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EXTENDED HISTOCOMPATIBILITY: GENOMIC PREDICTION OF KIDNEY TRANSPLANT OUTCOMES

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ACADEMIC DISSERTATION
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TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I 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 Int Rep. Sep 6;7(11):2484-2494

II Markkinen S[*], Lokki I[*], Helanterä I, Ritari J, Partanen J, Meri S, Hyvärinen K (2024) The impact of complement factor H-related protein gene deletions on kidney transplantation. Manuscript. [*]equal contribution

III Collins K, Gilbert E, Mauduit V, Benson K, Elhassan E, O'Seaghdha C, Hill C, McKnight A, Maxwell A, van der Most P, de Borst M, Guan W, Jacobson P, Israni A, Keating B, Lord G, Markkinen S, Helanterä I, Hyvärinen K, Partanen J, Madden S, Limou S, Cavalleri G, Conlon P (2023) Genetic burden for cerebrovascular risk and kidney transplant outcome. J Nephrol. 2024 May 29. Epub ahead of print. PMID: 38809363

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ABBREVIATIONS

ADPKD	Autosomal dominant polycystic kidney disease
AIM1L	Absent in melanoma 1-like
AP3D1	Adaptor Related Protein Complex 3 Subunit Delta 1
APC	Antigen-precenting cell
APOL1	Apolipoprotein L1
ARPKD	Autosomal recessive polycystic kidney disease
BCC	Basal cell carcinoma
C3G	Complement 3 glomerulopathy
CCDC67	Coiled-coil domain-containing protein 67
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CDC123	Cell Division Cycle 123
CDYL2	Chromodomain Y Like 2
CFH	Complement Factor H
CFHR	Complement Factor H-related protein
CFHR1	Complement Factor H-related protein 1
CFHR3	Complement Factor H-related protein 3
CFHR4	Complement Factor H-related protein 4
CFHR5	Complement Factor H-related protein 5
CHRNA10	Cholinergic Receptor Nicotinic Alpha 10 Subunit
CI	Confidence Interval
CKD	Chronic kidney disease

CNV	Copy number variant
COD	Cause of death
CSMD3	CUB And Sushi Multiple Domains 3
CTS	Collaborative Transplant Study
DCGL	Death-censored graft loss
DGF	Delayed graft function
DN	Diabetic nephropathy
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme-linked immunosorbent array
ESKD	End-stage kidney disease
eQTL	Gene expression quantitative trait loci
FAM129B	Family With Sequence Similarity 129 Member B
FDR	False detection rate
FIMM	Finnish Institute for Molecular Medicine
FRCBS	Finnish Red Cross Blood Service
FSGS	Focal segmental glomerulosclerosis
GCC2	GRIP And Coiled-Coil Domain Containing 2
GTEx	Genotype-Tissue Expression Consortium
GWAS	Genome-wide association study
HLA	Human Leukocyte Antigen
HLA I	Human Leukocyte Antigen Class I
HLA II	Human Leukocyte Antigen Class II
HLA-A	Human Leukocyte Antigen A locus

HLA-B	Human Leukocyte Antigen B locus
HLA-C	Human Leukocyte Antigen C locus
HLA-DPB1	Major Histocompatibility Complex, Class II, DP Beta 1
HLA-DQA1	Major Histocompatibility Complex, Class II, DQ Alpha 1
HLA-DQB1	Major Histocompatibility Complex, Class II, DQ Beta 1
HLA-DRB1	Major Histocompatibility Complex, Class II, DR Beta 1
HR	Hazard ratio
HSCT	Hematopoietic stem cell transplantation
IA	Intracranial aneurysm
IgAN	Immunoglobulin A nephropathy
IQR	Interquartile range
KiT-GENIE	Kidney Transplantation – Genomic Investigation of Essential clinical concerns
LD	Linkage disequilibrium
mHA	Minor histocompatibility antigen
MHC	Major histocompatibility complex
MM	Mismatch
MUC3A	Mucin 3A, Cell Surface Associated
MYOM2	Myomesin-2
NMSC	Non-melanoma skin cancer
NODAT	New-onset diabetes after transplantation
nsSNP	Nonsynonymous single nucleotide polymorphism
OR	Odds ratio
OR51F1	Olfactory Receptor Family 51 Subfamily F Member 1

OR8G1	Olfactory Receptor Family 8 Subfamily G Member 1
OR8G5	Olfactory Receptor Family 8 Subfamily G Member 5
pIBD	Proportion of identity-by-descent
PKD	Polycystic kidney disease
PNPLA6	Patatin like phospholipase domain containing 6
pQTL	Protein expression quantitative trait loci
PRA	Panel-reactive antibody
PRS	Polygenic risk score
PSEN2	Preselinin 2
PTDM	Post-transplant diabetes mellitus
PTPRO	Protein tyrosine phosphatase receptor type O
QUB	Queen's University Belfast
RASA3	RAS P21 Protein Activator 3
SCC	Squamous cell carcinoma
SD	Standard deviation
SLC	The solute carrier
SNP	Single nucleotide polymorphism
T2D	Type 2 diabetes
TAC	Tacrolimus
TCR	T-cell receptor
TGFB1	Transforming growth factor beta 1
TL	Transplant Lines
TX	Transplantation

UKIRTC	United Kingdom and Ireland Renal Transplant consortium
VEP	Variant effect predictor
WES	Whole exome sequencing
WGS	Whole genome sequencing
ZNF280D	Zinc Finger Protein 280D

ABSTRACT

Kidney transplantation is an established, cost-effective treatment for patients with an end-stage kidney disease. The need for kidney transplantation is constantly growing because of the aging population and increasing number of metabolic diseases, such as diabetes and cardiovascular diseases, which predispose to chronic kidney disease. The annual number of kidney transplantations performed in Finland is approximately 250. A hurdle in all transplantations between two individuals is alloimmune reaction, that is, immunological recognition of the transplanted graft as foreign by the recipient. This often leads to immunological attack against the graft. For example, about 10–20% of kidney transplantation recipients suffer from acute rejection that needs intensive medication and may impair the kidney function and the quality of recipient's life. The goal of the prior histocompatibility testing between the recipient and organ donor is to find the immunologically most suitable transplant for each patient and this way to reduce complications and immunization against the graft. In kidney transplantation, the histocompatibility between donor and patient is currently determined by matching the ABO blood group and the human leukocyte antigen (HLA) alleles. In addition, a crossmatch test is performed prior to transplantation to detect the presence of anti-donor cell antibodies in the patient serum. Despite current histocompatibility testing, we are unable to adequately predict severe transplantation-related complications, such as different forms of rejection, graft survival, or delayed graft function.

Systematic genomic screenings of risk factors outside the HLA genes for organ transplantation complications and long-term survival started in the last decade. Genome-wide association studies (GWAS) have reported variants that are associated with kidney transplant outcomes, but follow-up studies to confirm the findings and to determine the functional effects of polymorphisms are still required. In addition to effects of single variants, the genomic risk can also be estimated by combining all low-level effects of GWAS results into polygenic risk scores (PRS). An approach particular to transplantation is to study genome-level matching between donor and patient. Transplantation involves an interaction of two independent genomes, and resulting mismatches have complex mechanisms and potentially larger effect sizes than conferred by donor or recipient variants alone. Each donor-recipient pair contains several non-synonymous amino-acid differences that can

serve as potential trigger of alloimmune response. Recent studies have examined mismatches in non-HLA genes in multi-ethnic and genetically heterogeneous kidney allograft cohorts. Yet another interesting approach is to investigate mismatches of homozygous gene deletions between donor and recipient. The idea is that some deletions in the genome are so common in the population that individuals inherit them from both parents, resulting in a complete lack of the protein-coding gene and thus the protein itself. When the missing protein is introduced into the recipients' body in transplantation, the protein can be expected to be readily recognized as immunologically non-self and attacked against. Based on these previous studies and considerations, we hypothesized that there could be some genetic variation between recipient and donor outside HLA loci that could possibly lead to alloimmune reaction and thus have an impact on transplant outcome.

The aim of the present thesis is to identify novel non-HLA genomic risk factors that could be utilized to predict more accurately the outcome of kidney transplantation. The genome variation between kidney transplantation recipient and brain-dead donor was determined in a retrospective adult kidney transplantation cohort. The Finnish cohort included 1,025 kidney transplantation patient-donor pairs transplanted at the Helsinki University Hospital between 2007 and 2017. The association of the genome-wide compatibility with the transplantation outcomes were estimated using statistical methods. This thesis also utilized PRS estimates for the major causes of death of the graft donor in predicting the outcomes of kidney transplantation. In the future, with the help of genomic information, we may be able to create better tools to predict transplant complications and long-term survival, and to understand the mechanisms behind extended histocompatibility.

In the first part of the thesis, we studied the genetic variation of the patient-donor pairs at the level of the whole genome by examining missense variant mismatches in genes encoding for transmembrane, secretory, and kidney-related proteins. In addition, we studied common variants in a strong linkage disequilibrium with known gene deletions. The association between mismatches and acute rejection was estimated using statistical methods. The results showed that increasing mismatch sum of kidney-related genes between the recipient and the donor resulted in decreased rejection-free survival probability and indicated that the high overall alloantigenic load of the transplanted graft may increase the risk of rejection. Furthermore, we showed that a mismatch in a rs7542235

variant, earlier reported to tag gene deletions in the complement factor H related (*CFHR*) locus, was associated with time-to acute rejection.

The second part of the thesis confirmed that the allele G of genomic variant rs7542235 indeed tagged gene deletions in the *CFHR* locus and fine-mapped the boundaries of the *CFHR* gene deletion using whole genome sequencing (WGS) and multiplex ligation-dependent probe amplification (MLPA) methods. The effects of the *CFHR* deletions on the expression levels of other genes and proteins were also investigated. Both WGS and MLPA results showed that the deletions were heterogeneous in length, but all shared the deletion of the *CFHR1* gene. In addition, plasma proteomics studies showed that the deletion tagging allele was associated with changes in the expression levels of no less than 23 plasma proteins in healthy blood donors. As was expected, the variant was associated with different expression levels of complement-related proteins, but also genes like *PRLR* and *KIRREL1*, which take part in immune system regulation and kidney filtration, respectively. There was no given diagnosis associated with the gene deletion. These results indicate that the mechanism by which the *CFHR* gene deletion is associated with rejection can be more complex than merely the alloimmune reaction against the missing FHR-1 protein.

The third part of the thesis was carried out as part of a research consortium, which investigated 6,666 deceased and living kidney donors origin from seven different European kidney transplantation cohorts. Donor age of death and kidney transplant outcome was predicted using donors' PRSs to the risk factors of cerebrovascular diseases. It has been shown that grafts from deceased donors who died of spontaneous intracranial hemorrhage or stroke have worse outcomes than those from donors who died from other causes. The results showed that deceased donors had an increased polygenic burden for hypertension and intracranial aneurysm compared to living donors and healthy controls. In addition, donor IA polygenic burden was associated with donor age of death in those donors who died of stroke and - relevant to transplantation - was also associated with reduced graft function.

To conclude, the results of the three studies of this thesis have expanded our understanding of the concept of histocompatibility in kidney transplantation. The results indicate that level of incompatibility of genome variation in kidney-related genes and homozygous gene deletions could be utilized in the prediction of kidney transplantation outcomes.

Independent replication studies are needed before the clinical value can be estimated. In the future, addition of genetic information can be useful when creating better prediction tools for the complications of transplantations and long-term survival of the transplant.

TIIVISTELMÄ

Munuaissiirto on hyvä ja kustannustehokas hoitomuoto potilaille, joilla on loppuvaiheen munuaissairaus. Munuaisensiirtojen tarve kasvaa jatkuvasti väestön ikääntymisen ja länsimaille tyypillisten sairauksien, kuten diabeteksen ja sydän- ja verisuonitautien yleistymisen myötä. Suomessa tehdään vuosittain noin 250 munuaisensiirtoa. Huomioitavana tekijänä kaikissa kahden henkilön välisissä kudosten ja elinten siirroissa on alloimmuunireaktio, jossa vastaanottajan immuunijärjestelmä tunnistaa siirteen vieraaksi. Tämä johtaa usein hyljintäreaktioon siirrettä vastaan, ja noin 10–20 % munuaisensiirron saajista kärsii akuutista hyljintäreaktiosta, joka vaatii intensiivistä lääkitystä ja voi heikentää siirteen toimintaa ja potilaan elämänlaatua. Potilaan ja elimen luovuttajan välisen siirtoa edeltävän kudossopivuustestauksen tavoitteena on löytää jokaiselle elinsiirtoa odottavalle potilaalle immunologisesti sopivin siirre ja vähentää komplikaatioita. Munuaissiirrossa luovuttajan ja potilaan välinen kudossopivuus määritetään tällä hetkellä ABO-veriryhmän ja ihmisen leukosyyttiantigeenien (HLA) alleelien perusteella. Lisäksi ennen siirtoa suoritetaan ristikoetesti, jolla tutkitaan onko potilaalla luovuttajaan kohdistuvia leukosyyttivasta-aineita. Nykyisistä kudossopivuustestauksista huolimatta emme pysty ennustamaan siirtoihin liittyviä komplikaatioita, kuten hyljintää ja siirteen hidasta käynnistymistä, tai siirteen pitkäaikaista toimintaa riittävän hyvin.

Viime vuosikymmenen aikana on alettu tutkia HLA-geenien ulkopuolisia geneettisiä riskitekijöitä. Genominlaajuisissa assosiaatiotutkimuksissa (GWAS) on havaittu päätetapahtumiin liittyviä variantteja, mutta löydökset vaativat toistoja ja lisätutkimuksia varianttien toiminnallisuudesta. Genomista riskiä voidaan nykyään arvioida myös yhdistämällä GWAS-tulosten matalan tason vaikutukset polygeenisiksi riskisummiksi. GWAS:ien lisäksi toinen lähestymistapa on tarkastella genomitason yhteensopivuutta luovuttajan ja potilaan välillä. Transplantaatioon liittyy kahden genomin vuorovaikutus, jolla voi olla suurempi vaikutus siirron komplikaatioihin kuin luovuttajan tai vastaanottajan perimällä yksinään. Jokainen luovuttaja-vastaanottaja-pari sisältää joukon aminohappoeroja, jotka voivat mahdollisesti toimia alloimmuunivasteen laukaisijana. Aminohappoerojen lisäksi toinen kiinnostava lähestymistapa on etsiä geenideleetioeroja luovuttajan ja vastaanottajan välillä. Ajatuksena on, että jotkut deleetiot ovat niin yleisiä

populaatiossa, että yksilöt perivät ne molemmilta vanhemmiltaan, mikä johtaa proteiinia koodaavan geenin ja siten itse proteiinin täydelliseen puuttumiseen. Kun puuttuva proteiini tuodaan kehoon siirrettävässä elimessä, voidaan odottaa, että proteiini tunnistetaan helposti immunologisesti vieraaksi ja sitä vastaan hyökätään.

Tämän väitöskirjan tavoitteena oli löytää uusia HLA-geenien ulkopuolisia geneettisiä tekijöitä, joita voitaisiin hyödyntää munuaissiirtojen riskien ennustemalleissa. Suomalainen tutkimusaineisto koostui 1025 munuaissiirtoparista, joiden siirto tehtiin vuosina 2007–2017 HUSissa. Tarkoituksena oli tutkia munuaissiirteen saajan, eli potilaan, sekä aivokuolleen luovuttajan välistä perinnöllistä vaihtelua ja verrata sitä tilastollisin menetelmin akuutin hyljintäreaktion päätemuuttujaan. Lisäksi väitöskirjan tavoitteena oli tutkia luovuttajan aivoverenkiertohäiriöihin liittyvien polygeenisten riskisummien merkitystä siirroissa; miten riskisummat liittyvät luovuttajan kuolinikään ja munuaissiirteen toimintaan. Genomitiedon avulla voimme tulevaisuudessa luoda nykyistä parempia ennustetyökaluja siirtojen komplikaatioille sekä pitkäaikaiselle toiminnalle ja toisaalta ymmärtää kudossopivuuteen liittyviä mekanismeja entistä paremmin.

Väitöskirjan ensimmäisessä osatyössä tarkastelimme potilas-luovuttajaparin välisiä geneettisiä eroja koko genomin tasolla tutkimalla variantteja, jotka johtavat eri aminohapon koodaamiseen, transmembraanisissa, erittyvissä sekä munuaisiin liittyvissä proteiineissa. Lisäksi tutkimme variantteja, joiden tiedetään olevan vahvassa kytkentäepätasapainossa tunnettujen geenideleetioiden kanssa. Vertasimme geneettisiä eroja tilastollisin menetelmin akuuttiin hyljintäreaktioon. Tulokset osoittivat, että potilaan ja luovuttajan välisten erojen määrä munuaisiin liittyvissä proteiineissa voi altistaa potilaan hyljintäreaktiolle. Lisäksi osoitimme, että yhteensopimattomuus immuunipuolustukseen liittyvien komplementtitekijä H:n sukulaisproteiinien (complement factor H-related proteins, *CFHRs)* geenideleetiota osoittavassa variantissa, rs7542235, kytkeytyy akuuttiin hyljintäreaktioon.

Väitöskirjan toisessa osatyössä tutkimme tarkemmin ensimmäisessä osatyössä löydetyn *CFHR*-geenideleetion rakennetta koko genomin sekvensoinnin (WGS) sekä multiplex ligation-dependent probe amplification (MLPA)-tekniikan avulla. Lisäksi selvitimme kuinka deleetiohin linkittyvä variantti liittyy muiden geenien sekä proteiinien ilmentymiseen. Sekä

WGS- että MLPA-tulokset vahvistivat, että variantin rs7542235 GG-genotyyppi linkittyy homotsygoottiseen deleetioon *CFHR1*-geenissä. Plasman proteomiikkatulokset osoittivat, että G-alleeli vaikuttaa 23 proteiinin ekspressiotasoihin. Variantti oli odotettavasti yhteydessä komplementtitekijä H:n sekä sen sukulaisproteiinien ilmentymistasoihin, mutta myös mm. *PRLR* ja *KIRREL1* geenien ilmentymiseen, joista ensimmäinen on osana immuunijärjestelmän säätelyssä ja jälkimmäinen munuaisten suodatuksessa. Nämä tulokset osoittavat, että mekanismi, jolla *CFHR*-geenin deleetio liittyy hylkimiseen, voi olla monimutkaisempi kuin pelkkä alloimmuunireaktio puuttuvaa FHR-1-proteiinia vastaan.

Väitöskirjan kolmas osatyö on tehty osana suurempaa kansainvälistä tutkimusprojektia, joka koostui seitsemän eurooppalaisen tutkimuskohortin yhteensä 6666 munuaisen luovuttajasta. Tutkimuksessa ennustettiin luovuttajien kuolinikää sekä siirteen onnistumista luovuttajan GWAS-genomitiedosta luotujen aivoverenkiertohäiriöiden riskitekijöihin liittyvien polygeenisten riskisummien avulla. On osoitettu, että siirteillä, jotka tulevat aivoverenvuotoon tai aivohalvaukseen menehtyneiltä luovuttajilta on huonompi ennuste kuin siirteillä, jotka tulevat luovuttajilta jotka ovat kuolleet toisesta syystä. Tulokset osoittivat, että aivohalvaukseen kuolleilla luovuttajilla oli kohonnut aivoaneurysman riskisumma verrattuna eläviin luovuttajiin sekä terveisiin kontrolleihin, ja että aivokuolleilla luovuttajilla oli kohonnut aivohalvauksen riskisumma verrattuna eläviin luovuttajiin. Lisäksi aivohalvaukseen kuolleilla luovuttajilla oli kohonnut korkean verenpaineen riskisumma verrattuna terveisiin kontrolleihin. Korkean verenpaineen sekä aivoaneurysman riskisumma olivat yhteydessä alentuneeseen munuaissiirteen toimintaan.

