TRANSLATIONAL GENETICS

Transplant genetics and genomics

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Abstract | Ever since the discovery of the major histocompatibility complex, scientific and clinical understanding in the field of transplantation has been advanced through genetic and genomic studies. Candidate-gene approaches and recent genome-wide association studies (GWAS) have enabled a deeper understanding of the complex interplay of the donor–recipient interactions that lead to transplant tolerance or rejection. Genetic analysis in transplantation, when linked to demographic and clinical outcomes, has the potential to drive personalized medicine by enabling individualized risk stratification and immunosuppression through the identification of variants associated with immune-mediated complications, post-transplant disease or alterations in drug-metabolizing genes.

Tolerance
A state of immune unresponsiveness and quiescence towards specific antigens. In the case of transplantation, tolerance is directed towards donor-specific antigens.

Allogeneic
A term that describes tissues that are of distinct genetic origins and thus often immunologically incompatible.

Acute rejection
An episode of sudden deterioration in allograft function as a result of either antibody-mediated rejection or T cell-mediated rejection, which result from different molecular processes.

Isograft
A graft between two individuals who are genetically identical, such as in the case of monozygotic twins.

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That genetic factors participate in the mechanisms of transplant tolerance and rejection was established even before the first successful solid-organ transplantation was carried out in humans in 1954 (REF. 1). From the discovery in the 1900s that susceptibility to the growth of allogeneic tumours is genetically determined to the discovery of human leukocyte antigen (HLA) genes and allelic variants, genetics has been at the forefront of both scientific and clinical advances in the transplantation field[2]. These developments have been facilitated by the early adoption of genetic and genomic technologies, from PCR through to high-throughput, next-generation sequencing (NGS). Both PCR and sequencing technologies have contributed tremendously to the field's understanding of the roles of genetic polymorphisms and mismatched alleles in transplantation outcomes, which range from acute rejection to long-term interstitial fibrosis.

Despite the 'man-made' nature of organ transplantation, much remains to be understood about the combination of factors that leads to graft dysfunction or tolerance (BOX 1). Before the advent of immunosuppressive drugs, transplantation was mostly unsuccessful, with the exception of isograft transplantation between identical twins. Nowadays, the vast majority of organ transplants in the United States are allografts, which has spurred significant research into the consequences of genetic dissimilarities between donor and recipient. Allograft transplantation is in many ways more complex than other medical scenarios, as consideration must be given to more than one genome — that of the donor and that of the recipient — as well as to their epigenomic differences[3] (FIG. 1). Differences between donor and recipient can encompass between 3.5 million and 10 million genetic variants, as well as substantial epigenetic variation, dependent on ethnicity and geographical region[4,5]. Both animal and human studies, including recent genome-wide association studies (GWAS), have established a foundation of genes and variants that are associated with transplant outcomes, but follow-up studies to determine the functional effects of polymorphisms remain in their infancy. An understanding of the causal factors and an integrated 'omics' approach are, however, essential to achieve the goals of discovering novel drug targets, repositioning existing drugs and developing interventional treatments. Pre-transplant genetic analysis of donors can identify organs that are more susceptible to ischaemic and reperfusion damage, and help to reduce the risk of delayed graft function[6], whereas the identification of recipients who are at a higher risk of negative outcomes, such as new-onset diabetes after transplantation (NODAT) and cancer, or those with alterations in drug-metabolizing genes, enables targeted post-transplant monitoring and immunosuppressive therapy.

In this Review, we summarize how recent advances in genetics and genomics have led to improvements in the understanding of organ transplantation diagnostics and graft outcomes. We begin by discussing the basis of alloimmunity in the context of transplantation, recent insights into HLA and non-HLA factors, and studies that have used newer genetic approaches, such as GWAS and NGS. We describe advances in immunosuppressant pharmacogenomics and the evolution of diagnostic methods for monitoring and predicting transplant outcomes, as well as the roles of microRNAs and epigenetics in transplantation. Finally, we outline how novel technologies will both help to solve current challenges and set the future directions for the field.

Principles of alloimmunity

The genomic region encoding the major histocompatibility complex (MHC) — known as the HLA complex in humans — is the most gene-dense region of the

human genome, and one of the most immunologically and clinically relevant regions in the context of transplantation. MHC proteins enable the immune system to distinguish between 'self' and 'other'. Hence, these proteins and their genes determine histocompatibility — that is, the ability of an individual to accept tissue or cells from another person without generating an immune response[7,8]. In humans, the MHC region comprises more than 200 HLA loci, which are located in proximity on chromosome 6. Proteins encoded by the three main MHC class I genes — *HLA-A*, *HLA-B* and *HLA-C* — are present on the surface of most cells bound to immunogenic peptides that are exported from inside the cell. The six main MHC class II genes — *HLA-DPA1*, *HLA-DPB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DRA* and *HLA-DRB1* — encode cell-surface proteins that display peptides derived from circulating, extracellular proteins to the immune system. MHC class II molecules are expressed only on antigen-presenting cells (APCs), such as dendritic cells.

Graft rejection — which can be hyperacute, acute or chronic — has traditionally been understood to be mediated via the direct and indirect pathways of allorecognition (FIG. 2), the processing and presentation of donor antigens to recipient cells. Naive T cells activated after transplantation develop into either pro-inflammatory or anti-inflammatory subtypes, triggering characteristic immune response profiles that ultimately lead to cytotoxic T cell-mediated or antibody-mediated destruction of the graft, respectively. Hyperacute rejection, which develops within minutes to hours after transplantation, is caused exclusively by the humoral immune response acting through pre-existing antibodies against donor antigens that activate the complement system. By contrast, acute rejection, which can occur days to years after transplantation, and chronic rejection, which develops over months to years, can result from T cell-mediated or antibody-mediated mechanisms. Chronic injury to grafts by non-immune mechanisms — such as infection, ischaemia, ageing, drugs or recurring disease — can also occur.

Allografts
Grafts from another member of the same species, such as in the case of organ transplantation, as opposed to grafts from a member of a different species (xenograft) or from the recipient themselves (autograft).

Ischaemic and reperfusion damage
Damage to an organ as a result of a transient inadequate blood supply.

Delayed graft function
A state in which renal failure persists after transplantation, thus necessitating dialysis.

New-onset diabetes after transplantation
(NODAT). The occurrence of diabetes mellitus after transplantation in a patient who did not have the disease before. This occurs in 2–53% of all solid-organ transplants and is due in part to the immunosuppressive medications that are used to prevent transplant rejection.

Alloimmunity
An immune response to antigens that are both non-self and from the same species.

Allorecognition
The ability of a host to recognize allogeneic tissue as distinct from its own.

Complement system
A component of the innate immune system that can be activated by antigen-bound antibodies.

Central tolerance
The mechanisms by which T cells and B cells are rendered non-reactive to an antigen, typically a self-antigen, in the primary lymphoid organs.

Peripheral tolerance
The mechanisms by which T cells and B cells are rendered non-reactive to an antigen outside the primary lymphoid organs.

Regulatory T cells
(T_{reg} cells). A subpopulation of T cells that are generally immunosuppressive rather than pro-inflammatory.

Box 1 | **Tolerance**

Achieving immunological tolerance has been a goal for the field of transplantation ever since the Nobel Prize-winning discovery of actively acquired tolerance of skin grafts in mice in 1953 (REF. 164). In these experiments, exposure to foreign cells at a fetal stage led to the establishment of central tolerance to the foreign antigens. Although originally hypothesized to be a result of a specific failure of the host's immunological response, recent research has shown that tolerance is instead the result of an active immunological response, albeit one involving different factors. Operational tolerance is defined as the acceptance of a human leukocyte antigen-incompatible allograft in the absence of receiving drugs to maintain immunosuppression; operational tolerance is generally accepted to be robust when immunosuppressive therapy has been discontinued for at least 1 year and the patient is free of any infections or malignancies that may otherwise drive an immunocompromised clinical state. The underlying mechanisms of operational tolerance are based on peripheral tolerance, a state in which there is a complex homeostatic interplay of suppressor cells dominating over cytotoxic cells.

Although the true identities and roles of these suppressor cells is still an active area of research, studies have increasingly focused on the active part that regulatory T cells (T_{reg} cells) play in suppressing the anti-allograft immune response[165]. More recently, myeloid-derived suppressor cells (MDSCs), another cell type that negatively regulates the immune response, have been found to have a role in transplantation, and they synergize with T_{reg} cells in this context[166]. Infusions of MDSCs and T_{reg} cells have been shown in initial studies to extend allograft survival and establish tolerance to a transplanted organ after a brief period of adjunctive immunosuppression[167,168]. Other recent studies suggest that these cells are supported by an expansion of naive B cells[169,170], or possibly by a lower number of $CD4^-$ T cells and an expanded pool of plasmacytoid and myeloid dendritic cells[171]. It is very likely that all of these cell types have complex, intertwined roles in the induction and maintenance of operational tolerance.

Studies into the induction of tolerance have shown promise in the application of depletion approaches. For example, by transplanting donor haematopoietic cells and infusing specific facilitator cell pools, chimerism-based mechanisms for transplant tolerance could be induced[172,173]. Initial studies using gene transfer and editing have found that modification of bone marrow or hepatic cells so that they express specific allotypes of major histocompatibility complex class I or II proteins can lead to the tolerance of allografts[174,175]. These studies are promising, but their scope and utility currently remain limited by the low number of patients who undergo simultaneous solid-organ and bone marrow transplants, and by the infancy of gene-editing technologies for clinical use.

The ideal scenario would be the ability to individually monitor the evolution of the immune response profile of the recipient to the donor organ or cells, such that the timing of immunosuppression withdrawal can be customized to each donor–recipient pair. In patients who 'fail' to develop tolerance, further withdrawal of immunosuppressive therapy could then be stopped. The small trial sizes and the even smaller number of patients who demonstrate true clinical success with complete and safe drug withdrawal are even fewer than would support the study of this group of patients for the development of biomarkers for stable and robust induced transplant tolerance. The underlying mechanisms of graft acceptance in these trials are based on central deletional tolerance as well as possible adjunctive peripheral mechanisms that are under study[176]. As such, challenges remain in the prediction of which patients would achieve transplant tolerance and the time it takes to develop this accommodative response after engraftment. It is for this reason that our group, and others, have turned to the identification of transcriptional perturbations in peripheral blood in patients with operational or spontaneous tolerance to kidney[169,177–179] or liver[180,181] transplants. The utility of any biomarker that can reflect the development of peripheral transplant tolerance lies in its ability to represent changes in the alloresponse so as to predict the immunological safety of minimizing immunosuppression and provide a barometer for monitoring immune reactivity versus quiescence over the course of the life of the allograft. Robust validation of the tolerance-specific biomarkers that have been developed remains difficult to accomplish given the low frequency of stable clinical tolerance in any organ setting.

