Mismatches in Gene Deletions and Kidney-related Proteins as Candidates for Histocompatibility Factors in Kidney Transplantation

Markkinen, Salla

2022-11

Markkinen , S , Helanterä , I , Lauronen , J , Lempinen , M , Partanen , J & Hyvärinen , K 2022 , ' Mismatches in Gene Deletions and Kidney-related Proteins as Candidates for Histocompatibility Factors in Kidney Transplantation ' , Kidney International Reports , vol. 7 , no. 11 , pp. 2484-2494 . https://doi.org/10.1016/j.ekir.2022.08.032

http://hdl.handle.net/10138/354751
https://doi.org/10.1016/j.ekir.2022.08.032

KI REPORTS
KIReports.org

Mismatches in Gene Deletions and Kidney-related Proteins as Candidates for Histocompatibility Factors in Kidney Transplantation

Salla Markkinen[1], Ilkka Helanterä[2], Jouni Lauronen[1], Marko Lempinen[2], Jukka Partanen[1] and Kati Hyvärinen[1]

[1]Finnish Red Cross Blood Service, Helsinki, Finland; and [2]Transplantation and Liver Surgery, Helsinki University Hospital and University of Helsinki, Helsinki, Finland

Introduction: The genomic mismatch level between donor and recipient may be associated with the risk of rejection and graft survival. We determined the association of genome-level matching with acute rejection in deceased-donor kidney transplantation.

Methods: The study cohort consists of 1025 recipient-donor pairs transplanted in a single center from 2007 to 2017 in Helsinki. The associations between the sums of whole-genome missense variant mismatches and missense mismatches in transmembrane, secretory, and kidney-related proteins, with acute rejection were estimated using Cox model. In addition, we analyzed 40 deletion-tagging variants using Cox model.

Results: The association analysis between mismatch sums of kidney-related proteins and acute rejection resulted in an unadjusted hazard ratio (HR) of 1.15 (95% confidence interval [CI], 1.01–1.30; $P = 0.029$) and adjusted HR of 1.13 (95% CI, 0.99–1.28; $P = 0.071$). In deletion analysis, a mismatch in rs7542235 genotype GG tagging a homozygous deletion at the complement factor H-related (*CFHR*), proteins locus, predisposed to acute rejection with an unadjusted HR of 3.10 (95% CI, 1.53–6.29; $P = 0.002$) and adjusted HR of 2.97 (95% CI, 1.46–6.05; $P = 0.003$).

Conclusion: In conclusion, analyses of genome-level mismatches may be useful tools in prediction of transplantation outcome. The relative importance differs between populations, because we found evidence for *CFHR* deletion but could not replicate the finding of previously reported *LIMS1* deletion.

Kidney Int Rep (2022) **7,** 2484–2494; https://doi.org/10.1016/j.ekir.2022.08.032
KEYWORDS: acute rejection; genome-wide mismatching; genomics; histocompatibility; transplantation

Despite a good survival rate of kidney transplants and effective immunosuppressive treatments, up to 10% of recipients worldwide suffer from acute rejection in which the recipient's immune system recognizes nonself antigens in the allograft and elicits alloimmune reaction.[1]

The histocompatibility matching for transplantation in most centers relies on 3 major criteria, namely ABO-compatibility, donor-recipient matching at the human leukocyte antigen (HLA) genes, and a cross-matching test to evaluate the preformed antibodies against donor HLA molecules. In genetic terms, transplantation and matching can be regarded as a multifactorial trait in which the matching of HLA and ABO is known to be a critical but clearly not a sufficient factor to fully characterize the risk of alloimmune response.

In recent years, genome-wide association studies that search novel genetic factors for complications of kidney transplantation have been performed.[2-7] A few genetic associations have been reported[3,7] but replication of findings has proven to be difficult. By far the largest genome-wide association studies, including 2094 kidney transplant-pairs with replication in 5866 pairs, found no genome-level significant association with graft survival or acute rejection.[5]

An alternative approach to genome-wide association studies is to search at the whole genome level for matching of genes or genetic variation that would, in addition to HLA and ABO matching, be important in predicting immunological complications after transplantation. This kind of whole genome-level histocompatibility matching

Correspondence: Salla Markkinen, Research and Development, Finnish Red Cross Blood Service, Biomedicum Helsinki 1, Haartmaninkatu 8 00290 Helsinki, Finland. E-mail: salla.markkinen@bloodservice.fi

Received 11 July 2022; revised 23 August 2022; accepted 29 August 2022; published online 6 September 2022

Table 1. Characteristics of the study population

Characteristic	All recipients	Rejection	No rejection	*P* value[a]
Age (median, range)	57 (18–79)	56 (21–77)	57 (18–79)	0.258[c]
Sex, n (%)				0.394[d]
Male	703 (69)	142 (71)	561 (68)	
Female	322 (31)	58 (29)	265 (32)	
Rejection, n (%)	199 (19)	199 (100)		
T cell-mediated, n (%)	178 (17)	178 (89)		
Antibody-mediated, n (%)	21 (3)	21 (11)		
Primary diagnosis, n (%)				
Polycystic kidney disease	202 (20)	42 (21)	160 (19)	
Diabetic nephropathy with type I diabetes	150 (15)	31 (16)	119 (14)	
IgA nephropathy	106 (10)	18 (9)	88 (11)	
Chronic kidney disease, unspecified	105 (10)	21 (11)	84 (10)	
Diabetic nephropathy with type II diabetes	84 (8)	16 (8)	68 (8)	
Other	378 (37)	71 (35)	307 (38)	
		Median (IQR)		
Follow-up time, mo	37 (18–63)	35 (16–59)	38 (18–63)	0.087[c]
PRA I > 0, %[b]	22 (5–55)	17 (2–58)	23 (5–54)	0.353[c]
PRA II > 0, %[b]	27 (12–60)	45 (17–77)	26 (12–50)	0.074[c]
Cold ischemia, h	20 (17–23)	20 (17–23)	20 (17–23)	0.300[e]
HLA eplet mismatch sum, n	27 (19–36)	31 (23–39)	26 (18–35)	<0.001[c]
HLA I eplet mismatch sum, n	11 (7–15)	12 (8–16)	11 (7–15)	0.031[c]
HLA II eplet mismatch sum, n	16 (7–24)	19 (12–27)	15 (6–23)	<0.001[c]

HLA, human leukocyte antigen; IgA, Immunoglobulin A; IQR, interquartile range; PRA, panel-reactive antibody.
[a]The significance of variation was calculated between rejection-group and nonrejection-group.
[b]The medians of PRA values are calculated only from patients with PRAI > 0 and PRAII > 0
[c]The Mann–Whitney U-test.
[d]The Pearson chi-square test
[e]The *t*-test

has produced some promising results in hematopoietic stem cell transplantation[8,9] and lately in kidney transplantation.[10-14]

We investigated the association between acute rejection and genome-level matching among kidney transplant donor-recipient pairs. We analyzed a retrospective, single-center cohort of 1025 pairs of Finland. The results indicate that mismatches in deletions and kidney-related proteins may be novel histocompatibility factors associated with acute rejection.

