**COVER LETTER**

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Re: “HLA Loci and Recurrence of Focal Segmental Glomerulosclerosis In Pediatric Kidney Transplantation”

Dear Dr. Chapman:

Thank you so much for the opportune to revise our manuscript entitled: “HLA Loci and Recurrence of Focal Segmental Glomerulosclerosis In Pediatric Kidney Transplantation.” We believe we have made significant improvements to the manuscript and addressed all reviewer concerns. Specifically, we have:

* Clarified information about the cohort as a whole
* Added clinically relevant data regarding recurrence at one year
* Clarified information regarding the method of HLA typing
* Added information in the discussion regarding further mechanistic experiments

Please find below our responses to specific review comments in the lettered bullet points in blue.

We hope that you find these revisions appropriate and look forward to hearing from you soon.

Best regards,

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Brian Shaw

Executive Editor:

1. The authors of this manuscript have used STRR data to study the association of HLA antigens with FSGS recurrence in children. Their results did reveal associations of different haplotypes with FSGS recurrence and with risk of rejection. The reviewers have identified a number of issues that should be addressed. Please respond to their specific comments.
   1. Thank you for the opportunity to respond to these queries for potential publication in Transplantation Direct. Please see our responses below.

Reviewer 1:

1. The authors investigated the association of HLA antigens with recurrence of FSGS in children using the STRR database. They found the high risk haplotype of HLA-DR7, DR53, and DQ2 was associated with increased risk of recurrence, while C3, B58, an dDQ7 match was associated with decreased risk of rejection. The manuscript is very well written, the object clearly stated, the analysis appropriate, and the conclusions justified. I think this is an important contribution to the literature in pediatric kidney transplantation. Overall, I have the following suggestions, which I hope will further improve a manuscript that is already quite good.
   1. We appreciate your laudatory comments and helpful insights.

Methods

1. Could the authors please just add what test was used for the correlation matrix (Pearson, Spearman, etc?). Is this an r value?
   1. The correlation coefficient used in the matrix is a Pearson’s correlation coefficient. We have amended the figure legend for Figure 4 to reflect this.
2. This is a suggestion, to which I would be interested to solicit the authors’ opinion. In general, I am accustomed to seeing time-to-event analysis used to evaluate risk of a particular variable over an extended time (Cox PH). This study uses logistic regression. I am not sure this is entirely inappropriate, but wonder if the authors could opine on why they chose logistic regression, as opposed to a time to event analysis.
   1. We agree that time-to-event analyses are helpful when determining the hazard of an event (i.e. risk over time). Because our objective was only to determine the association of HLA with the risk of *any* recurrence event, we chose to use logistic regression to give us the greatest ability to detect an association.
3. This leads me to a second question, which is the nature of the outcome variable of FSGS recurrence. Looking at the K-M curves, you notice a sharp drop early in the time period, followed by a gradual leveling off of the curves, but they still continue to show additional recurrences of FSGS several years after transplant. I wonder if these latter recurrences are more of a secondary FSGS recurrence, or a different type of recurrence than the aggressive recurrence that usually, in my experience, happens immediately after transplant or certainly in the first 6 months. That is, is a recurrence of FSGS diagnosed 5 years after transplant the type of FSGS recurrence the authors are seeking to predict? To select for this specific outcome (early, aggressive FSGS recurrence), an outcome of FSGS recurrence by 6 months (which all of the patients are stated to have at least 6 months follow up), may be more appropriate. Also, I think this is the first follow-up data point in the UNOS data set. This would lend itself more to a logistic regression type analysis as well, as all patients would be evaluated at 6 months follow up. Finally, identifying this type of recurrence (early) is usually the main clinical concern.
   1. Thank you for the helpful comment. We agree that there may indeed be clinically relevant biologic differences between recurrence that occurs early in the course—as is classically seen with recurrent FSGS—and that which occurs later. We performed a sensitivity analysis to examine recurrence at one year. In this analysis, many of the same HLA which were associated with increased or decreased risk of recurrence (including HLA B13, DR7, DR52, DR53, and DQ2) were identified (**Page 9, Paragraph 2**) suggesting that the associations hold true for early recurrence. Additionally, we have modified the discussion to note this association and highlight the clinical importance of early recurrence (**Page 13, Paragraph 1**). We chose 1 year as opposed to 6 months in order to capture those individuals who may not have had their recurrence reported exactly at the 6 month timepoint.

Results

1. Could the authors provide some general p-values for the significance of HLA antigens with recurrence? They provide 95% CI's, which is fine. However, due to the nature of the study having multiple tests using the Benajamini-Hochberg method, p value information would be informative.
   1. Thank you for the suggestion. The Benjamini-Hochberg method is indeed a method of adjusting for multiple comparisons with a set false discovery rate (FDR). Because of this, the “adjusted” p-values are relatively arbitrary and influenced by the set FDR as well as the number of comparisons. Therefore, we elected not to present either raw p-values (due to the large number of comparisons) or “adjusted” p-values as they are not interpretable in the standard sense (i.e. the probability that one would obtain the same result, or a more extreme one, assuming the null hypothesis is correct).
2. I believe the HLA antigen DR53 was inadvertently left out of Figure 4.
   1. Thank you for the query. In Figure 4, the correlation matrix is asymmetric so DR53 is only represented on the X-axis and not the Y.
3. In the results, it would be clinically useful to provide some explicit numbers for recurrence. For example, for select high risk or low risk groups based on HLA antigens, state something like "Those who had the high-risk haplotype had a 50% recurrence rate at 6 months. Conversely, low with recipient C3 antigen only had a 10% recurrence risk at 6 months". Or something like this. This would provide very clinically useful information.
   1. Thank you for the comment. We have added the following on **Page 10 Paragraph 1:** “Among those with the risk haplotype, 27% recurred within the first year whereas among patients with the C3 allele, only 6% recurred.”