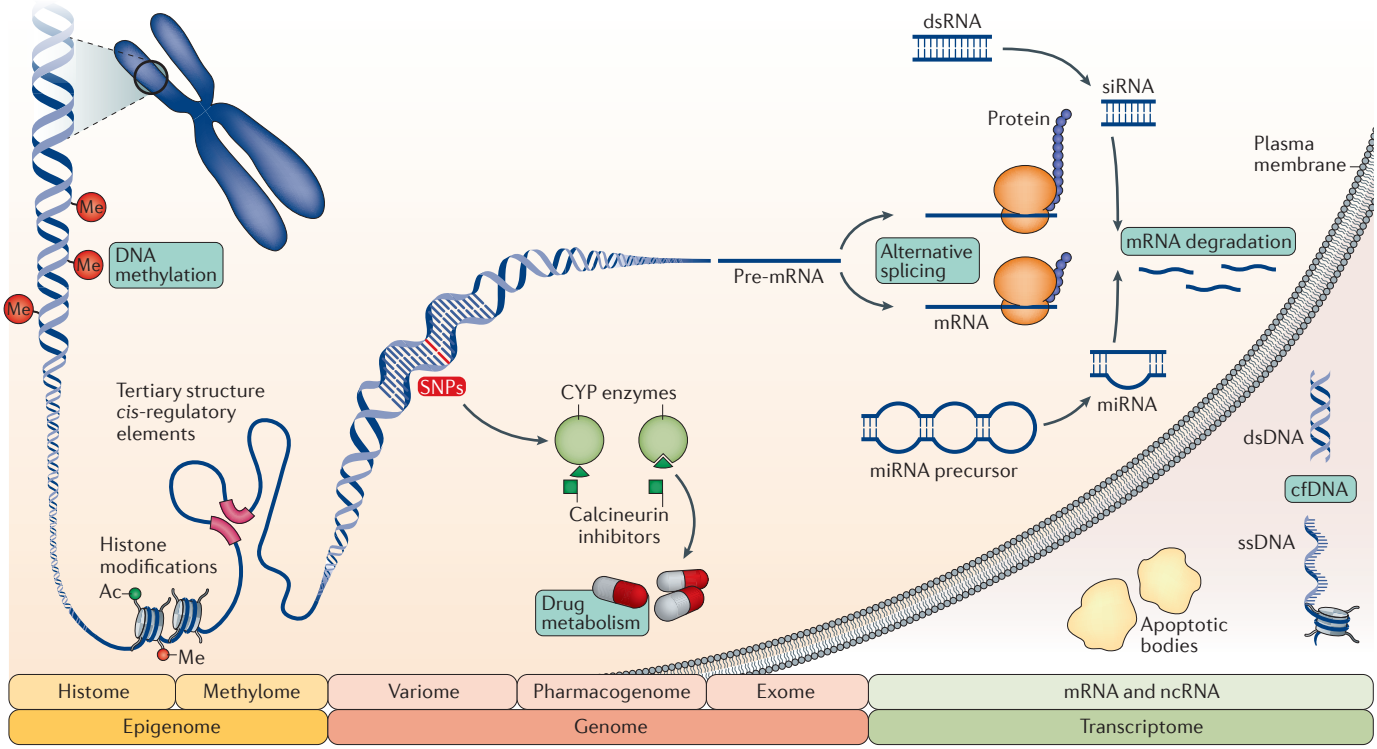

Figure 1 | **The omics of transplantation genetics.** Both the donor and the recipient contribute to the diversity of the types and modifications of nucleic acids that are relevant to transplantation genetics. The tertiary structure of DNA is determined by the degree of packing of the chromosome, which is dependent on histones. The 3D structure enables regulatory elements to perform their functions at sites that are *trans* to their location. Both histones and the DNA itself can be modified by, for example, acetylation ('Ac' in the figure) or methylation ('Me'). These epigenetic marks can influence the transcription of certain gene products and, unlike the sequence of the genome, may change in response to environmental factors. Transplantation-specific phenomena such as ischaemia–reperfusion injury and long-term low-level immune burden can alter gene regulation to promote tolerance or rejection. DNA variants in the donor and recipient may be present in the form of single-nucleotide polymorphisms (SNPs). Such SNPs can influence the conformation and function of proteins, for example, the drug metabolizing cytochrome P450 (CYP) family of enzymes, or they may affect promoters and enhancers to alter the transcription of their respective genes. These may in turn affect the pharmacodynamics and kinetics of immunosuppressive drugs. Furthermore, differences between the donor and recipient protein conformations and amino acid sequences can serve as a source of antigens for the host immune response against the allograft. DNA is transcribed into precursor mRNA (pre-mRNA), which is then spliced to produce mRNA. Alternative splicing may produce multiple mRNA transcripts from a single pre-mRNA. Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and short interfering RNAs (siRNAs), can function to modulate the translation of mRNA into gene products. Genetic material can also appear outside the cell and the nucleus in the form of cell-free DNA (cfDNA), either as single-stranded DNA (ssDNA) or double-stranded DNA (dsDNA), and may still be bound to histones (that is, the DNA present in nucleosomes). cfDNA is often released when cell death occurs as a result of apoptotic or necroptotic processes, and its levels in various bodily fluids may serve as a sensitive biomarker of transplant rejection. The fields of genomics, transcriptomics and epigenomics comprise even more specialized research fields, the scope of which can overlap; for example, the variome is a subset of the exome, and the coding portion of the pharmacogenome is contained within the exome. dsRNA, double-stranded RNA.

Genetics of allograft outcomes

Many clinical advances in transplantation have resulted from the identification of associations between polymorphisms in genes or their regulatory regions and transplant outcomes[9]. Data generated in sequencing projects[10,11] such as the Human Genome Project and the 1000 Genomes Project have formed the basis for the identification of regions of genetic variability between donors and recipients. Sequencing technologies have also driven the ENCODE project, which has assigned biochemical activities to more than 80% of the genome and identified more than four million regulatory regions[12]. This has enabled the mapping of genetic variants to gene regulatory regions and the indirect assessment of links to disease[13].

HLA genotyping. The HLA class I and class II loci are the most polymorphic genes in the human genome[14]. Moreover, almost half of the genes located in the MHC region have paralogous copies on chromosomes 1, 9 and 19, which may have enabled the swift evolution of novel functionalities[15]. Allelic variations in MHC genes underpin histocompatibility in transplantation, and *HLA-A*, *HLA-B* and *HLA-DR* are recognized to have the

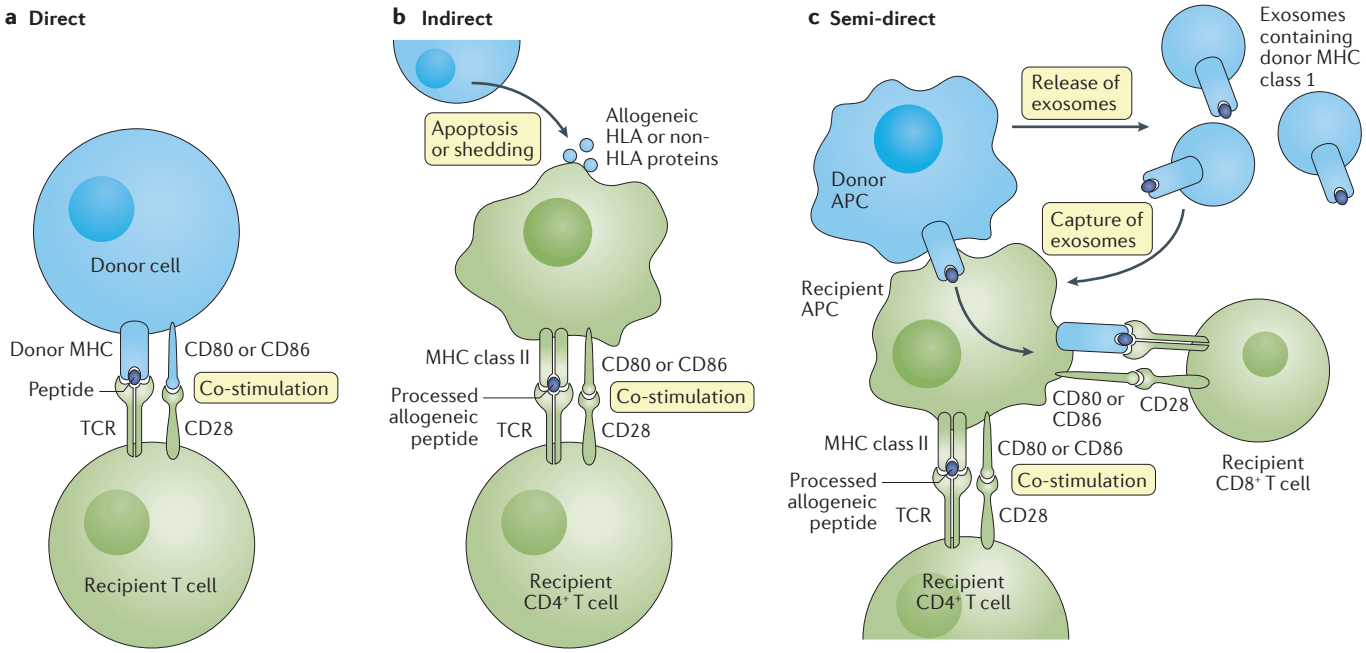

Figure 2 | **Pathways of allorecognition.** Graft rejection is mediated by T cells via the direct and the indirect pathways of allorecognition. **a** | In the direct pathway, which is important in the early phase of allorecognition of host antigens and graft rejection, polyclonal recipient T cells recognize intact donor major histocompatibility complex (MHC) molecules directly via their T cell receptors (TCRs). **b** | By contrast, the indirect pathway is oligoclonal and dependent on a restricted set of T cells that display a specific repertoire of TCRs. These T cells recognize only a limited number of dominant peptides that are displayed on the MHC of recipient antigen-presenting cells (APCs), and they play an important part in late and chronic rejection[213,214]. The indirect pathway is also responsible for the alloantibody responses seen in patients who have received organ transplants[215]. Consistent with the role of these pathways in transplantation, pre-transplant treatment with tolerogenic donor-derived MHC peptides can protect a graft from rejection in rodents[216]. **c** | In semi-direct allorecognition, donor MHC class I and/or class II molecules are acquired and presented by recipient APCs. Acquisition is achieved either through cell-to-cell contact-mediated transfer of the plasma membrane and cytoplasm from donor dendritic cells or endothelial cells to recipient APCs, or through the capture of donor exosomes containing MHC molecules (only MHC class I acquisition is shown in the figure). The transfer of MHC and peptide complexes in this manner can enable a recipient APC to simultaneously present donor-specific peptides via self-MHC class II molecules to CD4$^+$ T helper cells andstimulate CD8$^+$ cytotoxic T cells via donor MHC class I molecules. HLA, human leukocyte antigen.

greatest importance for successful HLA matching. When HLA is mismatched, graft survival decreases in a manner that is dependent on the number of mismatches[16]. As such, HLA typing is crucial for the identification of incompatibilities and enables the adequate matching of donor and recipient pairs.

