Exome Sequencing, Histoincompatibility and Long-Term Kidney Allograft Function

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Abstract

BACKGROUND

Kidney transplantation is the treatment of choice for most patients with end-stage renal disease and existing data suggest that post transplant graft function is a predictor of kidney graft failure.

METHODS

Exome sequencing of DNA from kidney graft recipients and their donors was used to determine recipient and donor mismatches at the amino acid level. The number of mismatches that are more likely to induce an immune response in the recipient was computationally estimated and designated the allogenomics mismatch score. The relationship between the allogenomics score and post transplant kidney allograft function was examined using linear regression.

RESULTS

A significant inverse correlation between the allogenomics mismatch score and kidney graft function at 36 months post transplantation was observed in a discovery cohort of kidney recipient-donor pairs (r^2>=0.57, P<0.05, the score vs. level of serum creatinine or estimated glomerular filtration rate). This relationship was confirmed in an independent validation cohort of kidney recipient-donor pairs. We observed that the strength of the correlation increased with time post-transplantation. This inverse correlation remained after excluding HLA loci from the calculation of the score. Exome sequencing yielded allogenomics scores with stronger correlations with graft function than simulations of genotyping assays which measure common polymorphisms only.

CONCLUSIONS

The allogenomics mismatch score, derived by exome sequencing of recipient-donor pairs, facilitates quantification of histoincompatibility between the organ donor and recipient impacting long-term post transplant graft function. The allogenomics mismatch score, by serving as a prognostic biomarker, may help identify patients at risk for graft failure.

Impact statement

Prediction of post transplant kidney graft function with the allogenomics mismatch score to quantify histoincompatibilty between the kidney recipient-donor pair.

Major subject areas, keywords, and research organism(s)

Genomics, kidney transplantation, organ transplantation, immunology, Human Leukocyte Antigen, long-term graft function.

Introduction

Survival of patients afflicted with End Stage Renal Disease (ESRD) is superior following kidney transplantation compared to dialysis therapy. The short-term outcomes of kidney grafts have steadily improved since the early transplants (performed in the 1960s) with refinements in immunosuppressive regimens, use of DNA-based HLA typing, and better infection prophylaxis(1–3). Despite these advances, data collected across the USA and Europe show that 40-50% of kidney allografts fail within ten years of transplantation(4). This observation strongly suggests that as yet uncharacterized factors including genomic loci may adversely impact long-term post-transplant outcome.

Observational studies have demonstrated the importance of matching for the HLA-determined proteins on kidney graft outcome. Therefore, in many countries, including the USA, donor kidney allocation algorithm includes consideration of HLA matching. With widespread incorporation of HLA matching in organ allocation decisions, it has become clearer that HLA mismatching represents an important risk factor for kidney allograft failure but fails to fully account for the invariable decline in graft function and failure in a large number of cases over time. Indeed, 40-50% of the grafts fail at 10 years post transplantation despite HLA matching at the HLA-A, B and DR loci, and only a 15% survival difference exist between the fully matched kidneys and the kidneys mismatched for both alleles at the HLA-A, B and DR loci.(5) These

observations suggest that mismatches at non-HLA loci in the genome could play a role in influencing long-term graft outcome. The current clinical practice of prescribing life-long immunosuppressive therapy to recipients of fully HLA matched living related donor kidneys, but not to recipients of monozygotic identical twin kidneys, also suggests a role for non-HLA related genomic factors on graft outcome.

While tests of allelic frequencies are a hallmark of genetic research, transplantation has none of the Mendelian characteristics for which genetic tests have been developed. Therefore, the assumption of the Mendelian transmission model seems inadequate to develop predictors of graft function following transplantation. Indeed, previous attempts at using this methodology have identified small genotype effects on graft function in cohorts of hundred of transplant patients, but often could not be replicated in independent cohorts (reviewed in Ref(6)).

