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Non-HLA Genetic Factors and Their Influence on Heart Transplant Outcomes: A Systematic Review

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Background. Improvement of immunosuppressive therapies and surgical techniques has increased the survival rate after heart transplantation. Nevertheless, a large number of patients still experience complications, such as allograft rejection, vasculopathy, kidney dysfunction, and diabetes in response to immunosuppressive therapy. Variants in HLA genes have been extensively studied for their role in clinical outcomes after transplantation, whereas the knowledge about non-HLA genetic variants in this setting is still limited. Non-HLA polymorphisms are involved in the metabolism of major immunosuppressive therapeutics and may play a role in clinical outcomes after cardiac transplantation. This systematic review summarizes the existing knowledge of associations between non-HLA genetic variation and heart transplant outcomes. **Methods.** The current evidence available on genetic polymorphisms associated with outcomes after heart transplantation was identified by a systematic search in PubMed and Embase. Studies reporting on polymorphisms significantly associated with clinical outcomes after cardiac transplantation were included. **Results.** A total of 56 studies were included, all were candidate gene studies. These studies identified 58 polymorphisms in 36 genes that were associated with outcomes after cardiac transplantation. Variants in *TGFB1*, *CYP3A5,* and *ABCB1* are consistently replicated across multiple studies for various transplant outcomes. **Conclusions.** The research currently available supports the hypothesis that non-HLA polymorphisms are associated with clinical outcomes after heart transplantation. However, many genetic variants were only identified in a single study, questioning their true effect on the clinical outcomes tested. Further research in larger cohorts with well-defined phenotypes is warranted.

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Heart transplantation is still considered to be the therapy of choice for patients with end-stage heart failure refractory to optimal medical and surgical therapy.[1] Every year, over 4000 heart transplantations are performed worldwide, the majority being in the United States and Europe.[2] The survival rate after heart transplantation has increased greatly in the last decades, mainly due to evolving immunosuppressant therapies and improvement in surgical techniques. The current 1-year survival is being reported at greater than 85%.[3] However, ~15% of recipients suffers at least 1 episode of acute cellular rejection in the first year after transplantation.[3] High doses of immunosuppressive drugs are not only needed to prevent rejection, but are also associated with an increased risk of infections, malignancies, and renal failure.[4]

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HLA (mis)matching and major histocompatibility antigen have been studied extensively for their role in the occurrence of acute and chronic rejection in solid organ transplantation.[5] In contrast, the effect of minor histocompatibility antigens (mHA) on transplant outcomes is largely unknown. These mHA polymorphisms could potentially lead to genetic differences between donors and recipients, activating the immune system of the recipient and consequently cause acute allograft rejection. This is supported by allograft rejection being observed in kidney transplants and stem cell transplantations between HLA identical siblings.[6] Recent findings suggest that mHA polymorphisms are not only involved in the development of acute rejection, but also determine the renal function posttransplantation and play a role in the development of chronic rejection.[7-9] Another group of non-HLA polymorphisms that may be involved in transplant outcomes are those genetic variants involved in drug metabolism. Pharmacogenomics studies have identified dozens of polymorphisms influencing plasma levels of a wide variety of drugs and other substances, some of which may influence metabolism of drugs commonly prescribed to transplanted patients.

This systematic review provides an overview of the published research on non-HLA genetics in heart transplantation. We included all studies that identified 1 or more significant associations between genetic variants and any heart transplant outcome, regardless of study design.

MATERIALS AND METHODS

An overview of the literature search and study selection is shown in Figure 1.