METHODS

The study follows the STREGA recommendations (STrengthening the REporting of Genetic Association Studies) in order to enhance the transparency of the report.[15]

Study Cohort and Design

The characteristics of recipients are presented in Table 1 and in the Supplementary Material. The flow of study cohort is described in Figure 1. A total of 1025 adult kidney transplant recipients (>18 years old) who received a first kidney transplantation during 2007 to 2017 in a single transplant center, at the Helsinki University Hospital, Helsinki, Finland, and 730 HLA-matched adult deceased donors were included in this study. Of the transplanted kidneys, 295 were partner kidneys from 1 donor to 2 recipients. The primary outcome examined was biopsy-proven acute rejection based on the Banff classification,[16] and included both antibody-mediated and T-cell-mediated rejections.[16] The borderline rejections were also included in this study.

DNA samples from recipients and donors were extracted from whole blood samples at the time of histocompatibility testing for transplantation at Finnish Red Cross Blood Service, Helsinki, Finland. At organ allocation, the donor-recipient pairs aimed to match the low-resolution level at the *HLA-A*, *HLA-B* and *HLA-DRB1* loci, with the *DRB1* locus being the most important. All transplantations were negative for complement-dependent cytotoxicity crossmatch test.

The clinical data of recipients and donors were extracted from the Finnish Transplant Registry, which is a national follow-up registry obliged by law.

Genotyping and Imputation

Genotyping was performed at the Finnish Institute of Molecular Medicine, Helsinki, Finland using Infinium Global Screening array-24 v2.0 with multidisease drop-in (Illumina).

Before imputation, genotyping chip data in GRCh37 build was lifted over to GRCh38/hg38 build following a protocol version 2.[17] The postliftover genotype data were imputed using a Finnish SISu v3 reference panel

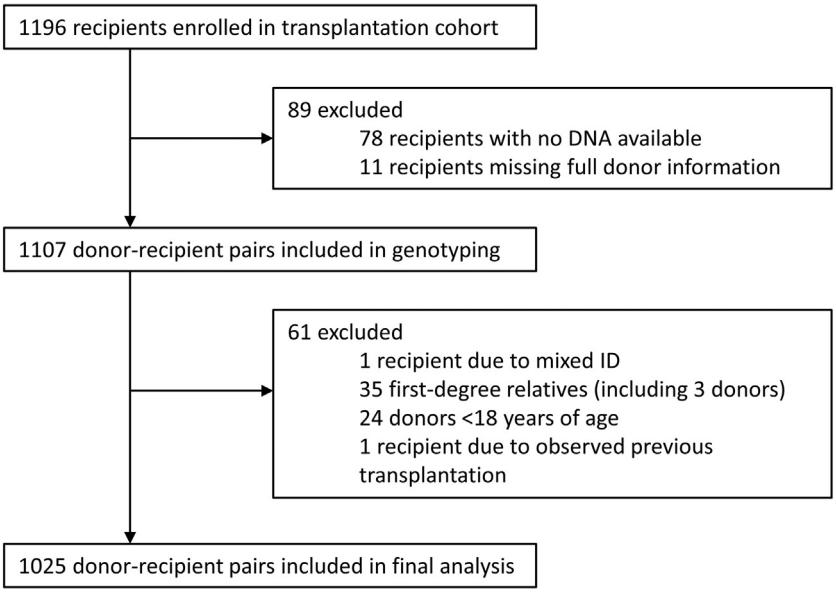

Figure 1. Flow of recipients. The enrolled recipients were ≥18 years of age and received the first kidney from deceased donor between 2007 and 2017. ID, identification detail.

consisting of high-coverage (read depth 25–30x) whole genome sequence (WGS) data from THL Biobank cohorts ($N = 1768$) (http://sisuproject.fi/). Genotype-wise, sample-wise, and variant-wise quality control filtering procedures for the SISu reference panel were applied by an iterative manner on the high-coverage WGS (hcWGS) data using the Hail framework (https://github.com/hail-is/hail) v0.1. The imputation procedure followed the genotype imputation pipeline version 2.[18] The flow of genetic variants after imputation is presented in Figure 2. After imputation, 32,321,074 variants were available for quality control procedures (Supplementary Material).

In addition to the clinical low-resolution HLA typing of the individual samples, HLA type was imputed to high resolution using HIBAG v1.0.3 with the Finnish HLA reference for *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, *HLA-DPB1*, *HLA-DQA1*, and *HLA-DQB1* gene alleles.[19]

Genome-wide Mismatch Analyses

We carried out a replication analysis of a recently reported mismatch study by Reindl-Schwaighofer *et al.*[12] The HLA eplet mismatch was calculated based on imputed high resolution HLA genotypes using HLA-Matchmaker (http://www.epitopes.net/).[20]

Detailed Method for variant selection is provided in the Supplementary Material. Briefly, the online tool Ensemble Variant Effect Predictor release 103 (https://www.ensembl.org/Homo_sapiens/Tools/VEP) was used for functional annotation of observed and imputed variants.[21] The flow of genetic variants is summarized in Figure 2. Transcripts for transmembrane and secreted proteins, transmembrane proteins, and kidney-related proteins were retrieved from UniProt (https://www.uniprot.org/).[22]

The variant mismatch was defined as the donor carrying an allele that was not present in the recipient. We calculated the sum of missense variant mismatches across the genome between donor and recipient for transmembrane and secreted proteins, transmembrane proteins and kidney-related proteins using R v3.6.2. In addition, we calculated the overall missense mismatch sum. Because of the adjustment for HLA eplet mismatch, the variants in the major histocompatibility complex region (28,510,120–33,480,577) on chromosome 6 and on sex chromosomes were excluded from the present study.

