ORIGINAL ARTICLE

In the print version of this article, the key in Figure 3 is incorrect. The key is correct in this electronic version.

Association of Kidney Function and Metabolic Risk Factors With Density of Glomeruli on Renal Biopsy Samples From Living Donors

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OBJECTIVE: **To test the hypothesis that kidney function and metabolic risk factors are associated with glomerular density on renal biopsy samples from healthy adults.**

PATIENTS AND METHODS: **This study compared glomerular density with predonation kidney function, blood pressure, and metabolic risk factors in living kidney donors at Mayo Clinic in Rochester, MN, from May 10, 1999, to February 4, 2009. During implantation of the kidney allograft, an 18-gauge core needle biopsy sample of the renal cortex was obtained, sectioned, and examined by pathologists. Glomerular density was determined by the number of glomeruli (normal and sclerotic) divided by area of cortex.**

RESULTS: **The study sample of 1046 kidney donors had a mean of 21 glomeruli (0.8 sclerotic glomeruli) and a glomerular density of 2.3 glomeruli per square millimeter. In a subset of 54 donors, glomerular density inversely correlated with the mean glomerular area (r_s=–0.28). Independent predictors of decreased glomerular density were older age, increased glomerular filtration rate, family history of end-stage renal disease, increased serum uric acid, and increased body mass index. Increased urine albumin excretion, hypertension, decreased high-density lipoprotein cholesterol, and metabolic syndrome were also associated with decreased glomerular density after age-sex adjustment. These associations were not explained by the presence of glomerulosclerosis, tubular atrophy, interstitial fibrosis, or arteriosclerosis on the renal biopsy sample. In older donors, decreased glomerular density was attenuated by an increased prevalence of glomerulosclerosis and tubular atrophy.**

CONCLUSION: **Decreased glomerular density is associated with many different kidney function and metabolic risk factors among relatively healthy adults and may represent an early state of increased risk of parenchymal injury.**

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BMI = body mass index; CKD = chronic kidney disease; ESRD = end-stage renal disease; GFR = glomerular filtration rate; HDL = high-density lipoprotein

The size of individual nephrons can reflect important elements of metabolic regulation. Persons with a low nephron endowment or with certain stress states (eg, obesity, pregnancy, or unilateral nephrectomy) develop glomerular hypertension and increased single-nephron filtration (hyperfilter) with compensatory glomerulomegaly.[1-5] Glomerulomegaly from hyperfiltration also occurs in response to nephron loss, perhaps due to a shift in perfusion from nonviable to viable nephrons.[6,7] In addition to glomerulomegaly, hyperfiltration leads to tubular hypertrophy and hyperplasia.[8-10] Much of the volume increase of viable nephrons in response to hyperfiltration may be in the proximal tubule rather than the glomerulus.[11-13] Unfortunately, there is no safe and practical method to directly measure the average volume occupied by nephrons in living humans.

Normal kidney parenchyma consists only of nephrons and supporting vessels, with a trivial amount of interstitium (Figure 1), and thus glomerular density on sectioned biopsy samples of renal cortex is inversely proportional to average nephron size to some extent. We hypothesized that glomerular density is associated with kidney function and metabolic characteristics of the kidney. Specifically, among persons with risk factors for glomerular hypertension and hyperfiltration, the glomerular density would be decreased. The rationale for this hypothesis is that any process that increases the volume occupied by each nephron would effectively push glomeruli apart, leading to decreased glomerular density. For example, decreased glomerular density has been associated with low birth weight in neonates and, in this instance, has been attributed to low nephron endowment with compensatory hypertrophy.[14] Alternatively, kidney function and metabolic risk factors may be associated with global glomerulosclerosis (complete scarring of glomerulus), and reabsorption of these sclerotic glomeruli[15] could decrease glomerular density.

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In the current study, we used adult living kidney donors with implantation (intraoperative) renal biopsy samples to study glomerular density. Although kidney donors are selected on the basis of good health, they still demonstrate substantial variation in kidney function,

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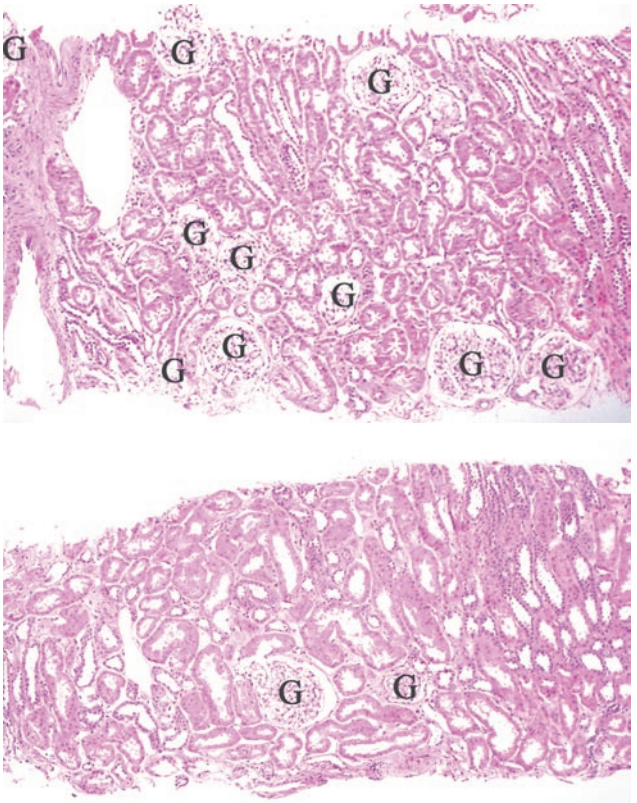