Väitöskirjani kolmen osatyön tulokset laajentavat käsitystä kudossopivuudesta munuaisensiirrossa. Tulokset osoittavat, että munuaisiin liittyvien geenien genomivaihtelun ja homotsygoottisten geenideleetioiden yhteensovittamista voidaan käyttää munuaisensiirron lopputuloksen ennustamiseen. Löydösten kliininen merkitys voidaan kuitenkin arvioida vain jatkotutkimusten avulla. Genomitiedon monipuolinen hyödyntäminen voi edesauttaa ennustetyökalujen kehitystä elinsiirtojen komplikaatioihin ja elinsiirron pitkäaikaiseen onnistumiseen.

1 REVIEW OF THE LITERATURE

1.1 Kidney transplantation

When kidney function is lost because of end stage kidney disease (ESKD), kidney replacement therapy is needed. In these cases, either dialysis or kidney transplantation is considered. Kidney transplantation serves as a life-saving intervention for individuals facing the debilitating consequences of diseases affecting kidney function. The decision for transplantation is made following a thorough assessment of the individual's health, the progression of the underlying condition, and the potential benefits of the transplant in restoring kidney function and improving overall quality of life. The need for kidney transplantation may arise from kidney failure due to, for example, autoimmune or metabolic conditions, or inherited diseases. Type 2 diabetes has been the leading cause of end-stage kidney disease since 1999 in Finland, and glomerulonephritis and type 1 diabetes have been the next most common causes. [1]

As kidney transplantation improves both quality of life and life expectancy, and is the most cost-effective therapy for ESKD, it is the preferred modality of treatment, rather than dialysis [2]. Since the kidneys play a pivotal role in filtering waste products and maintaining fluid and electrolyte balance in the body, impaired kidney function typically leads to several health complications, particularly cardiovascular diseases.

Since the first human kidney transplantation by Joseph Murray in 1954 [3], improvements in morbidity and mortality have been attributed to advances in surgical technique, peri-operative management, immunosuppressive regimens, and histocompatibility of donor-recipient pairs. The first kidney transplantation in Finland was performed in 1964 [4], and in recent years the average annual number has been between 250 and 300 transplantations [5]. **Figure 1** illustrates the rate of kidney transplantations per million population in Europe and the UK in the year 2022.

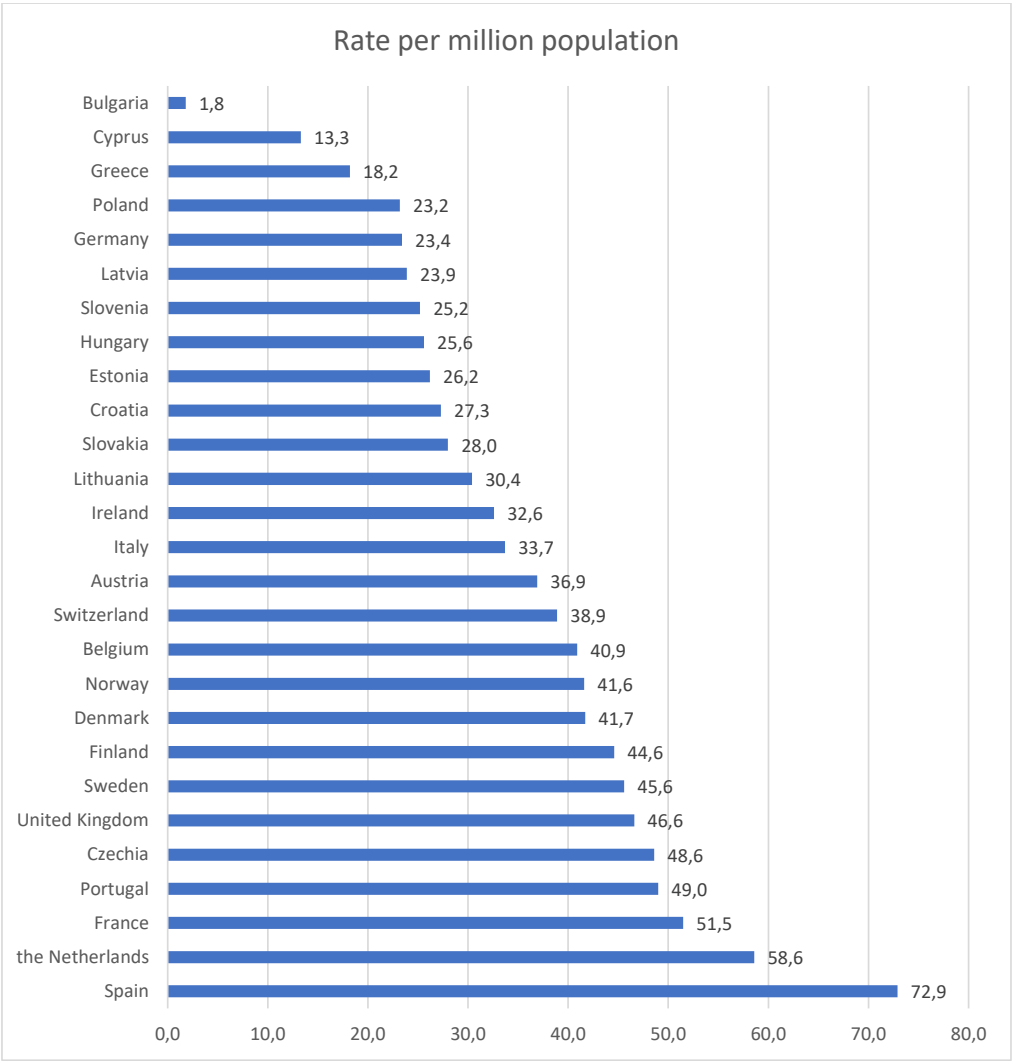

Figure 1. Rate of kidney transplant procedures per million population in Europe in 2022, by country. https://www.statista.com/statistics/537935/kidney-transplant-rate-in-europe/

The kidney transplantation procedure commences with a rigorous evaluation process to identify compatible donors and recipients, aiming to minimize the risk of rejection and ensure a successful outcome. Advances in medical science have expanded the options for donors, enabling both living and deceased individuals to contribute organs, thereby increasing the pool of available kidneys for transplantation. Kidney transplants are generally very successful at extending life for people who are eligible to undergo the transplantation. A recent study based on the Collaborative Transplant Study, including 108,787 recipients of

brain-dead kidney donors from 135 hospitals across 21 European countries showed that kidney graft survival has improved over time. According to the study, the 1- and 5-year death-censored graft survival rates were, 86.8% and 74.6% in patients transplanted between 1986 and 1995, respectively. The rates for patients transplanted between the years 1996 and 2005 were 91.1% and 82.5%, respectively, and 92.0% and 84.4% in patients transplanted between 2006 and 2015.[6]

The outcome of kidney transplantation is, however, influenced by several factors. For example, the age of donor and recipient can affect the success rate of transplantation, and in fact, a study including 853 kidney transplant recipients established that both donor and recipient age were risk factors for predicting of kidney transplantation outcome [7]. The study stated that transplantations between older adult donors and older adult recipients had the highest graft loss risk and mortality, and that the eGFR of the recipients from donors aged 70 to 89 years were significantly lower than those from younger donors. Another important factor is the cold ischemic time, meaning the time between the beginning of the cold perfusion of the donated kidney and restoring its blood supply. Several studies have shown that a shorter cold ischemic time is associated with higher rates of successful transplantation in deceased donation [8,9]. A recent meta-analysis of 164,179 living donor transplantations also showed a significantly lower incidence of delayed graft function, and significantly higher 1-year graft survival and 5-year graft survival, for cold ischemic time of less than four hours [10]. Based on these findings, it is important to keep the cold ischemic time as short as possible in both deceased donor and living donor transplantations.

One particularly important, and well-studied factor in predicting of transplant outcome is the eGFR [7,11–13], which is an estimate of function of the transplanted kidney, based on laboratory measurements of serum creatinine levels. A recent study including 139,363 kidney transplants showed that the durability of kidney graft survival after transplantation was associated with the amount of kidney function gained, as evaluated by eGFR [13]. Another study involving 754 transplant patients reported that eGFR three months after transplantation was a strong predictor of the long-term return to dialysis for kidney transplant patients [11].

Other factors for transplant outcome include donor type [14], donor cause of death [15,16], HLA mismatch [17], organ quality [18], and immunosuppressive treatment used after kidney

transplantation [19]. It is well established that organs from living donors generally have a better outcome compared to organs from deceased donors [14]. Organs from deceased donors who died of intracranial hemorrhage or stroke also have reported worse outcomes than those from donors who died from other causes, such as trauma. [15,16]. The reason for this is not fully understood, but brain injury and eventual brain death leads to pathophysiological changes, to impaired function and survival of transplanted organs [16]. Brain death is also usually more common in older people than among young, and an older age itself is a risk factor for worse transplant outcome [15]. The immunosuppressive medication used can also have an impact on long-term graft survival. A study including 1,645 kidney transplant recipients examined the effects of different immunosuppressive regimens to eGFR 12 months post-transplantation, acute rejection, and allograft survival. The results showed that treatment for 12 months with a regimen containing daclizumab, mycophenolate mofetil, corticosteroids, and low-dose tacrolimus provided sufficient immunosuppression with better kidney function and less acute rejection, when compared to daclizumab induction plus low dose cyclosporine or low dose sirolimus containing regimens or to a regimen containing standard dose cyclosporine without daclizumab induction. The results also showed that low dose tacrolimus provided better allograft survival than standard dose cyclosporine or low dose sirolimus. [20]

1.2 Conditions predisposing to end stage kidney disease

Kidney transplantation or dialysis becomes necessary when individuals face an end-stage kidney disease (ESKD) [21]. ESKD is the final, permanent stage of chronic kidney disease (CKD), where kidney function has declined to the point that kidney replacement therapy is needed. CKD is a progressive and often silent medical condition characterized by the gradual loss of kidney function over time. [22,23] In the early stages of CKD, individuals may remain asymptomatic, making early detection challenging. However, as the disease advances, impaired kidney function can lead to a range of complications, including fluid retention, electrolyte imbalances, hypertension, anemia, and cardiovascular issues. CKD may evolve from various underlying conditions, with diabetes and hypertension emerging as leading causes (**Figure 2**). Other diseases and factors include glomerulonephritis, polycystic kidneys,

infections, and genetic predisposition. As these provoke damage to the delicate structures of the kidneys, the organs gradually lose their ability to function.

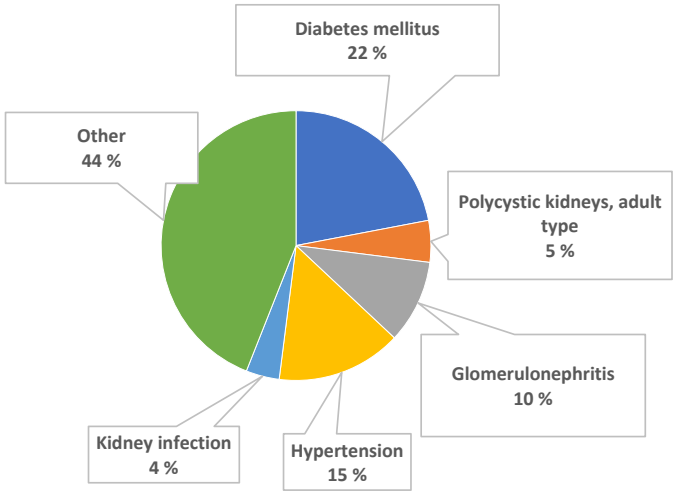

Figure 2. Primary kidney disease in patients accepted for kidney replacement therapy in 2021 according to The European Renal Association registry. https://www.era-online.org/wp-content/uploads/2023/12/ERA-Registry-Annual-Report-2021_231206.pdf

Diabetes mellitus

Diabetes mellitus, particularly type 1 and type 2, is a chronic metabolic disorder characterized by elevated blood glucose levels, and a leading cause of chronic kidney disease (CKD), known as diabetic nephropathy (DN) based on KDIGO [24]. DN is characterized by changes in the structure and function of the kidneys due to prolonged exposure to high levels of glucose, associated with diabetes, in the blood. DN does not develop in all individuals with diabetes, and progression is variable for those that do develop it. [25] The main risks are hypertension, glycemic control, obesity, smoking, and dyslipidemia, while the main unmodifiable risks are age, race, and genetic profile [26,27].

As the prevalence of diabetes continues to rise globally, the incidence of diabetes-related kidney disease has become a significant driver for dialysis treatment and for kidney transplantation.

Polycystic kidney disease

Polycystic kidney disease (PKD) is a genetic disorder that causes many fluid-filled cysts to grow in kidneys. Unlike the usually harmless simple degenerative cysts that can form in the kidneys later in life, PKD cysts grow gradually and typically change the shape of the kidneys, making them much larger. PKD reduces kidney function and typically eventually leads to kidney failure. Other complications, such as high blood pressure, liver cysts, and arterial aneurysms (especially cerebral arteries) are also often associated with PKD [28].

There are two known main types of PKD: autosomal dominant PKD (ADPKD), which is diagnosed in adulthood [29], and autosomal recessive PKD (ARPKD), which can be diagnosed already in the uterus or shortly after birth [30]. ADPKD is usually caused by mutations of either the *PKD1* gene on chromosome 16 or the *PKD2* gene on chromosome 4, and the gene responsible for ARPKD, *PKHD1*, has recently been identified on chromosome 6. [31]

Immunoglobulin A nephropathy (IgAN) and other glomerulonephritis

IgA nephropathy is the most common form of glomerulonephritis, caused by the deposition of IgA immunoglobulins in the glomerular basement membrane. The presence of IgA immune complexes leads to inflammation in the glomeruli, which can then cause damage to the kidney tissue. Immune-mediated damage to the glomerular basement membrane results in hematuria, the presence of blood in the urine (which can be either microscopic, meaning that the urine is normal in color, or macroscopic, meaning that there is visible blood in the urine), proteinuria, and renal insufficiency. [32] Clinical course of IgAN varies a lot between individuals, ranging from indolent intermittent hematuria to rapidly progressing kidney failure. The typical course is slowly declining kidney function leading eventually to ESKD. The exact cause of IgAN remains to be fully elucidated, but some genetic loci [33,34] and clinical risk factors [35] have been identified.

Additionally, several other chronic glomerulonephritis can lead to ESKD, primary or secondary to other disease conditions. Most common types include focal segmental glomerulosclerosis (FSGS) [36], membranous nephropathy (MN) [37], glomerular diseases

related complement disorders, e.g. Complement 3 glomerulopathy (C3G) [38], or glomerular lesions related to systemic lupus erythematosus [39].

1.2.1 Complement-mediated kidney diseases

Complement-mediated kidney diseases are rare, complex, and progressive diseases causing damage to the kidneys when a part of the immune system becomes overly active. This generates an inflammatory response that leads to kidney damage, resulting in proteinuria and impaired kidney function. [40] Complement-mediated kidney diseases include C3G [38], IgAN [32], atypical hemolytic uremic syndrome (aHUS) [41] and MN [37].

The role of complement is well established in aHUS. Mutations in complement factor H (*CFH*) gene are the most common cause of inherited complement-mediated aHUS, accounting for around 25% of cases [42]. FH protein is the most important fluid-phase regulator of the alternative pathway (AP) of complement. Most of the *CFH* mutations seen in aHUS occur in the C-terminal domains [42], which mediates FH self-surface binding via its interaction with C3b, sialic acid, and glycosaminoglycans [43,44]. Anti-FH antibody associated aHUS is an important subset of aHUS. These patients develop autoantibodies that bind to the C-terminus of FH thus impairing the interaction of FH with C3b and thereby causing dysregulation and overactivity of the complement pathway. This compromised interaction of FH and C3b is a significant step in the pathogenesis of this disease because it disrupts the alternative pathway [45]. Anti-FH antibody associated aHUS can arise due to homozygous deletion of the complement factor H-related genes, *CFHR1* and *CFHR3*, with *CFHR1* being more importantly linked, along with the presence of anti-factor H antibodies. Studies have, however, shown that not all patients with *CFHR1* deletion will display anti-FH antibodies [46,47].

1.3 Transplantation genomics and histocompatibility

The current histocompatibility matching for transplantation in most centers consists of three major components. First, the blood group must be compatible between the donor and the recipient. This is measured by ABO blood group matching. Donors with blood group O are known as universal donors because they can donate a kidney to people with any blood group, and people with blood group AB are universal in that they can receive a kidney from a donor in any other blood group. Second, alleles of the HLA genes are matched between

patient and donor. HLA matching in kidney transplantation is usually determined by three genes: HLA-A, -B and -DRB1. However, here no strict identity between patient and donor is assumed but mismatches are tolerated, with emphasis on matched DRB1 alleles [48,49]. Patients having a suitable HLA and ABO match with the donor will proceed to the third step of the histocompatibility testing, which is the crossmatch test. The purpose of the crossmatch is to detect the presence of preformed anti HLA antibodies against the donor. The preformed anti-HLA antibodies binding to the HLA molecules in the transplant readily trigger hyperacute rejection leading to the graft loss.

1.3.1 Matching of HLA alleles

HLA molecules are encoded by genes of the major histocompatibility complex (MHC) located on chromosome 6p21.3. The *HLA* genes are highly polymorphic, showing both allelic and gene copy number variation [50]. Classical HLA molecules are divided into two classes of molecules: HLA I and HLA II. HLA class I molecules include HLA-A, -B and -C, and they are heterodimers formed by two subunits: the membrane-bound α chain encoded by the HLA gene and the non-polymorphic β2 microglobulin. They are expressed by all nucleated cells and by thrombocytes. HLA class II molecules include HLA-DR, -DQ and -DP, and they are heterodimers formed by two transmembrane chains, α and β (**Figure 3**). These molecules are expressed only by the professional antigen presenting cells (APC), particularly dendritic cells and macrophages. [51]

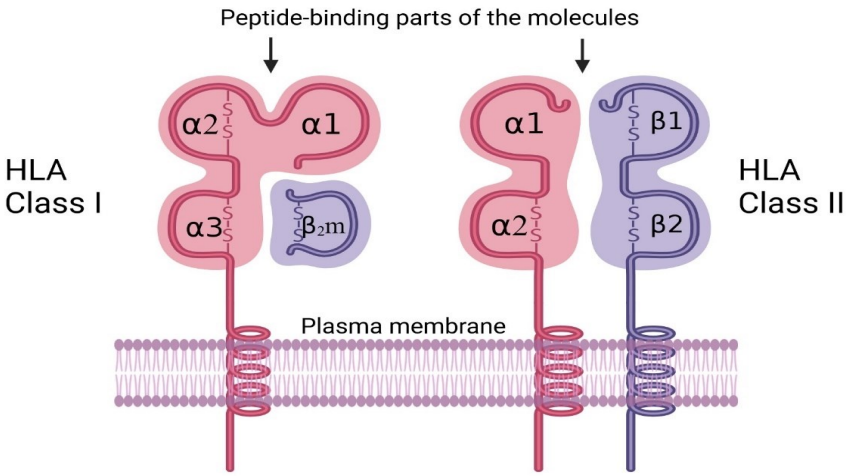

Figure 3. Structure of HLA molecules. The HLA class I molecule is a heterodimer consisting of the α chain and the non-polymorphic β2-microglobulin. The peptide-binding part of the HLA class I molecules is formed by α1 and α2 domains. The HLA class II molecule is a heterodimer consisting of α and β chains. The peptide-binding part of the HLA class II molecule is formed by α1 and β1 domains encoded by different HLA genes. HLA, human leukocyte antigen. Created with BioRender.com.