Numerous methodologies with low ambiguity rates have been developed for the high-throughput, multiplex and efficient typing of HLA genes[17,18]. These methods have just begun to see adoption within the field of solid-organ transplantation. NGS methods sequence millions of single DNA molecules in parallel, enabling complete HLA class I and II typing for 24 or 48 individual DNA samples in a single sequencing run. The very large number of sequencing reads generated in a single run (300,000–400,000) enables the detection of rare sequence variants that are present in individual samples. NGS panels targeted at HLA loci have become routinely available in the clinic within the past few years, partially due to the recent availability of commercial NGS HLA typing kits, as well as reductions in the cost of sequencing technologies over time to a level comparable to that of Sanger sequencing[19,20]. Although whole-exome sequencing and whole-genome sequencing are available in research settings, these are generally still not cost-efficient or widely available in clinical laboratories.

Although the coding regions of the HLA loci have been well studied, noteworthy discoveries in regulatory, non-coding regions of these genes remain to be made. For example, in a recent study of haematopoietic stem cell transplantation (HSCT), *HLA-DPB1* expression was dependent on the regulatory variant rs9277534, with rs9277534G being associated with higher *HLA-DPB1* expression than rs9277534A[21]. The risk of acute graft-versus-host disease (GVHD) was higher for recipients with the G allele if the donor had the A allele, even when donor and recipient were matched for *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1* and *HLA-DQB1* alleles. Findings such as these highlight the need for more comprehensive and deeper sequence analysis of the HLA region. The IPD–IMGT/HLA Database (Immuno Polymorphism Database–International Immunogenetics Information System/HLA Database) contains sequences for all the HLA alleles that are officially recognized by the

Tolerogenic
The quality of being able to induce immunological tolerance.

Survival
In the context of this Review, an outcome measured as the time until either graft failure (when referring to allograft survival) or patient mortality (when referring to recipient survival).

Ambiguity rates
The rates at which sequenced regions have a low level of confidence in excluding possible allelic variations.

Whole-exome sequencing
Sequencing of all protein-coding regions in the genome.

Whole-genome sequencing
Sequencing of the complete DNA genetic material in a cell or organism.

Graft-versus-host disease
(GVHD). Largely specific to haematopoietic stem cell transplantation, it is a medical complication in which immune cells in the donated tissue reject and attack the host cells.

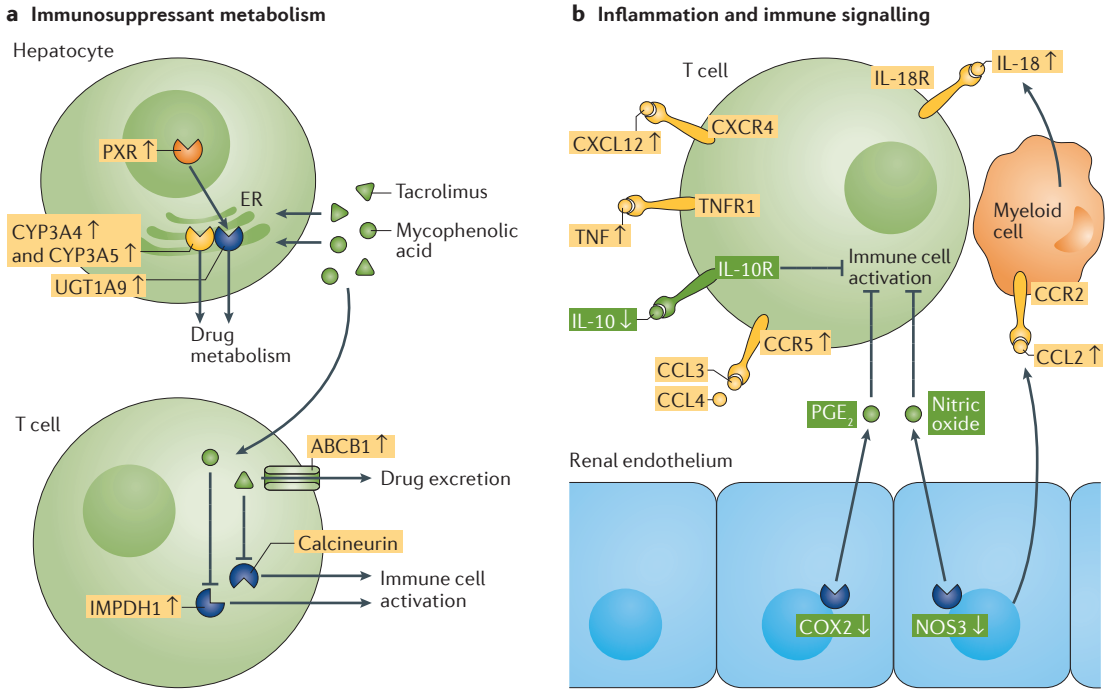

Figure 3 | **The effect of transplant-associated variants on immune-mediated rejection.** A schematic representation of the proposed integration of mechanisms by the protein products encoded by transplantation-associated loci that are involved in immune-mediated rejection and for which the direction of the functional effect of the variants is known or predicted. Genetic variants shown were selected on the basis of the presence of known or predicted mechanisms, and reproducibility in the literature for those investigated in multiple studies. Only kidney transplant studies were selected to minimize the divergent effects of variants in different organs. Molecules and proteins in green actively reduce the risk of rejection, graft dysfunction or graft failure, whereas those in yellow act to increase the risk. Cells in green represent recipient cells, whereas blue cells are graft cells. Arrows show the functional effect of the related genetic variant on protein activity, whether increased or decreased. This may be either through altering the functionality of the protein, or through increased or decreased expression, such as through altered gene transcription or mRNA stability. **a** | The effects of immunosuppressants are heavily altered by changes in serum levels and functionality. Increased drug degradation through the increased translation or activity of the metabolizing protein, and through the increased efflux of drugs from target cells, are ways in which the effectiveness of immunosuppressants may be decreased. **b** | Lymphoid and myeloid cell activation leads to increased inflammation and negative graft outcomes. Numerous genetic variants associated with cytokines and cytokine receptors alter either the binding affinities or the transcriptional activity of these inflammatory mediators. In the transplanted organ, the decreased activity of proteins that negatively regulate the inflammatory process may also contribute to the enhanced recruitment or activity of inflammatory cells. Note that this is a simplified picture, only taking into consideration gene products for which modulation by genetic variants is known or suspected. ABCB1, multidrug resistance protein 1; CCL, C-C motif chemokine; CCR, C-C chemokine receptor; COX2, cyclooxygenase 2 (also known as PTGS2); CXCL12, C-X-C motif chemokine 12; CXCR4, C-X-C chemokine receptor type 4; CYP, cytochrome P450; ER, endoplasmic reticulum; IL-10, interleukin-10; IL-10R, IL-10 receptor; IL-18, interleukin-18; IL-18R, IL-18 receptor; IMPDH1, inosine-5′-monophosphate dehydrogenase 1; NOS3, endothelial nitric oxide synthase; PGE_2, prostaglandin E_2; PXR, pregnane X receptor (also known as NR1I2); TNF, tumour necrosis factor; TNFR1, TNF receptor 1; UGT1A9, UDP-glucuronosyltransferase 1-9.

Killer-cell immunoglobulin-like receptors
(KIRs). Receptors that are expressed on the surface of natural killer cells and modulate their cytotoxic activity by recognizing major histocompatibility complex class I allelic variants.

MHC class I polypeptide-related sequence A
(MICA). A cell-surface antigen that is recognized by the receptor NKG2D, which is found on natural killer cells, T cells and macrophages.

Minor histocompatibility antigens
(miHAs). The distinct peptide products of polymorphic genes that distinguish the recipient from the donor.

WHO Nomenclature Committee for Factors of the HLA System, and provides users with online tools, allele reports, alignment tools and detailed descriptions of the source cells[22]. Researchers are encouraged to submit new HLA allele sequences directly to the IPD–IMGT/HLA Database before publication for curation and assignment of an official name to ensure consistency in nomenclature and rapid access to new sequence information.

The increasing importance of non-HLA loci. Even patients who receive HLA-identical transplants can undergo acute or chronic rejection[23], which suggests a role for non-HLA factors in alloimmunity (FIG. 3). Studies have found that graft loss at 10 years among recipients of cadaveric organs was caused by non-HLA factors in 38% of cases, whereas only 18% were caused by HLA-associated factors and 43% by non-immunological factors (for example, surgical complications, drug toxicity or infections)[24,25].

Potential non-HLA mediators include the killer-cell immunoglobulin-like receptors (KIRs), MHC class I polypeptide-related sequence A (MICA) and minor histocompatibility antigens (miHAs). Mismatches between a donor and recipient, either through incompatible receptor–ligand interactions or through polymorphic

genes that encode non-self-peptides, may trigger an alloimmune response. For example, in kidney transplantation, mismatches between the recipient KIRs and donor ligands in donor–recipient pairs matched for *HLA-A*, *HLA-B* and *HLA-DR* alleles were initially reported to be associated with reduced long-term graft survival[26], although a larger study by an independent group did not find any effect on graft survival after 10 years[27]. Another study found that MICA-specific antibodies were present at a significantly higher percentage in patients whose grafts failed than in those with functioning grafts[28]. In a large study of 1,910 kidney transplant recipient–donor pairs with varying *HLA-A*, *HLA-B* and *HLA-DR* matching, MICA antibody-positive patients and those who were sensitized against MICA pre-transplant had significantly poorer allograft survival than did control patients[29]. For miHAs, the most studied gene mismatch is that in the H-Y antigen, a product encoded by the Y-chromosome-specific gene *MEA1* (male-enhanced antigen 1). Two large studies, one including more than 195,000 patients, found that male-donor allografts in female recipients had the highest rate of graft failure relative to any other sex pairings[30,31]. Antibodies against H-Y were found in the sera of male-to-female transplant recipients and were associated with acute rejection[32].

