REVIEW

Using omics to explore complications of kidney transplantation

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SUMMARY

The importance of genetic and biochemical variation in renal transplant outcomes has been clear since the discovery of the HLA in the 1950s. Since that time, there have been huge advancements in both transplantation and omics. In recent years, there has seen an increased number of genome-, proteome- and transcriptome-wide studies in the field of transplantation moving away from the earlier candidate gene/protein approaches. These areas have the potential to lead to the development of personalized treatment depending on individual molecular risk profiles. Here, we discuss recent progress and the current literature surrounding omics and renal transplant complications.

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Background

Over the past number of decades, there have been considerable improvements in renal allograft survival, largely due to improved immunosuppressive protocols [1]. However, with better survival comes increased immunosuppression-associated co-morbidities such as cancer, infection and diabetes. For over 50 years, it has been recognized that genetic factors, such as allelic matches at the HLA, impact graft outcome [2]. Many studies have examined the effect of single genetic loci on allograft outcomes and complications. However, until recent years, little has been done to investigate these outcomes on a genome, proteome or transcriptome-wide scale (see Box 1). Study of these systems, together known as 'omics', has huge potential to advance our knowledge of disease pathogenesis and complex traits. In this review, we will discuss the current applications of omics methods to understanding kidney transplant outcome, focusing specifically on allograft function and two of the most common long-term complications of transplant – post-transplant diabetes (PTDM) and cancer. We will also briefly discuss how omics may help us reach the ultimate goal of transplantation – tolerance.

Omics of kidney transplant function

Clinicians strive for long-term well-functioning allografts for their patients. However, most transplants ultimately fail due to a combination of chronic immunological and nonimmunological injury. This is indicated histologically by of glomerular lesions with varying degrees of interstitial fibrosis and tubular atrophy (IF/TA). Some allografts attain varying degrees of operational tolerance with excellent function and an absence of histological injury despite minimal or even

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no immunosuppression. We need tools to predict these phenotypes pretransplant, test for them post-transplant and ultimately to create the conditions for allograft accommodation.

Allograft function

While the HLA system is critical to long-term transplant outcome, close matching between donor and recipient for HLA antigens does not guarantee good allograft outcome and the 'HLA effect' on allograft outcome may have diminished in the current era potent immunosuppression [3,4]. This has led researchers to explore genetic effects outside of the HLA locus, initially using a candidate gene approach. These candidate gene studies have mostly been performed in recipients (excluding donor genotype) and usually with acute rejection as the primary outcome, although several studies have also examined long-term allograft function, and sometimes eGFR [5,6]. Candidate gene studies have been criticized for the small number of subjects, lack of correction for multiple testing, lack of standardized techniques and analysis methods, lack of proper normalization, and a likely positive reporting bias. Moreover, those results from most studies that reported positive findings have never been independently replicated [7–11] and in the few cases where it was attempted, results have usually not been validated in the secondary cohort. A widely reported example is the association between donor and recipient C3 allotype combinations, named C3F (fast) and C3S (slow) on the basis of their electrophoretic motility. An initial study in 662 donor/recipient pairs suggested that in C3S/S recipients, receipt of a C3F/F or C3F/S donor kidney, was associated with a significantly better long-term outcome [12]. However, a subsequent study of 1,147 donor/recipient pairs in a both clinically and ethnically similar population demonstrated no association with transplant survival or allograft function [13].

Box 1. Explanation of common terms and methodologies used in omics.

Genomics:
The study of all the genetic material (or genome) of a cell or organism. This approach includes such methodologies as genome wide association studies (GWAS) and whole-genome sequencing and is a shift away from the classic candidate gene approach. Candidate gene association studies analyse associations between predefined genes or genetic variants and the given trait of interest. GWA studies scan millions of common genetic variants across the entire genome to find variants that associate with the trait of interest whereas whole-genome sequencing examines both rare and common variation. GWAS scans are significantly cheaper than whole-genome sequencing however are limited by their low affinity for capturing rare variants. Another common approach is whole-exome sequencing which examines specifically the coding regions of all genes present in the given organism.