Search

MEDLINE (PubMed) and Embase databases were searched for all relevant literature published on non-HLA polymorphisms associated with clinical outcome after heart transplantation. The search strategy included the terms "heart," "transplantation," "gene," and their synonyms and related terms. Searches were restricted to human studies:

MEDLINE: human[MeSH Terms] AND (heart[Title/Abstract] OR cardiac[Title/Abstract]) AND (transplant [Title/Abstract] OR transplantation[Title/Abstract]) AND (gene[Title/Abstract] OR SNP[Title/Abstract] OR polymorphism[Title/Abstract])

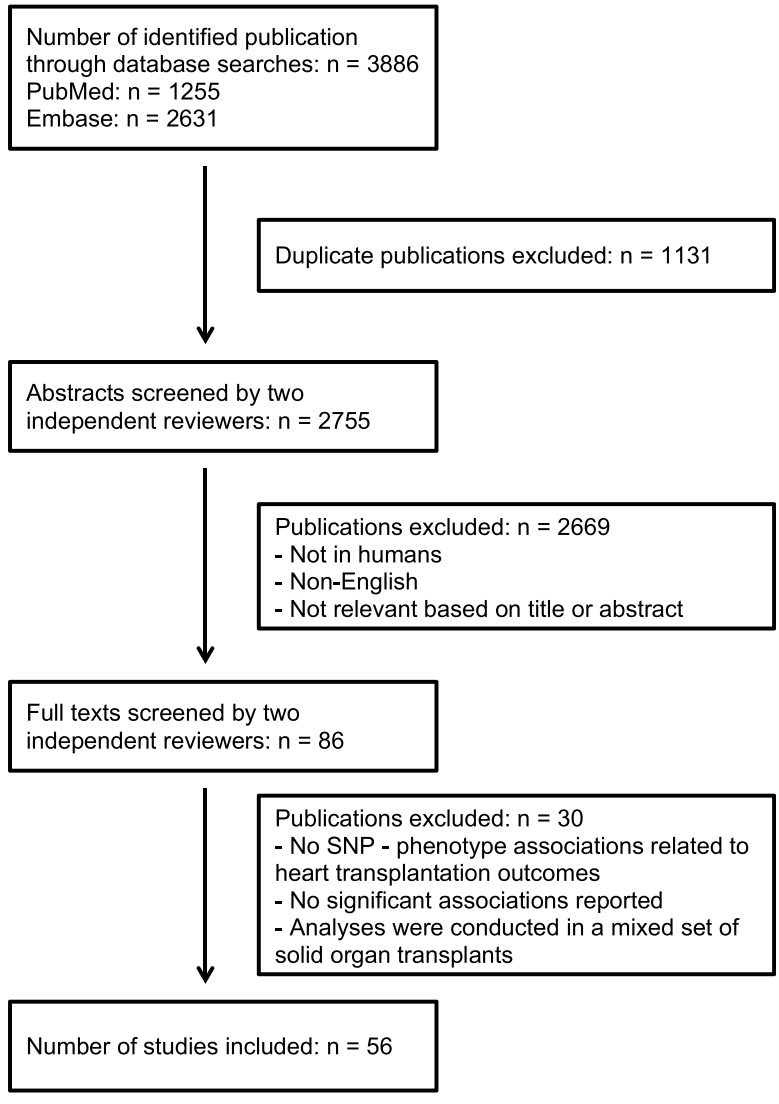

FIGURE 1. Literature search and study selection flowchart. SNP, single nucleotide polymorphism.

Embase: human AND (heart:ab,ti OR cardiac:ab,ti) AND (transplant:ab,ti OR transplantation:ab,ti) AND (gene:ab,ti OR SNP:ab,ti OR polymorphism:ab,ti). Reference lists of included articles, and previous reviews were manually searched for additional relevant studies. Databases were searched from their inception to July 10, 2018.

Inclusion Criteria

We included original research article published in peer-reviewed scientific journals, reporting on the association of genetic polymorphisms with clinical outcomes after heart transplantation in pediatric and adult patients. Only articles written in English were included.

Study Selection

Two reviewers independently screened titles and abstracts to identify potentially relevant articles. Two reviewers obtained and independently screened full text versions of potentially eligible studies to determine their suitability for inclusion. Studies were included on the basis of consensus agreement.

Data Extraction

Two investigators independently retrieved data from each included study. The data collected for each study included study name; first author; publication year; number of recipients, donors and controls in the study; the number of pediatric patients included; mean duration of follow-up; polymorphism/single nucleotide polymorphism (SNP) investigated; gene; reference SNP cluster ID (rsID) or primers for SNP; location of SNP; investigated phenotype(s); use of immunosuppressive drugs; statistical test; and *P* value. In those studies that reported primers instead of rsIDs, we queried the University of California, Santa Cruz Genome Browser and Basic Local Alignment Search Tool of the National Center for Biotechnology Information to try identifying the rsIDs.

