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Graft Growth and Podocyte Dedifferentiation in Donor-Recipient Size Mismatch Kidney Transplants

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Background. Kidney transplantation is the treatment choice for patients with end-stage renal diseases. Because of good long-term outcome, pediatric kidney grafts are also accepted for transplantation in adult recipients despite a significant mismatch in body size and age between donor and recipient. These grafts show a remarkable ability of adaptation to the recipient body and increase in size in a very short period, presumably as an adaptation to hyperfiltration. **Methods.** We investigated renal graft growth as well as glomerular proliferation and differentiation markers Kiel-67, paired box gene 2 and Wilms tumor protein (WT1) expression in control biopsies from different transplant constellations: infant donor for infant recipient, infant donor for child recipient, infant donor for adult recipient, child donor for child recipient, child donor for adult recipient, and adult donor for an adult recipient. **Results.** We detected a significant increase in kidney graft size after transplantation in all conditions with a body size mismatch, which was most prominent when an infant donated for a child. Podocyte WT1 expression was comparable in different transplant conditions, whereas a significant increase in WT1 expression could be detected in parietal epithelial cells, when a kidney graft from a child was transplanted into an adult. In kidney grafts that were relatively small for the recipients, we could detect reexpression of podocyte paired box gene 2. Moreover, the proliferation marker Kiel-67 was expressed in glomerular cells in grafts that increased in size after transplantation. **Conclusions.** Kidney grafts rapidly adapt to the recipient size after transplantation if they are transplanted in a body size mismatch constellation. The increase in transplant size is accompanied by an upregulation of proliferation and dedifferentiation markers in podocytes. The different examined conditions exclude hormonal factors as the key trigger for this growth so that most likely hyperfiltration is the key trigger inducing the rapid growth response.

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Kidney transplantation should be the treatment of choice for patients with end-stage renal diseases because it has the lowest mortality and best quality of life for the patients compared with all other forms of renal replacement therapy.[1,2] Especially for children, transplantation has significant advantages also for physical growth and mental development.[3-5] Due to the discrepancy between the lack of donors and the increasing number of patients on waiting lists, donor recipient conditions have been extended during the last years. In an older study, recipients from donors younger than 24 months had significantly poorer outcome, with no kidney surviving longer than 2 months.[6] However, with improving transplant techniques, pediatric kidney transplantation is the treatment of choice even in very small children. Becker et al[7] published a study with kidney transplantations in children with a body weight less than 11 kg. This study also included 3 cases of donors younger than 1 year with good clinical outcome.

A child can receive a kidney from an adult donor, because there is usually enough space in the belly to fit the new kidney when body weight is more than 7 kg.[7] However, adult donor kidneys in pediatric recipients decrease glomerular filtration rate (GFR) in the early stages and lack an increase in GFR with growth of the child.[8]

On the other hand, pediatric kidneys have been transplanted in adult recipients for many years.[9,10] Pediatric kidney transplantation into adult recipients can be done as en bloc kidney transplantation, transplanting both kidneys, or as single kidney transplantation.[11,12] When pediatric kidneys are transplanted in adults, there is usually a mismatch between the transplant kidney size and the body size of the recipient that might raise concerns. Life table analysis revealed no difference in graft survival in recipients of kidneys from donors aged 2 to 15 years compared with adult donors.

Low weight of the kidney graft to the weight of the recipient, however, was described to be an independent risk factor for poor long-term graft survival in a multicenter cohort study.[13] Moreover, Tan et al[14] reported that also donor-recipient sex mismatch affects renal allograft survival due to immune responses to sexually determined minor histocompatibility antigens. Similarly, Miller et al[15] found that a mismatch in donor-recipient weight and donor-recipient sex is associated with a higher risk of death-censored graft loss in kidney transplantation.[16]

In our center, we also accepted pediatric kidney grafts for transplantation in adult recipients in body size mismatch conditions, and we noticed a rapid increase in size in the transplanted pediatric kidneys already detectable shortly after transplantation. In child recipients, the size of pediatric grafts doubled in the first years after transplantation, whereas adult grafts had a stable size.[17]

Growth of kidney grafts in the recipient has already been described many years ago,[9,10] but how rapid these adaptations occur and the mechanistic orchestration of this growth has never been studied. It is also not known if different donor recipient age conditions effect transplant growth. In addition, it is unclear if the growth is a result of proliferation or hypertrophy of cells.

