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iTRAQ-based quantitative proteomics reveals reduced expression of KRT19, KRT7, and PTGDS in cutaneous specimens after kidney transplantation

Ichiro Tsuboi[1], Yosuke Mitsui[1]✉, Kasumi Yoshinaga[1], Tomoaki Yamanoi[1], Takanori Sekito[2], Yuki Maruyama[2], Takuya Sadahira[1], Shingo Nishimura[1], Kensuke Bekku[1] & Motoo Araki[1]

Clinical improvement in pigmentation is frequently observed after kidney transplantation. However, the underlying molecular and histological mechanisms remain unclear. We conducted a study to quantify the skin color change using a handheld reflected light colorimeter and to investigate protein expression changes in the skin before and after kidney transplantation. Paired skin biopsies were obtained from three patients who underwent kidney transplantation before and one month after transplantation. Protein expression was analyzed using iTRAQ-based quantitative proteomics. Differentially expressed proteins were identified and visualized using hierarchical clustering and volcano plots. Histopathological evaluation included hematoxylin and eosin (H&E), Masson's trichrome, and immunohistochemical (IHC) staining for keratin (KRT) 7, KRT19, and MelanA. Skin pigmentation of the arms, ankles, and abdomen had significant L-value improvement after kidney transplantation. Proteomic profiling identified 2148 proteins, with six proteins showing significant differential expression after transplantation. Among them, KRT7, KRT19, and prostaglandin D2 synthase (PTGDS) were significantly downregulated, potentially reflecting reduced epithelial stress and systemic inflammation. H&E and Masson's trichrome staining revealed a post-transplantation reduction in dermal pigmentation and collagen content. IHC showed decreased KRT7, KRT19, and MelanA expression after transplantation. Our results suggest that targeting KRT or prostaglandin pathways may offer new treatments for ESRD-related skin symptoms.

Keywords Cutaneous manifestations, Keratin, Skin color, Pigmentation, Prostaglandin D2 synthase, Renal transplantation, Dialysis

In the advanced stage of chronic kidney disease (CKD), known as end-stage renal disease (ESRD), kidney function declines to the point where life-sustaining treatment becomes essential. Hemodialysis is commonly used as a primary therapy to replace lost renal function. However, despite its life-preserving role, long-term dialysis often leads to a range of complications, including systemic disturbances and cutaneous manifestations.

Cutaneous manifestations, such as pigmentary change, xerosis, and pruritus, are a well-known problem in ESRD patients with hemodialysis. Previous studies have reported that the rate of skin pigmentary change in ESRD patients undergoing maintenance hemodialysis ranged from 17 to 43%[1,2,3]. Additionally, recent study reported that cutaneous pigmentary change was associated with diabetes mellitus (DM) duration and low albumin level ($p = 0.03$ and $p = 0.006$)[3].

In our institution's experience, kidney transplantation seems to improve skin color dramatically. However, the quantification of color change after transplantation has not yet been studied. Moreover, the mechanism of cutaneous pigmentation remains unclear. Therefore, this study aims to investigate the quantification of skin

[1]Department of Urology Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Okayama, Japan. [2]Department of Inflammation and Immunity, Lerner Research Institute Cleveland Clinic, Cleveland, OH, USA. ✉email: m28949825@gmail.com

color change and the biomarkers related to skin pigmentary change using cutaneous biopsy tissues obtained before and after renal transplantation in maintenance dialysis patients.

Materials and methods

This study consists of two parts. (a) The first part aims to quantitatively assess changes in skin color before and after kidney transplantation, as our institutional experience suggests a marked improvement in skin pigmentation following transplantation, although this has not been objectively evaluated. (b) The second part seeks to explore the underlying mechanisms of pigmentary change by analyzing biomarkers associated with skin pigmentation using cutaneous biopsy specimens collected before and after transplantation in patients undergoing maintenance dialysis.

Patients

(a) We retrospectively identified 182 patients who had undergone kidney transplantation between March 2018 and December 2024 at our institution. Patients without skin pigmentation data before or after transplantation were excluded. Written and informed consent was given by the people shown in the images to publish these in this online open-access publication.
(b) This study was conducted as a retrospective pilot study involving three patients with ESRD who underwent kidney transplantation. To specifically investigate chronic skin changes associated with prolonged uremia, we selected patients with a dialysis history of more than 15 years. To investigate molecular changes associated with skin pigmentary alteration, paired cutaneous biopsy specimens were collected from each patient at two time points: immediately before kidney transplantation and one month after transplantation.

Measurement of skin color

Skin color was measured with a handheld reflected light colorimeter (CR-20, Konica Minolta, Tokyo, Japan). The colorimeter was automatically set to white balance before each measurement. We measured skin color on the inner forearm, the face, the inner ankle and the abdomen near the umbilicus before transplantation and 1 month after transplantation. Measurements were performed twice at each time point, and the average values were calculated. Skin color was recorded by using the Commission Internationale de l'Eclalarge-recommende (CIE) L*a*b system[4]. This measurement system is widely used for quantification of skin color and has been used in patients with skin disorders[5,6] as well as patients with ESRD[7]. Per this system, skin color is defined by its reflectance (L*) and chromaticity (a* and b*). The L* value indicates brightness ranging from black (L* = 0) to white (L* = 100). The a* value represents the balance between red and green, and the b* value represents the balance between yellow and blue. To clarify the effect of dialysis, we further divided our cohort into a preemptive kidney transplantation (PEKT) group and non-PEKT group, and compared the skin color changes between the two groups.

Cutaneous biopsy and blood test

Skin specimens were obtained at two time points: prior to and one month after renal transplantation. Pre-transplant samples were collected intraoperatively at the time of surgery. A 4–5 mm section of skin was excised from the incision site immediately after the initial surgical cut, under sterile conditions. Post-transplant samples were acquired during the scheduled protocol renal biopsy, one month after transplantation. A 4-mm punch biopsy was performed at the anticipated site of needle insertion for the renal biopsy. The procedure was carried out under sterile conditions following local infiltration with 1% lidocaine without epinephrine. Care was taken to minimize tissue trauma and ensure consistent sampling across all patients. Blood tests were also performed at the almost same two points.

Protein extraction based on iTRAQ

Skin biopsy tissues were collected from each patient at both pre- and post-transplantation time points for protein extraction. Samples were sent to Integrale Inc. (Tokushima, Japan) for iTRAQ-based proteomic analysis under a contract agreement.Each tissue sample was briefly rinsed in phosphate-buffered saline to remove residual blood and contaminants, then homogenized in lysis buffer containing 7 M urea, 0.1% NP-40, and 500 mM triethylammonium bicarbonate (TEAB). The homogenates were further subjected to ultrasonic disruption (10 s ON, 10 s OFF) for 10 min on ice to minimize protein degradation and improve extraction efficiency. After centrifugation at 15,000 × g for 15 min at 4 °C, the supernatants containing soluble proteins were collected. Residual pellets were subjected to a second extraction using a modified RIPA buffer (25 mM TEAB, pH 8.5, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS), followed by the same ultrasonic treatment and centrifugation steps. The resulting supernatants from both extractions were combined. Protein concentrations were determined using the bicinchoninic acid (BCA) assay (Thermo Fisher Scientific, Waltham, MA, USA). Equal amounts of protein (typically 15 µg per sample) were subjected to downstream processing. Proteins were reduced with 5 mM dithiothreitol at 56 °C for 30 min, followed by alkylation with 15 mM iodoacetamide at room temperature for 30 min in the dark. Trypsin digestion was performed overnight at 37 °C at a trypsin-to-protein ratio of 1:50 (w/w). The resulting peptides were desalted using C18 spin columns (Pierce, Thermo Fisher Scientific), dried, and labeled with iTRAQ 8-plex reagents (AB Sciex, Framingham, MA, USA) following the manufacturer's protocol. Labeling was performed according to sample-specific assignments. Labeled peptides were pooled and fractionated using a cation exchange cartridge system (AB Sciex, Cat No. 4326747). Each fraction was desalted and dried by vacuum centrifugation prior to mass spectrometric analysis. LC-MS/MS analysis was performed on a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, https://www.thermofisher.com) coupled with an EASY-nLC 1200 system. Peptide separation was conducted using a 15 cm × 75 µm

ID PepMap C18 column (3 µm particles, 100 Å pore size) with a linear acetonitrile gradient in 0.1% formic acid at a flow rate of 300 nL/min. Electrospray ionization (ESI) was used in positive ion mode. MS/MS data were acquired in data-dependent acquisition mode with full MS scans over an m/z range of 350–1600, followed by MS/MS on the top 10 precursor ions using higher-energy collision dissociation (HCD) with dynamic exclusion enabled. Raw data were processed using Proteome Discoverer software (version 3.1, Thermo Fisher Scientific, https://www.thermofisher.com), employing the Mascot search engine (Matrix Science, London, UK) against the SwissProt human protein database (release as of November 7, 2021). Search parameters included a precursor mass tolerance of 10 ppm and a fragment mass tolerance of 0.02 Da. Fixed modifications included iTRAQ 8-plex tags on N-termini and lysine residues, and carbamidomethylation of cysteine; oxidation of methionine was set as a variable modification. Protein identifications were accepted if at least one unique peptide with a > 95% confidence level was detected. Quantification was based on the relative intensities of iTRAQ reporter ions.