Accumulating evidence indicates that another non-HLA source of alloimmunity is the action of antibodies against non-HLA targets, which triggers a response that is both alloimmune and autoimmune in nature, and links the HLA and non-HLA immune responses[33]. Antibodies have been identified in recipients that target both self-antigens and donor-specific MHC class I molecules, and lead to the activation of the complement system[34,35]. These antibodies may pre-exist owing to immunological challenges that occurred before transplantation, or owing to the exposure of cryptic antigens that were released from apoptotic and necrotic cells during the transplantation of the organ[36].

Although the association of antibodies that target non-HLA proteins with different diseases is not straightforward, they can serve as excellent surrogate biomarkers of transplant injury and rejection. That is, a number of studies have reported an association of different antibodies that target non-HLA proteins with varying injury types, including acute rejection[23,37]. For instance, a seminal study discovered that the transplanted kidney has differential immunogenicity in a kidney compartment-dependent manner, with the highest immunogenicity identified within the renal cortex, glomerulus and deep renal pelvis[37]. In particular, antibodies against RHO guanine nucleotide exchange factor 6 and stathmin 3 were found in the majority of renal transplant recipients who were undergoing rejection, and an integrated genomics approach identified an over-representation of the corresponding genes (*ARHGEF6* and *STMN3*, respectively) in specific kidney compartments.

Antibodies that target other non-HLA factors have also been shown to correlate with or, in some cases, even contribute mechanistically to graft dysfunction[33,38–40]. Using unbiased protein array technology on pre-transplant sera, one study demonstrated that antibodies that target non-HLA molecules — specifically those that target C-X-C motif chemokine 9 (CXCL9), CXCL11, interferon-γ (IFNγ) and glial-derived neurotrophic factor (GDNF) — correlated with post-transplant organ function and biopsy histology; that is, high levels of these antibodies were indicative of rapid post-transplant evolution to chronic allograft injury[23]. Thus, perturbed heterologous immunity before organ engraftment seems to be a primary driver of subclinical, injurious alloimmune responses in chronic transplant injury and rejection. Similar applications of protein arrays have identified antibodies that target other epitopes of non-HLA molecules, including protein kinase Cζ-type (PRKCζ) in patients with steroid-resistant acute rejection[40]; glomerular antigens (such as CD40) in patients who show a recurrence of focal segmental glomerulosclerosis after kidney transplantation[41]; and endoglin, Fms-related tyrosine kinase 3 ligand (FLT3LG), EGF-like repeat and discoidin I-like domain-containing protein 3 (EDIL3) and intercellular adhesion molecule 4 (ICAM4) in the case of anti-endothelial cell antibodies (AECAs)[42]. With the advent of high-throughput immunoglobulin sequencing, identifying sequence-specific antibodies and understanding the time-dependent development of the humoral immune response will enable greater distinction of the hallmarks of immune dysregulation in transplantation[43,44].

Variability in cytokine and cytokine receptor activity and expression in the donor and/or the recipient has also been shown to affect the immune response against allografts (FIG. 3b). Recipient genetic variation in cytokine genes — for example, those encoding tumour necrosis factor (TNF), interleukin-6 (IL-6), IFNγ and complement proteins — can increase the risk of graft rejection[45]. For example, renal transplant recipients who carried the *TNF* –308 G allele as well as the *IL10* –1082 G allele (who are known as 'high producers' of these cytokines) had a higher rate of acute rejection of grafts from *HLA-DR*-mismatched donors than did those carrying either variant alone or neither of the two variants. By contrast, patients who carried the *TNF* –308 G allele alone had more steroid-resistant acute rejection episodes than did those without[46]. Single-nucleotide polymorphisms (SNPs) in various genes, such as those encoding vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), have been associated with an increased risk of graft fibrosis[47,48].

Taken together, the investigations into non-HLA factors described above reflect the recent recognition of their importance in allograft outcomes.

GWAS in transplantation

GWAS have revolutionized the means and the ability to search for genetic influences on transplant outcomes, although only a few such studies have been conducted to date owing to the low number of patients undergoing transplants. A summary of transplant-associated GWAS is shown in TABLE 1, excluding non-human, non-outcomes-related studies.

Cryptic antigens
Self-antigens that are not clonally deleted in the thymus owing to low surface presentation on antigen-presenting cells (APCs). These self-antigens can be expressed by APCs following differential processing by inflammatory proteases.

Trough levels
The lowest levels of a pharmaceutical present in the blood before the next dose.

Long-term graft function
Refers to the functional characteristics of the transplanted organ, which typically decrease over time owing to immune injury and subsequent fibrosis. Biomarkers of function include serum creatinine levels for kidney transplants, pulmonary function tests for lung transplants, and bile or specific enzyme levels for liver transplants.

GWAS in solid-organ transplantation. The first GWAS of acute rejection in solid-organ transplantation in a sufficiently powered study of 778 renal transplant recipients was published online in 2016, and identified two loci as being associated with T cell-mediated rejection in discovery and replication cohorts[49]. Three SNPs were identified: rs10765602, which is located upstream of *CCDC67* (which encodes coiled-coil domain-containing 67; also known as *DEUP1*), and rs10846175 and rs7976329, which are located in the first intron of *PTPRO* (which encodes protein tyrosine phosphatase receptor type O). Although the study did not determine whether these SNPs act in *cis* to their nearby loci, the lymphocytic and ciliary associations and functions of CCDC67 and PTPRO suggest that these variants may be involved in transplant outcomes, as proposed by the authors of the study[49]. However, all study participants were of European descent, and thus the applicability of these findings to a more diverse patient population remains to be determined. In addition, of particular note is the use of pooled DNA samples in this GWAS and others; each pool had three replicates[49], which is insufficient to reduce the standard deviation of an allele frequency estimate to below 0.01 as a result of random experimental errors[50]. Furthermore, orthogonal validation of the positively associated loci, which provides a means of quality control and allows the assessment of marker predictiveness, was not undertaken in the original discovery cohort but can be achieved by individual genotyping.

Other solid-organ transplantation GWAS have included more modest cohort sizes. In a GWAS of 388 renal or heart transplant recipients, SNPs that had previously been shown to be associated with malignancy in other studies were identified as being significantly associated with cutaneous squamous cell carcinoma[51]. However, due to the small size of the discovery cohort, none of these SNPs reached genome-wide significance. In another study of 256 adult renal transplant recipients, 26 SNPs were associated with NODAT. Only one of these SNPs was validated in second-stage genotyping, although logistic regression found associations of eight SNPs with NODAT[52]. In a study of 357 African-American kidney transplant recipients, three allelic variants of *CYP3A5* (which encodes cytochrome P450 family 3 subfamily A member 5) — namely, *CYP3A5*3, CYP3A5*6* and *CYP3A5*7* — were found to explain a great proportion of the variability in the trough levels of tacrolimus[53], which is an immunosuppressive drug that is used to lower the risk of organ rejection. In addition, two previously identified variants within *CYP3A4* and *ABCB1* (which encodes multidrug resistance protein 1), rs355993567 and rs1045642, were found not to contribute to the variation observed in this analysis.

In a GWAS of 326 Irish renal transplant recipients, researchers assessed associations between recipient genotype and 5-year allograft function, and identified two variants (rs3811321 and rs6565887) that together explained 17.4% of the 5-year creatinine variance and were thus predictors of long-term graft function[54]. However, significance was borderline, which was reflective of the small cohort size. A follow-up study involving more than 1,600 patients from the Assessment of Lescol in Renal Transplant (ALERT) trial found no associations between the identified SNPs and long-term graft function or serum creatinine levels[55], which highlights the

Table 1 | **A brief history of genome-wide association studies in the transplantation field**

GWAS	Sample size and cohort type	Transplant type	Transplant-associated outcome or aspect of interest	Ethnicity of recipients	Associated genes or regions	Refs
O'Brien *et al.* (2013)	326 discovery	Kidney	Long-term graft function	Caucasian	TRA‡ and *ZNF516**	54
McCaughan *et al.* (2014)	256 discovery and 441 replication	Kidney	NODAT	Caucasian	*ATP5F1P6*‡	52
Sanders *et al.* (2015)	388 discovery	Heart and kidney	Post-transplant cancer	Caucasian	*LINC00882*‡, *CACNA1D*‡ and *CSMD1*‡	51
Oetting *et al.* (2016)	197 discovery and 160 replication	Kidney	Pharmacokinetics (tacrolimus)	African American	*CYP3A5* and *ZSCAN25*	53
Ghisdal *et al.* (2017)	778 discovery and 844 replication	Kidney	Acute rejection	Caucasian	*PTPRO* and *CCDC67*	49
Sato-Otsubo *et al.* (2015)	1,589 discovery	HSC	Acute GVHD	Japanese	*HLA-DPB1* and *KRAS*	65
Bari *et al.* (2015)	68 discovery and 100 validation	HSC	Acute GVHD	Mostly Caucasian	*SUFU*‡	67

ATP5F1P6, ATP synthase, H⁺-transporting mitochondrial Fo complex subunit B1 pseudogene 6; *CACNA1D*, calcium voltage-gated channel subunit α1 D; *CCDC67*, coiled-coil domain-containing 67; *CSMD1*, CUB and Sushi multiple domains 1; *CYP3A5*, cytochrome P450 family 3 subfamily A member 5; GVHD, graft-versus-host disease; GWAS, genome-wide association study; *HLA-DPB1*, human leukocyte antigen DP1; HSC, haematopoietic stem cell; *LINC00882*, long intergenic non-protein coding RNA 882; NODAT, new-onset diabetes after transplantation; *PTPRO*, protein tyrosine phosphatase receptor type O; TRA, T cell receptor-α locus; *ZNF516*, zinc finger protein 516; *ZSCAN25*, zinc finger and SCAN domain-containing 25. *Loci were significant in an initial study, but a follow-up study found no association. ‡Loci were the most significant association but did not reach the threshold significance of $P < 5 \times 10^{-8}$.

need for sufficiently powered studies and large cohort sizes in order to avoid bias in the estimated effect of novel variants, which is otherwise known as 'the winner's curse' (REF. 56).

