Original Article

Impact of differentially expressed genes and proteins in donor arterial plaque on renal function recovery following allogeneic kidney transplantation

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Background: Donor kidney arteriosclerotic plaque is a risk factor for renal allograft functional injury. The aim of this study was to screen, identify, and verify differentially expressed genes (DEGs) and proteins in arteriosclerotic plaques and analyze their correlation with renal transplant function recovery.

Methods: This study included 82 deceased donor allogeneic kidney transplant recipients and analyzed 20 eligible donor arterial plaque specimens. Patients were divided into "no or mild" and "moderate or severe" donor arteriosclerotic plaque groups. The latter group was further divided into impaired and normal kidney function groups. Correlations between target DEGs and proteins and transplanted kidney function recovery were determined using immunofluorescence assay, polymerase chain reaction (PCR), and western blotting.

Results: We identified 40 intersecting DEGs. Matrix metallopeptidase 12 (*MMP12*) and aquaporin 9 (*AQP9*) were identified as target DEGs, with their expression found to be higher in the "moderate or severe" group compared with that in the "no or mild" group. Additionally, *MMP12* and *AQP9* expression was higher in the impaired group than it was in the normal kidney function group. *MMP12* and *AQP9* are associated with transplanted kidney function recovery.

Conclusions: Our study contributes valuable information to the development of clinical strategies for kidney transplant maintenance.

Keywords: Arteriosclerotic plaque; allogeneic kidney transplantation; kidney function; differentially expressed gene (DEG)

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Introduction

Background

Renal allograft transplantation is considered the best treatment strategy for patients with end-stage renal disease. Recent studies have shown that renal allograft transplantation increases life expectancy in patients with renal failure compared with that in patients undergoing long-term dialysis (1,2). In recent years, immunosuppressive regimens, organ quality, and allocation policies have changed (3,4). Owing to the general shortage of organ donors to facilitate allogeneic kidney transplantation, kidneys from older donors (≥65 years old) and donors with comorbidities have been transplanted into recipients (5). In particular, the proportion of expanded criteria donor (ECD) kidneys has increased considerably over the past decade (6). However, expanding the standard donor criteria is associated with adverse outcomes, such as impaired graft function after allogeneic kidney transplantation (7), psychological effects on patients, and an immense social and economic burden on the healthcare system (8). Macroscopic arteriosclerosis of the renal arteries of ECD kidneys is likely to occur, and arteriosclerotic plaques of transplanted renal arteries not only impose technical challenges in establishing arterial anastomosis in the recipients, but also affect the allograft function of the transplanted kidneys (9). However, not all patients with macroscopic renal arteriosclerotic plaques exhibit impaired graft function after surgery.

Highlight box

Key findings

- Matrix metallopeptidase 12 (MMP12) and aquaporin 9 (AQP9) were identified as critical biomarkers in donor arterial plaques, showing strong associations with impaired renal function recovery after kidney transplantation.

What is known and what is new?

- Not all patients with macroscopic renal arteriosclerotic plaques exhibit impaired graft function after surgery.
- This study is the first to link specific differentially expressed genes and proteins in donor arterial plaques to transplant outcomes, providing novel insights beyond traditional visual assessments.

What is the implication, and what should change now?

- MMP12 and AQP9 may be used as molecular markers for donor selection and post-transplant management, potentially guiding targeted interventions to improve graft function.

Rationale and knowledge gap

To our knowledge, no study has explored the gene involved in the formation of arteriosclerotic plaques of transplanted kidneys that may affect transplanted kidney function. Transcriptomics can be used to explore specific genes that lead to impaired graft function due to graft arteriosclerotic plaques and identify relevant genes to provide novel biomarkers for improving transplant management.

Objective

Considering the lack of data on genes related to arteriosclerotic plaque formation in the context of kidney function post-transplant, we aimed to integrate data on arteriosclerotic plaques and normal vascular tissue from the Gene Expression Omnibus (GEO) database to identify differentially expressed genes (DEGs) and verify these data using samples from our center, combined with graft function recovery follow-up 1 year after allogeneic kidney transplantation. Clinical data were used to identify DEGs in renal arteriosclerotic plaques that may functionally impair transplanted kidneys after allogeneic kidney transplantation. We present this article in accordance with the MDAR reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-2024-736/rc).

Methods

Data collection

RNA sequencing data of atherosclerotic plaque and normal artery samples were obtained from the GSE100927 and GSE43292 datasets from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). We retrospectively collected data regarding allograft kidney transplants performed at Beijing Hospital between June 2019 and June 2021. In addition, we obtained the transplanted renal artery specimens of the donors, and clinical data of the donors and recipients for further screening and analysis. All study protocols were approved by the Research Ethics Committee of Beijing Hospital (No. 2024BJYYEC-KY006-01) and informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The inclusion criteria were as follows: (I) aged ≥18 years; and (II) recipients who had completed a 1-year follow-up and had creatinine recovery data. The exclusion criteria were as follows: (I) those who underwent combined transplantation; (II) immune rejection occurred

during follow-up; and (III) infections such as BK CMV occurred during follow-up. Immune rejection and infection are common complications after renal transplantation that directly affect the functional recovery of the transplanted kidney. Excluding the above factors can prevent confusing results. Samples were collected immediately after surgical resection, snap-frozen in liquid nitrogen, and stored at –80 ℃ until RNA extraction. Fresh samples were subjected to immunofluorescence, quantitative real-time polymerase chain reaction (qPCR), and western blotting. Baseline data of the donor and recipient, organ preservation demographic variables, and relevant post-transplant outcome measures were collected. Donor renal arteriosclerotic plaques visible to the naked eye were divided into "no or mild" and "moderate or severe" donor arteriosclerotic plaque groups. In this study, three senior urological surgeons participated in the assessment and grading of donor renal artery plaque severity. All physicians had extensive clinical experience and reached a consensus on the evaluation criteria before the study commenced to ensure consistency and reproducibility. Macroscopic assessment was performed immediately after donor nephrectomy. The renal artery was examined, and arteriosclerotic plaques were classified into "no or mild" and "moderate or severe" groups based on plaque thickness, extent, and texture. The no or mild arteriosclerotic plaque group was characterized by thin plaques (<25% arterial lumen stenosis) with largely intact tissue structure, whereas the moderate or severe arteriosclerotic plaque group exhibited significantly thickened plaques (≥25% arterial lumen stenosis) accompanied by pathological features such as fibrosis and calcification. To minimize confounding variables, strict recipient selection criteria were applied, ensuring that only transplant recipients without significant iliac vascular atherosclerosis were included, thereby eliminating the potential influence of recipient vascular factors on post-transplant renal function recovery. According to the estimated glomerular filtration rate (eGFR) of the recipients 1 year after allogeneic kidney transplantation (cutoff 50 mL/min/1.73 m^2) (9,10), the "moderate or severe" donor arteriosclerotic plaque group was further divided into a plaque group with impaired graft renal function (eGFR <50 mL/min/1.73 m^2) and another with normal transplanted kidney function (eGFR ≥50 mL/min/1.73 m^2). *Figure 1* shows the experimental procedure. The baseline immunosuppressive regimens included calcineurin inhibitors (tacrolimus and cyclosporine), antimetabolites (mycophenolate mofetil and azathioprine), and steroids. In this study, all patients received standard induction therapy with basiliximab and methylprednisolone. Dosages of tacrolimus and cyclosporine were adjusted based on therapeutic drug monitoring to balance immunosuppression and drug toxicity post-kidney transplantation. Blood levels of tacrolimus and cyclosporine were measured. Tacrolimus was initiated at 0.05–0.15 mg/kg/day, administered orally in two divided doses (every 12 hours), with target trough concentrations (C_0) of 8–12 ng/mL during postoperative months 0–1, 6–10 ng/mL during months 1–3, 5–10 ng/mL during months 4–12, and 5–8 ng/mL beyond 1 year. Cyclosporine was initiated at 4.0–6.0 mg/kg/day, administered twice daily, with therapeutic monitoring using either trough concentration (C_0) or 2-hour post-dose concentration (C_2). The target levels were C_0 150–300 ng/mL (C_2 1,000–1,500 ng/mL) during months 0–1, C_0 150–250 ng/mL (C_2 800–1,200 ng/mL) during months 1–3, C_0 120–250 ng/mL (C_2 600–1,000 ng/mL) during months 4–12, and C_0 80–150 ng/mL (C_2 >400 ng/mL) beyond 1 year. The specific dosage regimen was dynamically adjusted based on individual patient conditions, therapeutic drug monitoring results, and clinical presentation to optimize long-term graft survival.

