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Omics-based biomarkers for diagnosis and prediction of kidney allograft rejection

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Kidney transplantation is the preferred treatment for patients with end-stage kidney disease, because it prolongs survival and improves quality of life. Allograft biopsy is the gold standard for diagnosing allograft rejection. However, it is invasive and reactive, and continuous monitoring is unrealistic. Various biomarkers for diagnosing allograft rejection have been developed over the last two decades based on omics technologies to overcome these limitations. Omics technologies are based on a holistic view of the molecules that constitute an individual. They include genomics, transcriptomics, proteomics, and metabolomics. The omics approach has dramatically accelerated biomarker discovery and enhanced our understanding of multifactorial biological processes in the field of transplantation. However, clinical application of omics-based biomarkers is limited by several issues. First, no large-scale prospective randomized controlled trial has been conducted to compare omics-based biomarkers with traditional biomarkers for rejection. Second, given the variety and complexity of injuries that a kidney allograft may experience, it is likely that no single omics approach will suffice to predict rejection or outcome. Therefore, integrated methods using multiomics technologies are needed. Herein, we introduce omics technologies and review the latest literature on omics biomarkers predictive of allograft rejection in kidney transplant recipients.

Keywords: Omics; Biomarkers; Graft rejection; Kidney transplantation

INTRODUCTION

Kidney transplantation (KT) is the best treatment option for patients with end-stage kidney disease to prolong survival and improve quality of life [1-4]. Since the first KT was conducted in 1954, the surgical technique and immunological knowledge have advanced markedly [5,6]. However, due to increased life expectancy and organ shortages, loss of graft function remains an issue in transplantation [7,8]. A variety of factors are linked to graft survival. However, acute or chronic allograft rejection is the most important risk factor for graft failure [9-11]. Therefore, to avoid rejection and

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maintain graft function, personalized immunosuppression based on individual monitoring of kidney transplant recipients (KTRs) is required [12-16].

Allograft biopsy and histopathological examination are the gold standard for diagnosing allograft rejection in KTRs; however, allograft biopsy is an invasive, reactive procedure that is challenging to monitor continuously [17]. Moreover, there is marked interobserver disagreement in interpretations of histopathological findings [18]. Other markers of rejection include elevation of serum creatinine and proteinuria, but these are not sensitive or specific for early diagnosis of rejection.

Major recent advances in molecular biology have enabled highresolution sequence mapping of human structural variation [19,20]. Omics-based immunological monitoring and rejection-predicting technologies for KTRs have attracted interest over the last two decades. In this article, we introduce omics technologies for early diagnosis of allograft rejection in KTRs and review the related literature.

OMICS TECHNOLOGIES

Omics technologies are based on a holistic view of the molecules that constitute an individual [21]. They aim primarily at universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics), and metabolites (metabolomics) [21]. Generally, omics technologies assess cellular macromolecules in biological samples (e.g., blood, urine, and tissue) in a nonbiased and nontargeted manner [21]. Advances in high-throughput technologies combined with large-scale data acquisition and computational analysis enable study of complete sets of molecules with improved speed, high accuracy, and lower cost compared with repeated single-molecule experiments [20]. Therefore, the omics approach can be applied to disease screening, diagnosis, and prognosis prediction, providing insight into the pathophysiologic basis of disease [21,22]. Omics technologies have accelerated biomarker discovery and provided insight into the multidimensional biological processes implicated in transplantation [20].

Genomics involves sequencing and analyzing the genome of an organism or cell (complete set of DNA). The human genome contains about 3 billion base pairs and approximately 30,000 to 40,000 protein-coding genes [23]. To analyze the genetic contribution to complex diseases,

traditionally, genes have been individually analyzed (e.g., candidate gene association studies of the associations between predefined genes and a trait of interest) [24]. However, advanced genomic methodologies such as genome-wide association studies (GWAS) and whole-genome sequencing can simultaneously scan and analyze many genetic variants. GWAS scans using single nucleotide polymorphism (SNP) array data have the benefit of lower cost compared with whole-genome sequencing; however, they have low affinity for rare variants.

The transcriptome refers to all transcripts (both coding RNAs [mRNA] and noncoding RNAs—long noncoding RNA, microRNA [miRNA], small interfering RNA [siRNA], and piwi-interacting RNA [piRNA]) in an individual, and it is the template for protein synthesis via a process termed translation. mRNA plays an intermediary role in protein translation [24]. Therefore, according to its spatial and temporal specificity, the mRNA profile reflects the functions of cells and organisms under specific physiologic or pathophysiologic conditions [25]. Transcriptomics analyzes the whole set of RNA in each cell or organism. This enables identification of active and inactive genes [26]. There are two transcriptomic methodologies—microarrays and RNA sequencing. Microarray technologies are primarily used to analyze predefined RNA targets, whereas RNA sequencing uses deep-sequencing technologies to analyze all sequences in a sample. RNA sequencing, which uses next-generation sequencing, is now widely used for transcriptomics.

The proteome is defined as the set of all proteins in a cell, tissue, or organism [27]. Proteomics analyzes the presence, activity, and interactions of the proteome, and provides insight into the functional network and relevance of proteins [28,29]. This requires the ability to detect thousands of proteins simultaneously, by analyzing the entire proteome of a cell, tissue, or organism [30]. Mass spectrometry-based proteomics allows identification and quantification of proteins and posttranslational modifications, such as phosphorylation and ubiquitination, in complex biological samples [31,32]. There are numerous mass-spectrometric techniques for large-scale relative or absolute proteome quantification. These techniques differ in the level of proteome quantification, accuracy, and reproducibility. Therefore, it is essential to select the most suitable mass-spectrometric technique for the investigation of pathophysiologic tissue [30].

Metabolomics is the study of the global metabolite profile of a cell, tissue, or organism under physiologic or patho-

physiologic conditions [21]. Metabolomics analyzes chemical processes involving small metabolites (< 1,500 Da) in a sample. Metabolites are the final downstream products of gene transcription, so changes in metabolites are amplified relative to those in the upstream transcriptome and proteome [33]. Theoretically, this represents an advantage of metabolomics over other omics approaches. Moreover, the metabolome, as the downstream product, is the closest to the phenotype of the biological system and can be affected by the environment and microbiome [21]. Nuclear magnetic resonance (NMR) spectrometry, gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, and matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry are metabolomic methodologies. NMR spectrometry is useful in clinical practice because it does not require separation or ionization during sample preparation and yields results within a few hours. However, compared with mass spectrometry, the sensitivity is low and fewer metabolites that can be analyzed [34].

OMICS-BASED BIOMARKERS FOR ALLOGRAFT REJECTION IN KIDNEY TRANSPLANT RECIPIENTS

Regarding immunological monitoring in KTRs, the most widely used biomarker is donor-specific human leukocyte antigen (HLA) alloantibodies (DSAs). However, because of advances in immunosuppression, the incidence of *de novo* DSA is relatively low (15% to 25%) [35-38]. Furthermore, most *de novo* DSA production cases cause negative allograft outcomes several years after the initial occurrence, so may be associated with subclinical rejection during the early period [24]. Hence, research has focused on identifying non-HLA biomarkers predictive of allograft rejection using omics technologies (Fig. 1). To date, various potential biomarkers have been developed using omics technologies, which we summarize in the sections below.