Enrichment analysis

Enrichment analysis was performed using the Metascape (http://metascape.org), an online tool that integrates multiple authoritative data sources such as GO Biological Processes, KEGG Pathways, Reactome Gene Sets, and WikiPathways[8,9]. Visualization included bar plots of top enriched terms and network diagrams showing clustering of related biological processes.

Histopathological study

Formalin-fixed, paraffin-embedded (FFPE) skin biopsy specimens were sectioned at 4 µm thickness. Routine hematoxylin and eosin (H&E) staining was performed for general histological assessment. To evaluate changes in dermal collagen content, Masson's trichrome staining was carried out. Immunohistochemical (IHC) staining was performed to assess the expression of cytokeratin 7 (KRT7), cytokeratin 19 (KRT19), and MelanA. After deparaffinization and rehydration, tissue sections underwent antigen retrieval in citrate buffer (pH 6.0), and endogenous peroxidase activity was quenched with 3% hydrogen peroxide. The sections were incubated overnight at 4 °C with the following primary antibodies: anti-KRT7 (mouse monoclonal, Dako, Code: M7018), anti-KRT19 (mouse monoclonal, Leica, Code: NCL-L-CK19), and anti-MelanA (mouse monoclonal, Dako, Code: M7196). Subsequently, slides were incubated with appropriate secondary antibodies and visualized using the DAB chromogen system. Hematoxylin was used for nuclear counterstaining. All histological and immunohistochemical procedures were performed by Morphotechnology Co., Ltd. (Sapporo, Japan). Digital images of representative slides were obtained using a virtual slide scanner, and both low- and high-power fields were analyzed to evaluate epidermal and dermal alterations.

Statistical analysis

(a) All values are expressed as mean ± standard deviation (SD). Statistical comparisons of the patient characteristics and skin color were performed using Wilcoxon and paired t-tests, as appropriate. Statistical significance was set at $P < 0.05$ for all analyses. All statistical analyses were performed using JMP software (version 13.2; SAS Institute Inc., Cary, NC, USA; https://www.jmp.com).

(b) To assess differential protein expression, statistical analyses were performed using Proteome Discoverer software (version 3.1, Thermo Fisher Scientific, https://www.thermofisher.com), which incorporates integrated quantification and statistical modules. Proteins were considered differentially expressed if they exhibited a fold change > 2 or < 0.5 with a p-value < 0.05, calculated using paired Student's t-tests. A p-value < 0.05 was considered statistically significant. Hierarchical clustering and heatmap visualization of significantly altered proteins were generated within the Proteome Discoverer environment. Volcano plots were also constructed based on fold change and p-value results output from the software.

Results

Characteristics of the patients

A total of 21 patients were included in this retrospective study. Table 1 shows the patient characteristics of the PEKT and non-PEKT groups. Four patients were classified as PEKT (19.0%).

Three patients with ESRD who underwent deceased- or living-donor kidney transplantation were included in our study. Patient characteristics are summarized in Table 2. The patients included two males and one female, with ages ranging from 36 to 62 years. The primary causes of ESRD were focal segmental glomerulosclerosis, chronic glomerulonephritis, and unknown etiology. Dialysis durations varied from 15 to 18 years, and all patients had been on maintenance HD, with one patient having also undergone PD. None of the patients had diabetes mellitus. The renal transplantations were ABO identical, minor mismatch, or incompatible. Serum calcium, phosphate, albumin, and blood urea nitrogen levels before and after transplantation were also measured and are presented in Table 2. All patients showed improvements in serum phosphate and BUN levels following transplantation.

Skin color changes pre and post renal transplantation

Figure 1 shows skin color change before and after transplantation at 4 distinct body parts. At the arm, ankle and abdomen, there were significant improvements in the L-value after kidney transplantation ($p = 0.0011$, $p = 0.00019$ and $P = 0.037$, respectively) In the face, although there was no significant improvement of the L value, the lightness increased after transplantation ($P = 0.090$). Figure 2 depicts a representative case from the cohort in which L-values were measured, a 52-year-old male on dialysis for 10 months prior to transplantation. This patient was not included in the MS analysis cohort, and the image is presented for illustrative purposes only. The

	Total		PEKT		Non-PEKT		P
	(n = 21)		(n = 4)		(n = 17)		
Age, years, median (IQR)	48	(31-57)	31.5	(18.8-50.3)	52	(32.0-65.0)	0.13
Female, n (%)	9	(42.8)	2	(50.0)	7	(41.2)	1.00
HD duration, months, median (IQR)	14	(3.5-64)	0	(0)	14	(3.5-64)	<0.01
HT	11	(52.4)	2	(50.0)	9	(52.9)	1.00
DM	1	(4.8)	0	(0)	1	(7.1)	1.00
Transplantation from Brain-dead donor	2	(9.6)	1	(25)	2	(11.8)	
ABO blood groups (%)							0.33
Identical	13	(62.0)	2	(50)	11	(64.7)	
Minor mismatch	5	(23.8)	2	(50)	3	(17.6)	
Incompatible	3	(14.3)	0	(0)	3	(17.6)	
Rejection, n (%)	0	(0)	0	(0)	0	(0)	1.00
BKV, n (%)	0	(0)	0	(0)	0	(0)	1.00
CMV, n (%)	10	(47.7)	3	(75)	7	(41.2)	0.31

Table 1. Patient characteristics (quantification of skin color change) *PEKT* Preemptive kidney transplantation, *HT* Hypertension, *DM* Diabetes mellitus, *BKV* BK virus, *CMV* Cytomegalovirus

color of the face and arm dramatically changed. Preoperative L-values of the face and arm were 47.15 and 40.45, respectively, which increased to 53.55 and 53.25 after transplantation.

Skin color change between PEKT and non-PEKT patients

Figure 3 shows the comparison of skin color changes between PEKT and non-PEKT patients. Interestingly, there were no significant improvements in PEKT patients between before and after transplantation. On the other hand, non-PEKT patients showed dramatically improved lightness except for the face after transplantation.

Protein identification and differential expression analysis

A total of 2148 proteins were identified by iTRAQ-based quantitative proteomic analysis from skin biopsy samples collected before and after renal transplantation (Supplementary Table S1). A subset of top-ranking proteins with the most significant fold changes is shown in a volcano plot (Fig. 4A). Six proteins, including KRT7, KRT19, STAT5B, PTGDS, AMBP, and PEBP4, were found to be significantly differentially expressed between pre- and post-transplantation samples, based on a fold change threshold of >2 or <0.5 and a p-value <0.05. These differentially expressed proteins were visualized using heatmap and hierarchical clustering (Fig. 4B, C). The expression patterns showed clear separation between pre- and post-transplantation samples.

Visualization of protein expression patterns

To further investigate the global patterns of protein expression, hierarchical clustering analysis was performed based on the differentially expressed proteins. As shown in Fig. 4C, heatmap visualization revealed distinct clustering between pre- and post-transplantation samples, indicating a consistent pattern of protein regulation associated with renal transplantation. In addition, a secondary heatmap was generated focusing on selected keratin- and prostaglandin-related proteins, which were hypothesized to be involved in skin changes based on the result of iTRAQ data (Fig. 5). Proteins upregulated after transplantation were predominantly involved in immune response and metabolic processes, while those downregulated included structural and cytoskeletal components.

Enrichment analysis

Functional enrichment analysis reveals cytoskeletal and immune-related changes following kidney transplantation. Using Metascape, enrichment analysis was performed on proteins that were significantly downregulated after kidney transplantation (abundance ratio <0.67). The bar graph of enriched terms highlights several significant biological processes, including "Intermediate filament cytoskeleton organization" (GO:0045104), "Carbohydrate biosynthetic process" (GO:0016051), and "Neutrophil degranulation" (R-HSA-6798695) (Fig. 6A). Enrichment network visualizations cluster related terms, showing interconnected modules centered on cytoskeletal remodeling, immune regulation, and metabolic processes (Fig. 6B). Terms were grouped by functional similarity (color-coded clusters) and also ranked by statistical significance (colored by $-\log_{10}$ (p value)).