We evaluated the association between missense variant mismatch in transmembrane and secretory proteins, and graft loss using Cox proportional hazards model. We analyzed the mismatch sum both as a continuous variable, and also by dividing the sum into quartiles. The models were adjusted with additional covariates of recipient and donor sex, recipient and donor age, cold ischemia time, and HLA I (*HLA-A*, *HLA-B*, *HLA-C*) and HLA II (*HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1* and *HLA-DPB1*) eplet mismatch. Because of low amount of graft loss events observed in our data, we also performed the analyses using acute rejection endpoint. We evaluated the association of missense variant mismatch with acute rejection using logistic regression with recipient and donor sex, recipient and donor age, cold ischemia time, panel-reactive antibody (PRA) PRA I, PRA II, HLA I, and HLA II eplet mismatch as covariates. The missense mismatch sum was also tested in a univariate model that

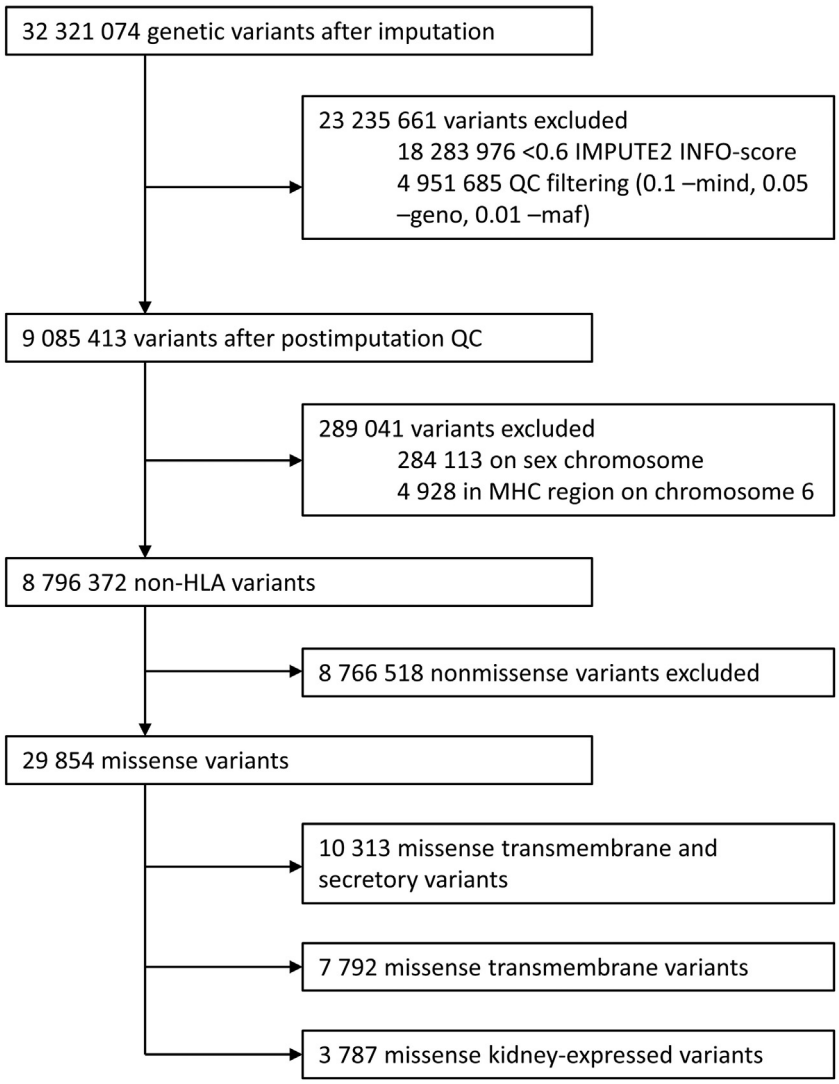

Figure 2. Flow of genetic variants. HLA, human leukocyte antigen; MHC, major histocompatibility complex; QC, quality control.

was not adjusted for additional covariates. Cox proportional hazards model was utilized to analyze time-to-acute-rejection with recipient and donor sex, recipient and donor age, cold ischemia time, PRA I, PRA II, and HLA I, and HLA II eplet mismatch as covariates. Two patients were excluded because of missing follow-up data. Recipients were divided into quartiles based on their mismatch sum, and the relationship of each quartile with time-to-acute-rejection was evaluated with Kaplan-Meier curves and tested with both Cox proportional hazards and logistic regression models.

Deletion Analysis

Steers *et al.*[13] reported a significant association with allograft rejection in the *LIMS1* gene deletion. We carried out a replication study with 40 deletion-tagging variants that were available in our dataset (Supplementary Table S1). The tagging variants analyzed had a global minor allele frequency of >10% and were all in strong linkage disequilibrium with the deletions.[13]

The dependent variable was time-to-acute-rejection, defined from the date of first transplant to the date of rejection event. We used Kaplan-Meier survival curves and Cox proportional hazards models to calculate the estimates for both the recipient-donor pairs and recipients only. In the recipient-donor analysis (collision model), the risk group was defined when a recipient who was homozygous for a deletion-tagging variant received a kidney from a donor who was either nonhomozygous or homozygous for the reference variant. The mismatch status was used as an independent variable (Supplementary Table S1). In the Cox proportional hazards model, the other covariates were recipient and donor sex, recipient and donor age, cold ischemia time, as well as HLA I and HLA II eplet mismatch. Two patients were excluded because of missing follow-up data. We conducted a Bonferroni correction threshold for statistical significance (level of 0.05/40, or 1.25×10^{-3}).

We calculated the mismatch sum of all homozygous deletions in donor-recipient pairs among the 40 deletion-tagging variants and evaluated the association of mismatch sum to time-to-acute-rejection with Kaplan-Meier and Cox proportional hazards models. The sum of all homozygous deletions in recipient-only data was assessed. All the associations were evaluated with logistic regression.

