2023

HLA epitope mismatch analysis in kidney transplant patients

BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

HLA EPITOPE MISMATCH ANALYSIS IN KIDNEY TRANSPLANT PATIENTS

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B.A., University of Maryland, College Park, 2021

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2023

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ACKNOWLEDGMENTS

I would like to thank Dr. Krummey and Dr. Henderson for their help in this project. I would like to further thank Gregory Cohen and the Johns Hopkins Immunological HLA Laboratory for their help in data collection and analysis.

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ABSTRACT

End Stage Renal Disease is the most common cause of kidney failure and is the most common diagnosis for the receival of a kidney transplant. Improvements in diagnostics, immunosuppression, post-operative monitoring, and organ allocation have allowed the procedure to become widespread in treating End Stage Renal Disease and have led to excellent post-operative outcomes. However, a disparity exists in access to and success of kidney transplantation between different racial and social groups. Success 10 years post-op has also been variable and demonstrates the need for further research in donor/recipient immune matching and immunosuppressive therapies. Human Leukocyte Antigen (HLA) Mismatch Analysis is the study and comparison of biomarkers between donors and recipients to ensure a "match" to prevent an immune response to foreign tissue. Eplet mismatch analysis further studies specific regions of these HLA proteins, which can help further ascertain the risk associated with matches. While epitope mismatch analysis has shown promise in predicting the risk of rejection post-transplant, there are many questions about its clinical use with low-resolution genotyping that is used for kidney transplantation. Comparing two surrogate groups that underwent either method of analysis, it was found in greater than 90% of pairs analyzed the immunological risk categorization resolution from molecular mismatch remained the same. A comparison was also made to understand the effect of racial classification on the number of epitope mismatches, which was also found not to have a significant difference among

racially concordant and discordant groups. Such information provides an important look into the utility of tests done to determine the most effective donor/recipient match. The minimal difference between imputation and high-resolution genotyping has shown that low resolution genotyping can be used to provide clinical risk classification based on epitope mismatches.

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BACKGROUND

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According to the Centers for Disease Control and Prevention, 1 in 7 people in the United States are estimated to have chronic kidney disease (CKD), and according to the United States Renal Data System Annual Report of 2020, it is estimated of that 37 million about 786,000 patients progress to ESRD.[1] [2] Kidney transplants, the most common procedure for the treatment of ESRD has provided a renewed quality of life and chance of survival for these patients.[3] About 90% of transplants survive past one year, with this trend improving over time. [4]

ESRD is most commonly caused by diabetic nephropathy, with nearly 30% of all diabetic patients worldwide having at least one type of this nephropathy. This is due to the persistent hyperglycemia present in the body that gradually overwhelms the body's ability to process toxins and other wastes through our body leading to "glomerular lesions".[5] In addition to these lesions, the build-up of waste in the body leads to additional injury. Diabetes remains the leading cause of CKD, but other secondary causes include hypertension, vascular disease, glomerular disease, urinary tract infections, recurrent kidney stones, and acute kidney injury.[6] Regardless of the cause, over time, the build-up of toxins and lesions leads to a gradual decrease in kidney function and then renal failure.[6] At first, this progression is asymptomatic despite injury to the nephron, due to the kidney compensating through hypertrophy and hyperfiltration. Ultimately this

adaptive mechanism will further renal dysfunction through the destruction of capillaries and other structures that further comprises kidney function.[6] As it progresses, CKD is associated with a low glomerular filtration rate and a high amount of albumin in the urine (proteinuria). It is also associated with a glomerular filtration rate of 60 ml/min. ESRD is associated with a glomerular filtration rate of less than 15 ml/min. [6] Ultimately, the disease can progress to complete renal failure, where the kidney can no longer process toxins and waste from the body. At this point, patients must rely on replacement therapy such as dialysis or a kidney transplant to survive.

At the point of ESRD, patients present with a characteristic set of symptoms, including increased water/volume retention, hypertension, mineral imbalances, and metabolic acidosis.[6] These symptoms may also present as edema, high blood pressure, anemia, fatigue, and cognitive deficits.[6] Preventing the reduction in the quality of life that comes from the progression of CKD into ESRD is an important reason to manage these symptoms as they appear, ideally in the earlier stages of the disease. Clinicians achieve this by managing hypertension and edema that is caused by hyperfiltration and fluid retention, as well as monitoring glucose levels to prevent further damage to the kidneys, especially in diabetic patients who are at higher risk of developing complications from CKD/ESRD. [5] Metabolic acidosis, which is characteristic of later stages of CKD transitioning into ESRD, must also be treated as soon as possible.

At a certain point, for some patients, the symptoms associated with their progression of kidney disease reach a point of no return, making long-term renal replacement therapy, or renal dialysis necessary. These indications include severe metabolic acidosis, pericarditis, encephalopathy, and peripheral neuropathy.[6] The most common form of this is either through dialysis or a kidney transplant, with the latter being the most effective. Currently, approximately 71% of patients with ESRD are currently being treated with dialysis, while only 29% have received a kidney transplant. [2] Kidney transplants are an excellent and cost-effective method in treating irreversible chronic kidney disease (ESRD Stage 5), that allows an increase in quality of life for the patient and dramatically prolongs life expectancy. [2 7] It is especially effective the earlier in treatment a transplant is received.[8]

ADVANCEMENTS IN SOLID ORGAN TRANSPLANTATION

While transplantation has not always been the gold standard in treating kidney failure, over the past sixty years, tremendous advancements have been made in the field of solid organ transplantation. Advances in surgical techniques, clinical management, and laboratory testing have dramatically improved outcomes of transplantation of kidneys from deceased and living donors.[7]

The idea of using tissue from one's own body or another has existed for hundreds of years, with many early physicians claiming successful transplants of ears, arms, and legs. Early unsuccessful attempts in 1906 were made at transplanting kidneys from pigs

and goats into humans.[9] However, it was not until 1869 when the potential of successful grafts was discovered by Jacques-Louis Reverdin, who uncovered the potential for grafts to heal on their own and thus be used in treating burns, ulcers, and open wounds.[10] Into the twentieth century, the fundamentals of transplant immunology began to shape the reasoning around graft matching. Transplant immunologist Georg Schöne discovered that after initial graft rejection by the body, subsequent grafts from the same donor would be rejected more rapidly than the first. [10] This demonstrated how the body would recognize foreign matter, attack it, and retain this memory for re-exposure. Once re-exposed to the same foreign bodies, the body responded better in attacking that tissue leading to faster rejection. This anchored the idea that the survival of a graft depended on the "lymphoid" system, which attacks this foreign tissue. To remove the cells that attack the graft is to prolong the survival of such graft and this is where researchers turned to by the end of the 1920s.

The "prelude" to the hallmark discoveries of transplant immunology was a discovery made by scientists Peter Medawar and Rupert Billingham. They transplanted a skin graft from fraternal twin cows that had remarkable success This was hypothesized to be because of the exchange of red and white blood cells in utero, which allowed each to establish immunologic tolerance.[10] Further investigations set up to induce tolerance in graft transplantation made it evident that the immune system plays a central role in graft tolerance. Other studies published at the same time demonstrated the role of lymphocytes in moving throughout the body to target specific tissues for attack.[10]

The first successful human kidney transplant was done in 1954 using an identical twin donor that bypassed the barrier of rejection seen with other "non-self" grafts.[9] Soon after, it was found that weakening the immune system of mice allowed better acceptance of bone marrow grafts. This prompted physicians and researchers to explore this mechanism to prevent transplant rejection in humans.[10] Joseph Murray, the physician who performed the first human kidney transplant, took this idea and irradiated human kidney recipients with "lethal total body irradiation" and donor bone marrow. It proved to be largely unsuccessful, as 11 out of 12 patients died soon after irradiation and receipt of the bone marrow transplant. However, the sole survivor maintained the "adequate" function of a transplanted kidney he had received from his fraternal twin brother. Soon after, in 1960 and 1962, transplants between unrelated donors and recipients were accomplished using total body irradiation.[10] Such studies demonstrated that the need for a total genetic matching was not necessary.