Histopathological changes

H&E and Masson's trichrome staining demonstrated a reduction in dermal melanin-related pigmentation and decreased dermal collagen density in post-transplant specimens compared to pre-transplant samples (Fig. 7A). Immunohistochemical staining in three patients revealed a visible decrease in KRT7, KRT19, and MelanA expression following kidney transplantation, particularly within the basal epidermal layer (Fig. 7B). Positive staining regions were identified and quantified using Fiji software (https://fiji.sc)[10], and are marked in red. Quantification of positive area is shown in the bar graphs below each marker, normalized to the expression level

Patients	Age, y	Sex	Primary cause of ESRD	Age at diagnosis, y	Type of dialysis and dialysis duration, y	Type of renal transplantation	ABO blood groups	DM	Before and after renal transplantation				Occupation
									Ca (mg/dL)	*P* (mg/dL)	Alb (g/dL)	BUN (mg/dL)	
No.1	36	M	Focal segmental Glomerulosclerosis	15	PD 11, HD 7, total 18	Living	Minor mismatch	No	8.9/9.5	4.8/1.4	4.3/3.4	39/21	Office worker
No.2	49	M	Unknown	33	HD 17	Deceased	Identical	No	9.1/10.6	4.7/2.3	4.1/3.7	43/31	Cleaning service
No.3	62	F	Chronic glomerulonephritis	42	HD 15	Living	Incompatible	No	8.9/10	6.4/1.2	4.4/4.2	57/24	Teacher

Table 2. Patient characteristics of three cases (skin biopsy for investigate the protein expression). *DM* Diabetes mellitus, *ESRD* End-stage renal disease, *HD* Hemodialysis, *PD* Peritoneal dialysis.

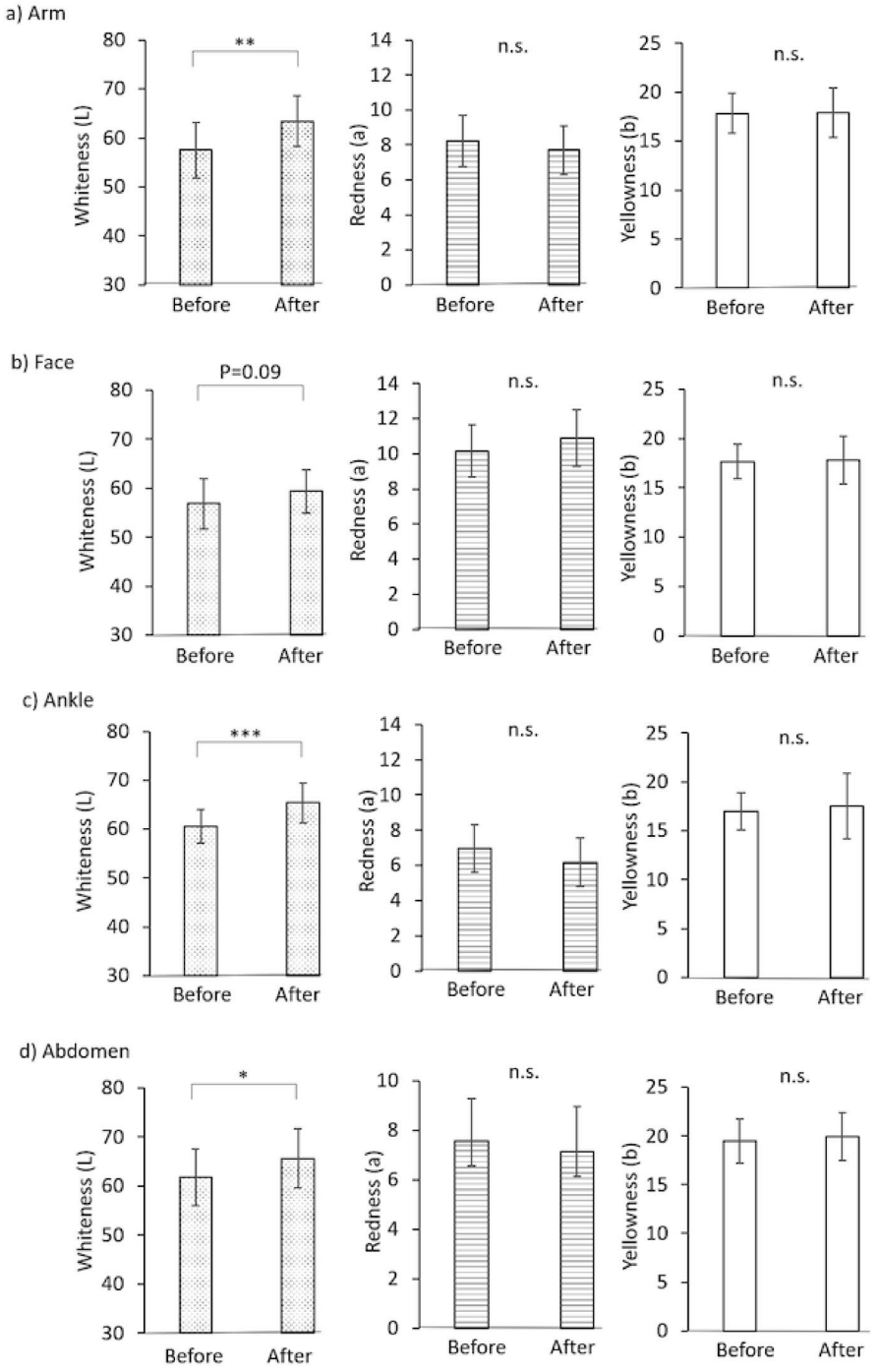


Fig. 1. Skin color change between before and after renal transplantation. Skin pigmentation changes before and after kidney transplantation. (**A**) Skin changes at the arm; (**B**) Skin changes at the face; (**C**) Skin changes at the inner ankle; (**D**) Skin changes at the abdomen.

before transplantation (set as 1.0). KRT7, KRT19, and MelanA decreased by approximately 80%, 70%, and 40%, respectively ($n = 3$) (Fig. 7D).

Discussion

To our knowledge, this is the first study to quantify color change before and after renal transplantation and to investigate differentially expressed proteins in skin biopsy specimens collected from ESRD patients before and after kidney transplantation using iTRAQ-based quantitative proteomics. Distinct clustering patterns between pre- and post-transplantation samples suggest consistent alterations in the skin proteome following transplantation. These findings support the hypothesis that kidney transplantation not only restores renal function but also contributes to the normalization of cutaneous molecular signatures associated with uremic