Whole Genome Sequencing

Detailed information about the WGS is provided in the Supplementary Material (Supplementary Table S2). Briefly, to validate the deletions in complement factor H (*CFH*) region, WGS was performed on 3 recipients who are homozygous for the deletion-tagging variant rs7542235.

Detection of CFH and CFH Antibodies

For determination of CFH and to assess *de novo* antibody formation, blood serum samples were analyzed with enzyme-linked immunosorbent assays. Details are provided in the Supplementary Material (Supplementary Table S3).

Other Statistical Analyses

Characteristics of recipients were non-normally distributed and thus described by medians and ranges, medians and interquartile ranges (IQRs) for continuous variables, and frequencies and percentages for binary variables (Table 1). The significance of variation between characteristics in the 2 cohorts (rejection and nonrejection) was analyzed using the nonparametric Mann–Whitney U-test for non-normally distributed data (recipient age, follow-up time, PRA I and II, HLA eplet mismatch, HLA I eplet mismatch, and HLA II eplet mismatch), Pearson's chi-square test for categorical data (recipient sex), or *t*-test for normally distributed data (cold ischemia). P values <0.05 were considered statistically significant.

We assessed the association of clinical covariates with acute rejection using a logistic regression model (Supplementary Table S4). P values <0.05 were considered statistically significant.

Statistical tests were carried out in R v3.6.2 using the R function glm (package stats) for logistic regression and survival packages (survival, survminer) for survival analyses.

Because we had only a few relevant cases in enzyme-linked immunosorbent assay analyses to compare the differences between pretransplantation and post-transplantation serum samples, we did not perform any statistical tests.

RESULTS

Analysis of Characteristics and Covariates

The clinical, laboratory, and demographic characteristics are shown in Table 1. A total of 199 rejection events were observed in the present cohort of 1025 recipients. Of these, 178 were T-cell-mediated and 21 were antibody-mediated rejections. The overall median follow-up time was 37 months (IQR 16–59 months) and the median follow-up time to rejection was 35 months (IQR 16–59 months). The overall HLA eplet mismatch and HLA II eplet mismatch sums were significantly different between the rejection and nonrejection groups ($P < 0.001$).

Donor sex, donor age, recipient age, PRA II, and mismatches in HLA II between donor and recipient emerged from the logistic regression model as significant predictors of acute rejection (Supplementary Table S4). Female donors were associated with an increased risk of acute rejection, as well as older donors, younger recipients, and an increase in PRA II value and HLA II mismatch sum.

Whole-genome Mismatch Analyses

After postimputation filtering and exclusion of major histocompatibility complex regions, sex chromosomes and nonmissense variants, 29,854 amino acid changing missense variants were available for analysis at the whole genome level. Of these amino acid differences, 10,313 were located in transmembrane or secretory proteins, 7792 in transmembrane proteins and 3787 in kidney-related proteins (Figure 2).

The median number of amino acid level mismatches between donor and recipient in transmembrane and secretory proteins was 1765 (IQR 1724–1812). For the transmembrane proteins, the median mismatch sum was 1334 (IQR 1292–1671), and for kidney-related proteins, it was 605 (IQR 585–627). The overall genome-wide missense variant mismatch sum was 4935 (IQR 4861–5012).

No evidence for the association of missense variant mismatch in transmembrane and secretory proteins with time-to-graft loss was found with adjusted HR of 1.00 (95% CI, 1.00–1.01, $P = 0.3$) (Supplementary Table S5). We also did not find an association when dividing the mismatch sum into quartiles with adjusted HR of 0.96 (95% CI, 0.77–1.19, $P = 0.7$) (Supplementary Table S6). No evidence for a whole-genome-level association of missense variants with acute rejection was found in either the Cox proportional hazards or logistic regression model (Supplementary Tables S7–S8). We performed an identical missense mismatch study of transmembrane and secretory proteins as reported by Reindl-

Schwaighofer *et al.*,[12] and analyzed missense mismatch of kidney-related proteins. Our results showed that increasing mismatch sum in kidney-related proteins increased the risk for acute rejection with an unadjusted HR of 1.15 (95% CI, 1.01–1.30, $P = 0.029$) (Figure 3a) and adjusted HR of 1.13 (95% CI, 0.99–1.28; $P = 0.071$) (Supplementary Table S9) when dividing the mismatch sum into quartiles.

We found no statistically significant association between the missense mismatch sum of transmembrane and secretory proteins and time-to-acute-rejection in the Cox proportional hazards model (Supplementary Table S10) or in the logistic regression model (Supplementary Table S11). The missense mismatch sums of transmembrane or kidney-related proteins were not significant predictors of time-to-acute-rejection or acute rejection outcome in our data (Supplementary Tables S12–S15).

In the Kaplan-Meier and Cox proportional hazards model analyses of the quartiles of mismatch sum, we found no statistically significant associations between the transmembrane and secretory proteins, transmembrane proteins or in all missense variants and acute rejection (Supplementary Figures S1–S3).

Deletion Analysis

In total, 40 of the deletion-tagging variants analyzed by Steers *et al.*[13] were available in the present dataset (Supplementary Table S1), and they were tested in the donor-recipient mismatch analysis. A mismatch refers to cases in which a recipient who is homozygous for a deletion-tagging variant received a graft from a donor with a nonhomozygous or homozygous nondeletion genotype.

The rs7542235 genotype GG has been reported to tag for deletions in CFHR proteins 1–3.[23,24] We observed that a deletion-tagging mismatch in rs7542235 was significantly associated with a higher risk for rejection than the no mismatch status with unadjusted HR of 3.10 (95% CI, 1.53–6.29; $P = 0.002$) and adjusted HR of 2.97 (95% CI, 1.46–6.05; $P = 0.003$) (Table 2). The number of recipients with rs7542235 genotype GG in the rejection group was 8 (4%) and the number in the nonrejection group was 8 (1%). None of the donors were homozygous for the deletion-tagging variant. The Kaplan-Meier plot of the association of the rs7542235-tagged deletion mismatch on rejection-free graft survival is shown in Figure 3b. The statistical significance of the association, however, failed to pass the Bonferroni-corrected threshold of 0.00125. The same results were observed in recipient-only data. No other deletion-tagging variants showed statistically significant association with these outcomes.