FIGURE 1. Representative fields of implantation biopsies (original magnification x100; hematoxylin-eosin) for kidney donors with (top) high glomerular density (10 glomeruli in a representative field of a biopsy section) and (bottom) low glomerular density (2 glomeruli in a representative field of a biopsy section). G = glomerulus.

blood pressure, and metabolic profiles.[16] Our goal was to compare glomerular density against predonation clinical characteristics, particularly those that have previously been implicated in glomerular hyperfiltration and glomerular hypertension or are risk factors for chronic kidney disease (CKD).

PATIENTS AND METHODS

We studied a series of adult living kidney donors at Mayo Clinic in Rochester, MN, between May 10, 1999, and February 4, 2009, who had implantation needle core biopsies of their kidney allografts.[16] The study protocol was approved by the Mayo Clinic Institutional Review Board. Donors who declined the Minnesota research authorization were excluded.[17] All potential kidney donors underwent a standardized evaluation with a protocol-based battery of tests. Approval for kidney donation was determined by a donor-selection committee, as well as guidelines that evolved during the study period. Potential candidates were usually excluded from donation if they had a measured glomerular filtration rate (GFR) (iothalamate clearance) below the age-specific fifth percentile[18] or albuminuria (>30 mg/24 h). Patients with diabetes or a fasting plasma glucose level greater than 110 mg/dL (to convert to mmol/L, multiply by 0.0555) were also excluded. Persons with hypertension were permitted as kidney donors if their blood pressure was controlled with minimal antihypertensive therapy (up to 2 agents if 1 was a thiazide diuretic). Obesity was not considered an absolute contraindication to donation. There were no exclusion criteria based on serum lipids or uric acid levels.

Donor Clinical Characteristics

The donor characteristics analyzed were kidney function test results, risk factors for CKD, and characteristics that may be affected by early CKD. Donor characteristics were obtained from the medical record, including only test results that were part of the predonation evaluation. If multiple test results were present, the result temporally closest to kidney donation was used in the analysis. The kidney function tests obtained included serum creatinine, estimated GFR by the Modification of Diet in Renal Disease Study equation,[19] measured GFR by iothalamate clearance,[18] and 24-hour urine protein and albumin excretion. The serum creatinine assay was standardized[19] in October 2006, and prior test results were adjusted by subtracting 0.14 mg/dL (the mean change in donor serum creatinine levels with the switch to a standardized assay). An ambulatory blood pressure monitor obtained systolic and diastolic blood pressure readings every 10 to 20 minutes during an 18-hour period. The mean overall blood pressure, mean active blood pressure (4 PM to 9 PM), and mean nocturnal blood pressure (12 AM to 5 AM) were obtained.[20,21] Hypertension was defined by treatment with antihypertensive therapy. Family history of end-stage renal disease (ESRD) was defined by the recipient being related to the donor. Metabolic characteristics obtained from a fasting morning blood draw were levels of total cholesterol, triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein (HDL) cholesterol, glucose, uric acid, calcium, and phosphorus. Body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared. Metabolic syndrome was defined by a BMI greater than 30 kg/m^2 (because waist circumference was unavailable) and any 2 of the following: (1) triglycerides ≥150 mg/dL (to convert to mmol/L, multiply by 0.0113), (2) HDL cholesterol <40 mg/dL in men and <50 mg/dL in women (to convert to mmol/L, multiply by 0.0259), (3) fasting glucose ≥100 mg/dL, (4) 18-hour mean systolic blood pressure ≥130 mm Hg or 18-hour mean diastolic blood pressure ≥85 mm Hg or antihypertensive therapy.[22]