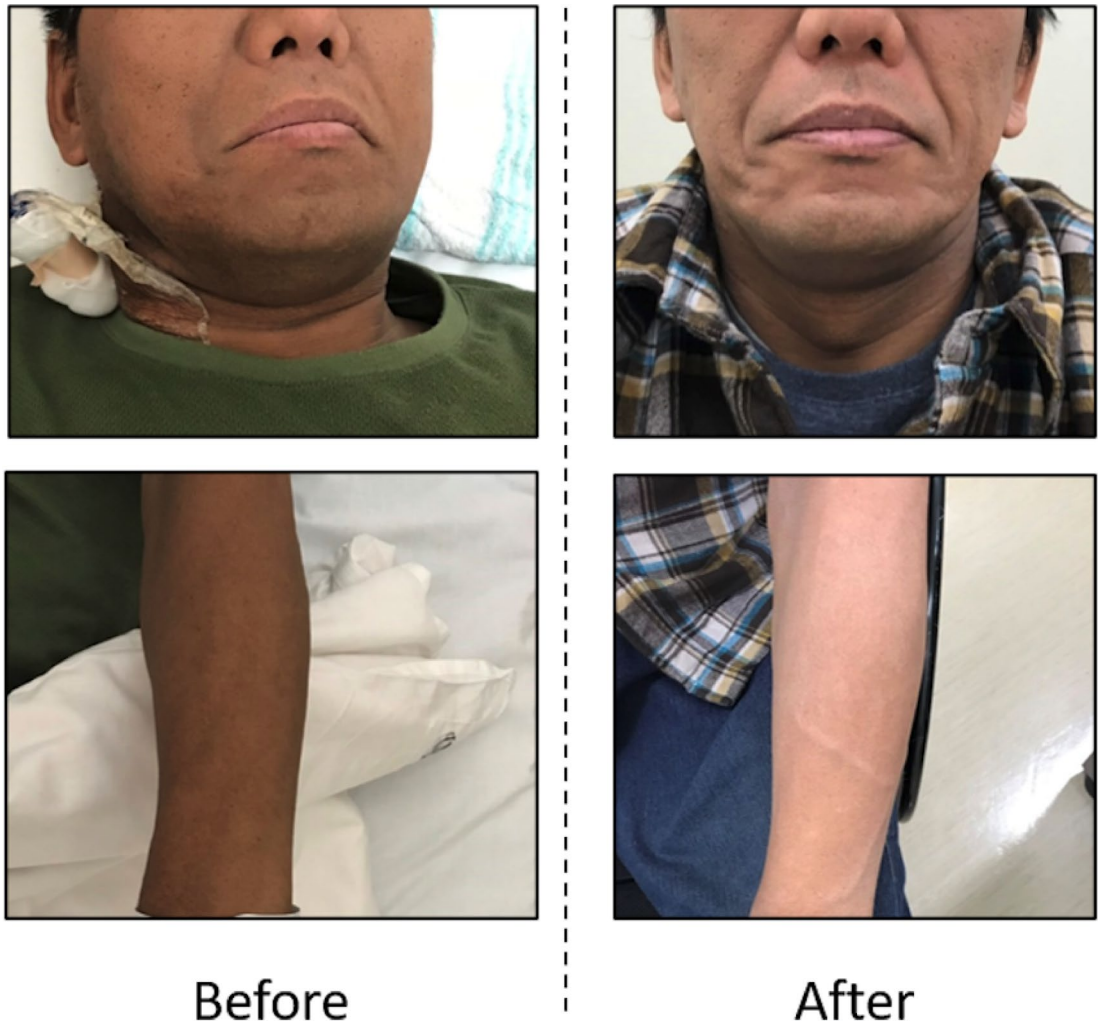


Fig. 2. Skin color change of the patient from the cohort in which L-values were measured. 52-year-old male: preoperative L-value in face and arm were 47.15 and 40.45 respectively. After renal transplantation, L-values improved to 53.55 and 53.25, respectively.

skin changes. Among the differentially expressed proteins, KRT7, KRT19, and PTGDS showed a marked decrease in expression levels after kidney transplantation based on the iTRAQ. The downregulation of these proteins suggests that they may accumulate in the skin of ESRD patients due to impaired renal function and subsequently decline following restoration of renal function through transplantation.

No significant improvement in skin color was observed among PEKT patients, in contrast to the significant changes detected in the non-PEKT group. This discrepancy may be attributed to the absence of prolonged dialysis exposure in PEKT patients, which likely limited the development of uremia-associated cutaneous pigmentation. As a result, the potential for visible reversal of pigmentation following transplantation may have been minimal in this subgroup. Additionally, the limited sample size of the PEKT cohort may have contributed to insufficient statistical power to detect modest changes.

KRT7 is a type II keratin expressed in simple epithelia and is mapped to chromosome 12, while KRT19 is a type I keratin expressed in epithelial cell and is mapped to chromosome 17. Previous study revealed that KRT1, KRT5, KRT10, and KRT14 are the major keratins expressed in normal epidermis, while KRT7 is not[11]. Previous study showed that KRT7 and KRT19 proteins are specifically expressed in the principal cells of the collecting ducts and the ascending limbs of the loops of Henle, while KRT7 and KRT19 showed diffuse expression in atrophic and cystically dilated tubules in patients with ESRD. This aberrant expression reflects impaired terminal differentiation and increases epithelial plasticity, possibly contributing to tumorigenesis[12]. The study have also shown that epithelial cells shift their keratin expression from KRT8 and KRT18 to KRT7 and KRT19 in kidneys with ESRD, reflecting a disruption of terminal differentiation and activation of stress-responsive programs[12]. Based on these findings, we hypothesize that a similar mechanism may occur in the skin, whereby epithelial cells under chronic stress or inflammation in patients with ESRD may upregulate KRT7 and KRT19 expression. This potential keratin switch in the skin may indicate impaired differentiation or a reparative phenotype in response to systemic or local stress. Furthermore, our study showed that not only the expression of KRT7 and KRT19 was reduced after kidney transplantation, as shown by immunohistochemistry, but also that the expression of

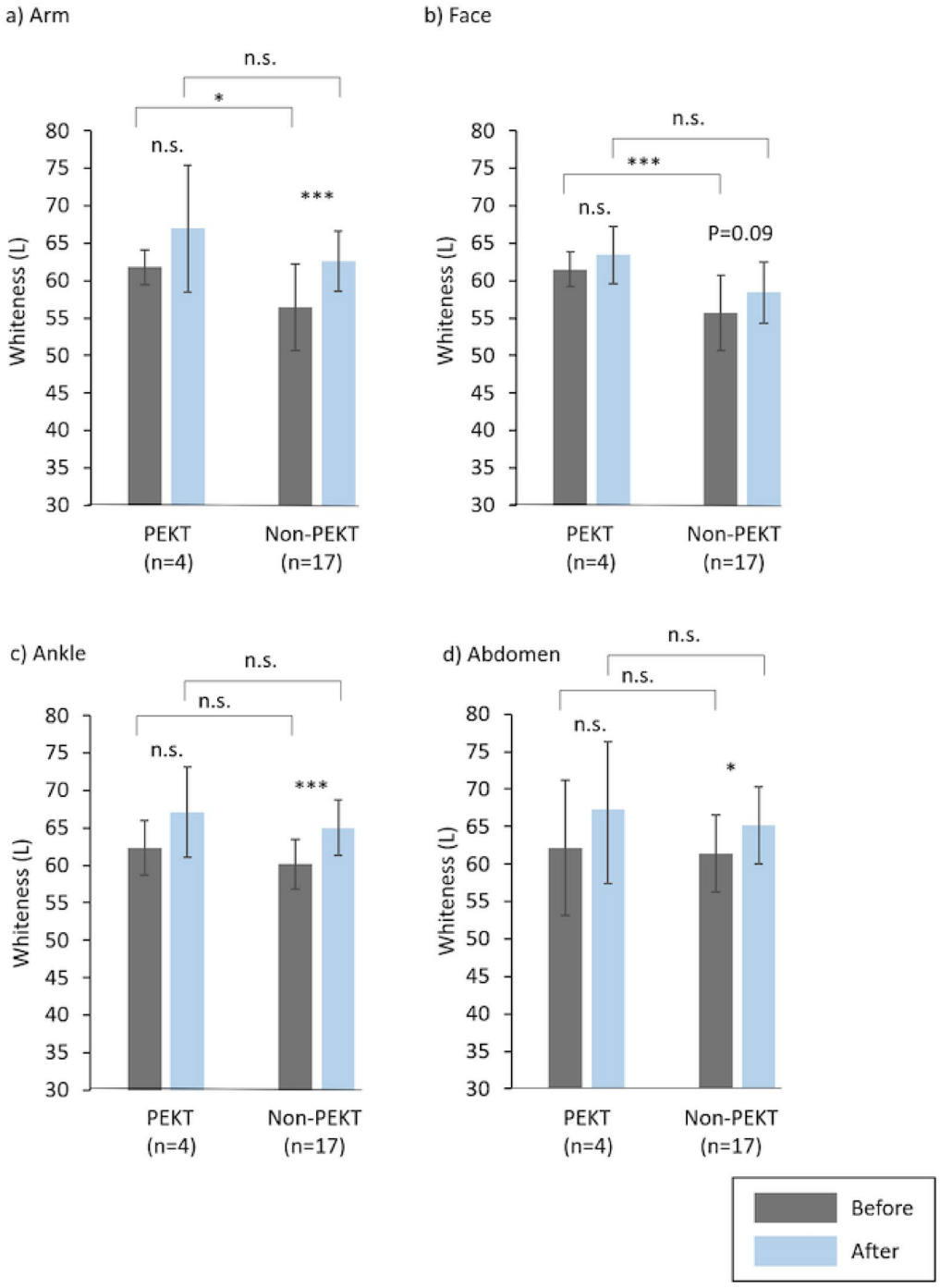


Fig. 3. Skin color change between PEKT and non-PEKT patients. (**a**) arm, (**b**) face, (**c**) ankle and (**d**) abdomen.

MelanA decreased. Based on these findings, we hypothesized that increased expression of KRT7 and KRT19 in patients with ESRD may be involved in the enhancement of cutaneous hyperpigmentation.