THE ALLOGENOMICS CONCEPT

In this report, we present a new method to estimate the genomic compatibility between the organ graft recipient and donor. This approach, designated as allogenomics in this communication, considers the entire coding sequence of both recipient and donor genomes, as determined by exome sequencing. The allogenomics concept makes it possible to estimate a quantitative compatibility score between the genomes of a recipient and potential donor and is calculated from genotypes and genome annotations available before transplantation. The allogenomics approach does not assume a Mendelian inheritance model but integrates the unique features of transplantation such as the existence of two genomes in a single individual and the recipient's immune system mounting an immune response directed at antigens displayed by the donor kidney. In this report, we show that this new concept helps predict long-term kidney transplant function from the genomic information available prior to transplantation.

Methods

Complete method descriptions are provided in supplementary materials. This human study was reviewed and approved by the Weill Cornell Medical College Institutional Review Board (protocol #1407015307 "Predicting Long-Term Function of Kidney Allograft by Allogenomics Score", approved 09/09/2014). All subjects gave written consent. Briefly, genotypes of donor and recipients are assayed with exome sequencing (Illumina TruSeq enrichment kit for the Discovery Cohort and Agilent Haloplex kit for the Validation cohort). Reads were aligned to the human genome with the Last(7) aligner integrated as a plugin in GobyWeb(8). Genotype calls are made with Goby(9) and GobyWeb(8). Prediction of polymorphism impact on the protein sequence were performed with the Variant Effect Predictor(10). The allogenomics score is estimated with the allogenomics scoring tool, available at (http://allogenomics.campagnelab.org) and implemented according to the model shown in Figure 1C Equations 1 and 2. Association between the allogenomics score and graft function is tested with linear regression, using blood creatinine levels or estimated glomerular filtration rate (eGFR: estimated with the MDRD equations(11)). Figures were constructed with MetaR (http://metaR.campagnelab.org).

Results

The allogenomics concept is the hypothesis that interrogation of the coding regions of both the organ recipient and organ donor DNA can identify the number of incompatible amino-acids (recognized as non-self by the recipient) that inversely correlates with long-term graft function post transplantation. Figure 1A is a schematic illustration of the allogenomics concept. Because human autosomes have two copies of each gene, we consider two possible alleles in each genome of a transplant pair. To this end, we estimate allogenomics score contributions between zero and two, depending on the number of different amino acids that the donor genome encodes for at a given protein position. Figure 1B shows the possible allogenomics score contributions when the amino acids in question are either an alanine or a phenylalanine or an

aspartate amino acid. The allogenomics mismatch score is a sum of amino acid mismatch contributions. Each contribution represents an allele coding for a protein epitope that the donor organ may express and that the recipient immune system could recognize as non-self (see Equation 1 and 2 in Fig. 1C and in Materials and Methods).

We have developed and implemented a computational approach to estimate the allogenomics mismatch score from genotypes derived for pairs of recipient and donor genomes. (See Material and methods for a detailed description of this approach and its software implementation, the allogenomics scoring tool, available at http://allogenomics.campagnelab.org.) Our approach is designed to consider the entire set of protein positions measured by a genotyping assay, or restrict the analysis to a subset of positions *P* in the genome. In this study, we focus on the subset of genomic sites P that encode for amino acids in transmembrane proteins.

THE ALLOGENOMICS MISMATCH SCORE CORRELATES WITH GRAFT FUNCTION POST TRANSPLANTATION

In order to test the allogenomics hypothesis, we isolated DNA from 10 kidney graft recipients and their living donors (Discovery Cohort), performed whole exome sequencing and analyzed genotype data for these recipient and donor genome pairs (10 pairs, 20 exomes). These patients were a subset of patients enrolled in a multicenter Clinical Trial in Organ Transplantation-04 (CTOT-04) study of urinary cell mRNA profiling and from whom tissue/cells were collected for future mechanistic studies(12). Table S1 provides demographics information about the patients included in the Discovery Cohort. Exome data were obtained with the Illumina TrueSeq exome enrichment kit v3. Primary sequence data analyses were conducted with GobyWeb(8) (data and analysis management), Last(7) (alignment to the genome) and Goby(9) (genotype calls).

Kidney graft function is a continuous phenotype and is clinically evaluated by measuring serum creatinine levels or using estimated glomerular filtration rate (eGFR)(11). In this study, kidney graft function was evaluated at months 12, 24, 36 or 48 following transplantation using serum creatinine levels and eGFR, calculated using the 2011 MDRD(13) formula. We examined whether the allogenomics mismatch score is associated with post transplant allograft function.