Genomic biomarkers of acute rejection

In a GWAS of European KTRs, Ghisdal et al. [39] used a DNA-pooling approach to compare 275 acute T cell-mediated rejection (TCMR) cases and 503 hyper-controls (KTRs
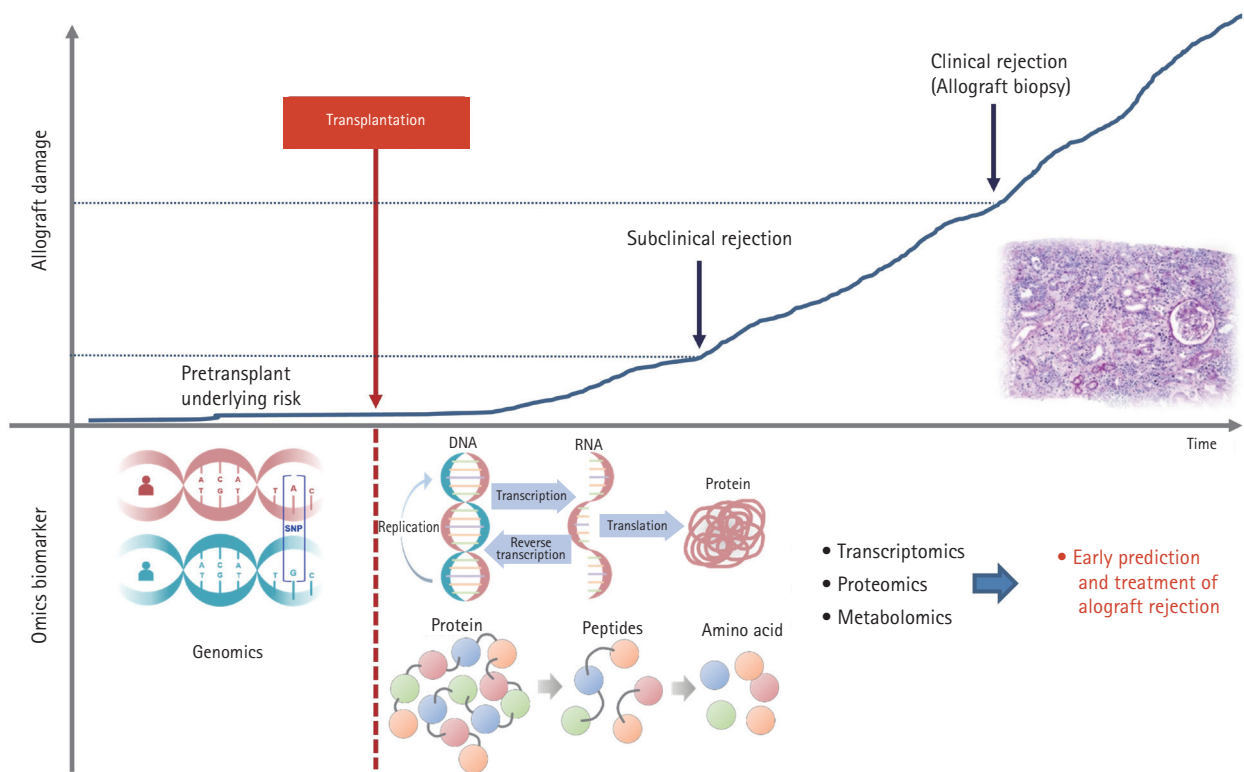

Figure 1. Advantages of omics biomarkers for prediction and treatment of acute rejection. SNP, single nucleotide polymorphism.

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Table 1. Genomic biomarkers of acute rejection

Study	Sample type	Biomarkers	Groups	AUC	Validation set	Results
Ghisdal et al. [39]	Blood	PTPRO and CCDC67	TCMR (n = 275) Hypercontrol without rejection (n = 503)	NA	Yes	Two SNPs had significant association with biopsy-proven acute TCMR.
Oetting et al. [40]	Blood	rs2910164 for miR-146a	TCMR and ABMR (n = 492) No rejection (n = 2,380)	NA	Yes	Among 75 candidate SNPs, only rs2910164 that alters the expression of miR-146a was a/w AR in the African American cohort.
Steers et al. [41]	Blood	*LIMS1* (rs893403)	TCMR and ABMR (n = 800) No rejection (n = 1,909)	0.91 (IgG3 subclass)	Yes	*LIMS1* locus appeared to encode a minor histocompatibility antigen and *LIMS1* was a/w AR.

AUC, area under the curve; TCMR, T cell-mediated rejection; NA, not available; SNP, single nucleotide polymorphism; ABMR, antibody-mediated rejection; AR, acute rejection; a/w, associated with; IgG3, immunoglobulin G3.

who did not show acute rejection [AR] although they had a less favorable HLA match at baseline) from a total of 4,127 KTRs (Table 1). The DNA-pooling approach decreases the number of tests needed and reduces the cost. For validation, 313 TCMR cases and 531 hyper-controls from 2,765 KTRs were used. Among 14 candidate SNPs, two had significant associations with AR—one locus in *PTPRO* coding for a receptor-type tyrosine kinase essential for B cell receptor signaling and the other encompassing the ciliary gene *CCDC67*.

American investigators validated 75 candidate SNPs reportedly associated with AR [40]. They used DNA from two multicenter cohorts of KTRs. GWAS data were identified and divided into two subcohorts consisting of 2,390 European American and 482 African-American KTRs, which were analyzed separately. Among 75 candidate SNPs, only rs2910164, which alters the expression of the miRNA *miR-146a*, showed a significant association with AR in the African-American cohort.

Steers et al. [41] focused on genomic collision situations, wherein transplant recipients carried two copies of a deletion that was not homozygous in the organ donor. They conducted a two-stage (discovery and replication) genetic association study to uncover high-priority copy number variants influencing AR. In the 705 discovery cohort, the *LIMS1* locus represented by rs893403 showed a significant association with allograft rejection. This was replicated among the

2,004 donor-recipient pairs after adjustment for the age, sex, ethnicity, and HLA status mismatch of the recipient.

GWAS studies have identified different SNPs associated with AR. The difference may be associated with the heterogeneity and complexity of AR, small size of the cohort, and genetic differences among ethnicities [42]. Also, there may be variation among transplant centers in the SNPs associated with AR [43]. Therefore, a large-scale, multicenter study including various ethnicities is required to evaluate the associations between SNPs and AR.

Transcriptomic biomarkers of acute rejection
The Suthanthiran group has suggested that urine mRNA levels could be used to detect AR [44-46]. They analyzed 4,300 urine samples from 485 KTRs in the prospective observational Clinical Trials in Organ Transplantation 04 (CTOT-04) study (Table 2) [46]. The combination of the 18S ribosomal RNA, *CD3ε,* and *IP-10* mRNA levels in urinary cells showed utility as a diagnostic and prognostic biomarker of TCMR. The diagnostic signature markedly increased for up to 20 days before histopathological diagnosis of TCMR.