Epigenomics:
The study of epigenetic modifications, such as histone modifications and DNA methylation, across the entire genome. There is a huge variety of methods for analysis of epigenomic modifications the choice of which depends on a number of factors including quality and type of the tissue sample available, cost, aims of the studies and computational/laboratory resources available. Such methods include quantification of global methylation via high-performance liquid chromatography and DNA methylation analysis via whole-genome bisulphite-sequencing [14]. Global methylation analysis can assess the methylation status of a tissue sample as a whole whereas DNA methylation analysis via whole-genome bisulphite-sequencing gives a site specific methylation status across the entire genome.

Proteomics:
Analyses the presence, activity and interaction of the complete set of proteins of a cell, tissue or organism [15]. Mass spectrometry has proven to be a hugely successful tool in this field, not only to quantifying and measuring the abundance of different proteins but also for detecting post-translational modifications such as phosphorylation and ubiquitination [16]. Methods for analysing protein interactions with DNA such as ChIPSeq have allowed for the detection of transcription factor binding sites along the genome [17].

Transcriptomics:
Examines the entirety of the mRNA in a given cell or organism [18]. mRNA acts as the intermediary component of the central dogma that translates the gene into the expressed protein. Transcriptomic techniques can examine both the quantity and type/sequence of RNA in the given sample [15]. There are two main methodologies applied in this field – probe-based microarrays and RNA sequencing (RNA-seq). Microarrays are used to analyse predefined targets, whereas RNA-seq uses deep-sequencing technologies to examine all sequences present in the given sample [19].

A notable feature of these studies was the investigation of the interaction between donor and recipient polymorphisms. Most transplant studies have examined the recipient genome although some robust, replicated studies have been performed using donor variants to assess allograft outcome. An *ABCB1* donor polymorphism, known to influence calcineurin inhibitor (CNI) metabolism, was associated with allograft failure in a discovery analysis ($n = 811$) and one of two replication cohorts treated mostly with cyclosporine [20]. Another validated study was performed in a donor variant of the gene encoding caveolin-1 (plasma membrane protein involved in G-protein signalling) which demonstrated an association with allograft failure [21]. Variants in *APOL1*, termed G1 and G2, are strongly associated with kidney disease in individuals of West African ancestry and have been associated with faster time to graft failure [22,23]. The mutations likely arose in prevalence in sub-Saharan Africa around 10,000 years ago, after the large-scale migration to Europe, due to positive selection resulting from resistance to Trypanosoma brucei rhodesiense infection [22]. Donor kidneys harbouring two *APOL1* risk alleles had significantly shorter allograft survival in a study of 106 African American donors (136 kidney transplants) [23], a finding since confirmed in larger studies [24,25]. This has major implications for assessment of deceased and living donors although a recent expert panel has not suggested routine assessment of *APOL1* status for African American donors [26].

Genomewide approaches do not rely on *a priori* hypotheses on specific genes, and allow for rapid and affordable assessment of many SNPs. Genomewide association studies (GWAS) have proved to be an invaluable tool for gene discovery in the context of complex disease. Our group performed the first GWAS in renal transplantation (see Table 1), examining the association between renal function at 5 years and genotype in a relatively small discovery cohort of 326 first-time, kidney-alone transplant recipients [27]. The study revealed two SNPs with genomewide levels of significance, one located in an intergenic region of the T-cell receptor alpha locus, and the second variant in an intron of a zinc finger protein of unknown function. Both variants were also predictors of long-term allograft survival. However, the study did not include a validation cohort. Attempted validation of the significance of these SNPs was performed in a separate Caucasian cohort of 1638 participants from the ALERT study, using the outcomes of death-censored graft survival or mortality [28]. No association was demonstrated in this study for either endpoint. This may be due to a population specific effect in the discovery cohort (Irish), a difference in the measured outcome (serum creatinine at 5 years versus graft failure/mortality) or type 1 error in the initial report [27]. It certainly highlights the need for robust replication in GWAS. Further analyses of long-term outcomes, such as eGFR, are warranted to aid development of predictive biomarkers of graft survival.

