gianpiero Cavalleri⁶, Brendan Clarke⁷, Menna Clatworthy⁸, John Feehally⁹, Susan Fuggle¹⁰, Sarah A. Gagliano¹¹, Sian Griffin¹², Abdul Hammad¹³, Robert Higgins¹⁴, Alan Jardine¹⁵, Mary Keogan³², Timothy Leach¹⁶, Iain MacPhee¹⁷, Patrick B. Mark¹⁵, James Marsh¹⁸, Peter Maxwell¹⁹, William McKane²⁰, Adam McLean²¹, Charles Newstead²², Titus Augustine²³, Paul Phelan²⁴, Steve Powis²⁵, Peter Rowe²⁶, Neil Sheerin²⁷, Ellen Solomon²⁸, Henry Stephens²⁴, Raj Thuraisingham²⁹, Richard Trembath²⁸, Peter Topham³⁰, Robert Vaughan³¹, Steven H. Sacks^{1,3}, Peter Conlon^{6,32}, Gerhard Opelz³³, Nicole Soranzo^{2,34}, Michael E. Weale^{28§†}, and Graham M. Lord^{1,3§} for the United Kingdom and Ireland Renal Transplant Consortium (UKIRTC) and the Wellcome Trust Case Control Consortium (WTCCC)-3.

* Equal contributors

§ Equal senior contributors

Affiliations:

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/ajt.14594](https://doi.org/10.1111/ajt.14594)

1. King's College London, MRC Centre for Transplantation, London, UK
2. Wellcome Trust Sanger Institute, Human Genetics, Cambridge, UK
3. NIHR Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London, London, UK
4. Renal Institute of Birmingham, Department of Nephrology and Transplantation, Birmingham, UK
5. Nottingham Renal and Transplant Unit, Nottingham University Hospitals NHS Trust, Nottingham, UK
6. Royal College of Surgeons in Ireland, Dublin, Ireland
7. Transplant and Cellular Immunology, Leeds Teaching Hospitals NHS Trust, Leeds, UK
8. Department of Medicine, University of Cambridge, Cambridge, UK
9. Leicester General Hospital, Leicester, UK
10. Transplant Immunology & Immunogenetics, Churchill Hospital, Oxford, UK
11. Center for Statistical Genetics, Department of Biostatistics, University of Michigan, USA
12. Cardiff & Vale University Health Board, Cardiff University, Cardiff, UK
13. The Royal Liverpool and Broadgreen University Hospitals, Liverpool, UK
14. University Hospitals Coventry and Warwickshire NHS Trust, Coventry, UK
15. School of Medicine, Dentistry and Nursing, University of Glasgow, Glasgow, UK
16. Queen Alexandra Hospital, Portsmouth, UK
17. St Georges' Hospital NHS Trust, London, UK
18. Epsom and St Helier University Hospitals Trust
19. School of Medicine, Dentistry and Biomedical Sciences, Queens University Belfast, Northern Ireland, UK
20. Sheffield Kidney Institute, Sheffield Teaching Hospitals NHS Foundation Trust, Sheffield, UK
21. Kidney and Transplant, Imperial College Healthcare NHS Trust, London, UK
22. Leeds Teaching Hospitals NHS Trust, Leeds
23. Central Manchester University Hospitals NHS Trust, Manchester, UK
24. NHS Lothian, Edinburgh, UK
25. Division of Medicine, University College London, London, UK
26. Plymouth Hospitals NHS Trust, Plymouth, UK

27. The Medical School, Newcastle University Newcastle, Newcastle upon Tyne, UK
 28. Division of Genetics & Molecular Medicine, King's College London
 29. Barts Health NHS Trust, London, UK
 30. Leicester General Hospital, Leicester, UK
 31. Clinical Transplantation Laboratory at Guy's Hospital, Guy's and St Thomas' NHS Trust, London UK
 32. Beaumont Hospital, Dublin, Ireland
 33. University of Heidelberg, Transplantation Immunology, Heidelberg, Germany
 34. Department of Haematology, University of Cambridge
- † Current address: UCB Celltech, Slough, UK
- ‡ Current address: Genomics plc, Oxford, UK

Corresponding author: Prof Graham Lord

email: graham.lord@kcl.ac.uk

I hereby confirm that had full access to all the data in the study and I had the final responsibility for the decision of submitting the manuscript for publication.

Abbreviations:

UK: United Kingdom

UKIRTC: The United Kingdom and Ireland Renal Transplant Consortium

WTCCC3: Wellcome Trust Case Control Consortium 3

NIHR: The National Institute for Health Research

ABSTRACT

Improvements in immunosuppression have modified short-term survival of deceased-donor allografts, but not their rate of long-term failure. Mismatches between donor and recipient HLA play an important role in the acute and chronic allogeneic immune response against the graft. Perfect matching at clinically relevant HLA loci does not obviate the need for immunosuppression, suggesting that additional genetic variation plays a critical role in both short-and long-term graft

outcomes. By combining patient data and samples from supranational cohorts across the UK and EU, we performed the first large-scale genome-wide association study analysing both donor and recipient DNA in 2,094 complete renal transplant-pairs with replication in 5,866 complete pairs. We studied deceased-donor grafts allocated on the basis of preferential HLA matching, which provided some control for HLA genetic effects. No strong donor or recipient genetic effects contributing to long or short term allograft survival were found outside the HLA region. We discuss the implications for future research and clinical application.

INTRODUCTION

Kidney transplantation is a highly successful treatment for end-stage renal failure, with significant benefits for recipients both in survival and quality of life. Early outcomes have steadily improved over the last 10 years,⁽¹⁾ with risk-adjusted and death censored, 1-year renal graft survival rates of 94% and 97% for deceased and living donor transplants respectively.⁽²⁾ However, both late allograft loss and increased mortality among transplant recipients remain key challenges for the transplant community. There are a wide number of factors that are known to influence long-term transplant outcome, including donor factors such as age and comorbidity, recipient factors such as comorbidity and response to immunosuppression, as well as allograft ischaemic time, the degree of HLA mismatch and the development of donor-specific antibodies (DSA).⁽³⁻⁵⁾ However, a comprehensive understanding of the pathophysiology of graft failure has remained elusive, with the observed variation in patient outcomes still inadequately explained by our current understanding of risk factors. An improved understanding of the determinants of transplantation outcome would allow the development of truly personalized approaches to the management of transplant recipients.

