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DOI: 10.1111/ajt.14877

Reply to Hernandez et al. - GWAS of acute renal graft rejection

To the Editor:

Hernandez et al recently reported on several large genome-wide association studies (GWAS) in kidney transplantation.[1] In particular, they investigated the effect of either donor single nucleotide polymorphisms (SNPs), recipient SNPs, or combined donor-recipient SNPs on graft survival and acute rejection. Despite impressive patient numbers, no SNP signal emerged. The authors concluded that "both phenotype heterogeneity and sample size may have contributed." We sought to understand the lack of genetic association with graft rejection, considering our recent findings.[2]

In a recipient-GWAS published in 2016, we were able to identify and validate the association of 2 genetic loci (CCDC67 and PTPRO) with biopsy-proven T cell–mediated rejection (TCMR)[2] in 2 independent European cohorts. However, Hernandez and colleagues did not retrieve our SNPs, nor did they discover other SNPs in larger cohorts of the same origin. We looked at study design elements that may have been decisive in obtaining our positive results (see Table 1).

First, we concentrated our efforts on building a discovery cohort of cases with very low immunological risk. Kidney graft–rejecting cases were all European adults (>18 years of age), not or minimally sensitized, transplanted with a first kidney allograft under

TABLE 1 Comparison of the eligibility criteria of the 2 largest recipient-GWAS studies relative to acute rejection published to date

Ghisdal et al	**Hernandez et al**
Discovery: n = 275 cases vs 503 hypercontrols (from 4127 single recipients from France and Belgium); Replication: n = 313 cases vs 531 controls (from 2765 single recipients from Belgium and Czech Republic)	**Discovery: n = 441 cases vs 941 controls (from 2689 single recipients from United Kingdom and Ireland); Replication: n = 575 cases vs 2573 controls (from 5866 single recipients from Germany)**
European adults > 18 y	European patients >16 y
First renal transplantation	Any rank renal transplantation
Isolated kidney transplantation only	Double and combined transplantations allowed
All donors	Deceased donors only
Follow-up >1 y (or earlier loss from rejection)	Graft survival time >3 mo
Treatment	
1. Induction (antilymphocyte serum or anti-IL2 receptor antagonist)[c]	No specific requirement
2. CNI therapy at baseline	
Immunization	
Absence of anti-HLA antibodies by Luminex or PRA of maximum 5%	No specific requirement
Cases	
≥1 first-year biopsy proven T cell–mediated rejection (Banff criteria)[a]	≥1 first-year rejection (clinician reported treated rejection)
Controls	
Recorded as not having acute or a chronic rejection (first year)	Recorded as not having an acute rejection (first year)
1. Stable function[b]	No further specific requirement
2. Center-matching (2 center controls/case)	
3. Exclusion of patients older than 55 y receiving antilymphocyte serum	
4. Hypercontrols who did not reject despite a less favorable HLA match[b c]	

CNI, calcineurin inhibitor; PRA, panel reactive antibody.
[a]Exclusion of untreated borderline rejections, unexpected histological findings, pure antibody-mediated rejections.
[b]Defined with formal criteria, as detailed in the original publication.
[c]Not required for the replication cohort.

a calcineurin inhibitors-based immunosuppressive regimen including induction. In parallel, we carefully selected nonrejecting controls based on a high risk of rejection (*hypercontrols)*: poorly HLA-matched recipients were preferentially included, whereas low-risk older patients treated with anti-lymphocyte serum were excluded. Doing this, we assumed that the genetic contrast between groups was enhanced.

Second, we ensured that phenotypes were correct and precise. Rejection was of TCMR type and histologically proven. We agree with Hernandez et al that the phenotypic heterogeneity they allowed in their study (cellular and humoral rejections were not distinguished, biopsy was not required, and combined transplantations were accepted) may have diluted a true genetic association with TCMR in kidney recipients.

In the absence of significant results in their discovery cohort, Hernandez et al specifically tested 139 SNPs selected from their top candidates or from the literature. Strangely, although they did investigate various non–genome-wide and/or non-replicated associations, they omitted to investigate the sole associations ever validated in the context of recipient-GWAS pertaining to acute rejection (our study).[2] Although a negative result might have been related to disparate study designs, a further confirmation of our findings would have been of the highest interest for the transplant community and patients.

Finally, the absence of donor-recipient SNP genotype interaction influencing acute rejection in this study echoes with a recent observation by Mesnard et al.[3] By the calculation of a genetic score based on the sum of non-HLA donor-recipient mismatches among transmembrane proteins, these authors noticed that many genetic variants composing the score were actually rare alleles, which are not captured in SNP-array–based GWAS. Together, the works of Hernandez and Mesnard call for sequencing-based studies that can detect both common and rare variants for deciphering non-HLA donor-recipient interactions.

These negative results should not discourage further investigations in transplant genetics; novel findings may come from deep phenotyping of patients coupled with whole-genome sequencing.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Keywords

genetics, genomics, kidney disease: immune/inflammatory, kidney transplantation/nephrology, rejection: acute, rejection: T cell mediated (TCMR), translational research/science

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