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Kidney Histologic Features

At Mayo Clinic, implantation renal biopsy samples are routinely obtained to provide baseline histologic features for comparison with future biopsy samples of the allograft. After vascular and ureteral anastomoses of the allograft and unclamping of vessels, biopsy samples were obtained from the antihilar border of the most accessible pole with an 18-gauge Bard Monopty biopsy instrument with a 1.7-cm specimen slot (Bard Peripheral Vascular, Inc, Tempe, AZ). Formalin-fixed, paraffin-embedded longitudinal sections stained with hematoxylin and eosin, periodic acid–Schiff, methenamine silver, and Masson trichrome were examined by dedicated renal pathologists who were blinded to donor characteristics. Biopsy reports were manually reviewed and abstracted for number of glomeruli and number of globally sclerotic glomeruli. Chronic histologic abnormalities of global glomerulosclerosis (defined when >10% of the glomeruli were globally sclerotic), any tubular atrophy, interstitial fibrosis (defined by >5% interstitial fibrosis),[23] and any arteriosclerosis were also identified from these reports. The biopsy sections (on slides) were subsequently examined (by M.H.S.) in a blinded manner to measure length and width of the sections. A ruler was used to measure the length of the biopsy section to the nearest 1 mm, summing the length of all sections observed on the slide. Under light microscopy, the width of the biopsy section was determined to the nearest 0.1 mm, averaging the width along the length of the section or sections. At low magnification, the proportion of the biopsy section that was cortex was estimated to the nearest fifth percentile. In a previously reported subset, the sectional area of each manually outlined glomerulus with a vascular pole was determined using a pixel-counting technique.[24]

Statistical Analyses

Donors without biopsy slides for reexamination or with an implantation biopsy sample of only medulla were excluded. The area of cortex for the sectioned renal biopsy sample from each kidney donor was determined from the product of length multiplied by width multiplied by proportion cortex. Donors without at least 4 mm^2 of cortex were excluded; this threshold was chosen a priori on the basis of the 10th percentile for cortex area. Glomerular density was calculated from the number of glomeruli (both normal and sclerotic) divided by the area of cortex (mm^2) and normalized by logarithmic transformation. The Spearman rank correlation between glomerular density and the mean glomerular area was assessed in 54 donors with at least 6 mm^2 of cortex to ensure an adequate number of glomeruli. The correlation between the number of glomeruli reported in the medical record by many different pathologists and a recount by one pathologist (L.D.C.) was also assessed in 39 donors. Logarithmic glomerular density was regressed on donor clinical characteristics with and without adjustment for age and sex. Independent predictors of glomerular density were identified using backwards stepwise selection in multivariable models. Regression coefficients from logarithmic models were converted to estimates that predicted percent differences in glomerular density. Continuous variables were also polychotomized for age (18-29, 30-39, 40-49, 50-59, and 60-77 years), quartile of BMI (<24, 24-27, 28-31, >31 kg/m^2), measured GFR (<99, 99-112, 113-128, >128 mL/min), and serum uric acid level (<4.1, 4.1-5.0, 5.1-6.1, >6.1 mg/dL; to convert to μmol/L, multiply by 59.5), and the associations with glomerular density were assessed with analysis of variance.

To account for the possibility of bias in determining the proportion cortex from biopsies, analyses were repeated after limiting the data to biopsies with only cortex present. Estimates of glomerular density may lack adequate precision even after excluding biopsy sections with less than 4 mm^2 of cortex; therefore, analyses were repeated after excluding sections with less than 6 mm^2 of cortex. Because glomerulosclerosis, tubular atrophy, interstitial fibrosis, and arteriosclerosis may affect glomerular density, analyses of the association between clinical characteristic and glomerular density were repeated with adjustment for these chronic histologic abnormalities. Analyses were also repeated with adjustment for percent glomerulosclerosis (number of globally sclerotic glomeruli divided by total number of glomeruli). We previously found chronic histologic abnormalities to strongly increase with age.[16] To further explore the U-shaped association between age and glomerular density, we included in statistical models a test for interaction between chronic histologic abnormalities and age in the prediction of glomerular density. All statistical tests were 2-sided, with $P<.05$ considered statistically significant.

RESULTS

In this study, 1221 living kidney donors with an implantation biopsy of the allograft cortex were identified. Of these, 53 were excluded because biopsy slides were missing for rereview. After further exclusion of 122 donors with less than 4 mm^2 of cortex, the study sample consisted of 1046 donors. The mean ± SD length and width of the sectioned biopsy samples were 13.7±3.5 mm and 0.73±0.10 mm, respectively. The mean proportion cortex was 93%, and 791 (76%) of the biopsies were only cortex with no medulla. The mean ± SD number of glomeruli present was 21±8 in an area of cortex averaging 9.3±2.9 mm^2, for a glomerular density of 2.3±0.8 glomeruli per square millimeter. The de-

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TABLE 1. **Clinical Characteristics and Renal Biopsy Findings of 1046 Living Kidney Donors at Mayo Clinic, 1999-2009**[a,b]