APCs express HLA-peptide complexes, which T-cells bind and recognize as self or non-self [51]. In general, the class I molecules bind and present to T cells peptides from endogenous sources, while class II molecules bind and present peptides from exogenous sources. On an uninfected cell surface, the HLA molecules bind hundreds of thousands of self-peptides.

The T cell receptor (TCR) recognizes the HLA molecule-peptide complex on the surface of APCs in the thymus. Immature double-positive T cells, expressing both CD4 cluster of differentiation and CD8, interact with their TCR, as well as CD4 and CD8 co-receptors to HLA molecules. If TCR binds to HLA class I, the T cell starts to downregulate CD4 receptors and upregulate CD8 receptors, and if TCR binds to HLA class II, the T cell starts to downregulate CD8 and upregulate CD4 (**Figure 4**). [52,53]

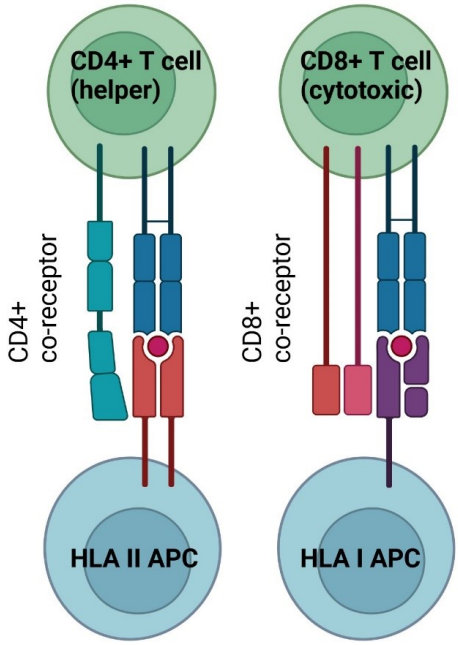

Figure 4. HLA I and HLA II and their role as mediators during antigen presentation and recognition. Antigen presented via HLA I can be recognized by the TCR and the CD8 co-receptor which is expressed by primarily cytotoxic T cells while antigen presented via HLA II is recognized by the TCR and the CD4 co-receptor which is mainly found on helper T cells. According to their co-receptors, T cells are classified as CD8 + or CD4 + T cells. The red dots between HLAs and TCRs (blue) represent peptides. Created with BioRender.com. APC, antigen-presenting cell; HLA, human leukocyte antigen; TCR, T cell receptor.

Development and mechanisms of immunological tolerance are crucial for understanding alloimmune response against non-self-antigenic structures. The central tolerance is formed in the thymus wherein the developing T-cells travel from the bone marrow where they are born. Creation of T-cell and B-cell antigen receptors include many unique and complex steps. The final T cell antigen receptor (TCR) unique to each T cell clone is formed of genomic rearrangements of DNA fragments. Initially each T cell clone produces by gene rearrangement a unique TCR. During T cell maturation in the thymic cortex, T cell clones encounter surface HLA molecules bound with extensive variety of self-peptides, peptides produced by our own genes that are widely expressed in the thymus. In the thymus T cells

are selected at two steps against autoimmune reactions. In the positive selection step only those T cell clones that are able to recognize weakly by their TCR the self-HLA molecules will survive. Second, in the negative selection step taking place in the thymic medulla, T cells with TCRs strong binding to self-peptides presenting HLA molecules undergo apoptosis. All the other T cell clones are released to the periphery where they screen the HLA plus peptide complexes on the cell surfaces. T cell clone that recognizes an HLA plus peptide complex strongly enough in the periphery becomes activated, proliferate, and elicit an immune response against the peptide. In transplantation the recipient T cells encounter both non-self HLA alleles and peptides in the graft. [54]

One HLA allele can bind thousands of different peptides if they all share certain critical amino acids at particular positions. The amino acids that form the pockets of the peptide binding part of the molecule and thus determine the peptides able to bind therein, show high genetic variation between HLA alleles. For that reason, different HLA alleles bind and present to the immune system different sets of peptides. [55]

There are two main forms of allorecognition, the ability of an individual to distinguish its own tissues from those of another, direct and indirect [56]. In direct allorecognition, the donor-derived HLA-peptide complexes presented by donor's APCs are recognized directly by recipient's cytotoxic CD8 T cells, resulting in response against the cells expressing the foreign molecule complex. This response can be formed against the allopeptide presented by the foreign HLA, or the foreign HLA molecule itself [57]. In indirect allorecognition, the allopeptides from the transplant are presented by the recipient's own antigen presenting CD4 helper T cells [58].

HLA plays a major role for graft rejection and long-term outcome in kidney transplantation. The matching of HLA alleles between the donor and recipient decreases immune activation against the graft. Also, the antibodies against the HLA molecules of the graft are harmful for the long-term graft survival. [59]

HLA-A, -B, and DRB1 are considered as the most important genes in kidney transplantation. Large registry reports including analysis from Collaborative Transplant Study (CTS) have demonstrated a strong association between HLA-matching at the *HLA-A, -B* and *-DBR1* loci and graft and patient outcomes, independent of donor type, initial immunosuppression,

transplant era and even the presence of donor specific antigens. [59,60] Logically the fewer the HLA mismatches, the more successful transplant outcome. The initial CTS analysis reported that HLA-DR and HLA-B antigens contribute the most for the alloimmune burden [61]. Eurotransplant and United Kingdom transplant data suggest that *HLA-DR* matching has a far greater effect than those of *HLA-A* or *HLA-B* matching [62,63]. The United Network for Organ Sharing registry further highlighted the significance of paying attention to having the least number of mismatches possible, independent of the locus [64]. They evaluated the risk of transplant failure with HLA mismatch in patients who had their first adult kidney allografts from deceased donors. The study revealed that having six HLA mismatches translated to a 64% higher risk when compared to risk of 13% when having just one HLA mismatch.

1.3.2 non-HLA genes in kidney transplantation

The highly polymorphic HLA system has been considered the main target for alloimmunity, but the non-HLA antibodies have gained a lot of importance in kidney transplantation for the past years. Although studies of completely HLA-matched grafts between siblings have been shown to have superior graft outcomes compared with grafts with at least one HLA-mismatch, a proportion remains complicated by acute rejection, possibly reflecting potential allorecognition of incompatibilities outside the HLA loci. [65,66]

Recent transplant genetic studies [67–70] have proposed gene regions outside of the HLA region that may act as genetic modifiers for transplant outcomes. These so-called minor histocompatibility antigens (mHAs) may be important regions of interest to examine further to improve transplant outcomes. Variants in non-HLA regions could affect outcomes depending on their presence in donor organs or recipients or their presence as mismatches between given donor-recipient pairs. Hypothesis-based, targeted analyses have primarily identified SNPs that have been associated with predefined phenotypes (eg, acute allograft rejection, graft survival) [71,72]. Recent GWASs have reported novel non-HLA loci associated with graft outcomes [73–75]. Of note, only one of these studies included both donor and recipient variation [74] whereas the other two included only kidney transplant recipients. Besides single nucleotide polymorphisms (SNPs), the relevance of interindividual non-HLA variations from copy number variants (CNVs) that can span exons or entire genes has been previously reported in bone marrow transplantation [76] and in kidney transplantation [69].

Table 1 and following chapters summarize some of the most utilized methods to study the genetics of kidney transplantation.

Table 1. Methods to study the genetics.

Method	Description
Targeted-gene approach	Screening of pre-selected subset of genes
Genotyping	Rapid screen of entire genome using array of common variant SNPs
Genome-wide association study (GWAS)	Screen of entire genome using genotyping array and imputation tool to look for associations between the variants and disease/non-disease outcomes
Polygenic risk score (PRS)	Derived from GWAS by summing the minor effects of several common variants to generate an overall estimate of risk
Whole genome sequencing (WGS)	Simultaneous screen of all coding and noncoding DNA in an individual
Whole exome sequencing (WES)	Simultaneous screen of all exons (i.e. coding sequence) in and individual
Quantitative trait loci (QTL)	
Expression quantitative trait loci (eQTL) analysis	Seeks to identify genetic variants that affect the expression of one or more genes
Protein quantitative trait loci (pQTL)	Seeks to identify genetic variants that affect the expression of one or more proteins

eQTL, expression quantitative trait loci; GWAS, genome-wide association study; PRS, polygenic risk score; pQTL, protein quantitative trait loci; SNP, single nucleotide polymorphism; WES, whole exome sequencing; WGS, whole genome sequencing; QTL, quantitative trait loci.

1.3.2.1 Candidate gene analyses

Several targeted-gene analyses studies in kidney donors and recipients have been performed during the past years. The candidate gene approach is one of the first approaches to test causal genes behind different clinical endpoints, directly testing the effects of genetic variants of potentially contributing gene. Studies including donors have, however, been limited as donor DNA is often unavailable for retrospective analysis. Studies including recipients have been more successful. **Table 2** summarizes the important data regarding recipient candidate genes and allograft outcomes. Many of the identified loci in targeted analyses are in genes with an immunomodulatory role, or in proteins involved in drug metabolism pathways.

Table 2. Recipient candidate genes and allograft outcomes.

Gene/SNP	Clinical outcome	Number of recipients	Reference
ITGB3	Acute rejection	119	Salido *et al.,* 1999 [77]
TNFA	Rejection episodes, rejection severity, steroid responsiveness	100	Sankaran *et al.,* 1999 [78]
CCR2, CCR5	Acute rejection	163	Abdi *et al.,* 2002 [79]
VEGFA	Acute rejection	173	Shahbazi *et al*., 2002 [80]
F2, F5, MTHFR	Acute rejection	165	Heidenreich *et al.,* 2003 [81]
IL2	Acute rejection	63	Morgun *et al.,* 2003 [82]
IL10, TGFB, TNF	Acute rejection	291	Alakulppi *et al.,* 2004 [83]
FCGR2A	Acute rejection	99	Yuan *et al.,* 2004 [84]
INFG, TGFB	Acute rejection	118	Tinckam *et al.,* 2005 [85]
CYP3A	Tacrolimus dose	80	Thervet *et al.,* 2003 [86]
CCL5	Acute rejection	261	Krüger et al., 2007 [87]
ABCB1, IL10, IMPDH2, TNF	Acute rejection	237	Grinyó *et al.,* 2008 [72]
CCL2	Acute rejection	167	Kang *et al.* 2008 [88]
IL1B, TNF	Acute rejection	100	Manchanda *et al*., 2008 [89]
CYP3A5	Acute rejection	136	Quteineh *et al*., 2008 [90]
IMPDH1	Acute rejection	191	Wang *et al*., 2008 [91]
ACE, AT1R	Acute rejection	206	Zhang *et al.,* 2008 [92]
ATR, CHEK2, DUSP13, PRDM1, IL15RA	Acute rejection	990	Israni *et al.*, 2010 [93]
IL3	Acute rejection	330	Lee *et al*., 2010 [94]
STAT4	Acute rejection	453	Yang *et al*., 2011 [95]
CXCL8	Acute rejection	296	Singh *et al.,* 2009 [96]
IFNG	Acute rejection, chronic allograft nephropathy	74	Crispim *et al.,* 2010 [97]
UGT1A9	Acute rejection	100	Pazik *et al*., 2011 [98]
CTLA4	Acute rejection	167	Gao *et al.,* 2012 [99]
APOL1	Long-term allograft outcomes	119	Lee *et al.,* 2012 [100]
FOXP3	Acute rejection, death-censored allograft loss	599	Engela *et al.,* 2013 [101]
AIF, PTGS2	Acute rejection	458	Vu *et al.,* 2013 [102]
TP53	Acute rejection	100	Azarpira *et al*., 2014 [103]
CYP2E1	Acute rejection	347	Kim *et al*., 2014 [104]
EPHX2	Acute rejection	259	Gervasini *et al.,* 2015 [105]

MBL2	Acute rejection	710	Golshayan *et al.,* 2016
F0XP3, NFKB1	Acute rejection	292	Misra *et al.,* 2016 [106]
CD46	Acute rejection	334	Park *et al*., 2016 [107]
LIMS1	TCMR, ABMR, allograft survival	841	Caliskan *et al*., 2021 [108]
APOL1	TCMR, death-censored allograft loss	507	Zhang *et al*., 2021 [109]

ABCB1, ATP Binding Cassette Subfamily B Member 1; ACE, angiotensin I converting enzyme; AIF, apoptosis-inducing factor; ATR, serine/threonine kinase; AT1R, angiotensin II type 1 receptor; APOL1, apolipoprotein L1; CCL5, chemokine ligand 5; CCL2, chemokine ligand 2; CCR2, C-C chemokine receptor type 2; CCR5, C-C chemokine receptor type 5; CD46, complement regulatory protein; CHEK2, checkpoint kinase 2; CTLA4, cytotoxic T-lymphocyte associated protein 4; CYP2E1, cytochrome P450 family 2 subfamily E member 1; CYP3A, cytochrome P450 family 3 subfamily A; CXCL8, interleukine 8; DUSP13, dual specificity phosphatase 13B; EPHX2, epoxide hydrolase 2; F2, coagulation factor 2; F5, coagulation factor 5; FCGR2A, Fc Gamma Receptor IIa; FOXP3, forkhead box P3; DUSP13, Dual Specificity Phosphatase 13; IFNG, interferon gamma; IL2, interleukine 2; IL3, interleukine 3; IL10, interleukine 10; IL15RA, Interleukin 15 receptor, alpha subunit; IL1B, interleukine 1beta; IMPDH1, inosine monophosphate dehydrogenase 1; IMPDH1, inosine monophosphate dehydrogenase 2; INFG, interferon gamma; ITGB3, Integrin Subunit Beta 3; LIMS1, LIM and senescent cell antigen-like-containing domain protein 1; MBL2, mannan-binding lectin; MTHFR, methylenetetrahydrofolate reductase; NFKB1, nuclear factor kappa B subunit 1; PRDM1, PR/SET Domain 1; PTGS2, prostaglandin-endoperoxide synthase 2; STAT4, signal transducer and activator of transcription 4; TGFB, transforming growth factor beta; TNF, tumor necrosis factor; TNFA, tumor necrosis factor alpha or TNF-α; TP53, tumor protein 53; UGT1A9, UDP-glucuronosyltransferase 1-9; VEGFA, Vascular endothelial growth factor A

Candidate gene studies can be performed relatively quickly and inexpensively and may allow identification of genes with small effects. However, the candidate gene approach is limited by how much is known of the biology of the disease being investigated.

1.3.2.2 Genome-wide association studies

Unlike candidate gene studies that focus on studying targeted genes, GWAS represents an increasingly popular approach to identify genetic variants among the entire genome associated with human diseases. The approach enables the analysis of millions of SNPs scattered across the genome.

Table 3. Genome-wide association studies in the field of kidney transplantations.

Associated gene/SNP	Clinical outcome	Sample size and cohort type	Reference
TRA, ZNF516	Long-term graft function	326 discovery	O'Brien *et al*., 2013 [75]
ATP5F1P6	New-onset diabetes after transplantation	256 discovery 441 replication	McCaughan *et al*., 2014 [110]
LINC00882, CACNA1D, CSMD1	Post-transplant cancer	388 discovery	Sanders *et al.,* 2015 [111]
CYP3A5, ZSCAN25	Tacrolimus trough	197 discovery, 160 replication	Oetting *et al.,* 2016 [112]
PTPRO, CCDC67	Acute rejection	4127 discovery, 2765 replication	Ghisdal *et al.,* 2017 [73]
No association	Death-censored graft loss	1638 discovery	Pihlstrom *et al.* 2017 [113]
No association outside HLA loci	Long- and short-term allograft survival	2094 discovery, 5866 replication	Hernandez-Fuentes *et al.,* 2017 [74]
APOL1	Kidney allograft survival	532 discovery	Divers *et al.,* 2020 [114]

ATP5F1P6, ATP Synthase, H+ Transporting, Mitochondrial Fo Complex, Subunit B1 Pseudogene 6; CACNA1D, Calcium Voltage-Gated Channel Subunit Alpha1 D; CCDC67, Coiled-Coil Domain-Containing Protein 67; CSMD1, CUB And Sushi Multiple Domains 1; CYP3A5, cytochrome P450; LIMS1, LIM and senescent cell antigen-like-containing domain protein 1; LINC00882, Long Intergenic Non-Protein Coding RNA 882, PTPRO, Protein Tyrosine Phosphatase Receptor Type O; TRA, T-cell receptor alpha; ZNF516, Zinc Finger Protein 516; ZSCAN25, Zinc Finger And SCAN Domain Containing 25

Table 3 summarizes the recent GWASs performed in the field of kidney transplantation. The first recipient-only GWAS by O'Brien *et al.* (2013) included 326 European transplant recipients and the endpoint was kidney allograft survival [75]. The study hypothesized that genetic variation in kidney transplant recipients contributed to post-transplant kidney function as measured by serum creatinine level at five years post-transplantation. The study identified two variants showing borderline genome-wide significance: *TRA* and *ZNF516*, encoding for proteins for the T-cell receptor alpha chains and intronic variant of zinc finger protein 516, respectively. The SNPs individually explained variance in creatinine levels and were predictors of long-term allograft function. This finding was not, however, confirmed by a subsequent validation by Pihlström *et al.* (2017) [113].

In a GWAS of 256 adult kidney transplant recipients by McCaughan *et al.* (2014), in total of 26 SNPs were associated with new-onset diabetes after transplantation (NODAT) [110]. In a study of 357 African-American kidney transplant recipients by Oetting *et al.* (2016), three allelic variants of *CYP3A5* encoding for cytochrome P450 family 3 subfamily A member 5,

were found to explain a great proportion of the variability in the trough levels, that is, the lowest level of the drug in the patient's body, of tacrolimus [112].

In 2017, Ghisdal *et al.* used a DNA pooling approach in a recipient-only GWAS to analyze 275 European cases of T-cell-mediated rejection and an additional 503 controls. [73] In their replication cohort, two loci remained significantly associated with acute rejection in both univariate and multivariate analysis. One of the loci encompassed the *PTPRO* gene coding for a receptor-type tyrosine kinase essential for B cell receptor signaling, the other involving ciliary gene *CCDC67,* essential in the functions of the immune synapse and primary cilium. These findings could not be replicated in external cohorts, however [115]. Hernandez-Fuentes *et al.* (2017) reported the largest genome-wide association study to date, involving 2094 kidney transplant recipient/donor pairs and replication of 5866 pairs; no strong donor or recipient genetic effects were found to contribute to long- or short-term allograft survival outside the HLA region [74].

In addition to recipient's genetic background, donor's genetic background and its association to kidney transplant outcome has also been studied. For example, it is known that *APOL1* risk genotypes can increase the chance of kidney disease among people who are of Western and Central African ancestry [114,116]. Several retrospective studies have reported that donor *APOL1* risk genotypes were associated with worse allograft outcomes [117–119], whereas recipient risk genotypes for the same gene were not correlated with graft survival [120]. Moreover, a retrospective study recently showed a rapid decline of eGFR and increased risk of ESKD among donors with the risk genotype, and raised concerns about using grafts from *APOL1* risk genotype carriers [121].