PTGDS is a protein belonging to the lipocalin family. Lipocalins are a large family of small extracellular proteins involved in various functions, including the transport of small hydrophobic molecules[13]. PTGDS serves a dual role: as an enzyme that catalyzes the conversion of prostaglandin H_2 to prostaglandin D_2, and as a transporter for various lipophilic substances, such as retinoids, thyroid hormones, and bile pigments[13,14]. The PGD_2 produced by PTGDS is involved in diverse physiological processes, including sleep regulation, pain modulation, and allergic responses. PTGDS can also bind to amyloid-beta peptides, functioning as a major chaperone in cerebrospinal fluid[13]. PTGDS is primarily synthesized in the central nervous system, specifically in the choroid plexus and leptomeninges, and is a major constituent of cerebrospinal fluid.

PTGDS is also present in human serum and urine[13]. Previous study revealed that the serum PTGDS level in patients with renal failure showed an approximately 35- to 150-fold increase compared to those with normal

renal function[15,16]. A significant elevation in serum PTGDS levels has also been observed in patients undergoing dialysis, including those receiving HD both before and after treatment, as well as those undergoing PD. The serum PTGDS level increases in parallel with serum creatinine as renal failure progresses. The serum PTGDS level is not significantly affected by HD, although serum creatinine levels significantly reduce after HD[16]. This difference in clearance efficiency is most likely due to the difference in molecular size. Creatinine can easily diffuse through the pores of the HD membrane, whereas PTGDS (with a molecular weight of approximately 20–30 kDa), like many other similarly sized macromolecules, is not efficiently cleared by conventional HD membranes[15,16]. Therefore, we hypothesize that the improved kidney function following kidney transplantation contributes to reduce the serum PTGDS levels and may also enhance the clearance of PTGDS from peripheral tissues, including the skin, via renal excretion. This mechanism may, in turn, lead to improvements in hyperpigmentation, pruritus, and other cutaneous symptoms associated with ESRD.

The findings of this study provide new molecular insights into the mechanisms underlying skin pigmentary changes observed in patients with ESRD. While hyperpigmentation and xerosis are well-recognized cutaneous manifestations of chronic kidney disease, their molecular basis has remained poorly understood. The differential expression of proteins before and after transplantation suggests that both systemic accumulation and local tissue responses contribute to these skin changes. Our data support a model in which the restoration of renal function after transplantation leads to the clearance of uremic metabolites and proteins, thereby reducing their deposition in peripheral tissues such as the skin. In parallel, improved nutritional, metabolic, and immune conditions may activate reparative processes and restore skin homeostasis. These molecular changes not only explain the clinical improvement in skin pigmentation but also offer potential biomarkers for monitoring cutaneous and systemic recovery after transplantation. Furthermore, if the proteins identified in our study, particularly PTGDS, can be selectively removed by hemodialysis in patients with ESRD, it may contribute to the improvement of skin hyperpigmentation and other cutaneous symptoms. In addition to selective removal by hemodialysis, the possibility of pharmacological interventions, such as medicine to suppress PTGDS expression, warrants further investigation. These insights may open avenues for the development of novel therapeutic strategies and future experimental validation.

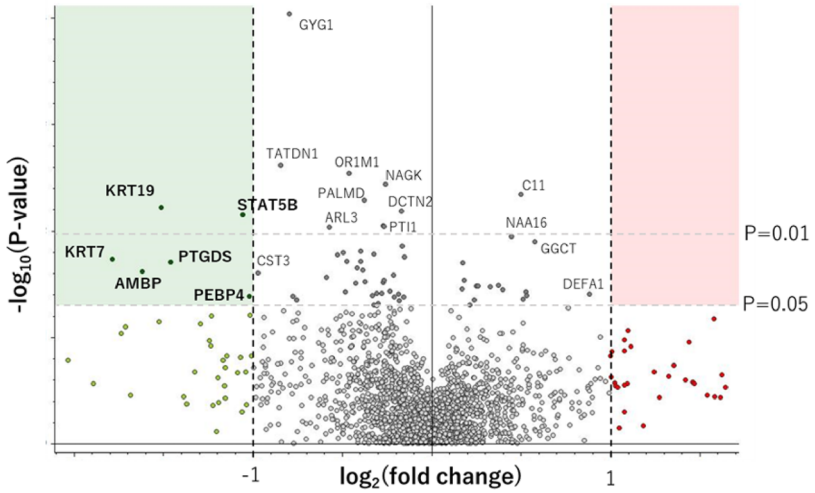
Based on our findings and previous literature, we hypothesize that the interaction between PTGDS and keratin-related proteins such as KRT7 and KRT19 may play a role in promoting pigmentary changes, skin hardening, and fibrosis in patients with ESRD. Keratinocytes are known to regulate prostaglandin synthesis in dermal fibroblasts through cytokine-mediated signaling, including interleukin-1, suggesting a potential cross-talk between epithelial stress pathways and prostaglandin signaling[17,18]. Elevated expression of both PTGDS and keratins under uremic conditions may therefore create a pro-inflammatory and pro-fibrotic microenvironment in the skin. The downregulation of these proteins following kidney transplantation could reflect not only systemic metabolic improvement but also a reduction in local epithelial–mesenchymal interactions that contribute to skin pathology.

This study has several limitations that should be acknowledged. First, the sample size was small, consisting of only three patients, which limits the generalizability of the findings. As a pilot study, the results should be interpreted with caution and validated in larger samples. Second, the precise mechanisms by which the identified proteins influence skin pigmentation remain speculative, and functional validation through in vitro or in vivo studies is necessary. Despite these limitations, our findings provide a valuable molecular perspective on the reversible skin changes associated with renal transplantation. The observed alterations in protein expression offer potential leads for identifying biomarkers of skin changes and for understanding how systemic metabolic status influences skin physiology. Future studies should focus on expanding sample size, incorporating control groups such as healthy individuals or non-transplanted ESRD patients, and integrating proteomic findings with histological and clinical assessments.

Conclusion

Our study suggested that renal transplantation is the most effective treatment to improve the skin darkness which derived from long term dialysis. We found that differential expression of skin proteins, including KRT7, KRT19, and PTGDS, in ESRD patients before and after kidney transplantation using iTRAQ-based proteomics. These findings provide molecular insight into the reversible skin pigmentary changes observed after transplantation and suggest potential biomarkers for cutaneous and systemic recovery in ESRD. Our results raise the possibility that targeting KRT or prostaglandin synthesis may represent novel therapeutic strategies to alleviate chronic skin symptoms, such as hyperpigmentation and pruritus, in long-term dialysis patients. Further studies are warranted to validate these targets and explore their therapeutic potential.

(A)


GYG1
TATDN1
OR1M1
NAGK
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PALMD
DCTN2
C11
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PTI1
NAA16
KRT7
PTGDS
CST3
GGCT
AMBP
DEFA1
PEBP4
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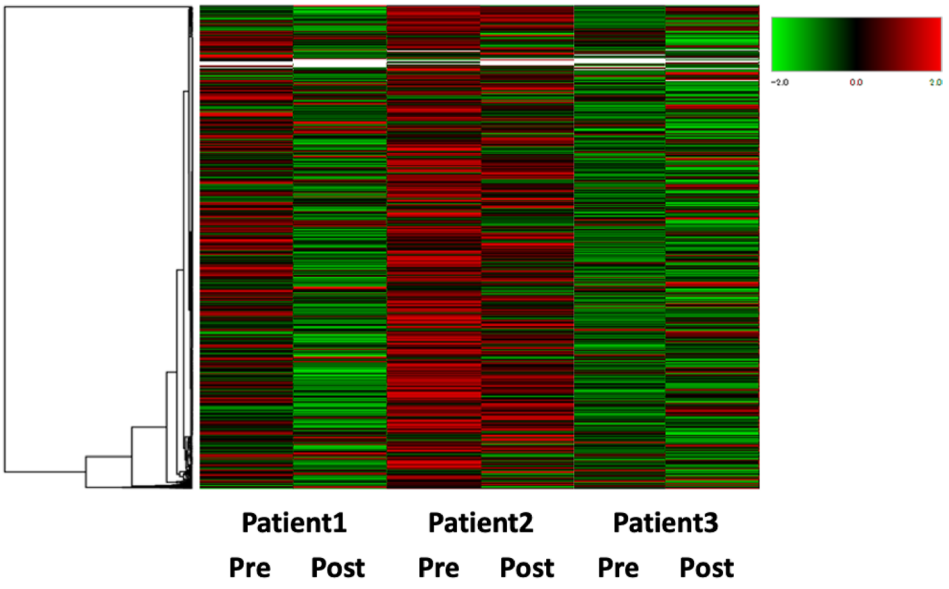
-1
log2(fold change)
1

(B)