Demographics	Mean ± SD or No. (%)
Age, y	43±12
Male	448 (43)
Kidney function	
24-h urine protein excretion, mg	45±26
24-h urine albumin excretion, mg	6.1±8.2
Serum creatinine, mg/dL	0.90±0.16
Estimated GFR, mL/min/1.73 m^2	81±14
Measured GFR, mL/min/1.73 m^2	103±18
Measured GFR, mL/min	116±24
Blood pressure	
18-h mean overall SBP, mm Hg	119±9
18-h mean active SBP, mm Hg	122±10
18-h mean nocturnal SBP, mm Hg	108±10
18-h mean overall DBP, mm Hg	73±7
18-h mean active DBP, mm Hg	75±8
18-h mean nocturnal DBP, mm Hg	63±8
Hypertension (with therapy)	61 (6)
Other clinical characteristics	
Family history of ESRD	599 (57)
Total cholesterol, mg/dL	196±37
Triglycerides, mg/dL	127±99
HDL cholesterol, mg/dL	56±16
LDL cholesterol, mg/dL	116±36
Glucose, mg/dL	95±9
Uric acid, mg/dL	5.2±1.4
Calcium, mg/dL	9.5±0.3
Phosphorus, mg/dL	3.6±0.5
BMI, kg/m^2	28±6
BMI >30	323 (31)
Metabolic syndrome	123 (12)
Renal biopsy findings	
No. of glomeruli	21±8
No. of globally sclerotic glomeruli	0.9±1.4
Area of cortex, mm^2	9.3±2.9
Glomerular density, glomeruli per mm^2	2.3±0.8
Percent global glomerulosclerosis	4.4%±6.6%
Global glomerulosclerosis >10%	130 (12)
Any tubular atrophy	239 (23)
Interstitial fibrosis >5%	53 (5)
Any arteriosclerosis	326 (31)

[a] BMI = body mass index; DBP = diastolic blood pressure; ESRD = end-stage renal disease; GFR = glomerular filtration rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SBP = systolic blood pressure.

[b] SI conversion factors: To convert cholesterol values to mmol/L, multiply by 0.0259; to convert triglyceride values to mmol/L, multiply by 0.0113; to convert serum creatinine values to μmol/L, multiply by 88.4; to convert glucose values to mmol/L, multiply by 0.0555; to convert uric acid values to μmol/L, multiply by 59.5; to convert calcium values to mmol/L, multiply by 0.25; to convert phosphorus values to mmol/L, multiply by 0.323.

mographics, clinical characteristics, and biopsy findings of the kidney donors are shown in Table 1. In a subset of 54 donors, glomerular density was inversely correlated with mean glomerular area (r_s=–0.28; *P*=.04). In another subset of 39 donors, the initial and repeat count of the number of glomeruli was highly correlated (r_s=0.97; *P*<.001).

Glomerular density decreased with older age (Table 2). After adjustment for age and sex, glomerular density decreased with increased urine albumin excretion, increased GFR, hypertension, family history of ESRD, decreased HDL cholesterol, increased uric acid, increased BMI, and metabolic syndrome (Table 2). Older age, increased measured GFR, family history of ESRD, increased uric acid, and increased BMI were independently associated with decreased glomerular density in the reduced model that contained only the statistically significant predictors (Table 3). The association between these polychotomized characteristics and glomerular density is displayed in Figure 2. In the sensitivity analysis, findings were similar (Table 4).

Chronic histologic abnormalities were also associated with glomerular density. Global glomerulosclerosis was associated with decreased glomerular density (unadjusted, –11.7%; *P*<.001; age-adjusted, –9.6%; *P*=.009), whereas tubular atrophy was associated with increased glomerular density (unadjusted, 5.2%; *P*=.05; age-adjusted, 7.7%; *P*=.005). Interstitial fibrosis had no association with glomerular density (unadjusted, –3.4%; *P*=.48; age-adjusted, 0.4%; *P*=.94), and the association of arteriosclerosis with decreased glomerular density was not independent of age (unadjusted, –6.4%; *P*=.005; age-adjusted, –4.5%; *P*=.06). We also assessed whether the association between glomerular density and age differed when glomerulosclerosis was present. As shown in Figure 3, among donors with less than 10% glomerulosclerosis (n=916), glomerular density decreased by 3.5% per decade of age; but among donors with more than 10% glomerulosclerosis (n=130), glomerular density increased by 4.6% per decade of age (*P*=.004 for interaction). There was no evident age interaction with tubular atrophy, interstitial fibrosis, or arteriosclerosis (*P*>.05 for all).