We performed ultrasound follow-ups of kidney grafts transplanted in different donor to recipient age combinations to investigate transplant growth within the first 6 months after transplantation and stained transplant biopsies for different proliferation and podocyte differentiation markers early after transplantation as well as 6 months later.

An infant was defined as being between 1 month and 2 years of age, a child was defined as being between 2 and 18 years of age, and adults were patients older than 18 years.

MATERIALS AND METHODS

Kidney Transplantations

In total, 9 renal transplant patients with different transplant conditions were investigated. The following kidney donor-recipient conditions were investigated: kidney from an infant donor for infant recipient (n = 1), kidney from an infant donor for child recipient (n = 1), kidney from an infant donor for adult recipient (n = 1), kidney from a child for child recipient (n = 2), kidney from a child for adult recipient (n = 2), and kidney graft from an adult for adult recipient (n = 2).

Patients' Inclusion and Exclusion Criteria

A transplant biopsy around the time of transplantation (t0 biopsy, < 1.5 month after transplantation) and 6 months after transplantation (t1 biopsy) had to be available. Patients or their parents had to give consent about the use of biopsy sections and clinical data for research analysis. Biopsies with any type of rejections, recurrence of disease or significant interstitial fibrosis, and tubulus atrophy (IFTA greater than 30%) in t0 or t1 biopsy were excluded.

Immunohistochemistry of Transplant Biopsies for Different Proliferation Marker

Transplant kidney biopsies were formalin-fixed and paraffin-embedded. Two-micron thick sections were rehydrated in a graded series of alcohol, blocked with 1% bovine serum albumin and stained with paired box gene 2 (PAX2), Wilms tumor protein (WT1), and Kiel-67 (Ki67). The following antibodies and concentrations were used: WT1 (Leica Biosystems, Newcastle, UK), 1:20, heat retrieval citrate buffer, pH 8.2; Ki67 (Thermo Scientific, Warm Springs, CA), 1:100, citrate buffer pH 8.2; PAX2 (Zeta Corporation, Arcadia, CA), 1:20, citrate buffer pH 8.2.

Statistical Analysis

Length of kidney transplant at time of transplantation (t = 0) and 6 months later (t1) was given as absolute values in cm and growth in kidney transplant between both time points was given in percent of increase. WT1-, PAX2-, and Ki67-positive podocytes and parietal cells were counted on the whole kidney biopsy section in a blinded fashion and given as positive podocytes and parietal cells per glomerular section surface area (mm^2). Mean and SD were given. Analysis of variance was used to test for significance with $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

RESULTS

Graft Size Increases Rapidly in Small for Recipient Constellations

Ultrasound measurements of kidney grafts directly after transplantation (t0, > 1.5 months after transplantation) and 6 months later (t1) detected an increase in transplant size. The increase in kidney graft length correlated with donor-recipient age mismatch with highest rate of growth when a kidney from an infant donor was transplanted to a child recipient. Within 6 months, a kidney from an infant donor increased by 7% when transplanted into another infant, by 49% after transplantation in a child, and by 28% after transplantation into an adult recipient. Kidney grafts from children increased by 13% and 15% after being transplanted in children and by 22% and 35% after transplantation in adults. Kidney grafts from adults only slightly increased in size 6 months after transplantation in adult recipients. Absolute numbers in growth and recipient body surface/graft length at t0 can be found in Table 1 and Figure 1. Recipient body surface/graft length at t0 was about 8 if a kidney from a child was transplanted in another child. This ratio increased to 15 if a kidney graft from an infant was transplanted in a child 6 months later. A recipient body surface/graft length of 16 in adult donor for adult recipient at transplantation increased to 22 and 25 when a kidney graft from a child or rather an infant was transplanted in an adult recipient 6 months after transplantation. To investigate the glomerular changes coming along with the increase in kidney transplant size, we stained kidney graft biopsies early after transplantation and 6 months later for different proliferation and podocyte differentiation marker.

TABLE 1.