The CTOT-08 study was designed to develop molecular biomarkers for clinical phenotypes in KTRs [47]. Among 253 KTRs, the investigators used 530 paired peripheral blood samples to discover a novel gene-expression profile predictive of subclinical AR. The classifier consisted of 61 probesets that mapped 57 genes. The gene expression profile was

Table 2. Transcriptomic biomarkers of acute rejection

Study	Sample type	Biomarkers	Groups	AUC/ sensitivity/ specificity	Validation set	Results
Suthanthiran et al. [46] CTOT-04 study	Urine	Three-gene signature (*CD3ε* mRNA, *IP-10* mRNA, and *18S* rRNA)	TCMR (n = 36) No rejection in biopsy (n = 150) STA without biopsy (n = 202)	0.74/ 71%/ 72%	Yes	It increased up to 20 days before histopathologic diagnosis of acute TCMR.
Friedewald et al. [47] CTOT-08 study	Blood	Panel of 57-gene signature[a]	Subclinical rejection (n = 143) No rejection (n = 239)	0.85/ 64%/ 87%	Yes	It was a/w with clinical and histopathologic outcomes and with *de novo* DSA.
Roedder et al. [48] AART study	Blood	17-Gene signature, named as kSORT[b]	TCMR and ABMR (n = 187) No acute rejection (n = 254) Pre-acute rejection (n = 65) Post-acute rejection (n = 52)	0.94/ 83%/ 91%	Yes	kSORT predicted AR up to 3 months before histopathologic diagnosis in 62.9% of stable KTRs.
Zhang et al. [49] GoCAR study	Blood	17-Gene signature[c]	Subclinical rejection at 3 months (n = 46) Non-subclinical rejection at 3 months (n = 145)	0.98/ NA/ NA	Yes	It identified ongoing subclinical TCMR and correlated with long-term risk of graft loss.
Christakoudi et al. [50] KALIBRE study	Blood	Seven-gene signature (*IFNG, IP-10, ITGA4, MARCH8, RORc, SEMA7A*, and *WDR40A*)	TCMR (n = 47) No rejection (n = 128)	0.84/ 67%/ 85%	Yes	It increased 7 weeks before histopathologic diagnosis of TCMR and decreased after treatment.
El Fekih et al. [51]	Urine	15-Gene signature[d]	TCMR and ABMR (n = 59) No rejection (n = 133)	0.93/ 85%/ 94%	Cross-validation	It effectively discriminated AR. Another five-gene signature could distinguish TCMR from ABMR.
Seo et al. [52] ARTKT study	Urine	Nine-gene signature (*CXCL9, CD3ε, IP-10, LCK, C1QB, PSMB9, Tim-3, Foxp3*, and *FAM26F*)	TCMR (n = 38) ABMR (n = 11) STA (n = 153)	0.89/ 91%/ 80%	Yes	Expression of nine genes were significantly different between AR and STA with normal pathology.
Anglicheau et al. [53]	Blood and graft tissue	*miR-142-5p, miR-155, miR-223, miR-30a-3p, miR-10b*, and *let-7c*	TCMR and ABMR (n = 12) No rejectin in biopsy (n = 21)	0.73–0.99/ 67%–100%/ 61%–95%	Yes	MicroRNA levels in intragraft and PBMCs were significantly altered in AR; thus, they could predict graft status.

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Table 2. Continued

Study	Sample type	Biomarkers	Groups	AUC/ sensitivity/ specificity	Validation set	Results
Tao et al. [54]	Blood	miR-99a	TCMR and ABMR (n = 12) No rejectin in biopsy (n = 11) Delayed graft function (n = 15)	0.75/ NA/ NA	Yes	Serum *miR-99* discriminated AR from STA and delayed graft function.
Lorenzen et al. [55]	Urine	miR-210	TCMR (n = 62) No rejection (n = 19) STA with urinary tract infection (n = 13)	0.7/ 74%/ 52%	Yes	Urinary *miR-210* level was a/w TCMR and predict long-term graft function.
Vitalone et al. [56]	Graft tissue	*miR-25, miR-181a, miR-204, miR-192, miR-10b, miR-142-3p, miR-215, miR-342-3p,* and *miR-615-3p*	TCMR and ABMR (n = 47) No rejection in biopsy (n = 120)	NA	Yes	The expression of miRNAs significantly a/w the intensity of the Banff-scored interstitial inflammation and tubulitis.

AUC, area under the curve; CTOT, Clinical Trials in Organ Transplantation; TCMR, T cell-mediated rejection; STA, stable graft function; a/w, associated with; DSA, donor-specific human leukocyte antigen alloantibody; AART, Assessment of Acute Rejection in Renal Transplantation; kSORT, Kidney Solid Organ Response Test; ABMR, antibody-mediated rejection; AR, acute rejection; KTR, kidney transplant recipient; GoCAR, Genomics of Chronic Allograft Rejection; NA, not available; KALIBRE, Kidney Allograft Immunological Biomarkers of Rejection; ARTKT, Assessment of immunologic Risk and Tolerance in Kidney Transplantation; PBMC, peripheral blood mononuclear cell.

[a]57 genes: *AARSD1, AP2M1, ARHGDIB, ASB6, BTD, C20orf27, C9orf16, CFL1, CIAO1, CNDP2, Cxorf56, DDX39B, EMP3, EXOC4, FAM103A1, FCGR2B, GNAI2, HLA-J, HMGXB3, HSPB1, IFNAR1, ILK, KCMF1, KIAA0141, KLHDC4, LOC101928595, LRWD1, MIB2, MYO19, MYO1C, MYPOP, OS9, PFN1, PKM, PKNOX1, PTK2B, RBBP9, RBM3, RBM5, RLIM, RPUSD3, RUSC1, SARNP, SH3BGRL3, SLC25A19, SLC35D2, SNX19, SNX20, STN1, TMEM62, TPMT, TRAPPC1, TTC9C, TWF2, UCP2, UQCR11,* and *UQCR11.*

[b]17 genes: *CFLAR, DUSP1, IFNGR1, ITGAX, MAPK9, NAMPT, NKTR, PSEN1, RNF130, RYBP, CEACAM4, EPOR, GZMK, RARA, RHEB, RXRA,* and *SLC25A37.*

[c]17 genes: *ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, SENP7, CLK1, SENP6, C1GALT1C1, SPCS3, MAP1A, EFTUD2, AP1M1, ANXA5, TSC22D1, F13A1,* and *TUBB1.*

[d]15 genes: *CXCL11, CD74, IL32, STAT1, CXCL14, SERPINA1, B2M, C3, PYCARD, BMP7, TBP, NAMPT, IFNGR1, IRAK2,* and *IL18BP.*

significantly correlated with the clinical and histopathologic outcomes, as well as with *de novo* DSA in two validation sets.