Steers *et al.*[13] reported a significant association with allograft rejection when recipients had *LIMS1* gene deletion. We carried out a replication study in both recipient-donor and recipient-only settings but could not find evidence for the association of *LIMS1* deletion or other deletion-tagging variants when using the Kaplan-Meier curve and Cox proportional hazards model (Supplementary Tables S16–S17).

The median number of all homozygous deletions in donor-recipient pairs was 3 (IQR 2–4, range 0–9), and in recipient-only data, it was 4 (IQR 3–5, range 0–10). We did not find a significant association between the deletion sum and acute rejection between donor-recipient pairs or among recipients (Supplementary Figures S4–S5, Supplementary Tables S18–S21).

Whole Genome Sequencing

WGS confirmed that each of the 3 rs7542235 GG recipients had homozygous deletions of different sizes at the *CFHR1* region, and 2 of them also had a homozygous deletion completely or partly at the *CFHR3* locus (Figure S6).

Detection of CFH and anti-CFH Antibodies

The results are presented in Supplementary Results (Supplementary Tables S22–S23). All serum samples showed normal levels of CFH. We were not able to find evidence for anti-CFH antibody formation between pretransplantation and post-transplantation serum samples.

DISCUSSION

The results of the present study indicate that genetic donor-recipient mismatches due to homozygous gene deletions in patients and amino acid changing genetic mismatches in kidney-related proteins may predispose to acute rejection. As similar results have been indicated by a few other recent studies,[10-14] these mismatches are potential histocompatibility factors in kidney transplantation and merit large systematic studies. The present study was conducted in a relatively large single center and single population cohort of 1025 kidney transplantation recipient-donor pairs, thereby reducing the impact of confounding factors. In the study, we investigated to what extent differences or matching between donor-recipient pairs (i) at the overall genome level, (ii) in graft-expressed proteins, and (iii) in common gene deletions were associated with the increased risk for acute rejection. Modern genome tools enable us to expand the analysis of all genetic variation between transplant pairs and address the role of non-HLA variation. In principle, we can assume that any immunogenic protein-level difference found in donors and missing in recipients can lead to alloimmune reactions

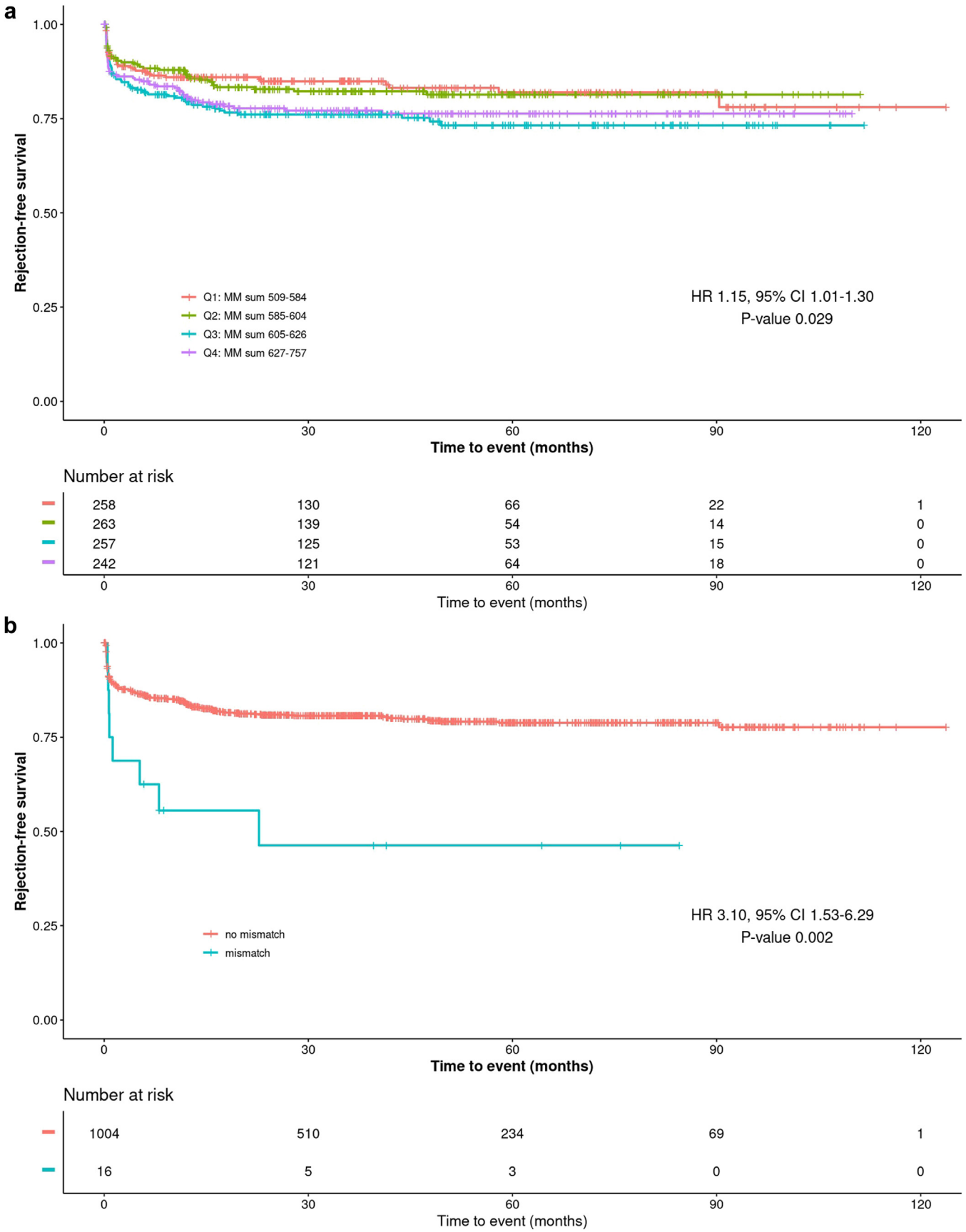

Figure 3. The effect of quartiles of missense variant mismatch sum coding for kidney-related proteins and rs7542235 mismatch on rejection-free graft survival in recipient-donor pairs. (a) The effect of quartiles of missense variant mismatch sum coding for kidney-related proteins on rejection-free graft survival. The quartile 1 (Q1) represents the lowest number of mismatches between recipient and donor, quartile (continued)

and increase the risk of, for example, rejection. There is indeed emerging evidence that genetic differences between recipient and donor, in for example, graft-expressed or cell surface-expressed proteins or even at the overall genomic level, associate with the risk of transplantation complications such as acute rejection or long-term graft loss.[10-14]