Data Analysis

The studies were searched for polymorphisms that were significantly associated ($P < .05$) with 1 or more clinical outcomes after heart transplantation. Associations were categorized based on their phenotype: immunosuppressive therapy, rejection and survival, cardiac function, kidney function, lipids, and other phenotypes (**Tables S1–S6, SDC**, http://links.lww.com/TXD/A172). Studies were excluded if they did not report a significant association related to heart transplantation outcomes, or if analyses were conducted in a mixed set of solid organ transplants with only a small percentage of cardiac transplants. We assessed quality of the individual studies by the following criteria: (1) selection bias, (2) phenotype definition, (3) population structure taken into account, (4) accuracy of genotyping measurement, (5) Hardy-Weinberg equilibrium assessed, (6) multiple testing correction, and (7) results consistent with other studies.

RESULTS

The literature search included 2755 published results until July 10, 2018, after removal of duplicates. A total of 86 articles were screened full text. After screening, 56 articles with statistically significant associations between SNPs and clinical outcomes after heart transplantation were eligible. Together, these 56 studies contained 107 significant SNP-phenotype associations, and included a total of 7030 recipients, 3059 donors, and 1308 controls. Some studies cohorts published multiple studies using the same samples, so the number of unique samples is lower. Forty-five studies included adult patients only, 9 studies included only pediatric patients and 2 studies included patients of all ages. All included studies were candidate gene studies. A total of 58 polymorphisms in 36 genes were identified to be associated with clinical outcomes after heart transplantation. Results per phenotype group can be found in **Tables S1–S6, SDC** (http://links.lww.com/TXD/A172). None of the studies fulfilled all of our quality criteria. Especially selection bias, population structure, Hardy-Weinberg equilibrium, accuracy of genotyping measurement, and multiple testing correction were hardly ever taken into account. Phenotype definition quality varies across clinical outcomes. For example, immunosuppressive drug level measures were explained in detail in all studies, whereas rejection type or grade was not specified in approximately 25% of studies. Therefore, we decided to only use

TABLE 1.

Single nucleotide polymorphisms (SNPs) in *ABCB1*, *CYP3A5*, and *TGFB1* are associated to a diverse range of clinical outcomes after cardiac transplantation

Genes	SNPs	Phenotype	References
ABCB1	rs1045642, rs1128503, rs2032582, rs2235013, rs2235033	Cyclosporin levels	10
ABCB1	C3435T	Steroid dependency, steroid weaning	11,12
ABCB1	C3435T, G2677T	Tacrolimus levels	13
ABCB1	C3435T, G2677T	Acute rejection	14
ABCB1	rs2066844	Graft rejection	15
ABCB1	rs9282564	Renal function	15
ABCB1	rs1045642	Infections	15
CYP3A5	rs776746	Tacrolimus levels, dose, clearance	13,16-19
CYP3A5	rs776746	Renal function	20
TGFB1	rs1800471	Acute rejection	21
TGFB1	rs1800471	Cardiac allograft vasculopathy	22-26
TGFB1	rs1800471	All rejection (chronic and acute)	24
TGFB1	rs1800471, rs1800470	End-stage renal failure, renal function	27-29

ABCB1, ATP binding cassette subfamily B member 1; CYP3A5, cytochrome P450 family 3, subfamily A, member 5; TGFB1, transforming growth factor beta-1.

consistency of findings across the 56 studies to identify 3 genes, *ABCB1*, *CYP3A5*, and *TGFB1*, associated with heart transplant outcomes (Table 1).

DISCUSSION

This literature review gives a comprehensive overview of all non-HLA polymorphisms that have been implicated to affect outcomes after heart transplantation. A total of 56 studies were included, all were candidate gene studies. Fifty-eight polymorphisms in 36 different genes were identified. Even though the field of genetics in heart transplantation remains largely unexplored, the identification of multiple polymorphisms supports the theory that non-HLA polymorphisms are involved in clinical outcomes after heart transplantation.