The importance of genetic factors in transplantation has been clear since the inception of the technique, with the first successful kidney transplant having been performed between identical twins in 1954. Renal transplantation between identical twins continues to show excellent long-term outcomes^(6, 7) and HLA matching has a large impact on graft survival even in the modern era of immunosuppression.⁽⁸⁾

HLA genes are highly polymorphic, and demonstrate the importance of genetic variation in donor-recipient pairing which impacts on long-term outcomes. However over recent decades, our ability to assay human genetic variation beyond the HLA region has increased considerably.

A number of studies have been published over recent years exploring the association between genotypes of interest and renal transplant outcomes.(9, 10) A large proportion of these studies have concentrated on immune-related genes, based on the hypothesis that the risk of acute rejection or late allograft loss may be modulated by genetic variation in the immune response. As summarized in Table S1, associations have been described between various transplant phenotypes and single nucleotide polymorphisms (SNPs) in a number of genes including those encoding tumour necrosis factor (TNF)- α , interleukins-1, -6 and -10, and interferon- γ . Of note, many of these studies have reported inconsistent findings. For example, analysis of DNA from donor-recipient pairs in the Collaborative Transplant Study failed to replicate an earlier finding that particular combinations of C3 genotypes in the donor and recipient were associated with reduced graft survival.(11, 12) While some of this discrepancy might be explained by methodological or populational differences between these studies, it is difficult to draw firm conclusions about the role of these genetic variations.(13)

More recently, attention has also focused on non-immune-related genetic risk variants. Donor genetic variation in *CAV1* (caveolin-1)(14), *APOL1* (apolipoprotein-L1)(15, 16), or *ABCB1* (ATP-binding cassette, subfamily-B, member-1, expressed in the kidney) genes (17, 18) has been reported to be associated with increased risk of allograft failure or poorer recipient survival. Recipient genetic variation effects on graft and patient survival have only been reported in one cohort for *CAV1*(14). In addition to effects of donor genetic variants, it might be expected that recipient genotypes in other pharmacometabolic pathways might also impact on transplant outcomes such as increased risk of acute rejection.(19)

In general, candidate gene studies in renal transplantation have so far failed to provide consistent and reproducible results. Some of the reasons for this may include small sample sizes, variations in genotyping methodology and strategy, and, perhaps most importantly, a lack of consistency in clinical phenotyping.(20) Genome-

wide association studies (GWAS) have contributed greatly to an increased understanding of complex common conditions such as inflammatory bowel disease, hypertension, type 2 diabetes and schizophrenia.(21) A small number of GWAS have been reported in the field of renal transplantation, describing SNPs associated with cardiovascular adverse events in recipients taking calcineurin inhibitor (CNI) immunosuppression,(22) two SNPs associated with serum creatinine levels at 5 years post-transplant,(23) and a number of SNPs associated with the development of new-onset diabetes after transplantation.(24) Recently, a GWAS using pooled DNA of recipient-only origin found variation in two new loci associated with acute rejection in both univariate and multivariate analysis.(25) However, these studies were underpowered for discovery of genetic variants with small effect sizes.

The Wellcome Trust Case Control Consortium (www.wtccc.org.uk/ccc3) has led the deployment of GWAS in a wide range of conditions. As part of WTCCC-3, all renal transplant centres in the UK and Ireland formed the UK and Ireland Renal Transplant Consortium (UKIRTC; www.ukirtc.org). Collaborative initiatives such as these are essential for the collection of adequate sample numbers, for the sharing of expertise, standardization of techniques, and building consensus on accurate phenotyping of clinical data. Through this consortium, 3,936 samples comprising 2,094 complete donor-recipient pairs were tested in the GWAS discovery phase, and an additional 5,866 complete donor-recipient pairs in the replication phase, making this the largest GWAS conducted to date in renal transplantation outcomes.

METHODS

Discovery study participants: The large multicentre United Kingdom and Ireland Renal Transplant Consortium (www.UKIRTC.org), coordinated by King's College London in partnership with the Wellcome Trust Case-Control Consortium-3 and the National Health Service Blood and Transplant database (NHS-BT), sourced all available good-quality stored DNA samples and pre-existing GWAS data from both recipients and their corresponding donors from all renal transplantation centres in the United Kingdom and the Republic of Ireland (listed in Table S2). The study was approved by the Hammersmith and Queen Charlotte's & Chelsea Research Ethics Committee REC No 08/H0707/1, on 14th October 2009. All samples and anonymised data for the replication cohort were provided by the University of Heidelberg.

Following genotyping and GWAS QC (see below), there were 2,689 unique recipients, 2,204 unique donors and 2,094 complete donor-recipient transplantation pairs available for analysis. Figure 1 describes the study design and analysis steps. Table 1 provides additional information for the complete donor-recipient pairs (discrepancies between Figure 1 and Table 1 are because some recipients received more than one graft, some donors donated two kidneys to different recipients, and not all recipients had matching donor GWAS data and vice versa). The samples and data referred to transplants that took place between December 1981 and December 2007.

Inclusion criteria for the study were as follows: (1) deceased donor kidney transplants only; (2) recipient is an adult (>16 years old); (3) reported European ancestry for recipients; (4) graft survival time greater than 3 months. Donor-recipient allocation followed NHS-BT standard UK-protocols during the study period.

This study was approved by the Hammersmith and Queen Charlotte's & Chelsea Research Ethics Committee REC No 08/H0707/1, approval for the collection of samples and clinical information from donors and recipients was obtained on 14th October 2009. Third party anonymised clinical data has been provided by NHS-BT UK.

Replication phase participants: A cohort of 5,866 complete donor-recipient pairsⁱ, with similar ethnicity to that of the discovery study were obtained from the Collaborative Transplant Study (CTS) DNA Biobank held at the University of Heidelberg, Germany (Table 1).

Clinical variables, datasets and analysis are described in supplementary methods.

Discovery phase genotyping and analysis. A whole genome amplification step was undertaken (Source BioScience, Nottingham) for samples containing 5 – 40 μ L of DNA (n=990 samples). Samples were assayed via Illumina 670 Quad Custom GWAS chips, and subjected to standard post-genotyping quality control procedures before being imputed to the 1000-genomes reference dataset. Imputation of HLA alleles from SNP genotype data was undertaken using HLA*IMP software (26) and compared to serologically typed alleles. A series of GWAS analyses were performed to investigate different genetic models and the renal transplant outcomes of interest. Graft survival genome-wide analyses were performed (using Cox proportional

hazards modelling) for (1) donor SNP genotype main effects; (2) recipient SNP genotype main effects; (3) donor*recipient SNP genotype interaction effects (1df and 3df tests); and (4) CNV-tag-SNPs genotype mismatch effects (two different models). Acute rejection genome-wide analyses were performed (using logistic regression) for models (1)-(3). An end-stage renal failure genome-wide analysis was performed (using logistic regression) for model (2), and to take advantage of the opportunity an intracranial haemorrhage genome-wide analysis was performed (using logistic regression) for model (1).

