Supplementary Appendix for

Exome Sequencing, Histoincompatibility and Long-Term Kidney Allograft Function

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Supplementary Methods

Discovery cohort: Transplant recipients and DNA samples.

We selected 10 kidney transplant recipients from those who had consented to participate in the Clinical Trials in Organ Transplantation-04 (CTOT-04), a multicenter observational study of noninvasive diagnosis of renal allograft rejection by urinary cell mRNA profiling. We included only the recipients who had a living donor transplant and along with their donors, had provided informed consent for the use of their stored biological specimens for future research. The demographic information is shown in Supplemental Table 1. DNA was extracted from stored peripheral blood using the EZ1 DNA blood kit (Qiagen®) based on the manufacturer recommendation.

Discovery cohort: Whole exome sequencing.

DNA was enriched for exome regions with the TruSeq exome enrichment kit v3. Sequencing libraries were constructed using the Illumina TruSeq kit DNA sample preparation kit. Briefly, 1.8 µg of genomic DNA was sheared to average fragment size of 200 bp using the Covaris E220 (Covaris, Woburn, MA, USA). Fragments were purified using AmpPureXP beads (Beckman Coulter, Brae, CA, USA) to remove small products (<100 bp), yielding 1 µg of material that was end-polished, A-tailed and adapter ligated according to the manufacturer's protocol. Libraries were subjected to minimal PCR cycling and quantified using the Agilent High Sensitivity DNA assay (Agilent, Santa Clara, CA, USA). Libraries were combined into pools of six for solution phase hybridization using the Illumina (Illumina, San Diego, CA, USA) TruSeq Exome Enrichment Kit. Captured libraries were assessed for both quality and yield using the Agilent High Sensitivity DNA assay Library Quantification Kit. Sequencing was

performed with six samples per lane using the Illumina HiSeq 2000 sequencer and version 2 of the sequencing-by-synthesis reagents to generate 100 bp single-end reads (1×100SE).

Validation cohort: Transplant recipients and DNA samples.

We studied 24 kidney transplant recipients who had a living donor transplant at the NewYork-Presbyterian- Weill Cornell Medical Center. This was an independent cohort and none of the recipients had participated in the CTOT-04 trial. Recipients were selected randomly based on the availability of archived paired recipient-donor DNA specimens obtained at the time of transplantation at our Immunogenetics and Transplantation Laboratory. The Institutional Review Board at Cornell approved the study. DNA extraction from peripheral blood was done using the EZ1 DNA blood kit (Qiagen®) based on the manufacturer recommendation.

Validation cohort: Whole exome sequencing.

The validation cohort was assayed with the Agilent Haloplex exome sequencing assay. The Haloplex assay enriches 37 Mb of coding sequence in the human genome and was selected for the validation cohort because it provides a strong and consistent exome enrichment efficiency for regions of the genome most likely to contribute to the allogenomics contributions in protein sequences. In contrast, the TrueSeq assay (used for the Discovery Cohort) enriches 63Mb of sequence and includes regions in untranslated regions (5' and 3' UTRs), which do not contribute to allogenomics scores and therefore do not need to be sequenced to estimate the score. Libraries were prepared as per the Agilent recommended protocol. Sequencing was performed on an Illumina 2500 sequencer with the 100bp paired-end protocol recommended by Agilent

for the Haloplex assay. Libraries were multiplexed 6 per lane to yield approximately 30 million PE reads per sample.

Allogenomics Site Minor Allele Frequencies.

We determined the minor allele frequency of sites used in the calculation of the allogenomics mismatch score using data from the NHLBI Exome Sequencing Project (ESP) release ESP6500SI-V2. We downloaded the data file ESP6500SI-V2-SSA137.protein-hgvs-update.snps_indels.txt.tar.gz and extracted MAF in the European American population (EA) and in the African American population (AA) [1]. The ESP measured genotypes in a population of 6,503 individuals across the EA and AA populations using an exome-sequencing assay[1]. This resource made it possible to estimate MAF for most of the variations that are observed in the subjects included in our discovery and validation cohort.

Overlap with EVP variants.

Of 12,457 sites measured in the validation cohort with an allogenomics contribution strictly larger than zero (48 exomes, sites with contributions across 24 clinical pairs of transplants), 9,765 (78%) have also been reported in EVP (6,503 exomes).