Univariate and multivariate analysis

The donor age, sex, body mass index (BMI), diabetes status, hypertension status, cause of death, pump variables (flow and resistance), and cold ischemia time, as well as recipient age, sex, BMI, diabetes status, creatinine before kidney procurement, dialysis time, human leukocyte antigen (HLA) mismatches, panel reactive antibody (PRA), and hypertension status were included in the univariate analysis. The outcome measure was defined as impaired graft function (eGFR <50 mL/min/1.73 m^2). Covariates with P<0.05, as determined using univariate analysis, were included in the multivariate analysis. P<0.05 was considered to indicate a significant difference.

Screening of DEGs and intersecting DEGs

The data in the GSE100927 and GSE43292 datasets were normalized using the "limma" package in R to identify DEGs between atherosclerotic plaque and normal artery samples. The filtering conditions were false discovery rate <0.05 and $|\log_2 FC| \geq 1.0$, where FC is the fold change between the groups. Subsequently, the GSE100927 and GSE43292 datasets were combined to identify intersecting DEGs, and the R package VennDiagram was used to

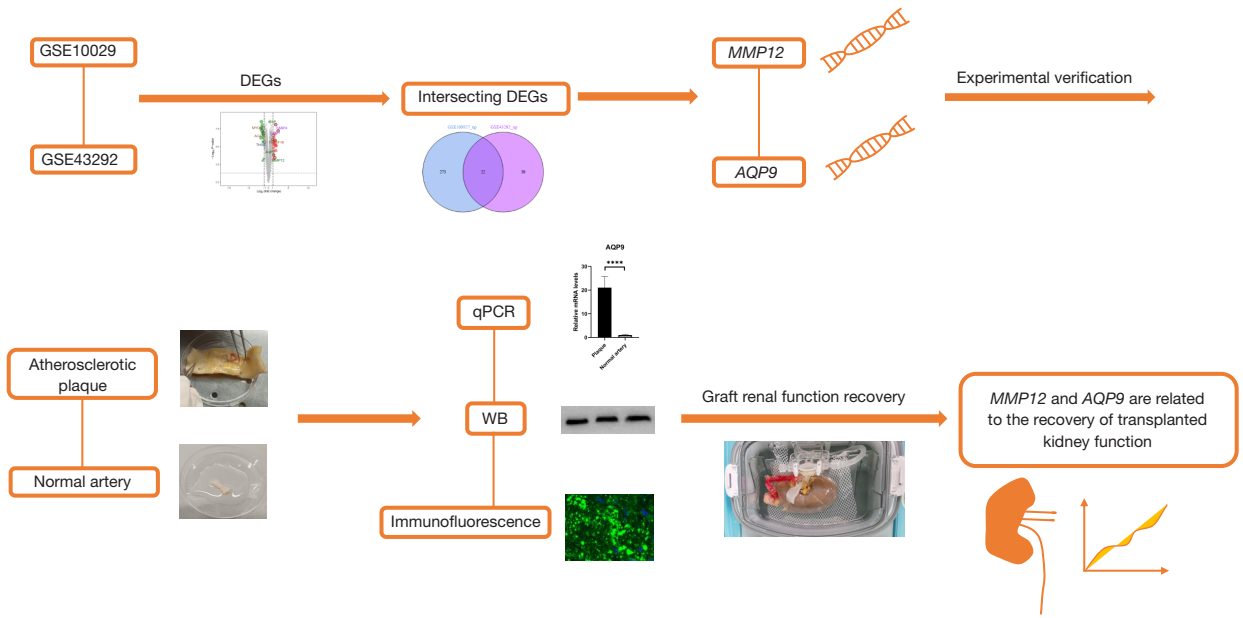

Figure 1 Experimental procedure. ****, P<0.0001. DEGs, differentially expressed genes; *MMP12*, matrix metallopeptidase 12; *AQP9*, aquaporin 9; qPCR, quantitative real-time polymerase chain reaction; WB, western blot.

visualize them.

Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses

The "clusterprofiler" package in R was used to conduct GO term (biological process, molecular function, and cellular component) and KEGG pathway enrichment analyses of the intersecting DEGs. Significance was defined as P<0.05, and the data are presented graphically.