We found positive linear associations between the allogenomics mismatch score and serum creatinine levels at 36 months post transplantation (R^2 adj.=0.78, P<0.01, n=10, at 36 months) but not at 12 or 24 months following kidney transplantation (Fig. 2A, B, C). We also found a negative linear relationship between the score and eGFR at 36 months post transplantation (R^2 adj.=0.57, P=0.02) but not at 12 or 24 months following kidney transplantation (Fig. 2D, E, F). These findings suggest that the allogenomics score is predictive of long-term graft function rather than short-term function.

THE ALLOGENOMICS MISMATCH SCORE ASSOCIATES WITH GRAFT FUNCTION IN AN INDEPENDENT COHORT OF KIDNEY RECIPIENT-DONOR PAIRS

We sought to validate the observation that the allogenomics mismatch score is associated with post-transplant kidney graft function by testing the association in an independent cohort of kidney transplant patients. To this end, we sequenced DNA collected from 24 additional kidney recipient-donor pairs (see Table S1 for demographic information of subjects included in the Validation cohort). DNA sequencing was performed using the Agilent Haloplex assay covering 37Mb of the coding sequence of the human genome. We called the genotypes and estimated the allogenomics mismatch score as described for the discovery cohort (see Methods).

Figure 3 shows that, as observed with the Discovery cohort, the allogenomics mismatch score correlates progressively better with kidney graft function at longer times following transplantation. At 36 months post-transplantation, a small to moderate positive association was

observed between the allogenomics mismatch score and the serum creatinine levels (R^2 adj. 0.139, P=0.049) (Fig. 3C) and eGFR (R^2 adj. 0.078, P=0.11) (Fig.3G). The association between the score and graft function was stronger and reached significance at 48 months post-transplantation for both creatinine levels (R^2 adj. 0.394, P<0.01) (Fig.3D), and eGFR (R^2 adj. 0.284, P=0.02) (Fig.3H), further validating the association in the Validation cohort.

In order to test whether models trained on one cohort would generalize to another cohort, we trained models on the Discovery cohort and used the fixed model to predict graft function in the Validation cohort. Figure S1 shows that such a fixed model does generalize when presented with new recipient-donor pairs, and also exhibited better fit to the longer 48 months time point compared to the earlier time point (Fig. S1B vs. S1C). Similarly, models trained on the Validation cohort generalize to the Discovery cohort (Figure S2). These results establish that the parameters of the models are stable, despite the relatively small numbers of kidney recipient-donor pairs included in the Discovery and Validation cohorts.

RELATION TO HLA MISMATCHES

Figure 4 presents an analysis where we combined the Discovery and Validation cohorts (32 transplant kidney recipient-donor pairs) and compared the allogenomics scores to the number of mismatches at the HLA-A, B and DR loci. We find that the allogenomics mismatch score is moderately correlated with the number of mismatch at the HLA loci (Fig. 4A, R^2 adj.=0.36, P<0.001). However, the number of HLA mismatches correlates poorly with an allogenomics score estimated from exome data when restricting the sites to the HLA A, B and DR loci (Fig. 4B, R^2 adj.=0.09, P=0.051. Furthermore, the allogenomics mismatch score estimated outside of the HLA A, B and DR loci significantly associates with serum creatinine levels (Fig. 4C, R^2 adj.=0.358, P<0.001 and eGFR (Fig. 4D, R^2 adj.=0.175, P=0.012). These data indicate that the

allogenomics mismatch score ability to predict future graft function is mostly independent of the HLA loci.

FINAL MODELS

We fit models across the combined cohorts to yield final models with fixed parameters. These models are trained across the 32 pairs of the Discovery and Validation cohorts against serum creatinine levels at 36 months (creatinine_at_36_months = 0.3823513 + 0.0009216* allogenomics_mismatch_score) and eGFR at 36 months (eGFR_at_36months = 83.802675 - 0.0254203*allogenomics_mismatch_score). The equations and parameters are provided to enable testing these models on independent cohorts of transplant pairs genotyped with exome sequencing. Fit parameter values were estimated with JMP Pro release 11, Fit X by Y, Fit Line. We note that the fixed parameters of this model can be sensitive to the exact analysis pipeline used to align reads to the genome and to call genotypes and that an objective test of this model should strictly follow the analysis protocols used to analyze data for this report.