A number of drugs have been studied for their utility in transplantation. Immunosuppressants such as 6-Mercaptopurine (6-MP) and nitrogen mustard have been used as potential drugs in oncology. In 1959 Robert Schwartz and William Dameshek at Tufts University found that 6-MP reduced antibody responses and that the drug could extend the survival of skin homografts. [10] A 6-MP derivative, azathioprine, was successfully used to prolong kidney transplant survival in dogs, however similar experiments in humans proved unsuccessful.[9]

Scientist Tom Starzl successfully combined azathioprine with prednisone, leading to over 70% one-year survival of kidney grafts in humans.[10] The key was to find a balance in targeting the immune response that prevented the acceptance of a donor graft while protecting the body's essential immune functions to survive.[9] This significant advancement led to the opening of fifty other transplant centers in the US within a year, with the "Starzl cocktail immunosuppression" used for almost the next two decades. [11]

As kidney transplants entered the mainstream, developments made in organ preservation and donor organ allocation also allowed for better outcomes. Such progress allowed for the successful transplants of other solid organ transplants such as liver, heart, and pancreas.[10] Tissue matching for kidney transplants began in 1958 when the first human leukocyte antigen (HLA) was discovered. Antibodies for these antigens were further identified, and a methodology was developed to test for these antibodies.[10] Scientist Paul Terasaki developed a micrototoxicity assay that became the standard for all transplant centers in the United States. The assay predicted the chances of hyperacute rejection and greatly advanced the assessment of donor-recipient compatibility.[10]

Developments in immunosuppression furthered the success of solid organ transplantation with the improvement of different drugs. Anti-lymphocyte serum, first clinically used In 1963, was used to mitigate cellular immunity and prolong skin graft survival significantly. The calcineurin-inhibitor cyclosporine prevented T cell

proliferation and considerably dampening the immune response to allogeneic tissues. [9] In the 1990s, tacrolimus replaced cyclosporine as the standard for immunosuppression into the modern era. [10] Drugs such as cyclosporine proved to be more effective than azathioprine, improving the one-year survival rate from 50% to 90% and leading to a significant decline in acute rejection episodes.[12]

While advancements in procedure, technique, organ cultivation, and immunosuppression have led to successful post-year outcomes of kidney transplants, yet such regimens necessary for graft survival come with significant side effects. Recent efforts have been made to enhance current diagnostics for the evaluation of using "big data" and non-invasive techniques.[7] Greater understanding of the immune response in graft rejection can help prolong kidney graft survival while minimizing the risk of side effects in the future.

SHORT AND LONG TERM OUTCOMES AFTER KIDNEY TRANSPLANTATION

The current standard of care has allowed kidney transplant recipients to have excellent short-term and long-term outcomes, with the five-year survival rate of kidney transplant recipients jumping to 74%.[13] With expanding eligibility and more patients receiving the procedure, mortality and graft loss have decreased, especially in older populations.[14] However, despite this, kidney transplant failure is the fourth leading cause of ESRD, citing the importance of rejection in determining the prognosis of the disease.[15]

North Americans have the world's highest share of ESRD patients, and about 4% of all kidney transplant recipients reside in North America or Europe.[13] In 2020, about 22,817 kidney transplants were conducted in the United States, with another 90,201 on the waiting list for a kidney.[2] Transplants conducted with live donor kidneys produced better outcomes than the more common deceased donor transplant. [4] The current five-year survival rate for patients with live donor kidneys is at 86.1%. [4] In fact, patients who undergo a re-transplant do not face lower survival rates.[4] Preemptive transplants have better post-transplant outcomes than patients who have undergone dialysis first, with even six months on dialysis leading to a 17% higher risk of rejection. [4]

Long-term outcomes for kidney transplant patients are worse in the United States than in other high-income nations. [4] Attention has thus turned towards what leads to chronic rejection and how to prolong the longevity of such grafts. Many factors contribute to short-term and long-term graft survival, such as patient age, patient comorbidities, use of dialysis, and CKD progression before kidney transplant. Chronic graft loss (can be attributed to patient death, delayed acute rejection, drug nephrotoxicity, and recurrent kidney disease. [12] Chronic Allograft Neuropathy (CAN) comprises chronic rejection and associated donor vascular disease and is seen as the leading cause of renal dysfunction and ultimate graft loss in such patients. Often CAN is characterized as a progressive decline in renal function associated with hypertension and proteinuria.[12] It was found that upwards of 81% of graft failures 10 years after were due to CAN. For

patients that returned to dialysis, CAN was seen as the root cause for 86.3% of these cases.[12] In a prospective cohort study examining the causes of kidney failure in transplant patients, the four major causes include chronic rejection (CAN), glomerulonephritis, polyomavirus nephropathy, and intercurrent events. Among these four CAN was seen to be a leading causes of kidney failure[15] [16]

CAN is a disease with multifactorial origins, including immunological and non-immunological risk factors promoting chronic rejection.[17] Examples of immune risk factors include HLA mismatch, inadequate immunosuppression, elevated levels of antibodies, re-transplant and multiple episodes of acute rejection.[12] A study published in the *American Journal of Transplantation* found that T cell-mediated rejection, acute kidney injury, drug toxicity, fibrosis, and patient non-compliance all played a role in ultimate kidney transplant failure.[15]

CAN can occur in the absence of immunological risk factors, meaning that risk factors such as race, age, sex of recipient, donor status, hypertension, high triglycerides, infection, and neurotoxicity can all play a role in chronic graft rejection.[12] For example, based on demographics alone, Caucasian and African American recipients, males, older individuals, and teenagers are more likely to face graft rejection than their counterparts.[12] The status of a donor compared to the recipient also matters in what researchers call a "fit-match" in which donor kidneys should go to a patient who is similar in age and size, with donor size playing a crucial role in graft survival.[12] Prolonged time on dialysis is

also connected to a decreased chance of graft survival with patients on dialysis for 24-36 months at a 68% increased risk of graft rejection. [12]

Prevention and treatment for the related conditions that contribute to both short-term and long-term rejection of kidney grafts must be addressed to maximize the benefit of kidney transplants for ESRD patients. In addition to medical factors that go into determining the success of a transplant, non-medical factors such as patient non-compliance, allocation of organs, and healthcare coverage play a role in the disparity of success between different patients and different nations. [4] [15]

RACIAL DISPARITIES WITHIN ESRD AND KIDNEY TRANSPLANTS

There remains a tremendous racial disparity in the prevalence of ESRD and kidney transplantation. According to the Centers for Disease Control and Prevention, CKD is more common in non-Hispanic Black adults (16%) compared to their white (13%) and Asian (13%) counterparts.[2] [1] For every Caucasian person that develops ESRD, three African Americans have also developed the disease.[2] Even in organ allocation, African American patients have an average longer wait time than their Caucasian counterparts.[2] However, the gap between Black and white patients is narrowing with the rate of deceased kidney transplantation increasing among Black patients bringing it to the level among white patients. Nevertheless, Black patients still lag in the rate of living donor transplantations done.[18] Black patients are less likely than their white counterparts to receive a kidney transplant despite having a higher incidence of CKD.[19] Barred from

the positive outcomes of a kidney transplant, Black patients and other health minorities have a high mortality rate because of ESRD. [20] [21] Even after controlling for other social determinants of health, Black patients are less likely to receive a kidney transplant.[19] Factors such as low income, being Black, being on public insurance, higher body mass index (BMI), high religiosity, and poor social support are all associated with lower kidney transplant rates. [19]

In addition to larger systemic issues such as genetic, occupational, social, behavioral, and environmental factors that affect the likelihood of an ESRD patient being able to access a kidney transplant, immunologic factors also play a role in contributing to the health disparities between different ethnic groups.[20] For example, proper HLA matching is a primary reason for success in any graft transplant; however, Black patients (controlled for other comorbidities) lagged in success from all other racial groups.[20] Due to racial differences in the number of alleles found at each loci and the basis of HLA compatibility based on specific racial populations, white patients are more likely to find a more accurate match than other racial and ethnic groups.[20] It was also demonstrated from bone marrow registries that African Americans are more "polymorphic" regarding HLA and are less likely to find an optimal match dues due to the current small registry size.[20] It is also seen that irrespective of donor type, differences in outcomes between racial/ethnic groups become more pronounced further out from surgery. Previous studies showed that Black patients have a more potent immune response, with higher levels of co-stimulatory molecules being suggested.[20] [21] Research into the differences in immune response among different racial groups is currently incomplete, contributing to the racial disparity seen

with kidney transplants. It will be important in the future that efforts are made to bridge the gap in access to kidney transplants and shift away from a "one size" fits all approach in determining optimal HLA matching and immunosuppression protocols. While immunological disparities play a significant role in acute graft rejection, non-immunological factors are more prominent in chronic graft rejection.[21] Addressing both by increasing access to treatments and furthering research into race-specific graft outcomes will help close the gap in the future.