Immunofluorescence

The specimens from the "no or mild" and "moderate or severe" donor arteriosclerosis groups were paraffin-embedded, fixed, and sectioned. The prepared paraffin-embedded tissue sections were dried in an oven (37 ℃), followed by deparaffinization in 100% xylene three times (10 minutes each) and gradient ethanol rehydration using 100% ethanol (3 minutes, twice), 95% ethanol (3 minutes, twice), 80% ethanol (3 minutes), and 70% ethanol (3 minutes). After washing with phosphate buffered saline (PBS) three times (5 minutes each) to remove excess ethanol, antigen retrieval was performed by placing the sections in sodium citrate antigen retrieval buffer, heating them in a microwave for 15 minutes, allowing them to cool to room temperature, and performing another PBS wash (three times, 5 minutes each). Blocking was carried out in a humidified chamber using goat serum blocking solution at 37 ℃ for 1 hour, after which the sections were incubated overnight at 4 ℃ primary antibody of AQP9 (Santa Cruz Biotechnology, Danvers, USA) and MMP12 (ab314017). The next day, sections were washed with 1× PBS (three times, 5 minutes each), then incubated with an appropriate fluorescent secondary antibody (1:300 dilution) at 37 ℃ for 1 hour in the dark. After removing the secondary antibody solution [Cell Signaling Technology (CST), Danvers, USA], the sections were washed again with 1× PBS (three times, 5 minutes each) under light-protected conditions. For multiplex fluorescence labeling, steps 5–9 were repeated to incubate with a second primary and secondary antibody. Finally, the sections were mounted using fluorescent mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) and stored in the dark to preserve fluorescence. The sections were observed under a fluorescence microscope (Polaris multispectral tissue imaging quantitative analysis system).

qPCR

Tissue samples from the "no or mild" and "moderate or severe" donor arterial plaque groups were collected. Total RNA was subsequently extracted using the Tissue RNA Purification Kit Plus (ESscience Biotech, Shanghai, China). RNA concentration and purity were assessed using the NanoDrop® ND-1000 spectrophotometer (Thermo Fisher, Shanghai, China). Total RNA was reverse transcribed to cDNA, and the expression of each gene was detected using qPCR with cDNA as a template. For polymerase chain reaction (PCR) amplification of targeted and housekeeping genes, a cDNA template known to express the target gene was selected. The PCR reaction mixture consisted of 5 µL 2× Master Mix (Yeasen Biotech Co., Shanghai, China), 0.5 µL 10 µM forward specific primer, 0.5 µL 10 µM reverse specific primer, and 2 µL cDNA, with water added to a final volume of 10 µL. The mixture was briefly centrifuged (5,000 rpm) and subjected to PCR with an initial denaturation at 95 ℃ for 10 minutes, followed by 40 cycles of 95 ℃ for 10 seconds and 60 ℃ for 60 seconds (fluorescence collection). The PCR products were then analyzed using 2% agarose gel electrophoresis with ethidium bromide staining with a 100 bp DNA ladder to verify the presence of a single specific amplification band. Subsequently, the PCR products were diluted in a 10-fold gradient, with concentrations ranging from 1× (undiluted) to 1×10^{-9}. For real-time PCR, all cDNA samples were prepared with a reaction system containing 5 µL 2× Master Mix, 0.5 µL 10 µM forward specific primer, 0.5 µL 10 µM reverse specific primer, and water up to a total volume of 8 µL. The mixture was briefly centrifuged (5,000 rpm) before sample loading. In a 384-well PCR plate, 8 µL of the reaction mix was added to each well, followed by 2 µL cDNA, then sealed with Sealing Film (YuanMu Bio-Technology Co., Shanghai, China), briefly centrifuged, and placed on ice before PCR setup. The plate was then loaded onto the real-time PCR machine for amplification with the following conditions: 95 ℃ for 10 minutes, followed by 40 cycles of 95 ℃ for 10 seconds and 60 ℃ for 60 seconds (fluorescence collection). To generate a melting curve, the reaction was extended post-amplification at 95 ℃ for 10 seconds, 60 ℃ for 60 seconds, 95 ℃ for 15 seconds, and a gradual temperature increase from 60 to 99 ℃. The following primers were used: aquaporin 9 (*AQP9*) forward, 5'-CTCTCTGAGTTCTTGGGCACG-3'; *AQP9* reverse, 5'-CCCAGGGAGGAAGCAATGAC-3'; matrix metallopeptidase 12 (*MMP12*) forward, 5'-GATGTGGAGTCCCCGATGTC-3'; *MMP12* reverse, 5'-GGATTTGGCAAGCGTTGGTT-3'; *GAPDH* forward, 5'-GACAGTCAGCCGCATCTTCT-3'; and *GAPDH* reverse, 5'-GCGCCCAATACGACCAAAT-3'.

Western blotting

Pre-cooled radioimmunoprecipitation (RIPA) protein extraction reagent was used to extract the proteins from tissues, and the supernatant was used for protein quantification using the bicinchoninic acid method. RIPA lysis buffer was used to adjust the protein concentration. An 8% separating gel and 5% stacking gel were prepared according to the molecular weight of the target protein. The protein samples were loaded, electrophoresed, transferred onto a membrane, and blocked. The primary and secondary antibodies were then incubated with the membrane and reacted with the ECL reagent (Millipore, Shanghai, China). Finally, data analysis was performed using the Gel Image System ver.4.00 (Tanon, China).

Surgical procedure

In this study, we employed the following standardized donor kidney transplantation surgical techniques to optimize hemodynamics and functional recovery of the transplanted kidney. (I) Donor kidney procurement and processing: the donor kidney was retrieved by an experienced organ retrieval team at the donor hospital using rapid organ perfusion techniques, with University of Wisconsin solution as the perfusion fluid. During the procedure, the arterial condition of the donor kidney was assessed, and arteriosclerotic plaques were graded. The donor kidney was transported in cold preservation solution to the transplant center, where, prior to surgery, arterial reconstruction was performed under an ice bath (e.g., branch vessel suturing or intimal dissection). (II) Recipient preparation and vascular anastomosis: preoperative abdominal imaging [e.g., computed tomography (CT) or ultrasound] was performed to assess the vascular conditions of the recipient and to avoid severe arteriosclerosis that could impair the blood supply to the transplanted kidney. The kidney was typically implanted in the right iliac fossa, with the external iliac artery used as the blood supply artery and the iliac vein as the drainage vein. The arterial anastomosis was performed using end-to-side anastomosis, while venous anastomosis was performed using end-to-side anastomosis, with continuous suturing using 6-0 or 7-0 Prolene sutures (Johnson & Johnson, Shanghai, China). The ureteroneocystostomy

Table 1 Demographic and clinical characteristics of transplant donors and recipients

Demographic and clinical characteristics	No/mild	Moderate/severe	P
Donor demographics			
Age (years)	46.0±10.9	54.6±11.2	0.002
Sex			0.59
Female	5 (8.6)	3 (12.5)	
Male	53 (91.4)	21 (87.5)	
BMI (kg/m^2)	23.7±3.4	24.7±4.5	0.25
Diabetes mellitus	4 (6.9)	6 (25.0)	0.02
Hypertension	18 (31.0)	15 (62.5)	0.008
Cause of death			0.75
Intracranial bleeding	39 (67.2)	17 (70.8)	
Trauma	19 (32.8)	7 (29.2)	
Creatinine (μmol/L)	48 (37, 61.8)	62 (32.2, 77.8)	0.57
Pump variable			
Flow (mL/h)	114.5±29.1	89.3±22.3	<0.001
Resistance	0.27 (0.21, 0.31)	0.35 (0.26, 0.41)	0.001
Recipient demographics			
Age (years)	47.3±9.8	48.9±11.6	0.51
Sex			0.11
Female	10 (17.2)	8 (33.3)	
Male	48 (82.8)	16 (66.7)	
BMI (kg/m^2)	23.7±2.7	23.2±4.0	0.50
Diabetes mellitus	14 (24.1)	4 (16.7)	0.46
Hypertension	55 (94.8)	20 (83.3)	0.09
Cold ischemia time (hours)	5.4 (4.5, 7.4)	6.0 (5.1, 8.9)	0.19
Dialysis time (months)	19.2 (5.75, 24)	27.0 (6, 45)	0.19
HLA mismatches	2.4 (2, 3)	2.5 (2, 3)	0.47

