Supplementary discussion

Potential roles of the dysregulation of the five genes in DCD kidneys

CHST3 is an enzyme catalyzing the synthesis of chondroitin 6-sulfate proteoglycan, which belongs to the chondroitin sulfate proteoglycan family serving as key drivers or mediators in inflammation and leukocyte infiltration (27,34,35). Interestingly, Kai *et al.* reported that silencing CHST3 in a murine pulmonary emphysema model could significantly inhibit TNF-α and MMP-9 expression and macrophage accumulation promoting lung recovery (27). Activation of matrix metalloproteinases (MMPs) plays crucial roles in renal IRI in KT by facilitating extracellular matrix (ECM) degradation and subsequent leukocyte movement across endothelial cells and the ECM with the release of cytokines and free radicals (36,37,38). Thus, there is a simple hypothesis that CHST3 upregulation in transplanted kidneys could increase the expression of MMPs promoting ECM degradation and leukocyte recruitment, finally leading to renal IRI and DGF after DCD KT. GOLPH3 is a Golgi-associated protein, and its role in some pathological conditions (especially tumors) has been widely reported (28,39). Importantly, its protective role in IRI has been preliminarily revealed in a finding that the antioxidative stress effects of salvianolate on brain IRI rely on activation of the GOLPH3-Akt-mTOR signaling pathway (28). In addition, its role in the modulation of mTOR-related pathways (including the PI3K-AKT-mTOR and MAPK-ERK-mTOR pathways) also deserves attention (28,40-42). It has been found that excessive autophagy is common in DCD allografts suffering prolonged CIT, which could aggravate renal IRI by depriving the cell necessary energy and further increase the risk of DGF (25). In this light, excessive autophagy via mTOR inhibition induced by GOLPH3 downregulation may be one of the mechanisms underlying renal IRI after DCD KT. Moreover, it has been demonstrated that PI3K-Akt-mTOR pathway is responsible for HIF-1α accumulation, which plays a protective role during IRI (25). Thus, it is possible that GOLPH3 downregulation could also influence the accumulation of HIF-1α by inhibiting the PI3K-Akt-mTOR pathway, thereby promoting renal IRI. Emerging evidence suggests that EGFR (epidermal growth factor receptor), a transmembrane receptor with intrinsic tyrosine kinase activity, plays a dual role in renal IRI (43). Previous studies have indicated that proper activation of EGFR contributes to the tubular reparative response in the early phase of IRI (2 days), whereas its sustained or overactivation triggers renal fibrogenesis and potentiates the kidney IRI (43,44). Therefore, balanced modulation of EGFR is crucial for renal functional recovery during kidney IRI. Zhou *et al.* found that GOLPH3 could inhibit Rab5-mediated endocytosis and degradation of EGFR, thereby activating EGFR-related pathways (45). And Chen *et al.* identified that ERRFI1, a known negative feedback regulator of EGFR, could reduce proinflammatory mediator production (such as TNF-α and IL-1β) by controlling excessive EGFR activation in LPS-induced endotoxemia or LPS-treated nucleus pulposus cells (46,47). Importantly, Ma *et al.* reported that downregulation of ERRFI1 in AKI of sepsis targeted by miR-152-3p could promote the activation of the STAT3 signaling pathway, thereby aggravating cell apoptosis and the inflammatory response (31). It seems that both dysregulation of GOLPH3 and ERRFI1 in DCD kidneys may lead to disorder of EGFR expression resulting in enhanced renal IRI and DGF in DCD KT. AKR1C4 is a member of the aldo-keto reductases that plays a vital role in NADPH-dependent reductions, and its isoforms have been implicated in anti-inflammatory effects and alleviation of oxidative stress (OS) damage in some disease models, which strongly indicates that AKR1C4 downregulation in transplanted kidneys may aggravate renal IRI via reactive oxygen species (ROS) generation and uncontrolled OS damage (30,48,49). ZBED5 belongs to the ZBED gene family originating from domesticated hAT DNA transposons (29). Based on the identified roles of its homologs in the regulation of diverse functions, we speculate that the dysregulation of ZBED5 may be implicated in the transcriptional reprogramming of transplanted kidneys during IRI (25,29). Overall, the correlations between the dysregulation of the above five genes and renal IRI or DGF occurrence deserve in-depth studies in the future.

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Table S1 The clinical characteristics of the patients enrolled in the study (14)

Variable	DCD-C1 (n=29)	DCD-C2 (n=35)	DBD-C1 (n=67)	DBD-C2 (n=38)
Donor age (years)*	52 (18 to 66)	42 (9 to 65)	52 (10 to 76)	53 (17 to 72)
ECD donor (%)	14	17	16	32
Donor gender (% female)	41	51	51	58
Cause of death (%)				
CVA	45	40	75	71
Trauma	31	34	15	18
Other	24	26	10	11
Duration of BD (min)	NA	NA	602 (184 to 3,325)	607 (220 to 2,850)
WIT (min)*	16 (9 to 35)	18 (9 to 33)	NA	NA
Recipient age (years)*	59 (25 to 68)	59 (22 to 75)	56 (19 to 73)	56 (23 to 71)
Recipient gender (% female)	24	37	46	47
Recipient transplants (% first)	97	94	90	84
CIT (min)*	956 (517 to 1,500)	998 (579 to 2,092)	1096 (461 to 1,817)	1,005 (600 to 1,430)
DGF (%)	79	77	33	34

*, median (range). DBD, donation after brain death; DCD, donation after cardiac death; C, cohort; WIT, warm ischemia time; CIT, cold ischemia time; DGF, delayed graft function; NA, not applicable.