DISCUSSION

Our results indicate that glomerular density is associated with kidney function and other metabolic characteristics in a relatively healthy population. As expected, glomerular density had an inverse correlation with mean glomerular area. Decreased glomerular density was associated with older age, increased urine albumin excretion, increased GFR, hypertension, family history of ESRD, decreased HDL cholesterol, increased uric acid, increased BMI, and metabolic syndrome. Importantly, these characteristics are recognized risk factors for CKD and/or cardiovascular disease. Histologic findings for CKD (glomerulosclerosis, tubular atrophy, interstitial fibrosis, and arteriosclerosis) have previously not been associated with GFR, family history of ESRD, HDL cholesterol, uric acid, BMI, or metabolic syndrome in this population sample.[16] To the extent that metabolic risk factors for CKD affect glomerular density, decreased glomerular density may be a precursor

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TABLE 2. **Percent Difference in Glomerular Density (No. of Glomeruli per Cross-sectional Cortex Area) by Donor Characteristics Among 1046 Living Kidney Donors at Mayo Clinic, 1999-2009**

Donor characteristic (per SD if continuous)	Unadjusted		Adjusted for age and sex	
	% Difference	*P* value	% Difference	*P* value
Demographics				
Age, y	−3.8	<.001		
Male	−4.0	.06		
Kidney function				
24-h urine protein excretion	−1.7	.10	−1.2	.29
24-h urine albumin excretion	−2.5	.02	−2.4	.02
24-h urine albumin excretion >15 mg	−12.2	.003	−12.0	.003
Serum creatinine	−0.7	.49	1.8	.22
Estimated GFR, mL/min/1.73 m^2	−0.7	.44	−2.4	.01
Measured GFR, mL/min/1.73 m^2	−0.9	.44	−3.1	.01
Measured GFR, mL/min	−3.9	<.001	−5.8	<.001
Blood pressure				
18-h mean overall SBP	−2.8	.009	−1.7	.12
18-h mean overall DBP	−2.2	.06	−1.0	.40
18-h mean active SBP	−2.7	.01	−1.7	.13
18-h mean active DBP	−1.4	.24	−0.4	.71
18-h mean nocturnal SBP	−2.8	.02	−2.2	.06
18-h mean nocturnal DBP	−2.4	.04	−1.5	.23
Hypertension (with therapy)	−12.4	.004	−9.2	.04
Other clinical characteristics				
Family history of ESRD	−5.2	.01	−6.6	.002
Total cholesterol	−1.3	.24	−0.3	.78
LDL cholesterol	−1.5	.17	−0.8	.49
HDL cholesterol	2.5	.02	3.0	.01
Triglycerides	−1.1	.28	−0.7	.49
Glucose	−3.5	.001	−2.2	.06
Uric acid	−4.6	<.001	−4.9	<.001
Calcium	−1.2	.19	−0.9	.32
Phosphorus	0.0	.99	−0.6	.53
BMI, kg/m^2	−6.3	<.001	−6.0	<.001
BMI >30 kg/m^2	−10.6	<.001	−10.1	<.001
Metabolic syndrome	−12.9	<.001	−12.3	<.001

BMI = body mass index; DBP = diastolic blood pressure; ESRD = end-stage renal disease; GFR = glomerular filtration rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; SBP = systolic blood pressure.

state for chronic parenchymal injury that parallels the increase in glomerular area observed in early stages of overt kidney disease.[25-27]

What underlying biologic process does glomerular density represent? An interstitial infiltrative process might reduce the density of glomeruli in the cortex; however, such an infiltrative process was not seen except for a mild interstitial fibrosis that occurred in only 5% of donors, and there was no association between interstitial fibrosis and glomerular density. Interestingly, although glomerular density was associated with global glomerulosclerosis, tubular atrophy, and arteriosclerosis, the association between glomerular density and clinical characteristics was independent of these histologic abnormalities. Reabsorption of sclerotic glomeruli might be expected to decrease glomerular density,[15] but associations with glomerular density did not change substantially with adjustment for severity of glomerulosclerosis. Thus, it is less plausible that glomerulosclerosis leading to reabsorption of sclerotic glomeruli could explain these kidney function and risk factor associations with glomerular density. Depth of the biopsy in the cortex may also affect glomerular density, but this would not explain the association between donor clinical characteristics and glomerular density in a renal biopsy sample obtained by a surgeon who was effectively blinded to these characteristics. Measurements of biopsy depth were not available; however, excluding biopsy samples with medulla present did not substantially change the findings.

We suspect that decreased glomerular density on sectioned renal biopsy specimens primarily represents dispersed glomeruli from increased average nephron size secondary to both glomerular hypertrophy and hyperplasia and renal tubular hypertrophy and hyperplasia. This hypothesis was supported by the inverse correlation between glomeru-

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TABLE 3. **Multivariable Model Estimating Percent Difference in Glomerular Density (No. of Glomeruli per Cross-sectional Cortex Area) With Donor Characteristics Among Living Kidney Donors at Mayo Clinic, 1999-2009**[a]