Discussion/Conclusions:

1. Overall well-written. The only suggestion I would make concerns the abstract. I think the conclusion could be strengthened to summarize the findings, rather than making the general comment about further study. Perhaps something like "HLA profiles can be used to risk stratify the recurrence of FSGS in pediatric kidney transplant recipients".
   1. Thank you for the positive comment. We agree that these are exciting findings and have updated the abstract to read “HLA profiles may be used for risk stratification of recurrence of FSGS in pediatric kidney transplant recipients and deserves further study.”

Reviewer 2:

1. This paper by Shaw et al studies a registry of pediatric kidney transplant patients to show associations of FSGS with HLA loci. Outstanding description of numbers of inclusion criteria. Paper is well written and easy to follow. The discovery can be very important in driving future research to stratify patients for future inclusion of studies; however, it is unclear how much use it has clinically. Discussion would be better fit to describe how this data can be used moving forward. It is very unclear what adding HLA into predictive model would change patient management when the cohort is narrowed from 463,766 to 1,196. If this is truly a biomarker paper, which table 3 frames it as, it needs to include both a discovery and testing cohort to avoid overfitting the narrowed patient subtypes.
   1. Thank you for the overall positive and helpful comments to enhance our manuscript. We agree that there are a large number of candidates in the STAR file. However, as shown in Figure 1, only 15,000 are pediatric transplant recipients in the modern era (after 2000), our general population of interest. Therefore, the 1200 patients represents 8% of all pediatric recipients in the modern (after 2000) era.
   2. We also agree that if we were looking to validate a biomarker, then a training and testing cohort would be appropriate. However, this is a hypothesis generating study and we stressed this in the Discussion section. We do not seek to determine the absolute risk conferred by certain HLA but rather discover associations between HLA and our outcome of interest (disease recurrence). We included Table 3 to give a rough estimate of the strength of the HLA association when controlling for other factors.
2. Methods: As all of the results are dependent on the HLA genotyping, a statement on the approaches used within the cohort for determining these needs to be included. Were they a standard clinical test or research level determination. How many of the patients had molecular testing vs serological? Can the molecular testing patients be used to assess rare genotypes of HLA? Readers should not have to wait until the discussion to have a better understanding of the methods used for HLA typing.
   1. Thank you for your comment. As this is a large, retrospective, registry study, there are various methods used. Molecular (DNA) HLA typing was in broad use in the US in the post-2000 era: however, the OPTN’s inability to accept HLA allele group designations (NMDP codes or P groups) and the requirement for HLA serologic equivalent reporting at time of waiting list activation limits the number of laboratories that update molecular typing nomenclature at time of transplantation. As described on **Page 7, Paragraph 1**: “Of note, generally only serological typing is reported to UNOS. Where molecular data were available, they were converted to serologic types for consistency25.”

Nevertheless, a strength of our analysis is that we are using clinical HLA typing information and not HLA imputation.

* 1. Given the small number of patients, we cannot assess the impact of rare HLA genotypes.

1. Results: Table 1-2: A brief description of the cohort following inclusion criteria should be put into the text (age, gender, race, BMI). Unclear what inclusion of insurance status in table 1 shows and why it is included. Why is the No Recurrence N not the same in both tables (862 vs 857)?
   1. Thank you for your comment. We have included the following sentence: “Our cohort consisted of 1,196 patients. In the cohort as a whole, the median age at listing was 15 (Interquartile Range [IQR] 11-17), 43% were female, 58% were white, 36% were black, 27% were Latino, and the median BMI was 20 (IQR 17-24).”
   2. We have removed insurance status from Table 1.
   3. The n was different as a single donor could contribute multiple kidneys. Of note, there was a small coding error that was fixed leading to slightly different numbers in Table 2. However, there were no changes in any of the overall trends or statistical comparisons.
2. Discussion: These findings don't seem very surprising and the discussion limits the excitement of the findings. How does this discovery change management of patients? While it is predictive of pathology, it does not change management, which is the main stated goal. At least some speculations in the discussion on follow up experiments for the community to identify mechanisms, such as blood biomarker analysis targeted to patients with these HLA to identify pathways of FSGS recurrence would help readers better frame the importance of this work.
   1. Thank you for your comment, we have added a paragraph (**Page 14, Paragraph 1**) detailing a possible mechanistic experiment to understand the role of class II HLA expression in mediating the observed increase in recurrence with some HLA.
3. "Finally, it is possible that the associations we see here implicate not HLA in the pathogenesis of FSGS but rather other genes in linkage disequilibrium with those HLA." Genes are not in linkage disequilibrium, variants are, this is an odd statement that needs cleaned up.
   1. We have amended the sentence to state “Finally, it is possible that the associations we see here implicate not HLA in the pathogenesis of FSGS but rather other variants in linkage disequilibrium with those HLA.”