Reindl-Schwaighofer *et al.*[12] reported a statistically significant association in unadjusted Cox proportional hazards model between a transmembrane and secretory nonsynonymous donor-recipient mismatch sum and time-to-10-years and death censored graft loss in a cohort of 477 transplant pairs. We tested graft loss but could not replicate the findings of Reindl-Schwaighofer *et al.*[12] Instead, results showed that increasing mismatch sum in kidney-related proteins was associated with time-to-acute-rejection when the sum is divided into quartiles. The proteins expressed in the kidney might be exposed to the surface of the allograft in stressful conditions, such as delayed graft function, infections, and cold ischemia, and could therefore be recognized as foreign. The endpoint used is not identical to that of Reindl-Schwaighofer *et al.*[12] but may reflect a similar long-term effect because the acute rejection has been associated with a decrease in long-term survival. The study of Mesnard *et al.*[10] also found an association between long-term outcome and overall genetic mismatch score. It is plausible that the overall alloantigenic load of the donor graft is associated with longterm rather than acute effects more readily handled by medication.

Steers *et al.*[13] investigated the association of common deletion-tagging variants and kidney allograft rejection. The researchers hypothesized, along with the concept introduced by McCarroll *et al.*[8] in the hematopoietic stem cell transplantation setting, that a recipient whose genome lacks a kidney-related gene product due to a gene deletion should raise alloimmune reaction to graft from a donor whose genome carries the functional gene, hence expressing the protein. Steers *et al.*[13] found that kidney recipients with a homozygous *LIMS1* gene deletion had a significantly higher risk of rejection when the donor had at least 1 functional copy of the same gene. In the present study cohort, we were not able to confirm the effect of the *LIMS1* deletion. We instead found an association between an acute rejection and *CFHR1–3*-deletion-tagging variant. rs7542235 GG-homozygotes are assumed to be homozygotes for deletion of these genes. We confirmed the tagging by genomic sequencing; interestingly, the deletions were not identical in size and encompassed different genes. The homozygous deletion in the *CFHR* locus was associated with a decreasing probability of rejection-free graft survival in recipient-donor pairs, albeit we must note that the actual numbers of cases were low. The same finding was observed in recipient-only data as well, most likely because it is unlikely that an unrelated organ donor would have the same homozygous deletion. In fact, if deletions indeed prove to be novel risk markers for transplantation complications as now indicated by a few studies, including the present study,[8,13] it may be possible to estimate the complication risk due to gene deletions directly from patient genome data before transplantation if unrelated donors are used.

To further study the effect of *CFHR1*-locus deletion on the protein level and its possible alloantigenic role, we performed anti-CFH antibody enzyme-linked immunosorbent assay for pretransplantation and post-transplantation serum samples of 5 deletion homozygotes. In this small set of samples available, we could not find evidence for anti-CFH formation post transplantation. We had no tools to investigate whether antibody responses were raised against other proteins expressed by the genes located in the deleted *CFHR1* locus. Previous studies have shown that anti-CFH antibody formation is associated with *CFHR1* and *CFHR3* deletions,[25-27] and later is has been suggested that the deletion of *CFHR1* alone could be involved in anti-CFH antibody formation.[26]

CFH is a control protein of the complement system, inhibiting the alternative pathway of complement activation. Depending on the individual *CFHR* genes, it has been associated with a number of diseases, including atypical hemolytic uremic syndrome, C3 glomerulopathies, IgA nephropathy, age related macular degeneration, and systemic lupus erythematosus.[25-29] The functions of CFHR1 and CFHR3 are still unclear, but these 2 proteins have been suggested to be complement regulators as well.[28] It has been proposed that complement activity is determined by a homeostatic balance between CFHR1, CFHR3 and CFH.[29] The present finding of the deletion may be related to both primary kidney disease and outcome of transplantation.

Figure 3. (continued) 4 (Q4) the highest number of mismatches. Unadjusted HR with CI shown. (b) The effect of deletion-tagging variant rs7542235 mismatch on rejection-free graft survival in recipient-donor pairs. In recipient-donor analysis the recipient who was homozygous for a deletion-tagging allele G received a transplant from a donor with AG or AA genotype. The orange curve represents the no mismatch status and the light blue curve represents the mismatch status. An event (acute rejection) occurs each time the curve drops. The tick marks indicate censored data (end of follow-up time). Unadjusted HR with CI shown. CI, confidence interval; HR, hazard ratio; MM, mismatch.

Table 2. Cox proportional hazards model on the association of rs7542235 mismatch to time to acute rejection

Covariate	Hazard ratio (95% CI)	*P* value
rs7542235 mismatch	2.97 (1.46–6.05)	0.003
Recipient age	0.98 (0.97–0.99)	0.001
Donor age	1.03 (1.01–1.04)	<0.001
Recipient sex	0.91 (0.67–1.24)	0.561
Donor sex	2.00 (1.50–2.67)	<0.001
Cold ischemia	1.00 (1.00–1.00)	0.102
HLA I eplet mismatch	1.01 (0.99–1.04)	0.396
HLA II eplet mismatch	1.03 (1.02–1.04)	<0.001

CI, confidence interval; HLA, human leukocyte antigen.

There are limitations in the present study. All acute rejections in this study were biopsy- confirmed, and borderline rejections were included only if the patient received treatment for rejection (with pulse steroid). Detailed information of the Banff grades of the rejections were unfortunately not available for the purpose of this study, which is a limitation in the data. Our study cohort included 295 partner-kidneys from 1 donor to 2 recipients which was not considered nonindependently in the genetic analysis. The overall coverage of genetic variants in the missense mismatch analyses was lower in our data than that of Reindl-Schwaighofer *et al.*[12] We also had a limited amount of graft loss cases in our data. The significance of the kidney-related mismatch analysis was lost after adjusting the data with additional covariates. The *P* value of our findings in the deletion mismatch study did not achieve the Bonferroni-corrected level of significance. The Bonferroni correction, however, can be very conservative when several tests are included. The ideal control group for a deletion mismatch would be those patients who were deletion homozygous and received a deletion-matched graft. Unfortunately, such cases are low in number or not found at all. There are also differences in gene deletion frequencies between populations. The frequency of risk rs7542235 genotype GG in the present study cohort was 1.6%. We had access to only a few pretransplantation and post-transplantation samples; a prospective collection of serum samples from deletion homozygotes should be performed.