Although many variants and genes were only significantly associated to heart transplant outcomes in a single study, some were identified in multiple studies. Especially SNPs in *ABCB1*, *CYP3A5*, and *TGFB1*, summarized in Table 1, were consistently replicated for a subset of traits.

ATP Binding Cassette Subfamily B Member 1

The ATP binding cassette subfamily B member 1 (*ABCB1)* gene on chromosome 7 encodes for a transporter protein, P-glycoprotein, which is expressed in the liver, pancreas, kidney, and intestines. This protein is involved in multidrug resistance and is often linked to the development of resistance to anticancer drugs.[30,31] Together with CYP3A, it is involved in the transportation and biotransformation of cyclosporine and tacrolimus. It is suspected to be associated with inflammatory bowel disease[32] and colchicine resistance.[33] Several polymorphisms, rs1045642 within exon 26, rs1128503 within exon 12, rs2032582 within exon 21, rs2235013, and rs2235033, both within an intron, were associated with cyclosporine levels in heart transplant recipients.[10] Two reviews on several *CYP3A* and *ABCB1* SNPs show conflicting results on the influence of rs1045642, rs2032582, and rs1128503 on both cyclosporine and tacrolimus pharmacokinetics and drug response solid organ transplant recipients.[34-36] It is hypothesized that these SNPs could induce a lower P-glycoprotein activity, which could lead to less cyclosporine to be removed from the cells, thus increasing the bioavailability.[37] Two studies found associations between *ABCB1* SNPs and acute rejection after heart transplantation, which may be attributed to altered cellular levels of immunosuppressants.[14,15]

Cytochrome P450 Family 3, Subfamily A, Member 5

The cytochrome P450 family 3, subfamily A, member 5 (*CYP3A5*) gene on chromosome 7 encodes an enzyme of the cytochrome P450 family, mostly expressed in the liver, which is involved in the metabolism of a large variety of drugs and the synthesis of cholesterol and other lipids.[38] *CYP3A5* is known to be involved in the development of essential hypertension in humans[39] and to influence the required dose of the calcineurin inhibitors cyclosporin and tacrolimus in solid organ transplantation recipients. The rs776746 G allele is a loss-of-function allele, which disrupts a splice site. This leads to expression of a nonfunctional enzyme, resulting in reduced metabolization of tacrolimus and thus higher concentrations.[40,41] Multiple studies demonstrate an association between the SNP rs776746 and the tacrolimus level or dose for heart transplant recipients.[13,16-18] Renal dysfunction after cardiac transplantation is an adverse outcome frequently observed, mainly due to the prescription of calcineurin inhibitors; they are known to be nephrotoxic and involved in the development of hypertension, which can also result in renal dysfunction.[42] Two genomewide association studies (GWAS) in African American and European kidney transplant recipients, respectively, identified several *CYP3A5* SNPs to be associated with tacrolimus through concentrations, including rs776746.[43,44] One study found this SNP to be associated with renal function postheart transplantation.[20]

Transforming Growth Factor Beta-1

The transforming growth factor beta-1 (*TGFB1)* gene on chromosome 19 encodes a cytokine that has a wide range of functions and is involved in many biological processes, such as embryogenesis, carcinogenesis and immune response.[45] TGF-ß1 plays an especially large role in the regulation of T-lymphocytes[46] and is known to be of importance in a large variety of disorders in humans, including coronary artery disease (CAD) and rejection after kidney transplantation.[47-49] Two different polymorphisms in the *TGFB1* gene were identified in relation to outcomes after heart transplantation: rs1800470 in codon 10 and rs1800471 in codon 25. Several studies found an association between rs1800471 and acute cellular rejection, and/or CAD posttransplantation.[21-24,49] However, these findings were inconsistent because 3 studies showed no significant association between acute cellular rejection and rs1800471,[50-52] and another 3 studies failed to find an association between this SNP and chronic rejection.[25,52,53] Two studies found rs1800471 to be associated with renal function after transplantation[27,28] and 3 found no significant association.[54-56] The SNP rs1800470 was also associated with accelerated CAD after transplantation[25] and with renal function posttransplantation.[29]

