The Substantial Loss of Nephrons in Healthy Human Kidneys with Aging

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ABSTRACT

Nephron number may be an important determinant of kidney health but has been difficult to study in living humans. We evaluated 1638 living kidney donors at Mayo Clinic (MN and AZ sites) and Cleveland Clinic. We obtained cortical volumes of both kidneys from predonation computed tomography scans. At the time of kidney transplant, we obtained and analyzed the sections of a biopsy specimen of the cortex to determine the density of both nonsclerotic and globally sclerotic glomeruli; the total number of glomeruli was estimated from cortical volume×glomerular density. Donors 18–29 years old had a mean 990,661 nonsclerotic glomeruli and 16,614 globally sclerotic glomeruli per kidney, which progressively decreased to 520,410 nonsclerotic glomeruli per kidney and increased to 141,714 globally sclerotic glomeruli per kidney in donors 70–75 years old. Between the youngest and oldest age groups, the number of nonsclerotic glomeruli decreased by 48%, whereas cortical volume decreased by only 16% and the proportion of globally sclerotic glomeruli on biopsy increased by only 15%. Clinical characteristics that independently associated with fewer nonsclerotic glomeruli were older age, shorter height, family history of ESRD, higher serum uric acid level, and lower measured GFR. The incomplete representation of nephron loss with aging by either increased glomerulosclerosis or by cortical volume decline is consistent with atrophy and reabsorption of globally sclerotic glomeruli and hypertrophy of remaining nephrons. In conclusion, lower nephron number in healthy adults associates with characteristics reflective of both lower nephron endowment at birth and subsequent loss of nephrons.

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It is generally accepted that the number of nephrons relates to the functional capacity of the kidney. With normal aging, nephron loss occurs and is detectable to some extent by the age-related decrease in GFR.[1] In addition, low nephron endowment at birth may lead to hypertension and CKD in adult life.[2–5]

Prior studies have used precise methods to count glomeruli. The disector/fractionator technique applied to kidneys obtained at autopsy has identified significant variability in nephron number per kidney, ranging from 200,000–2,000,000.[6–11] A decline in nephron number with aging is consistently evident in autopsy series, but the extent to which this decline in nephron number can be detected in living patients has been unclear. It is unknown whether the decrease in cortical volume with aging[12] or the increase in glomerulosclerosis on biopsy with aging[13,14] accurately detects the decline in nephron number. Whether nephron number associates with kidney function and risk factors is not easily determined from autopsy series.

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Few studies have attempted to estimate nephron number in living humans. One approach is to estimate the whole-kidney K_f calculated from renal plasma flow *via* para-aminohippurate clearance and then divide by the single-nephron K_f estimated from electron microscopic measures of the glomerular filtration barrier on kidney biopsy.[15–17] This approach only estimates the number of functional (nonsclerotic) glomeruli and does not count the number of nonfunctional (globally sclerotic) glomeruli. Alternatively, the density of nonsclerotic glomeruli (NSG) and globally sclerotic glomeruli (GSG) on a kidney biopsy specimen can be multiplied by the volume of the kidney cortex measured with imaging to estimate the number of glomeruli. To our knowledge, this approach has only been applied to a small series of 39 patients.[18]

Living kidney donors at some centers, including ours, have both a predonation contrast computed tomography (CT) scan that delineates kidney cortex and an intraoperative biopsy of the kidney cortex at the time of transplantation, allowing for estimation of nephron number. Our primary hypothesis was that the decline in nephron number with aging is proportional to the decline in cortical volume on CT scan and to the increase in glomerulosclerosis on biopsy. Our secondary hypothesis was that lower nephron number would associate independently with multiple clinical characteristics that are reflective of lower nephron endowment at birth and subsequent loss of nephrons later in life.