Donor characteristic (per SD if continuous)	Full model (n=848)[b]		Reduced model (n=967)[b]	
	% Diff	*P* value	% Diff	*P* value
Age	−4.7	.001	−5.6	<.001
Male	1.3	.67		
24-h urine albumin excretion	−3.0	.06		
Measured GFR, mL/min	−3.1	.03	−3.9	.002
18-h mean overall SBP, mm Hg	1.0	.39		
Hypertension (with therapy)	−7.4	.11		
Family history of ESRD	−5.9	.01	−6.2	.004
HDL cholesterol	−0.2	.88		
Glucose	−1.0	.44		
Uric acid	−3.2	.03	−2.6	.02
BMI	−3.8	.007	−4.4	<.001

[a] BMI = body mass index; Diff = difference; ESRD = end-stage renal disease; GFR = glomerular filtration rate; HDL = high-density lipoprotein; SBP = systolic blood pressure.
[b] Sample sizes that are less than 1046 differ between models because of missing data.

lar density and glomerular area in the subset analysis. Although we could not assess for inverse correlation between average tubular volume of each nephron and glomerular density, hypertrophy of proximal tubules has been shown to occur with glomerulomegaly via tubuloglomerular feedback in animal models.[10]

Increased GFR, BMI, and uric acid and family history of ESRD were independent predictors of decreased glomerular density. These associations with decreased glomerular density are consistent with those that other investigators have seen with increased glomerular area. With increased single glomerulus filtration there is compensatory glomerulomegaly,[1-5] and the sum expected effect would be an increase in GFR. Obesity has been associated with glomerulomegaly[24,28,29] and with risk of CKD.[30,31] Hyperuricemia is also a risk factor for CKD,[32,33] and raising uric acid levels in rats leads to intraglomerular hypertension[34] and glomerular hypertrophy.[35] A family history of ESRD is also a risk factor for CKD and may reflect a familial trait