Even though GWAS has proven its value for researching various multifactorial traits, its application in studies of transplant outcomes requires further study, mainly due to the lack of powered cohorts with genome-wide genotype or sequence data.

1.3.2.3 Polygenic risk scores

Common disease risks are polygenic in nature [122,123]. Polygenic risk score (PRS) is a way of utilizing the existing GWAS findings to determine summary-level disease risks [124]. The purpose is to summarize the individual genotype findings into a single variable that produces an individual-level risk score for genetic liability. The PRS approach has been

introduced for a variety of traits, including heart disease [124,125], diabetes [124,126], hypertension [127], obesity [128], and cancers [129,130]. For example, a study by Khera et al. (2018) [124] developed and validated PRS for five common diseases. Their approach identified the relative percentage of the population at greater than three-fold increased risk for coronary artery disease (8.0%), atrial fibrillation (6.1%), type 2 diabetes (3.5%), inflammatory bowel disease (3.2%), and breast cancer (1.5%).

PRSs have also been utilized in outcomes, such as CKD [131,132] and eGFR measuring kidney function [133]. The 2017 study by Gorski *et al.* [133] used summary statistics from their GWAS of eGFR to create a PRS which was then tested against eGFR in an independent cohort of 1017 individuals. They found the PRS explained 2.2% of the trait variance compared to 1.3% when just considering genome-wide significant loci. Khan *et al.* (2022) [132] combined APOL1 risk genotypes with GWAS data of kidney function, and designed, optimized and validated a PRS for CKD. The PRS was then tested in 15 independent cohorts, including 3 cohorts of European ancestry (n = 97,050), 6 cohorts of African ancestry (n = 14,544), 4 cohorts of Asian ancestry (n = 8,625), and 2 admixed Latinx cohorts (n = 3,625). The top 2% of the PRS was associated with a nearly threefold increased risk of CKD across ancestries.

With the availability of large-scale GWAS for kidney function, PRS may become a useful tool for prediction of kidney transplantation outcomes [134]. PRS application could be of potential value when predicting long-term allograft function and other risks after transplantation. To date, a relatively low number of PRS studies have been performed in the field of kidney transplantation (**Table 4**).

Table 4. PRS studies in the field of kidney transplantations.

PRS trait	Clinical outcome	Sample size and cohort type	Reference
Non-transplant NMSC, SCC, and BCC	Time to post-transplant NMSC	899 kidney recipients	Stapleton *et al*., 2018 [135]
Non-transplant eGFR	1-year and 5-year after transplantation, and Δ (change between 1 and 5 year) eGFR as measures of kidney function	10,844 kidney recipients and donors	Stapleton *et al*., 2019 [67]
Non-transplant BCC and SCC	Post-transplant BCC and SCC	1,272 kidney recipients	Seviiri *et al.,* 2021 [136]
Non-transplant type 2 diabetes	New-onset diabetes after transplantation	2,062 kidney recipients and 533 donors; 1,581 liver recipients and 1,555 donors	Shaked *et al*., 2022 [137]

BCC, basal cell carcinoma; eGFR, estimated glomerular filtration rate; NMSC, non-melanoma skin cancer; SCC, squamous cell carcinoma.

One of the first PRS studies in the field of kidney transplantations was performed by Stapleton *et al*. in 2019 [135]. They analyzed the impact of PRS on transplant eGFR in a cohort of 10,844 donor-recipient pairs, calculated using genetic variants associated with non-transplant eGFR [67]. In this study, PRS was defined as the sum of the alleles associated with a given trait weighted by the effect size of that allele as determined by a previous GWAS. Risk alleles reaching P value of $<1 \times 10^{-4}$ were included in the calculations. This risk score was applied on both donors and recipients to predict allograft function at 1 and 5 years and change in eGFR post-transplant. They discovered that PRS calculated using the recipient's genotype alone was 1significantly associated with eGFR at 1-year post-transplant.

In another study, Stapleton *et al.* examined the risk of non-melanoma skin cancer (NMSC) among 889 European ancestry kidney transplant recipients [135]. They calculated PRS from GWAS of NMSC, and genetic variants reaching pre-defined P value thresholds were chosen from previously published squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) non-transplant GWAS. Using these GWASs, calculated PRSs for BCC and SCC were shown to be predictive of case-control status and time to NMSC post-transplant, respectively.

In 2021 Seviiri *et al.* generated PRSs from the general non-transplant population and used those to predict and stratify the risks of BCC and SCC in an independent population of 1,272 kidney transplant recipients. Kidney transplant recipients in the highest genetic risk stratum

(top 20% of the PRSs), exhibited a greatly elevated BCC risk compared with those in the lowest genetic risk group (bottom 20%). The study reported that the lowest genetic risk group, while being immunosuppressed after organ transplantation, had a BCC risk profile similar to that of the non-transplant general population in the UK Biobank. [136]

A study by Shaked and colleagues investigated the association of post-transplant diabetes mellitus (PTDM) using type 2 diabetes (T2D) derived PRS in 1,581 liver transplantation recipients and their 1,555 donors, and 2,062 kidney transplantation recipients and their 533 donors [137]. They examined whether recipient genomics contributed to PTDM development regardless of the specific organ transplanted. The results demonstrated that, irrespective of the type of solid organ transplanted, recipient T2D PRS is a good predictor of PTDM. Multivariate analyses also confirmed that recipient T2D PRS, with adjustment of known prognostic clinical variables, is an independent contributor to PTDM risk in both liver and kidney transplants.

The PRS studies have not yet resulted in a clinically meaningful prediction, however, this approach is becoming even more powerful as the methods to model PRS are improving and larger GWAS studies for chronic kidney disease and other kidney transplantation-related endpoints continue to grow [138].

1.3.2.4 Gene and protein expression studies

Although GWASs are not usually directly informative with respect to disease mechanisms since many associated variants are in intergenic regions, the effect sizes of the variants are small, and the associations might not be linked to direct biological relevance to disease, the functional studies of genetic variants might reveal novel targets for therapeutic intervention. Expression quantitative trait loci (eQTL) are genetic variants associated with changes in gene expression and are identified by linking variations in transcript levels with variations in genotypes. Utilizing public resources, such as the Genotype-Tissue Expression (GTEx) Consortium (https://gtexportal.org/home/)[139], eQTL analysis could be applied to hundreds of individuals with dozens of tissues retrieved by the expression levels of given SNPs [140]. For example, Pineda et al. [68] studied the impact of the 123 antibody-mediated rejection-associated mismatched variants on gene expression using GTEx. They hypothesized that a mutation in the gene would result in different mRNA expression in the

same gene (cis) or at another locus (trans), which would then produce a change in the expression of a protein in the donor kidney, and, consequently triggering an antibody response in the recipient, and drive kidney allograft rejection and injury. The study found eighteen differentially expressed genes associated with rejection to be biologically relevant: *AP3D1, CDC123, CDYL2, CSMD3, FAM129B, MUC3A, MYOM2, OR51F1, OR8G1, OR8G5, PNPLA6, PSEN2, RASA3, ZNF280D, AIM1L, CHRNA10, KIAA1755,* and the *SLC*-family. Fifteen are related to antibody-mediated rejection and three to T-cell mediated rejection after kidney transplantation.

Transcriptomic profiling has emerged over the past decade as a powerful approach for revealing unbiased biological information useful for posttransplant management. The largest high-throughput transcriptomic analysis to date identified differential pretransplant transcriptional profiles between kidneys with low and high function at 24 months, providing a deeper insight into the early biological processes leading to graft dysfunction. This study employed 174 deceased donor pretransplant biopsies to assess the utility of pretransplant gene expression profiles in predicting 24-month outcomes after kidney transplantation. Almost 600 differentially expressed genes were associated with 24-month graft function. The gene expression for a subset of candidate genes was then measured in an independent set of 96 pretransplant biopsies, and a risk calculator was created using thirteen genes with three donor characteristics (age, race, body mass index). Results showed that grafts progressing to low function at 24 months exhibited upregulated immune responses and downregulated metabolic processes at pretransplantation. [141]

MicroRNAs (miRNAs) are 22 nucleotides short, non-coding, and tissue-specific single-stranded RNA which regulate target gene expression. To date, several studies have confirmed that different miRNAs play a key role in kidney pathophysiology [142–145]. Moreover, since these molecules are stable and can be easily detected in body fluids, such as blood and urine, they are even more interesting and promising as potential prognostic and diagnostic markers for non-invasive monitoring of kidney, and various other diseases [146,147].

A number of miRNAs and messenger RNA (mRNA) profiles in urine and peripheral blood cells are associated with acute kidney allograft rejection [147]. One of the first studies of kidney allograft biopsies with acute rejection found a strong association between intra-graft expression of miRNAs and mRNAs. Acute rejection and kidney allograft function can be

predicted with a high degree of precision using intra-graft levels of miRNAs. The expression analyses of miRNAs showed that immune regulators miR-142-5p, miR-155, and miR-223 are overexpressed not only in biopsies from patients with acute rejection but also in their peripheral blood mononuclear cells. [148]. In another study, mRNA and miRNA expression was profiled in allograft biopsies with and without acute rejection. For acute rejection, specific dysregulations of both miRNAs and mRNAs were identified: 1035 mRNAs that correlated with nine relevant miRNAs and were regulated by p53 and FoxP3. There is a highly regulated interplay between specific mRNA/miRNAs in allograft rejection, which influences immune-mediated injury during acute rejection. [149]

In addition to transcription studies, proteomic analysis of cells, tissues, and body fluids has generated valuable insights into the complex processes influencing human biology. Proteins represent intermediate phenotypes for disease and provide insight into how genetic and non-genetic risk factors are linked to clinical outcomes. Associations between protein levels and DNA sequence variants that colocalize with risk alleles for common diseases can expose disease-associated pathways, revealing novel drug targets and translational biomarkers [150].

1.3.2.5 Genome-wide mismatch studies

Based on the assumption that mHAs are products of allograft-expressed genes with nonsynonymous SNPs (nsSNP), mismatches in nsSNPs between donors and recipients have been hypothesized to increase the risk of graft rejection and failure [151]. mHAs are defined as any non-HLA encoded gene product that can stimulate alloreactive T cells and trigger an immune response when transplanted into another individual. [151] Martin and colleagues [152] were one of the first to use genome-wide SNP arrays to predict amino acid differences between hematopoietic stem cell transplantation (HSCT) donors and recipients based on 19 104 coding single nucleotide variants. In HLA-matched sibling transplants, mismatches in coding SNPs were associated with an increased risk of severe and acute graft-versus-host disease.

Several studies have evaluated the non-HLA mismatches between kidney donor and recipient using genome-wide analysis (**Table 5**). The study by Mesnard et al. in 2016 [155] was one of the first to analyze the importance of non-HLA donor-recipient mismatch in a cohort of 53 kidney transplantation donor-recipient pairs. They performed a whole exome

sequencing (WES) for kidney transplant recipients and their living donors and estimated all cell surface protein mismatches for each donor-recipient pair by calculating the number of amino acid mismatches in transmembrane proteins. The authors showed that their allogenomics mismatch score was predictive of long-term graft function, independent of *HLA-A, -B* and *-DRP1* matching.

Table 5. Donor-recipient mismatch studies in the field of kidney transplantations.

Mismatches studied (found gene)	Clinical outcome	Sample size and cohort type	Reference
Amino-acid mismatches in transmembrane proteins	Long-term allograft outcome measured by eGFR	53 donor-recipient pairs	Mesnard *et al*., 2016
All genome-wide mismatches	ABMR, TCMR	28 donor-recipient pairs	Pineda *et al*., 2017 [68]
nsSNPs mismatches in transmembrane and secretory proteins	Graft loss	477 donor-recipient pairs	Reindl-Schwaighofer *et al.,* 2019 [70]
Mismatches in genome-shared identity-by-descent SNPs	Death-censored allograft loss	385 donor-recipient pairs	Zhang *et al.,* 2020 [153]
50 deletion-tagging SNPs (LIMS1)	Kidney allograft rejection	705 kidney transplant recipients and 2,004 donor-recipient pairs	Steers *et al*., 2019
Non-HLA mismatches at variant-, gene-, and genome-wide scales (LIMS1)	Death-censored graft loss	385 and 146 donor-recipient pairs	Sun *et al*., 2023 [154]

ABMR, antibody-mediated rejection; eGFR, estimated glomerular filtration rate; nsSNP, nonsynonymous single nucleotide polymorphism; TCMR, T-cell mediated rejection

Pineda and colleagues [68] tested the role of non-HLA donor-recipient mismatches in kidney graft rejection in a cohort of 28 pairs, using WES and gene expression data. They identified 123 non-HLA variants associated with antibody-mediated and T-cell mediated rejections and with no rejection at all. From the 123 variants, 94 were described to be biologically relevant for rejection and 15 among them were associated with the risk of post-transplant antibody-mediated rejection.

Reindl-Schwaighofer *et al*. (2019) [70] genotyped 477 kidney transplant recipients and their deceased donors, and evaluated genome-wide mismatches in nsSNPs. They analyzed 59,268 nsSNPs in transmembrane or secretory proteins. The degree of nsSNP mismatch, adjusted

for HLA eplet mismatch, was independently associated with graft loss. Furthermore, they utilized a peptide array, including self and non-self-peptides, to test the alloimmune response against the donor and to predict mismatched epitopes in a subset of 25 patients with chronic antibody-mediated rejection. They detected 16 non-HLA donor-specific antibodies against genetically mismatched peptides in patients with chronic antibody-mediated rejection. Hence, they were able to show that genome mismatches indeed resulted in alloimmune antibody response.

In a cohort of 385 donor-recipient pairs of multiethnic origins, Zhang *et al.* (2020) [153] analyzed the role of genetic differences between a donor and a recipient in graft histology and survival, using genome-wide SNP array data, and excluding the HLA region. They estimated the ancestry in each donor-recipient pair and proportion of genome-shared identity-by-descent (pIBD) between donor-recipient pairs. In donor-recipient pairs of similar ancestry, pIBD was significantly associated with allograft survival, independent of HLA mismatches. Moreover, pIBD was significantly associated with early vascular intimal fibrosis, which was an independent predictor of graft survival.

In addition to studying mismatches at SNP level, one interesting approach is to study gene deletions and their associations to kidney transplantation outcomes. The hypothesis is that some gene deletions are so common in population that individuals inherit both deleted alleles from their parents, thus lacking the functional gene and the protein product itself. When an individual lacking the gene receives a graft from a donor who carries at least one functional copy of that gene, the recipient's immune system recognizes the protein as foreign, which may lead to allograft rejection. A relatively recent study by Steers *et al*. (2019) tested this hypothesis in a discovery cohort of 705 kidney transplant recipients [69], and in the 'collision model' of 2004 donor-recipient pairs of a replication cohort. The collision model was defined as the recipient who was homozygous for a deletion-tagging allele received a graft from a donor carrying at least one reference allele. They examined the associations of 50 SNP alleles that were known to be in a strong linkage disequilibrium (LD) with a known deletion, shown to tag the deletion. They found that rs893403 genotype G on a chromosome 2q12.3 locus, tagging a deletion of *LIMS1* gene, was associated with rejection independently of the HLA mismatch and other clinical factors in both the recipient only and the collision models.

The most recent study by Sun *et al.* (2023) [154] discovered in two cohorts of 385 and 146 donor-recipient pairs that mismatches in the LIM Zinc Finger Domain Containing 1 *(LIMS1)* gene were associated with death-censored graft loss (DCGL). After first confirming that donor-recipient differences resulting from SNP mismatches associate with DCGL, they searched for the mismatches across all annotated gene loci to identify individual gene-level mismatches that significantly associated with an increased risk of graft loss. The screening confirmed *LIMS1* as a top-ranked gene associated with DCGL, independent of genome-wide mismatches. They further screened SNP-wise donor-recipient mismatches, and identified 30 SNPs in high LD, distinct from the previously reported *LIMS1* deletion tagging rs893403 [69], which were significantly associated with increased risk of graft loss. They also performed eQTL analysis for both the previously reported rs893403 and the identified 30 SNPs and identified GRIP And Coiled-Coil Domain Containing 2 *(GCC2)*, a gene adjacent to *LIMS1*. *GCC2* promotes transforming growth factor beta (*TGFB1*) signaling in lymphocytes/epithelial cells by regulating the trafficking of mannose-6-phosphate receptors, which cleave the latency-associated peptide and activate *TGFB1*. It is currently unclear whether the primary factor is the *LIMS1* deletion or variation in the adjacent *GCC2* gene.

1.3.2.6 Pharmacogenetics

Pharmacogenetics studies how genetic variation at the individual level affects drug metabolisms, levels, or other responses. Immunosuppressive therapy could be optimized according to the patient genotype, thus ensuring maximum efficacy with minimal adverse effects. Advances in immunosuppression have been the cornerstone of long-term allograft survival [156]. Most transplant recipients are prescribed tacrolimus (TAC) or cyclosporine, both of which have target trough levels. These levels are ideally set at an optimal balance to avoid too much immunosuppression, which can lead to drug toxicity, increased cancer risk, and opportunistic infections, and too little immunosuppression, which may result in subclinical or acute rejection [157].

Pharmacogenetic differences in immunosuppression could explain the risks of transplantation complications in some immunologically well-matched patient-donor pairs. Studies have shown that indeed certain specific genetic variants alter metabolisms of many drugs [158,159].

Calcineurin inhibitors are one of the most broadly used classes of immunosuppressive medications in solid organ transplantation [160]. Among them, TAC is metabolized through the cytochrome P450 proteins encoded by *CYP3A4* and *CYP3A5* [161]. Studies have shown that there is high level of variability in TAC blood levels depending on individuals, and that a common loss-of-function variant in *CYP3A5*3* allele associates with TAC metabolism [162,163]. Additionally, two recently published GWASs [164,165] studied the genetic factors associated with dose-adjusted TAC levels in African American and European kidney transplant recipients. Both confirmed that *CYP3A5* was the main genetic locus controlling TAC metabolism.

1.4 Complications after kidney transplantation

There is a considerable risk of developing complications with kidney transplantation, despite good histocompatibility between recipients and donors, modern immunosuppression, and surgical skills. Surgical complications include vascular and urological complications, lymphocele and wound infection, for example [166,167]. Other complications include delayed graft function, and infections or malignancies due to weakened immune system caused by immunosuppressive medication.

1.4.1 Delayed graft function

Delayed graft function (DGF) is a frequent post-transplant complication after kidney transplantation and is related to ischemic cold storage of the donor organ before transplantation, leading to ischemia – reperfusion injury, histologically characterized by acute tubular necrosis. Risk factors include donor age, recipient's immunological response and immunosuppressive medication [168]. DGF is usually defined as the need for dialysis treatment in the first post-transplantation week. [169] Studies have shown that not only the presence, but also longer duration of DGF is associated with a risk of acute rejection and worse long-term graft survival [170,171].

1.4.2. Rejection

After transplantation, the recipient's immune system recognizes the transplanted tissue as foreign and activates an immune response to destroy it unless prevented with immunosuppressive medication. Immunosuppressive drugs inhibit the immune system from

attacking the transplanted organ and thus lower the ability of the immune system to reject the graft.