Patient1
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Pre
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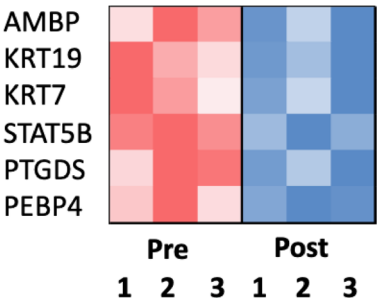
(C)


AMBP
KRT19
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Post
1 2 3 1 2 3

◂**Fig. 4**. (**A**) Volcano plot of differentially expressed proteins in skin biopsy specimens before and after kidney transplantation. Each dot represents an individual protein. The x-axis shows the $\log_2$ fold change ($\log_2$FC) of protein expression after transplantation compared to before transplantation, and the y-axis indicates the $-\log_{10}$ adjusted p-value (FDR-corrected). Proteins significantly upregulated post-transplantation ($\log_2FC > 1$, $p < 0.05$) are marked in red, while those significantly downregulated ($\log_2FC < -1$, $p < 0.05$) are marked in green. Selected proteins of interest, including KRT7, KRT19, and PTGDS, are annotated. (**B**) Hierarchical clustering heatmap of differentially expressed proteins in paired skin biopsy samples pre- and post-kidney transplantation. Each column represents a tissue sample, and each row corresponds to a differentially expressed protein. The color scale ranges from green (low expression) to red (high expression), indicating relative protein expression levels. Samples were collected at two time points: before transplantation (Pre) and one month after transplantation (Post). The clustering pattern illustrates distinct global proteomic profiles between Pre and Post samples, suggesting transplantation-induced molecular changes in the skin. (**C**) Heatmap of selected differentially expressed proteins (DEPs) pre- and post-transplantation. The heatmap shows the expression patterns of representative DEPs across paired skin samples collected before and after kidney transplantation. Rows represent proteins, and columns represent individual patient samples. Red and blue colors indicate upregulation and downregulation, respectively, relative to the mean expression. The color intensity reflects the magnitude of differential expression (log2 scale).

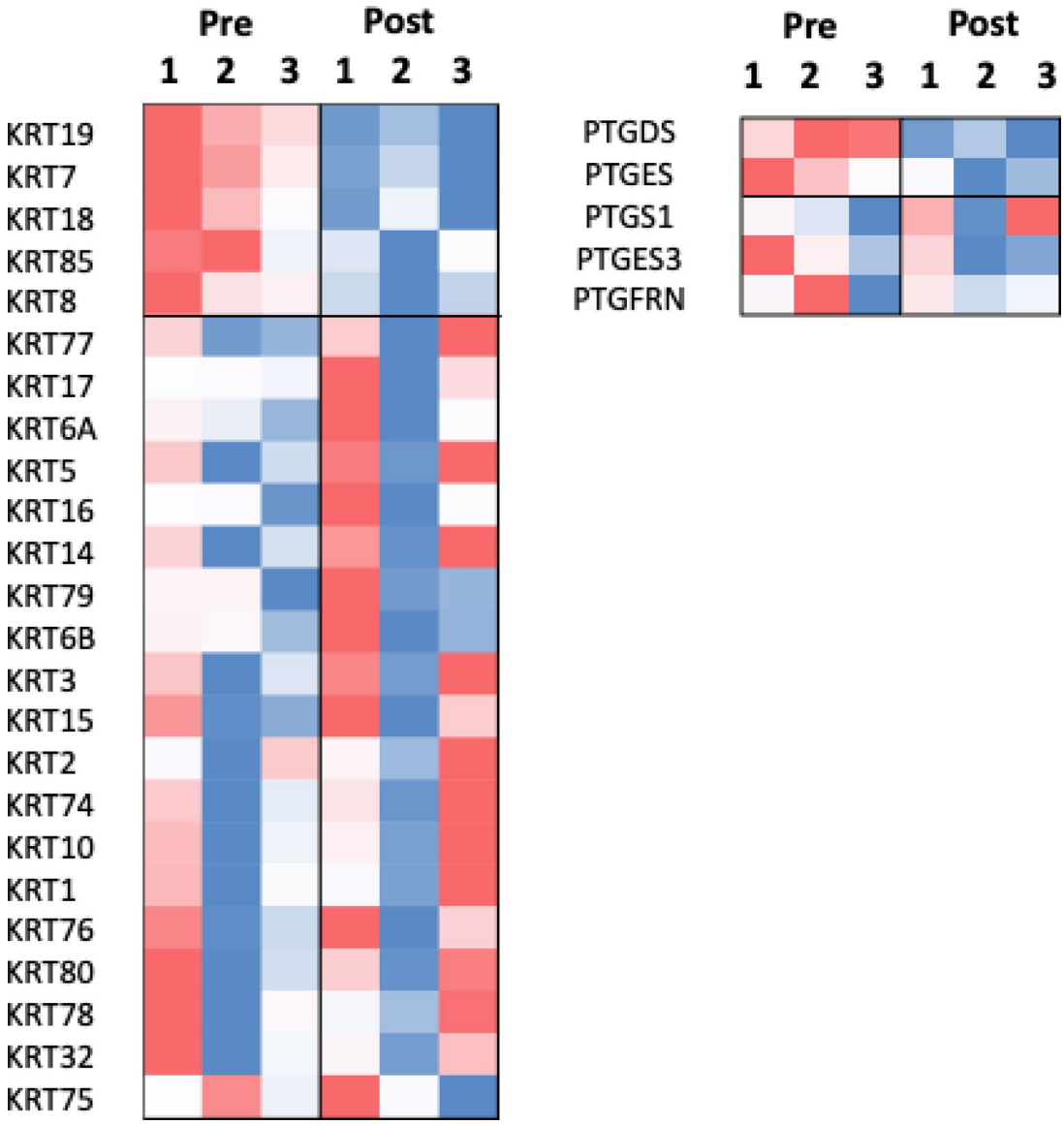

Fig. 5. The heatmap shows KRT and prostaglandin in pre- and post-transplantation skin samples. Red indicates higher expression and blue indicates lower expression.

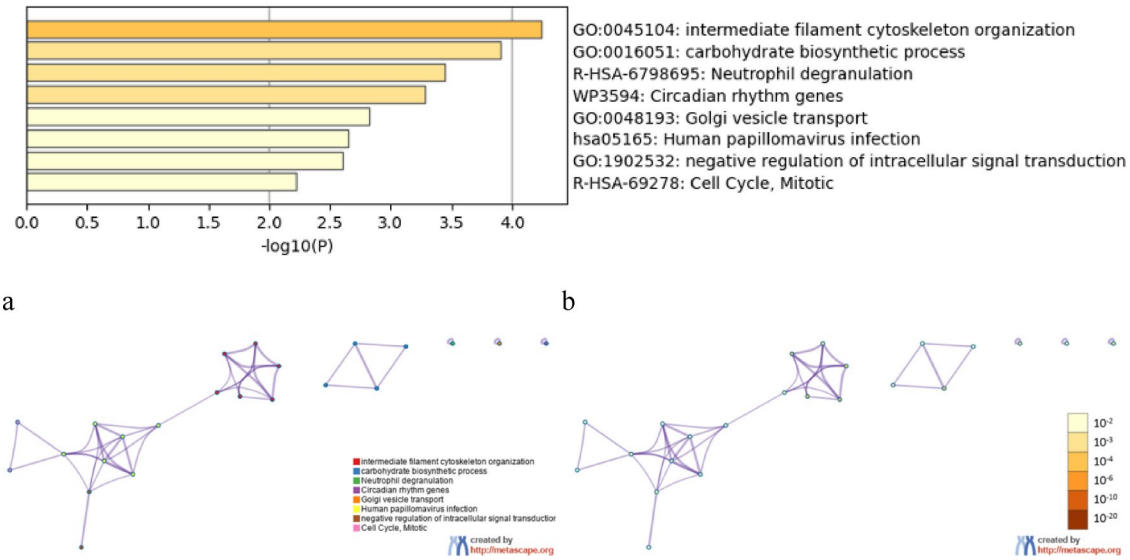

Fig. 6. Functional enrichment analysis of differentially expressed proteins using Metascape. (A) Enrichment bar graph: gene lists, colored by p-values. (B) Enrichment network: (**a**) colored by cluster ID, where nodes that share the same cluster ID are typically close to each other; (**b**) colored by p-value, where terms containing more genes tend to have a more significant p-value.