Data are presented as mean ± standard deviation, n (%), or median (IQR). BMI, body mass index; HLA, human leukocyte antigens; IQR, interquartile range.

was performed using the Lich-Gregoir technique for anti-reflux, and a double J stent (BARD, Shanghai, China) was placed for drainage. A total of four urological surgeons participated in the kidney transplantation procedure and strictly followed the standardized surgical protocol to ensure consistency and reproducibility.

Statistical analysis

Normally distributed data are expressed as the mean ± standard deviation, and comparisons between groups were performed using the paired *t*-test. Non-normal data are expressed as the median [interquartile range (IQR)], and comparisons between groups were performed using the Wilcoxon rank sum test. Unordered variables were compared using the Chi-squared test. First, univariate logistic analysis was performed on all included factors to identify potential predictors. Variables demonstrating statistically significant associations (P<0.05) in the univariate analysis were then selected as candidate variables. Second, these significant variables were subsequently incorporated into a multivariate logistic regression model to assess their independent effects. Results with P<0.05 were considered statistically significant.

Results

Demographic characteristics

A total of 82 renal allograft recipients were included in this study, and qualified specimens were collected from 20 patients for experimental analysis. *Table 1* summarizes the recipient and donor characteristics. All recipients received their first renal allografts. The average age of donors was 46.0±10.9 years in the no/mild group and 54.6±11.2 years in the moderate/severe group, while the average age of recipients was 47.3±9.8 and 48.9±11.6 years, respectively. Male donors accounted for 91.4% and 87.5% of the no/mild and moderate/severe groups, respectively, while male recipients accounted for 82.8% and 66.7%. The donor and recipient BMIs were 23.7±3.4 and 23.7±2.7 kg/m^2 in the no/mild group and 24.7±4.5 and 23.2±4.0 kg/m^2 in the moderate/severe group, respectively. Brain death among donors was primarily caused by intracranial bleeding, occurring in 67.2% of the no/mild group and 70.8% of the moderate/severe group. Among the donors, 31.0% in the no/mild group and 62.5% in the moderate/severe group had hypertension, while 6.9% and 25.0% had diabetes. Among the recipients, 94.8% in the no/mild group and 83.3% in the moderate/severe group had hypertension, while 24.1% and 16.7% had diabetes. The median cold ischemia time was 5.4 (IQR, 4.5–7.4) hours in the no/mild group and 6.0 (IQR, 5.1–8.9) hours in the moderate/severe group. Median

Table 2 Univariate and multivariate Cox proportional hazards analysis of graft renal function based on baseline histology and donor demographic variables

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Donor demographics				
Age	1.081 (1.013–1.152)	0.02	0.976 (0.884–1.077)	0.63
Sex				
Female	Reference			
Male	0.469 (0.083–2.653)	0.39		
BMI	0.951 (0.799–1.131)	0.57		
Diabetes mellitus	31.267 (6.130–159.483)	<0.001	13.768 (1.799–105.339)	0.01
Hypertension	24.000 (2.914–197.640)	0.003	8.526 (0.705–103.121)	0.09
Cause of death				
Intracranial bleeding	Reference			
Trauma	0.681 (0.168–2.758)	0.59	–	–
Arterial plaque	11.000 (2.643–45.779)	0.001	10.359 (1.352–79.391)	0.02
Creatinine	1.006 (0.999–1.012)	0.07		
Pump variable				
Flow (mL/h)	1.000 (0.979–1.021)	0.98		
Resistance	0.361 (0.001–249.138)	0.76		
Recipient demographics				
Age	0.976 (0.920–1.036)	0.43		
Sex				
Female	Reference			
Male	0.319 (0.087–1.167)	0.08		
BMI	0.927 (0.758–1.134)	0.46		
Diabetes mellitus	1.222 (0.294–5.087)	0.78		
Hypertension	0.385 (0.066–2.258)	0.29		
Cold ischemia time (hours)	1.119 (0.904–1.385)	0.30		
Dialysis time (months)	1.009 (0.988–1.029)	0.41		
HLA mismatches	1.005 (0.408–2.476)	>0.99		

HR, hazard ratio; CI, confidence interval; BMI, body mass index; HLA, human leukocyte antigen.

donor kidney perfusion flow was 114.5±29.1 mL/h in the no/mild group and 89.3±22.3 mL/h in the moderate/severe group, while median donor kidney perfusion resistance was 0.27 (IQR, 0.21–0.31) and 0.35 (IQR, 0.26–0.41), respectively. Median recipient dialysis duration was 19.2 (IQR, 5.75–24) months in the no/mild group and 27.0 (IQR, 6–45) months in the moderate/severe group, and the median number of HLA mismatches was 2.4 (IQR, 2–3) and 2.5 (IQR, 2–3), respectively. All recipients had negative PRA.

Relationship between clinical and histological characteristics and graft renal function recovery

In the univariate Cox proportional hazards analysis, diabetes mellitus in donors [hazard ratio (HR) =31.267, 95% confidence interval (CI): 6.130–159.483, P<0.001], older donors (HR =1.081, 95% CI: 1.013–1.152, P=0.02) was associated with atherosclerotic plaques (HR =11.000, 95% CI: 2.643–45.779, P=0.001), and hypertension in

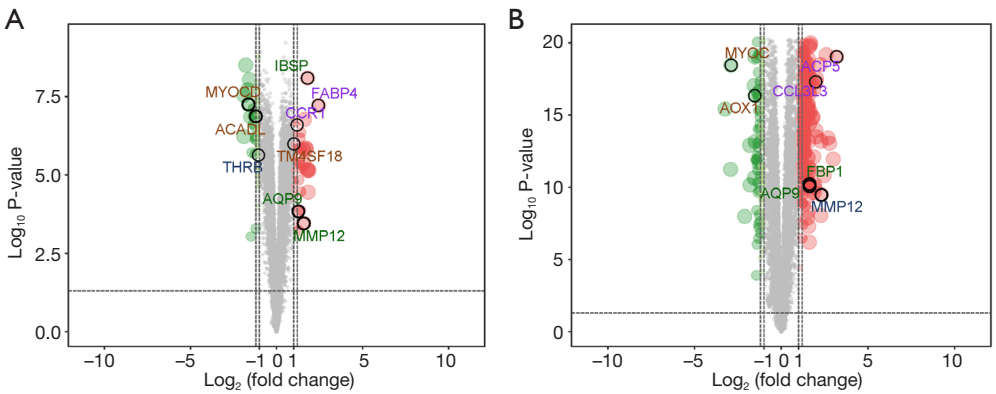

Figure 2 Determination of DEGs. Volcano plots for (A) GSE43292 and (B) GSE100927. DEGs, differentially expressed genes.