ALLOCATION OF ORGANS FOR TRANSPLANT

With the rise of CKD and subsequent ESRD, the demand for kidney transplants has steadily risen. For many patients, access to a kidney transplant is the only way to reverse their course from kidney failure. As transplantation has become the standard of care since it provides the best health outcomes, getting access to donor organs has become more complex. The demand is evident because living donor kidney transplants have increased from 3668 in 1996 to 6563 in 2005 in the United States.[14] In 2022, 25,498 total kidney transplants were carried out, demonstrating how the demand will continue to rise in the coming years. [22] For this reason matching healthy and viable donors to their optimal recipients is especially important. Researchers in the community have been trying to find ways to expand the donor pool, not only in the number of donors available but also in the number of recipients a donor kidney can reach.[14]

Currently, the most common reason for a kidney transplant is glomerular disease; however, the rates of diabetes mellitus and hypertensive nephrosclerosis have increased among kidney transplant recipients.[14] Generally those that receive kidney transplants tend to veer towards older recipients. Between 1990 and 2011 the percentage of recipients aged 50-64 had increased from 33% to 41%, and for patients aged 65 and older, that figure had increased from 6% to 11%.[14] In terms of race the amount of deceased-donor kidney transplants done has equaled out between white, Black, and Hispanic patients.[18] However white recipients make up the majority of live-donor kidney transplants accounting for 66% of such procedures in 2005 where African Americans and Hispanics make up only 6% and 11% of live kidney transplants respectively.[14] As previously discussed, the disparity in access to kidney transplants is especially evident for African Americans with a higher incidence of disease.[14]

Due to the scarcity of these procedures, transplantation used to be done with donors local to each individual transplant center.[23] As the popularity of kidney transplants started to spread and to deal with the demand for kidney transplants and to make sure organs are allocated ethically and effectively, the United States Congress passed the National Organ Transplant Act in 1984, which led to the creation of the Organ Procurement and Transplantation Network (OPTN) under the management of the United Network for Organ Sharing (UNOS).[24] This system stayed in practice for the better part of three decades, had lead to a nationwide network of organ sharing making it easier for

patients to find proper matches. However with an increase in demand for the procedure and stagnant supply of kidneys the system struggled to keep up.[24] [25]

In 2014 the OPTN devised its framework to be more equitable, decrease wait times in receiving a kidney, and produce optimal matches that ultimately prolong the longevity of a kidney transplant.[26] The new system has ultimately led to greater utilization of available kidneys, increased the number of transplants in general and prolong the half-life of a kidney graft.[26] In addition, the changes have led to an increase in the number of African American patients receiving kidneys helping to close the disparity seen beforehand. The focus went from providing a kidney to the patient waiting for the longest to incorporating other factors, such as HLA matching criteria and prioritizing more urgent cases.[23] Another shift included maximizing the utility of organ allocation with equitable access for all patients involved.[23] Other factors taken into consideration include donor age, height, weight, ethnicity, history of hypertension, diabetes, cause of death for donors, serum creatinine, Hepatitis C status, and donation factor circulatory death status.[22]

The process of donating a kidney starts with the donor, where hospital staff and the organ procurement organization (OPO) refer patients to become donors. Legal consent is taken from the patient or family of patients for deceased donor donations.[24] Testing is then done to determine the donor's ABO blood group status, infectious disease, and histocompatibility results. Matching is then done by the OPTN

where a "Kidney Donor Risk Index" score is given to determine the success and viability of the donor organ. [24] A matching algorithm that considers various favors such as immune sensitivity, location, and blood type matching is run. Centers are then notified, and organ-matching information is further narrowed to the specific patient. The kidney is then shipped to the transplant center as fast as possible in order to minimize cold ischemic time .[24] Multiple factors and processes come together to match a donor with a recipient to ensure patients can get off the waitlist as soon as possible while maximizing the success of the transplant by ensuring an effective match.

THE ALLOIMMUNE RESPONSE

A hallmark of any solid organ transplant's success is the understanding and manipulation of the body's immune response to foreign tissue. The response is initiated when a major histocompatibility complex present a peptide antigen to T cells, which allows the immune system to detect foreign protein whether it be from non-self tissue or pathogens. The activate of T cells is what leads to the creation of antibodies, and further proliferation of the immune response by other immune cells that leads to rejection.[25] While matching major histocompatibility complexes (MHCs) can help decrease the rejection rate, differences in genetic polymorphisms can be recognized as foreign and trigger an immune response.[14] It is by understanding this mechanism further that immunosuppressive therapies can be further developed to promote the success of such transplants.

The MHCs or human leukocyte antigens (HLA) are genes that code for transplant antigens located on chromosome 6 which also follows a co-dominant pattern of inheritance.[25] HLA cells comprise of class I and class II molecules. Class I molecules include A, B, and C and are present on all nucleated cells.[14] [26] Class II antigens include DP, DQ, and DR and are present on all antigen-presenting cells such as dendritic cells, macrophages, and B cells. [25] [26] These different antigen presenting cells are responsible for the proliferation of cytokines that cause an inflammatory response in endothelial and vascular tissue that leads to rejection.[25] The matching of these antigens in donor and recipient is essential for the success of such a graft. Regarding kidney transplants, specific attention is paid to matching at HLA-A, HLA-B, and HLA-DR loci. The best long-term outcomes arise from living donors that are HLA identical to the recipient.[25] Specifically, a study was done that showed 10-year graft survival was higher in transplants with 0 antigen mismatch at HLA-A, -B, and -DDR versus 2 antigen mismatched transplants at each loci.[26] The loci most sensitive to rejection includes HLA-DR followed by HLA-B and HLA-A, but post-op 10 years, the differences in sensitivity between these three loci are negligible and additive.[26] [27]

Other antigens that may lead to an immune response in the response against transplanted tissue that includes ABO blood group antigens where blood incompatibility leads to acute rejection as well as minor histocompatibility antigens that can be recognized by CD8+ cytotoxic T cells and lead to rejection. [25] [28] Specifically, CD8+ T cells will act upon minor histocompatibility antigens where the recipient and donor have

identical polymorphic antigens that are identical but vary at particular loci. The immune response triggered by minor H antigens is less potent than a typical immune response but still requires immunosuppressive therapies to prevent prompt rejection.[14] While this immune response is similar in the way the body produces an anti-viral response, in viral infections only the infected cells are targeted, but since minor H antigens are expressed on all graft cells the entire graft is a threat to be destroyed.[14]

After transplantation, donor HLAs are recognized and targeted by T cells. Targeting these donor cells with immunosuppressants will minimize rejection and survival of the transplanted organ.[25] [14] One such mechanism . [25] [14] In the direct pathway, the number of alloreactive T cells will be high. It is by this mechanism that cytotoxic T cells attack graft cells as they will recognize them directly. [14] The indirect pathway is primarily responsible for the chronic rejection of grafts and involves T cells recognizing peptides that are derived from donor HLA that are presented by the recipient's antigen-presenting cells this will then activate other immune cells such as macrophages to cause injury to graft tissue.[25] [14] Donor antigen-presenting cells from the graft can travel to the lymph nodes where they activate host T cells that send effector T cells back to the target graft for attack. However, the resulting attack can be delayed by reducing the amount of antigen-presenting cells in the graft or if the graft does not have access to the lymphatic system.[14]

The T cell receptor cell recognizes the HLA peptide complexes which leads to the proliferation of multiple pathways including the calcium-calcineurin, RAS-mitogen protein kinase, and IKK nuclear factor κB. [25] These pathways trigger the T cell cycle, leading to clonal expansion, greater production of cytokines and effector T cells, which produces CD8+ T cell-mediated toxicity.[25] [28] CD8+ T cells will lyse and release cytotoxic granules that will induce apoptosis in the target tissue.[28] This cascade also leads to a macrophage-induced delayed hypersensitivity reaction, B cell antibody production, and the creation of alloantigen-specific memory T cells.[25] [28] This multiplies the inflammatory effect of the current immune response against the graft which ultimately leads to failure of function for the graft.