Replication phase genotyping and analysis. Replication DNA samples were received at King's College London and sent to the Wellcome Trust Sanger Institute for replication analysis. A replication panel of 139 SNPs (post-QC) was tested based on a combination of low p-value ($< 10^{-6}$) from the discovery phase, plus good support of association signals from SNPs in local linkage disequilibrium (LD), or on prior candidature from previous association studies. SNPs were tested according to the same model as motivated their inclusion in the replication panel (for example, if nominated based on a low p-value for acute rejection in recipients, then that was also the test of interest in the replication analysis). Meta-analysis of discovery and replication results was carried out using inverse variance meta-analysis (27).

For further details see Figure 1 and the Methods section in the Supplementary Appendix.

Role of the funding source: The funding sources did not participate in the study design, collection, analysis or interpretation of the data, nor did they have a role in writing the report or the decision to submit for publication.

RESULTS

1) SNP association analysis

Despite the large size of our study (Table 1 & Table S2), none of the phenotypes and genetic models tested in the discovery phase produced any linkage disequilibrium-supported single-SNP results of genome-wide significance (p-value $\leq 5 \times 10^{-8}$). We also performed a partitioned heritability analysis via stratified LD score regression

(28), which failed to reveal any significant enrichment of heritability in genomic regions marking tissue- or cell-type-specific activity (Figure 2).

We pursued a second 'replication' phase in the hope that the most significant SNPs would be enriched for true association signals. 139 SNPs (post-QC) were tested, based either on a low p-value from the discovery phase plus LD support or on prior candidature from previous association studies. In general, the distribution of replication p-values for all the tests did not depart appreciably from that expected under a global null hypothesis (Table S3). There was some enrichment for low p-values in the recipient genotype main effect tests for acute rejection in the 12 months following transplantation. However, the p-values from meta-analysis (across both discovery and replication phases) did not reach genome-wide significance (p-value $\leq 5 \times 10^{-8}$). The single SNP with strongest evidence for association was rs2289887 (Figure S1 and Table S4), which had consistent effects in both cohorts but a meta-analysis p-value of only 0.00011, indicating that further studies are needed to establish the validity of this signal.

We collated all previously published association signals for early graft rejection and long term allograft survival and none of these replicated in our study (Table S1).

2) HLA mismatch analysis

We took advantage of recorded serological information to check for known associations with donor-recipient mismatch levels and to compare recorded information with imputed mismatches based on SNP genotype information (Figure 3). As expected for cadaveric donor transplants, the number of non-zero mismatch transplants in our data was low, reducing our power to detect associations. Nevertheless, we confirmed significant associations with HLA-A (p=0.022) and HLA-DRB (p=0.00049) mismatches using the recorded data.

On the other hand, the imputed mismatch results did not reproduce the expected associations. We attribute this to two factors: (1) the true mismatch=2 frequency was low, due to donor selection; (2) as with any statistical noise, the imputation inaccuracy tended to act with disproportionate effect in the extremes of the distribution (here, the mismatch=2 group), resulting in higher relative errors in that group. Thus, although the overall imputation accuracy was high (Tables S5 and S6),

the rate of false positives was disproportionately high in the mismatch=2 categories, swamping our ability to detect the true association signals (Table S7).

DISCUSSION

In this paper, we report the results of the first large scale GWAS in renal transplantation. Despite our considerable sample size, we did not replicate any proposed findings from previous candidate gene studies nor did we discover any convincing new variants in our own analyses. There are a number of plausible reasons that may explain this.

Firstly, although this was a study involving thousands of individuals, by GWAS standards it was at the lower end of the range of sample sizes that have been employed for other human traits.(29, 30) A decade of GWAS across multiple complex traits has shown that single effect sizes for any one causal variant are typically low, and thus for some traits even bigger sample sizes than ours are needed to discover them. For example, the first robustly associated locus for schizophrenia was found in a discovery GWAS cohort of 3,322 cases and 3,587 controls.(31) The number of reliably associated signals for schizophrenia has now grown to 108, providing new biological insights into the disease, thanks to a meta-analysis that involved 40,000 cases and 113,000 controls.(32)

Secondly, part of our study sought to look for recipient-donor genetic interactions. Interaction effects require even larger sample sizes to be reliably discovered. With a few notable exceptions,(33) GWAS studies on other traits have been unsuccessful in discovering reliable interaction effects.

Thirdly, and perhaps most importantly, the transplantation outcomes we considered were relatively crude measures obtained retrospectively from national registry data, collected over many years for reasons other than for acting as endpoints in a genome-wide association study. Our outcomes were therefore heterogeneous, subject to missingness, and subject to environmental factors which likely weakened the genetic signals. Graft survival time, is expected to be subject to a range of

factors including graft quality, drug regimen, patient compliance, changes in standard-of-care over time, between-centre differences, and underlying biological causes of renal dysfunction. Additionally, a substantial proportion of the survival time data was right-censored (60.5%-76.4% depending on model being fitted), reducing the power for this endpoint.

Acute graft rejection is also a heterogeneous phenotype arising from a various immune and non-immune biological causes. Furthermore, this phenotype was derived from a field that in many records was left blank, resulting in a high degree of missingness (56.7%-58.3% depending on model being fitted). The high missingness rate reduced the power of our study, and non-random missingness would complicate the interpretation of any positive association signals we might have found (although we note the non-random missingness bias would need to be the same in both the discovery and replication cohorts for a signal to be replicated).

Batch effects are also a concern. Both the discovery and replication data were obtained from multiple different collection points in different countries over many years, and thus our phenotypes may be subject to batch effects, for example arising from different treatment protocols over time and space. Genetic data can also be subject to batch effects, for example arising from differences in sample collection protocols and unknown differences in population structure. We applied both covariate selection and genetically derived principal component axes to try and mitigate such effects. We also note that, as with non-random missingness, the batch effect structure would need to be the same in both the discovery and replication cohorts in order for false positive association signals to be replicated. Nevertheless, together these extraneous factors may well have increased the noise in our data, and so reduced (and made less detectable) the genetic effects in our data.