Rejections can be classified as hyperacute, acute, or chronic. Hyperacute rejection is usually caused by pre-existing specific antibodies against the graft and occurs within minutes to hours after transplantation procedure. Hyperacute rejection is rare with the current crossmatching and histocompatibility testing but may occur if the prior HLA immunization of the patient has been missed in the pre-transplant crossmatching and antibody screening tests. Hyperacute rejection cannot usually be treated and results in the loss of the transplanted kidney. [172] Acute rejection is the most common form of rejection. About 15–25% of kidney transplant recipients have at least one mild to moderate episode of acute rejection within the first three months after transplantation [173]. It can be caused by lymphocytes in the recipient that recognize mismatched HLA molecules in the grafted organ. A T-cell mediated response against foreign HLA molecules is the key feature in acute T-cell mediated rejection, which is histologically characterized by interstitial inflammation, tubulitis, and arteritis in the cortical kidney. In an acute antibody-mediated rejection, donor-specific HLA antibodies attach to capillaries, and activate complement and immune responses, resulting in damage of the grafted organ. This rejection is characterized by peritubular capillaritis, glomerulitis, and arteritis. [174] Acute T-cell mediated rejection can be treated with steroids, or in steroid-resistant rejection with lymphocyte antibodies, among others. Antibody-mediated rejection is typically also treated with plasma exchange, intravenous immunoglobulins, and drugs that inhibit B-cell function and antibody production. Rejection episode length and response to therapy determine the resulting graft damage. Permanent damage may lead to long-term deterioration of graft function. Therefore it is important to recognize patients with high rejection risk and diagnose rejection early to limit damage to the graft and improve the transplant prognosis [175]. Chronic rejection usually occurs months or years after transplantation. It involves various mechanisms, including chronic inflammation, antibody-mediated and T-cell mediated immunity. [176] Chronic rejection may occur if the acute rejection process fails to completely resolve, or if it continues slowly over time [177]. It is more difficult to treat as more permanent chronic changes have already occurred in the kidney tissue, and it usually leads to eventual loss of graft function.

When kidney transplantation results in rejection, three steps are common: (i) the recognition of the transplanted kidney as foreign; (ii) an initiation of immune response; and (iii) destruction of the transplant [172]. The rejection mechanism can be either T-cell mediated or antibody-mediated. In T-cell mediated immunity, the system occurs inside the infected cells and is mediated by T-lymphocytes. The pathogen's antigens are expressed on the cell surface or on an antigen-presenting cell. Helper T-cells release cytokines that help activated T-cells bind to the infected cells' HLA-antigen complex and differentiate the T-cell into a cytotoxic T-cell. The infected cell then undergoes lysis. [178]

In antibody-mediated immunity, with the help of helper T-cells, B-cells will differentiate into plasma B cells that can produce antibodies against a specific antigen. The antibody-mediated system deals with antigens from pathogens that are freely circulating, or outside the infected cells. The produced antibodies will then bind to antigens, neutralizing them, or augmenting their phagocytosis. [179]

1.4.3 Graft loss

Sometimes the transplantation may result in complete graft failure. Graft failure is defined as the loss of allograft function, resulting in the need to restart dialysis, the need for re-transplantation, or death of the patient. [180]

The use of immunosuppressive drugs that are needed to prevent graft loss is directly associated with an increased risk of infections and cancers, which are among the main causes of morbidity and mortality in transplanted patients [181]. Post-transplant malignancy is the third most common cause of death in kidney transplant recipients, with some malignancies occurring at much higher rates compared to the general population [182]. Death with a functioning graft due to cardiovascular diseases is also a major cause of graft loss and is a competitive risk factor for all other causes of graft failure, particularly in the elderly [183].

2 AIMS OF THE STUDY

The general, long term aims of transplantation genomics studies are: (i) evaluating whether addition of genome-level information from patient-donor pairs and modern data analysis tools increase accuracy of pre-transplant histocompatibility testing, and (ii) setting up foundations for integrated and comprehensive prediction tools for transplantation outcomes.

The specified aims for the present thesis were:

1. To investigate the association between acute rejection and genome-level mismatching among kidney transplant donor-recipient pairs.
2. To fine-map the *CFHR*-deletion boundaries and to investigate the impact of deletion-tagging allele on gene and protein expression levels, to better understand the mechanisms behind the association of the *CFHR*-deletion with rejection.
3. To investigate the role of polygenic burden for cerebrovascular disease risk factors on donor age of death, graft survival, and graft function.

3 MATERIALS AND METHODS

All materials and detailed information about the methods have been described in the original publications (I–III). Only short descriptions are provided here.

3.1 Study ethics (I–III)

Studies I, II, and III conform to the principles of the Declaration of Helsinki and have 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). Study III was approved by the Hammersmith and Queen Charlotte's & Chelsea Research Ethics Committee REC No 08/H0707/1, on October 14, 2009, as well as by the local ethical boards of each collaborator.

3.2 Study subjects (I–III)

Characteristics of the cohort in study I are provided in **Table 6**. In total, 1,025 kidney transplant recipients over the age of 18 years, and their 730 HLA deceased donors (>18 years) were included in this study. The recipients received their first kidney transplantation between 2007 and 2017 in a single transplant center, at the Helsinki University Hospital, Helsinki, Finland. DNA samples from participants were extracted from whole blood at the time of histocompatibility testing for transplantation, and serum samples were collected for antibody detection for complement-dependent cytotoxic crossmatch at the Finnish Red Cross Blood Service, Helsinki, Finland. The clinical endpoint for this study was biopsy-proven acute rejection (19.4%) based on the Banff classification [184], and included both antibody-mediated and T-cell-mediated rejections. We also studied graft loss, but due to a low number of graft loss events, 68 (6.6%) observed in our data, we focused on analyses using acute rejection endpoint.

Table 6. Characteristics of the study population in Study I.

Characteristic	All recipients	Rejection	No rejection	P[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, months	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, hours	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 non-rejection-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 Student's t-test.
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All 15 patients with rs7542235 GG-genotype tagging for the homozygous deletion in the *CFHR3–1* locus from Study I were included in study II. The clinical endpoint was biopsy-proven acute rejection. Characteristics of the study cohort in Study II are provided in **Table 7.**

Table 7. Characteristics of the 15 patients with rs7542235 GG-genotype tagging for homozygous *CFHR3–1* deletion in Study II. Rejection vs. non-rejection group.

Characteristic	Patients with rs7542235 GG genotype, n = 15	Patients with rs7542235 GG genotype with rejection, n = 8	Patients with rs7542235 GG genotype without rejection, n = 7	P-value[a]
Age (median, range)	58 (36–74)	52 (36–73)	61 (53–74)	0.064[c]
Sex, n (%)				0.854[d]
Male	10 (67)	6 (75)	4 (57)	
Female	5 (33)	2 (25)	3 (43)	
Rejection, n (%)	8 (50)	8 (100)	0 (0)	
T-cell mediated, n (%)	6 (38)	6 (75)	0 (0)	
Antibody mediated, n (%)	2 (12)	2 (25)	0 (0)	
Primary diagnosis, n (%)				
Polycystic kidney disease	3 (20)	0 (0)	3 (43)	0.076[e]
Diabetic nephropathy with type I diabetes	2 (13)	1 (12.5)	1 (14)	1[e]
IgA nephropathy	2 (13)	2 (25)	0 (0)	0.466[e]
Chronic kidney disease, unspecified	2 (13)	2 (25)	0 (0)	0.466[e]
Glomerular disorder in amyloidosis	1 (7)	1 (12.5)	0 (0)	1[e]
Glomerular disorder in polyarteritis nodosa	1 (7)	0 (0)	1 (14)	0.466[e]
Glomerular disorder in granulomatosis with polyangiitis	1 (7)	1 (12.5)	0 (0)	1[e]
Hydronephrosis with ureteropelvic junction obstruction	1 (7)	0 (0)	1 (0)	0.466[e]
Other chronic kidney disease	1 (7)	0 (0)	1 (14)	0.466[e]
Polycystic kidney disease, unspecified	1 (7)	1 (12.5)	0 (0)	1[e]
	Median (IQR)			
Follow-up time, months	37 (9–50)	29 (9–40)	41 (24–70)	0.189[c]
PRA I >0, %[b]	52 (20–64)	52 (36–62)	36 (22–50)	NA[f]
PRA II >0, %[b]	17 (17–58)	17 (17–17)[g]	99 (99–99)[h]	NA[f]
Cold ischemia, hours	21 (19–22)	22 (19–23)	21 (19–22)	0.491[f]
HLA eplet mismatch sum, n	28 (17–46)	38 (23–49)	27 (14–33)	0.182[c]
HLA I eplet mismatch sum, n	9 (7–13)	12 (7–19)	9 (8–11)	0.521[c]
HLA II eplet mismatch sum, n	22 (8–28)	23 (14–30)	8 (7–25)	0.269[c]

HLA, human leukocyte antigen; IgA, Immunoglobulin A; IQR, interquartile range; PRA, panel-reactive antibody.
[a]Significance of variation was calculated between rejection and non-rejection group of patients with rs7542235 GG-genotype.
[b]The medians of PRA values are calculated only from patients with PRAI >0 and PRAII >0.
[c]Mann–Whitney U-test.
[d]Pearson chi-square test.
[e]Fisher's exact test.
[f]Significance of variation was not analyzed due to the low frequency count.
[g]n = 2
[h]n = 1
[f]Student's t-test.

Study III consisted of 6,666 deceased and living donors and their recipients from seven independent European ancestry cohorts. Only donors over the age of 18 were included in

this study. Three subgroups of donors were studied: 1,582 (24%) living donors; 3,113 (47%) donors died of stroke; and 1,971 (30%) donors died of other causes of death. Detailed descriptions of these study cohorts are provided in **Table 8**.

Table 8. Detailed descriptions of study cohorts included in Study III.

Donor type	DeKAF	FRCBS	GEN03	KiT-GENIE	QUB	TL	UKIRTC
Living	684 (100%)	0 (0%)	476 (100%)	319 (17%)	0 (0%)	103 (15%)	0 (0%)
Stroke cause of death	0 (0%)	618 (67%)	0 (0%)	879 (47%)	78 (59%)	344 (49%)	1194 (63%)
Other cause of death	0 (0%)	311 (33%)	0 (0%)	655 (35%)	55 (41%)	249 (36%)	701 (37%)
All	684	929	476	1853	133	696	1895

DeKAF, Deterioration of Kidney Allograft Function [185]; FRCBS, Finnish Red Cross Blood Service [186]; Kit-GENIE, Kidney Transplantation - Genomic Investigation of Essential clinical concerns [187]; TL, Transplant Lines [188]; QUB, Queen's University Belfast [69]; UKIRTC, United Kingdom and Ireland Renal Transplant consortium [74].

3.3 Genotyping, imputation, and data QC (I–III)

Genotyping of all samples for studies I and II was performed at the Finnish Institute of Molecular Medicine, Helsinki, Finland, using Illumina's Infinium Global Screening array-24 v2.0 + multidisease drop-in. Before imputation, the genotyped data was lifted over to a newer genome build of GRCh38/hg38 following a liftover protocol version 2 [189]. The imputation was performed using a Finnish SISu v3 reference panel consisting of high-coverage whole genome sequence data from THL Biobank cohorts (N = 1768) (http://www.sisuproject.fi/, Pärn et al. manuscript in preparation). In the quality control after imputation, individuals with a minor allele frequency of <0.01, missingness >0.05 and genotyping rate >0.1 were excluded. The final percentage of imputed SNPs was 92% of the total amount of 9,085,413 variants. (PLINK1.9 [190] IBD analysis was performed to identify first-degree relatives (n=35), which were then excluded from the cohort. The *HLA* types of individuals were imputed to high resolution using HIBAG v1.0.3[191] with the Finnish *HLA* reference for *HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DPB1, HLA-DQA1,* and *HLA-DQB1*. In addition to imputation of HLA genes, HLA eplet mismatch was calculated based on imputed high resolution HLA genotypes using online tool HLAMatchmaker (http://www.epitopes.net/) [192].

The detailed information about genotyping of all cohorts in study III are presented in the original publications.[69,74,185–188,193] The individuals from study cohort "Transplant Lines" from the Netherlands were imputed using the 1000 Genomes imputation panel[194]. The individuals from study cohort "FRCBS" were imputed as described above (Study I). All other individuals were imputed using the Haplotype Reference Consortium r1.1 2016 on the Sanger imputation server, with phasing using Eaglev2.4. In the quality control after imputation, the following parameters were used: minor allele frequency of 0.02, missingness 0.05, and geno-typing rate 0.05. KING software[195] was used to determine the relatedness of participants, and 3rd degree relatives were excluded. All participants were of European ancestry, and this was determined by principal components analysis (PCA) with the reference European ancestry population from 1000 Genomes data.

3.4 Mismatch analysis of transmembrane, secretory, and kidney-related missense variants (I)

Mismatch analysis of missense variants was performed for subsets related to: (i) transmembrane and secretory; (ii) transmembrane; and (iii) kidney-related proteins. Specific query strings were used to retrieve the protein groups based on their annotation type, location, and tissue specificity. A more detailed description can be found in the supplementary data of the original publication[186]. We also calculated the overall mismatch sum of missense variants between donor and recipient. A mismatch was defined whenever the donor carried an allele that was not present in the recipient (**Table 9** shows the definition for mismatch for bi-allelic SNP). An online tool, Ensemble Variant Effect Predictor (https://www.ensembl.org/Homo_sapiens/Tools/VEP), was used for functional annotation of genotyped and imputed variants [196]. Only missense variants, e.g., variants that change the amino acid, were included in this study. Uniprot database (https://www.uniprot.org/) was used for retrieving transcripts for all subsets of proteins [197].

Table 9. A definition for mismatch for bi-allelic SNP. A mismatch was defined whenever the donor carried an allele that was not present in the recipient.

Recipient	Donor
AA	AB
AA	BB
BB	AA
BB	AB

The missense variant mismatch sum between donor and recipient was evaluated both as a continuous variable, and by dividing the sum into quartiles. Time-to graft loss and time-to acute rejection were used as clinical endpoints for survival analyses, graft loss and acute rejection were used as clinical endpoints for logistic regression analysis. The mismatch sum was calculated, and all analyses were performed using R v3.6.2. [198]. We used *glm* function from *stats* package [198] for logistic regression model, and *survival* package [199] for the survival analyses; *coxph* function for cox proportional hazards model for adjusted data, and *survfit* function for univariate Kaplan-Meier analysis. The models were adjusted with the same covariates as a previous study by Reindl-Schwaighofer *et al*., which included recipient and donor sex, recipient and donor age, cold ischemia time, panel reactive antibody I and II, and HLA I (HLA-A, -B, -C) and HLA II (HLA-DRB1, -DQA1, -DQB1, -DPB1) eplet mismatch. Because of the adjustment for HLA eplet mismatch, and interest in the effect of non-HLA incompatibility, the major histocompatibility complex from chromosome 6 (position 28,510,120–33,480,577) was excluded. Sex chromosomes were also excluded from the data because of the unique analytical challenges they present.

3.5 Deletion analysis (I)

We performed a deletion analysis on previously reported deletion-tagging variants that have been reported to be in strong linkage disequilibrium with the deletions [69]. Our dataset provided us with 40 common variants that had a global minor allele frequency of >10%. The clinical endpoint of interest was acute rejection, and we estimated the association of deletions to time-to acute rejection in both donor-recipient pairs and recipients only. In the donor-recipient analysis, so-called collision model, the kidney transplant recipient was

homozygous for a deletion-tagging variants and received a kidney from a donor who had at least one reference allele. This mismatch status was used as an independent variable in both univariate Kaplan-Meier survival curve and multivariate Cox proportional hazards model. The associations were also evaluated using logistic regression with acute rejection endpoint. Deletion analyses were performed using R v3.6.2. The function *glm* from package *stats* [198] was used for logistic regression, *survival* package [199] for the survival analyses; *coxph* function for cox proportional hazards model for adjusted data, and *survfit* function for univariate Kaplan-Meier analysis. The covariates for adjustments were the same as in the mismatch analysis; recipient and donor sex, recipient and donor age, cold ischemia time, panel reactive antibody I and II, and HLA I (HLA-A, -B, -C) and HLA II (HLA-DRB1, -DQA1, -DQB1, -DPB1) eplet mismatch. Problems with multiple comparison were addressed by using Bonferroni-corrected threshold for statistical significance (α level of $0.05/40 = 1.25 \times 10^{-3}$).

In addition to evaluating the association of each variant to acute rejection separately, we also calculated the overall mismatch sum of all homozygous deletions among the 40 deletion-tagging variants in both donor-recipient pairs and recipients only. All the deletion-tagging variants, their associated genes, and minor allele frequencies in our data are provided in the supplementary material of the original publication [186].

3.6 Whole genome sequencing (I, II)

To validate the deletions in complement factor H (*CFH*) region, whole genome sequencing was performed first on three recipients (Study I) homozygous for the *CFHR3–1* deletion-tagging variant rs7542235 (GG), and on seven other GG-recipients in Study II. The sequencing was performed at FIMM, Helsinki, Finland, and the samples were sequenced with Illumina's NovaSeq S4 NS4-300 run.

In Study I, the total number of aligned bases was ~110 gigabases. The input reads for each sample were approximately 1 billion, and the length for each read was 151 base pairs. The coverage depth for these samples was 34–36x. In Study II, the total number of aligned bases was 20–50 gigabases. The input reads for the samples were around 200–500 million, and

the length for each read was 151 base pairs. The coverage depth for the sequenced samples was 7–17x.

3.7 Enzyme-linked immunosorbent assay (I)

For determination of the expression of FH and to assess *de novo* antibody formation against FH, blood serum samples of recipients were used in enzyme-linked immunosorbent assays (ELISA). We analyzed all serum samples that were available for the *CFHR* deletion homozygous patients, and a set of additional control samples. The detection of FH from the samples was performed by using FH ELISA kit (Abnova, Taipei, Taiwan) according to the manufacturer's instructions. Dilution 1:200,000 was optimal for the analysis. In addition, we also used FH IgG ELISA kit (Abnova, Taipei, Taiwan) to detect *de novo* antibodies against the FH. The hypothesis was that recipients homozygous for the *CFHR1*-deletion could potentially develop anti-FH antibodies [200]. For anti-FH kit, we used a dilution of 1:50 of the serum samples according to manufacturer's recommendations.

3.8 Multiplex ligation-dependent probe amplification (II)

Multiplex ligation dependent probe amplification (MLPA) was performed for 15 homozygous rs7542235 GG patients (eight with rejection, seven without rejection) to confirm and fine map the deletions in the *CFH/CFHR* genomic region in more detail. We also performed the MLPA analysis for control patients including eight individuals with heterozygous AG-genotype (four with rejection, four without rejection) and eight individuals with homozygous AA-genotype (four with rejection, four without rejection) (**Table 10**).

Table 10. Number of samples in MLPA analysis.

rs7542235 genotype	Rejection	Nonrejection
GG	8	7
AG	4	4
AA	4	4

MLPA, multiplex ligation-dependent probe amplification

MLPA is a variation of the multiplex polymerase chain reaction which detects copy number changes at the molecular level. Readily available SALSA MLPA kit P236-A3 and SALSA MLPA

Probemix P236-B1 (MRC Holland) containing 53 probes for *CFH/CFHR* genes (including 16 probes for *CFH*, 8 probes for *CFHR3*, 6 probes for *CFHR1*, 4 probes for *CFHR4*, 4 probes for *CFHR2*, and 5 probes for *CFHR5*) were used for detection of copy number variations in the *CFH*/*CFHR* locus. The software program Coffalyser provided online by the manufacturer (www.mlpa.com) was used for analysis of the PCR products.