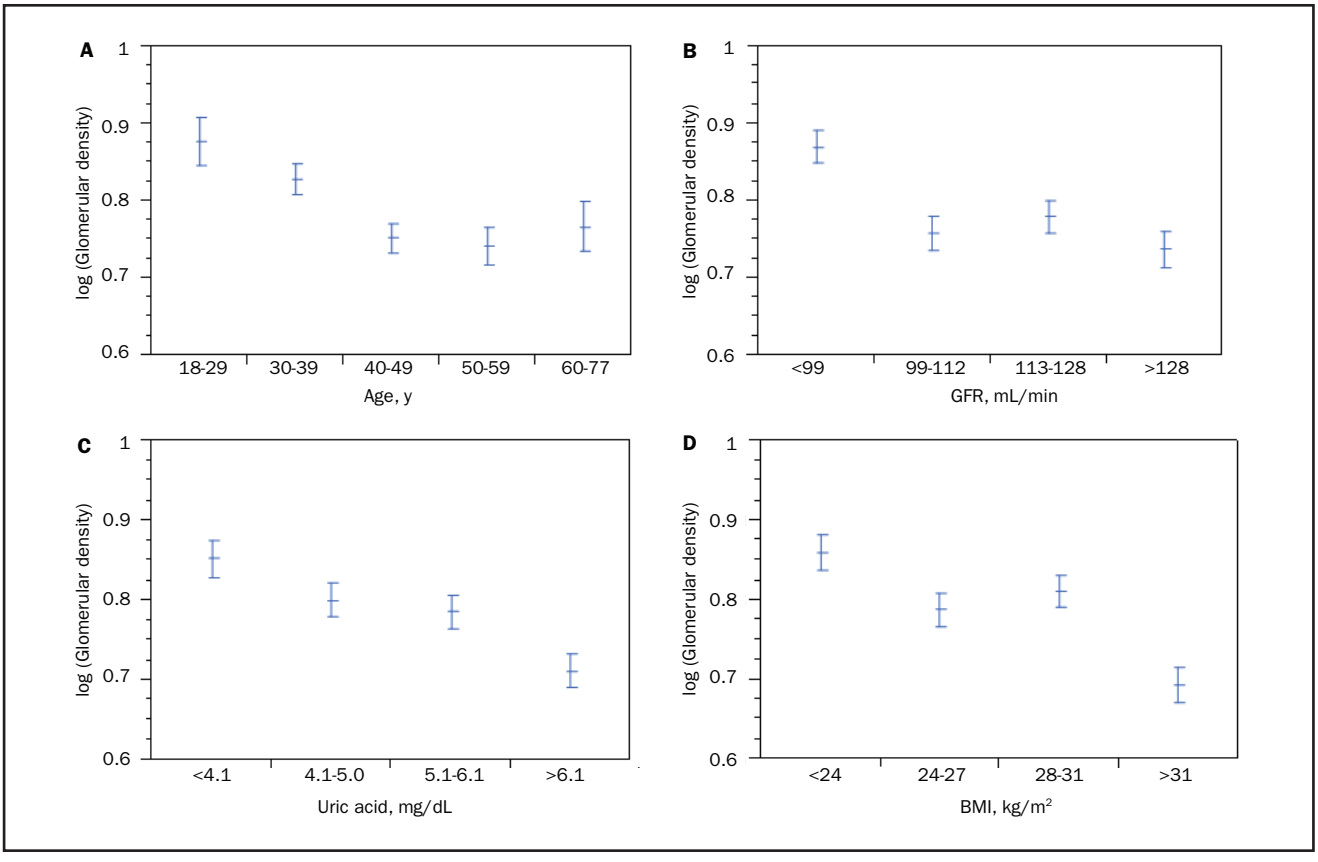

FIGURE 2. Logarithmic glomerular density by (A) age groups, (B) measured glomerular filtration rate (GFR) quartiles, (C) serum uric acid level quartiles, and (D) body mass index (BMI) quartiles among 1046 living kidney donors. The mean with standard error bars is shown for each group (analysis of variance, *P*<.001 for all).

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TABLE 4. **Sensitivity Analyses for Estimating Percent Difference in Glomerular Density With Donor Characteristics in a Multivariable Model Among Living Kidney Donors at Mayo Clinic, 1999-2009**[a]

Donor characteristic (per SD if continuous)	Original analysis (n=967)		Data limited to biopsies that were all cortex (n=728)		Data limited to biopsies with >6 mm^2 of cortex (n=837)		Adjustment for global glomerulosclerosis, tubular atrophy, interstitial fibrosis, and arteriosclerosis (n=966)		Adjustment for percent glomerulosclerosis (n=966)	
	% Diff	*P* value	% Diff	*P* value	% Diff	*P* value	% Diff	*P* value	% Diff	*P* value
Age per 12 y	−5.6	<.001	−5.1	<.001	−5.0	<.001	−5.0	<.001	−4.4	<.001
Measured GFR per 24 mL/min	−3.9	.002	−2.9	.03	−2.7	.06	−3.6	.004	−3.8	.002
Family history of ESRD	−6.2	.004	−4.1	.08	−3.9	.12	−6.4	.003	−6.1	.004
Uric acid per 1.4 mg/dL[b]	−2.6	.02	−3.4	.005	−4.3	.001	−2.9	.01	−2.7	.02
BMI per 6 kg/m^2	−4.4	<.001	−3.8	.006	−3.3	.02	−4.5	<.001	−4.4	<.001

[a] BMI = body mass index; Diff = difference; ESRD = end-stage renal disease; GFR = glomerular filtration rate.
[b] SI conversion factor: To convert uric acid values to μmol/L, multiply by 59.5.

for lower nephron endowment at birth, leading to increased glomerular area.[36,37] Hypertension, increased urine albumin excretion, and lower HDL cholesterol level were also predictors of decreased glomerular density independent of age and sex. Glomerulomegaly has been associated with hypertension in autopsy studies.[1,38] Glomerular area has also been found to correlate with urine albumin excretion in kidney donors.[24] Intrauterine growth retardation could also link these metabolic risk factors to decreased glomerular density from a reduction in total number of nephrons (Barker and Brenner hypothesis).[39]

We found that glomerular density decreased with age, as others previously reported in an autopsy study.[40] The reason for this finding is unclear, but increased glomerular area with age[40-42] might result from increased metabolic burden (eg, oxidative stress) leading to hypertrophy and hyperplasia of viable nephrons. In our study, the age-related decrease in glomerular density also appeared to level off around 40 to 49 years of age. Further analysis showed that this age-related decrease in glomerular density did not level off but continued to decline when glomerulosclerosis was 10% or less, but there was paradoxically an increase in glomerular density with age when glomerulosclerosis was greater than 10%. This may be explainable by the increased prevalence of glomerulosclerosis with tubular atrophy in older compared with younger donors.[16] In this study, among donors aged 18 to 29 years, only 3% (4/131) had greater than 10% glomerulosclerosis, none of whom had tubular atrophy, whereas among donors aged 60 to 77 years, 36% (35/96) had greater than 10% glomerulosclerosis, 63% of whom had tubular atrophy. Glomerulosclerosis is a volume-losing lesion that may have more impact on glomerular density in older kidneys, where it is more prevalent. Furthermore, tubular atrophy can result from glomerulosclerosis in the same nephron and, as expected, is a volume-losing lesion associated with increased glomerular density. Finally, hypertrophy of the unaffected nephrons occurs to compensate for glomerulosclerosis,[43,44] and this compensatory hypertrophy will decrease glomerular density; however, younger kidneys may have more of this compensatory reserve than older kidneys.[45]

Our study data have several strengths and limitations that should be noted. The living kidney donors underwent protocol-driven evaluations (ie, there was no medical complaint), and the renal biopsy sample was obtained by protocol as part of a transplant operation. This avoids the problem of "confounding by indication" in the interpretation of renal biopsy findings. Nonetheless, kidney donors are selected on the basis of good health such that the spectrum of kidney function and metabolic risk factors is restricted compared with that of the general population. In particular,