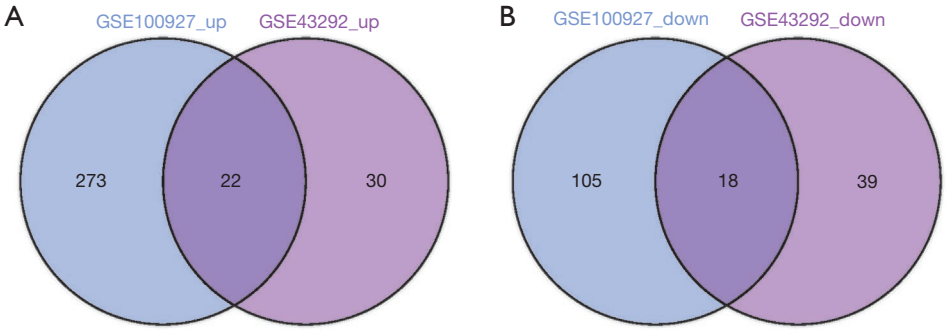

Figure 3 Determination of intersecting DEGs. Venn diagrams of (A) upregulated and (B) downregulated DEGs. DEGs, differentially expressed genes.

donors (HR =24.000, 95% CI: 2.914–197.640, P=0.003) was an important predictor of impaired renal allograft function (*Table 2*). No other donor or recipient factors were associated with impaired graft function.

In the multivariate Cox proportional hazards analysis, only diabetes mellitus (HR =13.768, 95% CI: 1.799–105.339, P=0.01) with atherosclerotic plaques (HR =10.359, 95% CI: 1.352–79.391, P=0.02) in donors was an important predictor of impaired renal allograft function (*Table 2*).

Determination of DEGs and intersecting DEGs

Using $|\log_2 FC| \geq 1.0$ and adj P<0.05 as the standards, a total of 418 and 109 DEGs were identified in the GSE100927 and GSE43292 datasets, respectively (*Figure 2A*,*2B*). As shown in *Figure 3* and Table S1, 40 intersecting DEGs, including *MMP12* and *AQP9*, were identified between the two datasets. A literature search revealed that the expression of *MMP12* and *AQP9* could affect the recovery of renal function, which may be related to the recovery of renal function after allogeneic kidney transplantation. These two genes were selected for further analysis.

GO term and KEGG pathway enrichment analyses

GO term analysis showed that these intersecting genes could be divided into several basic biological processes, including regulation of smooth muscle cell proliferation and sarcomere, and enzyme-related activities (*Figure 4A*). KEGG pathway analysis showed that the 40 intersecting genes are mainly related to the regulation of smooth muscle cell proliferation, extracellular matrix disassembly, and transport signaling pathways (*Figure 4B*).

Immunofluorescence

To further study the expression levels of genes in “no or mild” and “moderate or severe” donor arteriosclerosis

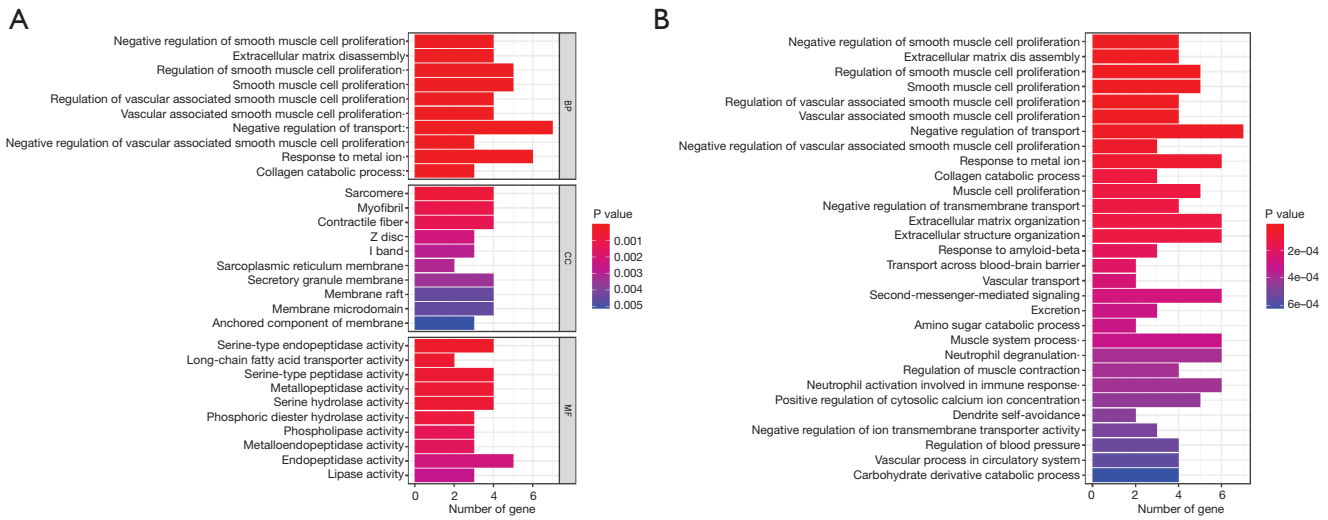

Figure 4 GO and KEGG analyses. Enriched GO terms (A) and KEGG pathway analyses (B) among the DEGs. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function; DEGs, differentially expressed genes.

tissues, we collected 10 samples of "no or mild" and 10 samples of "moderate or severe" donor arteriosclerosis plaque tissues. The expression of *MMP12* and *AQP9* in "moderate or severe" donor arteriosclerosis plaque tissue was significantly increased compared with that in "no or mild" donor arteriosclerosis plaque tissue (*Figure 5*). Concurrently, the immunofluorescence results showed that there were differences in the expression of *MMP12* and *AQP9* between the plaque group with impaired kidney function and that with normal kidney function.

qPCR

The expression of *MMP12* and *AQP9* in "moderate or severe" donor arteriosclerosis plaque tissue was significantly higher than that in "no or mild" donor arteriosclerosis plaque tissue (P<0.001; *Figure 6*). These two genes were more highly expressed in the plaque group with impaired kidney function than they were in the plaque group with normal kidney function (P=0.02 and P=0.02, respectively; *Figure 6*).