3.9 Protein expression levels using Western blot (II)

To investigate the protein expression levels of FH, FHR-1, and FHR-2 of recipients with homozygous *CFHR1* deletion, in total of 15 serum samples from nine rs7542235 GG patients (five with rejection, four without rejection) were studied with western blot analysis. Of the serum samples, nine were collected before transplantation and six were collected after.

3.10 eQTL, pQTL, gene ontology, and Reactome analyses (II)

By using the expression quantitative trait loci (eQTL) data, we wanted to see whether the *CFHR3–1* deletion tagging variant rs7542235 has impact on gene expression levels. We utilized: (i) the FIVEx browser (https://fivex.sph.umich.edu/) eQTL data from blood vessel and kidney samples from the EBI eQTL catalogue [201]; (ii) the eQTLGen Consortium database (https://www.eqtlgen.org/) including data from 37 datasets [202] from blood samples; and (iii) the Human Kidney eQTL Atlas (https://susztaklab.com/Kidney_eQTL/index.php) consisting of human kidney tubule samples [203]. All results were included regardless of false detection rate (FDR).

In addition to eQTL analyses, we utilized Olink (n = 1225) (https://olink.com/)[204] and SomaScan (n = 865) (https://somalogic.com/somascan-platform/)[205] pQTL plasma proteomics data from healthy blood donors in FinnGen project (https://www.finngen.fi/en) to investigate the impact of *CFHR3–1* deletion tagging variant rs7542235 on protein expression. FDR of <0.010 were considered statistically significant. Gene ontology (GO) enrichment analysis (https://geneontology.org/)[206,207] for three aspects of biological process, molecular function and cellular component, and Reactome pathway database

(https://reactome.org/)[208] were also utilized. All proteins from plasma proteomics data with FDR <0.010 were included in the GO and Reactome analyses.

3.11 Genetic burden for cerebrovascular risk (III)

The PRSs were created for three different traits that are known to have an impact on graft outcome; stroke[209], intracranial aneurysm[210] and hypertension using published GWASs of European ancestry for each trait [211–213]. The created PRSs estimate the cumulative effect of common genetic variation on an individual's disease status weighted by estimated effect size. Further details of the used GWASs are presented in **Table 11.**

Table 11. Details of studies utilized to generate PRSs.

GWAS Trait	Study	Sample size	SNPs used (after pruning)*
Intracranial aneurysm (IA)	Bakker et al. 2020 [211]	7,495 cases and 71,934 controls	50,377
Hypertension	Wenjian et al. 2020 [212]	76,566 cases and 206,305 controls	63,640
Stroke	Malik et al. 2018 [214]	67,162 cases and 454,450 controls	65,266

*SNPs used refers to the number of Single Nucleotide Polymorphisms used by PRSice in calculating the PRSs after pruning and thresholding.

3.11.1 Power calculations (III)

A power analysis was performed with the function power.t.test from the *stats* package [198] in R v4.2.1. to determine the smallest effect size that could be reliably detected using our variables of interest; donor age in donors that died of stroke, graft survival, eGFR at 1-year post-transplant, and eGFR at 5 years after transplantation. The calculation was performed for each variable separately. For each variable we calculated the number of non-missing individuals along with the standard deviation of the variable. We used 95% power and a significance level of 5% to generate a value for delta (the smallest effect size reliably detectable) using a two-sample t-test power calculation for each variable.

3.11.2 Polygenic risk score calculations (III)

PRSs for each trait (stroke, intracranial aneurysm, and hypertension) were calculated in R v4.2.1 using polygenic risk score software PRSice2 [215]. Alleles with a p-value threshold greater than 0.5, physical distance threshold for clumping of 250 kb, and linkage disequilibrium threshold of 0.1 were selected for calculations.

3.11.3 Donor polygenic burden analyses (III)

Kruskal-Wallis and Dunn test were used to investigate whether there was any difference in polygenic burden between four groups of individuals: healthy controls, living donors, deceased donors who died of stroke, and deceased donors who died of other causes. These tests were performed separately for each trait of interest: stroke, intracranial aneurysm, and hypertension.

Meta-regressions were created with the R package *meta* [216] to investigate the role of polygenic burden for each trait on donor age of death among donors who died of stroke. Separate models were constructed for each PRS trait (stroke, intracranial aneurysm, and hypertension). These models were adjusted with additional covariates of donor sex and the first four principal components of genetic ancestry. Donors who died of stroke were split into three groups for each polygenic trait: high risk (top 10% of the risk for each trait), intermediate risk (middle 80% of the risk), and low risk (bottom 10% of the risk). Median donor age of death was compared between each of these groups.

Meta-regressions were created with the R package *meta* [216] to investigate the role of polygenic burden for each trait on graft survival. Cox proportional hazard models were performed for each PRS trait. These models were adjusted with donor and recipient sex, donor and recipient age, year of transplant, whether it was the recipient's first transplant or not, and the first four principal components of genetic ancestry. All recipients were split into three groups for each polygenic trait: high risk (top 10% of the risk for each trait), intermediate risk (middle 80% of the risk), and low risk (bottom 10% of the risk). Median graft survival was compared between each of these groups.

Recipient graft function was measured as eGFR at 1 and 5 years after transplantation. Meta-regressions were created with the R package *meta* to predict recipient eGFR at 1 year after transplantation for each trait. These models were adjusted with donor and recipient sex, donor and recipient age, donor type (living donors, deceased donors who died of stroke, and deceased donors who died of other causes), year of transplant, and whether it was the recipient's first transplant or not. A similar process was carried out to predict eGFR at 5 years after transplantation. All donors were then split into three groups for each polygenic trait: high risk (top 10% of the risk for each trait), intermediate risk (middle 80% of the risk), and low risk (bottom 10% of the risk). Median eGFR at 1 and 5 years after transplantation was compared between each of these groups with student's t-test. The P-values were then adjusted for multiple testing using a FDR correction.

3.12 Other statistical analyses (I, II)

Characteristics of recipients were described by medians and interquartile ranges (IQR) or ranges for continuous variables, and frequencies and percentages for binary variables. The comparison between the two groups of recipients with rejection and without rejection were 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 recipient sex, Fisher's exact test for primary diagnosis, or Student's t-test for normally distributed data (cold ischemia). P-values <0.05 were considered statistically significant. The same statistical tests were used when comparing the two groups of recipients with rs7542235 GG-genotype and patients with AA/AG-genotype, and GG-genotype with rejection and GG-genotype without rejection in Study II. All statistical tests were carried out in R v3.6.2.

4 RESULTS

Figure 4 shows the workflow for each study.

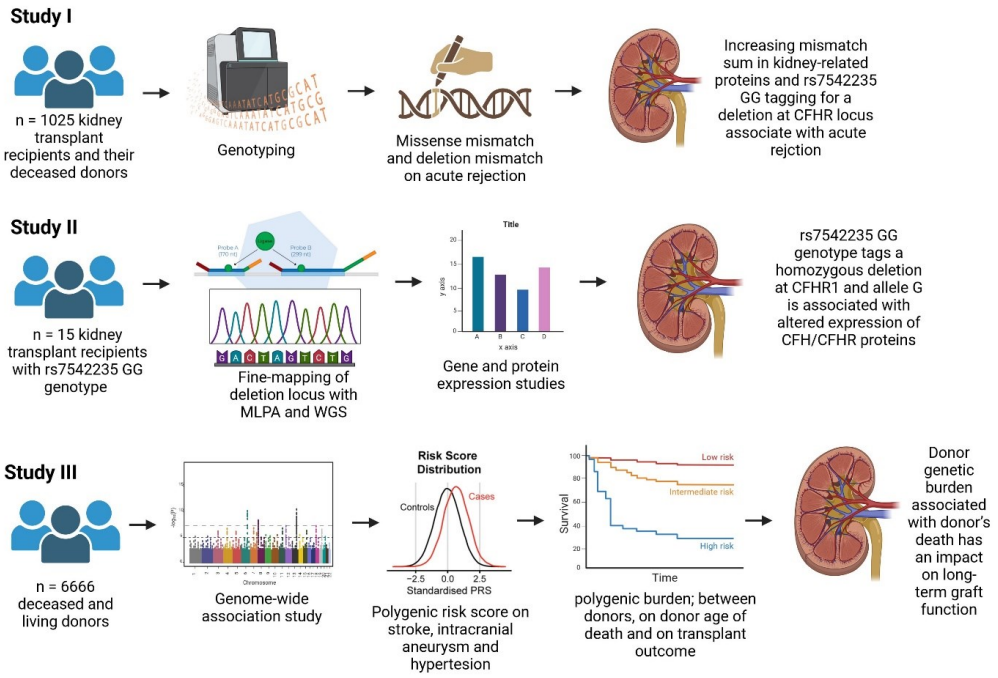

Figure 4. Workflow of Studies I–III. In Study I, we explored the genetic variation of the patient-donor pair at the level of the whole genome by examining missense variants in transmembrane, secretory, and kidney-related genes. Additionally, we studied common variants in donor-recipient pairs, tagging for known gene deletions, and examined their association to acute rejection using statistical methods. In Study II, we fine-mapped the *CFHR* gene deletion found in the first subproject using whole genome sequencing and multiplex ligation-dependent probe amplification methods and studied its effects on the expression levels of other genes and proteins. In Study III, we predicted the donor age of death and kidney transplant outcome with polygenic risk scores related to the risk factors of cerebrovascular diseases created from the donor's genome-wide association data. Figure created with BioRender.com.

4.1 The effect of genome-level mismatching (I)

Mismatch analysis of transmembrane, secretory, and kidney-related missense variants

To understand effect of donor-recipient matching outside the HLA and ABO types, variation of 1025 transplantation pairs was determined at the genome level using SNP array. After post-imputation QC and the exclusions of (i) MHC region that by default was matched, (ii) sex chromosomes, and (iii) non-missense variants 29,854 amino acid changing missense variants were available for analysis at the whole genome level. Of these missense variants, 10,313 have been mapped in the transmembrane or secretory proteins; 7,792 in transmembrane only proteins; and 3,787 in kidney-related proteins (**Table 12**).

The median number of missense variant mismatches between donor and recipient in transmembrane plus secretory proteins was 1,765 (IQR 1,724–1,812) and for the transmembrane proteins the median mismatch sum was 1,334 (IQR 1,292–1,671). For the kidney-related proteins, the median mismatch sum was 605 (IQR 585–627), showing that, indeed, the alloantigenic load is high in each transplantation pair. The overall genome-wide missense variant mismatch sum was 4,935 (IQR 4,861–5,012) (**Table 12**).

Table 12. Number of missense variants, and mismatch sum of each protein group.

Missense variants	Number of variants	Median mismatch sum (IQR)
Transmembrane and Secretory	10,313	1,765 (1,724–1,812)
Transmembrane	7,792	1,334 (1,292–1,671)
Kidney-related	3,787	605 (585–627)
Total	29,854	4,935 (4,861–5,012)

IQR, interquartile range

We found no statistically significant association for transmembrane plus secretory, transmembrane only, or kidney-related proteins and time-to graft loss or time-to acute rejection when analyzing the mismatch sums as continuous variables. No evidence was found for transmembrane plus secretory, or transmembrane missense variants when the

mismatch sums were divided into quartiles and evaluated the association of these quartiles to time-to acute rejection (**Table 13**).

Table 13. Cox proportional hazards model for mismatch sums as continuous variables and divided into quartiles.

Covariate	Clinical endpoint	HR (95% CI)	P value
Missense mismatch sum of transmembrane and secretory variants	Time-to graft loss	1.00 (1.00–1.01)	0.30
Quartiles of missense mismatch sum of transmembrane and secretory variants	Time-to graft loss	0.96 (0.77–1.19)	0.70
Missense mismatch sum of transmembrane and secretory variants	Time-to acute rejection	1.00 (1.00–1.00)	0.58
Quartiles of missense mismatch sum of transmembrane and secretory variants	Time-to acute rejection	1.00 (0.89–1.14)	0.90
Missense mismatch sum of transmembrane variants	Time-to acute rejection	1.00 (1.00–1.00)	0.39
Quartiles of missense mismatch sum of transmembrane variants	Time-to acute rejection	1.02 (0.90–1.16)	0.70
Missense mismatch sum of kidney-related variants	Time-to acute rejection	1.00 (1.00–1.01)	0.41

HR, hazard ratio; CI, confidence interval

Our results, however, showed that increasing mismatch sum of missense variants in kidney-related proteins showed a trend for reducing the probability of rejection-free survival with 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) when dividing the mismatch sum into quartiles (**Figure 5**).

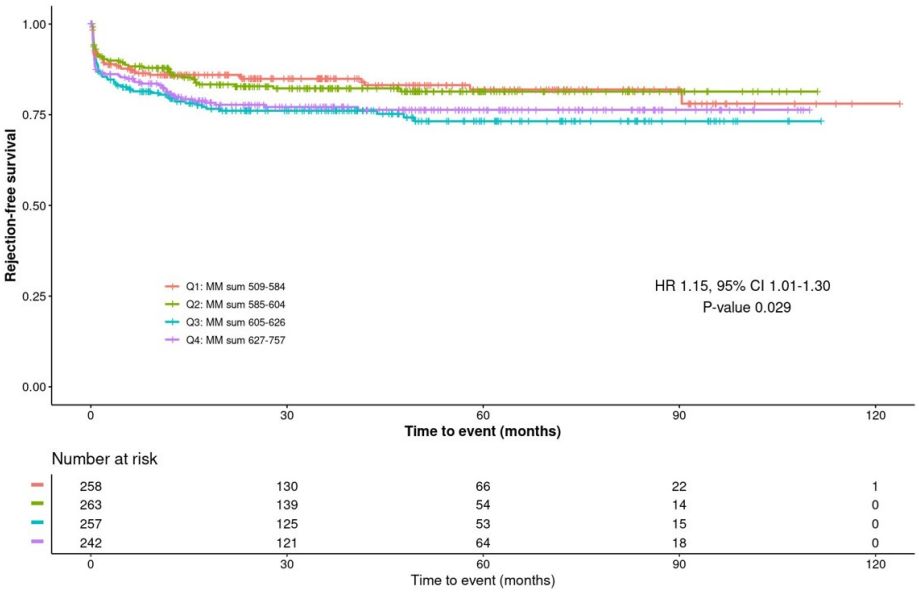

Figure 5. The effect of quartiles of missense variant mismatch sum coding for kidney-related proteins on rejection-free graft survival in recipient-donor pairs. The effect of quartiles of missense variant mismatch sum coding for kidney-related proteins on rejection-free graft survival. The quartile 1 (Q1) represents the lowest number of mismatches between recipient and donor, quartile 4 (Q4) the highest number of mismatches. An event (acute rejection) occurs each time the curve drops. The tick marks indicate censored data (end of follow-up time or death of the patient). Unadjusted hazard ratio with confidence interval shown. CI, confidence interval; HR, hazard ratio; MM, mismatch. (Markkinen *et al.* 2022 [186]). Figure reproduced from Publication I with permission of Kidney International Reports.

Deletion analysis

Our imputed genotype data included 40 common deletion-tagging variants that were previously analyzed by Steers et al. [69], and reported an association of *LIMS1* gene deletion mismatch with acute rejection. We tested the variants in a collision-model, where a recipient who was homozygous for a deletion-tagging variant received a graft from a donor with a functional gene.

Out of the 40 deletion-tagging variants, rs7542235 genotype GG associated with time-to acute rejection with the p value of <0.05. Previous studies have reported that the rs7542235 allele G tags deletions in *CFH*-related proteins (*CFHR*) 1–3 locus [217,218]. We observed that the mismatch rs7542235 GG versus GA/AA was associated with a reduced probability of rejection-free survival, with an unadjusted HR of 3.10 (95% CI, 1.53–6.29; P=0.002) and an adjusted HR of 2.97 (95% CI, 1.46–6.05; P=0.003) (**Figure 6**). The actual numbers of cases were low, however: There were 8 (4%) recipients with rs7542235 genotype GG in the rejection group and 8 (1%) in the non-rejection group, making it only 1.6% of the whole study population. None of the donors were homozygous for the deletion-tagging variant, hence, the deletion homozygous patients were always mismatched with the donors. The statistical significance of the association, however, failed to pass the Bonferroni-corrected α level threshold of 0.00125.

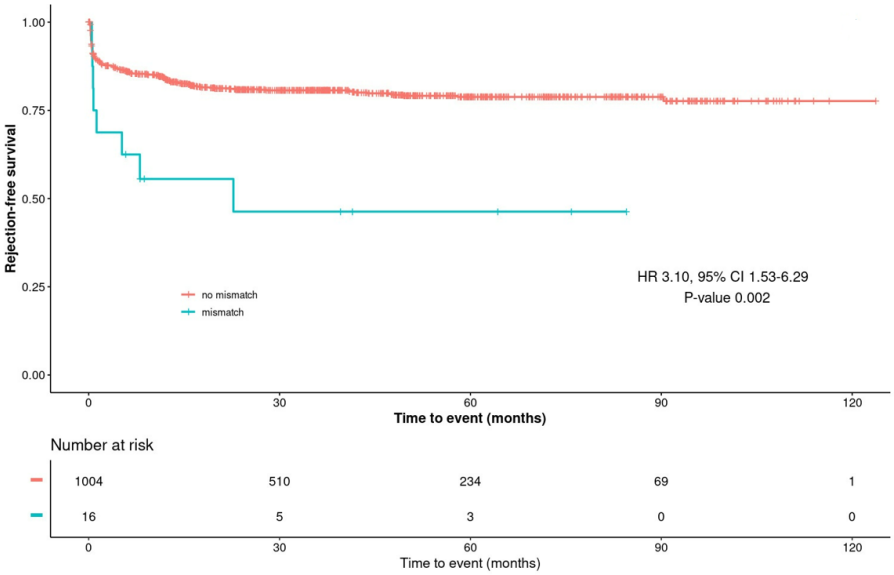

Figure 6. The effect of rs7542235 mismatch on rejection-free graft survival in recipient-donor pairs. In recipient-donor analysis the recipient who was homozygous for a deletion-tagging allele G received a transplant from a donor with AG or AA genotype. The orange curve represents the no mismatch status, 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 hazard ratio with confidence interval shown. CI, confidence interval; HR, hazard ratio; MM, mismatch. (Markkinen *et al.* 2022) [186]. Figure reproduced from Publication I with permission of Kidney International Reports.