In summary, we found potential evidence for association between the mismatches in gene deletion and increasing mismatch sum in kidney-related proteins and acute rejection. Nevertheless, to find valid genetic associations, larger, prospective collaborative and meta-analysis studies should be performed to obtain sufficient power.

ACKNOWLEDGMENTS

We want to thank doctors Satu Koskela, Jarmo Ritari and Mikko Arvas for their help, Ms Sisko Lehmonen for help in collecting the samples and the staff of the HLA laboratory for collaboration. The data used for the research was imputed with the THL Biobank's SISu v3 Imputation reference panel obtained from THL Biobank. We thank all study participants for their generous participation in the FINRISK, Health 2000 and Migraine Family studies. We also thank the Sequencing Informatics Team, Finnish Institute of Molecular Medicine Human Genomics, University of Helsinki for the work done in preparation of the reference panel data.

DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the Journal of Medical Genetics. I.H. reports receiving research funding from MSD and has ongoing consultancy agreements with Novartis and Hansa Biopharma.

Funding

The study was partially supported by funding from the Government of Finland VTR funding, Munuaissäätiö (to SM), Suomen Transplantaatiokirurginen yhdistys ry (to SM), and Academy of Finland (to JP).

Data Availability Statement

The code for variant selection and statistical testing is publicly available in GitHub (https://github.com/FRCBS/Kidney_genome_wide_mismatch). Genetic data are not publicly available due to restrictions issued by the ethical committee and current legislation in Finland that do not allow the distribution of pseudonymized personal data, including genetic and clinical data.

Ethics Approval Statement

The study conforms to the principles of the Declaration of Helsinki and has been approved by the ethics committee of Helsinki University Hospital (HUS/1873/2018) and the Finnish National Supervisory Authority for Welfare and Health (V/9161/2019).

AUTHOR CONTRIBUTIONS

SM, JP, and KH planned the study, interpreted the results and prepared the manuscript; SM and KH carried out the genome data analysis. IH, ML, and JL collected the clinical data. All authors read, accepted, and contributed to the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Detailed Methods.

Detailed Information.

Detailed Results.

Figure S1. The effect of quartiles of missense variant mismatch sum coding for transmembrane and secretory proteins on rejection-free graft survival

Figure S2. The effect of quartiles of missense variant mismatch sum coding for transmembrane proteins on rejection-free graft survival
Figure S3. The effect of quartiles of the overall genome-wide missense variant mismatch sum on rejection-free graft survival
Figure S4. The effect of deletion mismatch sum on rejection-free graft survival in donor-recipient pairs
Figure S5. The effect of deletion sum on rejection-free graft survival among recipients
Figure S6. The results of whole genome sequencing of CFH-related and CFH-related protein loci on chromosome 1
Table S1. Deletion-tagging variants and their frequencies in the study cohort
Table S2. General statistics of the whole genome sequencing with Illumina's NovaSeq S4 NS4-300 run
Table S3. The number of pre- and post-transplantation serum samples in CFH- and CFH-IgG ELISA
Table S4. Logistic regression model on the association of clinical covariates and acute rejection
Table S5. Cox proportional hazards model on the association of mismatch sum of secretory and transmembrane variants to graft loss
Table S6. Cox proportional hazards model on the association of quartiles of mismatch sum of secretory and transmembrane variants and graft loss
Table S7. Cox proportional hazards model on the association of mismatch sum of all missense variants to time to acute rejection
Table S8. Logistic regression model on the association of mismatch sum of all missense variants and acute rejection
Table S9. Cox proportional hazards model on the association of quartiles of mismatch sum of kidney-related variants and acute rejection
Table S10. Cox proportional hazards model on the association of mismatch sum of secretory and transmembrane variants to time to acute rejection
Table S11. Logistic regression model on the association of mismatch sum of secretory and transmembrane variants and acute rejection
Table S12. Cox proportional hazards model on the association of mismatch sum of transmembrane variants to time to acute rejection
Table S13. Logistic regression model on the association of mismatch sum of transmembrane variants and acute rejection
Table S14. Cox proportional hazards model on the association of mismatch sum of kidney-related variants to time to acute rejection
Table S15. Logistic regression model on the association of mismatch sum of kidney-related variants and acute rejection
Table S16. Cox proportional hazards model on the association of all deletion tagging variant mismatches to time to acute rejection
Table S17. Cox proportional hazards model on the association of all deletion tagging variants to time to acute rejection in recipient data
Table S18. Cox proportional hazards model on the association of the sum of deletion mismatches between donor-recipient to time to acute rejection
Table S19. Logistic regression model for the sum of deletion mismatches between donor-recipient pairs and acute rejection
Table S20. Cox proportional hazards model on the association of the sum of deletions and time to acute rejection in recipient data
Table S21. Logistic regression model for the sum of deletions and acute rejection in recipient data
Table S22. CFH ELISA results for serum samples
Table S23. CFH IgG ELISA results for serum samples
STREGA Checklist.