B cell maturation plays a role in the alloimmune response by creating antibodies when binding to donor HLA, specifically to Ig receptors (IgD and IgM). [25] [28] Activation of B cells by binding to the B cell receptor is mediated by complement proteins and T helper cells. The B cells will differentiate into plasma cells that secrete antibodies and when binding to antigens, can lead to graft injury via complement cascade and the response from natural killer cells, neutrophils, and eosinophils. [25] [28] [29] Other B cells will mature into memory B cells carrying antibodies ready for re-exposure to the same or similar antigens presented by graft tissue. [28] The vascular integrity of endothelial cells of the target tissue will be comprised of an antibody-mediated complement cascade that promotes coagulation and the formation of the membrane attack complex (MAC) that will trigger mast cell degranulation and inflammation to cause further injury.[28] If a graft

is linked to the blood supply, acute rejection may occur through destruction and fibrosis of the surrounding endothelial tissue, causing a loss in blood supply and, thus, death of the tissue.[14 29] Antibody dependent cell-mediated toxicity (ADCC) will also lead to damage of graft tissue by the release of nitric oxide, tumor necrosis factor (TNFα), and reactive oxygen species. [14 28 29] Such mechanisms contribute to about 60% of late graft failure; in fact, it seems to be a controlling factor in the success of grafts and the level of immunosuppression patients may need to maintain the health of their transplanted organ.[28] A study sought to seek the cause of graft rejection in kidney transplant cases and had seen that every kidney rejection was associated with evidence of antibody-mediated rejection at the time of failure. [8]

T effector cells will also secrete chemokines and cytokines that recruit elements from the innate immune system, including complement activation and leucocyte migration to the response site.[25] Cells of the innate immune system will express pattern-recognition receptors (PRR) that allow for the recognition of pathogen-associated molecular patterns (PAMPs).[28] PAMPs are associated with damage-associated molecular patterns (DAMPs) that typically arise in the presence of tissue injury due to rejection, which further exacerbates the chronic rejection immune response. [28] A diverse array of B and T cells play a role in the adaptive immune system such that immune cells gain robust memory and upon re-exposure, an even stronger response is generated to an allograft. It can be seen that patients develop sensitivity to more than 50% of the available donor

population after transplant failure, contributing to the poor outcomes found in re-transplanted patients.[28] [26]

CLINICAL EVALUATION OF HLA COMPATIBILITY

As previously discussed, the HLA genes encode a highly polymorphic set of proteins located on the short arm of chromosome 6.[25] HLAs are particularly at risk of targeting leading to an immune response that mediates graft rejection due to the facilitation of T cell, maturation of B cells and the production of donor-specific antibodies (DSA).[26] [30] The HLA system has evolved to become exceptionally diverse to provide excellent protective immunity. HLA represents a fraction of the different complexes that are presented for and potentially targeted by T cells in the immune response. However, this diversity also increases the likelihood of alloimmunity after transplantation and complicates the identification of compatible donor-recipient pairs. [14] [26] These polymorphisms are also why there is a slight chance of finding a match between siblings, despite such genes being inherited as a haplotype. [26] [29] Historically, HLA typing came from observations made around leucoagglutination antibodies in patients with leukopenia. The differences in reaction patterns were studied between different cell panels and revealed a system of similarities and differences that helped to further the understanding of allograft rejection.[26]

One of the first methods of identifying HLA polymorphisms includes the use of complement-dependent lymphocytotoxicity (CDC) assays which are classified as "cell

membrane" or "membrane-dependent" assays. [26] [31] In this method, if wells coated in antibodies with specificity to HLA complements are expressed on the target cells, the rabbit complement would be activated leading to lytic action resulting in cell death.[26] Cell death is recorded as a positive reaction, and this pattern of reactions will help to characterize an HLA phenotype.[26] The CDC method of HLA typing is now consistently used for class I and class II monoclonal antibody typing as the HLA molecules are present in their natural configuration but may lack specificity due to false positive reactions that may occur as a result of non-HLA antibodies.[26] [30]

Other techniques include solid phase or "membrane-independent" assays using flow technology on beads coated with HLA antigens. These techniques allow for HLA molecules to be purified and bound to plates or beads and do not influence the use of lymphotoxic immunosuppressive drugs.[31] However, membrane-based assays may also lead to false positives by detecting denatured HLA antigens and specific epitopes lost due to changes in the configuration of the HLA molecule. [31] Both solid and cell-based methods can be used to determine clinically significant levels of anti-HLA antibodies. Further monitoring of antibodies can provide valuable information on the clinical need for immunosuppression and the risk of chronic and acute rejection post-transplant. [31]

The earliest method of HLA was Southern Blots, which provided genotyping for a limited number of class II HLA alleles. [26] Later techniques include the amplification of HLA alleles via PCR-sequences-specific priming (SSP) and locus-specific amplification

of HLA genes through PCR-sequence-specific oligonucleotide probes (SSOP).[26] In SSP typing, In SSP typing, multiple simultaneous reactions are performed, and the overall pattern of amplification allows for the assignment of an HLA genotype..[26]

Compared to SSP, SSOP relies on the amplification of exons in HLA genes containing hypervariable regions that confer specificity Current methods hybridize amplified DNA to bead-bound probes. Positive binding can be detected using multiplexed bead arrays and an HLA genotype can be assigned. The advantages of the SSOP technique versus SSP include the ability to run multiple samples simultaneously. It is easier to conserve the sample, and PCR amplification is only needed once per sample, making it more economical. [26] However, genetic sequencing is favored at the best method for analysis due to specificity and economical practicality.

HLA antibody detection is another critical component of assessing donor-recipient compatibility. Screening for HLA-specific antibodies enables the avoidance of incompatible donors that express the HLA proteins that are targeted by the antibodies. Flow cytometry has recently been used to provide a more sensitive cross-matching method to identify mismatches with heightened risk for rejection. [30 32]

More recent advancements have allowed for epitope mismatch analysis at the amino acid level, further minimizing the chance of mismatch.[33] HLAs contain multiple epitopes that contain polymorphic amino acid residues that affect the structure of the HLA and

therefore its antibody accessibility, recognition, and reactivity in situations of an immune response.[34] Methods such as single antigen bead assays and high-resolution typing have been used to determine these epitope mismatches and the configuration of that specific HLA. It shows options in which there is and is not accessibility to the antigen by a B cell, preventing the production of DSAs and allowing for greater graft success.[33] Multiple studies have demonstrated that the number of eplet mismatches is correlated to the development of donor-specific antibodies, plays a role in transplant rejection, and has been proven to be a better predictor of graft loss than classic HLA antigen mismatch analysis. [33] With this, the development of the HLA Matchmaker algorithm has improved our ability to find patterns of HLA antibodies in transplant patients.[31] Determining the load of epitope mismatch will provide valuable information to clinicians when determining candidacy for certain immunosuppressive regimens post-transplant as well as further minimizing transplants done with a high risk of rejection.[35]

With current technology and previous health history for patients of interest, it has become easier to assess for HLA sensitivity and characterization of the donor/recipient mismatch profile. [31] HLA matching plays a pivotal role in assessing the risks of immunosuppression in a specific case and considering the geographical constraints of organ allocation. It is balancing these multitudes of factors that clinicians must balance in providing patients with the optimal outcome.

HLA EPITOPE MISMATCH ANALYSIS

Developments in mismatch analysis have allowed researchers to ascertain further specific categories of risk associated with different donor/recipient pairs.[35] The mismatches at the amino acid level between donor/recipient HLA alleles have been used to determine the level of immune response generated from transplantation and the extent of DSA development. Groups of three amino acids that were "on or near the surface of HLA molecules" are discontinuous but near each other in the quaternary structure of the protein are named "eplets" or "epitopes" .[35] [36] Epitopes have been used to determine the functional binding of antibodies to HLAs, and the degree to which different amino acids differ is the basis for epitope mismatch analysis to determine immune risk and optimal donor-recipient pairs. HLA Matchmaker is an algorithm that identifies the different HLA epitopes between donor and recipient, termed Epitope Mismatch Analsysis.[35] Alternative methods in characterizing epitopes include TerEps, where alloantibody is eluted and compared to HLAs on reactive beads. Others have also tested for the physical and chemical properties of specific epitopes and their associated binding, evaluating factors such as hydrophobicity and electrical charge when determining the consequences of epitope mismatch in allografts. [35]