4.2 Fine-mapping of the *CFHR*-deletion (I, II)

WGS and MLPA analyses (I, II)

In Study I, WGS confirmed that each of the three recipients with an rs7542235 GG genotype had homozygous deletions at the *CFHR* locus, however, the deletions were of different sizes. All encompassed the *CFHR1* gene, and two of them also had a homozygous deletion completely or partly at the *CFHR3* locus (three lowest lines in **Figure 7**). In Study II, WGS of seven more recipients with the rs7542235 GG genotype supported the previous WGS findings: all individuals had homozygous deletions of different sizes. All ten patients shared a deletion of the *CFHR1* gene. The deletion-tagging variant rs7542235 was located in the intergenic region between *CFHR1* and *CFHR4*. (**Figure 7**)

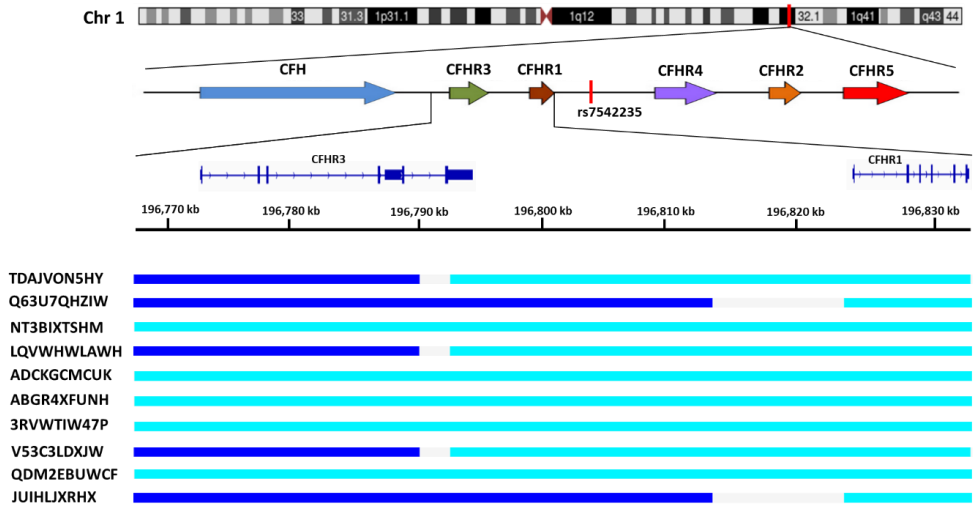

Figure 7. The results of whole genome sequencing of *CFHR3–1* loci on chromosome 1 for 10 rs7542235 GG genotype patients. The turquoise color represents a homozygous deletion, and the dark blue a heterozygous deletion. The three lowest samples were sequenced at the time of Study I, other samples were sequenced at the time of Study II. The IDs on the left indicate the pseudonyms for each patient. The variant rs7542235 tagging for the deletion is located in the intergenic region between *CFHR1* and *CFHR4* (red mark).

MLPA confirmed the findings of WGS: All 15 patients with rs7542235 GG genotype had homozygous deletions of different sizes at the *CFH*/*CFHR* locus. The deletions invariably encompassed the whole *CFHR1* gene. Three different deletions here called type 1, 2, and 3, each encompassing different genomic fragments but sharing the deletion of *CFHR1*, could

be deduced from the results (**Figure 8**). Patients with type 1 deletion had homozygous deletion at *CFHR3* exons (ex) 1–6 and *CFHR1* ex 1–6. Patients with type 2 deletion had homozygous deletion at *CFHR1* ex 2–6 and heterozygous deletions at *CFHR3* ex 1–6 and *CFHR4* ex 1–10. Patients with deletion type 3 had homozygous deletion at *CFHR3* ex 6 and *CFHR1* ex 1–6 and heterozygous deletions at *CFHR3* ex 1–4 and *CFHR4* ex 1–6. We found no obvious association between the deletion sizes or types and the diagnoses of the patients.

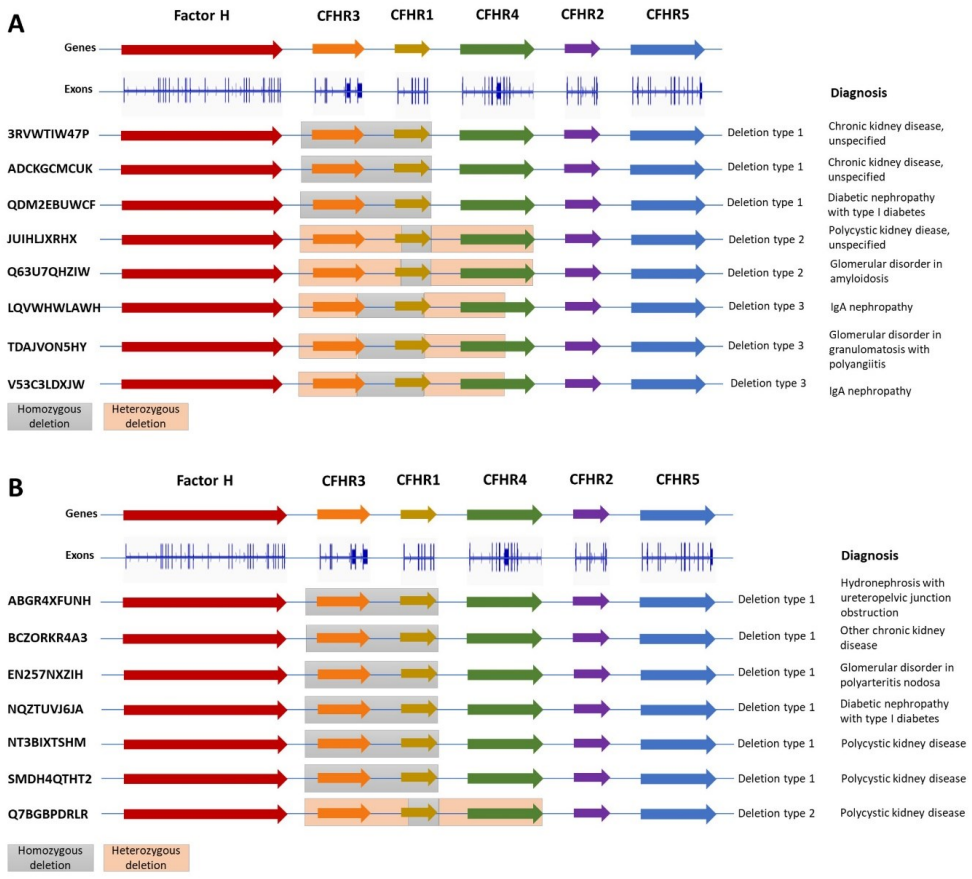

Figure 8. MLPA results for rs7542235 GG genotype patients with rejection (A) and without rejection (B). Deletion type 1 indicates homozygous deletion at *CFHR3* exons (ex) 1–6 and *CFHR1* ex 1–6. Deletion type 2 indicates homozygous deletion at *CFHR1* ex 2–6 and heterozygous deletions at *CFHR3* ex 1–6 and *CFHR4* ex 1–10. Deletion type 3 indicates homozygous deletion at *CFHR3* ex 6 and *CFHR1* ex 1–6 and heterozygous deletions at *CFHR3* ex 1–4 and *CFHR4* ex 1–6. *CFHR*, complement factor H related; MLPA, multiplex ligation-dependent probe amplification. The IDs on the left indicate the pseudonyms for each patient.

Figure 9 combines the results from WGS and MLPA data, giving a more detailed illustration and description about the borderlines of homozygous and heterozygous deletions in patient with deletion type 3.

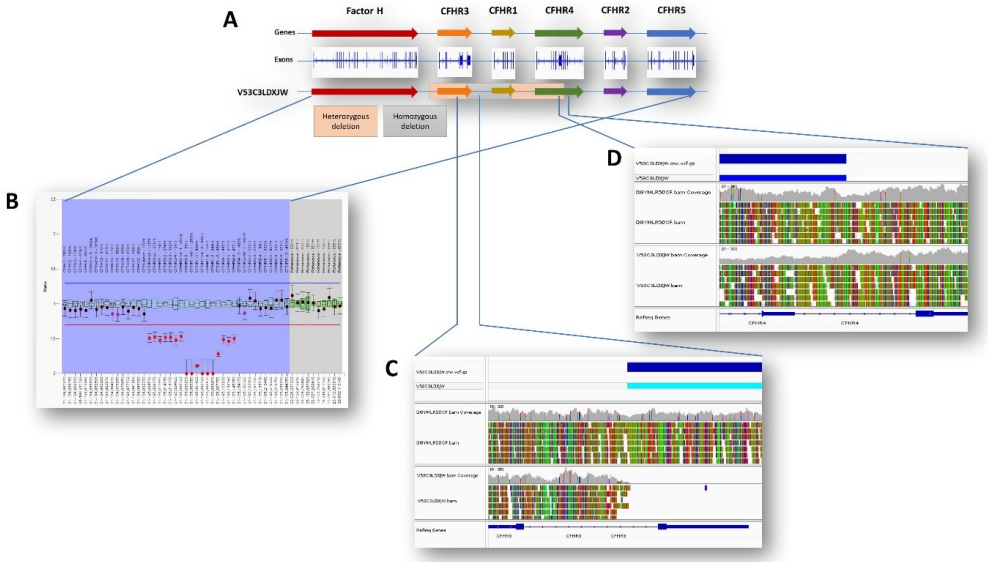

Figure 9. Detailed example of MLPA and WGS results for Deletion type 3. Patient with homozygous deletion covering *CFHR3* exon 6 and *CFHR1* exons 1–6, and heterozygous deletions covering *CFHR3* exons 1–4 and CFHR4 exons 1–4. A) *CFH*/*CFHR* gene locus, exons and deletions of patient V53C3LDXJW. B) MLPA result for the *CFH*/*CFHR* locus. The deletion is denoted by the red spots below the deletion cut-off line (red) in the ratio chart. The red line represents the 0.7 ratios, and the blue line represents the 1.3 ratios. Above the blue line are the genes covered by the SALSA MLPA probemix, P236 *CFH* Region B1. C) Visualization of homozygous deletion in WGS data at the beginning of *CFHR3* gene exon 6. Control sample without deletion above, the patient sample below. D) Visualization of heterozygous deletion in WGS data at the end of *CFHR4* gene. The coverage (gray) halves in size at the heterozygous deletion region. CFH, complement factor H; CFHR, complement factor H related; MLPA, multiplex ligation-dependent probe amplification; WGS, whole genome sequencing.

Expression levels of CFHR proteins and the effect deletion-tagging allele on gene and protein expression (II)

Western blot analysis confirmed that all nine serum samples from individuals with the homozygous *CFHR1* deletion also lacked protein expression of FHR-1 in their serum, as was assumed.

To see whether the *CFHR3–1* deletion tagging variant rs7542235 regulates the expression of other genes, we searched the eQTL databases for effects of rs7542235 variant. When searching in the FIVEx database, including data from blood vessel and kidney samples, the allele G was associated with lower expression levels of *CFHR1, -3,* and *-4* genes with P-values from 7.59×10^{-06} to 1.17×10^{-23}. In the eQTLGen Consortium database consisting of only blood samples, rs7542235 allele G was associated with the increased expression level of FH with P-value 8.88×10^{-24}. When screening the Human Kidney eQTL Atlas having samples from kidney tubule, we found two statistically significant associations in genes *CFHR1* and *CFHR3*, with reduced expression and P-values of 2.73×10^{-58} and 4.01×10^{-10}, respectively.

To see whether the *CFHR3–1* deletion tagging variant rs7542235 has an impact on protein expression, we used FinnGen plasma proteomics data consisting of Olink and SomaScan measures. Olink results showed increased expressions of FH, FHR-2, and FHR-5 to be associated with the *CFHR*-deletion tagging variant with FDR <0.010. Similar to the Olink results, SomaScan showed an increase in expression of FH and FHR-5 proteins, and as was assumed, the *CFHR3–1* deletion tagging variant resulted in a decreased expression of *CFHR1* protein. Other differentially expressed proteins with FDR <0.010 were prolactin receptor (*PRLR*) with decreased levels and Kirre Like Nephrin Family Adhesion Molecule 1 (*KIRREL1*) with increased levels.

In the GO analysis of molecular function annotation, FH, FHR-1, FHR-2, and FHR-5 were associated to the complement component C3b binding, opsonin binding, and complement binding at FDR < 3.7×10^{-05} level of significance. The Reactome pathway analysis showed that FH, FHR-1, FHR-2, and FHR-5 were associated with the regulation of the complement cascade, and with the complement cascade in general at FDR 1.0×10^{-03} level of significance.

4.3 The effect of donor polygenic burden for cerebrovascular diseases (Study III)

Study III focused on applying PRS of graft donors to understand how much donor genome composition affects the outcome of kidney transplantation. The study was executed as part of an international consortium that was able to analyze 6,666 kidney donors. Previous studies have established that organs from living donors generally have a superior outcome compared to deceased donors, so we hypothesized that the polygenic burden of kidney donors is significantly different between living and deceased donors, and between deceased kidney donors who died from different causes. We also hypothesized that distinct donor genetic architecture would have an impact on different clinical outcomes in transplant recipients based on the genetic architecture of kidney donors.

Cerebrovascular polygenic burden across donor types, and its impact on donor age of death

When comparing the mean PRS to living donors, we observed that donors who had died of stroke had a significantly higher polygenic burden for intracranial aneurysm (IA) and stroke (p-values of 3.733×10^{-08} and 7.562×10^{-07}, respectively) (**Table 14**). When comparing to healthy controls, we observed that donors who died of stroke also had a higher polygenic burden for IA and hypertension (p-values 2.525×10^{-06} and 9.453×10^{-05}, respectively). Also, when comparing to living donors, donors who had died of another cause had a significantly higher polygenic burden for stroke with p-value of 5.429×10^{-09}.

Table 14. Donor polygenic burden for each trait and donor type, and comparison of polygenic burden within each trait between donor types.

	PRS for each trait and each donor type Median (IQR)			
Trait	Healthy control	Living donor	Deceased donor (stroke COD)	Deceased donor (other COD)
IA	-0.005 (-0.677–0.683)	-0.055 (-0.713–0.628)	0.126 (-0.572–0.817)	0.012 (-0.682–0.631)
Stroke	0.002 (-0.671–0.678)	-0.163 (-0.695–0.448)	0.019 (-0.613–0.695)	0.096 (-0.622–0.774)
Hypertension	0.001 (-0.688–0.680)	0.106 (-0.498–0.599)	0.106 (-0.581–0.804)	-0.023 (-0.621–0.679)
Comparison of polygenic burden for IA between donor types among donors who died of stroke				

Comparison	Z	P value	FDR-adjusted P value
Deceased donor (other COD) - Deceased donor (stroke COD)	-4.298	1.726×10^{-05}	1.035×10^{-04}
Deceased donor (other COD) - Healthy control	-0.045	0.9638	1.000
Deceased donor (stroke COD) - Healthy control	4.729	2.525×10^{-06}	1.351×10^{-05}
Deceased donor (other COD) - Living donor	1.369	0.1711	1.000
Deceased donor (stroke COD) - Living donor	5.503	3.733×10^{-08}	2.240×10^{-07}
Healthy control - Living donor	1.518	0.1291	0.7744

Comparison of polygenic burden for stroke between donor types among donors who died of stroke

Comparison	Z	P value	FDR-adjusted P value
Deceased donor (other COD) - Deceased donor (stroke COD)	1.535	0.124	0.748
Deceased donor (other COD) - Healthy control	2.577	0.009	0.059
Deceased donor (stroke COD) - Healthy control	1.206	0.227	1.000
Deceased donor (other COD) - Living donor	5.833	5.429×10^{-09}	3.258×10^{-08}
Deceased donor (stroke COD) - Living donor	4.946	7.562×10^{-07}	4.530×10^{-06}
Healthy control - Living donor	3.881	1.041×10^{-04}	6.252×10^{-04}

Comparison of polygenic burden for hypertension between donor types among donors who died of stroke

Comparison	Z	P value	FDR-adjusted P value
Deceased donor (other COD) - Deceased donor (stroke COD)	-3.048	0.002	0.014
Deceased donor (other COD) - Healthy control	0.455	0.649	1.000
Deceased donor (stroke COD) - Healthy control	3.904	9.453×10^{-05}	1.001
Deceased donor (other COD) - Living donor	-1.151	0.249	1.000
Deceased donor (stroke COD) - Living donor	1.583	0.113	0.680
Healthy control - Living donor	-1.665	0.095	0.575

COD, cause of death; FDR, false detection rate; IA, intracranial aneurysm; IQR, interquartile range; PRS, polygenic risk score

The smallest effect size that could be reliably detected in donor age among donors that died of stroke, at 95% power and 0.05 level of significance, was 1.17 years (~14 months). Our results showed that increasing IA PRS resulted in a 0.57 year (~7 month) decrease in the age of death (95% CI: 1.04 – 0.11 years) among the donors who died of stroke (**Figure 10**). The result for the Finnish cohort (FRCBS in the Figure) was -0.84 (-1.71 – 0.03). Stroke and hypertension PRSs appeared to have no significant impact on donor age of death.

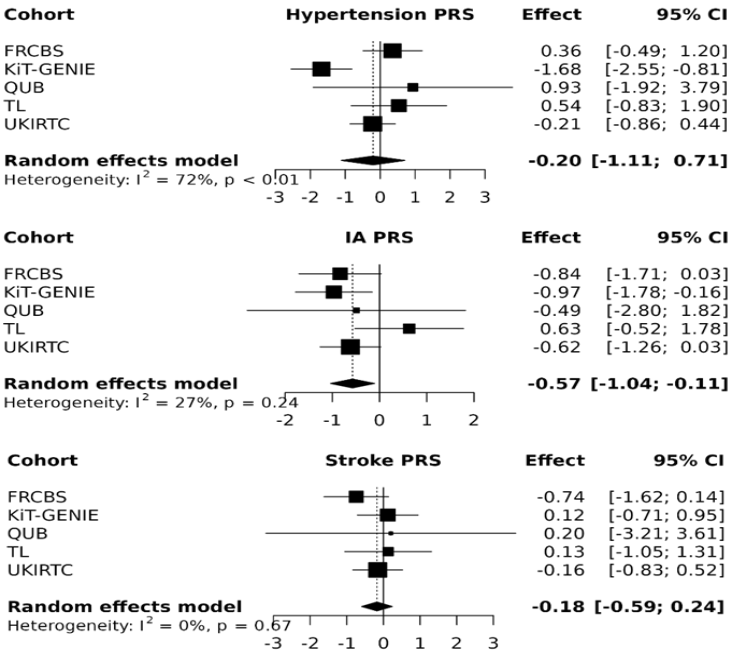

Figure 10. The influence of donor polygenic risk score on donor age of death among donors who died of stroke. Linear models for donor age of death in deceased donors who died of stroke (one model for each PRS: hypertension, IA, and stroke). CI, confidence interval; FRCBS, Finnish Red Cross Blood Service; IA, intracranial aneurysm; Kit-GENIE, Kidney Transplantation - Genomic Investigation of Essential clinical concerns; PRS, polygenic risk score; TL, Transplant Lines; QUB, Queen's University Belfast; UKIRTC, United Kingdom and Ireland Renal Transplant consortium. Figure reproduced from Publication III with permission of Journal of Nephrology.

Donor polygenic burden and impact on graft outcome and function

When investigating whether donor polygenic burden had an impact on recipient graft function, the smallest effect size that could be reliably detected in eGFR at 1-year after transplantation, at 95% power and 0.05 level of significance, was 1.39 mL/min/1.73m2. We found that both hypertension and IA PRSs were significant predictors of graft function, with estimated effect sizes of -0.96 (95% CI: -1.45 – -0.48) and -0.70 (95% CI: -1.16 – -0.24) (**Figure 11**). The increasing PRS value of hypertension resulted in an 0.96 mL/min/1.73m^2 decrease in eGFR at 1-year post-transplant, and an increasing IA PRS resulted in an 0.7 mL/min/1.73m^2 decrease in eGFR at 1-year post-transplant. The results for the Finnish cohort (FRCBS) were -0.59 (-1.94 – 0.77) for hypertension, and -0.83 (-2.29 – 0.62) for IA.