Western blotting

The expression of *MMP12* and *AQP9* in "moderate or severe" donor arteriosclerosis plaque tissue was significantly higher than that in "no or mild" donor arteriosclerosis plaque tissue (*Figure 7*). Similar to the immunofluorescence results, there were differences in the levels of the two proteins between the plaque group with impaired kidney function and that with normal kidney function (*Figure 7*).

Discussion

Kidney transplantation exhibits significant advantages over dialysis in multiple clinically relevant outcomes. Studies have shown that over time, the relative benefit of kidney transplantation in reducing mortality risk compared to dialysis increases further. Data indicate that the relative risk of death for kidney transplantation in 1985 was 0.44, whereas by 2005, this value had decreased to 0.17, suggesting continuous improvement in the long-term outcomes of kidney transplantation. The research also found that kidney transplantation not only significantly reduces the occurrence of cardiovascular events but also substantially improves patients' quality of life, with notable benefits in both physical and mental health. These findings further support kidney transplantation as the preferred treatment option for end-stage renal disease (11). Although ECD kidneys have been used to alleviate the shortage of deceased donor organs in clinical practice, expanding the use of standard donor organs inevitably results in complications (12).

Kon *et al.* showed that the formation of renal arteriosclerotic plaques can lead to the progression of chronic kidney disease and that it indicates the involvement

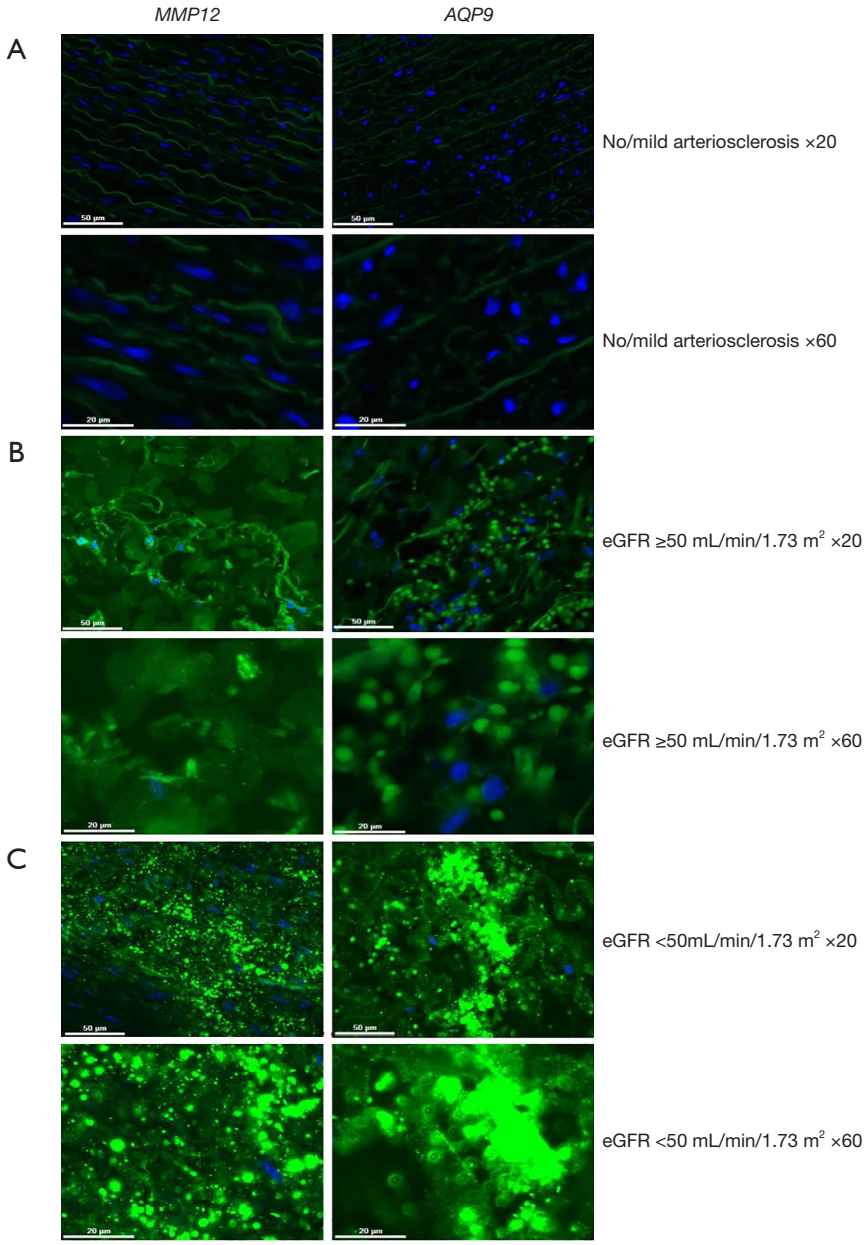

Figure 5 Immunofluorescence analysis. Expression of *MMP12* and *AQP9* in no/mild arteriosclerosis (A), the moderate/severe arteriosclerotic plaque group with normal renal transplant function (B), and the arterial plaque group with impaired renal transplant function (C). Blue, DAPI for nuclei; green, MMP12/AQP9 expression. *MMP12*, matrix metallopeptidase 12; *AQP9*, aquaporin 9; DAPI, 4',6-diamidino-2-phenylindole; eGFR, estimated glomerular filtration rate.

of renal microvessels (13). In addition, some studies have shown that the presence of atherosclerotic plaques in renal arteries is associated with the worsening of chronic kidney disease and microalbuminuria (14-16). These findings suggest that the presence of large-vessel atherosclerotic plaques may be an indicator of chronic kidney injury, which may contribute to poor long-term renal allograft function. By mining the GEO database and verifying our samples, this study confirmed that two DEGs in arteriosclerotic plaques are related to the recovery of transplanted kidney function.