IMPACT OF GENOTYPING PLATFORM AND RARE POLYMORPHISMS

We studied the impact of the genotyping platform on the estimation of the allogenomics mismatch score (Figure S3). Large cohorts of matched recipient and donor DNA are being assembled and genotyped with SNP chip array technology such as the Illumina 660W bead array platform(14). We asked whether such platforms would be appropriate to validate the allogenomics model in large cohorts. Figure S3A documents the number of sites that contribute to the allogenomics score on each platform. Figure S3B indicates that the exome assay captures many more sites with rare polymorphisms (minor allele frequency <5%) than the GWAS array platform. This is expected because exome assays directly sequence an individual DNA, while GWAS platforms are designed with a fixed set of polymorphisms and will not include many of the rare polymorphisms any given individual may carry. Figure S3C compares the

correlations measured with the exome assay or that could have been obtained if we had measured the allogenomics mismatch score with the Illumina 660W assay. The weak correlations obtained suggest that GWAS platforms are not ideal for future tests of the allogenomics model.

234

235 # Discussion

236

237 In this study, we introduce the allogenomics concept to quantitatively estimate the
238 histoincompatibility between an organ donor and recipient. We tested the simplest model
239 derived from this concept to calculate an allogenomics mismatch score. We demonstrated that
240 the allogenomics mismatch score, which can be estimated before transplantation, correlates
241 with post-transplant kidney graft function. Interestingly, the strength of the correlation increases
242 with the time post transplantation, an observation we make in both the discovery and validation
243 cohorts.

244

245 We chose to focus this study on living donors because these surgeries can be planned in
246 advance and because differences in cold ischemia times and other covariates common in
247 deceased donor transplants are negligible when focusing on living donors. We expect that the
248 allogenomics approach will also be predictive in cohorts with deceased donors, but that
249 potentially much larger cohorts may be required in such settings to achieve sufficient power to
250 detect the allogenomics contribution in the presence of many covariates known to impact future
251 graft function.

252

253 While several case-control studies have been conducted with large organ transplant cohorts,
254 the identification of genotype/phenotype associations has been limited to the discoveries of
255 polymorphisms with small effect, reviewed in(15), which have often not been replicated(16–18).
256 Such studies have observed small average effects measured across groups of transplants when
257 our study is measuring moderate effects in individual transplants. Rather than focusing on
258 specific genomic sites, the allogenomics concept sums contributions of many mismatches that
259 can impact protein sequence and structure and could yield an immune response in the recipient.

260

Multiple testing and statistical power considerations seemed to suggest that thousands of transplant pairs must be assayed and analyzed to find polymorphisms that associate with a transplantation-related endpoint(15). However, the allogenomics model, as presented in this report, can be tested with a single test of significance per cohort. This design results in the ability to test association in smaller cohorts.

In this study, we observed a strong correlation to predict serum creatinine levels and eGGR (Pearson r coefficient ~ 0.6 in individual transplant pairs of the combined Discovery and Validation cohorts, n=32 transplant pairs at 36 months). Since the signal appears to increase with the number of months post transplantation and a clinical useful objective is to predict long-term graft function, we expect that other transplant centers will be able to obtain matched recipient-donor genotypes and will independently assess whether the allogenomics mismatch score is predictive of long-term kidney allograft function. Several such multi-center independent validations will be essential to establish if prospective clinical trials are warranted. We distribute the software that we developed to estimate the allogenomics mismatch score to facilitate further studies by others (see http://allogenomics.campagnelab.org).