Ultrasound measurements of kidney graft length at time of transplantation (t0) and 6 months later (t1) as well as recipient body surface/graft length at t0

Donor-recipient	Infant-infant	Infant-child	Infant-adult	Child-child		Child-adult		Adult-adult	
Recipient body surface, m^2	0.59	1.05	1.83	0.75	0.72	1.82	1.78	1.81	1.79
Recipient body surface/graft length at t0, m	8.81	15.67	24.7	9.03	8.37	21.92	24.05	16.76	15.98
Kidney graft length (t0), cm	6.5	6.7	7.4	8.3	8.6	8.3	7.4	10.8	11.1
Kidney graft length (t1), cm	7.4	10.0	10.0	10.1	9.8	10.1	8.4	11.3	11.5

Altered WT1 Expression in Kidney Grafts Small for Recipient Size

The number of WT1 positive podocytes and parietal epithelial cells per mm^2 glomerular surface area of the biopsy section were quantified in t0 biopsies and transplant biopsies 6 months after transplantation. Podocyte WT1 staining in transplant biopsies from infant, child, and adult donors were comparable. Moreover, there was no significant change in podocyte WT1 expression in implant kidney biopsies in the different donor-recipient condition (Figures 2A, B). In contrast to that, parietal epithelial cell WT1 expression was significantly higher in a kidney graft from an infant donor compared with kidney grafts from child and adult donors. If a kidney graft from a child was transplanted in an adult patient, parietal epithelial cell WT1 expression was significantly increased 6 months after transplantation (Figures 2A, C).

Reexpression of Glomerular PAX2 in Kidney Grafts Small for Recipient Size

Next, we quantified PAX2-positive podocytes and parietal epithelial cells per mm^2 glomerular surface area on t0 and t1 transplant kidney biopsies. Glomerular PAX2 expression was absent in adult kidneys (t0 biopsies from adult donors), whereas it was only weakly detectable in glomeruli from infant and child donors (t0 biopsies from infant and child). Podocyte PAX2 expression was significantly induced if a kidney graft from an infant was transplanted in an adult recipient 6 months after transplantation compared with the t0 biopsy of the infant kidney graft. Moreover, PAX2 was also reexpressed in podocytes in kidney grafts from children transplanted in adults (Figures 3A, B).

In glomerular parietal epithelial cells, PAX2 expression was significantly more detectable in early life compared with adulthood (t0 biopsies of infant and children donors compared to adult donors). PAX2 expression in parietal epithelial cells was further upregulated if kidneys from infant or child donors were transplanted in older recipients (Figures 3A, C).

Increased Glomerular Ki67 Expression in Kidney Grafts Small for Recipient Size

We also examined proliferation marker Ki67 expression in glomerular cells per mm^2 glomerular surface area. We found that in general more Ki67 was detectable in t0 kidney biopsies from an infant compared with child or adult donors. Ki67 was also reexpressed in glomerular cells when the kidney graft from an infant was transplanted into an adult recipient (t1 biopsy). The most prominent change in Ki67 expression was detectable when the kidney graft of a child was transplanted in an adult recipient. Interestingly, staining also seems to be localized in podocytes. In contrast, there was only little Ki67 detectable in the glomeruli of adult kidney donors in t0 as well as t1 biopsies (Figures 4A, B).

Tubular Ki67 staining was comparable between the different transplant conditions except for the condition where a kidney from an infant was transplanted in an adult. Here, Ki67 was significantly upregulated 6 months after transplantation (Figure 4C).

DISCUSSION

In the past, there have been concerns about disparity of donor and recipient weight in pediatric to adult kidney transplantations regarding hyperfiltration syndrome, which can be seen when nephron number is insufficient in relation to body size. Hyperfiltration can cause hypertension, proteinuria, and glomerulosclerosis.[18,19] This phenomenon is seen most commonly when 1 kidney is congenitally absent or lost later in life, in diabetes, or obesity.[20] Hyperfiltration with shortened graft survival has also been reported when smaller

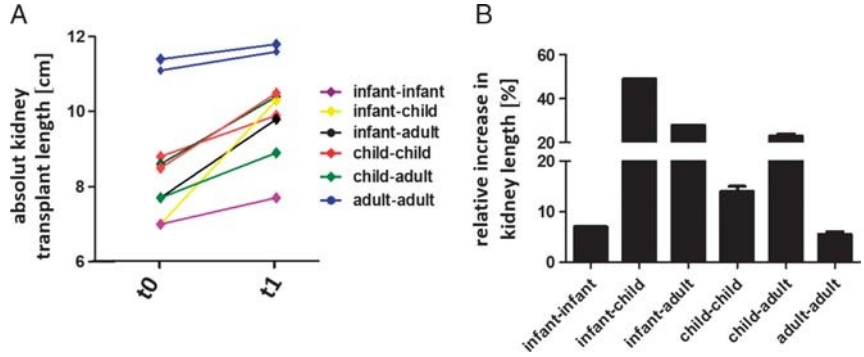

FIGURE 1. Transplanted kidneys grow if they are small for recipient size. A, Kidney graft length (cm) measured by ultrasound directly after transplantation (t0) and 6 months posttransplantation (t1) in different donor-recipient constellations: infant-infant, infant-child, infant-adult, child-child, child-adult, and adult-adult. Total, n = 9 patients. B, Growth in kidney transplant between t0 and t1 is given as percent of increase.