RESULTS

There were 1638 living kidney donors that had both a predonation CT scan to segment the kidney cortex and an intraoperative biopsy of the cortex during transplant surgery (1270 at Mayo Clinic, MN, 266 at Mayo Clinic, AZ, and 102 at Cleveland Clinic, OH) (Figure 1). Measured GFR was available in only 1523 (93%) and 24-hour urine albumin in only 1359 (83%). Luminal stenosis measures were available in the 1424 (87%) donors who had an artery present on biopsy. The baseline demographic, clinical, biopsy and CT characteristics of kidney donors are presented in Table 1. The cortex area, the presence of capsule, or the presence of medulla on the biopsy did not associate with age (data not shown).

Overall, there were a mean 873,696 NSG and 41,435 GSG per kidney. There were a mean 990,661 NSG and 16,614 GSG in 18–29 year olds that progressively decreased to 520,410 NSG and increased to 141,714 GSG in 70–75 year olds ($P<0.001$ for both trends). The decline in number of NSG was approximately proportional to the decline in GFR (Figure 2). Men had 85,204 more NSG than women ($P<0.001$), but the age-related decline in NSG number did not differ between men and women ($P=0.73$ for age×sex interaction). We compared the decrease in nephron number (number of NSG) across age groups to the decrease in cortical volume and the increase in the percentage of GSG across age groups (Supplemental Table 1). Compared with 18–29 year olds, 70–75 year olds had a 48% lower nephron number ($P<0.001$), a 16% smaller cortical volume ($P=0.01$), and a 15 percentage points higher percentage of GSG ($P<0.001$). The slope of structural changes by age showed nephron number to decrease by 7.3% per age decade ($P<0.001$), cortical volume to decrease by 3.7% per age decade ($P<0.001$), and the percentage of GSG to increase by 1.3 percentage points per age decade ($P<0.001$). The number of missing glomeruli increased with age (Supplemental Figure 1).

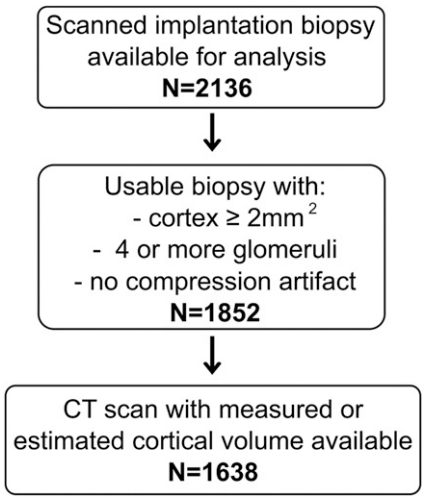

Figure 1. Selection of study sample.

Nephron number correlated with biopsy characteristics. Lower nephron number correlated with larger nephron size as determined by profile tubular area (Spearman rank coefficient [r_s] $=-0.273$, $P<0.001$; age–sex-adjusted $r_s=-0.28$, $P<0.001$) and by NSG volume ($r_s=-0.47$, $P<0.001$; age–sex-adjusted $r_s=-0.51$, $P<0.001$) (Figure 3). Lower nephron number also correlated with nephrosclerosis as determined by percentage of GSG ($r_s=-0.12$, $P<0.001$; age–sex-adjusted $r_s=-0.07$, $P<0.001$), percentage of fibrosis ($r_s=-0.05$, $P=0.04$; age–sex-adjusted $r_s=-0.01$, $P=0.59$), and percentage of luminal stenosis ($r_s=-0.11$, $P<0.001$; age–sex-adjusted $r_s=-0.07$, $P=0.01$).

Clinical characteristics independently associated with lower nephron number were older age, shorter height, family history of ESRD, higher uric acid level, and lower GFR. Female sex and mild hypertension did not associate with lower nephron number after multivariable adjustment (Supplemental Table 2). These associations with nephron number did not substantively change in analysis limited to either donors with at least ten glomeruli, donors <70 years, or donors with directly measured cortical volume. Male sex did become statistically significant for higher nephron number ($P=0.04$) in analyses limited to measured cortical volume (Supplemental Table 3). Characteristics that independently associated with nephron number differed from those that independently associated with larger nephron size (larger body mass index [BMI], family history of ESRD, and higher 24-hour urine albumin) and with larger cortical volume (younger age, male sex, larger BMI, taller height, higher GFR, and higher 24-hour urine

