Single nucleotide polymorphisms in renal transplantation: Can't see the wood for the trees.

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Invited Commentary related to "Analysis of 75 Candidate SNPs Associated with Acute Rejection in Kidney Transplant Recipients: Validation of rs2910164 in MicroRNA MIR146A"

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Studies on single nucleotide polymorphisms (SNPs), most notably genome-wide association studies, have been very important in identifying new mechanisms of disease. Also in transplantation, in the quest towards better long-term graft survival and personalized medicine, the field is focused on identifying individuals at increased risk for rejection and graft failure. The genetically determined donor-recipient mismatch is a proven determinant of outcome after transplantation, especially in the Human Leukocyte Antigen (HLA) gene, and potentially also in other genes.¹

As acute rejection is deleterious for long-term graft survival, despite powerful immunosuppression, and as the donor-recipient mismatches can often not be avoided, efforts are also centred on identifying gene polymorphisms that increase susceptibility for acute rejection, independent of the donor-recipient mismatch. This is represented by the abundance of genetic variants possibly associated with acute rejection, mainly single nucleotide polymorphisms (SNPs), that are reported in the large forest of studies on this topic. However, findings across publications are discordant and many studies are underpowered and lack thorough validation.

Importance of validation of candidate genetic variants

In this issue of *Transplantation*, Oetting et al address this ‘can’t see the wood for the trees’ situation in an attempt to validate 75 previously reported candidate SNPs in kidney recipients that associate with acute rejection.² Their large prospective cohort study of kidney allograft recipients used for this validation was composed of two multicentre cohorts enrolled in genome-wide association studies (DeKAF and GEN-03). Two groups were defined based on ancestry principal components: European-Americans (N=2390) and a much smaller group of African Americans (N=482). As the study focus lies on analysing only 75 candidate genes, with thus less multiple-testing burden compared to genome-wide association study (GWAS) design, their large cohort renders satisfactory power for validation analysis.

The main conclusion from the study by Oetting et al is that none but one of the 75 genetic variants previously reported to be associated with acute rejection were validated in this large multicentre study. This study clearly illustrates the indispensability of validation studies in SNP- and genome-wide association studies.

The one validated SNP – rs2910164 in the MIR146A gene - was only significantly associated with acute rejection in the smaller African American group with lesser power. This SNP was previously reported in a North Indian population (N=270 live-related renal allograft recipients) to be associated with lower overall survival and rejection.³ There is supporting evidence for an important role of the MIR146A gene based on its relevant immunological role and its association with both acute rejection and death-censored graft failure in this study. However, the inconsistency of associations in different populations (association with acute rejection in a small population of mostly male North Indians and African Americans but not European Americans versus association with graft failure in African Americans and European Americans) makes further validation of this genetic variant in other cohorts necessary to determine whether this is a true positive risk allele.

High heterogeneity confounding replication and comparisons

The results presented in the well-built study by Oetting et al are highly relevant and raise some important comments.

First, although this study had satisfactory overall power for candidate SNP testing, power in the African American group, where the SNP in the MIR146A gene was validated, was much lower. Second, as for many other studies, there was rather high heterogeneity in many aspects, namely population structure and clinical care as two multicentre studies were combined. The investigators addressed this through stratification per transplant centre.

More importantly, high heterogeneity in acute rejection definition confounds the outcome across different studies as well as within the same study. For example, in the current study by Oetting et al, acute rejection was defined as ‘T-cell, antibody-mediated and mixed rejection’, ‘as determined by the treating physician’ and was not biopsy-confirmed in all cases (96%). No differentiation between antibody-mediated or T-cell mediated rejection was made, although these rejection phenotypes are very likely to be genetically distinct. Acute rejection is subdivided in early and late but in the study by Oetting et al no data are provided on the proportion of early vs. late rejection episodes and the potential association with genetic risk factors. Also, as mentioned by the authors, no subclinical rejection is taken into account, despite its reported deleterious effect on graft outcome. As the authors indicate, adherence data was missing and could thus not be adjusted for. This could have been relevant as late acute rejection episodes are often related to non-adherence and could mitigate the associations with genetic risk factors.

Finally, Oetting et al rightfully adjusted the association studies between acute rejection and candidate SNPs for clinical variables, including the donor-recipient HLA mismatch. This was rarely the case in the previous studies, where only single univariate associations were analysed, raising the power of statistical testing and increasing the chance of finding a significant (but therefore not true positive) association. This adjustment for clinical variables, as was done by Oetting et al, is utterly relevant. Taking into account readily available information on known risk factors for acute rejection like donor-recipient HLA matching is very important to not oversimplify the problem. If one wants to find recipient risk factors that determine the risk for acute rejection independent of known risk factors, taking into account the known risk factors, including HLA match, shouldn’t be omitted from the analyses. Also this is a clear message in the study by Oetting et al.

Conclusion

In conclusion, the study by Oetting et al presented in this issue of *Transplantation* highlights the need for thorough validation of candidate genetic variants in kidney recipients that increase the risk for acute rejection outside of the donor-recipient HLA mismatch. This study illustrates the vast heterogeneity in the previous literature, which confounds comparison of the published studies. From the large inconsistency of findings and mostly negative findings in adequately powered studies, like the one by Hernandez-Fuentes et al⁴ and the current study by Oetting et al, one can solely infer that up to today, no acute rejection SNP has been validated in kidney recipients. Despite important efforts by large consortia and individual centers, no fundamental progress was made in this field over the past 20 years.

It is very ambitious to search for genetic risk factors for acute rejection in the recipient's genome, independent of clinical risk factors, independent of the donor-recipient genetic mismatch and in the presence of powerful immunosuppressive agents.

Could this perhaps be too ambitious? We hope not. Further research should focus on adequately powered genome-wide studies with thorough validation, adjustment for known clinical risk factors and rigorous phenotypic definition, as acute rejection is a complex entity with distinct subtypes and diverse underlying pathophysiology. All our hope to find and validate SNPs that associate with acute rejection and, from this, new avenues for research now lies with larger collaborative projects like DeKAF, GEN03, and iGeneTRAIN.^{2,5}

We seem to be lost in the forest of studies associating SNPs with acute rejection, and still can't see the wood for the trees.

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