The Assessment of Acute Rejection in Renal Transplantation (AART) study group developed the Kidney Solid Organ Response Test (kSORT) to detect KTRs with a high risk of AR [48]. They analyzed the gene expression data of 558 blood samples from 436 KTRs and selected 17 genes (*USP1, CFLAR, ITGAX, NAMPT, MAPK9, RNF130, IFNGR1, PSEN1, RYBP, NKTR, SLC25A37, CEACAM4, RARA, RXRA, EPOR, GZMK,* and *RHEB*) that could discriminate AR. kSORT predicted AR up to 3 months prior to histopathologic diagnosis

in 62.9% of stable KTRs, so could be used to predict subclinical rejection.

Zhang et al. [49] examined subclinical histologic and functional changes in KTRs from the prospective Genomics of Chronic Allograft Rejection (GoCAR) study. They used peripheral blood RNA from 191 KTRs who underwent surveillance biopsy 3 months after transplant. A 17-gene signature set (*ZMAT1, ETAA1, ZNF493, CCDC82, NFYB, SENP7, CLK1, SENP6, C1GALT1C1, SPCS3, MAP1A, EFTUD2, AP1M1, ANXA5, TSC22D1, F13A1,* and *TUBB1*) was identified as a candidate biomarker of ongoing subclinical TCMR. After extensive validation in an independent cohort of 110 KTRs,

this gene set was confirmed to be associated with subclinical TCMR 3 months after transplant, and to be related to an increased risk of long-term graft loss.

Christakoudi et al. [50] developed a multivariable gene-expression signature targeting TCMR using 1,464 peripheral blood samples from 248 patients in the Kidney Allograft Immunological Biomarkers of Rejection (KALIBRE) study. They selected a parsimonious gene-expression signature—the smallest set of genes showing satisfactory predictive performance in the development phase. The selected seven-gene signature (*IFNG, IP-10, ITGA4, MARCH8, RORc, SEMA7A,* and *WDR40A*) was confirmed using internal and external KT cohorts. The estimated probability of TCMR increased 7 weeks before histopathologic diagnosis and decreased after treatment.

El Fekih et al. [51] isolated urinary exosomal mRNAs of 192 KTRs (59 rejections) and developed rejection signatures based on differential gene expression. After cross-validation, a 15-gene signature (*CXCL11, CD74, IL32, STAT1, CXCL14, SERPINA1, B2M, C3, PYCARD, BMP7, TBP, NAMPT, IFNGR1, IRAK2,* and *IL18BP*) discriminated AR with an area under the curve (AUC) of 0.93. Moreover, a five-gene signature (*CD74, C3, CXCL11, CD44,* and *IFNAR2*) could differentiate TCMR from active antibody-mediated rejection (ABMR) with an AUC of 0.87.

The Korean Assessment of immunologic Risk and Tolerance in Kidney Transplantation (ARTKT) study group reported a transcriptomic biomarker of AR using the urinary mRNA signature of KTRs [52]. Initially, they searched for candidate genes for AR in the Gene Expression Omnibus (GEO) database and selected 10 genes based on a meta-analysis of four datasets in the GEO database, along with four genes from the literature. Among these 14 candidate genes for AR, the expression of nine (*CXCL9, CD3ε, IP-10, LCK, C1QB, PSMB9, TIM-3, FOXP3,* and *FAM26F*) differed significantly between AR and stable KTRs in the validation set. The final AUC value of the nine-gene signature was 0.84 in the validation model.

The associations between miRNAs and AR have been analyzed. Anglicheau et al. [53] reported alterations of miRNA expression in KTRs with AR. They analyzed the expression of miRNAs in allograft tissue and peripheral blood mononuclear cells in 33 KTRs (12 with AR) using the TaqMan low-density array. Among the identified miRNAs, *miR-142-5p, miR-155,* and *miR-223* were increased, and *miR-30a-3p, miR-10b,* and *let-7c* were decreased, in KTRs with AR

compared to KTRs with stable graft function. Therefore, patterns of miRNA expression can serve as biomarkers of allograft status.

Tao et al. [54] analyzed miRNA profiles in blood samples from 33 KTRs (12 with AR; 11 with stable control; and 15 with delayed graft function). The TaqMan miRNA assay showed that *miR-99a, miR-100, miR-151a, let-7a, let-7c,* and *let-7f* were altered in the serum of KTRs with AR. Among these six miRNAs, *miR-99a* and *miR-100* were significantly upregulated in KTRs with AR compared to the stable controls. In KTRs with delayed graft function, *miR-99a* could discriminate AR from both stable graft function and delayed graft function.

Lorenzen et al. [55] profiled the urinary miRNAs of 81 KTRs (62 TCMR and 19 stable control). The TaqMan miRNA assay showed that *miR-10a, miR-10b,* and *miR-210* were markedly deregulated in urine of KTRs with TCMR. Among them, only *miR-210* differed between KTRs with TCMR compared to stable control KTRs. In addition, a decreased *miR-210* level was associated with a greater rate of decline in the estimated glomerular filtration rate at 1 year after transplant. Therefore, the urinary *miR-210* level could identify KTRs with AR and predict long-term graft function.

Vitalone et al. [56] analyzed the graft tissue of 167 KTRs (47 with AR and 120 without rejection) to understand the molecular pathophysiology of alloimmune injury. They transcriptionally profiled miRNAs by multiplexed microfluidic quantitative polymerase chain reaction. Nine relevant miRNAs (*miR-25, miR-181a, miR-204, miR-192, miR-10b, miR-142-3p, miR-215, miR-342-3p,* and *miR-615-3p*) were identified only in the AR group. The expression levels of these miRNAs were significantly associated with histopathological interstitial inflammation and tubulitis intensity.

Proteomic biomarkers of acute rejection

Several plasma and urine proteomic studies have revealed proteomic biomarkers associated with AR (Table 3). O'Riordan et al. [57] analyzed the urinary proteome of 73 KTRs (34 with AR and 39 with stable graft function). As AR developed, β-defensin-1 was decreased and α-1-antichymotrypsin was increased. Therefore, the ratio of β-defensin-1 to α-1-antichymotrypsin in urine may be a novel proteomic biomarker for AR.