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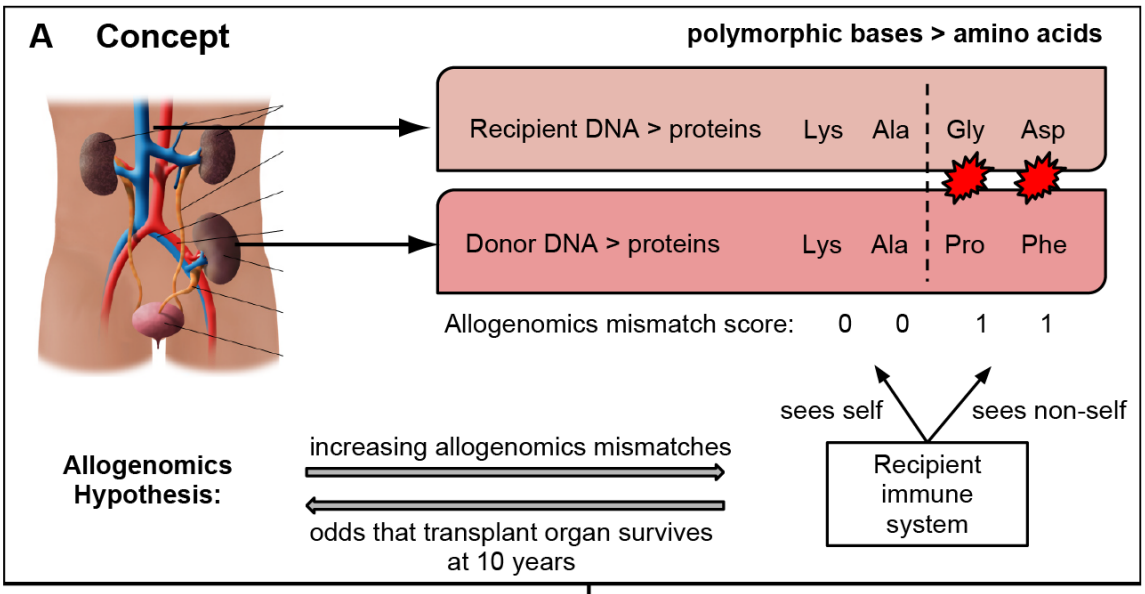

B Score Contribution Examples

Donor amino-acids	Recipient amino-acids	Allogenomics score contribution δ_p
{Ala,Phe}	{Ala,Phe}	0
{Ala}	{Phe}	1
{Phe}	{Ala,Phe}	0
{Ala,Phe}	{Asp}	2

C Allogenomics Model

$$\Delta(r,d) = \sum_{p \in P} \delta_p(G_{rp}, G_{dp}) \qquad \textbf{Eqn 1}$$

$$\delta_p(G_{rp}, G_{dp}) = \sum_{a \in G_{dp}} \begin{cases} 0 \text{ if } a \in G_{r,p} \\ 1 \text{ otherwise} \end{cases} \qquad \textbf{Eqn 2}$$

Figure 1. Recipient/Donor incompatibility quantified by exome sequencing and calculation of allogenomics mismatch score. (A) Hypothesis: Post-transplant kidney graft function is associated with the number of amino acids coded by the donor genome that the recipient's immune system could recognize as non-self. **(B)** Examples of donor/recipient amino-acid mismatches at one protein position, and resulting contributions to the allogenomics mismatch score. The allogenomics mismatch score is calculated by summing contributions over a set of genomic polymorphisms (see Methods for details). **(C)** Equations for the allogenomics model. Score contributions are summed across all genomic positions of interest (set P) to yield the allogenomics score $\Delta(r,d)$. $G_{r,p}$: genotype of the recipient r at genomic site/position p. $G_{d,p}$: genotype of the donor d at site p. Alleles of a genotype are denoted with the letter a.