REFERENCES

1. Cooper JE. Evaluation and treatment of acute rejection in kidney allografts. *Clin J Am Soc Nephrol*. 2020;15:430–438. https://doi.org/10.2215/CJN.11991019
2. O'Brien RP, Phelan PJ, Conroy J, et al. A genome-wide association study of recipient genotype and medium-term kidney allograft function. *Clin Transpl*. 2013;27:379–387. https://doi.org/10.1111/ctr.12093
3. Ghisdal L, Baron C, Lebranchu Y, et al. Genome-wide association study of acute renal graft rejection. *Am J Transplant*. 2017;17:201–209. https://doi.org/10.1111/ajt.13912
4. Pihlstrøm HK, Mjøen G, Mucha S, et al. Single nucleotide polymorphisms and long-term clinical outcome in renal transplant patients: a validation study. *Am J Transplant*. 2017;17:528–533. https://doi.org/10.1111/ajt.13995
5. Hernandez-Fuentes MP, Franklin C, Rebollo-Mesa I, et al. Long- and short-term outcomes in renal allografts with deceased donors: a large recipient and donor genome-wide association study. *Am J Transplant*. 2018;18:1370–1379. https://doi.org/10.1111/ajt.14594
6. Stapleton CP, Heinzel A, Guan W, et al. The impact of donor and recipient common clinical and genetic variation on estimated glomerular filtration rate in a European renal transplant population. *Am J Transplant*. 2019;19:2262–2273. https://doi.org/10.1111/ajt.15326
7. Divers J, Ma L, Brown WM, et al. Genome-wide association study for time to failure of kidney transplants from African American deceased donors. *Clin Transplant*. 2020;34:1–11. https://doi.org/10.1111/ctr.13827
8. McCarroll SA, Bradner JE, Turpeinen H, et al. Donor-recipient mismatch for common gene deletion polymorphisms in graft-versus-host disease. *Nat Genet*. 2009;41:1341–1344. https://doi.org/10.1038/ng.490
9. Ritari J, Hyvärinen K, Koskela S, et al. Computational analysis of HLA-presentation of non-synonymous recipient mismatches indicates effect on the risk of chronic graft-vs.-host disease after allogeneic HSCT. *Front Immunol*. 2019;10:1–9. https://doi.org/10.3389/fimmu.2019.01625

10. Mesnard L, Muthukumar T, Burbach M, et al. Exome sequencing and prediction of long-term kidney allograft function. *PLoS Comput Biol*. 2016;12:1–15. https://doi.org/10.1371/journal.pcbi.1005088

11. Pineda S, Sigdel TK, Chen J, et al. Novel non-histocompatibility antigen mismatched variants improve the ability to predict antibody-mediated rejection risk in kidney transplant. *Front Immunol*. 2017;8:1–17. https://doi.org/10.3389/fimmu.2017.01687

12. Reindl-Schwaighofer R, Heinzel A, Kainz A, et al. Contribution of non-HLA incompatibility between donor and recipient to kidney allograft survival: genome-wide analysis in a prospective cohort. *Lancet*. 2019;393:910–917. https://doi.org/10.1016/S0140-6736(18)32473-5

13. Steers NJ, Li Y, Drace Z, et al. Genomic mismatch at LIMS1 locus and kidney allograft rejection. *N Engl J Med*. 2019;380:1918–1928. https://doi.org/10.1056/nejmoa1803731

14. Zhang Z, Menon MC, Zhang W, et al. Genome-wide non-HLA donor-recipient genetic differences influence renal allograft survival via early allograft fibrosis. *Kidney Int*. 2020;98:758–768. https://doi.org/10.1016/j.kint.2020.04.039

15. Little J, Higgins JPT, Ioannidis JPA, et al. Strengthening the reporting of genetic association studies (STREGA): an extension of the STROBE Statement. *Hum Genet*. 2009;125:131–151. https://doi.org/10.1007/s00439-008-0592-7

16. Roufosse C, Simmonds N, Clahsen-Van Groningen M, et al. A 2018 reference guide to the Banff classification of renal allograft pathology. *Transplantation*. 2018;102:1795–1814. https://doi.org/10.1097/TP.0000000000002366

17. Pärn K, Fontarnau JN, Isokallio MA, et al. Genotyping chip data lift-over to reference genome build GRCh38/hg38 V. 1. FIMM HumGen Sequencing Informatics. 2; 2019. Accessed September 23, 2020. https://www.protocols.io/view/genotyping-chip-data-lift-over-to-reference-genome-n2bvjmbpvk5w/v1?version_warning=no

18. Pärn K, Isokallio MA, Fontarnau JN, et al. Genotype imputation workflow v3.0 V.2. FIMM HumGen Sequencing Informatics. 2; 2019. Accessed September 23, 2020. https://www.protocols.io/view/genotype-imputation-workflow-v3-0-e6nvw78dlmkj/v2

19. Ritari J, Hyvärinen K, Clancy J, et al. Increasing accuracy of HLA imputation by a population-specific reference panel in a FinnGen biobank cohort. *NAR Genom Bioinform*. 2020;2(lqaa030). https://doi.org/10.1093/nargab/lqaa030

20. Duquesnoy RJ, Askar M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. V. Eplet matching for HLA-DR, HLA-DQ, and HLA-DP. *Hum Immunol*. 2007;68:12–25. https://doi.org/10.1016/j.humimm.2006.10.003

21. McLaren W, Gil L, Hunt SE, et al. The Ensembl variant effect predictor. *Genome Biol*. 2016;17:1–14. https://doi.org/10.1186/s13059-016-0974-4

22. Bateman A, Martin MJ, O'Donovan C. UniProt: the universal protein KnowledgeBase. *Nucleic Acids Res*. 2017;45:D158–D169. https://doi.org/10.1093/nar/gkw1099

23. Soumya R, Stephan R, Mingyao L, et al. Associations of CFHR1-CFHR3 deletion and a CFH SNP to age-related macular degeneration are not independent. *Nat Genet*. 2010;42:553–556. https://doi.org/10.1038/ng0710-553.Associations

24. Gan W, Wu J, Lu L, et al. Associations of CFH polymorphisms and CFHR1-CFHR3 deletion with blood pressure and hypertension in Chinese population. *PLoS One*. 2012;7:e42010. https://doi.org/10.1371/journal.pone.0042010

25. Józsi M, Licht C, Strobel S, et al. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. *Blood*. 2008;111:1512–1514. https://doi.org/10.1182/blood-2007-09-109876

26. Moore I, Strain L, Pappworth I, et al. Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. *Blood*. 2010;115:379–387. https://doi.org/10.1182/blood-2009-05-221549

27. Skerka C, Chen Q, Fremeaux-Bacchi V, Roumenina LT. Complement factor H related proteins (CFHRs). *Mol Immunol*. 2013;56:170–180. https://doi.org/10.1016/j.molimm.2013.06.001

28. Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. *Nat Rev Immunol*. 2009;9:729–740. https://doi.org/10.1038/nri2620

29. Fritsche LG, Lauer N, Hartmann A, et al. An imbalance of human complement regulatory proteins CFHR1, CFHR3 and factor H influences risk for age-related macular degeneration (AMD). *Hum Mol Genet*. 2010;19:4694–4704. https://doi.org/10.1093/hmg/ddq399