Ling et al. [58] performed a urine proteomic analysis of 70 samples from 50 KTRs and 20 healthy controls. A panel of 40 peptides for AR was identified, which discriminated AR

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Table 3. Proteomic biomarkers of acute rejection

Study	Sample type	Biomarkers	Groups	AUC/ sensitivity/ specificity	Validation set	Results
O'Riordan et al. [57]	Urine	β-Defensin-1 and α-1-antichymotrypsin	TCMR and ABMR (n = 34) STA with normal biopsy (n = 39)	0.91/ 91%/ 77%	No	β-Defensin-1 was decreased and α-1-antichymotrypsin was increased as AR developed.
Ling et al. [58]	Urine	Panel of 40-peptides[a]	TCMR and ABMR (n = 20) STA with normal biopsy (n = 20) BKVAN (n = 10)	0.96/ NA/ NA	Yes	It discriminated AR in training and validation groups, and highlighted the changes in collagen remodeling in AR.
Ziegler et al. [59]	Blood	APOA1, α-1-antichymotrypsin, and the unidentified 5191 Da peptides	TCMR and ABMR (n = 16) No rejection in biopsy (n = 48)	0.79 (4,467 Da) and 0.98 (5,191 Da)/ NA/ NA	Yes	APOA1 and α-1-antichymotrypsin were decreased and 5191 Da peptides were increased in AR.
Sigdel et al. [60]	Urine	Fibrinogen beta (FGB), fibrinogen gamma (FGA), HLA-DRB1	TCMR and ABMR (n = 74) STA (n = 74) CAI (n = 58) BKVAN (n = 38)	0.8/ NA/ NA	Yes	Three progeins were highly specific for AR and discriminated from STA, BKVAN, or CAI.
Lim et al. [61] ARTKT study	Urine	Tetraspanin-1 (TSPAN-1) and Hemopexin (HPX)	TCMR (n = 25) STA (n = 22)	0.74/ 64%/ 73%	Yes	A total of 17 protein enriched in urinary exosome in KTRs with TCMR; tetraspanin-1 and hemopexin proposed as biomarkers in validation.

AUC, area under the curve; TCMR, T cell-mediated rejection; ABMR, antibody-mediated rejection; STA, stable graft function; AR, acute rejection; BKVAN, BK virus-associated ephropathy; NA, not available; CAI, chronic allograft injury; ARTKT, Assessment of immunologic Risk and Tolerance in Kidney Transplantation; KTR, kidney transplant recipient.
[a]The peptides mapped to nine different proteins, eight of which belonged to the collagen family (COL1A1, COL1A2, COL3A1, COL4A3, COL4A4, COL4A5, COL7A1, COL18A1) and uromodulin (UMOD).

in the validation cohort (AUC = 0.96). Moreover, a mechanistic analysis based on peptide sequencing implicated proteolytic degradation of uromodulin and collagens, such as COL1A2 and COL3A1.

Ziegler et al. [59] analyzed blood samples from 64 KTRs to identify biomarkers of AR. Among the 22 candidate proteins, APOA1, α-1-antichymotrypsin, and an unidentified 5191 Da peptide were associated with TCMR.

Sigdel et al. [60] used isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomic discovery and targeted enzyme-linked immunosorbent assay (ELISA) validation to discover and validate candidate urine proteomic biomarkers from 262 KTRs with biopsy-proven graft injury. Among the 69 urine proteins whose abundance differed significantly between the AR and stable graft function groups, nine (HLA-DRB1, FGG, FGB, FGA, KRT14, HIST1H4B, ACTB, KRT7, and DPP4) were associated with stable graft function, BK virus nephropathy, and chronic allograft injury ($p < 0.01$; fold increase > 1.5). Among these nine proteins, three (FBG, FGG, and HLA-DRB1) were validated as candidate biomarkers for AR.

Lim et al. [61] performed a proteomic analysis to identify candidate biomarkers for TCMR diagnosis in urinary extracellular vesicles using urine samples collected for the ARTKT

study. They performed a proteomic analysis by nanoliquid chromatography-tandem mass spectrometry and selected five proteins as candidate biomarkers for early diagnosis of acute TCMR. Subsequently, they validated the protein levels of the five candidate biomarkers by western blot analysis. Of them, the tetraspanin-1 and hemopexin levels were significantly higher in KTRs with TCMR.

Metabolomic biomarkers of acute rejection

Initially, metabolomic studies used NMR spectrometry, but this has been gradually replaced by mass spectrometry for blood and urine samples. The Blydt-Hansen group discovered urinary metabolomic biomarkers of TCMR by analyzing 277 urine samples from 57 KTRs (Table 4) [62]. Among 134

unique metabolites, the selection of the top 10 most important metabolites was internally validated (AUC = 0.88). They also identified urinary metabolomic biomarkers of ABMR [63] by analyzing 396 urine samples from 59 KTRs. They found 133 metabolites related to ABMR, and the top 10 metabolites were selected as a urine metabolomic signature for ABMR. The urine metabolite signature had an AUC of 0.84 for discriminating ABMR, and internal validation yielded an AUC of 0.76.

Sigdel et al. [64] analyzed the metabolomic profiles of biopsy-matched urine samples from 310 KTRs (106 AR). They identified 266 metabolites; a panel of 11 metabolites (glycine, glutaric acid, adipic acid, inulobiose, threose, sulfuric acid, taurine, N-methylalanine, asparagine, 5-aminovaleric

Table 4. Metabolomic biomarkers of acute rejection

Study	Sample type	Biomarkers	Groups	AUC/ sensitivity/ specificity	Validation set	Results
Blydt-Hansen et al. [62]	Urine	Classifier using top 10 metabolites[a]	TCMR (n = 19) No rejection in biopsy (n = 38)	0.88/ 89%/ 77%	Yes	The metabolite classifier could identify TCMR, and the metabolites overlap with those that identify borderline tubulitis in pathology.
Blydt-Hansen et al. [63]	Urine	Classifier using top 10 metabolites[b]	ABMR (n = 10) No ABMR (n = 49)	0.84/ 78%/ 83%	No	Exploratory analyses identified overlapping metabolite signatures between ABMR and TCMR, suggesting similar pathophysiology of tissue injury.
Sigdel et al. [64]	Urine	Panel of 11 metabolites[c]	TCMR and ABMR (n = 106) STA (n = 111) IF/TA (n = 71) BKVAN (n = 22)	0.99/ 93%/ 96%	No	A panel of 11 metabolites could detect AR and another panel of four metabolites differentiated AR from BKVAN.
Kim et al. [65] ARTKT study	Urine	Panel of five metabolites (guanidoacetic acid, methylimidazoleacetic acid, dopamine, 4-guanidinobutyric acid, and L-tryptophan)	TCMR (n = 14) STA (n = 17)	0.93/ 90%/ 85%	Yes	Among 17 putative metabolomic biomarkers, five-metabolite panel could effectively discriminate acute TCMR from STA.

AUC, area under the curve; TCMR, T cell-mediated rejection; ABMR, antibody-mediated rejection; STA, stable graft function; IF/TA, interstitial fibrosis and tubular atrophy; BKVAN, BK virus-associated nephropathy; AR, acute rejection; ARTKT, Assessment of immunologic Risk and Tolerance in Kidney Transplantation.

[a]Top 10 metabolites: proline, PC.aa.C34.4, kynurenine, sarcosine, methionine sulfoxide, PC.ae.C38.6, threonine, glutamine, phenylalanine, and alanine.

[b]Top 10 metabolites: proline, citrulline, PC.aa.C34.4, C10.2, lysine, methionine sulfoxide, hexose, threonine, tetradecanoylcarnitine, and acetylornithine.

[c]11 metabolites: glycine, glutaric acid, adipic acid, inulobiose, threose, sulfuric acid, taurine, N-methylalanine, asparagine, 5-aminovaleric acid lactam, and myo-inositol.

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acid lactam, and myo-inositol) enabled detection of AR. The AUC for discriminating AR from stable graft function was 0.99, with 92.9% sensitivity and 96.3% specificity.