Sequence Data Analysis.

Illumina sequence base calling was performed in the Weill Cornell Genomics Core Facility. Sequence data in FASTQ format were converted to the compact-reads format using the Goby framework [14]. Compact-reads were uploaded to the GobyWeb[2] system and aligned to the 1000 genome reference build for the human genome (corresponding to hg19, released in February 2009) using the Last[3,4] aligner (parallelized in a GobyWeb[2] plugin). Single nucleotide polymorphisms (SNPs) and small indels genotype were called using GobyWeb with the Goby[5] discover-sequence-variants mode and annotated using

the Variant Effect Predictor[6] (VEP version 75) from Ensembl. The data were downloaded as a Variant Calling format[7] (VCF) file from GobyWeb[2] and further processed with the allogenomics scoring tool (see http://allogenomics.campagnelab.org).

Estimation of the allogenomics mismatch score.

The allogenomics mismatch score $\Delta(r,d)$ is estimated for a recipient *r* and donor *d* as a sum of score mismatch contributions (see Figure 1C Equation 1).

Equation 1 (reproduced from Figure 1C).

$$\Delta(r,d) = \sum_{p \in P} \sigma_p[G_{rp} = genotype(r,p), G_{dp} = genotype(d,p)]$$

Contributions are observed for each polymorphic site *p* in a set *P*, where *P* is determined by the genotyping assay and analysis methods, and can be further restricted (*e.g.*, to polymorphisms within genes that code for membrane proteins). Score mismatch contributions $\sigma_p(G_{rp},G_{dp})$ is calculated using the recipient genotype G_{rp} and the donor genotype G_{dp} at the polymorphic site *p*. Here, we consider that a genotype can be represented as a set of alleles that were called in a given genome. For instance, if a subject has two alleles at one polymorphic site, and we denote each allele A or B, the genotype at *p* is represented by the set {A,B}. This representation is general and sufficient to process polymorphic sites with single nucleotide polymorphisms or insertion/deletions.

Equation 2 describes how the individual score mismatch contributions are calculated at a polymorphic site of interest.

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

Equation 2 (reproduced from Figure 1C).

A contribution of 1 is added to the score for each polymorphic site where the donor genome has an allele (a_{dp}) that is not also present in the recipient genome. When both donor and recipient genome are called at polymorphic site P, no contribution is added. For example, assuming a genomic site where the donor genome has two alleles, *i.e.*, G_{dp}={A,B}, and the recipient genome is homozygote with G_{rp}={A}. In this case, (G_{rp},G_{dp})=1. Figure 1B presents additional examples of donor and recipient genotypes and indicates the resulting score contribution (the subscript *p* is omitted for conciseness). Score contributions are summed across all polymorphism sites in the set *P* to yield the allogenomic mismatch score (see Figure 1C Equation 1).

Selection of informative polymorphisms.

The selection of the set of polymorphic sites *P* is important to the effectiveness of the approach. In the current method, we select exonic polymorphic sites that are (1) predicted to create non-synonymous change in a protein sequence, (2) are located in a gene that code for one or more membrane proteins (defined as any protein with at least one predicted transmembrane segment, information obtained from Biomart[8], Ensembl database 75). Additional filters can be applied to restrict *P*, which may lead to improved prediction of transplant clinical endpoints. Constructing additional filters will require the study of a larger training set of matched recipient and donor genotypes, which currently does not exist. It is possible that such study will indicate that other criteria than (2) also lead to predictive scores.

Implementation: the allogenomics scoring tool.

We developed the *allogenomics scoring tool* to process genotypes in the VCF format and produce allogenomics mismatch score estimates for specific pairs of genomes in the

input file. The *allogenomics scoring tool* was implemented in Java with the Goby framework and is designed to read VCF files produced by Goby and GobyWeb. The source code of the allogenomics scoring tools is distributed for academic and non-commercial purposes at http://allogenomics.campagnelab.org. The following command line arguments were used to generate the estimates described in this manuscript and can be run from the Allogenomics_Package file provided in supplementary. The genotype input file necessary to reproduce these results (GobyWeb tag: JEOHQUR) will be distributed through dbGAP (http://www.ncbi.nlm.nih.gov/gap) to control access to these private genotype human subject data. A copy of the file has been provided to the editors of the journal who can make it available to the reviewers upon condition of confidentiality.