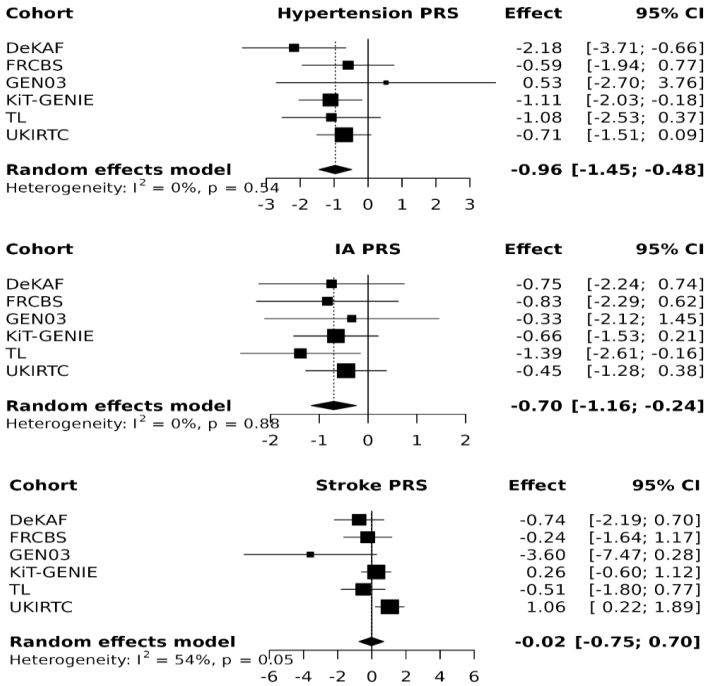

Figure 11. The influence of donor polygenic risk score on recipient estimated glomerular filtration rate at 1-year post-transplant. Linear models for recipient eGFR at 1-year post-transplant (one model for each PRS: hypertension, IA, and stroke). CI, confidence interval; eGFR, estimated glomerular filtration rate; FRCBS, Finnish Red Cross Blood Service; IA, intracranial aneurysm; Kit-GENIE, Kidney Transplantation - Genomic Investigation of Essential clinical concerns; PRS, polygenic risk score; TL, Transplant Lines; QUB, Queen's University Belfast; UKIRTC, United Kingdom and Ireland Renal Transplant consortium. Figure reproduced from Publication III with permission of Journal of Nephrology.

We also found significant differences in recipient eGFR at 1-year post-transplant between the patients with low, intermediate, and high polygenic burden for IA and hypertension (**Table 15**). The median value for eGFR in the high hypertension-burden patients was 51.14 mL/min/1.73m^2, whereas in the low hypertension-burden individuals the median was 55 mL/min/1.73m^2 (Bonferroni adjusted p-value: 0.005). For IA, the median values were 50.31 mL/min/1.73m2 and 54.66 mL/min/1.73m2 (Bonferroni adjusted p-value: 0.005). Donor kidneys with low polygenic burden for hypertension and IA resulted in a higher recipient eGFR at 1-year post-transplant by approximately 4 mL/min/1.73m2.

Table 15. The influence of donor polygenic risk score on recipient post-transplant eGFR at 1-year post-transplant compared between low, intermediate, and high risk for each trait (and 95% CI).

	PRS risk on recipient post-transplant eGFR at 1-year Median (IQR)		
Trait	Low	Intermediate	High
IA	54.66 (40.37–68.83)	52.89 (40.64–66.51)	50.31 (39.57–62.46)
Stroke	51.99 (50.60–62.91)	53.00 (40.39–66.68)	51.40 (41.09–66.08)
Hypertension	55.00 (42.17–70.00)	52.75 (40.60–66.16)	51.14 (37.97–64.09)

Comparison of polygenic burdens		
Comparison	P value	FDR adjusted P value
IA high-intermediate risk	0.004	0.008
IA high-low risk	0.002	0.005
Stroke high-intermediate risk	0.284	0.284
Stroke high-low risk	0.277	0.284
Hypertension high-intermediate risk	0.039	0.059
Hypertension high-low risk	0.001	0.005

CI, confidence interval; eGFR, estimated glomerular filtration rate; IA, intracranial aneurysm; IQR, interquartile range

5 DISCUSSION

This thesis examines genome level analyses as tools for extended histocompatibility. A Finnish, single-center cohort of kidney transplantation patients and their deceased graft donors was the primary study cohort, their genome variation was determined, and their roles in determining the outcomes of transplantation was estimated using various statistical approaches. The focus was on genome-level matching between recipients and donors, i.e., outside the HLA and ABO genes currently matched before transplantations, and on donor cerebrovascular genetic burden across donor types, and its impact on donor age of death and transplant outcome. Firstly, the current HLA and ABO matching is insufficient to predict transplant outcome; secondly, any difference in the kidney-related proteins may be recognized as immunologically foreign leading to harmful alloimmune attack against the graft; and finally, the current matching protocols do not reliably predict the severe complications of transplantation. Therefore, there is a clear need for novel, advanced prediction tools.

Study I was a genomic mismatch study that evaluated the impact of donor-recipient mismatch sum in transmembrane, secretory, and kidney-related proteins on rejection-free survival probability. It also evaluated the association between mismatches of gene deletions and time to acute rejection. Study II defined the boundaries of *CFHR* deletions, and additionally investigated gene and protein expression levels. Study III examined the effect of donor genetic burden of three cerebrovascular disease risk factors on donor age of death, graft survival, and graft function.

5.1 The effect of genome-level mismatching (I)

This study provided new information about genetic mismatches in kidney-related proteins and homozygous gene deletions in the *CFHR* locus, and their association with acute rejection. The relatively large cohort of adult recipients were transplanted in a single center in Helsinki, Finland covering the whole Finnish population.

Previous studies have already demonstrated that mismatches and gene deletions between donor and recipient have an impact on kidney transplantation outcomes and our study supports these findings. Genomic mismatches can indeed be potential, novel histocompatibility factors in kidney transplantation.

The hypothesis for this study was that any immunogenic protein-level difference between donor and recipient genome could lead to alloimmune reactions and increase the risk of complications after transplantation. In kidney transplantation, the focus must naturally be on differences in proteins expressed or occurring in the kidney. To further refine the analysis, one should include the HLA alleles and their ability to bind different antigenic peptides in each transplant pair. There are no such studies yet performed in kidney transplantation, however, Ritari *et al.* [219] introduced this type of approach in stem cell transplantation. Reindl-Schwaighofer *et al.* conducted a study on missense variant mismatches in transmembrane and secretory proteins, showing association with death censored graft loss in a cohort of 477 kidney transplant pairs. We replicated their approach but could not find support for their result in the Finnish cohort. The graft loss events observed in our study cohort were 68/1025 (6.6%), while the number was 52/477 (11%) in Reindl-Schwaighofer's study cohort. This is one possible explanation for discrepant results. It may also relate to various clinical and treatment differences between the populations. Instead, our results provided evidence that the higher mismatch sum in kidney-related proteins was associated with decreased acute rejection-free survival when dividing the mismatch sum into quartiles. It is possible that proteins expressed in the kidney could 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 non-self. In addition to the study of Reindl-Schwaighofer, others have reported that mismatches between donor and recipient were associated with long-term survival [70]. Based on these findings, it could be assumed that the overall alloantigenic load on the donor kidney could not only have an impact on acute rejection, but even more strongly on long-term outcomes. One example is the late antibody-mediated rejection and thus late graft failure, which is considered a multifactorial process that involves immunological factors related to the donor-recipient genetic mismatch [220].

Steers et al. [69] evaluated the association of common deletion-tagging variants with kidney allograft rejection. The hypothesis was that a recipient whose genome lacks a kidney-related gene product due to a gene deletion should raise an alloimmune reaction toward a graft from a donor whose genome carries at least one functional copy of that gene, hence expressing the protein in the graft. They reported an association of homozygous *LIMS1* gene

deletion and allograft rejection. We were unable confirm their *LIMS1* findings, instead, we found that rs7542235 GG genotype tagging for homozygous deletion in *CFHR* gene was associated with acute rejection in the Finnish cohort. It may be that many different single homozygous deletions confer susceptibility to alloimmune reaction, and the exact significant example varies between study cohorts. The genetic makeup across the Finnish population is also quite unique with a relatively small number of founder individuals and strong genetic isolation over centuries, and thus differs from other populations [221,222]. For example, the minor allele frequency (MAF) in our study cohort was 0.31 for the *LIMS1* variant rs893403, while it was 0.42 in the Columbian study cohort [69]. For *CFHR*, the MAF in our study cohort was only 0.14, while it was 0.22 in the Columbian cohort. Our study cohort also had only 15 (1.5%) individuals with rs7542235 GG genotype. Clearly, larger collaborative studies including different populations are needed. It would be possible to estimate the risks associated with gene deletions solely from the patient genome early before transplantation. Namely, those patients who have a homozygous deletion most likely are going to get a transplant from donor with a functional gene, hence resulting in a mismatch. In living, related donor transplantations the match can be better.

5.2 *CFHR1* deletion and expression studies (II)

As we found in Study I that rs7542235 variant mismatch predisposed to rejection, we wanted to dissect whether the effect is related to the missing protein as such and alloimmune reaction against it, or whether the gene deletion has more direct effects. The first option, alloimmune response would require prospective collection of blood samples from patients with both the homozygous deletion and rejection, something we could not execute within reasonable time limits. We hence restricted our analyses to the latter. We first confirmed that the rs7542235 indeed tagged the deletion by using genomic sequencing and MLPA techniques in samples assumed to have homozygous, heterozygous, and no deletions. The results confirmed that rs7542235 allele G was in a strong linkage disequilibrium with deletions at the *CFHR* locus. However, we do not know whether all deletions in that locus always occur with the tagging SNP. We then studied the effect of this variant on gene and protein expression levels to see whether the variant or gene deletion regulates expression levels of other genes.

The results showed that kidney transplantation patients with GG genotype of deletion-tagging variant rs7542235 have deletions of various sizes in the *CFHR* locus. All of them shared a deletion encompassing the whole *CFHR1* gene, however, pointing to its primary role. These patients also lacked the FHR-1 protein in their serum, as could be assumed. Based on the previous knowledge that SNPs and structural variations in the genome can have an impact on transcriptional regulation and the discovery of gene networks or other pathways [223], we utilized both eQTL databases and proteomics studies to see whether the rs7542235 allele G has an impact on gene expression and protein levels of not only the *CFH/CFHR* locus, but others as well. We found that rs7542235 G genotype was associated with decreased levels of FHR proteins and increase in FH levels. Additionally, proteomics studies showed that the variant was associated with levels of many proteins related to the immunology of kidney disorders as explained in more detail below.

The 15 patients with a *CFHR1* gene deletion shared no particular type of diagnosis, hence, we conclude that it was not associated with underlying diseases. Most of the diseases found in deletion homozygotes have been reported to lead to an increase, rather than decrease of FHR proteins. None of the diseases were strongly related to the complement system. Previous studies, however, have shown that *CFHR3—1* deletions are linked to several complement-mediated diseases [224–227].

Based on previously published results, deletion of *CFHR1* and *CFHR3* could lead to the formation of anti-FH antibodies [200,228]. Our first study showed no *de novo* anti-FH antibodies in the serum samples of recipients with rs7542235 GG genotype receiving a graft from a donor of either AG or AA genotype. One limitation of this study was, that we could not study the anti-FHR-1 antibodies, so there is a possibility that FHR-1 could become the target of alloimmunity.

As shown by the eQTL and pQTL results, we may assume that the homozygous deletion of *CFHR1* impacts the expression levels of other *CFHR* genes and proteins, and thus could alter the complement activation via other complement-related genes. The rs7542235 allele G was associated with an increased level of FH and decreased level of FHR-1. The function of FH is to inhibit the complement activation and to protect human endothelial cells from damage, FHR-1 has an opposite effect. In the absence of FHR-1 and FHR-3, local FH binding and

activity could be increased, resulting in suppression of complement activation, but also, on the other hand, possibly to an impaired ability to handle injured tissue components.

FH is a complement control protein, inhibiting the alternative pathway of complement activation. Like FH, the FHR-1 and FHR-3 proteins act as complement regulators. *CFHR* genes have been associated with several diseases, including atypical hemolytic uremic syndrome, C3 glomerulopathies and IgA nephropathy. Considering the function of *CFH/CFHR* genes, our finding might be related to either the primary kidney disease diagnosis of the transplant recipients or the outcome of transplantation.

Although the function of FH is well established, the detailed biological role of the other five FHR proteins is still under investigation. It is likely that FHR-5 has a role in the processing of complement within the kidney because it has complement regulatory activity in vitro [229], it co-localizes with complement deposits within the kidney [230], and a FHR-5 mutation is associated with familial complement-mediated glomerulonephritis [231]. While FH regulates the C3 convertase and promotes degradation of C3b and opsonization of foreign pathogens with iC3b that results in phagocytosis, FHR-1 competes with FH for these activities. FHR-1 thereby increases the complement activation, and thus indirectly promotes C3/C5 convertase activity and complement membrane attack complex assembly. By inhibiting FH binding to C3b, FHR-1 promotes C5a formation and consequent inflammation. In the absence of FHR-1 and FHR-3, local FH binding and activity would be increased, resulting in suppression of complement activation, but possibly also in an impaired ability to handle injured tissue components. Although FHR-1 and FHR-3 have complement regulatory activity [232,233], both have also been considered biologically redundant because their complete absence in healthy populations is common. For example, the frequency of combined homozygous deletion of both *CFHR1* and *CFHR3* genes (Δ*CFHR3–CFHR1*) is 4.7% in European-American and 15.9% in African-American populations [234].

The deletion of *CFHR3–1* has been associated with protection against IgAN [225], whereas a hybrid form of the *CFHR3–1* gene has been associated with an increased risk for C3G [224].

The capillaries of kidney glomerulus are continually perfused by large volumes of blood, so the FH/FHR complement proteins present in plasma are in direct contact with the membrane. Because of the filtration of water into urine, the concentration of complement

proteins increases as plasma flows through the kidney, thus exposing these glomerular cells and surfaces to higher concentrations of FH/FHR proteins than are found elsewhere in the body. This could explain why the kidney is particularly susceptible to damage by complement [235].

Because the capillaries of kidney glomerulus are continually perfused by large volumes of blood, the FH/FHR complement proteins present in plasma are in direct contact with the membrane. Since the kidneys filtrate water into urine, the concentration of complement proteins increases as plasma flows through the kidney, exposing these glomerular cells and surfaces to higher concentrations of FH/FHR proteins than are found elsewhere in the body. This could explain the relative sensitivity of kidney to any complement deficiencies. [235]

We identified other proteins of potential interest in the proteomics data. Prolactin (*PRL*) is an endocrine hormone that functions in immune system regulation and has anti-inflammatory effects. PRL can trigger the production of proinflammatory cytokines, but it can also have anti-inflammatory effects that can reduce hyperinflammation. It functions through PRL receptor (*PRLR*). [236] KIRREL1, or NEPH1, is a member of the NEPH protein family and is expressed in kidney podocytes, cells involved in ensuring size- and charge-selective ultrafiltration. Mutations in *KIRREL1* gene have been associated with hereditary nephrotic syndrome. [237] These findings could support the evidence that lack of *CFHR1* and deficiencies in complement function could have an impact on the function of other important immune- or kidney disease-related genes.

5.3 Donor polygenic burden (III)

We investigated in a large study cohort of 6,666 European ancestry adult kidney donors the impact of donor polygenic burden of cerebrovascular traits on donor age of death and recipient graft outcomes. The results showed that deceased donors have an elevated genetic burden for these traits compared to healthy subjects and living donors. This study is important considering that previous reports have shown the cause of death being independently associated with graft outcome [15,16]. Our results support these findings, demonstrating that ischemic stroke as the donor cause of death has an impact on worse transplant outcome. It is also well known that transplantation from living donors generally has a better outcome compared to those from deceased donors [14], so it is no surprise that

deceased donors had elevated burden for all cerebrovascular traits compared to living donors. Our study provides completely new results in this area, since this was the first time that cerebrovascular genetic burden was examined in transplant donors. We found that cerebrovascular polygenic burden significantly predicts the donor age of death among donors who died of stroke. It is of note that the donor PRS was associated with graft function at 1 year after transplantation.

As GWAS will continue to expand to more diverse and larger populations and rarer variants, PRS will also be able to explain more of the long-term survival of kidney transplants, and thus play a role in transplant allocation in the future. Especially those recipients who receive a kidney with a high PRS for hypertension or IA, for instance, would benefit from more detailed follow up after transplantation.

Immunological mechanisms have been the most plausible reason for graft loss and other complications of transplantation. Unlike most other studies, our current analysis is independent of immunological factors and demonstrates that donor genetic mechanisms associated with cerebrovascular diseases influence long-term graft function.

With support from the results of other two subprojects of this thesis, we may conclude that several genetic factors in the donor and recipient genomes might have an impact of different transplant outcomes.

5.4 Strengths and limitations (I-III)

Our study consisted of relatively large single-center and single population cohort of 1025 kidney transplantation recipient-donor pairs, thereby reducing the impact of confounding factors. We had a comprehensive view of patient clinical data, and great genomic data available. We also had a strong collaboration with clinicians and other research groups to gain even deeper knowledge on each study.

Our study also carries some limitations. In Study I, a relatively low number of recipients suffered a graft loss, which is an important clinical endpoint, so we could not reliably estimate the association of mismatches to it.

In the deletion mismatch analysis, we performed a multiple-comparison correction using Bonferroni correction like Steers *et al.* did in the previous study. The P-value of our result

did not meet the Bonferroni-corrected level of significance which might be due to overall low frequency of deletion-homozygous patients in our cohort, so larger cohorts are needed to gain more power. Also, when testing the multiple-comparison, the Bonferroni correction is one of the most conservative methods for probability thresholding. Instead, another method, like Benjamini-Hochberg, would be less sensitive to use.

Study II included only 15 individuals with the genotype we were interested in, which makes the interpretation of the results difficult. Also, as we could not investigate the anti-FHR-1 antibody response, we were unable to dissect whether it was a target of alloimmune reaction.

Study III included individuals who had received a kidney graft before the year 2000. On the other hand, it gave us more follow up time for some recipients, but also the protocols of transplantations have changed a lot during the years. There have been significant improvements from immunosuppression to organ preservation. However, the year of transplantation was accounted in the statistical analyses as one of the covariates. We also lacked some covariates, such as graft quality and eGFR data at 5 years. As several study cohorts were involved, it is understandable that all data was not similarly available from all centers. Larger cohort size would certainly result in more powered GWAS and PRS estimates but usually at the same time result in more heterogeneity.

6 CONCLUSIONS

To conclude the results of the three subprojects, this thesis offers information about new genetic risk factors associating with kidney transplant outcomes. It supports the idea that not only HLA and ABO genomic differences between a donor and a recipient influence the transplant outcome. A few other well conducted larger studies have suggested effects mediated by non-HLA genes and their mismatches. However, no single gene with well confirmed effect has been identified. The polygenic risk estimates may provide a more reliable and universal tool, but they also require further development. Large consortia, such as the iGeneTRAIN (http://igenetrain.org/), are needed—the transplantation genomics team is currently collaborating with the iGeneTRAIN.

The analyses of genome-level mismatches may be useful tools in prediction of transplant outcome. It could be possible to estimate the complication risk due to gene deletions directly from patient genome data before transplantation if grafts from unrelated donors who most likely lack the same homozygous deletion are transplanted.

Different deletion types found in patients with *CFHR*-deletion also shared the complete deletion of the *CFHR1* gene pointing to its primary role. Plasma proteomics studies showed that this deletion is associated with altered expression of not only FH/FHR proteins, but also other proteins related to immune mechanisms and kidney diseases. This leaves room for their role, rather than the deletion as such, in the associated rejection risk.

Our results also demonstrate the impact of inherited factors associated with donor death on long-term graft function. Furthermore, the genetic differences in non-immunological factors between living and different types of deceased donors explain some of the differences in long-term graft outcomes between these different donor types.

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Helsinki, September 27th

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