The ARTKT study group developed a urinary metabolomic biomarker for TCMR [65]. They identified 17 putative metabolites that were altered in TCMR compared with stable graft function, and discovered five urinary metabolomic biomarkers (guanidoacetic acid, methylimidazoleacetic acid, dopamine, 4-guanidinobutyric acid, and L-tryptophan) of TCMR. Their ability to predict TCMR was excellent, with an accuracy of 87.0% (AUC = 0.926, sensitivity = 90.0%, specificity = 84.6%).

Omics biomarkers for chronic allograft injury and chronic rejection

There are various omics biomarkers for chronic allograft injury and chronic active antibody-mediated rejection (CABMR) (Table 5). The GoCAR group prospectively analyzed the gene-expression profiles of 159 tissue samples from KTRs with stable graft function at 3 months after transplantation [66]. They identified a set of 13 genes (*CHCHD10, KLHL13, FJX1, MET, SERINC5, RNF149, SPRY4, TGIF1, KAAG1, ST5, WNT9A, ASB15,* and *RXRA*) independently predictive of allograft fibrosis 12 months after transplantation. The 13-gene graft signature was validated in an independent cohort (n = 45, AUC 0.87) and two independent expression datasets (n = 282, AUC = 0.83; n = 24, AUC = 0.97, respectively).

Li et al. [67] performed a meta-analysis of molecular datasets of interstitial fibrosis and tubular atrophy (IF/TA) in the GEO database from peripheral blood and allograft biopsy samples. They identified a robust typical transcriptional response in IF/TA involving 85 significantly differentially expressed genes compared with non-IF/TA.

The ARTKT study group identified potential urinary extracellular vesicle protein biomarkers of CABMR [68]. They

Table 5. Omics biomarkers of chronic allograft dysfunction and chronic rejection

Study	Sample type	Biomarkers	Groups	AUC/ sensitivity/ specificity	Validation set	Results
O'Connell et al. [66] GoCAR study	Graft tissue at 3rd months surveillance	13-Gene renal tissue signature[a]	High CAD score (n = 44) Low CAD score (n = 57)	0.97/ NA/ NA	Yes	It predicted graft fibrosis at 12 months among KTRs with normal histology at 3-month after transplantation.
Li et al. [67]	Blood and graft tissue	85-Gene signature[b]	IF/TA (n = 128) STA (n = 147)	NA	No	The meta-analysis of multicenter independent gene-expression data sets identified 85 genes that were a/w IF/TA.
Jung et al. [68] ARTKT study	Urine	Six proteins (APOA1, TTR, PIGR, HPX, CP, and AZGP1)	CABMR (n = 26) LGS (n = 57) Graft function matched control (n = 10)	0.93, 0.85, 0.76, 0.73, 0.86, 0.74/ 82%, 77%, 71%, 59%, 71%, 65%/ 83%, 75%, 71%, 58%, 71%, 67%	Yes	They were distinguishable between CABMR and long-term stable KTRs.

AUC, area under the curve; CAD, chronic allograft damage; NA, not available; KTR, kidney transplant recipient; IF/TA, interstitial fibrosis and tubular atrophy; STA, stable graft function; a/w, associated with; CABMR, chronic active antibody-mediated rejection; LGS, long-term graft survival.

[a]13 genes: *CHCHD10, KLHL13, FJX1, MET, SERINC5, RNF149, SPRY4, TGIF1, KAAG1, ST5, WNT9A, ASB15,* and *RXRA.*

[b]85 genes: *CASP1, CASP8, CBL, CD3D, CD48, CD53, CCR5, CTSS, CYBB, S1PR1, EVI2B, MS4A2, FCGR2A, FCGR2B, FPR3, FYB, GPR34, HCK, HLA-DPB1, PRMT2, HSPA9, SP110, IL7R, TNFRSF9, ISG20, JAK3, LY9, LYZ, MCL1, MNDA, NCF2, NCF4, PTGER3, PT-PRC, PTPRE, RNASE6, SELL, SLC22A5, THRB, TJP1, TLR2, TLR4, TTN, ZNF207, LAPTM5, ST8SIA4, LST1, CD84, BTRC, NMI, CD163, AIM2, ARHGAP25, PLXNC1, IGSF6, TNFSF13B, GLIPR1, RAB31, CD300A, SAMHD1, LAMP3, CYTH4, CKLF, C1RL, TLR8, MS4A4A, CHST15, TM6SF1, FAR2, ARHGAP15, MS4A7, NLRC4, MCCC2, MS4A6A, RGS18, SMAP2, AMICA1, TMEM71, KIAA2018, MPEG1, RNF144B, SCML4, ARHGAP30, PPP1R2P4,* and *CCR2.*

used a proteomic approach to measure changes in urinary extracellular vesicles in urine samples from 93 KTRs (CABMR, n = 26). Six proteins (APOA1, TTR, PIGR, HPX, AZGP1, and CP) enabled discrimination of the CABMR and long-term graft survival groups. AZGP1 was a CABMR-specific biomarker that enabled differentiation of the rejection-free control group based on age at transplant, time since KT, and graft function.

Limitations and future directions

Advances in high-throughput omics technologies have facilitated the discovery of biomarkers in the transplant field. However, there are several barriers to their clinical application. The key features of a biomarkers of AR in KTRs are noninvasiveness, ease of measurement and interpretation, reproducibility, high sensitivity and specificity, good prognostic performance, and cost-effectiveness [69-72]. Prospective randomized controlled trials to assess the efficacy and cost-effectiveness of candidate omics biomarkers compared with traditional diagnostic methods are required for clinical application of biomarkers. In addition, considering the complexity and heterogeneity of rejection, large cohorts are needed to overcome the problem of inconsistent omics biomarkers among studies. A meta-analysis using the same type of data from different cohorts would go some way to overcoming this limitation.

Another requirement for the clinical application of omics biomarkers is integration. Noninvasive omics biomarkers show promise not only for diagnosis of rejection, but also for early diagnosis of histopathological injuries, such as subclinical AR, as well as stratifying patients according to the risk of rejection to reduce the need for surveillance biopsies. However, rejections (TCMR and ABMR) are heterogeneous in terms of severity and underlying pathophysiologic mechanisms, so it is difficult to determine the status of an individual patient using a single omics method developed based on a small sample size. Moreover, the expression of a given gene set does not indicate the total amount of proteins produced, their biological activities, or the functions of metabolites [73]. Hence, the predictive performances of omics biomarkers differ among cohorts [74]. Therefore, to assess the pathophysiologic and immunological responses associated with AR and long-term stable graft function, multidimensional multiomics approaches are needed. Genomics, transcriptomics, proteomics, and metabolomics data are typically produced on an individual basis and ana-lyzed separately [24]. The application of artificial intelligence (AI) through machine-learning algorithms and neural networks may be the solution. AI models enable analysis and integration of large-scale molecular information [69,75,76], including complex multiomics data. This will provide insight into the mechanisms of AR and long-term graft survival.