Table 1. Baseline characteristics of 1638 kidney donors at Mayo Clinic (MN and AZ sites) and Cleveland Clinic

Characteristics	*N* (%) or Mean (SD)
Demographics and anthropometrics	
Mean age, yr	43.2 (11.7)
Female	951 (58.1%)
Height, cm	170.9 (9.6)
Race	
White or unknown	1517 (92.7%)
Black	38 (2.3%)
American Indian/Alaskan Native	25 (1.5%)
Asian	22 (1.3%)
Other	36 (2.2%)
Risk factors	
Family history of ESRD	853 (52.1%)
Mild hypertension	171 (10.4%)
BMI, kg/m^2	27.6 (4.9)
Uric acid, mg/dl	5.2 (1.4)
Kidney function	
Measured GFR, ml/min per 1.73 m^2	103.4 (19.9)
24-h urine albumin, mg	5.1 (8.4)
Biopsy measures	
Nonsclerotic glomerular volume, μm^3 $\times 10^6$	2.63 (1.01)
GSG volume, μm^3 $\times 10^6$	0.83 (0.46)
Profile tubular area, μm^2	4,486 (1,497)
Cortical area, mm^2	6.5 (2.7)
No. of NSG on biopsy section	16.7 (8.7)
No. of GSG on biopsy section	0.5 (1.0)
GSG, %	
0	1079 (65.9%)
1–5	176 (10.7%)
6–10	209 (12.8%)
11–15	96 (5.8%)
>15	78 (4.8%)
Luminal stenosis, %	
<50	1084 (76.1%)
50–75	293 (20.6%)
>75	47 (3.3%)
Interstitial fibrosis, %	
0	1303 (80.0%)
1–5	282 (17.3%)
6–10	34 (2.1%)
>10	9 (0.6%)
CT scan measure	
Cortical volume (left+right), mm^3	208,970 (43,232)

albumin) (Table 2). However, each characteristic's association with cortical volume was consistent with the net effect of the characteristic's association with nephron number and nephron size (profile tubular area in particular).

DISCUSSION

Loss of nephrons with aging is grossly underappreciated by both the degree of glomerulosclerosis on biopsy and the cortical volume decline on imaging. Glomeruli can sclerose, atrophy, and disappear; low nephron number in the absence of significant glomerulosclerosis is not necessarily due to low nephron endowment, as previously argued.[8] From young adulthood (18–29 years) to old age (70–75 years), healthy adults lose almost half of their nephrons, not the 15% loss inferred from glomerulosclerosis or cortical volume loss. Loss of nephrons is proportionally consistent with the decline in GFR with aging. There has been significant debate over the interpretation of the age-related decline in kidney function.[19] Current guidelines define CKD with a single GFR threshold,[20] which may over-diagnose CKD in older adults and underdiagnose CKD in younger adults.[1] The loss of nephrons with aging in a healthy population supports the argument that overt kidney disease should be distinguished from this age-related loss of kidney reserves.

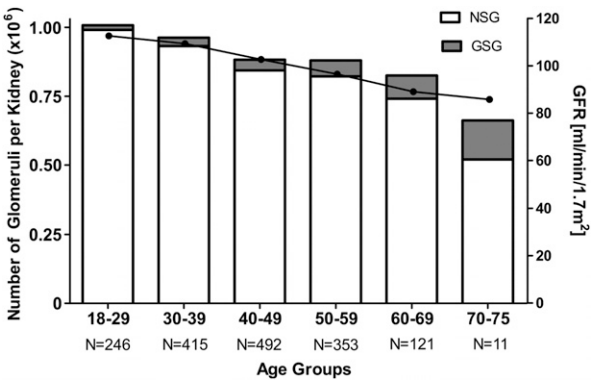

Figure 2. Nephron number and GFR progressively decrease even with healthy aging. The mean number of NSG and GSG per kidney are shown by age group (left *y*-axis). The mean GFR for each age group is shown by the connected black circles (right *y*-axis). GFR decline is proportional to the decline in NSG with aging except in the oldest age group. However, potential donors with a GFR<65 ml/min per 1.73 m^2 are excluded from donation which disproportionately impacts the oldest age group.