Genome-wide analysis of liver transplantation in humans has also been performed, although only a subset of 1,774 candidate SNPs from the genotyping array were analysed[57], in contrast to traditional GWAS.

GWAS in HSCT. A greater number of GWAS have been conducted in the setting of HSCT than in that of solid-organ transplantation, and HSCT GWAS have largely focused on GVHD. In 2008, a combined GWAS and cytotoxic T lymphocyte assay approach was used to identify associations in miHA-encoding genes and identified two loci, one previously characterized and one novel[58]. The same group identified additional miHA variants, the characterization of which is still an ongoing process[59].

The first outcomes-focused GWAS was conducted in 2009 in a cohort of 1,560 donor–recipient pairs, and sought to identify SNPs associated with Gram-negative bacteraemia and bronchiolitis obliterans syndrome, which is a manifestation of chronic allograft rejection and results from fibrosis of the bronchioles[60]. Although the study identified nine recipient allelic associations in the case of bacteraemia, as well as four recipient and three donor loci for bronchiolitis obliterans syndrome, a replication study of the bacteraemia SNP associations by the same group subsequently found only one of them to be significant[61]. Of note, these findings were published in review articles, and the actual SNPs themselves were not published in the primary literature. In 2012, the same group evaluated a series of candidate SNPs for acute GVHD using a genome-wide imputation-based approach in 1,424 donor–recipient pairs, and validated previous associations with SNPs in *IL10* and *IL6* (REF. 62). Although this was not a traditional GWAS approach, the authors of the study acknowledged the difficulties of an unbiased, non-candidate gene approach in HSCT.

Methods to detect copy number variants in a genome-wide fashion were limited until recently, when a hybrid genotyping array was designed to simultaneously detect SNPs and copy number variation in the human genome[63]. To identify associations with acute GVHD, the authors used this array to specifically interrogate deletion alleles in the protein-coding sequence that occurred with an allele frequency >10% and that were expressed in tissues involved in acute GVHD. Quantitative PCR (qPCR) assays in a total of three cohorts, comprising 1,345 donor–recipient pairs of HLA-identical siblings, revealed that mismatch of *UGT2B17* (which encodes UDP glucuronosyltransferase family 2 member B17) between the donor and recipient (specifically donor-negative and recipient-positive) was associated with acute GVHD and showed that multiple miHAs were derived from UGT2B17 (REF. 64).

The largest GWAS in HSCT to date was conducted in 1,589 patients who had received transplants from unrelated donors that were matched for *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1* and *HLA-DQB1*, and aimed to identify associations with severe acute GVHD[65,66]. Under the assumption of no HLA restriction, the researchers identified the rs6937034 SNP in the *HLA-DPB1* locus to be associated with grade 2–4 acute GVHD[65]. When they restricted for *HLA-DQB*061* or *HLA-B*4403*, signals in regions of chromosomes 12 and 9 were found to be associated with varying grades of acute GVHD. In a paediatric population, the development of acute GVHD was associated with recipient polymorphisms in *SUFU*, with CC homozygosity at rs17114808 (but not the CT or TT genotype) being significantly associated with acute GVHD[67], although the sample sizes were small (68 and 100 patients in the discovery and validation cohorts, respectively). Functional assays demonstrated that the SUFU protein suppresses allogeneic T cell proliferation through a reduction in HLA-DR expression on dendritic cells[67].

Follow-up studies using large cohorts of transplant recipients will be necessary to validate the findings of all the aforementioned GWAS. Sample size considerations have an important role in the analysis and interpretation of GWAS results, and cohort sizes will need to be adjusted to the disease being investigated and the outcome of interest, as assumptions with regard to disease prevalence, inheritance models and effect sizes will vary[68]. Tools such as the publicly available Genetic Power Calculator are available to help with the proper design of genetic mapping studies[69].

Population-specific genetic risk factors

One source of variation that underpins differences in transplant outcomes between population groups are unique, non-HLA population-specific variants from ethnically diverse donor–recipient pairings. The best studied of these are the two allelic variants G1 and G2 of *APOL1* (which encodes apolipoprotein L1), which are present in a large proportion of African Americans but absent in individuals without African ancestry[70]. Variants in *APOL1* were originally studied in the context of the higher rates of kidney failure in African Americans than in European Americans. Although *MYH9* (which encodes myosin heavy chain 9) was originally suspected to be responsible, in part because it encodes a myosin chain that is expressed in specialized epithelial cells of the kidney, linkage disequilibrium analysis revealed that *APOL1* — which is adjacent to *MYH9* — was under the greatest positive selective pressure[70]. The G1 and G2 variants of *APOL1* were subsequently shown to confer resistance to trypanosomiasis, a disease that is endemic to regions of Africa, and to mediate cellular injury and cytotoxicity through a variety of mechanisms[71,72]. In the context of transplantation, it had been previously established that the relative risk of kidney graft loss was greater when the donor was African American and the recipient either Caucasian or African American than in the setting of Caucasian-to-Caucasian donation[73]. In 2011, it was found that both the G1 and G2 coding variants in the donor are reproducibly and strongly associated with shorter renal allograft survival but not recipient survival[74,75]. These effects were independent of the adverse effects associated with younger recipient age and older donor age[76], two established risk factors for shorter

HLA restriction
An analytical condition in which association tests are confined to subgroups that share common HLA alleles.

allograft survival. In the recipient, however, the *APOL1* genotype was not correlated with allograft survival at 5 years post-transplant, which suggests either that the effect of *APOL1* variants on allograft status may be due to an intrinsically increased risk of kidney failure or that the allograft had already sustained subclinical damage by the time of donation[77]. This is in line with a mechanism of injury that is cell based rather than mediated by a circulating factor.

Of topical consideration in the transplant field is also the increased risk to living donors of African-American ancestry. Following living donor nephrectomy, it had been established that the highest risk group for post-donation end-stage renal disease (ESRD) is African Americans[78]. The increased frequency of the G1 and G2 allelic variants of *APOL1* in African-American populations may underlie this phenomenon, as these variants largely explain the increased rate of kidney failure in African Americans compared with European Americans[79]. Patients with two risk alleles had a hazard ratio of 1.88 for a composite primary outcome of ESRD or doubling of serum creatinine[79]. This poses a concern because living donors may themselves have an increased risk of renal failure after donation[80].

From a practical standpoint, genotyping for these variants is relatively inexpensive. However, ethical and policy considerations about *APOL1* screening have increasingly been the subject of discussion in the transplant community. Some groups advocate waiting for more rigorous, prospective studies to evaluate the true impact of *APOL1* renal risk variants on donors and recipients[81]. Ethically, such an approach can be justified by the principle of non-maleficence — the avoidance of harm[82]. Others advocate genotyping only deceased African-American donors to inform risk stratification measures for allocation while waiting for the resolution of the impact of these risk variants in living donors[83]. Meanwhile, some institutions have already started to screen potential living related donors for African-American recipients as well as African-American donors in general, with donor eligibility being relatively contraindicated in the presence of two risk alleles[80,84]. In these cases, offering counselling and genotyping would empower the donor to make an informed decision about their own health.

This creates a dilemma regarding how best to serve patients, both donors and recipients. If such policies reduce the availability of organs by reducing the number of donations, the time to transplantation for African Americans may increase, particularly as organs from African-American donors are given disproportionately to African-American recipients[85]. However, it is also possible that knowledge of one's genotype can enable more proactive, more vigilant monitoring of kidney health in the future, and reduce the rate of ESRD among these patients as well as the number of people on transplant waiting lists. Currently, there is no universal recommendation for the screening of donors, living or deceased, and further studies of the impact of *APOL1* risk variants on living donors, and of the impact of informed consent policies on organ supply and allocation, are warranted.

Other variants have been found to have gene–gene interactions with the *APOL1* gene variants in an ethnicity-dependent manner. The rs6466583 SNP in *CAV1* (which encodes caveolin 1) and the rs956825 SNP in *ABCB1* interacted with *APOL1* only in transplants from African-American donors, whereas the *ABCB1* rs1045642 SNP was associated with allograft failure only in European-American donors[86]. Moreover, additional variants have been found to be associated with transplant outcomes in a population-specific manner, such as associations between variants of *TLR4* (which encodes Toll-like receptor 4) and graft failure[87], between *CYP3A5* variants and tacrolimus trough concentrations[88] (discussed below), and between variants in mitochondrial DNA haplogroups and NODAT[89].

Connecting genotype to phenotype

More often than not, the functional implications of identified risk alleles are unknown. Whereas the clinical effects in the case of HLA genotype mismatch are clear, that is often not the case for mismatches in non-HLA loci. For example, in autoimmune disorders, the lead SNP is causal in only 5% of cases[90]. As such, one of the pressing challenges of the transplantation field is to elucidate the biological mechanisms through which identified and validated risk alleles function[91].

Existing functional insights come predominantly from the exploration of candidate genes; however, understanding the myriad data yielded by sequencing technologies will require greater investment in the functional exploration of newly discovered genetic associations in the future. Such mechanistic studies will be essential to understand the role that genetic variants have in the immunological processes relating to transplantation and to translate findings from genetic association studies into the clinic for truly personalized medicine. For example, the aforementioned G1 and G2 *APOL1* variants are predictive of renal graft failure, whereas their effect on other transplanted organs is mixed. Moreover, these variants have been implicated in liver necrosis in mouse studies[92], but they were found to have little effect on the outcomes of liver transplantation in a study of 639 human liver donors[93]. Although many immunological and cellular processes are shared between different allograft types, it is evident that many of the underlying mechanisms of transplant outcomes will need to be studied in an organ-specific manner.

In addition to *in vitro* and animal model studies, one approach is to use existing findings from related fields to provide mechanistic insights into the functional role of allelic variants in transplantation. For example, in one study analysing 23 genetic variants[94], the rs6025 SNP in the coagulation factor V-encoding gene *F5* could be validated by previous research on deep vein thrombosis. The variant had been shown to cause an arginine-to-glutamine substitution at codon 534 that leads directly to activated protein C resistance and subsequent endothelial damage in the kidney[94]. Approaches using existing knowledge will enable synthesis of novel insights and previously explored pathways in mechanistic studies.