CONCLUSIONS

Omics biomarkers are the cornerstone of precision medicine in KT; they can guide selection of the best treatment by integrating traditional clinical information and tailoring immunosuppression. Integration of individual omics analyses may be critical for deciphering complex biological and immunological systems in transplantation. Therefore, large-scale prospective studies using multiple omics biomarkers are warranted.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Wolfe RA, Ashby VB, Milford EL, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N Engl J Med 1999;341:1725-1730.
2. Port FK, Wolfe RA, Mauger EA, Berling DP, Jiang K. Comparison of survival probabilities for dialysis patients vs cadaveric renal transplant recipients. JAMA 1993;270:1339-1343.
3. Park S, Kim M, Kim JE, et al. Characteristics of kidney transplantation recipients over time in South Korea. Korean J Intern

Med 2020;35:1457-1467.

4. Park Y, Ko EJ, Chung BH, Yang CW. Kidney transplantation in highly sensitized recipients. Kidney Res Clin Pract 2021;40:355-370.

5. Morris PJ. Transplantation: a medical miracle of the 20th century. N Engl J Med 2004;351:2678-2680.

6. AbdElhalim MS, Kenawy AS, Demellawy H, et al. The impact of omeprazole on mycophenolate pharmacokinetics in kidney transplant recipients. Kidney Res Clin Pract 2020;39:479-486.

7. Jeon HJ, Bae HJ, Ham YR, et al. Outcomes of end-stage renal disease patients on the waiting list for deceased donor kidney transplantation: a single-center study. Kidney Res Clin Pract 2019;38:116-123.

8. Chung BH, Choi BS, Oh EJ, et al. Clinical impact of the baseline donor-specific anti-human leukocyte antigen antibody measured by Luminex single antigen assay in living donor kidney transplant recipients after desensitization therapy. Transpl Int 2014;27:49-59.

9. Jeong HJ. Diagnosis of renal transplant rejection: Banff classification and beyond. Kidney Res Clin Pract 2020;39:17-31.

10. Halloran PF. Immunosuppressive drugs for kidney transplantation. N Engl J Med 2004;351:2715-2729.

11. Chung BH, Yang CW, Cho ML. Clinical significance of Th17 cells in kidney transplantation. Korean J Intern Med 2018;33:860-866.

12. Naesens M, Anglicheau D. Precision transplant medicine: biomarkers to the rescue. J Am Soc Nephrol 2018;29:24-34.

13. Lim JH, Cho JH, Jung HY, et al. Excellent outcome after desensitization in high immunologic risk kidney transplantation. PLoS One 2019;14:e0222537.

14. Potter SR, Hinojosa R, Miles CD, O'Brien D, Ross DJ. Remote monitoring using donor-derived, cell-free DNA after kidney transplantation during the coronavirus disease 2019 pandemic. Kidney Res Clin Pract 2020;39:495-500.

15. Bae H, Na DH, Chang JY, et al. Usefulness of BK virus-specific interferon-γ enzyme-linked immunospot assay for predicting the outcome of BK virus infection in kidney transplant recipients. Korean J Intern Med 2021;36:164-174.

16. Kim T, Lee HJ, Kim SM, et al. Diagnostic usefulness of the cytomegalovirus (CMV)-specific T cell-based assay for predicting CMV infection after kidney transplant. Korean J Intern Med 2020;35:438-448.

17. Park WY, Kim Y, Paek JH, Jin K, Han S. Clinical significance of serum galactose-deficient immunoglobulin A1 for detection of recurrent immunoglobulin A nephropathy in kidney transplant recipients. Kidney Res Clin Pract 2021;40:317-324.

18. Glassock RJ. Con: kidney biopsy. An irreplaceable tool for patient management in nephrology. Nephrol Dial Transplant 2015;30:528-531.

19. Kidd JM, Cooper GM, Donahue WF, et al. Mapping and sequencing of structural variation from eight human genomes. Nature 2008;453:56-64.

20. Naesens M, Sarwal MM. Molecular diagnostics in transplantation. Nat Rev Nephrol 2010;6:614-628.

21. Horgan RP, Kenny LC. 'Omic' technologies: genomics, transcriptomics, proteomics and metabolomics. Obstet Gynaecol 2011;13:189-195.

22. Rhee EP. How omics data can be used in nephrology. Am J Kidney Dis 2018;72:129-135.

23. Baltimore D. Our genome unveiled. Nature 2001;409:814-816.

24. Stapleton CP, Conlon PJ, Phelan PJ. Using omics to explore complications of kidney transplantation. Transpl Int 2018;31:251-262.

25. Mele M, Ferreira PG, Reverter F, et al. Human genomics. The human transcriptome across tissues and individuals. Science 2015;348:660-665.

26. Gunesacar R, Opelz G, Erken E, et al. VEGF 936 C/T gene polymorphism in renal transplant recipients: association of the T allele with good graft outcome. Hum Immunol 2007;68:599-602.

27. Theodorescu D, Mischak H. Mass spectrometry based proteomics in urine biomarker discovery. World J Urol 2007;25:435-443.

28. Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol 2017;18:83.

29. Vlahou A, Fountoulakis M. Proteomic approaches in the search for disease biomarkers. J Chromatogr B Analyt Technol Biomed Life Sci 2005;814:11-19.

30. Wang D, Yang J, Fan J, Chen W, Nikolic-Paterson DJ, Li J. Omics technologies for kidney disease research. Anat Rec (Hoboken) 2020;303:2729-2742.

31. Liu Y, Beyer A, Aebersold R. On the dependency of cellular protein levels on mRNA abundance. Cell 2016;165:535-550.

32. Mann M, Jensen ON. Proteomic analysis of post-translational modifications. Nat Biotechnol 2003;21:255-261.

33. Urbanczyk-Wochniak E, Luedemann A, Kopka J, et al. Parallel analysis of transcript and metabolic profiles: a new approach in systems biology. EMBO Rep 2003;4:989-993.

34. Emwas AHM, Salek RM, Griffin JL, Merzaban J. NMR-based metabolomics in human disease diagnosis: applications, limitations, and recommendations. Metabolomics 2013;9:1048-

1072.

35. Everly MJ, Rebellato LM, Haisch CE, et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. Transplantation 2013;95:410-417.

36. Hourmant M, Cesbron-Gautier A, Terasaki PI, et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. J Am Soc Nephrol 2005;16:2804-2812.

37. Mohan S, Palanisamy A, Tsapepas D, et al. Donor-specific antibodies adversely affect kidney allograft outcomes. J Am Soc Nephrol 2012;23:2061-2071.

38. Loupy A, Jordan SC. Transplantation: donor-specific HLA antibodies and renal allograft failure. Nat Rev Nephrol 2013;9:130-131.

39. Ghisdal L, Baron C, Lebranchu Y, et al. Genome-wide association study of acute renal graft rejection. Am J Transplant 2017;17:201-209.

40. Oetting WS, Schladt DP, Dorr CR, et al. Analysis of 75 candidate SNPs associated with acute rejection in kidney transplant recipients: validation of rs2910164 in microRNA MIR146A. Transplantation 2019;103:1591-1602.