This estimated 873,696 nephrons per kidney among our 1638 living kidney donors is consistent with prior studies despite different methodologies for counting nephrons (Table 3).[6,8–10,16,18,21,22] We found an average loss of 6207 nephrons per year, similar to the loss of 6752 nephrons per year reported by Hoy *et al.* in an autopsy series.[7] Whereas the functional status of all remaining nephrons remains to be established,[23] smaller ischemic-appearing NSG (pericapsular fibrosis and capillary wrinkling) occur in only 0.6% of all NSG in this population.[14] There are several reasons why the loss of nephrons with aging is not sufficiently detected by increased glomerulosclerosis or cortical volume loss. Eight decades ago, Hayman *et al.* described nephron loss that was proportional to GFR decline in the normal range and suggested that "scars of destroyed glomeruli disappear without leaving recognizable traces."[24] Likewise, we found evidence that sclerosed glomeruli continue to atrophy (obsolescence) until they are missing (no longer detectable on light microscopy) and presumably reabsorbed. The number of these reabsorbed glomeruli in

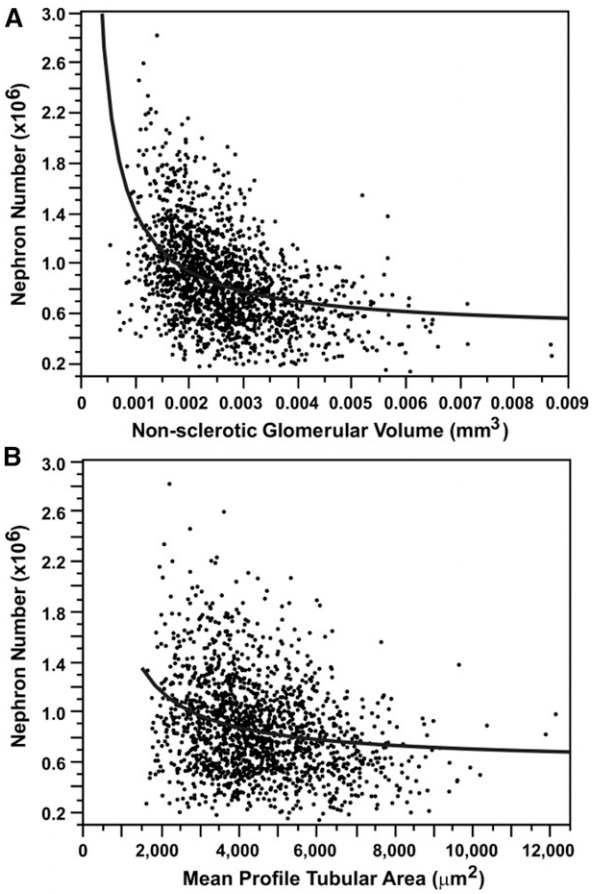

Figure 3. Nephron number has a reciprocal relationship with nephron size. Nephron size was measured by (A) nonsclerotic glomerular volume and (B) profile tubular area. The curve is a reciprocal regression fit.

70–75 year olds calculates to 345,151, which is 2.5-fold more than the number of GSG detected in this age group.

The rate of cortical volume loss with aging is proportionally less than the rate of nephron loss. Tubular, rather than glomerular volume is the major determinant of cortical volume. An increase in tubular volume with aging as detected by profile tubular area attenuates the cortical volume loss. Evidence of glomerular compensation for the age-related nephron loss is less evident; GFR decline with aging is proportional to the rate of nephron loss, and glomerular volume does not increase with healthy aging.[25] This suggests there may be less metabolic demand for glomerular function with aging. However, given the inverse correlation between nephron number and glomerular volume, other determinants of lower nephron number besides aging, such as low nephron endowment, likely increase glomerular volume.