Many loci were published in only a single study, or tested in multiple studies but could not be consistently replicated. For instance, the *IL10* SNP rs1800896 has been reported as significantly associated with rejection in 3 studies (**Table S2, SDC,** http://links.lww.com/TXD/A172).[50,57,58] However, 6 other studies were not able to find a significant effect for the same SNP on any form of rejection.[52,53,59-62] Publication bias, that is, the fact that negative findings are less likely to be published, makes it even more difficult to establish the true association between this SNP and rejection.

This lack of replication of findings could have various reasons. First, sample sizes of most studies are limited, which reduces statistical power to detect significant associations. Most transplant centers perform less than a few dozen of heart transplants per year, making it difficult to collect larger numbers of samples that are needed for genetic studies. Second, the definitions of the investigated phenotype varied greatly. For instance, the definition of acute rejection varied from "any histological evidence of rejection" to "acute cellular rejection grade 2R or higher." If the biological mechanisms leading to clinical outcomes are different, the underlying genes and loci involved may also vary. Combining clinical outcomes may lead to more noise and thus less statistical power, whereas testing a gene found for 1 outcome for association with a slightly different outcome, may lead to nonreplication. Third, patient characteristics of the included studies were heterogeneous, which is not unexpected because of the limited numbers of transplants performed each year. The studies samples differ for instance in ethnicity, sex, age, and underlying disease causing the need for heart transplantation.

Fourth, the vast majority of studies did not correct for potential confounding due to population structure or cryptic relatedness. Also, many studies did not correct for HLA type in the recipient, or HLA mismatches between donors and recipients. Lastly, many reports studied more than 1 polymorphisms and did not correct for multiple testing, most likely resulting in a number of false-positive results. Overall, the quality of the candidate gene studies was limited, with none of the studies fulfilling our quality criteria.

Candidate gene studies are hypothesis driven, assuming that prior knowledge of the gene function will lead to identification of genetic variants associated with clinical traits. Complex conditions, such as acute and chronic rejection or renal dysfunction posttransplantation, are believed to be influenced by multiple genetic polymorphisms that individually contribute only a small proportion to the overall risk. Various biological pathways are involved in the development of these conditions, but in many cases, it is unclear which pathways are involved or how important a specific pathway is. Thus, a candidate gene study may not be ideal to identify SNPs related to clinical outcomes after heart transplantation.

Genomewide association studies, incorporating millions of genetic variants, would likely be a more appropriate study design because it has an agnostic approach. However, the combination of a need for large sample sizes to be appropriately powered to detect significance in a GWAS and the limited amount of cardiac transplantations being performed could prove to be challenging. This is reflected by the lack of genomewide studies on heart transplant outcomes, and the small number of GWAS on solid organ transplant outcomes in general. Thus far, only 8 GWAS have been published in this field, and all involved renal transplants.[43,44,63-68] Outcomes investigated were medium-term graft function, new onset diabetes after transplantation, acute cellular rejection, and tacrolimus concentrations.

More research into the genetic factors associated with outcomes posttransplantation can lead to the discovery of novel pathways or biological processes involved and a better understanding of already known biological pathways. Large consortiums are key to allow these large-scale genetic analyses, potentially testing tens of millions of genetic variants. The International Genetics & Translational Research in Transplantation Network (www.igenetrain.org) was designed to this end, and now consist of more than 30 studies with extensive phenotypic and genomics and other -omics data.[69,70] The ultimate goal is to use the knowledge from this research to improve outcomes for transplant recipients, for example, by better individualized dosing and selection of immunosuppressive medication and possibly even risk stratification for adverse outcomes after heart transplantation.

Findings of this systematic review of current literature support the hypothesis that non-HLA polymorphisms are involved in outcomes after heart transplantation, but evidence for individual SNPs associated with specific phenotypes is limited. Larger studies incorporating genetic variation on a genomewide scale may be able to discover novel loci and to validate known genetic variation associated to outcomes after cardiac transplantation.

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