HLA imputation from chip-based genetic data might in time be of value as an adjunct to serological typing. However, our investigations found that the additional noise introduced by imputation uncertainty prevented the imputed data from picking up the well-known mismatch signals at HLA-A and HLA-DRB. This suggests that the accuracy of HLA imputation will need to be improved before it can be used reliably as an alternative to serotyping.

In contrast, our serological data successfully detected the HLA-A and HLA-DRB mismatch signals, despite the reduced power to do so resulting from HLA-matching of cadaveric allografts. This indicates that, despite the various shortcomings of our study, strong HLA signals were detectable in our data. The implication therefore, at least as far as our primary graft survival endpoint is concerned, is that signals outside the HLA region are weaker than those already established in the HLA region. We therefore anticipate that genetic effects outside of the HLA region are more likely to be of value in elucidating biological pathways than in direct clinical prediction.

We explored this last point further via formal power calculations. These indicated that we were well powered to detect any main effect graft survival association signals involving causal SNPs with allelic hazard ratios in the range 1.4 - 1.9 (log-additive risk model, $\alpha=5 \times 10^{-8}$, power=0.8, minor allele frequency > 0.05), and to detect main effect acute rejection association signals with allelic odds ratios in the range 1.7 - 2.9. We emphasise that these effect sizes are applicable to the traits investigated in this study, but that larger effects might be found in future studies under more precise phenotyping.

In summary, while our study was able to replicate known mismatch signals in the HLA region, we failed to find convincing association signals outside of the HLA region. Both phenotype heterogeneity and sample size may have contributed to this result. Looking ahead, we note that the general lessons from GWAS applied to multiple human traits over more than a decade have brought home three clear messages.^(29, 30) The first is that all complex traits contain a genetic component, and harbour a large number of causal variants throughout the genome. The second is that larger GWAS studies, often obtained via meta-analysis with previous studies, inevitably succeed in discovering some portion of these causal variants. The third is that when a large enough portion of these variants is discovered, new insights into the biology of the trait are gained. With these points in mind, we look forward to an international consortium (iGeneTrain⁽³⁴⁾), which has been formed to share and meta-analyse genetic and phenotypic data from most major transplant cohorts worldwide. We also look forward to efforts to collect more detailed phenotypes of relevance to transplant failure, which should provide greater genetic resolution. The data provided by our study form a foundation for ongoing efforts seeking to uncover the biology and improve the prospects for renal transplantation outcomes.

ACKNOWLEDGMENTS

Supported by grants awarded from the Wellcome Trust (090355/A/09/Z, 090355/B/09/Z and 088849/Z/09/Z, “WTCCC3”), the Medical Research Council (grants G0600698 and MR/J006742/1 to S.H.S; G0802068 to G.M.L. and MR/K002996/1 to G.M.L; grants G0801537/ID: 88245), Guy’s and St Thomas’ Charity (grants R080530 and R090782) to MHF and GML, from the European Union FP7 (grant agreement no HEALTH-F5–2010–260687 to MHF and project number 305147: BIO-DrIM to MHF and IRM); and by the National Institute for Health Research Biomedical Research Centre at Guy’s and St Thomas’ and King’s College London. Anonimised clinical data was also provided NIHR Health Informatics Collaborative. Nicole Soranzo's research is supported by the Wellcome Trust (Grant Codes WT098051 and WT091310), the EU FP7 (EPIGENESYS Grant Code 257082 and BLUEPRINT Grant Code HEALTH-F5-2011-282510) and the National Institute for Health Research Blood and Transplant Research Unit (NIHR BTRU) in Donor Health and Genomics at the University of Cambridge in partnership with NHS Blood and Transplant (NHSBT). The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, the Department of Health or NHSBT. The NIHR Health Informatics Collaborative includes: Bradley JR, University of Cambridge; Channon KM, University of Oxford; Lord GM, King’s College London; Weber J, Imperial College London; Williams B, University College London; Crichton C, University of Oxford; Davies J, University of Oxford; Welch J, University of Oxford; Woods K, Oxford University Hospitals NHS Foundation Trust.

AUTHORS AND CONTRIBUTIONS

| Name | Literature search | Study design | Data + Sample Collection | Data analysis and interpretation | Manuscript preparation and review | Grant provision & supervision |
|----------|-------------------|--------------|--------------------------|----------------------------------|-----------------------------------|-------------------------------|
| MPH | X | | X | | X | X |
| CF & IRM | X | | | X | X | |
| JM | X | | | | | |
| FD | | | X | | | |
| EP | X | | X | X | X | |
| CS | | | | X | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| RB, CB, BC, MC, JF, SF, SG, AH, RH, AJ, MK, TL, IMc, PBM, JM, PM, WMcK, AMcL, CH, TA, PP, SP, PR, HS, RT, PT, | | | X | X | | |
| NS, ES | | | | | | X |
| RT, | X | | | | | X |
| RV, SS | X | X | X | X | | |
| GC, PC | X | X | X | | X | X |
| GO | | | X | | | |
| NS | | | | | X | X |
| MW | X | | | X | X | X |
| GL | X | | | | X | X |

DISCLOSURE

Author MEW is an employee of Genomics plc, a company providing genomic analysis services to the pharmaceutical and health care sectors. MHF and IRB are employees of UCB Celltech, a pharmaceutical company. Their involvement in the conduct of this research was solely in their capacity as academics at King's College London.

Supporting Information

This article is protected by copyright. All rights reserved

Additional Supporting Information may be found online in the supporting information tab for this article.

Supplementary Methods

Table S1: Data on previously published SNPs. Partial table below for benefit of reviewers. For full table, including explanation of headers, refer to Excel file uploaded in the system.

Table S2. Sample distribution per Transplant centre, UK.

Table S3. Data on SNPs selected for replication, together with discovery-phase results and replication-phase results.

Table S4. SNP with evidence for an AR-recipient main effect in both GWAS and Replication.

Table S5: Overall imputation accuracy at the allele level using serological Broad Specificity as gold standard.

Table S6. Frequency distribution of correctly and incorrectly imputed alleles by broad serological specificity.

Table S7. Predictive performance of imputation compared to serological typing in determining the number of HLA mismatches (at 2-digit resolution) between donor and recipient.

Table S8. Missingness in the covariates considered for the multivariate analysis.

Figure S1. Regional association plot of the single SNP with main effect tests for acute rejection in the 12 months following transplantation, replication and meta-analysis.