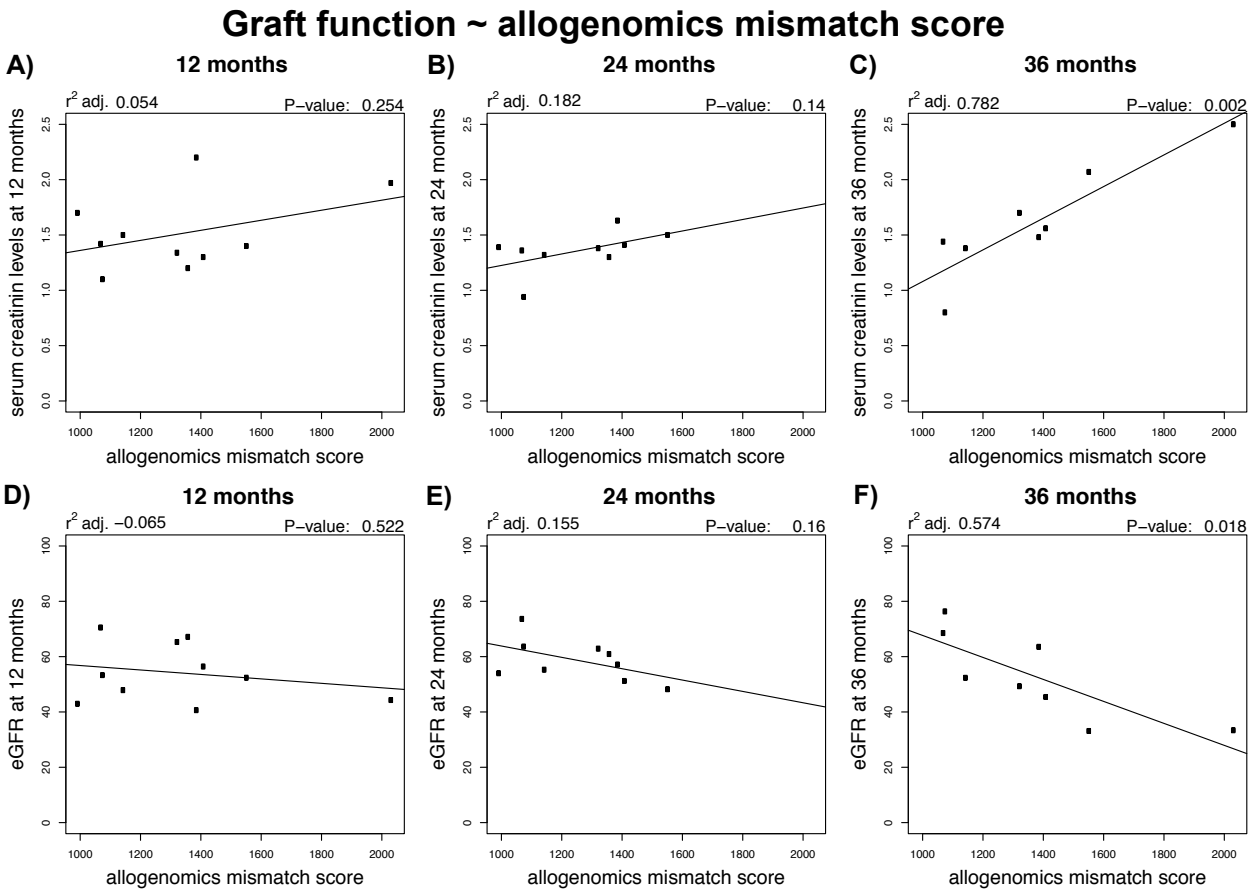

Figure 2. Relationship between the allogenomics mismatch score and kidney graft function at 12, 24 or 36 months following transplantation in the Discovery cohort. DNA was isolated from 10 pairs of kidney graft recipients and their living kidney donors (Discovery set). Whole exome sequencing of the donor genomes and recipient genomes was performed and the sequencing information was used to calculate allogenomics mismatch scores based on amino acid mismatches in transmembrane proteins. The panels depict the relationship between the allogenomics mismatch scores and serum creatinine levels at 12, 24 and 36 months following transplantation (Panels A, B and C, respectively) and the relationship between the allogenomics mismatch scores and estimated glomerular filtration rate at 12, 24 and 36 months following transplantation (Panels D, E and F, respectively). Both serum creatinine levels and eGFR correlate in a time dependent fashion with the allogenomics mismatch score with the strongest correlations being observed at 36 months post-transplantation.