Pre-requisite to running the command lines: (1) You must have the Java runtime environment installed on your computer (the software has been tested with version 1.6) (2) You must define the environment variable ALLO to the location where you have downloaded the distribution of the allogemomics scoring tool. (3) You must obtain the input VCF file and place it under: ${ALLO}/VCF_files_input/JEOHQUR-stats.vcf.gz

Estimating allogenomics mismatch scores on the discovery cohort:

```
java -Xmx4g -jar allogenomics-1.1.7-scoring-tool.jar \
        --input ${ALLO}/VCF_files_input/JEOHQUR-stats.vcf.gz \
        -p ${ALLO}/Pair_files/Discovery_cohort.pairs.tsv \
        -a Annotation_files/All_protein_coding_Ensembl_75.gtf \
        --output ${ALLO}/Output/TM-Discovery.tsv \
        --output-format TSV --only-non-synonymous-coding --vep \
        --consider-indels --minimum-depth 10 --max-depth 500 \
        -t ${ALLO}/Annotation_files/TrM-Transcript_Ensembl_75.tsv \
        --clinical
```

Estimating allogenomics mismatch scores on the validation cohort:

```
java -Xmx4g -jar allogenomics-1.1.7-scoring-tool.jar \
        --input ${ALLO}/VCF_files_input/JEOHQUR-stats.vcf.gz \
        -p ${ALLO}/Pair_files/Validation_cohort.pairs.tsv \
        -a ${ALLO}/Annotation_files/All_protein_coding_Ensembl_75.gtf \
        --output ${ALLO}/Output/TM-Validation.tsv \
        --output-format TSV --only-non-synonymous-coding --vep \
        --consider-indels --minimum-depth 10 --max-depth 500 \
        -t ${ALLO}/Annotation_files/TrM-Transcript_Ensembl_75.tsv \
        --clinical --measured-sites SitesHaloplexExome.tsv
```

Estimating allogenomics mismatch scores on merged discovery and validation cohorts:

```
java -Xmx4g -jar allogenomics-1.1.7-scoring-tool.jar \
        --input ${ALLO}/VCF_files_input/JEOHQUR-stats.vcf.gz\
        -p Pair_files/Discovery+Validation_cohort.pairs.tsv \
        -a ${ALLO}/Annotation_files/All_protein_coding_Ensembl_75.gtf \
        --output ${ALLO}/Output/TM-Discovery+Validation.tsv \
        --output-format TSV --only-non-synonymous-coding \
        --vep --consider-indels --minimum-depth 10 \
        --max-depth 500 \
        -t ${ALLO}/Annotation_files/TrM-Transcript_Ensembl_75.tsv --clinical
```

Estimating allogenomics mismatch score limited to Illumina GeneChip660W loci on the validation cohort:

```
java -Xmx4g -jar allogenomics-1.1.7-scoring-tool.jar \
        --input ${ALLO}/VCF_files_input/JEOHQUR-stats.vcf.gz \
        -p ${ALLO}/Pair_files/Validation_cohort.pairs.tsv \
        -a ${ALLO}/Annotation_for_660W/Human660W_Gene_Annotation_hg19-ilmn.tsv \
        --output ${ALLO}/Output/TM-Validation_Illumina660W.tsv \
        --output-format TSV --only-non-synonymous-coding --vep \
        --consider-indels --minimum-depth 10 --max-depth 500 \
        -t ${ALLO}/Annotation_for_660W/TM-as-gene-names_for_Illumina660W.tsv \
        --clinical --measured-sites sites-660W.tsv
```

Statistical Analyses.

Analyses were conducted with either JMP Pro version 11 (SAS Inc.) or metaR (http://metaR.campagnelab.org). Figures 2, 3 and 4, as well as SF1B, SF1C, SF2B, SF3C were constructed with metaR analysis scripts and edited with Illustrator CS6 to increase some font sizes or adjust the text of some axis labels.