Epitope mismatch analysis has been postulated as a better alternative to antigen-level (full protein) HLA mismatch analysis in determining the specificity and precision of mismatch between donor/recipient pairs and the level of immunological risk associated with mismatch. A prospective observational study has reported that the number of locus-specific epitope mismatches predicted post-transplant DSA development. [35] [37] Multiple

studies have confirmed the positive relationship between the degree of molecular mismatch and the level of DSA development post-transplant, which also serves as one of the most critical risk factors in graft rejection. [37] The goal of such assessments is to minimize the use of immunosuppression while assuring the greatest changes of allograft acceptance by the recipient post-transplant and the transplant's longevity. Epitope mismatch analysis has been utilized to determine the risk level associated with certain allograft donor/recipient combinations. It has been shown to predict the chance of transplant glomerulopathy independent of factors such as immunosuppression, induction therapy, and donor type.[35] The HLA Matchmaker algorithm has been used to show the predictive value of mismatch in HLA class II molecules in determining the risk of chronic graft rejection post-transplant.[38] Epitope mismatch analysis has also been used to determine the level of immunological risk associated with each donor/recipient pair combination at a greater level of precision when compared to their HLA mismatch analysis counterparts. A study published in the American Journal of Transplantation compared HLA-DR/DQ HLA and epitope level mismatch analysis and correlated it to specific serological, histologic, and clinical outcomes in 664 transplant patients.[36] It was seen that compared to traditional whole HLA mismatch analysis, single molecule eplet mismatch was better correlated with DSA development and allowed recipients to be classified in low, intermediate, and high-risk alloimmune categories. [36] Risk categories were associated with certain immunological events such as T cell-mediated rejection, DSA development, and antibody-mediated rejection in response to an allograft.[36]

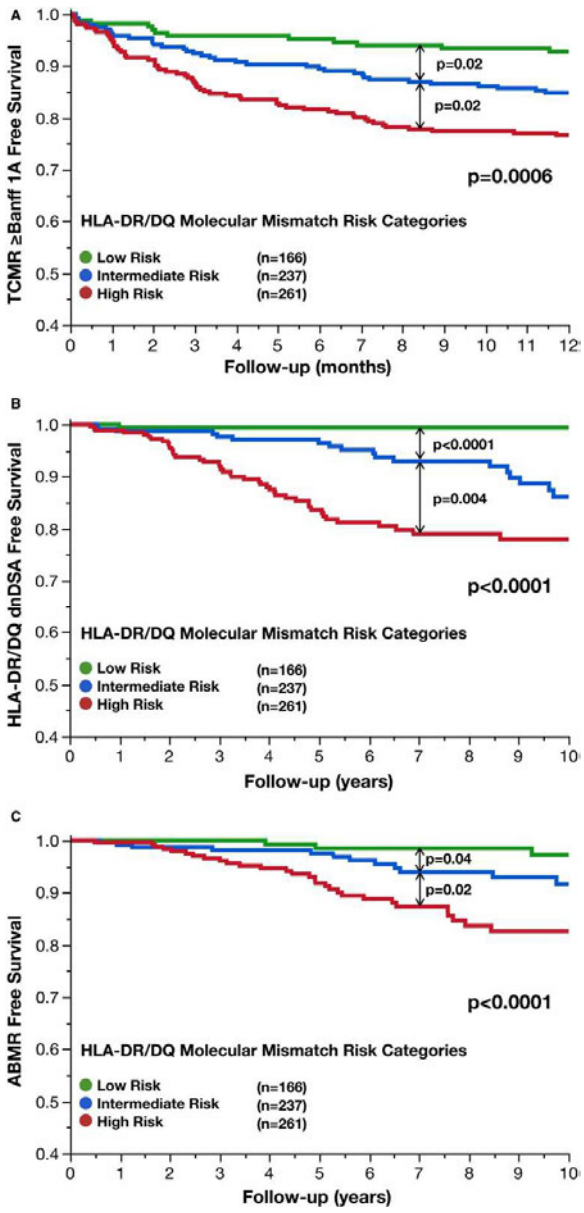

Figure 1: Rate of Immunological Events Based on Risk Categorization. Adapted from Wiebe et al [Wiebe C, Kosmoliaptsis V, Pochinco D, et al. HLA-DR/DQ molecular mismatch: A prognostic biomarker for primary alloimmunity. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2019;19(6):1708-1719. doi:10.1111/ajt.15177]. A cohort of x Canadian renal transplant recipients was categorized into low, intermediate, or high risk groups based on the number of HLA Class II Epitope Mismatches with the donor. The development of (A) T cell mediated rejection (TCMR), (B) de novo donor-specific HLA antibody (dnDSA), and (C) antibody-mediate rejection (ABMR) were evaluated for 10 years post-transplant. [38]

As seen in the figure above (Figure 1), alloimmune risk categories were determined T cell-mediated rejection events (A), DSA development (B), and antibody-mediated rejection (C), with low-risk recipients least likely to develop such immunological events and high-risk recipients more likely to develop an immune response post-transplant.[36] However, all risk categorizations are susceptible to post-transplant graft loss.[36] The positive correlation between risk categorization and the likelihood of graft loss and other immunological events makes single eplet mismatch analysis a valuable tool in determining the eligibility of specific donor/recipient graft cases. Eplet mismatch analysis served as a pre-transplant biomarker that can also be used for developing patient-specific immunosuppression protocols and as a tool in pharmaceutical development. Furthermore, the classification of graft recipients provides essential information about a specific graft's perceived clinical success and informs the necessary precautions that must be taken for that specific patient.

STUDY RATIONALE

Currently, solid organ transplantations have excellent short-term outcomes. However, the long-term survival of these organ transplants is limited and has not improved in recent decades. [39] HLA analysis has been used to determine the immunological compatibility of donors and recipients by assuring a close match between the two's HLA proteins which helps to further characterize the risk of rejection post-transplant for a specific donor-recipient pair.[40] Epitope mismatch analysis can provide

additional strength to organ allocation by examining the loci's specific amino acid residues and configuration on HLAs at which donor and recipients do not match.[33] Epitope mismatch analysis is currently done via high-resolution HLA genotyping; however, deceased donors typically have intermediate-resolution HLA genotyping that is then imputed. Current studies suggest that imputation introduces a number of inaccuracies into HLA haplotype identification such that it may impact clinical outcomes, particularly for non-white patients.[41]

This investigation seeks to determine the impact of the accuracy of low-resolution imputation in determining the number of epitope mismatches compared to high-resolution genotyping and whether the difference between the two are clinically relevant. [42] The epitope mismatch disparity will also be characterized into high, intermediate, and low-risk categorization for the likelihood of DSA formation post-transplant, which can help guide the clinical decision-making around specific transplant cases.[41]

In addition to determining the value of low-level imputation analysis in providing additional information for informed organ allocation, the imputation is evaluated for its accuracy within and between different racial groups. Historically solid-organ transplant data that serves as the basis for creating standards for organ allocation has primarily been determined on Caucasian donor/recipient data. Such disparity may lend itself to inconsistent results in epitope mismatch analysis among non-Caucasians. Determining the disparity in epitope mismatch between and within racial donor/recipient pairings can

provide further context on the additional risk factors between racially concordant and discordant pairs and maximize long-term organ/graft survival.