Lead SNP
The single-nucleotide polymorphism (SNP) within any given locus in a genome-wide association study that has the strongest statistical significance.

Interstitial fibrosis and tubular atrophy
(IFTA). Historically called chronic allograft nephropathy, it is the most common cause of long-term renal graft failure and is characterized by the gradual deterioration of graft function.

Pharmacokinetics
The study of how an organism affects a pharmaceutical agent, one aspect of which is the metabolism of the drug. The levels of immunosuppressive drugs are highly affected by individual variability in specific metabolizing enzymes.

GWAS to interrogate recipient-specific loss-of-function mutations and non-synonymous SNP mismatches in transplantation are currently under way[95,96]. Moreover, although the discoveries from GWAS in transplantation have yet to lead to the identification of therapeutic targets, advances are also being made on this front[23,24]. For instance, GWAS in patients with chronic kidney disease (CKD) previously identified the intronic rs17319721 SNP in *SHROOM3* as a risk factor for CKD[97]. Analysis of this variant in the Genomics of Chronic Allograft Rejection (GoCAR) study found that its presence in the donor kidney was associated with increased *SHROOM3* expression and increased allograft fibrosis[98]. Mechanistically, transforming growth factor β1 (TGFβ1), a key mediator of fibrosis, was shown to upregulate *SHROOM3* expression, whereas SHROOM3 facilitated TGFβ1 signalling and α1-collagen expression. Furthermore, cell-specific knockdown of *Shroom3* abrogated interstitial fibrosis, suggesting its potential as a target for therapeutic intervention.

Allogenomics

Whole-exome sequencing in transplantation is still in its infancy, but one study has provided proof of principle for its use in the identification of miHAs. Specifically, whole-exome sequencing was able to reveal that HLA-matched donor–recipient pairs in HSCT had significant differences in their exon sequences, with an average of 13,423 SNPs per pair[99]. As such, this approach identifies polymorphic regions that could encode antigens not present in the graft, for which tolerance is therefore lacking. In renal transplantation, exome sequencing was conducted in donor–recipient pairs to identify potential cell-surface antigen mismatches[100]. The authors created an allogenomics mismatch score that was based on the number of amino acid mismatches between the exomes of the paired donors and recipients. Strikingly, this score was associated with graft function independently of HLA matching, donor age and time post-transplant in three independent cohorts. Ongoing whole-exome sequencing studies are investigating the association of allelic variants with chronic lung allograft dysfunction[101] and with GVHD[102], and the association of copy number variants and loss-of-function mutations with alloantibody status in a variety of organ transplant types[103]. Studies investigating rearrangements of the B cell VDJ immune repertoire through NGS to predict allograft injury are also under way[44].

RNA sequencing (RNA-seq) has also seen rapid adoption in transplantation research. For example, RNA-seq on peripheral blood mononuclear cells was used to identify gene transcripts that were differentially expressed between the pre-transplant state and various time points after transplant[104]. RNA-seq has also been conducted in osteochondral allografts to identify differences between fresh and stored cartilage allografts[105], and in lung allografts from bronchoalveolar lavage (BAL) exosomes to identify differences between stable grafts and acute rejection[106]. RNA-seq on biopsy samples has also been used to identify differences in microRNA expression (BOX 2) in, for example, renal allografts to distinguish patients with interstitial fibrosis and tubular atrophy (IFTA) and those with stable phenotypes[107]. Although most of these studies have been done using a small number of patients, they have set the stage for future studies to investigate larger cohorts and other transplantation outcomes.

Pharmacogenetics of immunosuppression

Advances in immunosuppression have been the cornerstone of long-term allograft survival. The large majority of transplant recipients are prescribed tacrolimus or cyclosporine, both of which have target trough levels. Ideally, these levels are set at an optimal balance to avoid too much immunosuppression, which can lead to drug toxicity and opportunistic infections, and too little, which may result in subclinical or acute rejection[108,109]. In practice, this target is difficult to achieve because of the large variabilities in pharmacokinetics that exist owing to individual pharmacogenetics[110]. Efforts to collate the various results of pharmacogenetic studies of the functional effects of allelic variants resulted in The Pharmacogenetics Knowledgebase, which enables easy access to genomic, phenotypic and clinical information from ongoing and completed studies[111].

The best-established and best-studied genetic determinants of immunosuppressant trough variability have been in the cytochrome P450 3A family, particularly CYP3A4 and CYP3A5. These variants affect the pharmacokinetics of calcineurin inhibitors such as tacrolimus and mammalian target of rapamycin (mTOR) inhibitors[112,113]. A number of allelic variants of *CYP3A4* and *CYP3A5* have been identified that significantly alter the metabolism and clearance of tacrolimus, and thus affect the trough level that can be achieved. A large majority of these variants cause loss of function of the enzyme, leading to significantly increased tacrolimus concentrations in the sera. *CYP3A5*1* encodes a functional enzyme, whereas the three other best-studied alleles, *CYP3A5*3*, *CYP3A5*6* and *CYP3A5*7*, result in non-functional proteins owing to either a reading frameshift and subsequent premature stop codon, or alternative splicing and subsequent protein truncation[114,115]. For *CYP3A4*, the *CYP3A4*22* allelic variant causes decreased enzymatic activity[116]. The frequencies of these alleles vary broadly across different population groups. Whereas the non-functional *CYP3A5*3* is very common in white populations, at an estimated allele frequency of 0.82–0.95, *CYP3A5*1* is most common in African Americans, and >50% of African American individuals are estimated to have this allele, which encodes a highly metabolically active form of CYP3A5 (REFS 115,117). As a result, guidelines published by the Clinical Pharmacogenetics Implementation Consortium for tacrolimus dosing take into consideration *CYP3A5* genotype[118]. Attempts have been made to create dosing models that are based on these genetic factors as well as clinical factors to predict the dosage needed for optimal trough levels through retrospective validation in independent cohorts[119,120]. Although these results are promising from a technical standpoint, further prospective validation of their clinical utility

Acute pyelonephritis
A bacterial infection of the kidney. Individuals who are taking immunosuppressive medication are at an increased risk of developing this condition.

Box 2 | **MicroRNAs in transplantation**

The two earliest studies investigating the role of microRNAs (miRNAs) in allograft outcomes carried out miRNA expression profiling of renal allograft biopsy samples[182,183]. A subset of miRNAs that were differentially expressed could discriminate acute rejection from stable allografts or control tissue were associated with the expression levels of specific mRNAs[182,183], and were expressed differentially in resting or activated human renal epithelial cells or peripheral blood mononuclear cells (PBMCs)[182]. Since then, numerous studies have been published for a variety of organ types, outcomes and sample sources.

The kidney has been the subject of the largest number of studies, with experiments conducted in tissue biopsy samples, PBMCs and urine for the discrimination of outcomes such as acute rejection[182], interstitial fibrosis and tubular atrophy (IFTA)[184], or T cell-mediated rejection versus stable grafts[185], as well as the differentiation of viral pyelonephritis from acute rejection[186]. Recent studies in human liver[187], lung[188], heart[189], intestinal[190] and pancreatic islets[191] have revealed the diagnostic utility and potential part that miRNAs play in the processes underlying a diversity of transplant outcomes. Studies have also begun investigating the temporal dynamics of miRNAs; for example, the expression of miR-223p-3p and miR-93-5p was investigated in patients with chronic kidney disease (CKD) and was found to be associated with CKD stage[192]. Notably, the decreased levels of these miRNAs observed in late-stage CKD were reversed upon kidney transplantation, suggesting their use as biomarkers of transplant success.

In multiple organs and phenotypes, some miRNAs have been shown to be differentially regulated. For example, the two miRNAs miR-142-5p and miR-142-3p, which derive from the same precursor, have been found to be associated with acute rejection, chronic allograft dysfunction and IFTA in kidney, lung, liver and heart transplantation[107,182,184,190,193–195]. Circulating miR-21 levels in renal transplant recipients correlated with IFTA scores and were increased in tissue biopsy samples from patients with chronic allograft dysfunction, antibody-mediated rejection, delayed graft function or acute pyelonephritis[107,186,196–198]. Unlike many other miRNAs that show organ-specific enrichment, the differential regulation of miR-21 was found to be associated with acute rejection, fibrosis and cardiac allograft vasculopathy in heart allografts[189,199,200], and with graft dysfunction in lung allografts[188], suggesting a degree of universality in its response. The differential expression of miRNAs in multiple organs could reflect their role in overlapping processes related to rejection; for example, miR-142-5p has been reported to have a pro-fibrogenic role in both liver and lung fibrosis[201].

Although publications identifying miRNAs in transplantation have been mostly diagnostic, miR-21 is notable for numerous mechanistic studies into its role in fibrosis. In addition to the aforementioned studies in transplantation, it was found that miR-21 levels are increased in renal ischaemia–reperfusion injury, a condition that can lead to fibrosis if left untreated[202]. Mechanistic studies revealed that miR-21 promoted renal fibrosis in a peroxisome proliferator-activated receptor-α (PPARα)-dependent manner and that its ectopic expression in primary kidney fibroblasts promoted the differentiation of myofibroblasts, which are a key cell type in the fibrotic response. Notably, *mir-21*$^{-/-}$ mice were resistant to fibrosis development with ischaemia–reperfusion injury, and the treatment of ongoing fibrosis with anti-miR-21 oligonucleotides limited the fibrotic response[197]. Although *in vivo* human studies remain to be done, studies such as these present the possibility of using and modulating miRNAs in both a diagnostic and a therapeutic capacity.

miRNAs that are present in many organs or many tissues are in the minority, as many miRNAs show substantial organ-specific or tissue-specific enrichment. These 'enriched' miRNAs may enable the development of organ-specific monitoring tools for the quantitative assessment of graft health. One example is miR-375, which is specific to pancreatic β-cells and is only found in the circulation upon the death of these cells. Circulating miR-375 was detected in patients who had received pancreatic islet transplants, and levels were higher in transplant recipients who did not receive anti-inflammatory treatment than in those who received either tumour necrosis factor (TNF)-inhibition therapy, or combined treatment with TNF and interleukin-1β inhibitors[191]. Studies such as these show the clinical utility of monitoring circulating miRNA levels, which may prove useful for organ-specific immune monitoring in patients who receive multiple transplants.

is needed to confirm not only if these equations will work in diverse clinical settings but also whether the use of such equations will reduce the occurrence of adverse events. Initial studies so far suggest that dosing of tacrolimus that is based on *CYP3A5* genotype does not improve clinical outcomes in the short or long term[121,122].