Supplementary Discussion

Several Donor Recipient matching factors have been identified prior to this study as import for transplantation. For instance, blood group compatibility is a prerequisite unless pre-conditioning of the recipient is undertaken to facilitate blood group incompatible kidney transplantation. While HLA compatibility is a necessary requirement for successful bone marrow transplants, full HLA compatibility is not an absolute prerequisite for all types of transplantations as indicated by the thousands of solid organ transplants performed yearly despite lack of full matching between the donor and recipient at the HAL-A, B or the DR locus. In view of better patient survival following transplantation compared to dialysis, kidney transplants are routinely performed with varying degrees of HLA-mismatches including HLA mismatches for all HLA-class I and II antigens. Although, graft outcome is better with better HLA-matching, excellent long-term graft outcome with stable graft function have been observed in patients with 6 HLA mismatches. The success of these transplants clearly suggests that factors other than HLA compatibility may influence the long-term clinical outcome of Kidney allografts.

Case-control designs are appropriate when studying phenotypes that are expected to be associated with genotypes that follow a Mendelian inheritance mechanism. We note that transplant patients are not appropriate subjects for this experimental design. Patients who received a kidney transplant have two genomes in their body: their germline DNA, and the DNA of the donor. In cases when the transplanted kidney was from an unrelated donor (e.g., organs from deceased donors), it is clear that a Mendelian genetic transmission mechanism is not at play. Even in cases where the donor is one of the parents of the transplant recipient (familial transplant), the genome of the parent will break the assumptions of Mendelian inheritance. For instance, for genomic loci where

the two parents are heterozygotes (e.g., father A/B, mother A/B and the child was A/A by mendelian recombination), transplantation of the mother's kidney into the child will result in a transplanted individual with genotype A/A from birth to transplant and genotype A/A (in most of the body), and A/B in the transplanted kidney, after the transplant. Because the transplant recipient has two genomes after transplant, it is not appropriate to assume that genomic markers can be identified when assuming a Mendelian inheritance process. Yet, this assumption has been made in most of the few transplantation genomic studies published to date.

The allogenomics concept that we present in this manuscript assumes a different mechanism for the development of the immune response in the transplant recipient: immunological and biophysical principles strongly suggest that alleles present in the donor genome, but not in the recipient genome, would have a potential to produce epitopes that the recipient immune system would recognize as non-self. This reasoning explains why the allogenomics score is not equivalent to the genetic measures of allele sharing distance that have been used to perform genetic clustering of individuals[9].

Our results suggest that allogenomic mismatches in proteins expressed at the surface of the donor genome could explain why some recipients' immune systems mount an attack against the donor organ, while other patients tolerate the transplant for many years, when given similar immunosuppresive regimens. If the results of this study are confirmed in additional independent transplant cohorts (renal transplants, solid or hematologic transplants), they may prompt the design of prospective clinical trials to evaluate whether allocating organs to recipients with a combination of allogenomics mismatch scores and HLA mismatch scores improves long term graft outcome. A

positive answer to this question could profoundly impact the current clinical and regulatory framework for assigning organs to ESRD patients.

While we have not attempted to optimize the set of sites considered to estimate the allogenomics mismatch score, it is possible that reduced and more focused subsets could increase the predictive ability of the score. On the other hand, it is also possible that most polymorphisms that contribute to the score have a low frequency in the population (e.g., minor allele frequency less than 5%), which would make the identification of common sites of mismatches unlikely. In the event that frequent sites of allogenomics mismatches could be found, such studies would require access to large datasets of genotypes for matched recipient and donor genomes that are not available to us at this time.