Table 1. Condensed summary of GWAS for phenotypes related to transplant outcome.

Study	Transplant outcome studied	Discovery *N*	Replication *N*	Genomewide significant loci
O'Brien *et al.* (2013) [27]	Serum Creatinine at five years post-transplant	326	Independent replication study [28]	*ZNF516* – this signal did not replicate in independent study [28]
McCaughan *et al.* (2014) [33]	Post-transplant diabetes	256	441	None (most significant loci *ATP5F1P6*)
Sanders *et al.* (2015) [34]	Post-transplant SCC	388	No replication	None (most significant loci *LINC00882*)
Giri *et al.* (2016) [35]*	Post-transplant diabetes	302	No replication	None (most significant loci *PLXDC1*)
Ghisdal *et al.* (2017) [36]	T-cell mediated rejection	778	844	*PTPRO*, *DEUP1*†

Discovery *N*, number of individuals in discovery analysis; replication *N*, number of individuals in replication cohort; ZNF516, zinc finger protein 516, ATP5F1P6, ATP synthase, H+ transporting, mitochondrial Fo complex subunit B1 pseudogene 6; LINC0882, long intergenic nonprotein coding RNA 882; PLXDC1, plexin domain containing 1; PTPRO, protein tyrosine phosphatase, receptor type O; DEUP1, deuterosome assembly protein 1.

*Abstract presented, not full article.

†These GWAS were outside the remit of this review and therefore will not be discussed further [see review by (insert reference here for acute rejection review which will be in the same focused issue)]. However, it is of note that this study used pooled DNA which used different methods to the standard individually genotyped GWAS analysis.

The clinical setting of renal transplantation offers multiple heterogeneous outcomes for genetic mapping via GWAS, including renal function and allograft failure. One of the major challenges in studying transplant-associated outcomes is the differences in phenotypes across populations such as differences in serum creatinine across different ethnicities [29]. Another challenge comes with the changes in clinical definitions of these phenotypes over time such as the periodical changes in Banff pathological assignments [30]. It is vital that these differences are considered carefully and appropriate adjustments are worked into the study design. Transplant phenotypes are multifactorial, caused by the interplay of multiple genetic as well as environmental factors. The effect size of individual genetic variants is likely to be small and few, if any, may be obligatory for the outcome to occur. Donor and recipient genetic interactions add an additional layer of complexity when applying genetic approaches to transplantation. While we have discussed some donor polymorphisms which may be important to allograft function, the bioinformatic methods required to capture donor/recipient interactions on a genomewide scale remain unclear. A loss of function compatibility approach (effect of mismatch of the number of functioning copies of a gene between the donor and recipient) has been proposed by the iGeneTRAiN Consortium [31]. Another group has performed donor/recipient whole-exome sequencing (see Box 1) and used it to estimate all cell surface antigen mismatches, in effect the burden of presented epitopes potentially recognized as non-self by the recipient [32]. They did this by determining amino acid mismatches in transmembrane proteins, creating a so-called allogenomics mismatch score. This score significantly associated with eGFR, independent of HLA-matching and clinical covariates.

Late allograft failure

The major barrier to a lifelong functioning kidney transplant is chronic injury which may be the result of immunological or nonimmunological factors. Accruing evidence suggests that allograft failure after the first postoperative year is largely a result of chronic antibody mediated rejection [37,38], although nonimmunological injury may also be a factor [39]. Understanding these mechanisms of injury critically important as an alternative theory for late allograft dysfunction is calcineurin inhibitor nephrotoxicity, which motivated immunosuppression minimization strategies, an approach that in general has failed to improve long-term outcomes. The histological pattern of injury with late allograft loss is variable, commonly including glomerular lesions, IF/TA or both [39]. Many patients have no history of overt acute rejection and many late failing allograft biopsies reveal no evidence of inflammation [40]. IF/TA itself is of course not a diagnosis, merely a descriptive pattern, revealing nothing about aetiology. Moreover, transplant glomerulopathy is often considered to be a manifestation of immunological injury but frequently no objective alloimmune response may be determined. Methods to explain what is happening at a molecular level would be clinically invaluable. Omics techniques hold huge potential in this regard. Urine proteomics is an attractive approach to discover biomarkers associated with allograft dysfunction [41,42]. Peripheral blood has also been employed for proteomic as well as genomic profiling with some success [43], but we will focus our discussion on the transcriptomic assessment of allograft tissue.