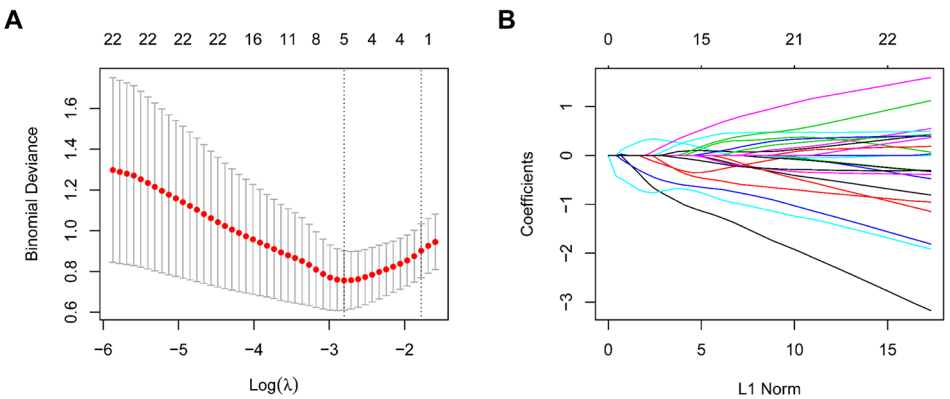

Figure S1 Selection of the optimal genes for modeling by LASSO-penalized logistic regression analysis. (A) The optimal gene group was chosen by 10-fold cross-validation and lambda.min; (B) LASSO coefficient profile of the genes.

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Table S2 Logistic regression model analyses of correlations between the expression of the five genes in T3 kidney biopsies and DGF occurrence following DCD KT

Variables	Univariate analysis		Multivariate analysis, coefficient (β)
	OR (95% CI)	P value	
CHST3	5.158 (1.873, 14.206)	0.002	1.685
GOLPH3	0.284 (0.124, 0.649)	0.003	–2.066
ZBED5	0.188 (0.072, 0.492)	0.001	–1.023
AKR1C4	0.315 (0.123, 0.806)	0.016	–3.831
ERRFI1	0.276 (0.123, 0.619)	0.002	–1.529

OR, odd ratio; CI, confidence interval.

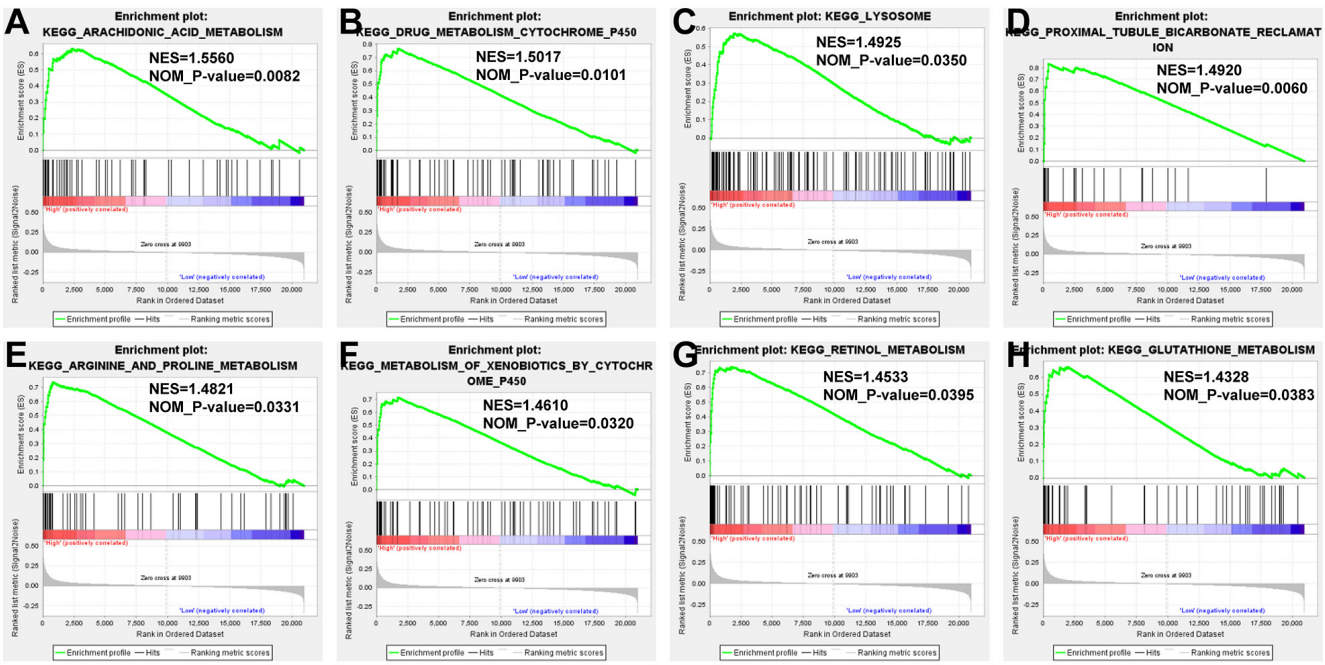

Figure S2 Gene set enrichment analysis based on the risk score using the GSE43974 dataset. A total of 8 KEGG signaling pathways were significantly enriched in the high-risk group defined by the genomic risk score, including "arachidonic acid metabolism" (A), "drug metabolism cytochrome p450" (B), "lysosome" (C), "proximal tubule bicarbonate reclamation" (D), "arginine and proline metabolism" (E), "metabolism of xenobiotics by cytochrome p450" (F), "retinol metabolism" (G), and "glutathione metabolism" (H). NES, normalized enrichment score; NOM p, normalized P value.

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