TABLES

Table 1. Indicative renal transplant demographics from WTCCC3 and the validation cohort. For consistency, numbers refer to transplants where both donors and recipients passed QC ('complete' donor-recipient pairs).

| | WTCCC3 (post QC) | % | Replication cohort | % |
|--|-----------------------------|----------|-------------------------------|----------|
| Total transplants where both donors and recipients passed QC | 2094 | 100% | 5866 | 100% |
| Total unique donors with a paired recipient | 1850 | 100% | 5027 | 100% |

| | | | | |
|--|-------------|-------|-------------|-------|
| Total unique recipients with a paired donor | 2086 | 100% | 5866 | 100% |
| Mean donor age +/- SD | 43 +/- 15.4 | | 43 +/- 16.7 | |
| Mean recipient age +/- SD | 45 +/- 13.3 | | 48 +/- 13.6 | |
| 0 previous grafts | 1864 | 89% | N/A | |
| 1 previous graft | 204 | 9.7% | N/A | |
| 2 or more previous grafts | 26 | 1.2% | N/A | |
| Graft survival time (days-to-uncensored-event, 25% quartile) | 902 | | 442 | |
| Graft survival time (days-to-uncensored-event, median) | 1866 | | 1769 | |
| Graft survival time (days-to-uncensored-event, 75% quartile) | 3165 | | 3162 | |
| 0 HLA mismatches | 223 | 10.7% | N/A | |
| 1 or 2 Class I HLA mismatches | 839 | 40.1% | N/A | |
| 1 or 2 Class II HLA mismatches | 20 | 0.96% | N/A | |
| 1 or 2 mixed Class I/II HLA mismatches | 124 | 5.9% | N/A | |
| 3 to 5 HLA mismatches | 612 | 29.2% | N/A | |
| 6 HLA mismatches | 8 | 0.4% | N/A | |
| N/A HLA mismatches | 268 | 12.8% | N/A | |
| Graft survival: total uncensored | 495 | 23.6% | 2951 | 50.3% |
| Graft survival: total censored | 1599 | 76.4% | 2915 | 49.7% |
| Total double-kidney transplants | 3 | 0.14% | N/A | |
| Total enpbloc kidney transplants | 1 | 0.05% | N/A | |
| Total kidney+pancreas transplants | 16 | 0.76% | N/A | |
| Total kidney-only transplants | 2074 | 99.0% | N/A | |
| Total rejections (first 3 mo.) | 259 | 12.4% | N/A | |
| Total no rejections (first 3 mo.) | 915 | 43.7% | N/A | |
| N/A rejections (first 3 mo.) | 920 | 43.9% | N/A | |
| Total rejections (3-12 mo.) | 221 | 10.6% | 575 | 9.8% |
| Total no rejections (3-12 mo.) | 946 | 45.2% | 2573 | 43.9% |
| N/A rejections (3-12 mo.) | 927 | 44.3% | 2718 | 46.3% |

N/A: data not available.