DNA is transcribed into messenger RNA (mRNA), and these mRNA transcripts provide a reflection of physiological and pathological processes occurring in the cell. A quantitative assessment of the complete set of these mRNA's (termed 'transcriptomics') can be done at a single cell level or in a high-throughput fashion on microarrays or RNA-seq (see Box 1), capturing gene expression of thousands of genes from many cells types. Transcriptomics of peripheral blood cells have been studied [44,45] but much of the work has been performed on allograft tissue. An exciting feature of this type of work is the potential to observe cellular transcriptional changes before tissue injury has manifested, which has been demonstrated for many phenotypes including ischaemia reperfusion injury [46], acute rejection and chronic allograft injury [47]. This creates a window whereby intervention could potentially alter the outcome or prevent injury. Much of this work has been focussed on the outcome of acute rejection, which is the focus of a separate article in this issue. However, other phenotypes have also been studied, such as allograft fibrosis, which as mentioned, may be due to both chronic immunological and nonimmunological injury. Studies have reported shared pathways with rejection phenotypes in biopsies with both inflamed and uninflamed IF/TA, suggesting a possible common aetiology of immunological injury across most cases of late allograft dysfunction [40].

Phil Halloran's laboratory has made significant strides in progressing the field of transcriptomics in transplantation. Initial work performed using animal models and

subsequently in human real-world indication biopsies has created so-called 'pathogenesis-based transcript sets' (PBTs). PBTs segregate together based on biological events evident on biopsy samples (e.g. T-cell infiltration, interferon-gamma expression, parenchymal deterioration) [48]. They used the PBTs to form classifiers, or machine learning algorithms from multiple resampling of different subsets of the data, which give a score used to predict the molecular phenotype. Their methods were refined based on additional samples and have been validated in prospective cohorts using indication biopsies, independent of the discovery population [49]. The group has examined IF/TA at varying time points post-transplant, reporting an absence of fibrosis initially after transplant but with a linearly increasing prevalence with time [50]. Early biopsy transcripts that were found to be associated with acute kidney injury pathways related to preservation and implantation injury. Later biopsies expressed rejection and glomerulonephritis-associated transcripts and also tended to be associated with progressive injury and allograft failure. Genes associated with late allograft loss indicate that the final common pathway is an active ongoing tissue response to injury regardless of the initial disease state. The study supports a nephron-centric model of fibrosis caused by continuing injury, rather than autonomous committed fibrogenesis, which cannot be abrogated. This suggests that when injury is shut off early, good function may be restored, and vice versa. The molecular classifier was also shown to outperform clinical and histological features in predicting allograft loss in late biopsies with IF/TA [51].

The Genomics of Chronic Allograft Rejection (GoCAR) consortium has also attempted to discover molecular signatures associated with chronic allograft injury. They prospectively examined transcripts from biopsies at 3 months post-transplantation in over 200 recipients with stable allograft function and then correlated these with chronic injury at 12 months [52]. They identified a set of 13 genes, independently predictive for the development of allograft fibrosis between 3 and 12 months, as well as early allograft loss (at 2 or 3 years). The transcript set had a high 'area under the curve' or AUC (in receiver-operating characteristics analysis), higher than that of baseline clinical characteristics and the combination of clinical and pathological factors. While the results of this study are encouraging, it is certain there are many more genes involved in chronic allograft injury. It is also quite likely that the reported genes are not intimately involved in injury pathogenesis, but may be biomarkers of early chronic allograft injury, predictive in these specific cohorts. However, this work holds great promise to predict allograft injury before it becomes clinically evident via traditional biomarkers and histology. Moreover, it is likely that the transcript sets become increasingly predictive when data from additional transplants and in different cohorts are added. The authors of the GoCAR study validated the findings internally and in two independent external data sets, although some criticism has been voiced regarding the replication methods in the study [53]. The authors refute this criticism and defend the robustness of their predictive gene set [54], highlighting the difficulty that the clinician has in interpreting the complex methodology of these studies, and their clinical applicability.