MMP12 belongs to the MMP family, which consists of

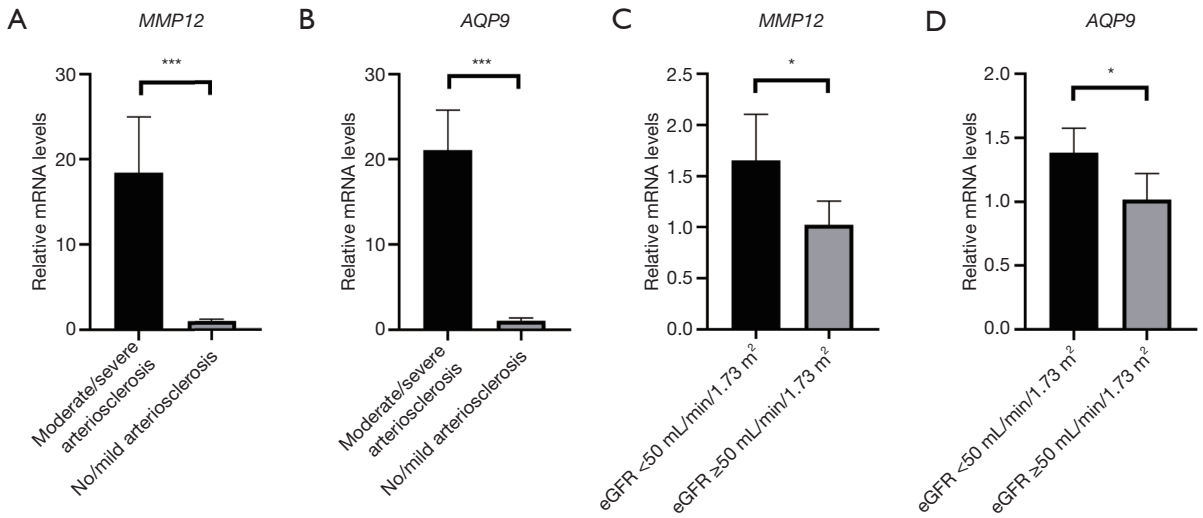

Figure 6 qPCR analysis of *MMP12* and *AQP9* mRNA levels in no/mild arteriosclerosis and moderate/severe arteriosclerotic plaque. (A) Expression of *MMP12* in no/mild arteriosclerosis and moderate/severe arteriosclerotic plaque. (B) Expression of *AQP9* in no/mild arteriosclerosis and moderate/severe arteriosclerotic plaque. (C) *MMP12* expression in the moderate/severe arteriosclerotic plaque groups with normal and impaired renal graft function. (D) *AQP9* expression in the moderate/severe arteriosclerotic plaque groups with normal and impaired renal graft function. *, P<0.05; ***, P<0.001. *MMP12*, matrix metallopeptidase 12; *AQP9*, aquaporin 9; qPCR, quantitative real-time polymerase chain reaction; eGFR, estimated glomerular filtration rate.

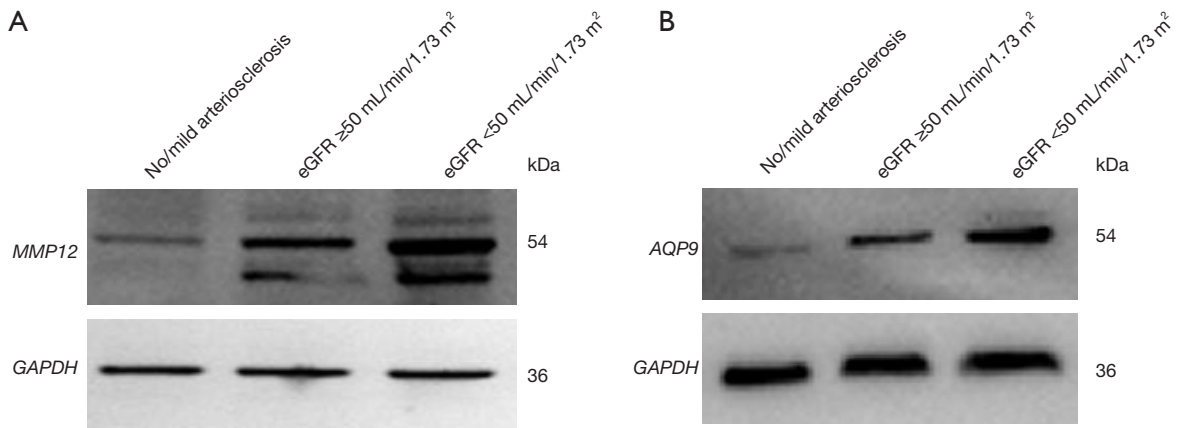

Figure 7 Western blot analysis. Expression of *MMP12* (A) and *AQP9* (B) in no/mild arteriosclerosis, the moderate/severe arteriosclerotic plaque group with normal renal transplant function, and the moderate/severe arteriosclerotic plaque with impaired renal transplant function. *MMP12*, matrix metallopeptidase 12; *AQP9*, aquaporin 9; eGFR, estimated glomerular filtration rate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

23 zinc-dependent endopeptidases that share a common multidomain structure (17). MMPs are involved in extracellular matrix degradation and renewal, and tissue remodeling (18). MMPs have been shown to play key roles in various biological processes, including metabolism and inflammation (19,20). *MMP12* is produced by macrophages and plays an important role in the development and instability of atherosclerotic plaques (21). Another study demonstrated that genetic deletion of *MMP12* may improve low-grade systemic inflammation and reduce the development of atherosclerotic plaques (22). *MMP12* is involved in MMP-mediated macrophage invasion, leading

to tissue damage and the rupture of atherosclerotic plaques (23). Niu *et al.* found that *MMP12* produced by macrophages infiltrating the glomeruli may be the main factor causing glomerular fibrosis, injury, and inflammation (24). Some studies have also indicated that the potential mechanism of renal pathology may involve macrophage infiltration and the subsequent expression of *MMP12* in the glomerular compartment, resulting in decreased renal function (25,26).

AQPs are a family of membrane water channels responsible for the transport of water and small solutes such as glycerol, gases, and ions (27). *AQP9* is a member of the AQP family and serves as a water-selective membrane channel (28). Recent research has shown that *AQP9* is primarily expressed in human hepatocytes and neutrophils. In addition to regulating cell migration and the inflammatory response, it regulates lipid uptake and energy metabolism (29). Zhang *et al.* conducted a biological analysis of 220 samples and showed that *AQP9* is involved in atherosclerotic plaque formation and can be used as a marker for acute myocardial infarction (30). Animal and cellular experiments have also shown that *AQP9* is involved in the development of atherosclerotic plaques (31). However, *AQP9* has not been extensively studied in the context of kidney disease. Studies have shown that *AQP9* is a potential prognostic gene in patients with clear-cell renal cell carcinoma. Concurrently, *AQP9* may play a key role in tumor microenvironment state transition through lipid metabolism and the P53 and JAK/STAT pathways, which affect M2 macrophage polarization (32). Notably, RG100204, a novel, potent, and selective *AQP9* inhibitor, attenuated the increase in serum creatinine levels caused by sepsis in a mouse model of polymicrobial infection, and significantly alleviated the risk related to sepsis-associated renal dysfunction (33). Our results show that *AQP9* can affect the recovery of renal function after transplantation; however, the underlying mechanism requires further investigation.

This study had some limitations. First, this was a retrospective, single-center study with a limited number of patients. Therefore, studies on graft function using larger samples are required to confirm our findings. Second, we used follow-up data on graft kidney function for only 1 year after transplantation; long-term follow-up data are needed to verify our results. Third, we failed to elucidate the mechanism by which the DEGs impair transplanted kidney function, and more in-depth research is needed.