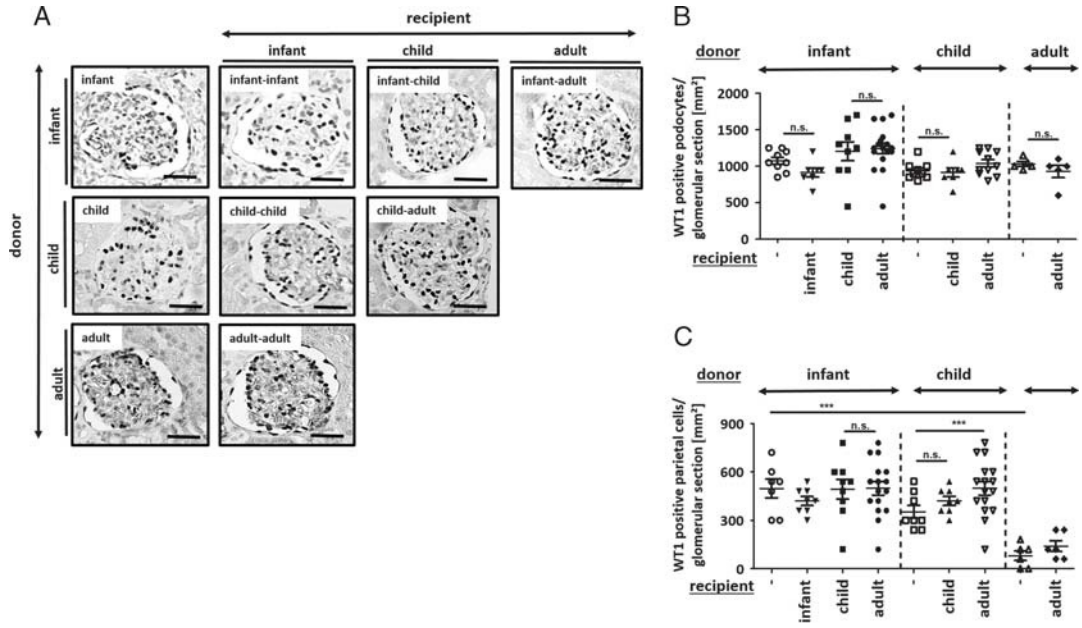

FIGURE 2. Altered WT1 expression in kidney grafts small for recipient size. WT1 staining of transplant biopsies shortly after transplantation (t0 biopsy, Aa, Ba, Ca) and 6 months after transplantation (t1 biopsy, Ab-d, Bb-c, Cb). Kidney from an infant donor transplanted in an infant (Ab, infant-infant, n = 1), in a child (Ac, infant-child, n = 1) or in an adult (Ad, infant-adult, n = 1). Kidney from child donors transplanted in children (Bb, child-child, n = 2) or in an adult (Bc, child-adult, n = 2). Kidney from adult donors transplanted in adults (Cb, adult-adult n = 2). Quantification of WT1 positive podocytes and parietal epithelial cells is given in the right panels as positive podocytes a.e. parietal cells per glomerular section (mm^2) for the different transplant constellations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Scale bar = 50 µm.

adult kidneys are transplanted into larger recipients.[21] Per a study by Andres et al, weight disproportion between donor and recipient, unfavorable to the recipient, can be a risk factor for the development of chronic allograft nephropathy.[22] Therefore, kidneys from pediatric donors have often been primarily allocated for children with exception of Eurotransplant allocation system where kidneys from pediatric donors are often not primarily allocated to pediatric recipients.[5,23]

However, Halldorson et al[24] reported that pediatric en bloc transplanted kidneys adapt to the workload of acquired obesity without evidence of hyperfiltration injury. They suggested that hyperfiltration syndrome results from weight gain