Complementary to these transcriptomic experiments, researchers are beginning to explore epigenetic modifications and their role in mediating response to injury and determinants of committed fibrogenesis. Bontha *et al.* [44] employed an integrative multi-omics approach by analysing DNA methylation, gene expression and microRNAs (miRNA) in transplant recipients with and without IF/TA on allograft biopsies as well as predonor biopsies. They demonstrated hypomethylated and highly expressed genes generally in pathways involved in immune responses. Moreover, the pattern of miRNA expression appeared distinct in IF/TA cases and miRNA themselves could be regulated by methylation/hypomethylation. While these experiments suggest epigenetic processes involved with immune injury and subsequent allograft dysfunction, further mechanistic studies are warranted to untangle the cause–effect relationship between DNA methylation and gene expression and to prove their effect on subsequent tissue injury.

Omics of tolerance

Tolerance, or allograft acceptance in the absence of immunosuppression, is a major goal in transplantation. However, there are no clinical biomarkers for guiding the safe reduction of immunosuppression. Discovery of operationally tolerant transplant recipients, through either nonadherence or physician directed cessation of immunosuppression, has allowed the study of tolerant individuals to identify their molecular signatures. Brouard *et al.* [55] reported a 33 gene peripheral blood gene expression panel from a discovery cohort of renal transplant recipients and normal individuals without a transplant that appeared to predict a tolerant state in a validation cohort of transplant patients. The signature

suggests a pattern of reduced costimulatory signalling, apoptosis, immune quiescence with memory T-cell responses. A greater numbers of regulatory T cells expressing the transcription factor Foxp3 (Foxp3 + Tregs) were also observed in the peripheral blood of tolerant patients versus those with a chronic rejection phenotype. This is a regular finding noted in tolerant states post-transplant with the loss of $Foxp3^{+}$Tregs often heralding a harmful effector T-cell phenotype [56]. Later work by several groups has demonstrated that tolerant patients demonstrate an increased repertoire of B cells, particularly naive cells, and related gene transcripts in peripheral blood [57–59].

Other investigators have attempted to characterize gene expression profiles to determine signatures of tolerance. Roedder *et al.* investigated peripheral blood samples from 348 HLA-mismatched renal transplant recipients and 101 nontransplant controls. They employed microarrays and quantitative PCR for gene discovery and then whole-genome lymphocyte expression data with subsequent flow cytometry to identify predicted and actual dominant cell types driving these signatures. A three-gene assay (*KLF6*, *BNC2* and *CYP1B1*) correlated significantly with operational tolerance (AUC 0.95) and a significant shift towards dendritic cells as well as B lymphocytes and NK cells [60]. This result was confirmed in multiple validation sets [60]. When applied to stable patients on immunosuppression, the panel identified 7% of patients with a similar, 'tolerant' signature, potentially highlighting patients who could be enrolled in trials of drug minimization. Brouard's group has recently reported similar findings for a composite score of a different set of six genes with an equally impressive reported predictive ability [61]. The panel also associated with the development of *de-novo* donor-specific antibody formation. Leventhal and Mathew [62] carried out global gene expression profiling of peripheral blood mononuclear cell in six tolerant and nine nontolerant HLA-identical nonchimeric kidney transplant recipients. They found a 357 gene expression signature that associated with tolerance (area under curve for tolerance versus nontolerance 0.807) which was replicated in an external cohort. They also found that a number of inflammatory and immune-related genes were downregulated in tolerant recipients when compared to normal controls, suggesting that that tolerant patients had a dampened immune-related gene expression. These techniques could potentially be used to monitor for donor-specific hyporesponsiveness and graft accommodation post-transplant, although such studies are awaited.