Glomerulosclerosis correlating with lower nephron number was evident and has also been reported in autopsy series.[26] Nephrosclerosis is a biopsy pattern of glomerulosclerosis, tubular atrophy with fibrosis, and arteriosclerosis (luminal stenosis) that occurs with aging.[13] Nephrosclerosis and, specifically, glomerulosclerosis leads to loss of nephrons with normal aging. We found that nephrosclerosis also associates with lower nephron number independent of age. This is consistent with factors besides normal aging also leading to nephrosclerosis and subsequent nephron loss. Unfortunately, detection of subclinical nephrosclerosis is currently not possible without a biopsy.[13] We also found an association of mild hypertension with lower nephron number, which was no longer evident after multivariable adjustment. Prior reports that associate hypertension with lower nephron number were of small sample size and lacked multivariable analysis.[8] Also, more severe hypertension than is permitted among kidney donors may be associated with reduced nephron number.

A conceptual schematic that summarizes the factors that influence nephron number is shown in Figure 4. Predictors of decreased nephron number can be divided into congenital factors that reduce nephron endowment and acquired factors that reflect loss of nephrons. Height and family history of ESRD are genetically determined factors that affect nephron endowment, as evidenced by their association with nephron number. The association of taller height with higher nephron number has been previously reported.[27–29] The novel association of family

Table 2. Clinical characteristics as predictors of nephron number per kidney, nephron size, and cortical volume of both kidneys adjusted for each other characteristic in multivariable linear regression models

Variable	Nephron No.		Nephron Size				Cortical Volume, mm^3	
			Profile Tubular Area μm^2		NSG Volume μm^3			
	Estimate	P Value	Estimate	P Value	Estimate	P Value	Estimate	P Value
Age per 10 yr	−45,885	<0.001	91.9	0.02	−20,083	0.45	−1718	0.03
Male	50,808	0.11	173.9	0.17	66,840	0.42	18,939	<0.001
BMI per SD	−9938	0.35	253.1	<0.001	240,487	<0.001	16,670	<0.001
Height per SD	42,407	<0.01	82.9	0.15	105,759	0.01	15,222	<0.001
Mild hypertension	−33,165	0.32	152.3	0.26	97,354	0.27	−1801	0.49
Family history of ESRD	−56,175	<0.01	269.0	<0.01	190,839	<0.001	1676	0.29
Uric acid per SD	−30,498	0.02	−93.1	0.07	47,196	0.16	−1698	0.09
Measured GFR per SD	58,164	<0.001	58.2	0.21	19,565	0.51	15,397	<0.001
24-h urine albumin per SD	6543	0.52	142.3	0.01	70,546	<0.01	4621	<0.001

SDs: 4.9 kg/m^2 for BMI, 9.6 cm for height, 1.4 mg/dl for uric acid, 19.9 ml/min per 1.73 m^2 for measured GFR, and 8.4 mg for 24-hour urine albumin.

Table 3. Estimated nephron number per kidney in adults across different studies

Study Population	Clinical Kidney Disease Present	Technique	Mean Nephron No. per Kidney	Sample Size	Clinical Characteristics Associated with Low Nephron Number	Year of Publication
Autopsy series						
Traumatic accidents	no	Acid maceration	908,333	18	Age	1973[43]
Autopsy cases	no	Acid maceration	1,309,280	32	Age	1977[44]
Autopsy of full term infants	no	Acid maceration	1,107,000	28	Low birth weight, low vitamin A levels	1999[21]
Autopsy cases	no	Disector/fractionator	617,000	37	Age	1992[6]
Traumatic accidents	yes	Disector/fractionator	702,379	10	Hypertension	2003[8]
	no		1,429,200	10	N/A	
Autopsy cases	no	Disector/fractionator	992,353	39	N/A	2010[11]
Autopsy cases	no	Disector/fractionator	901,902	420	Age, low birth weight, short height, Australian Aboriginal race, hypertension	2010[22]
Autopsy cases	some	MRI with cationized ferritin	1,236,667	3	N/A	2014[38]
Living patients						
Stable renal transplants	some	MRI and protocol biopsy (Weibel–Gomez model)	730,000	39	Age, low GFR	2003[18]
Older and younger kidney donors	no	Whole-kidney K_f	631,500	34	Age, low GFR	2010[15]
Healthy kidney donors	no	Whole-kidney K_f	641,730	19	Age	2015[16]
Normotensive and hypertensive kidney donors	no	Whole-kidney K_f	605,592	51	Age, hypertension	2015[17]
Healthy kidney donors	no	Renal CT angiogram and implantation biopsy (Weibel–Gomez model)	873,696	1638	Age[a] female sex, short height[a] family history of ESRD[a] high serum uric acid[a] and low GFR[a]	This study