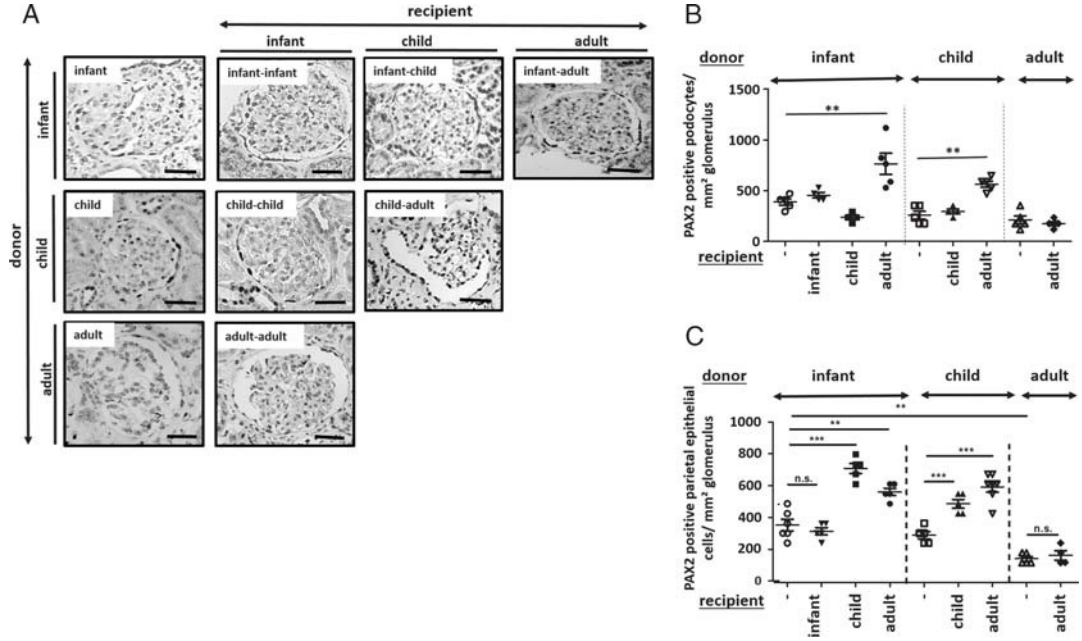

FIGURE 3. Reexpression of glomerular PAX2 in kidney grafts small for recipient size. PAX2 staining of transplant biopsies shortly after transplantation (t0 biopsy, Aa, Ba, Ca) and 6 months after transplantation (t1 biopsy, Ab-d, Bb-c, Cb). Kidney from an infant donor transplanted in an infant (Ab, infant-infant, n = 1), in a child (Ac, infant-child, n = 1) or in an adult (Ad, infant-adult, n = 1). Kidney from child donors transplanted in children (Bb, child-child, n = 2) or in an adult (Bc, child-adult, n = 2). Kidney from adult donors transplanted in adults (Cb, adult-adult n = 2). Quantification of PAX2 positive podocytes and parietal epithelial cells is given in the right panels as positive podocytes a.e. parietal cells per glomerular section (mm^2) for the different transplant constellations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Scale bar = 50 µm.