Omics of post-transplant diabetes mellitus

Post-transplant diabetes is a common transplant complication. It has an estimated prevalence of between 2% and 50% at 1 year post-transplant [63] and was 39% at 1 year when prospectively screened for in the steroid arm of the HARMONY Trial [69]. A number of clinical risk factors have been implicated in the development of PTDM including age, race, body mass index, hepatitis C infection and immunosuppression treatment regime [64]. There have been a limited number of studies on the molecular pathogenesis of PTDM. To date, the majority of genetic studies have been candidate gene studies which tend to have a number of caveats including differences in PTDM definitions used, inadequate size, lack of correction for multiple testing and failure to replicate across multiple cohorts. In a large meta-analysis of 18 SNPs (across 36 articles) previously associated with PTDM, three SNPs were found to be significantly associated across the studies highlighting the need for adequate replication [65]. These variants were found in *TCF7L2*, *CDKAL1* and *KCNQ1*, all of which have previously been implicated in diabetic mechanisms and associated with type 2 diabetes in nontransplant populations [66,67].

To date, two GWAS of PTDM have been published. The first GWAS implicated beta-cell dysfunction in the pathogenesis of PTDM [33], although no SNP reached genomewide significance, possibly due to sample size ($n = 256$). Of the top 26 most significant SNPs ($P < 10^{-5}$), they replicated associations with eight SNPs in a secondary cohort. The authors employed pathway analysis which implicated seven of the SNPs in beta-cell apoptosis. Pathway analysis may be a beneficial approach to use variants below strict levels of genome-wide significance to generate hypotheses. The second PTDM GWAS, of 302 kidney transplant patients of European ancestry, has only been presented in abstract form [35]. While again, no variants reached a genome-wide level of significance, there were a number of biologically plausible nominally associated loci including *CNTNAP2* which has been associated with diabetic kidney disease [68]. This study was also limited by the sample size and so larger, better powered studies will be needed to confirm the aforementioned findings.

Several immunosuppression agents have been associated with the development of PTDM, particularly calcineurin inhibitors and corticosteroids [69,70]. mTOR inhibitors, such as sirolimus and everolimus, may also be implicated. A number of *in vivo*, *ex vivo* and *in vitro* studies have shown that sirolimus leads to a reduction in both glucose stimulated insulin secretion and pancreatic β-cell

proliferation leading to hyperglycaemia [71]. Moreover, Fuhrmann *et al.* [72] found exposure of rats to either sirolimus or cyclosporine reduced expression of glucose metabolism genes and proteins including *GLUT4*. Pharmacogenomic studies such as these will be vital for the development of personalized drug therapies to reduce risk of PTDM.

Omics of post-transplant cancer

Cancer is a common complication of renal transplantation, largely due to prolonged immunosuppression. The most common post-transplant malignancies are nonmelanoma skin cancers (NMSC), with squamous cell carcinoma (SCC) and basal cell carcinoma (BCC), accounting for over 90% of post-transplant skin cancers [73]. Over half of transplant recipients will develop a skin lesion in the duration of their transplant and so it is vital that we develop methods for identifying those with increased risk [73,74]. Clinical risk factors for post-transplant NMSC include age of recipient, human papillomaviruses infection, cumulative sun exposure and immunosuppression treatment, with azathioprine being a particular risk [34,75].

Genome wide association studies of NMSC, conducted in nontransplant populations, have identified robust genetic associations with risk of these diseases. For example, a large GWAS (7404 cases and 292 076 controls) carried out on individuals of European ancestry found several genetic predictors of SCC [76]. Solid organ transplant recipients have a 65- to 250-fold and 10-fold increased risk of SCC and BCC, respectively [73]. Despite the increased risk of skin cancer in transplant populations, until recently limited attempts had been made at mapping the genetic predictors. Candidate SNP studies have implicated a number of genes including *GSTM1* [77] and *MTHFR* [78] in post-transplant NMSC but very few studies have examined skin cancer in transplant populations at a genomewide scale. A recent study by Asgari *et al.* [79] examined SNPs previously implicated in SCC in nontransplant populations in both candidate gene studies and GWAS [80,81]. Eight SNPs were analysed for associations with post-transplant SCC. Nominal associations were found with SNPs in *IRF4* and *SLC45A2*; however, no results were significant after correction for multiple testing. This study was limited by a small sample size and leaves us uncertain of the importance of these polymorphisms in post-transplant NMSC.