Supplementary Figures

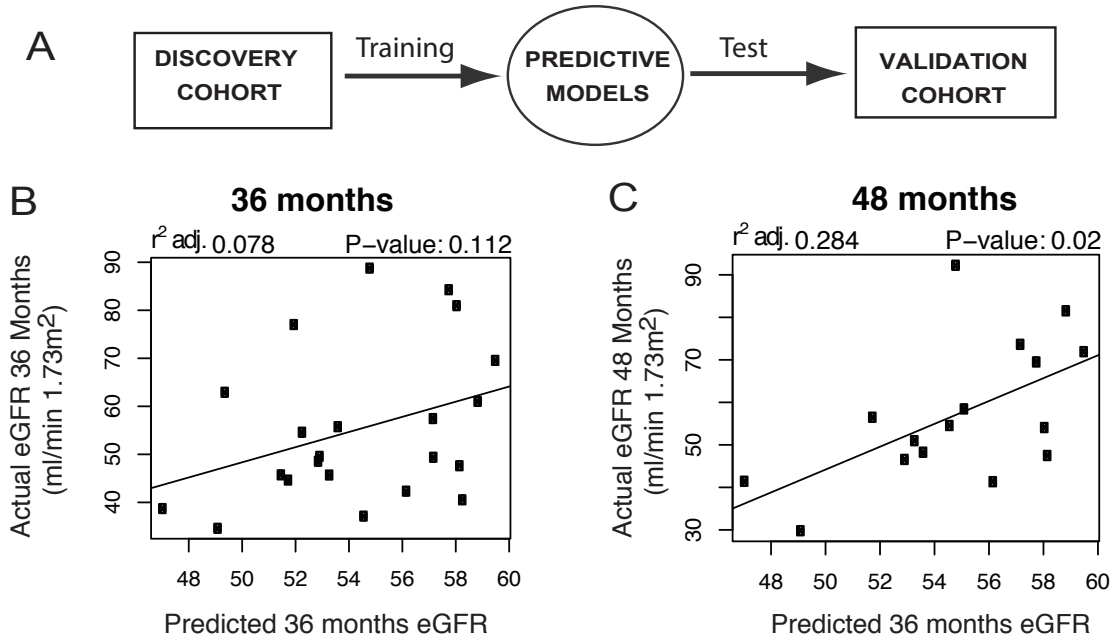

Figure S1. Model trained on the Discovery cohort applied to the Validation cohort. A) We trained a model to predict eGFR on the discovery cohort (using eGFR at 36 months) and used the trained, fixed, model to predict eGFR at 36 months and 48 months for recipients of the Validation cohort. The trained model was eGFR=109.031825038751 - 0.0404193475856964 * allogenomics_mismatch_score. Correlation between predicted eGFR and observed eGFR on the Validation cohort at 36 (B) and 48 (C) months post transplantation.