Conclusions

MMP12 and *AQP9* are DEGs in arteriosclerotic plaques in transplanted kidneys that can affect the functional recovery of transplanted kidneys after allogeneic transplantation. Based on this conclusion, further in-depth research should be conducted to improve the safety and quality of allogeneic kidney transplantation. In addition, a potential strategy for predicting the outcomes of allogeneic kidney transplantation has been proposed.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-2024-736/rc

Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-2024-736/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-2024-736/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All study protocols were approved by the Research Ethics Committee of Beijing Hospital (No. 2024BJYYEC-KY006-01) and informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Lenain R, Boucquemont J, Leffondré K, et al. Clinical Trial Emulation by Matching Time-dependent Propensity Scores: The Example of Estimating Impact of Kidney Transplantation. Epidemiology 2021;32:220-9.
2. Strohmaier S, Wallisch C, Kammer M, et al. Survival Benefit of First Single-Organ Deceased Donor Kidney Transplantation Compared With Long-term Dialysis Across Ages in Transplant-Eligible Patients With Kidney Failure. JAMA Netw Open 2022;5:e2234971.
3. Lehner LJ, Kleinsteuber A, Halleck F, et al. Assessment of the Kidney Donor Profile Index in a European cohort. Nephrol Dial Transplant 2018;33:1465-72.
4. Mannon RB. Delayed Graft Function: The AKI of Kidney Transplantation. Nephron 2018;140:94-8.
5. Süsal C, Kumru G, Döhler B, et al. Should kidney allografts from old donors be allocated only to old recipients? Transpl Int 2020;33:849-57.
6. Schrezenmeier E, Müller M, Friedersdorff F, et al. Evaluation of severity of delayed graft function in kidney transplant recipients. Nephrol Dial Transplant 2022;37:973-81.
7. Stewart DE, Foutz J, Kamal L, et al. The Independent Effects of Procurement Biopsy Findings on 10-Year Outcomes of Extended Criteria Donor Kidney Transplants. Kidney Int Rep 2022;7:1850-65.
8. Mezzolla V, Pontrelli P, Fiorentino M, et al. Emerging biomarkers of delayed graft function in kidney transplantation. Transplant Rev (Orlando) 2021;35:100629.
9. Pascual J, Berger SP, Witzke O, et al. Everolimus with Reduced Calcineurin Inhibitor Exposure in Renal Transplantation. J Am Soc Nephrol 2018;29:1979-91.
10. Zhi R, Zhang XD, Hou Y, et al. RtNet: a deep hybrid neural network for the identification of acute rejection and chronic allograft nephropathy after renal transplantation using multiparametric MRI. Nephrol Dial Transplant 2022;37:2581-90.
11. Tonelli M, Wiebe N, Knoll G, et al. Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes. Am J Transplant 2011;11:2093-109.
12. Metzger RA, Delmonico FL, Feng S, et al. Expanded criteria donors for kidney transplantation. Am J Transplant 2003;3 Suppl 4:114-25.
13. Kon V, Linton MF, Fazio S. Atherosclerosis in chronic kidney disease: the role of macrophages. Nat Rev Nephrol 2011;7:45-54.
14. Vashishtha D, McClelland RL, Ix JH, et al. Relation Between Calcified Atherosclerosis in the Renal Arteries and Kidney Function (from the Multi-Ethnic Study of Atherosclerosis). Am J Cardiol 2017;120:1434-9.
15. Roseman DA, Hwang SJ, Manders ES, et al. Renal artery calcium, cardiovascular risk factors, and indexes of renal function. Am J Cardiol 2014;113:156-61.
16. Chiu YW, Adler S, Budoff M, et al. Prevalence and prognostic significance of renal artery calcification in patients with diabetes and proteinuria. Clin J Am Soc Nephrol 2010;5:2093-100.
17. Overall CM. Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites. Mol Biotechnol 2002;22:51-86.
18. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92:827-39.
19. Cui N, Hu M, Khalil RA. Biochemical and Biological Attributes of Matrix Metalloproteinases. Prog Mol Biol Transl Sci 2017;147:1-73.
20. Guan C, Xiao Y, Li K, et al. MMP-12 regulates proliferation of mouse macrophages via the ERK/P38 MAPK pathways during inflammation. Exp Cell Res 2019;378:182-90.
21. Goncalves I, Bengtsson E, Colhoun HM, et al. Elevated Plasma Levels of MMP-12 Are Associated With Atherosclerotic Burden and Symptomatic Cardiovascular Disease in Subjects With Type 2 Diabetes. Arterioscler Thromb Vasc Biol 2015;35:1723-31.
22. Amor M, Bianco V, Buerger M, et al. Genetic deletion of MMP12 ameliorates cardiometabolic disease by improving insulin sensitivity, systemic inflammation, and atherosclerotic features in mice. Cardiovasc Diabetol 2023;22:327.
23. Newby AC. Metalloproteinase production from macrophages - a perfect storm leading to atherosclerotic plaque rupture and myocardial infarction. Exp Physiol 2016;101:1327-37.

24. Niu H, Li Y, Li H, et al. Matrix metalloproteinase 12 modulates high-fat-diet induced glomerular fibrogenesis and inflammation in a mouse model of obesity. Sci Rep 2016;6:20171.
25. Tan RJ, Liu Y. Matrix metalloproteinases in kidney homeostasis and diseases. Am J Physiol Renal Physiol 2012;302:F1351-61.
26. Abraham AP, Ma FY, Mulley WR, et al. Matrix metalloproteinase-12 deficiency attenuates experimental crescentic anti-glomerular basement membrane glomerulonephritis. Nephrology (Carlton) 2018;23:183-9.
27. Kozono D, Yasui M, King LS, et al. Aquaporin water channels: atomic structure molecular dynamics meet clinical medicine. J Clin Invest 2002;109:1395-9.
28. Yool AJ, Brown EA, Flynn GA. Roles for novel pharmacological blockers of aquaporins in the treatment of brain oedema and cancer. Clin Exp Pharmacol Physiol 2010;37:403-9.
29. da Silva IV, Garra S, Calamita G, et al. The Multifaceted Role of Aquaporin-9 in Health and Its Potential as a Clinical Biomarker. Biomolecules 2022;12:897.
30. Zhang L, Liu Y, Wang K, et al. Integration of machine learning to identify diagnostic genes in leukocytes for acute myocardial infarction patients. J Transl Med 2023;21:761.
31. Shan E, Yu Y, Tang W, et al. miR-330-3p alleviates the progression of atherosclerosis by downregulating AQP9. Funct Integr Genomics 2023;23:77.
32. Jing J, Sun J, Wu Y, et al. AQP9 Is a Prognostic Factor for Kidney Cancer and a Promising Indicator for M2 TAM Polarization and CD8+ T-Cell Recruitment. Front Oncol 2021;11:770565.
33. Mohammad S, O'Riordan CE, Verra C, et al. RG100204, A Novel Aquaporin-9 Inhibitor, Reduces Septic Cardiomyopathy and Multiple Organ Failure in Murine Sepsis. Front Immunol 2022;13:900906.

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