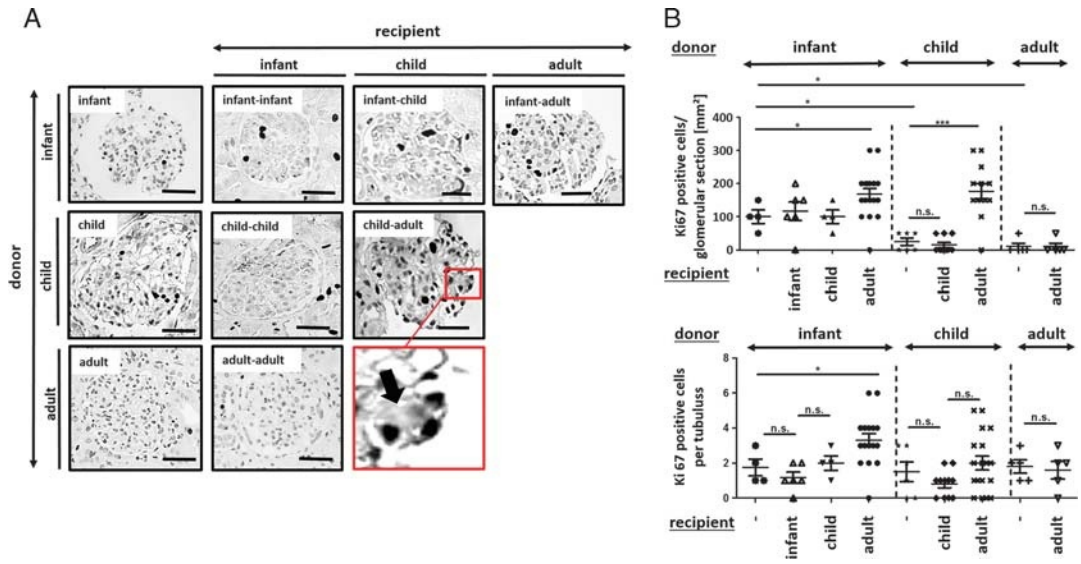

FIGURE 4. Increased glomerular Ki67 expression in kidney grafts small for recipient size. Ki67 staining of transplant biopsies shortly after transplantation (t0 biopsy, Aa, Ba, Ca) and 6 months after transplantation (t1 biopsy, Ab-d, Bb-c, Cb). Kidney from an infant donor transplanted in an infant (Ab, infant-infant, n = 1), in a child (Ac, infant-child, n = 1) or in an adult (Ad, infant-adult, n = 1). Kidney from child donors transplanted in children (Bb, child-child, n = 2) or in an adult (Bc, child-adult, n = 2). Kidney from adult donors transplanted in adults (Cb, adult-adult n = 2). Quantification of Ki67 positive glomerular cells is given in the right panels as positive podocytes per glomerular section (mm^2) a.e. positive cells per tubulus for the different transplant constellations. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bar = 50 µm. Insert: Magnification of Ki67 positive podocytes (arrow).

only in recipients with fully matured kidney grafts, which are unable to adapt to an increasing workload. An analysis of the outcome of kidneys from pediatric donors either en bloc or as a single kidney transplant into recipients older than 15 years revealed similar 1-year graft survival.[25] Thus, the use of pediatric cadaver kidneys can provide adequate graft function in both pediatric and adult recipients and their use increases the number of organs available for transplantation.

However, from a pediatric point of view, the main reason to use pediatric donors for children is that adult donor kidneys in pediatric recipients decrease GFR in the early stages and lack an increase in function with the growth of the child.[8,23,26] Therefore, we once suggested that the goal for the future may be an age-matched organ allocation of children because this also provides higher long-term GFRs and graft survival as well as better psychosocial rehabilitation, growth, and development of children.[5]

We and others noticed an increase in the transplanted pediatric kidney grafts size shortly after transplantation.[9,10,17] Rosenbaum et al[27] published that the formula for normal renal length in children older than 1 year is renal length (cm) = 6.79 + 0.22 × age (years). However, there is no formula available for proper growth of kidney transplants in children.

In our cohort, kidney transplant growth was most significant in grafts small for recipient body size. An increase in size after transplantation that remained constant during 5-year follow-up among renal allograft recipients has also been described.[28] Nghiem et al[29] demonstrated an average of threefold physical enlargement for pediatric en bloc kidneys in the first 6 months posttransplantation by ultrasound. However, the absolute nephron number is believed to be determined at birth and cannot increase.[30] Thus, the question arises on how transplanted kidneys can still grow.

In general, adult glomeruli are larger than glomeruli from children, and glomerular hypertrophy is usually a compensatory mechanism that serves to match physiological demands. In rats, the function of transplanted kidneys adapted to the body size of the recipient if recipient and donor had unequal body size.[31] Kidney transplants with low weight in relation to recipient body weight had a higher graft filtration rate compared with conditions with high renal graft weight.[13] Another study noticed that all pediatric renal allografts experienced significant hypertrophy over time.[25]

To correlate transplant growth to changes in glomerular expression, we analyzed podocyte differentiation and proliferation markers in transplant kidney biopsies with different donor-recipient conditions at the time of transplantation and 6 months later. Estimating podocyte numbers in renal biopsies is challenging. Different stereological approaches were described for quantification of podocyte number.[32] Novel methods that combine serial sectioning of paraffin-embedded tissue, immunohistochemistry, confocal microscopy were recently suggested to count glomerular podocytes even more precisely.[33,34] When whole kidneys or large tissue samples are available, stereological methods are considered as gold standard for the estimation of podocyte numbers. However, stereological approaches are time-consuming, expensive, not standardized, and inapplicable when dealing with small samples, such as biopsies. Therefore, the most commonly used method for the assessment of podocytes is counting podocyte nuclei per glomerular cross-section. In our approach with biopsy material only, we quantified podocyte and parietal epithelial cells positive for differentiation and proliferation markers by counting the cells per mm^2 glomerular surface on the biopsy sections.