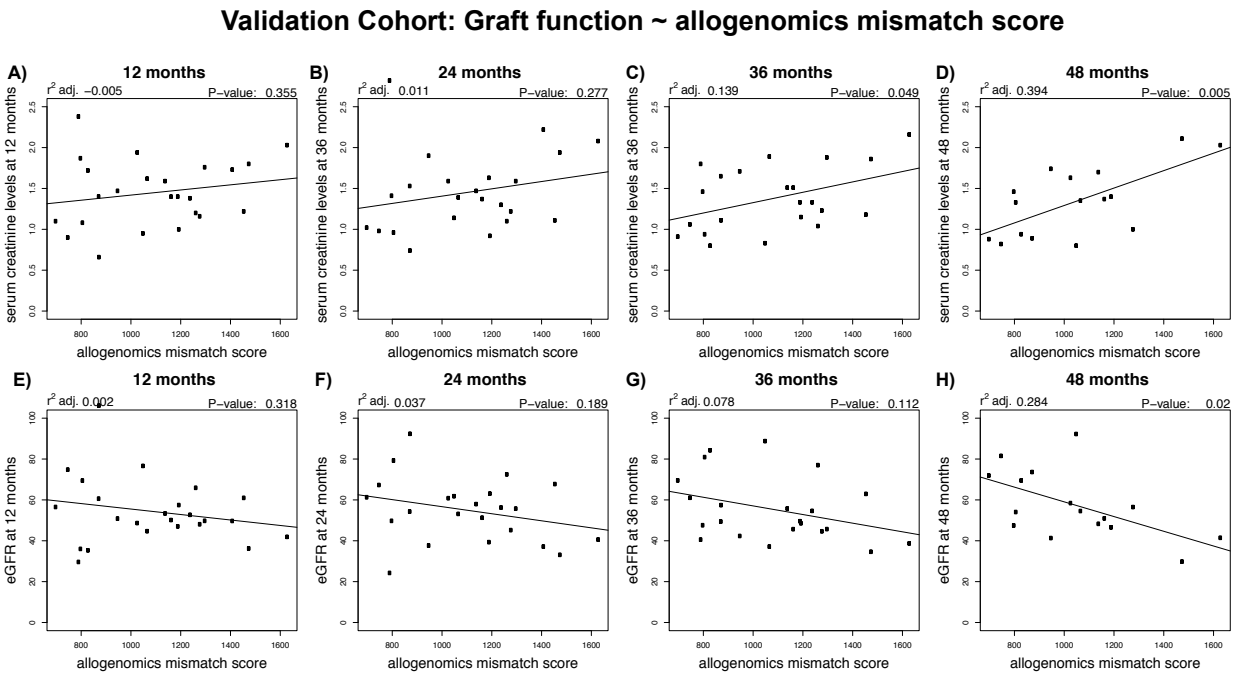

Figure 3. Relationships between allogenomics mismatch scores and kidney graft function at 12, 24, 36 or 48 months following transplantation in the Validation Cohort. DNA was isolated from 24-pairs of kidney graft recipients and their living kidney donors (Validation set). Whole exome sequencing of the donor genomes and recipient genomes was performed and the sequencing information was used to calculate allogenomics mismatch scores based on amino acid mismatches in transmembrane proteins. The relationships between allogenomics mismatch score and serum creatinine levels at 12, 24, 36 and 48 months following transplantation (Panels A, B, C, and D respectively) are shown. In panels E, F, G and H, the relationships between the allogenomics mismatch scores and estimated glomerular filtration rate at 12, 24, 36 and 48 months following transplantation are shown. Both serum creatinine levels and eGFR correlate in a time dependent fashion with the allogenomics mismatch score with the strongest correlations being observed at 48 months post-transplantation.