In 2015, the first GWAS of post-transplant SCC was published in a Caucasian kidney and heart transplant population (n = 388) [34]. Two of the most significant polymorphisms were found in genes previously associated with SCC and other cancers (*CACNA1D* and *CSMD1*) [82–85]. However, no variants reached genomewide significance, perhaps due to the small sample size, and there was no validation cohort so firm conclusion cannot be drawn from these results.

A number of studies have investigated associations with miRNA and gene expression profiles in NMSC. miRNAs are small RNA molecules (~22 nucleotides in length) that bind complementary sequences of target mRNAs and inhibit their translation [86]. They are key post-transcriptional gene expression regulators. The expression levels of four miRNAs with described functions in keratinocytes were examined in transplant patients, nonimmunocompromised individuals with SCC and normal controls [87]. They found significant differences in the expression levels of three of these miRNAs in the SCC lesions versus normal skin tissue, but no differences between the two groups with SCC. This suggests that gene expression in SCC lesions may be similar in transplant patients and nonimmunocompromised individuals; however, this study was relatively small (n = 37) and limited to a set of four miRNAs and so further analysis will be needed to validate these findings.

Hameetman *et al.* [88] examined genomewide expression profiles and chromosomal abnormalities from normal skin, SCC lesions and actinic keratosis (AK) lesions in 13 renal transplant recipients. In six of the SCC lesions, they found chromosomal aberrations including one complete loss of the short arm of chromosome 9. However, overall there were less copy number variations than expected. Large differences in gene expression profiles between normal skin and the SCC and AK lesions were seen. Genes involved in cellular differentiation and proliferations were found to be upregulated in SCC and AK when compared to normal skin. Many of the pathways upregulated in the SCC lesions were also found to be upregulated in AK lesions which is in line with the hypothesis that AKs are precursor SCC lesions. They also found that a number of oncogenic pathways that were activated in SCC were also active in AKs including the NFκB and TNF pathways. On the other hand, pathways such as RAS and MYC, which regulate a large number of molecular processes including apoptosis, tumorigenesis and cellular proliferation, were exclusively activated in SCC [89,90].

Laing *et al.* [91] performed a global methylation study of SCC lesions compared to normal skin in

transplant recipients and found genomewide hypomethylation in the SCC lesions. Methylation refers to the addition of a methyl group to DNA, usually at CpG islands. This process is a key epigenetic regulator as it suppresses the expression of target genes [92]. Global hypomethylation is associated with a large number of cancers in nontransplant populations, including head and neck SCC [93] and results in genomic instability [94]. These studies indicate the importance of epigenetic and transcriptome processes in post-transplant skin cancer. Understanding these mechanisms will be vital for the developing biomarkers and treatment strategies for this disease.

Future directions and conclusion

Technologies and resources in the field of omics are rapidly becoming more available and affordable. The evolution in next-generation sequencing technologies has made the once daunting task of whole-genome sequencing a realistic option for researchers. To date, however, little work has been done to examine the role of rare variants at a genomewide scale in transplant outcomes. Examining rare variants across the genome in transplantation could allow us to appreciate gene–gene interactions, both in recipients and in donor/recipient pairs, and potentially uncover genes associated with outcome that have a larger burden of rare variants. Understanding the role of genetics beyond the HLA, until recent years, has largely taken a candidate gene approach. Larger unbiased studies will be needed to further our understanding of genetic variation in the field of transplantation. This area, however, is looking bright. A number of transplant focused consortia have formed such as UK & Ireland Renal Transplant Consortium and more recently iGeneTRAiN which will allow us to upscale these omics efforts [31]. International consortia may also help to overcome the problem of population biases in genomic studies and may allow us to increase our numbers of poorly represented populations such as those of African ancestry.