MRI, magnetic resonance imaging.
[a]Characteristic was an independent predictors of low nephron number in the study.

history of ESRD with lower nephron number is consistent with genetic/familial factors leading to fewer nephrons and this likely contributes to ESRD risk. Compensation for fewer nephrons occurs with larger nephrons such that cortical volume does not associate with family history of ESRD. Lower birth weight is known to associate with fewer nephrons.[26,30] Birth weight data were not available, although height is somewhat reflective of birth weight.[31] Besides genetic causes, perturbations to the fetal/maternal environment during pregnancy have been implicated in reduced nephron endowment.[32,33] Acquired factors for lower nephron number are older age and possibly higher uric acid levels (hyperuricemia is rare in children). GFR is the sum of single-nephron GFRs affected by both nephron endowment and subsequent nephron loss. We have previously shown that larger NSG volume correlates with higher uric acid levels[25] and lower nephron number is inversely correlated with larger NSG volume. Uric acid is a marker of metabolic syndrome and a risk factor for CKD,[34] although the mechanism for this association is unclear. Notably, obesity and higher urine albumin excretion did not associate with nephron number, but did associate with larger nephron size and larger cortical volume in this relatively healthy population.

There are also potential limitations to this study. The Weibel–Gomez models used a coefficient of 1.382 that assumes glomeruli are spheres.[35] Although glomeruli are not perfect spheres, this is likely only a nondifferential bias because the same coefficient was applied to all glomeruli; the associations would not substantively change with a different coefficient. The Weibel–Gomez model may not accurately account for the within-person variation in glomerular size and may have underestimated the density of the progressively atrophying GSG. However, the key finding remains that nephron loss is not appreciated by the perceived amount of glomerulosclerosis on biopsy sections, even if the extent to which this is due to underdetection from atrophy versus complete disappearance remains uncertain. The number of NSG was estimated from the product of NSG density (within-person, between-biopsy coefficient of variation [CV] of 27%)[36] and cortical volume (within-person, between-scan CV of 6%).[12] This calculates to a CV for nephron number of 33%. The imprecision might make this approach for estimating nephron number less useful for individual-level inferences, yet it remains informative for population-level inferences made with a large sample size. Smaller sample sizes may be underpowered to detect associations by this approach. We could not fully account for the effect of biopsy depth on glomerular density, but this would