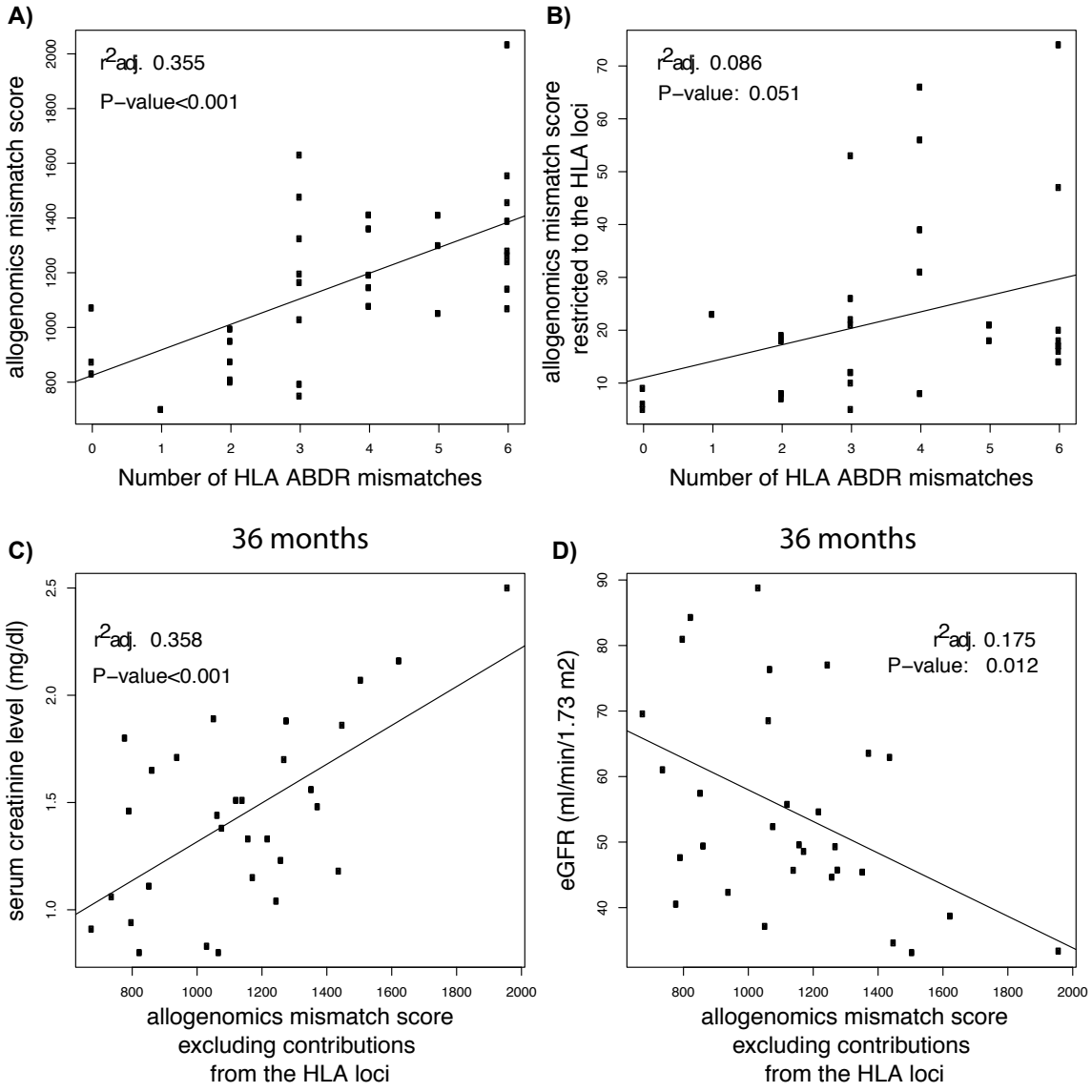

Figure 4. Relationships between allogenomics mismatch scores and the to HLA loci. We combined the Discovery and Validation cohorts to examine the relation of the allogenomics mismatch score to the HLA-A, B and DR mismatch between the recipient and the kidney donor. Allogenomics mismatch scores, either calculated over all transmembrane proteins (Panel A), or restricted to the HLA A, B DR loci (Panel B) correlate with the number of mismatches in the A,B, and DR HLA loci for the complete cohort. These correlations however do not explain the association with post-transplantation graft function because when the HLA loci (A/B/ DR/DQ) are excluded from the sites included in the calculation of the allogenomics mismatch score, a significant correlation is still observed between the allogenomics mismatch score and serum creatinine level (Panel C) and eGFR (Panel D) at 36 months post transplantation (similar results are obtained when excluding the A/B/C/DR/DQ/DP HLA loci, data not shown).