First, we examined the expression of WT1 which is one of the most important transcriptional regulators involved in nephrogenesis.[35] During kidney development, WT1 is expressed initially in mesenchymal cells and then becomes restricted to podocytes along with the maturation of the glomerulus. The expression of WT1 in the podocytes persists into adult life. In line with this, the number of WT1-positive

podocytes was comparable between kidney grafts from different age groups and after transplantation with different donor-recipient constellations. However, WT1 expression in parietal epithelial cells was significantly higher in kidneys from infants compared with children and adults. Furthermore, there was a significant increase in parietal cell WT1 expression in kidney grafts from children transplanted into adult recipients 6 months after transplantation. Thus, WT1 expression is altered in parietal epithelial cells in kidney grafts that are small for recipient size as a first hint for an adaptation in glomerular expression.

Even though WT1 is thought mainly as a podocytes differentiation, studies have shown that WT1 may also stain parietal epithelial cells either alone or together with claudin. Thus, another hypothesis for the altered WT1 expression is that parietal cells may transdifferentiate into podocytes.[36]

Next, we examined glomerular PAX2 expression in the different transplant conditions. During kidney development, podocytes express PAX2 at the renal vesicle stage, but this marker is downregulated and not detectable in mature podocytes. The nuclei of parietal epithelial cells of Bowman's capsule however remain strongly positive for PAX2.[37] A persistent expression of podocyte PAX2 was found in a variety of cystic and dysplastic renal diseases.[38] Moreover, ectopic expression of PAX2 in podocytes has been reported in various glomerular diseases. In cellular lesions of focal segmental glomerulosclerosis, reexpression of podocyte PAX2 and loss of podocyte WT1 expression was described.[37] Abnormal distribution of WT1 and PAX2 was observed in immune complex glomerulonephritis and HIV associated nephritis, and this dysregulation was associated with podocyte proliferation.[39]

PAX2 has also been identified in the undifferentiated metanephric mesenchyme and is required for proper differentiation of the metanephric kidney. Therefore, it is an early marker of kidney progenitor cells.[40] Bruno and Camussi[41] reported that human glomeruli deprived of the Bowman's capsule contained a population of CD133(–) CD146(+) cells that coexpress typical mesenchymal stem cells markers and renal-specific stem cell markers among which was PAX2. This cell population could differentiate into endothelial cells, epithelial cells expressing podocytes markers, and mesangial cells.

In our study, PAX2 was upregulated in parietal epithelial cells and podocytes if kidney grafts of infants or children were transplanted into older recipients. Therefore, we hypothesize that a mismatch in kidney transplant size to recipient size induces a dedifferentiation response of podocytes resulting in reexpression of PAX2 and proliferation. However, as mentioned above, PAX2 reexpression in parietal cells may also suggest activation of precursor/stem cell population.[42]

We also examined Ki67 expression in the different transplant conditions. Ki67 is a nuclear antigen that is only detectable in proliferating cells during all active phases of the cell cycle.

Glomerular Ki67 staining was strongest when kidney grafts from infants were transplanted into adult recipients 6 months after transplantation. This correlated with the most prominent increase in graft size in this transplant constellation. In contrast to that, we could not detect induction of Ki67 expression within the graft after kidney transplantation between adults.

In summary, kidney grafts grow after transplantation if they are small for recipient size. We speculate that the hyperfiltration of transplanted kidneys small for recipient body size leads to a dedifferentiation response in all glomerular epithelial cell types in an attempt to adapt to the volume expansion for the glomeruli. The constant podocyte numbers indicate that no "true" proliferation occurs but that the fast-necessary adaptation responses require a less "static" phenotype, resulting in dedifferentiation and reexpression of proliferation markers.

Our study is limited by the small case number and our simplified approach of counting cells per mm^2 glomerular surface area. However, this is the first systematic documentation of glomerular cell densities and marker expressions in different transplant age and size constellations.

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