METHODS

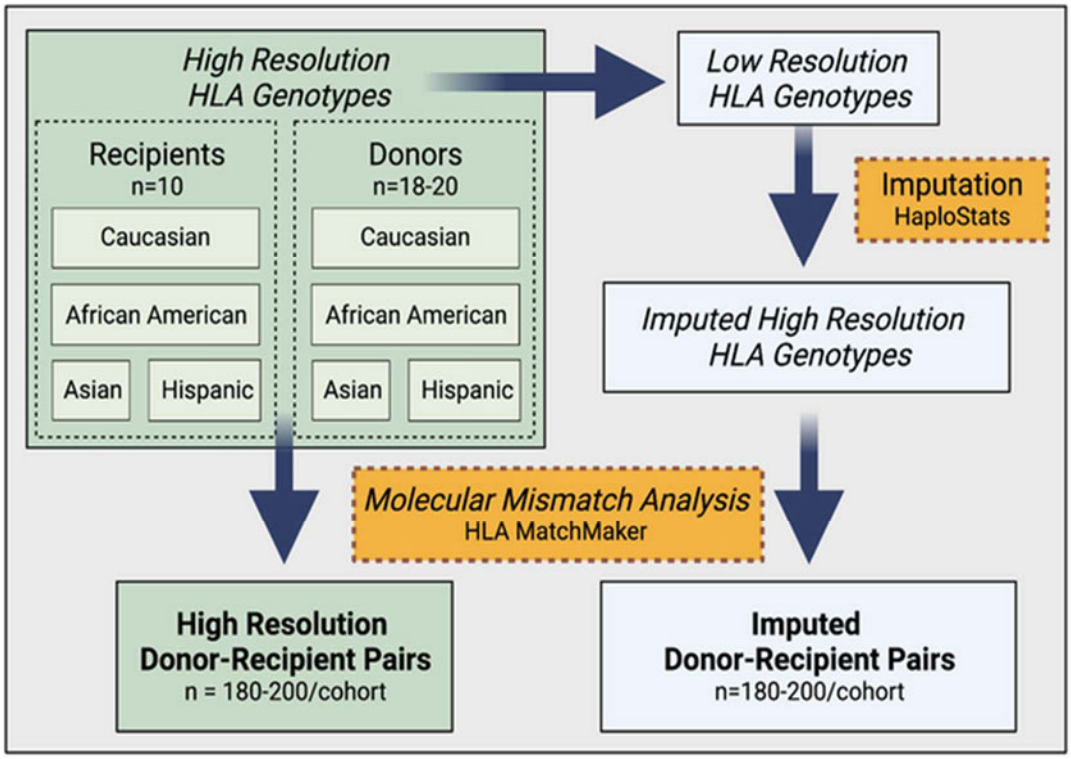

Figure 2: HLA Study Design. Renal transplant patients with confirmed high-resolution HLA genotypes were selected from the Johns Hopkins Immunogenetics Laboratory. High-resolution genotyping was transformed into low resolution genotyping, and then imputed back into high-resolution genotyping in order to mimic the use of imputation in the clinical setting. Two parallel cohorts of 180-200 surrogate pairs (High Resolution or Imputed) were evaluated using HLA MatchMaker to evaluate the Epitope Mismatch Analysis.

This study aims to evaluate the degree of difference in the molecular mismatch analysis between high-resolution and imputed donor-recipient pairs. The data starts with high-resolution HLA genotypes that are then transformed into low-resolution HLA genotypes, which are further imputed using HaploStats. The imputed data for the donor-recipient and high-resolution donor-recipient pairs undergo molecular mismatch analysis using the HLA Matchmaker algorithm. The number of mismatches in both groups are then categorized into a clinical risk category and compared. This comparison is analyzed for both differences at 11 different HLA loci as well as differences between racially concordant and racially discordant groups. This study was approved by the Johns Hopkins Institutional Review Board.

Population:

We selected renal transplant recipients and donors who underwent high-resolution HLA genotyping at the Johns Hopkins Immunogenetics Laboratory from 01/14/2021 through 06/30/2022. The patients were placed into the racial categories Caucasian, African American, Asian, and Hispanic through self-reporting. Individuals were randomly assigned as "donors" or "recipients" and used to construct surrogate donor/recipient pairs. Each racial group contained 10 donors and 20 recipients, with the exception of the Asian subgroup, for which only 18 recipients were available within the data set. The total cohort included 180-200 donor/recipient pairs. The average number of sets per cohort was set to approximately 200 to model the annual renal transplant volume of the average US transplant center.

Transformation of HLA Genotyping Data

The original data set used include high-resolution genotyping conducted by the Johns Hopkins Immunogenetics laboratory by next-generation sequencing, using CareDx Tx17 platform to create 2 field genotypes at each loci of interest. The high-resolution genotyping data was transformed in serological-level low-resolution genotyping by removing the second HLA type field, except in cases where the field is required to maintain a serological type. The exceptions were made for the following: HLA-B*14, HLA-B*15, HLA-B*40, HLA-B*55, HLA-B*56, HLA-C*03, HLA-DRB1*03, HLA-DRB1*103, DQB1*03. The HaploStats algorithm used to analyze "indirectly measured haplotypes" is then used to create the imputed data. HaploStats does not produce a result for the HLA-DQA1 locus, so high-resolution genotyping was used in its place for analysis.

Molecular Mismatch Analysis

About 200 "surrogate" pairs are made from 20 donor and 10 recipient pairs for each racial group for both the high-resolution and imputed groups. The HLA Matchmaker algorithm is run using the hlaR package in R to identify molecular mismatches at each loci studied, and a risk categorization is assigned to each mismatch for DRB1/DRB345/DQB1. [43] [36] The molecular mismatch analysis is conducted on both the high-resolution genotyping and imputed genotyping for identical cohorts to compare the level of mismatch.

Statistical Analysis

The high-resolution and imputed cohorts are compared for their level of mismatch by the number of total molecular mismatches, number of mismatches per loci, changes in epitope mismatches, and risk categorization from high resolution to imputed. The statistical significance of the mismatches was determined using a t-test with a p-value of (referenced needed). An odds ratio of changes between the two cohorts was determined using logistic regression. All statistical analyses were done using R.

Currently, solid organ transplantations have excellent short-term outcomes. However, the long-term survival of these organ transplants remains dismal, with a majority of these solid operations failing within the first two decades of use. [39] HLA analysis has been used to determine the immunological compatibility of donors and recipients by assuring a close match between the two's HLA proteins which helps to further characterize the risk of rejection post-transplant for a specific donor-recipient pair.[40] Epitope mismatch analysis can provide additional strength to organ allocation by examining the loci's specific amino acid residues and configuration on HLAs at which donor and recipients do not match.[33] Epitope mismatch analysis is currently done via high-resolution HLA genotyping; however, deceased donors typically have intermediate-resolution HLA genotyping that is then imputed. Current studies suggest that imputation introduces inaccuracies into HLA haplotype identification such that it may impact clinical outcomes, particularly for non-white patients.[41]

This investigation seeks to determine the impact of the accuracy of low-resolution imputation in determining the number of epitope mismatches compared to high-resolution genotyping and whether the difference between the two are clinically relevant. [42] The epitope mismatch disparity will also be characterized into high, intermediate, and low-risk categorization for the likelihood of DSA formation post-transplant, which can help guide the clinical decision-making around specific transplant cases. [41]

In addition to determining the value of low-level imputation analysis in providing additional information for informed organ allocation, the imputation is also evaluated for its accuracy within and between different racial groups. Historically solid-organ transplant data that serves as the basis for creating standards for organ allocation has primarily been determined on Caucasian donor/recipient data. Such disparity may lend itself to inconsistent results in epitope mismatch analysis among non-Caucasians. Determining the disparity in epitope mismatch between and within racial donor/recipient pairings can provide further context on the additional risk factors between racially concordant and discordant pairs and maximize long-term organ/graft survival.

RESULTS

HLA Loci	High-Resolution Genotyping		Imputed Genotyping		No. Molecular Mismatches Changed	
	AVG	STDEV	AVG	STDEV	AVG	STDEV
	Caucasian					
A	9.8	6.9	9.8	6.9	**0.00**	0.00
B	6.9	4.0	6.9	4.0	**0.00**	0.00
C	4.0	3.2	4.1	3.2	**0.15**	0.78
DRB1	7.8	5.5	8.4	5.9	**0.78**	1.86
DRB345	6.5	6.8	7.1	7.7	**0.89**	2.40
DQB1	12.3	8.2	12.8	8.9	**0.63**	2.88
DQA1	3.4	3.1				
DPB1	5.0	3.8				
DPA1	1.0	2.0				
	African American					
A	9.9	5.3	9.9	5.4	**1.07**	1.46
B	7.4	4.0	7.7	4.1	**0.25**	0.84
C	4.4	3.4	4.8	3.5	**0.32**	0.93
DRB1	10.0	6.2	10.2	6.2	**1.41**	2.21
DRB345	7.8	5.6	7.2	5.9	**2.39**	3.23
DQB1	11.1	6.7	12.3	8.0	**2.01**	3.74
DQA1	2.9	2.5				
DPB1	5.6	3.3				
DPA1	2.9	2.8				
	Asian					
A	8.9	6.7	9.7	6.8	**1.05**	2.00
B	7.4	4.3	7.6	4.4	**0.39**	1.24
C	6.5	4.5	6.5	4.7	**1.02**	2.22
DRB1	9.4	5.3	9.5	5.3	**0.74**	0.87
DRB345	8.9	7.6	9.6	8.5	**2.06**	3.07
DQB1	13.1	7.8	12.8	7.8	**0.77**	1.23
DQA1	4.2	2.9				
DPB1	5.2	3.7				
DPA1	0.6	1.4				
	Hispanic					
A	10.0	4.6	9.7	4.5	**0.29**	0.64
B	7.7	4.0	7.8	4.0	**0.20**	0.51
C	6.7	4.0	6.7	4.0	**0.02**	0.14
DRB1	9.2	6.4	9.1	6.7	**1.42**	2.06
DRB345	7.1	7.3	7.0	7.8	**0.87**	2.04
DQB1	11.7	9.0	11.7	9.0	**0.41**	1.32
DQA1	3.0	2.4				
DPB1	4.5	3.9				
DPA1	1.6	2.2				

Table 1: Molecular mismatch analysis by HLA loci for racially concordant high-resolution and imputed cohorts. The average (AVG) and standard deviation (STDEV) for each racial classification was determined for the loci HLA- A, B, C, DRB1, DRB45, DQB1, DQA1, DPB1, DPA1. The average and standard deviation for the change in number of molecular mismatch between high resolution and imputed cohorts was found.