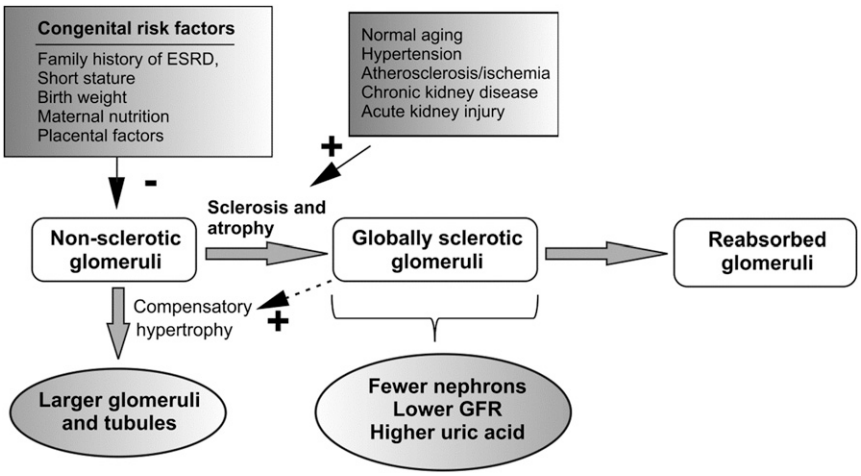

Figure 4. Kidney function and risk factors relate to loss of nephrons. Congenital risk factors lead to lower nephron endowment (fewer NSG) at birth. With aging, hypertension, ischemia, CKD, or AKI there is loss of nephrons to glomerulosclerosis with eventual reabsorption of the GSG. This loss of nephrons is detectable to some extent by lower GFR and higher uric acid levels. There is also evidence of compensatory hypertrophy of the remaining nephrons.

likely contribute to imprecision rather than bias. The estimated 43% tissue shrinkage due to formalin fixation and paraffin embedding[18] may actually vary somewhat between donors;[36] volume shrinkage due to loss of tissue perfusion pressure was based on a pig model, as such data are not available in humans.[37] The interpretation of missing glomeruli as reabsorbed glomeruli with aging assumes equal nephron endowment at birth for each age group. Finally, the population studied were all living kidney donors, predominantly white, and the oldest age group had only 11 subjects aged 70–75 years. Similar work in other populations could assess generalizability, although access to kidney biopsies is a limiting factor.

In summary, nephron number shows substantial decline with aging among healthy adults that is only partially appreciated by glomerulosclerosis on biopsy or cortical volume on imaging. Risk factors and kidney function associate with nephron number differently from their association with nephron size or cortical volume. However, kidney biopsies are too invasive for routine use. Novel and promising imaging methods that can count glomeruli in human kidneys obtained at autopsy may eventually be applicable to living humans.[38]

CONCISE METHODS

Study Population

The study population was living kidney donors who had undergone a predonation CT scan and a protocol core-needle biopsy of the donated kidney cortex during transplant surgery at one of three participating sites (Mayo Clinic, MN; Mayo Clinic, AZ; and Cleveland Clinic, OH) between the years 2000 and 2011. All kidney donors at the three sites underwent a thorough medical evaluation before donation with a prescheduled battery of tests. Acceptable criteria for donation varied by site and era, but in general included a 24-hour urine albumin <30 mg and a normal-for-age GFR; mild hypertension in older donors and moderate obesity (BMI<35 kg/m^2) were allowed.[39]

Kidney Function and Risk Factors

Hypertension was defined as office BP $>140/90$ mmHg or use of antihypertensive medication to lower BP. The hypertension in donors was considered "mild" as BP was either borderline elevated or controlled with one antihypertensive agent (with or without a thiazide diuretic). Potential donors with more severe hypertension were excluded from donation. Family history of ESRD was determined by the recipient being biologically related to the donor. The predonation evaluation also included BMI (kg/m^2), urinary iothalamate clearance to measure GFR, 24-hour urine albumin excretion, and serum uric acid.