Polygenic risk scores (PRS) go a step beyond the candidate gene approach. They have shown great promise in understanding the role of common genetic variation on neurological disorders [95]. PRS encompasses genetic variants previously found to be associated with a given trait and creates a score per individual based their alleles at these variants. This methodology could be applied readily in a transplant setting using results from nontransplant GWAS to understand transplant outcomes as well as complications such as PTDM and skin cancer.

A number of metabolomics and proteomic studies have been carried out in recent years. These studies are vital for the development of biomarkers to signal the onset of post-transplant morbidities as well as early predictors of graft failure. Currently used biomarkers such as serum creatinine and albuminuria are insensitive, nonspecific and signal graft dysfunction after the event [96]. A longitudinal study of microbiota of renal allograft recipients characterized changes in the early post-transplant months [97]. This study showed promise in predicting transplant outcomes with changes in microbiota correlating with poor transplant outcomes, albeit in a very small patient group. Another study showed the presence of certain bacteria in the gut microbiota of recipients correlated with tacrolimus dosing [98]. Understanding the composition and dynamic changes within a patient's microbiome could potentially act as a biomarker or be used to guide treatment, although this remains speculative.

Tolerance is the ultimate goal of any solid organ transplant. To reduce or completely abolish, a patient's reliance on immunosuppression could drastically reduce the risk of post-transplant complications such as diabetes and cancer and greatly improve their quality of life. An exciting new prospect for the area of tolerance is the idea of induced tolerance which has been successful experimentally using a variety of approaches including donor bone marrow transplants to induce hematopoietic chimerism [99]. Induced tolerance has also seen some success in clinical applications through the use of combined kidney and bone marrow transplants. Kawai *et al.* [100,101] could successfully induce long-term tolerance in a number of patients. An in-depth understanding of the genomic influences, regulatory process and molecular signatures of tolerance is key for the success of these approaches, which have the potential to drastically reduce immunosuppression-related complications post-transplant.

It is clear from the prior evidence presented in this review that transplant outcomes and the onset of transplant complications such as skin cancer and PTDM are affected by the behaviour of the immune system. This behaviour is heavily influenced by epigenetic mechanisms such as DNA methylation and histone modifications [102]. For example, the class II transactivator, CIITA, which induces MHC class II expression, is regulated by both DNA methylation and histone modification [103]. Epigenetic mechanisms are influenced by their surrounding environmental factors such as stress, chronic morbidities and drug exposure [104]. These mechanisms when combined with the

underlying genetic variants give rise to the presented phenotypes. Transplant phenotypes are even further complicated by the interplay between both recipient and donor genome and epigenome. Parker *et al.* [105] demonstrated in a rat model that cold ischaemic time of 4 h induced aberrant demethylation of the promoter of complement factor gene (C3), a key regulator of innate immunity, increasing its expression in the donor kidney. This study demonstrates the impact of the transplant setting on epigenetic mechanisms and how changes in these systems can have a massive impact on gene expression. The area of epigenetics, as discussed previously in this review, holds huge potential for understanding the dynamic processes and changes of the genomic landscape in a transplant setting.

Conclusions

The field of omics in transplantation is growing rapidly. With more and more data being created, there is a need for standardized, robust bioinformatics techniques that can be replicated easily and remove the problem of positive reporting biases. It is vital that large omics studies evaluate methods so that candidate predictive signatures and biomarkers can be sufficiently validated and brought forward into a clinical setting [106].

Furthering the field of omics in transplant is vital for understanding underlying dynamic genomic, transcriptomic and proteomic mechanisms which cause allograft dysfunction and post-transplant complications. Furthering our understanding of this area could massively aid the transplant community in developing improved, personalized treatment regimens to extend the life of the transplant while also reducing morbidities such as cancer and diabetes.

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Conflict of interest

The authors have declared no conflicts of interest.

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