FIGURES

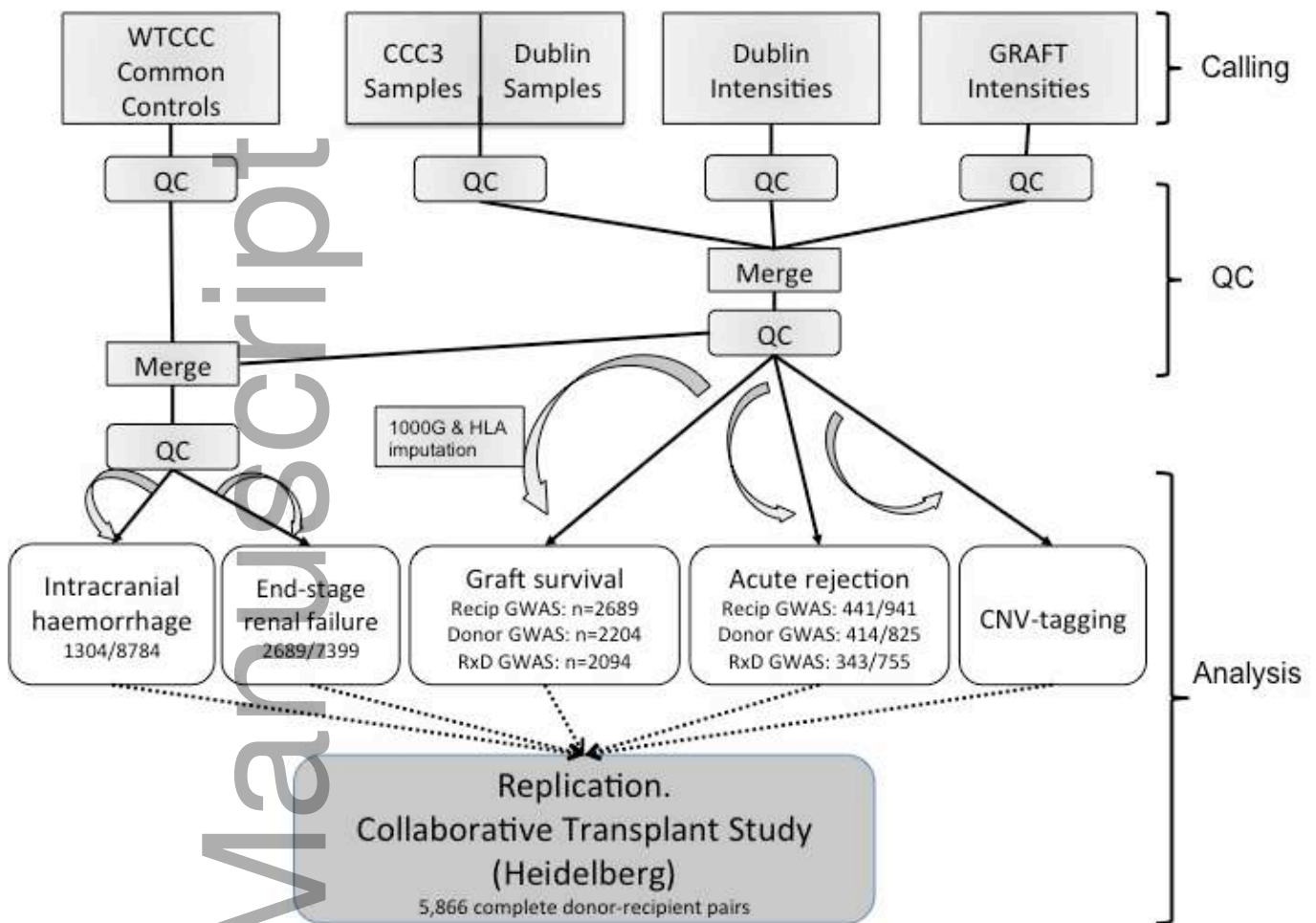
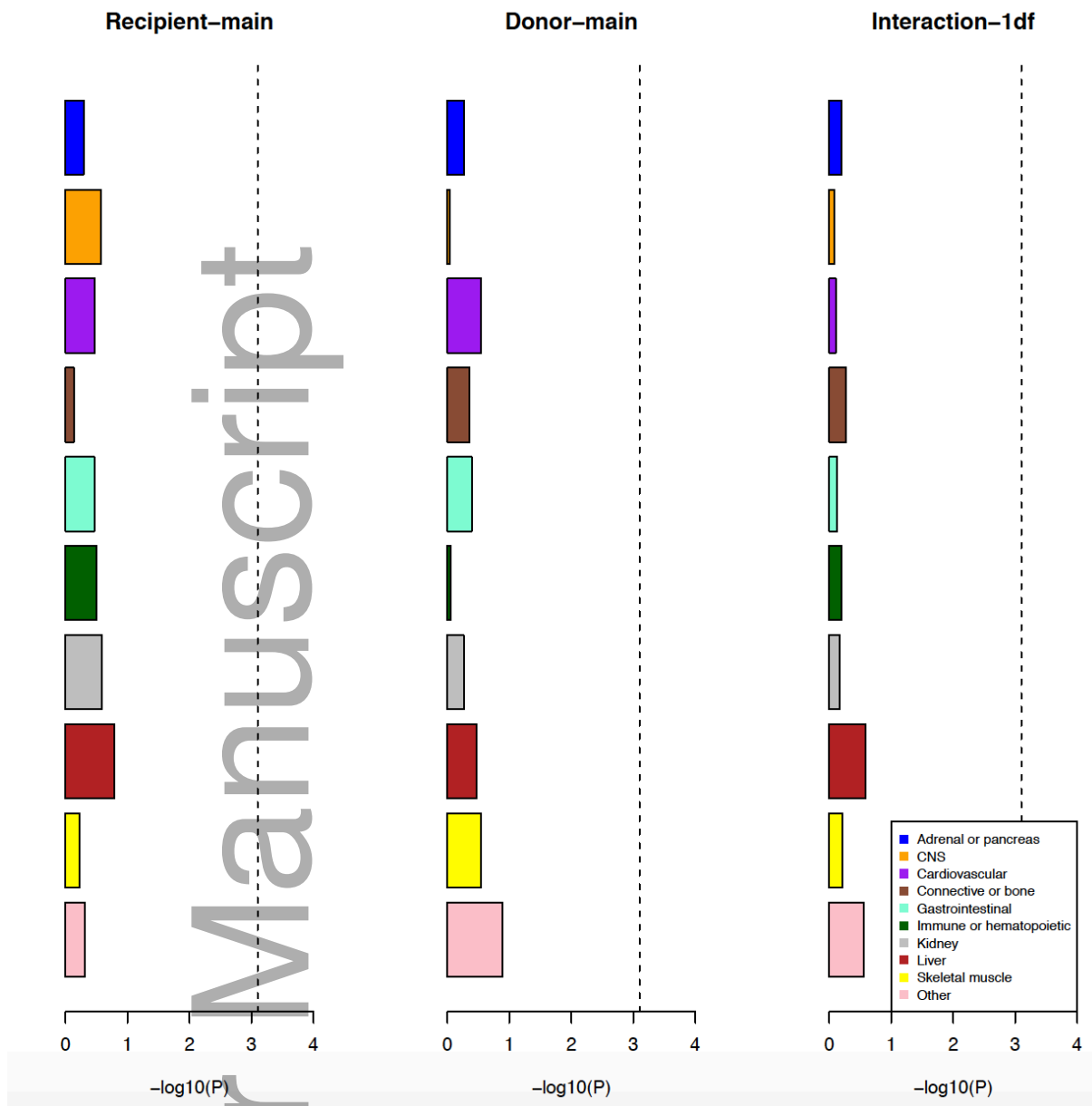


Figure 1. Analysis workflow and strategy. Main input cohorts, analysis methods and sample sizes are indicated. For binary traits, numbers indicate samples with/without the trait. For further details, see Methods and Supplementary Methods.

Figure 2. Partitioned heritability analysis of graft survival GWAS results.

X-axis indicates $-\log_{10}(\text{p-value})$ for a test for heritability enrichment within 10 cell/tissue-type categories of genomic annotations, marking tissue- or cell-type-specific activity. Dotted lines indicate Bonferroni significance level. (a) death treated as a censored event; (b) death treated as a failure event.

(a)



(b)