Donor-Recipient Pair Racially Concordant		High resolution Genotyping		Imputed Genotyping		Change in # Molecular Mismatches		
		AVG	STDEV	AVG	STDEV	**AVG**	STDEV	p value
Caucasian	Class I	20.7	8.9	20.8	8.9	**0.15**	0.8	0.0092
	Class II	25.9	16.6	27.6	17.6	**2.08**	5.0	0.00002
African American	Class I	21.8	8.3	22.31	8.8	**1.42**	2.09	0.0036
	Class II	28.5	12.3	29.3	13.0	**1.76**	2.83	0.070
Hispanic	Class I	24.4	7.4	24.1	7.3	**0.35**	0.69	0.00004
	Class II	27.9	16.7	27.8	17.5	**2.41**	3.11	0.55
Asian	Class I	22.9	10.4	23.9	10.9	**2.24**	3.28	0.00072
	Class II	31.4	15.7	31.9	16.5	**2.62**	3.24	0.16

Table 2. Molecular mismatch analysis by HLA Class and Class II loci using high-resolution and imputed HLA genotyping in racially concordant cohorts. Average (AVG) and standard deviation (STDEV) of the number of molecular mismatches using high-resolution and imputed genotyping for each racial cohort.

Table 2: Molecular Mismatch analysis of HLA Class I and Class II loci. Average (AVG) and standard deviation (STDEV) was found for high resolution and imputed genotyping and changes in molecular mismatches for racially concordant.

Donor-Recipient Pair Racially Concordant		Molecular Mismatch Risk Group			% Pairs Changed	Odds Ratio	95% CI	p-value
		High	Int	Low				
Caucasian	# Pairs High Res. Typing	81	86	33	0.5%	N/A	N/A	N/A
	# Pairs Changed with Imput.	0	0	1				
African American	# Pairs High Res. Typing	41	138	21	6.5%	13.8	1.8-106.6	0.012
	# Pairs Changed with Imput.	7	4	2				
Hispanic	# Pairs High Res. Typing	56	103	41	3.5%	7.2	0.9-59.1	0.065
	# Pairs Changed with Imput.	0	7	0				
Asian	# Pairs High Res. Typing	53	110	17	9.4%	20.8	2.7-157.4	0.003
	# Pairs Changed with Imput.	6	8	3				

Table 3: Impact of Imputation on Change of Molecular Mismatch Risk Group. The number of pairs changed with imputation were measured for racially concordant groups. The percent of pairs changes, odd ratio, confidence interval and p-value was measured for each group.

Donor-Recipient Pairs Racially Discordant			Molecular Mismatch Risk Group			% Pairs Changed	Odds Ratio	95% C.I.	p-value
Recipient Race	Donor Race		High	Int	Low				
Caucasian	African American	# Pairs High Res. Typing	100	83	17	3.5%	7.2	.9-59.2	0.066
		# Pairs Changed with Imput.	7	0	0				
	Asian	# Pairs High Res. Typing	95	74	11	2.8%	5.7	0.7-49.1	0.114
		# Pairs Changed with Imput.	4	0	1				
	Hispanic	High Res. Typing # Pairs	83	87	30	0.0%	0	0-Inf.	0.99
		# Pairs Changed with Imput.	0	0	0				
African American	Caucasian	# Pairs High Res. Typing	33	141	26	4.5%	0.7	0.3-1.6	0.383
		# Pairs Changed with Imput.	5	3	1				
	Asian	# Pairs High Res. Typing	41	124	15	4.4%	0.7	0.3-1.7	0.384
		# Pairs Changed with Imput.	4	2	2				
	Hispanic	# Pairs High Res. Typing	34	130	36	3.5%	0.5	0.2-1.3	0.175
		# Pairs Changed with Imput.	2	4	1				
Asian	Caucasian	# Pairs High Res. Typing	50	110	40	4.0%	0.4	0.2-0.9	0.038
		# Pairs Changed with Imput.	6	0	2				
	African American	# Pairs High Res. Typing	66	107	27	8.5%	0.9	0.4-1.8	0.748
		# Pairs Changed with Imput.	12	1	4				
	Hispanic	High Res. Typing # Pairs	51	112	37	7.5%	0.8	0.4-1.6	0.496
		# Pairs Changed with Imput.	3	6	6				
Hispanic	Caucasian	# Pairs High Res. Typing	42	128	30	2.0%	0.6	0.2-2.0	0.365
		# Pairs Changed with Imput.	0	4	0				
	African American	# Pairs High Res. Typing	57	126	17	4.5%	1.3	0.5-3.6	0.611
		# Pairs Changed with Imput.	6	3	0				
	Asian	# Pairs High Res. Typing	66	107	7	3.3%	1	0.3-2.9	0.929
		# Pairs Changed with Imput.	4	2	0				

Table 4: Impact of imputation on racially discordant cohorts. The number of changes per molecular mismatch risk group was measured as well as the percent change in, odds ratio, confidence interval and p value.

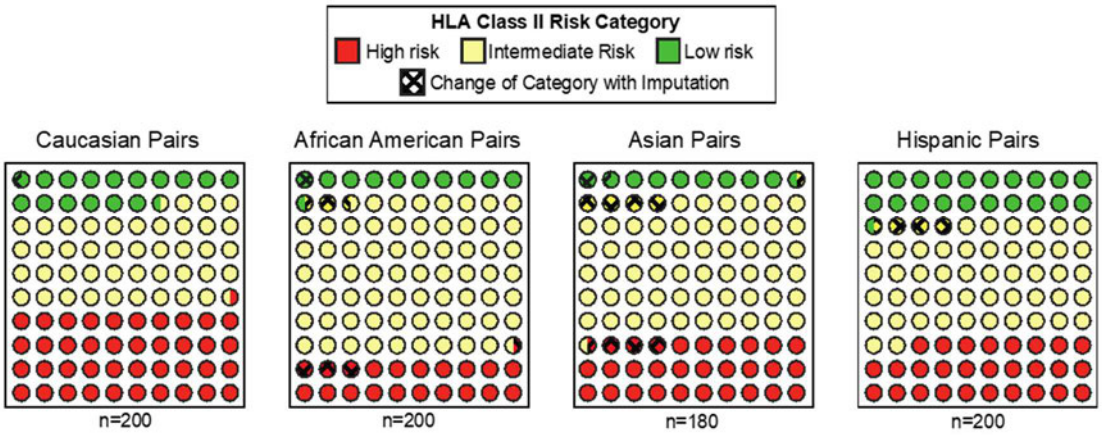

Figure 3: Impact of imputation on HLA class II risk categorization. This parts-whole analysis was done to visualize the measure and spread of the change in immunological risk categorization between high resolution and imputed HLA genotyping. Each dot represents 1% of the cohorts (n=180-200 pairs). The colors green, yellow and red represent low, intermediate and high risk categorization respectively. Dots with crosshatch represent pairs in which imputation changes immune risk categorization. This analysis was done for racially concordant groups. Most significant risk categorization changes were seen among the Asian cohort with changes present at both low, intermediate and high risk categories. The least amount of change was observed in the Caucasian cohort where imputation risk had changed in pairs previously labeled low risk.