41. Steers NJ, Li Y, Drace Z, et al. Genomic mismatch at LIMS1 locus and kidney allograft rejection. N Engl J Med 2019;380:1918-1928.

42. Dorr CR, Oetting WS, Jacobson PA, Israni AK. Genetics of acute rejection after kidney transplantation. Transpl Int 2018;31:263-277.

43. Israni A, Leduc R, Holmes J, et al. Single-nucleotide polymorphisms, acute rejection, and severity of tubulitis in kidney transplantation, accounting for center-to-center variation. Transplantation 2010;90:1401-1408.

44. Li B, Hartono C, Ding R, et al. Noninvasive diagnosis of renal-allograft rejection by measurement of messenger RNA for perforin and granzyme B in urine. N Engl J Med 2001;344:947-954.

45. Muthukumar T, Dadhania D, Ding R, et al. Messenger RNA for FOXP3 in the urine of renal-allograft recipients. N Engl J Med 2005;353:2342-2351.

46. Suthanthiran M, Schwartz JE, Ding R, et al. Urinary-cell mRNA profile and acute cellular rejection in kidney allografts. N Engl J Med 2013;369:20-31.

47. Friedewald JJ, Kurian SM, Heilman RL, et al. Development and clinical validity of a novel blood-based molecular biomarker for subclinical acute rejection following kidney transplant. Am J Transplant 2019;19:98-109.

48. Roedder S, Sigdel T, Salomonis N, et al. The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study. PLoS Med 2014;11:e1001759.

49. Zhang W, Yi Z, Keung KL, et al. A peripheral blood gene expression signature to diagnose subclinical acute rejection. J Am Soc Nephrol 2019;30:1481-1494.

50. Christakoudi S, Runglall M, Mobillo P, et al. Development of a multivariable gene-expression signature targeting T-cell-mediated rejection in peripheral blood of kidney transplant recipients validated in cross-sectional and longitudinal samples. EBioMedicine 2019;41:571-583.

51. El Fekih R, Hurley J, Tadigotla V, et al. Discovery and validation of a urinary exosome mRNA signature for the diagnosis of human kidney transplant rejection. J Am Soc Nephrol 2021;32:994-1004.

52. Seo JW, Lee YH, Tae DH, et al. Non-invasive diagnosis for acute rejection using urinary mRNA signature reflecting allograft status in kidney transplantation. Front Immunol 2021;12:656632.

53. Anglicheau D, Sharma VK, Ding R, et al. MicroRNA expression profiles predictive of human renal allograft status. Proc Natl Acad Sci U S A 2009;106:5330-5335.

54. Tao J, Yang X, Han Z, et al. Serum microRNA-99a helps detect acute rejection in renal transplantation. Transplant Proc 2015;47:1683-1687.

55. Lorenzen JM, Volkmann I, Fiedler J, et al. Urinary miR-210 as a mediator of acute T-cell mediated rejection in renal allograft recipients. Am J Transplant 2011;11:2221-2227.

56. Vitalone MJ, Sigdel TK, Salomonis N, Sarwal RD, Hsieh SC, Sarwal MM. Transcriptional perturbations in graft rejection. Transplantation 2015;99:1882-1893.

57. O'Riordan E, Orlova TN, Podust VN, et al. Characterization of urinary peptide biomarkers of acute rejection in renal allografts. Am J Transplant 2007;7:930-940.

58. Ling XB, Sigdel TK, Lau K, et al. Integrative urinary peptidomics in renal transplantation identifies biomarkers for acute rejection. J Am Soc Nephrol 2010;21:646-653.

59. Ziegler ME, Chen T, LeBlanc JF, et al. Apolipoprotein A1 and C-terminal fragment of α-1 antichymotrypsin are candidate plasma biomarkers associated with acute renal allograft rejection. Transplantation 2011;92:388-395.

60. Sigdel TK, Salomonis N, Nicora CD, et al. The identification of novel potential injury mechanisms and candidate biomarkers in renal allograft rejection by quantitative proteomics. Mol Cell Proteomics 2014;13:621-631.

61. Lim JH, Lee CH, Kim KY, et al. Novel urinary exosomal

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biomarkers of acute T cell-mediated rejection in kidney transplant recipients: a cross-sectional study. PLoS One 2018;13:e0204204.

62. Blydt-Hansen TD, Sharma A, Gibson IW, Mandal R, Wishart DS. Urinary metabolomics for noninvasive detection of borderline and acute T cell-mediated rejection in children after kidney transplantation. Am J Transplant 2014;14:2339-2349.

63. Blydt-Hansen TD, Sharma A, Gibson IW, et al. Urinary metabolomics for noninvasive detection of antibody-mediated rejection in children after kidney transplantation. Transplantation 2017;101:2553-2561.

64. Sigdel TK, Schroeder AW, Yang J, Sarwal RD, Liberto JM, Sarwal MM. Targeted urine metabolomics for monitoring renal allograft injury and immunosuppression in pediatric patients. J Clin Med 2020;9:2341.

65. Kim SY, Kim BK, Gwon MR, et al. Urinary metabolomic profiling for noninvasive diagnosis of acute T cell-mediated rejection after kidney transplantation. J Chromatogr B Analyt Technol Biomed Life Sci 2019;1118-1119:157-163.

66. O'Connell PJ, Zhang W, Menon MC, et al. Biopsy transcriptome expression profiling to identify kidney transplants at risk of chronic injury: a multicentre, prospective study. Lancet 2016;388:983-993.

67. Li L, Greene I, Readhead B, et al. Novel therapeutics identification for fibrosis in renal allograft using integrative informatics approach. Sci Rep 2017;7:39487.

68. Jung HY, Lee CH, Choi JY, et al. Potential urinary extracellular vesicle protein biomarkers of chronic active antibody-mediated rejection in kidney transplant recipients. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1138:121958.

69. Quaglia M, Merlotti G, Guglielmetti G, Castellano G, Cantaluppi V. Recent advances on biomarkers of early and late kidney graft dysfunction. Int J Mol Sci 2020;21:5404.

70. Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood) 2018;243:213-221.

71. Salvadori M, Tsalouchos A. Biomarkers in renal transplantation: an updated review. World J Transplant 2017;7:161-178.

72. Verhoeven J, Boer K, Van Schaik R, et al. Liquid biopsies to monitor solid organ transplant function: a review of new biomarkers. Ther Drug Monit 2018;40:515-525.

73. Zhang W, Li F, Nie L. Integrating multiple 'omics' analysis for microbial biology: application and methodologies. Microbiology (Reading) 2010;156(Pt 2):287-301.

74. Cao Y, Alexander SI, Chapman JR, Craig JC, Wong G, Yang J. Integrative analysis of prognostic biomarkers for acute rejection in kidney transplant recipients. Transplantation 2021;105:1225-1237.

75. Niel O, Bastard P. Artificial intelligence in nephrology: core concepts, clinical applications, and perspectives. Am J Kidney Dis 2019;74:803-810.

76. Briganti G, Le Moine O. Artificial intelligence in medicine: today and tomorrow. Front Med (Lausanne) 2020;7:27.