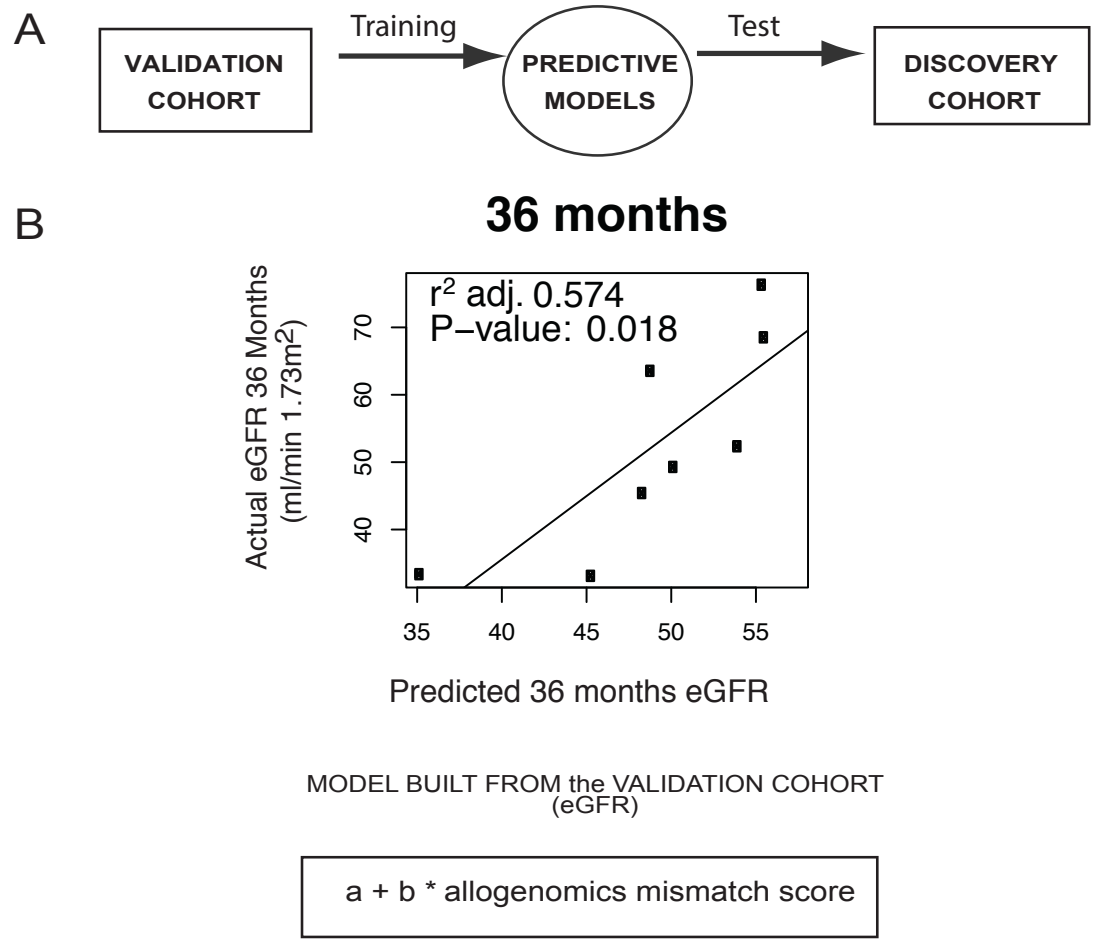

Figure S2. Model trained on the Validation cohort applied to the Discovery cohort. A) We trained models to predict serum creatinine and eGFR on the validation cohort and used the trained, fixed, model to predict serum creatinine and eGFR for recipients of the Discovery cohort. B) Correlation between the eGFR predicted by the fixed model and that observed in the Discovery cohort. Parameters of the trained models were: a=82.12310, b=-0.002528.