Recipient-main

Donor-main

Interaction-1df

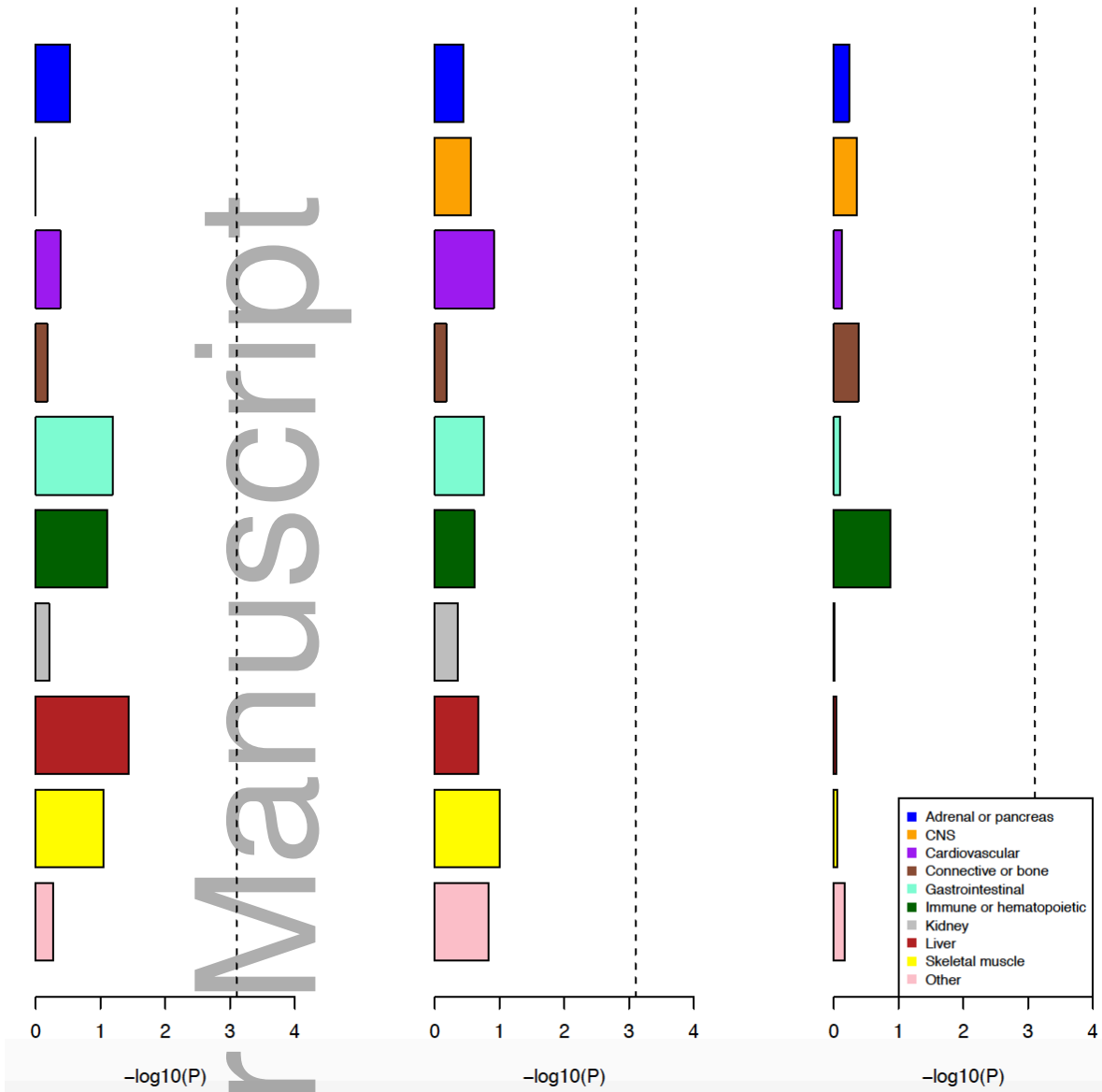
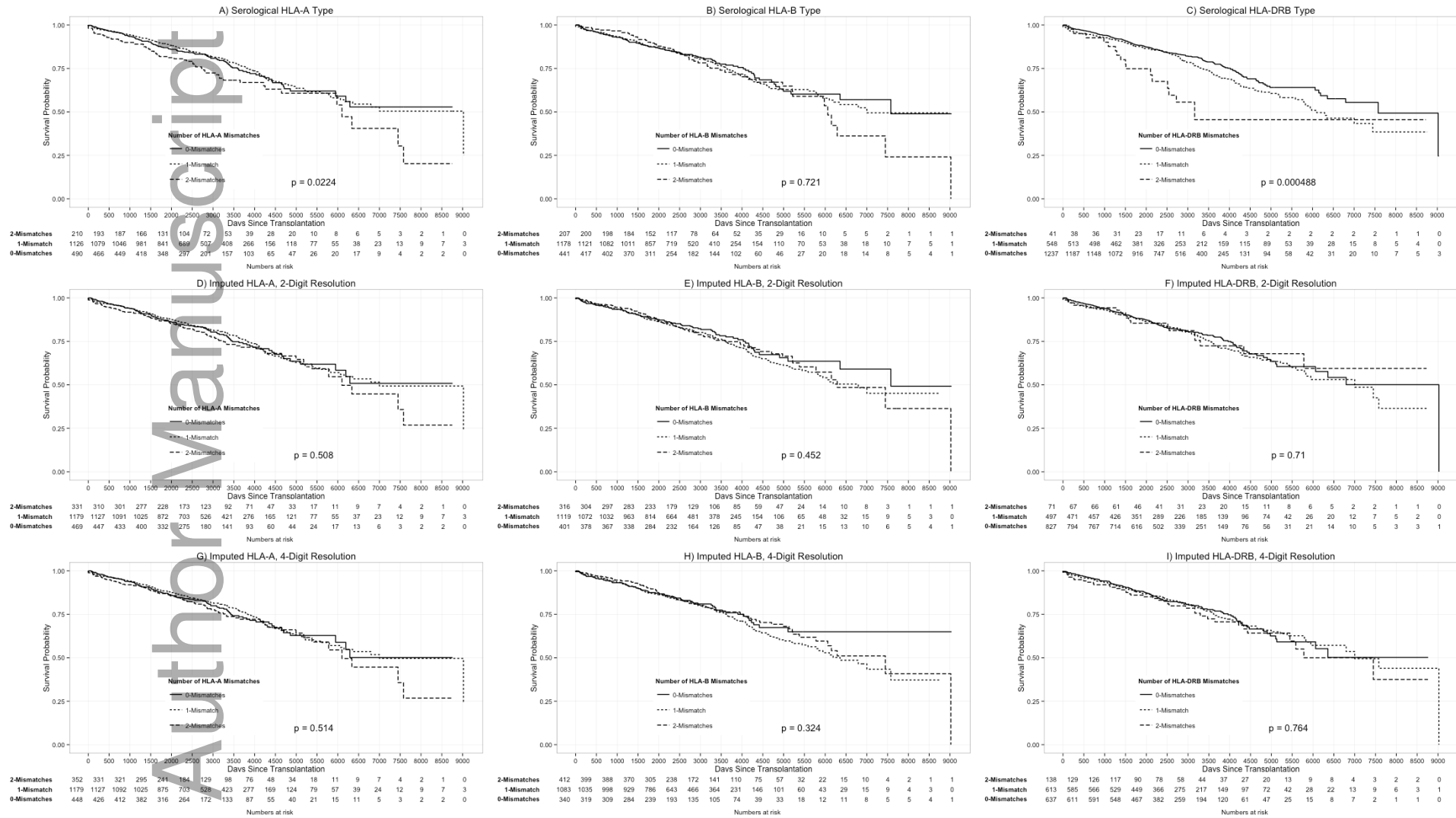


Figure 3 . Kaplan Meier plots of graft survival by number of mismatches by serological typing (A, B and C), imputed 2-digit resolution (D, E and F), and imputed 4-digit resolution (G, H and I). P-values were obtained from likelihood ratio tests on Cox proportional hazards models.