Post-transplant diagnostics

The early identification of highly sensitive and specific surrogate biomarkers of allograft injury enables timely intervention and facilitates patient-specific customization of immunosuppressive drugs to optimize graft outcomes. Understanding the correlation between the presence or lack of biomarkers, which include altered levels of nucleic acids (DNA, circulating cell-free DNA (cfDNA) and cfRNA), proteins and metabolites, offers valuable insights into post-transplant graft monitoring. For example, it was shown in the 1990s that donor-specific cfDNA is present in the plasma of transplant recipients through the analysis of Y-chromosome-specific PCR products in recipients of male-to-female transplants[123]. This approach has since been applied to plasma samples for the analysis of liver transplantation, and to plasma and urine samples for the analysis of kidney transplantation[123–125]. However, its limitation to cases of male-to-female transplantation has prevented this approach from being universally applicable.

The limitations of invasive biopsy and, in the case of renal transplantation, serum creatinine monitoring, are well established, and were the impetus for the development of more predictive and non-invasive diagnostics[126] (FIG. 4). Diagnostics comprising a single gene or a few gene transcripts have been proposed and tested on the basis of a priori knowledge of candidate gene product function (see REFS 127–129 for in-depth

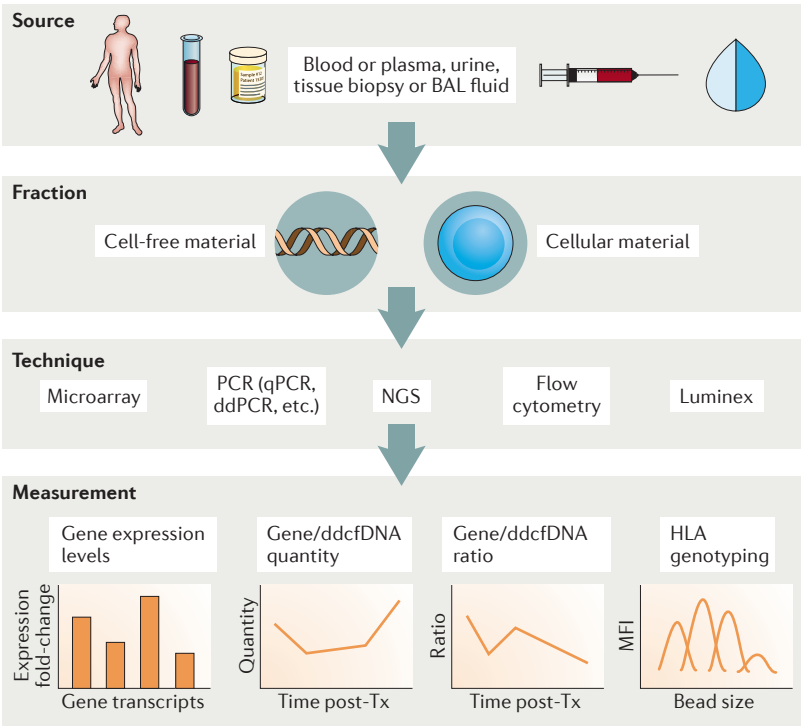

Figure 4 | **Diagnostic approaches in transplantation.** By analysing a variety of different biological samples from different sites, both cellular and acellular, of a single individual, many different biomarkers that are associated with graft outcome can be measured. These can be measured directly from a biopsy sample of the graft, peripheral blood and plasma, or — in the case of specific transplants — from urine (for kidney transplants) and bronchoalveolar lavage (BAL) fluid (for lung transplants). Investigators use different techniques depending on the biomarker of interest and the type of transplant, and these techniques include nucleic acid microarrays, various PCR modalities, next-generation sequencing (NGS), Luminex and flow cytometry. Measurements using these techniques include gene or microRNA expression levels, donor-derived cell-free DNA (ddcfDNA) quantification or ratios, and human leukocyte antigen (HLA) genotyping. ddPCR, digital droplet PCR; MFI, mean fluorescence intensity; qPCR, quantitative PCR; post-Tx, post-transplant.

reviews). A move towards the use of multi-transcript arrays and non-invasive testing of biological fluids such as plasma and urine is under way, and this has been enabled in part by the development of protocols and technologies that allow the uniform recovery and subsequent analysis of low concentrations of nucleic acids[130]. Unbiased, high-throughput gene expression profiling technologies (such as DNA microarrays) and novel analytical methods (such as the kidney Solid Organ Response Test (kSORT) analysis suite (kSAS)[131]) have accelerated the discovery of genes and gene sets that can be used to predict transplant outcomes. Gene expression profiles of the cells found in BAL fluid have been predictive of acute lung rejection[132], and profiles from endomyocardial biopsy samples were able to distinguish between no rejection, rejection and *Trypanosoma cruzi* infection[133]. In renal transplantation, gene expression microarray technology has also been used to distinguish between antibody-mediated rejection and T cell-mediated rejection, as well as between different subphenotypes of antibody-mediated rejection[134–136]. Furthermore, methods using multiple microarray platforms with subsequent real-time qPCR validation have been used to create gene signature models for the prediction and detection of acute rejection and IFTA in patients receiving kidney or heart transplants[137,138].

With the advent of high-throughput sequencing and digital droplet PCR (ddPCR) technologies, universal and non-invasive diagnosis of rejection episodes has become possible. Quantification of the relative levels of donor and recipient cfDNA through SNP analysis has enabled the post-transplant diagnosis of acute rejection in recipients of heart[139,140], lung[141] and liver[142] transplants, and of relapse in HSCT[143]. These approaches involve pre-transplant sequencing of both the donor and the recipient, analyses of polymorphic markers such as short tandem repeats, and probabilistic approaches testing multiple SNPs that have high minor allelic frequencies. Such tools have been extended to the identification of donor-specific mitochondrial cfDNA in organ transplantation[144].

Remaining challenges and future directions

Genetic advances have been the driver of 'success' and have advanced transplantation to its current state — the rate of acute rejection is at an all-time low, particularly in kidney transplantation, and the long-term quality of life post-transplantation has increased[145]. However, despite the progress that has been made, a number of challenges remain to be overcome.

Reproducibility and statistical power. Many studies have been conducted on specific polymorphisms. High confidence about the association of these SNPs with transplant outcomes (or lack thereof) can be determined through meta-analyses, such as in the case of *IL10*, where meta-analyses found no association between SNPs and acute rejection in renal and liver transplantation[146,147]. Nevertheless, the findings of many genetic association studies have been difficult to reproduce across independent studies. In one striking example, 26 different studies that enrolled a maximum of 394 patients (with an average of 175.5) found a total of 23 SNPs associated with acute rejection[94]. However, an independent and subsequent validation study involving 969 patients found only one of these SNPs to be significantly associated with acute rejection after multiple variable and testing corrections were made[94]. In another case, four SNPs were found to be associated with post-HSCT outcomes in a study of 470 patients, but a validation study by the same group in 928 patients did not confirm any of their original findings[148,149]. The reasons for this phenomenon are manifold, and many of them are statistical in nature. Different demographic and clinical variables and subsets in study groups may result in statistical heterogeneity and inaccurate conclusions. These variables should be assessed as clinical covariates in multivariate analyses to help to ascertain true associations. Particularly in transplantation, many studies have small sample sizes and thus insufficient statistical power, a problem that is compounded by the increasing use of assays that assess multiple variables at once. This problem holds true for GWAS, most of

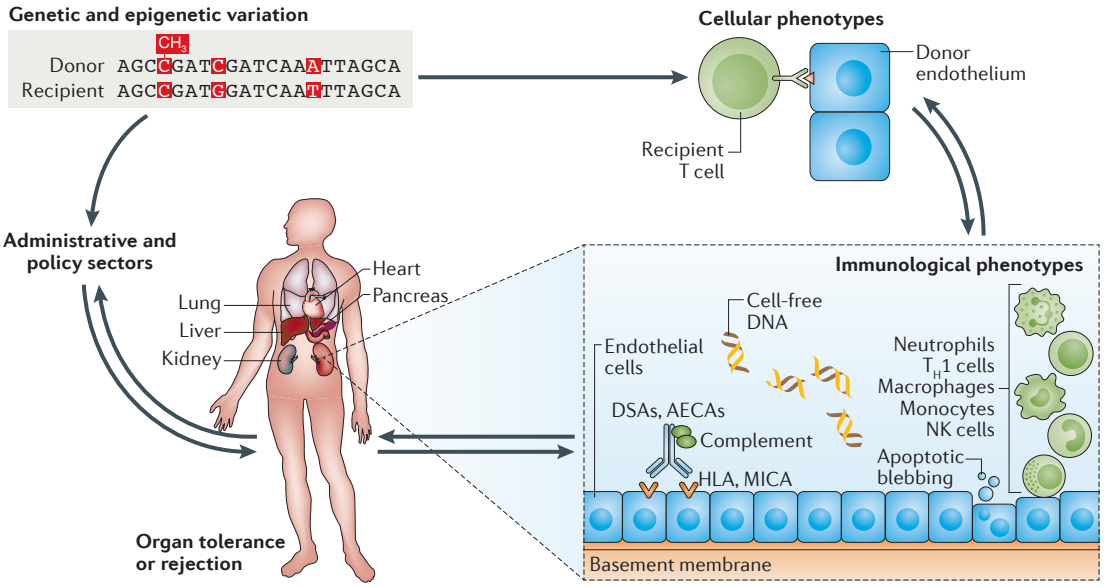

Figure 5 | **An integrated approach combining clinical phenotype with transplantomics.** Epigenetic or genetic differences (top left) between a donor and recipient can influence cellular phenotypes, such as mRNA transcription and degradation, alternative splicing and protein–protein interactions (top right). In turn, these processes can affect immunological processes such as cell–cell interactions and donor–recipient antibody binding (bottom right). Phenotypic differences influence the likelihood, type and severity of rejection in a manner that is also dependent on the specific transplanted organ (bottom left). In order for this knowledge to make clinical differences, this personalized genetic information will need to be used by organizations in the administrative and policy sectors that are responsible for the distribution and matching of organs in a manner that optimizes transplant outcomes. AECAs, anti-endothelial cell antibodies; DSA, donor-specific antibody; HLA, human leukocyte antigen; MICA, MHC class I polypeptide-related sequence A; NK, natural killer; T_H1, T helper 1.

which are insufficiently powered and include low numbers of transplant recipients, particularly in studies of transplanted organs other than kidneys.