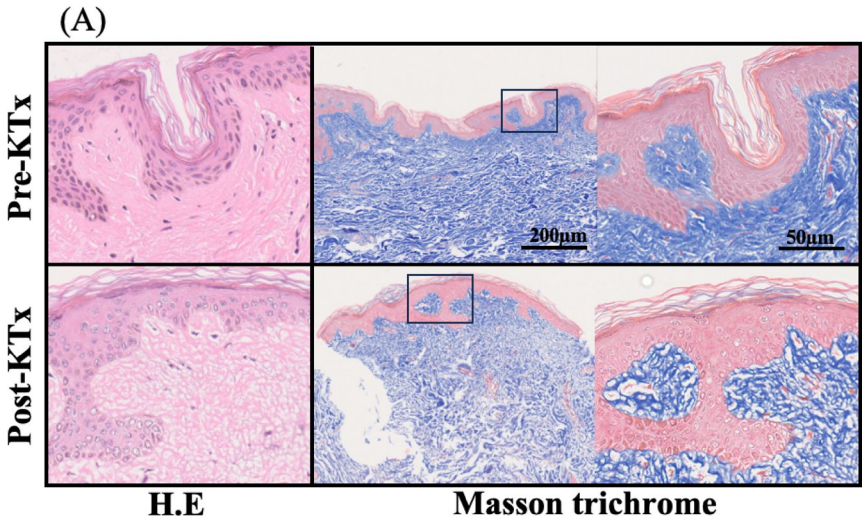

Fig. 7. Histological and immunohistochemical analysis of skin biopsy specimens before and after kidney transplantation. (**A**) Hematoxylin and eosin (H&E) staining and Masson's trichrome staining of representative skin sections. Upper panels show pre-transplantation samples, and lower panels show post-transplantation samples. Masson's trichrome staining indicates a visible decrease in dermal collagen density after transplantation (blue area), while H&E staining shows changes in epidermal thickness and dermal structure. (**B**) Immunohistochemical staining for cytokeratin 7 (KRT7), cytokeratin 19 (KRT19), and MelanA in matched skin samples in three patients. Positive signals appear as brown coloration. KRT7, KRT19, and MelanA expression was visibly reduced in the basal layer of the epidermis following transplantation. (**C**) Positive staining regions were identified and quantified using Fiji software, and are marked in red. (**D**) Quantification of positive area is shown in the bar graphs below each marker, normalized to the expression level before transplantation (set as 1.0) ($n = 3$).

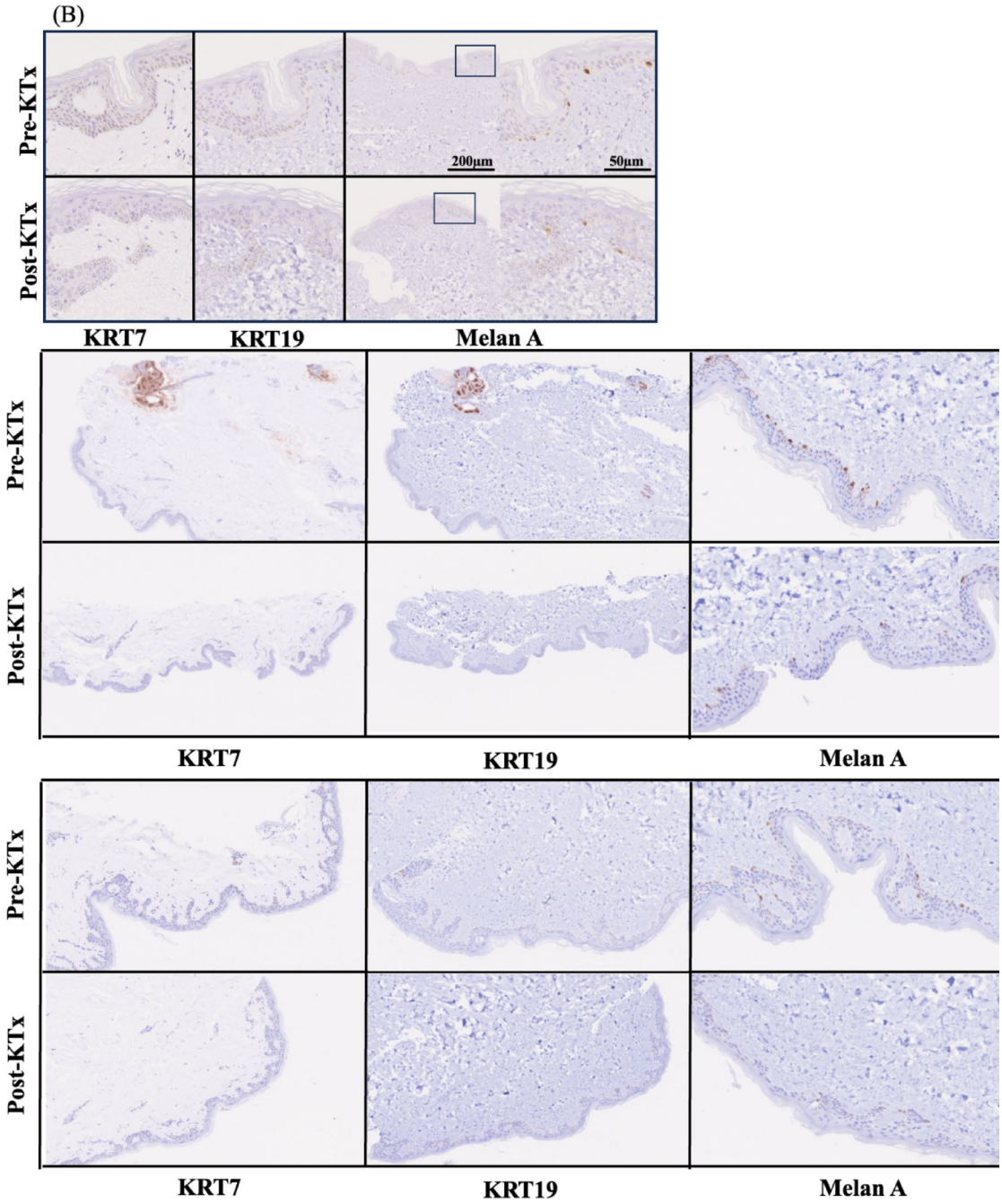

Fig. 7. (continued)

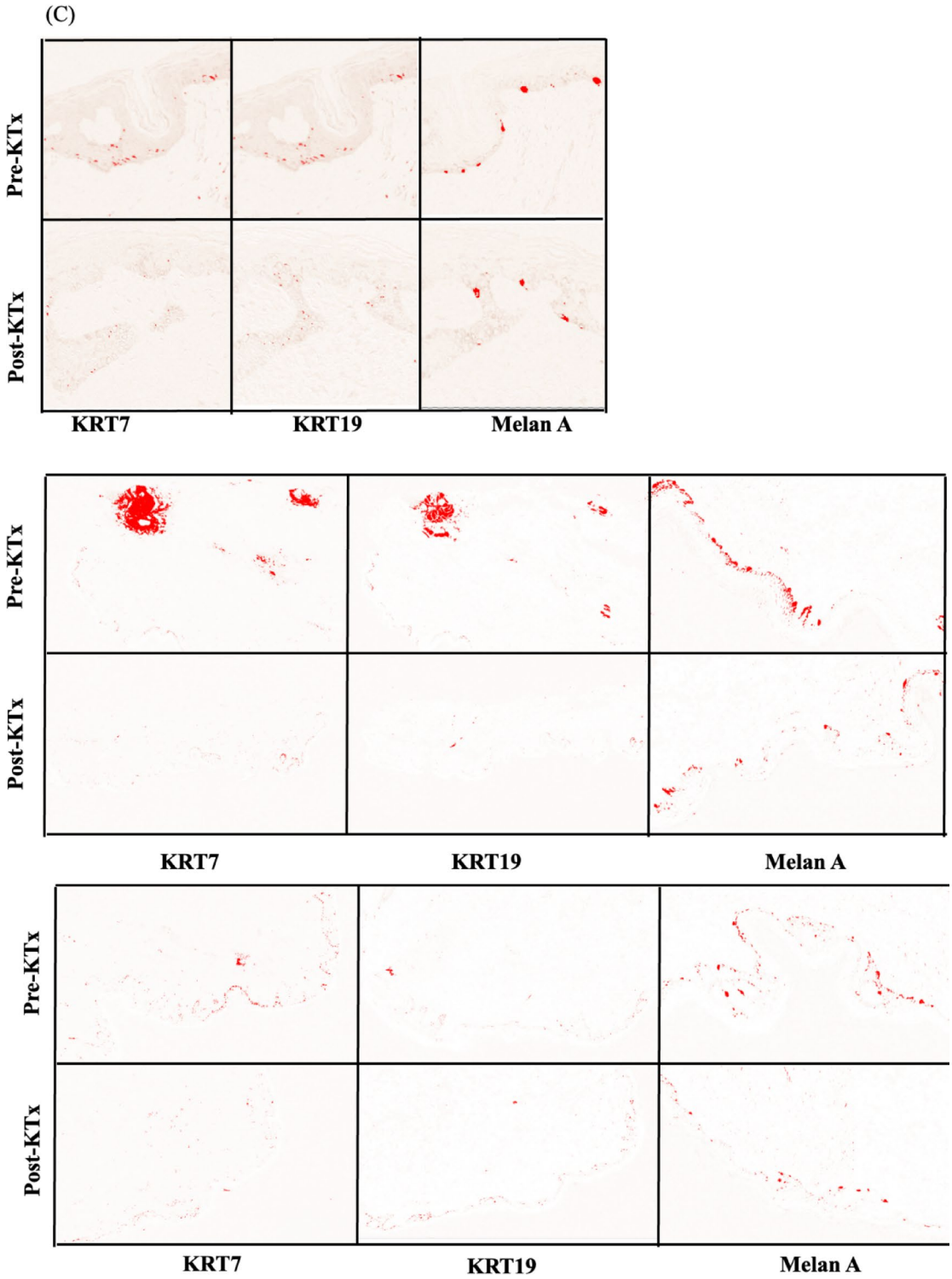

Fig. 7. (continued)