Kidney Biopsy Morphometry and Stereology

An intraoperative 18-gauge needle core biopsy of the kidney cortex was taken with a biopsy gun (1.7 cm specimen slot) during the transplant surgery. The tissue specimen was fixed in formalin and embedded in paraffin. Two sections of 3 μm in depth from the middle of the core were stained (one with periodic acid–Schiff and one with Masson trichrome). Subsequently, these sections were scanned into high-resolution digital images (Aperio XT system scanner, www.aperio.com). The inclusion criteria for all biopsy sections were: no compression artifacts, at least 2 mm^2 of cortex area, and at least four glomeruli.[40] The volume of NSG (mm^3), the NSG density (per mm^3), and the GSG density (per mm^3) were calculated using the Weibel–Gomez stereological models[35] as detailed in the Supplemental Material (Supplemental Figure 2 shows an example NSG density calculation). The mean profile tubular area in 1 mm^2 of cortex was also estimated as detailed in the Supplemental Material.[36] The percentage of luminal stenosis was determined from the artery in the cortex most orthogonal to its axis (if artery present) (Supplemental Figure 3, Supplemental Material). Presence of capsule or corticomedullary junction was also identified. The percentage of fibrosis was estimated in a semiquantitative manner by a renal pathologist on the basis of a visual inspection of the percentage of total cortex area that showed interstitial fibrosis (0%, 1%–5%, 6%–10%, or >10%) on the Masson trichrome–stained section.[25] Interstitial fibrosis was defined by interstitial expansion with increased collagen staining by trichrome.

CT Image Morphology

Cortical volume declines with age whereas medullary volume actually increases with age,[12] thus we focused on cortical volume as a surrogate for detecting nephron number decline. Transverse CT images of the kidneys from the angiogram/cortical phase were downloaded onto a workstation for processing. The entire kidney cortex and

medulla was segmented as previously described.[12] About 9% of donor CT scans had poor cortical–medullary differentiation. In these donors, the total kidney parenchymal volume was segmented and cortical volume was then estimated from total kidney parenchymal volume (on the basis of a linear regression model).

Estimation of Glomerular Number

Glomerular density is higher near the capsule and decreases toward the corticomedullary junction.[36,41] on the basis of a regression model to predict volumetric glomerular density from presence of capsule and corticomedullary junction, we found that biopsies with capsule had 2.4 (18%) more NSG per mm^3 and biopsies with corticomedullary junction had 1.5 (8%) more NSG per mm^3 compared with biopsies without capsule or corticomedullary junction. Thus, we standardized the glomerular density of all biopsies to the absence of capsule or corticomedullary junction. The total number of NSG (or GSG) per kidney was calculated by multiplying total cortical volume (mm^3) by the NSG (or GSG) volumetric density (per mm^3) and dividing by 2 (per kidney), dividing by 1.43 (tissue volume shrinkage due to paraffin embedding),[18] and dividing by 1.268 (volume shrinkage due to loss of tissue perfusion pressure)[37] (Supplemental Figure 4). The term "nephron number" specifically referred to the number of NSG.[9,28]

Statistical Analyses

Image analysis was centralized (Mayo Clinic, MN) with training data and reproducibility studies to ensure consistency between different analysts (needed for cortical volume estimates). Images were analyzed in a random order with masking to clinical characteristics to optimize the accuracy of measures.[12,36] Age was categorized as 18–29, 30–39, 40–49, 50–59, 60–69, and 70–75 years. The percentage of GSG was calculated from GSG density/(NSG density+GSG density). Using the youngest age group as the reference group, the difference in percentage of GSG, cortical volume, and nephron number was compared with each older age group. Missing glomeruli for each older age group were calculated from the difference in number of glomeruli (NSG+GSG) in the youngest age group compared with each older age group. Linear regression was also used to evaluate the relationship of nephron number with age (continuous), sex, height, GFR, urine albumin, family history of ESRD, mild hypertension, BMI, and serum uric acid in both univariable and multivariable models. To assess the robustness of these relationships, three sensitivity analyses were performed limiting the sample to donors: (*1*) with at least ten glomeruli on biopsy section, (*2*) $<$70 years of age, and (*3*) with directly measured cortical volume (not estimated from total parenchymal volume). The same multivariable predictors for nephron number were also applied to models for estimating nephron size (NSG volume or profile tubular area) and for cortical volume. Spearman rank correlations determined how nephron number correlated with nephrosclerosis (higher percentages of GSG, fibrosis, and luminal stenosis) and nephron hypertrophy (larger NSG volume or profile tubular area). These correlations were then age–sex-adjusted using partial Spearman rank correlations.[42] All statistical analyses were performed using JMP (SAS Institute, Cary, NC; version 10.0).

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DISCLOSURES

None.

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