In seeking to solve these problems, collaborative initiatives such as the Clinical Trials in Organ Transplantation consortium are being supported by the US National Institutes of Health to bring together investigators and to facilitate the addition of samples to biobanks from patients enrolling in transplantation-associated clinical trials in the United States. The combined validation and cross-validation of data emerging from these prospectively collected samples, and the re-analysis of data from completed trials — such as those archived in the ImmPort database — will enable the discovery of genetic associations that can be used in precision and personalized medicine to improve patient outcomes. The International Genetics and Translational Research in Transplantation Network (iGeneTRAiN) plans to combine genomic data with the corresponding heart, kidney, liver and lung allograft phenotypes of more than 32,000 patients, and include more than 800,000 genetic variants[150]. These individuals include cases with a variety of post-transplant complications and outcomes, and a sizeable proportion (>15%) of cases are of non-European ancestry. Furthermore, the iGeneTRAiN consortium developed TxArray — a genotyping array designed to capture variants within transplant-relevant loci, such as the HLA and KIR genomic regions — to identify genetic variants associated with organ transplant injury[151]. The iGeneTRAiN initiative is certain to reveal novel variants that will affect outcomes and to provide cohort sizes that will reduce biases.

Diagnostic assays. To date, many diagnostic studies conducted in transplantation have been designed to discriminate between two phenotypes, typically patients with stable transplants and those with acute rejection. To be clinically useful, diagnostic assays will need to discriminate between multiple phenotypes at once. Recent studies have begun to address this issue; for instance, by combining urinary proteome and transcriptome measurements, one study was able to discriminate between a number of different transplant outcomes, such as stable transplants, acute rejection, IFTA and BK virus nephropathy[152].

In the interest of increased physician experience with the performance of diagnostic assays in their patient populations, the availability of some of the more robustly validated assays as laboratory developed tests is warranted. This will offer physicians tools for more astute patient and graft monitoring. Although practical limitations for the expansion of these diagnostic technologies into the clinic remain with regard to the expense of and access to NGS and ddPCR equipment, as well as the need for the establishment and validation of individual thresholds for each type of organ, commercial assays based on some of the highlighted

Xenografts
Grafts from one species to a different species, such as in the case of heart valve replacement, which often involves the transplantation of heart valves from pigs into humans.

Sirtuins
A class of proteins implicated in processes such as ageing, apoptosis and inflammation.

approaches are already being offered by Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories (kSORT[131,153], AlloSure[154] and AlloMap[155]).

Assays that have become commercially available face many adoption challenges that are unrelated to their scientific merit. For example, although individualized diagnostics for rejection risk prediction have been validated through clinical research and prospective trials, most of these have yet to be put into routine clinical use, and currently, only a few of these assays have been launched for commercial use in heart transplantation and kidney transplantation[131,155]. Further advances in the field will require buy-in not only from clinicians and patients, but also from health insurance companies (such as Medicare) and reimbursement structures. To achieve the approval of such a wide variety of stakeholders, studies on newly developed therapeutics and diagnostics will need to demonstrate not only clinical utility but also cost-effectiveness relative to existing options.

Genomics and systems biology. In order to attain a deeper understanding of transplant outcomes, an approach that synthesizes knowledge from genomics, proteomics, transcriptomics, metabolomics and other omics approaches is essential (FIG. 5). Such a multi-omics approach in transplantation has been coined 'transplantomics' (REF. 156). Studies that involve more than one omics domain are starting to emerge: for example, those combining proteomics and transcriptomics[152,157], antibodyomics and transcriptomics[37], or proteomics and metabolomics[158]. Key to making the most of these datasets will be new tools to visualize and analyse omics data in an integrated fashion[159]. Only then will a systems biology-level understanding of transplantation begin to emerge. Synthesizing knowledge at all levels — from individual genetic or epigenetic differences (BOX 3) to cellular phenotypes, immunological responses and transplant outcomes — will be vital to gain a complete picture of transplant phenomena.

Beyond allografts. With more than 120,000 people waiting for solid-organ transplants in the United States and just 33,500 transplants occurring in 2016, according to the Organ Procurement and Transplantation Network, xenografts offer the possibility of solving the long-standing issue of organ shortage. Pig-derived xenografts are currently the most promising owing to their ready availability and their relative social acceptability compared with xenografts from other animals. However, xenografts have their own variety of problems that must be resolved, ranging from immunogenicity to potential pathogen transmission. The pig genome contains many porcine endogenous retroviruses (PERVs), the infection risk of which to humans remains an area of active concern and debate[160]. Furthermore, in pigs, the antigen α-1,3-galactose (α-gal) can cause organ rejection within minutes[161]. New genetic technologies are being used to address these issues. Using the CRISPR–Cas9

Box 3 | **Epigenetics in transplantation**

Although rare, case reports of isograft failure in identical twins do exist[203], suggesting a possible role of epigenetics in transplant outcomes. In the case of haematopoietic stem cell transplantation (HSCT), it was found that hypomethylation of *IFNG* (which encodes interferon-γ) and *FASL* (which encodes FAS ligand (also known as TNFSF6)), and hypermethylation of *IL10* (which encodes interleukin-10), were associated with more severe graft-versus-host disease (GVHD), although a correlation with gene expression was not established[204]. Another study involving a genome-wide approach using 450K arrays in HSCT identified a donor-specific DNA methylation signature that predicted the incidence of severe acute GVHD in human leukocyte antigen-matched sibling recipients[205]. Crucially, the detection of this signature was replicated using a low-cost assay, suggesting the utility of such an approach for pre-transplant testing of HSCT donors in the clinical setting.

Other studies have investigated the suitability of diagnostic assays that are based on the specific epigenetics of the transplanted tissue rather than on specific sequences that differ between a donor and recipient. The first proof of concept for the use of epigenetic features as a diagnostic came in 2006 when it was discovered that hypermethylation of the promoter of *CALCA* (which encodes calcitonin-related polypeptide-α) in urine sediment could distinguish between deceased and living kidney transplants[206]. Analyses of DNA methylation patterns specific to pancreatic β-cells have revealed that the levels of β-cell-derived cell-free DNA (cfDNA) in the plasma were elevated in patients who had received transplanted islets compared with controls[207]. A correlation between plasma C-peptide, a marker of β-cell damage, and this specific cfDNA suggests the utility of this approach for monitoring the efficacy of immune suppression. One study in liver and bone marrow transplantation took an epigenomics approach that used genome-wide bisulfite sequencing of plasma cfDNA and compared these data with methylation data from either liver tissue or white blood cells[208]. Comparison of the methylation deconvolution approach with a SNP-based approach found a correlation of $r = 0.99$, although the utility of this approach in the detection of rejection episodes remains undetermined.

Knowledge about the epigenetics of immune cell development and lineage specification may also have a clinical role in transplantation. For example, regulatory T (T_{reg}) cells, which are important in the development and maintenance of allograft tolerance (see BOX 1), and have multiple immunoregulatory roles[165], are currently being studied as a treatment for subclinical inflammation in renal transplantation[209]. However, the expansion of T_{reg} cells typically results in increased CpG methylation within *Foxp3* (which encodes forkhead box protein P3) and a concomitant emergence of pro-inflammatory cytokines[210]. As such, elucidation of the epigenetic underpinnings of the development of T_{reg} cells and the maintenance of *Foxp3* expression will be essential for the use of these cells as a therapeutic agent. Research into the pathways that affect T_{reg} cell development has shown that histone deacetylase inhibition promotes the production and suppressive functions of T_{reg} cells through *Foxp3* acetylation[211]. Nicotinamide, an inhibitor of sirtuins, increases the numbers of $FOXP3^+$ cells and the levels of FOXP3 per cell *in vitro*[212]. Studies such as these that synergize epigenetic knowledge with the testing of small-molecule compounds may enable the use of T_{reg} cells in transplantation.

system in the PK15 porcine kidney epithelial cell line, all copies of the PERV *pol* gene were disrupted, preventing PERV transmission to human cells[162]. CRISPR–Cas9 was also used to knock out *GGTA1* (which encodes α-1,3-galactosyltransferase), the product of which synthesizes the α-gal epitope[163]. Studies such as these are stepping stones towards a possible future in which organ transplants are not only of allograft origin but also of xenograft origin.

Conclusion

The fields of transplantation and genomic technology are expanding rapidly and side-by-side. Knowledge about genetic differences and histo-incompatibility have created clinical innovations that have pushed the boundaries in the transplantation field. Linking HLA and non-HLA genetic associations with key transplant outcomes, from graft survival to pharmacogenomics, will continue to improve donor–recipient pairing and facilitate the use of personalized immunosuppressive therapies in transplant recipients. Moreover, genome-based and transcriptome-based diagnostics have paved the way for individual, sensitive and predictive monitoring of the efficacy of these therapies. However, many problems remain to be solved within the field, such as the continued loss of transplants from chronic allograft dysfunction and immunosuppressive regimen complications[1]. The ability to identify genetic variants that can predict adverse outcomes in allograft transplantation is essential to enhance transplantation success through the individualization of clinical care, otherwise known as personalized medicine. However, much remains to be done in the identification of truly causal variants, particularly those with small effect sizes and a low allelic frequency. As evidenced by the majority of existing studies, moving beyond renal transplantation and the Caucasian populations to other types of organ transplant and other ethnic groups will be essential to gain a fuller understanding of transplantation outcome phenomena. Larger and more diverse cohorts than previously studied will be needed to detect such variants, and initiatives such as iGeneTRAiN will be essential for future variant discovery. There is, however, no doubt that genetics, genomics and subsequent related technological advances will be key not only in enhancing our mechanistic understanding of genetic variants and in developing novel and personalized therapeutics, but also in improving the quality of life and care of transplant patients.

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Competing interests statement
The authors declare competing interests: see Web version for details.

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ENCODE project: https://www.encodeproject.org/
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ImmPort database: http://www.immport.org
IPD–IMGT/HLA Database: http://www.ebi.ac.uk/imgt/hla/
Organ Procurement and Transplantation Network: https://optn.transplant.hrsa.gov
The International Genetics and Translational Research in Transplantation Network: http://www.iGeneTRAiN.org
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