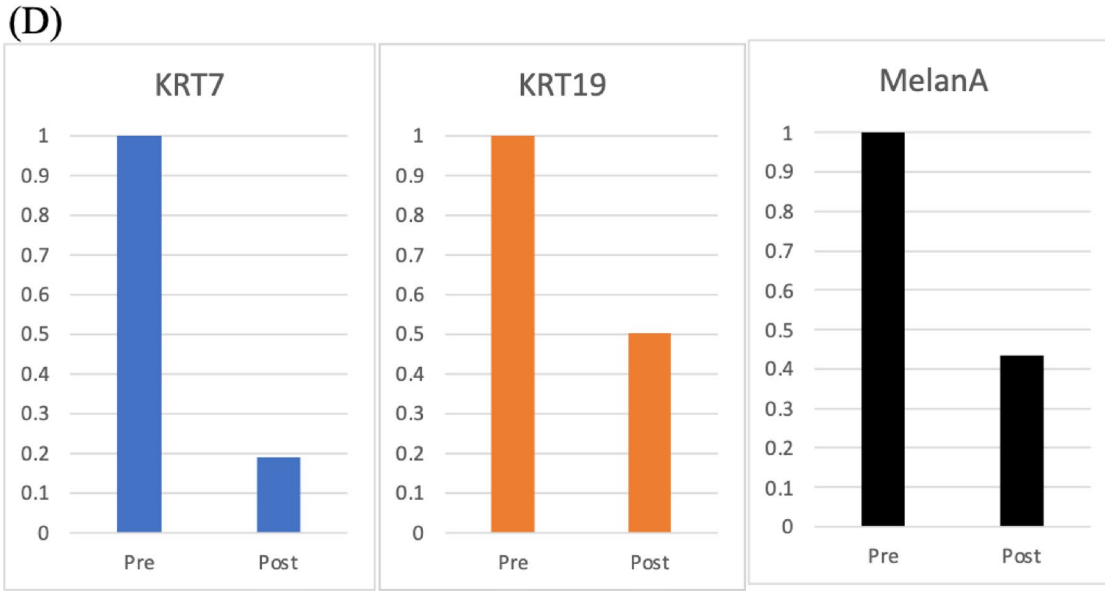

Fig. 7. (continued)

Data availability

The iTRAQ proteomics dataset is available in the Supplementary Information. All other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 27 June 2025; Accepted: 1 September 2025
Published online: 26 September 2025

References

1. Udayakumar, P. et al. Cutaneous manifestations in patients with chronic renal failure on Hemodialysis. *Indian J. Dermatol. Venereol. Leprol.* **72**, 119–125. https://doi.org/10.4103/0378-6323.25636 (2006).
2. Kolla, P. K. et al. Cutaneous manifestations in patients with chronic kidney disease on maintenance hemodialysis. *ISRN Dermatol* https://doi.org/10.5402/2012/679619 (2012).
3. Al-Thnaibat, M. H. et al. The skin manifestations in end-stage renal disease patients in Jordan, single-center experience. *BMC Nephrol.* https://doi.org/10.1186/s12882-024-03922-2 (2025).
4. Lai, C. F. et al. Quantitative comparison of skin colors in patients with ESRD undergoing different Dialysis modalities. *Am. J. Kidney Dis.* **48**, 292–300. https://doi.org/10.1053/j.ajkd.2006.04.087 (2006).
5. Porges, S. B., Kaidbey, K. H. & Grove, G. L. Quantification of visible light-induced melanogenesis in human skin. *Photodermatol* **5**, 197–200 (1988).
6. Takiwaki, H. Measurement of skin color: practical application and theoretical considerations. *J. Med. Invest.* **44**, 121–126 (1998).
7. Deleixhe-Mauhin, F., Krezinski, J. M., Rorive, G. & Piérard, G. E. Quantification of skin color in patients undergoing maintenance Hemodialysis. *J. Am. Acad. Dermatol.* **27**, 950–953. https://doi.org/10.1016/0190-9622(92)70292-n (1992).
8. Zhou, Y. et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* **10**, 1523. https://doi.org/10.1038/s41467-019-09234-6 (2019).
9. Kanehisa, M. & Goto, S. KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* **28**, 27–30. https://doi.org/10.1093/nar/28.1.27 (2000).
10. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. *Nat. Methods.* **9**, 676–682. https://doi.org/10.1038/nmeth.2019 (2012).
11. Rosenberg, M., Fuchs, E., Le Beau, M. M., Eddy, R. L. & Shows, T. B. Three epidermal and one simple epithelial type II keratin genes map to human chromosome 12. *Cytogenet. Cell. Genet.* **57**, 33–38. https://doi.org/10.1159/000133109 (1991).
12. Sarlos, D. P., Peterfi, L., Szanto, A. & Kovacs, G. Shift of keratin expression profile in End-stage kidney increases the risk of tumor development. *Anticancer Res.* **38**, 5217–5222. https://doi.org/10.21873/anticanres.12845 (2018).
13. Chandrasekaran, P., Weiskirchen, S., Weiskirchen, R. & Structure,. Functions, and Implications of Selected Lipocalins in Human Disease. *Int. J. Mol. Sci.* https://doi.org/10.3390/ijms25084290 (2024).
14. Urade, Y., Hayaishi, O. & Prostaglandin, D. Synthase: structure and function. *Vitam. Horm.* **58**, 89–120. https://doi.org/10.1016/s0083-6729(00)58022-4 (2000).
15. Melegos, D. N., Grass, L., Pierratos, A. & Diamandis, E. P. Highly elevated levels of prostaglandin D synthase in the serum of patients with renal failure. *Urology* **53**, 32–37. https://doi.org/10.1016/s0090-4295(98)00453-1 (1999).
16. Maesaka, J. K., Palaia, T., Fishbane, S. & Ragolia, L. Contribution of prostaglandin D2 synthase to progression of renal failure and Dialysis dementia. *Semin Nephrol.* **22**, 407–414. https://doi.org/10.1053/snep.2002.34726 (2002).
17. Conconi, M. T., Bruno, P., Bonali, A., De Angeli, S. & Parnigotto, P. P. Relationship between the proliferation of keratinocytes cultured in vitro and prostaglandin E2. *Ann. Anat.* **178**, 229–236. https://doi.org/10.1016/s0940-9602(96)80055-0 (1996).
18. Pentland, A. P. & Mahoney, M. G. Keratinocyte prostaglandin synthesis is enhanced by IL-1. *J. Invest. Dermatol.* **94**, 43–46. https://doi.org/10.1111/1523-1747.ep12873337 (1990).

Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP20K18141.

We would like to thank Ms. Satomi Yamashita and the organ transplant coordinators for their efforts in collection of data.

Author contributions

I.T. and Y.M. wrote the main manuscript text. I.T., Y.M., and Y.M. (Maruyama) collected and analyzed the clinical data. Y.M. (Maruyama) prepared Figs. 1, 2 and 3. K.Y., T.Y., T.S., S.T., S.N., K.B., and M.A. contributed to data interpretation and manuscript revision. All authors reviewed and approved the final manuscript.

Funding

The authors declare no funding for the study.

Declarations

Competing interests

The authors declare no competing interests.

Ethics

This clinical study was approved by the Okayama University Institutional Review Board prior to study initiation (Registration no. 951 and no. 2005-029). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-18391-2.

Correspondence and requests for materials should be addressed to Y.M.

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