REFERENCES

1. Opelz G. President's Address. The Transplantation Society--Berlin 2012. *Transplantation* 2013;95(1):4-7.
2. Bradbury L. Annual Report on Kidney Transplantation. Report for 2014/2015. UK: National Health Service - Blood and Transplant; 2015 September 2015.
3. Johnson RJ, Bradbury LL, Martin K, Neuberger J, Registry UT. Organ donation and transplantation in the UK-the last decade: a report from the UK national transplant registry. *Transplantation* 2014;97 Suppl 1:S1-S27.
4. Legendre C, Canaud G, Martinez F. Factors influencing long-term outcome after kidney transplantation. *Transpl Int* 2014;27(1):19-27.
5. Nabokow A, Dobronravov VA, Khrabrova M, Gröne HJ, Gröne E, Hallensleben M et al. Long-term kidney allograft survival in patients with transplant glomerulitis. *Transplantation* 2015;99(2):331-339.
6. MERRILL JP, MURRAY JE, HARRISON JH, GUILD WR. Successful homotransplantation of the human kidney between identical twins. *J Am Med Assoc* 1956;160(4):277-282.
7. Kessaris N, Mukherjee D, Chandak P, Mamode N. Renal transplantation in identical twins in United States and United Kingdom. *Transplantation* 2008;86(11):1572-1577.
8. Süsal C, Opelz G. Current role of human leukocyte antigen matching in kidney transplantation. *Curr Opin Organ Transplant* 2013;18(4):438-444.
9. Simmonds MJ. Using Genetic Variation to Predict and Extend Long-term Kidney Transplant Function. *Transplantation* 2015;99(10):2038-2048.
10. Chand S, McKnight AJ, Borrows R. Genetic polymorphisms and kidney transplant outcomes. *Curr Opin Nephrol Hypertens* 2014;23(6):605-610.
11. Brown KM, Kondeatis E, Vaughan RW, Kon SP, Farmer CK, Taylor JD et al. Influence of donor C3 allotype on late renal-transplantation outcome. *N Engl J Med* 2006;354(19):2014-2023.
12. Varaganam M, Yaqoob MM, Döhler B, Opelz G. C3 polymorphisms and allograft outcome in renal transplantation. *N Engl J Med* 2009;360(9):874-880.
13. Goldfarb-Rumyantsev AS, Naiman N. Genetic predictors of acute renal transplant rejection. *Nephrol Dial Transplant* 2010;25(4):1039-1047.
14. Moore J, McKnight AJ, Simmonds MJ, Courtney AE, Hanvesakul R, Brand OJ et al. Association of caveolin-1 gene polymorphism with kidney transplant fibrosis and allograft failure. *JAMA* 2010;303(13):1282-1287.
15. 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):841-845.

16. Freedman BI, Kopp JB, Langefeld CD, Genovese G, Friedman DJ, Nelson GW et al. The apolipoprotein L1 (APOL1) gene and nondiabetic nephropathy in African Americans. *J Am Soc Nephrol* 2010;21(9):1422-1426.
17. Woillard JB, Rerolle JP, Picard N, Rousseau A, Guillaudeau A, Munteanu E et al. Donor P-gp polymorphisms strongly influence renal function and graft loss in a cohort of renal transplant recipients on cyclosporine therapy in a long-term follow-up. *Clin Pharmacol Ther* 2010;88(1):95-100.
18. Moore J, McKnight AJ, Döhler B, Simmonds MJ, Courtney AE, Brand OJ et al. Donor ABCB1 variant associates with increased risk for kidney allograft failure. *J Am Soc Nephrol* 2012;23(11):1891-1899.
19. Wang J, Yang JW, Zeevi A, Webber SA, Girnita DM, Selby R et al. IMPDH1 gene polymorphisms and association with acute rejection in renal transplant patients. *Clin Pharmacol Ther* 2008;83(5):711-717.
20. Pallet N, Thervet E. The genetics of kidney transplantation. *Hum Genet* 2012;131(3):317-323.
21. Manolio TA. Genomewide association studies and assessment of the risk of disease. *N Engl J Med* 2010;363(2):166-176.
22. Mushiroda T, Saito S, Tanaka Y, Takasaki J, Kamatani N, Beck Y et al. A model of prediction system for adverse cardiovascular reactions by calcineurin inhibitors among patients with renal transplants using gene-based single-nucleotide polymorphisms. *J Hum Genet* 2005;50(9):442-447.
23. O'Brien RP, Phelan PJ, Conroy J, O'Kelly P, Green A, Keogan M et al. A genome-wide association study of recipient genotype and medium-term kidney allograft function. *Clin Transplant* 2013;27(3):379-387.
24. McCaughan JA, McKnight AJ, Maxwell AP. Genetics of new-onset diabetes after transplantation. *J Am Soc Nephrol* 2014;25(5):1037-1049.
25. Ghisdal L, Baron C, Lebranchu Y, Viklický O, Konarikova A, Naesens M et al. Genome-Wide Association Study of Acute Renal Graft Rejection. *Am J Transplant* 2016.
26. Dilthey AT, Moutsianas L, Leslie S, McVean G. HLA*IMP--an integrated framework for imputing classical HLA alleles from SNP genotypes. *Bioinformatics* 2011;27(7):968-972.
27. Evangelou E, Ioannidis JP. Meta-analysis methods for genome-wide association studies and beyond. *Nat Rev Genet* 2013;14(6):379-389.
28. Finucane HK, Bulik-Sullivan B, Gusev A, Trynka G, Reshef Y, Loh PR et al. Partitioning heritability by functional annotation using genome-wide association summary statistics. *Nat Genet* 2015;47(11):1228-1235.
29. Manolio TA. In Retrospect: A decade of shared genomic associations. *Nature* 2017;546(7658):360-361.
30. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA et al. 10 Years of GWAS Discovery: Biology, Function, and Translation. *Am J Hum Genet* 2017;101(1):5-22.
31. Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 2009;460(7256):748-752.

32. Consortium SWGotPG. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 2014;511(7510):421-427.
33. Strange A, Capon F, Spencer CC, Knight J, Weale ME, Allen MH et al. A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet* 2010;42(11):985-990.
34. (iGeneTRiN) IGTRiTN. Design and Implementation of the International Genetics and Translational Research in Transplantation Network. *Transplantation* 2015;99(11):2401-2412.

i

Author Manuscript