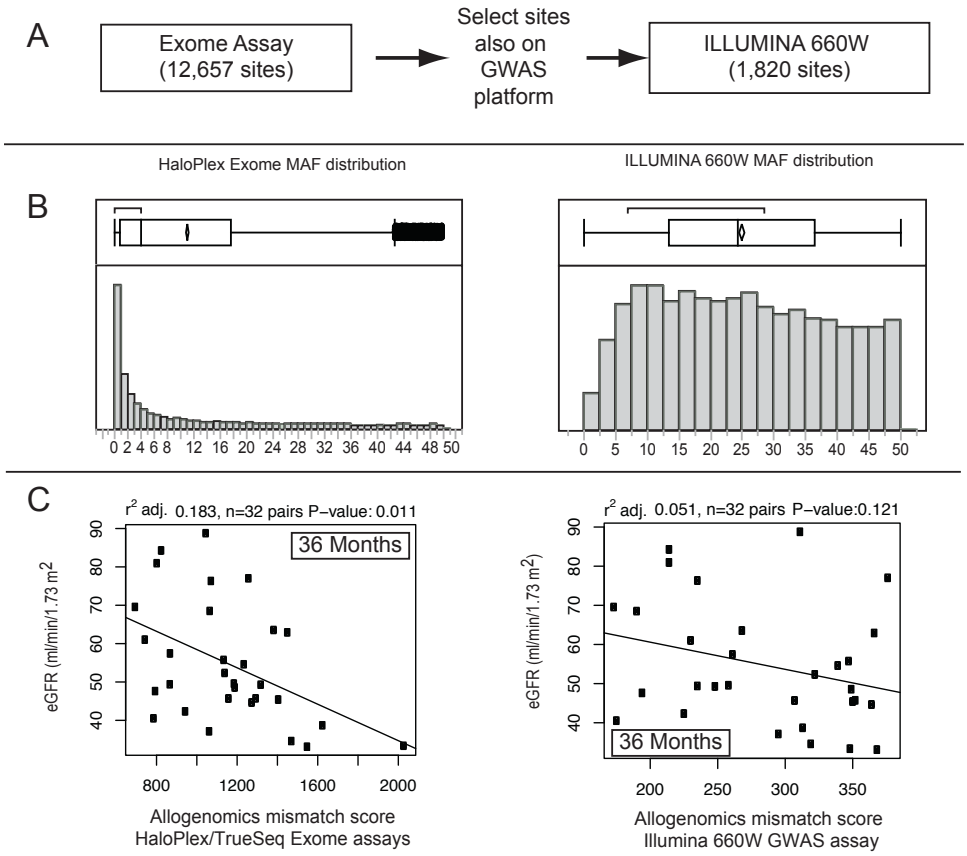

Figure S3. Effect of genotyping platform on future replication studies. In this simulation, we estimate how well the allogenomics mismatch score could be evaluated with the genotyping array technology frequently used in GWAS studies. Analysis are done on the Validation cohort (n=24 pairs, 48 exomes). A) The allogenomics mismatch score evaluated with the Agilent Haloplex exome platform takes advantage of 17,025 genomic sites to estimate allogenomic contributions in transmembrane proteins. Only sites were an allogenomics mismatch score contribution different from zero are counted. We filtered the exome genomic sites to exclude sites not found on the Illumina 660W genotyping platform (used in [10]). After filtering, the allogenomics score is estimated with 1,797 remaining genomic sites. B) The minor allele frequency (MAF) of the alleles described at each set of genomic site is shown as a histogram (MAF is estimated from the EVP database, see Methods). Exome sequencing is an assay that directly observes variations in an individual DNA sample. The MAF distributions confirm that exome sequencing help estimate contributions from many rare (MAF<5%) polymorphisms, whereas the chip genotyping platform estimates the score from contributions from frequent alleles. C) The correlation obtained with the score estimated from the exome sites and the subset of sites also measured by the GWAS platform. While some trend is still visible with sites measured on the GWAS platform, more samples would be needed to reach significance in the combined Discovery and Validation cohorts (n=34 pairs). Note that the magnitude of the scores is smaller on the GWAS platforms because fewer contributions are summed. In contrast, the exome assays (Illumina TrueSeq for the Discovery cohort or Agilent Haloplex for the Validation cohort) result in stronger and significant correlation on the same set of samples.

Supplementary Tables

Table S1. Characteristics of Kidney transplant recipients and their donors.

Characteristic	Discovery cohort	Validation cohort
Number of Transplant Pairs with living donors	10/10	24/24
Clinical factors		
Age		
Donor (SD)	41 (13)	46 (10)
Recipient (SD)	48 (10)	51 (13)
Living Donor type		
Living related N (allog. mism. score [SD])	4 (1116 [143])	13 (939 [218])
Living unrelated N (allog. mism. Score [SD])	6 (1481 [300])	11(1277 [170])
Donor sex		
Male (%)	2 (20%)	8 (33%)
Female (%)	8 (80%)	16 (67%)
Donor Race		
Black (%)	4(40%)	5 (21%)
Non-Black (%)	6(60%)	19 (79%)
Recipient sex		
Male (%)	9 (90%)	13 (54%)
Female (%)	1 (10%)	11 (46%)
Recipient Race		
Black (%)	4(40%)	7(29%)
Non-Black (%)	6(60%)	17(71%)
Number of HLA mismatches ABDR (SD)	3.9 (1.8)	3.6(1.93)
Functional Factors		
Number of Patients at 12 months	10	24
Serum creatinine level at 12 months mg/dL (SD)	1.51 (0.35)	1.45 (0.41)
eGFR at 12 months ml/min/1.73m^2 months (SD)	54.3(10)	54.3 (16.3)
Number of Patients at 24 months	9	23
Serum creatinine level at 24 months mg/dL (SD)	1.36 (0.19)	1.45 (0.49)
eGFR at 24 months ml/min/1.73m^2 months (SD	59 (7.7)	54.85 (15.7)
Number of Patients at 36 months	8	22
Serum creatinine level at 36 months mg/dL(SD)	1.62 (0.50)	1.38 (0.40)
eGFR at 36 months ml/min/1.73m^2 months (SD)	53.4(15)	55.3(15.9)
Number of Patients at 48 months	0	16
Serum creatinine level at 48 Months mg/dL(SD)	-	1.34 (0.43)
eGFR at 48 months ml/min/1.73m^2 months (SD)	-	57.4 (16.4)
Patients with an Acute Cellular rejection episode in the first year of transplantation, N (%)	3 (30%)	5(20%)
Immunosupression		
Calcineurin Inhibitors, n (%)	9 (90%)	24 (100%)
Corticosteroids, n (%)	0 (0%)	5 (21%)

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