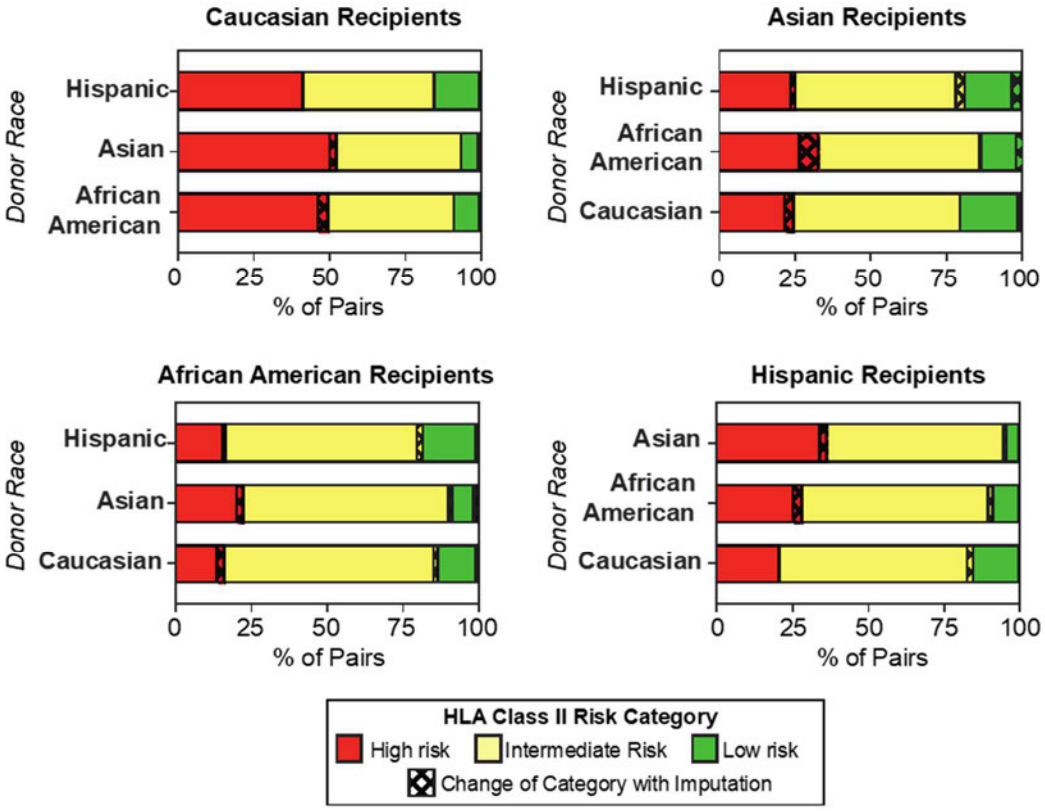

Figure 4: Impact of imputation on HLA class II risk categorization in racially discordant cohorts. This bar graph represents a parts-of-whole analysis in which crosshatch represents change in risk categorization. Greatest amount of change was observed in the group containing Asian recipients and the least was observed in the group with Caucasian recipients.

Changes in Molecular Mismatches by Loci

This analysis demonstrated modest changes in molecular mismatch between high-resolution genotyping and imputed cohorts at each locus studied with both racially concordant and discordant groups. When evaluating the number of changes at each HLA loci in the surrogate pairs of both cohorts (Class I: HLA-A, HLA-B, and HLA-C; Class II HLA-DRB1, HLA-DRB345, HLA-DQB1), it was seen for the racially concordant cohort the average change was between 0-2.39 in the number of mismatches. For all racial groups except Asians, HLA Class II molecular matches changed more by imputation than class I. For the Asian racial subgroup, the average number of changes per loci for class I and II were similar (as seen in table 1).

Examining the number of molecular mismatch changes for each class of loci (HLA class I and HLA class II), the changes are also small when going from high resolution to imputed; the range is seen to be 0.1-2.24. However, this difference was statistically significant, as seen in the paired t-test conducted across all four cohorts (Table 2). The average number of changes for particularly HLA Class II mismatches was between 1.76-2.62 within cohorts. However, this was only statistically significant for Caucasians. This overall trend has been demonstrated across all racially concordant groups for both Class I and Class II molecular mismatch analysis. This is especially important as mismatches in HLA class II loci have been associated with an increase in the generation of donor-specific antibodies leading to graft rejection. [44]

Changes in Risk Categorization

When determining the clinical significance of the degree of molecular mismatch analysis, the impact of the molecular mismatch is classified into low, intermediate, and high-risk categories. A change in molecular mismatches that would influence risk categorizations that could provide clinical relevant info regarding organ allocation, immunosuppressive regimens and post operative monitoring. The accuracy of the imputed data set correctly categorizing the mismatch pairs was evaluated by comparing it to the high-resolution data. The change of risk categorization in either direction (for example, high to low or intermediate to high) was tabulated. It was seen that the risk categorization between high-resolution and imputed pairs was maintained for 90.6-99.5% of the pairs measured (Table 3, Figure 2). The slightest change in risk categorization was seen in the Caucasian subgroup and was the basis of calculating the odds ratio for all other racial groups in reference to the Caucasian subgroup. There was an increase in odds for risk category change in the African American and Asian pairs relative to Caucasians ($p=0.0018$ African Americans, $p=0.003$ Asians). However, this was not seen for Hispanics. Overall, imputation maintained the correct molecular mismatch characterization for racially concordant pairs in almost all instances. However, there was an increase in the risk of category change for certain racial groups compared to Caucasians.

Differences between Racially Concordant and Discordant Groups

The differences in molecular mismatch were analyzed between racially concordant and discordant groups by examining these differences with different donor/recipient racial combinations (Table 4). It was seen that the molecular mismatch categorization did not change for 91.5-100% of the racially discordant pairs. There was a slightly more significant impact on change in risk categorization for Asian recipients with Hispanic donors (7.5%) and African American donors (8.5%). All other recipient groups saw a shift in molecular mismatch risk around 2.0-4.5%, apart from Caucasian recipients with Hispanic donors, for which there was little change in molecular mismatch risk categorization from high resolution to imputed. The odds ratio was also calculated for racially discordant donors relative to racially concordant counterparts to assess the impact of donor race on the imputation. It was observed that donor race does not significantly impact changing the risk categorization of molecular mismatches with imputation (Table 4). With the exception of the Asian-Caucasian recipient-donors that did have a statistically significant difference in risk categorization ($p= 0.038$), overall imputation did not lead to substantial changes in molecular mismatch risk categorization of racially discordant pairs.

DISCUSSION

This analysis was done to determine the practical difference in molecular mismatches between high-resolution genotyping data and imputed data for donor-

recipient candidates. It also sought to determine the difference in molecular mismatches between racial concordant and discordant groups and associated risk categorization to assess its clinical relevance. The findings reveal that imputation leads to minimal changes in molecular mismatches compared to high-resolution genotyping at each locus in racially concordant groups. This remains largely true for racially discordant donor-recipient pairs as well. The shift in risk categorization between imputed data and high-resolution typing is also minimal, thus demonstrating imputation's utility, especially when high-resolution typing is not available. As technology has advanced, techniques in HLA matching have become more sensitive such that specific residues can be compared and matched to provide better immunological assessment in transplant cases. Currently, deceased donor HLA genotyping is conducted at a low-resolution level, which at current is the standard required for matching in solid organ transplantation. For practicality, low-resolution or serological HLA antigen matching methods are readily available and low-cost, making it easier for transplant centers to follow through with organ allocation. This investigation seeks to assure of no significant clinical deficit by using imputed data instead of high-resolution genotyping, which is not always readily available.

Some limitations of this study include the nature and size of the study cohort. The investigation and resulting data were derived from a set of surrogate donors and recipients with the pairs set randomly. The size of the study cohort was limited due to limited HLA genotyping information available. In the future a power analysis could be conducted to determine the true size of a cohort needed for significant results. Racial classifications also serve as a weakness in this investigation. Comparing this study to

real-world donor-recipient pairs and their associated transplant outcomes will be essential in affirming the claims made by this investigation. It also provides an opportunity to compare HLA mismatch analysis-based assessment to real-world outcomes. Future studies can build upon this work by comparing the molecular mismatch analysis done on surrogate pairs to a validation cohort.

Historically, non-Caucasians have been underrepresented in research on HLA analysis and the immune response to allografts; because of this, less is known about the population-level HLA genotypes of racial groups such as African Americans. Taking race into account when examining HLA matches can provide more information on optimal organ allocation and immune risk for a potential donor-recipient match. This investigation sought to understand the role race plays in molecular mismatch, and changes were seen to be minimal. However, racial classifications were self-reported by patient which could affect the accuracy of the data collected. An argument can also be made that racial classifications themselves are imprecise and subjective. For example the racial classification of "Asian" may be too broad of a term to represent the genetic diversity of regions such as East Asia, South Asia and the Middle East. This may also explain the high levels of change in risk categorizations in racially concordant and discordant groups. Further research should base racial categorizations based on objective measures such as genetic biomarkers that may be associated with a specific group. Despite the variability in racial classification, the information presented in this study serves as a foundation for future research into the clinical implications of racial

classifications on differences in immune function and help to further address the issue of racial disparities within solid organ transplantation.

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