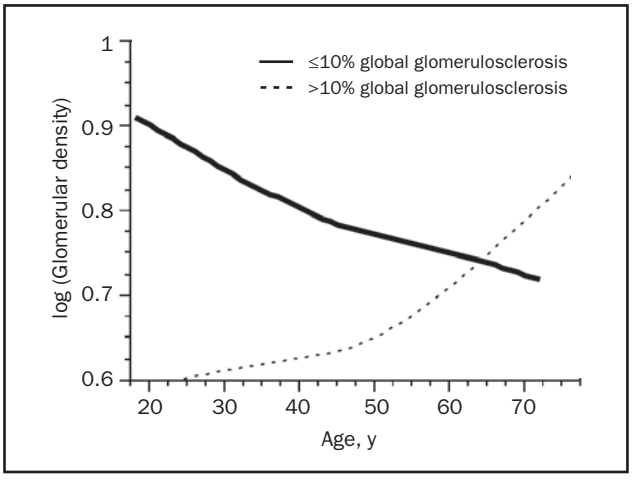

FIGURE 3. Logarithmic glomerular density by age for persons with ≤10% (solid smoothing spline, λ=100,000) or with >10% (dashed smoothing spline, λ=100,000) glomerulosclerosis (*P*=.004 for statistical interaction in the linear regression model) among 1046 living kidney donors.
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persons with reduced GFR or other conditions associated with significant interstitial fibrosis were excluded. Changes in glomerular density when patients develop overt CKD were not assessed. Despite this restriction, we found substantial evidence of clinicopathologic correlation between donor characteristics and glomerular density. How associations with glomerular density compare with associations with glomerular area requires further investigation because glomerular area data were available only for a small subset of the sample. Because less than 2% of the sample was any specific nonwhite race group, race differences in glomerular density could not be meaningfully assessed.

CONCLUSION

Our study indicates that glomerular density on sectioned renal biopsy samples is an informative measure in a relatively healthy population. Decreased glomerular density is associated with older age, increased urine albumin excretion, increased GFR, hypertension, family history of ESRD, decreased HDL cholesterol, increased uric acid, increased BMI, and metabolic syndrome. It is not practical or safe to biopsy the kidneys of most patients in clinical practice. However, this finding should be further explored because intervention to treat albuminuria, hyperfiltration, hypertension, low HDL cholesterol level, hyperuricemia, and obesity may be protective against the development and progression of CKD. Indeed, increased nephron size as detected by decreased glomerular density may be part of a common pathway for increased risk of CKD. Because increased nephron size may also be physiologic (eg, during pregnancy)[46] and is not always associated with adverse outcomes (eg, postnephrectomy kidney donors),[47,48] additional factors are likely necessary for progression to CKD. Nonetheless, novel biomarkers and risk factors for CKD may benefit from validation in living kidney donors to assess for an association with decreased glomerular density.

REFERENCES

1. Hoy WE, Hughson MD, Singh GR, Douglas-Denton R, Bertram JF. Reduced nephron number and glomerulomegaly in Australian aborigines: a group at high risk for renal disease and hypertension. *Kidney Int*. 2006;70:104-110.

2. Brenner BM, Chertow GM. Congenital oligonephropathy and the etiology of adult hypertension and progressive renal injury. *Am J Kidney Dis*. 1994;23:171-175.

3. Brenner BM, Garcia DL, Anderson S. Glomeruli and blood pressure. Less of one, more the other? *Am J Hypertens*. 1988;1:335-347.

4. Brenner BM. Hemodynamically mediated glomerular injury and the progressive nature of kidney disease. *Kidney Int*. 1983;23:647-655.

5. Zandi-Nejad K, Luyckx VA, Brenner BM. Adult hypertension and kidney disease: the role of fetal programming. *Hypertension*. 2006;47:502-508.

6. Newbold KM, Sandison A, Howie AJ. Comparison of size of juxtamedullary and outer cortical glomeruli in normal adult kidney. *Virchows Arch A Pathol Anat Histopathol*. 1992;420:127-129.

7. Samuel T, Hoy WE, Douglas-Denton R, Hughson MD, Bertram JF. Determinants of glomerular volume in different cortical zones of the human kidney. *J Am Soc Nephrol*. 2005;16:3102-3109.

8. Hayslett JP, Kashgarian M, Epstein FH. Functional correlates of compensatory renal hypertrophy. *J Clin Invest*. 1968;47:774-799.

9. Sigmon DH, Gonzalez-Feldman E, Cavasin MA, Potter DL, Beierwaltes WH. Role of nitric oxide in the renal hemodynamic response to unilateral nephrectomy. *J Am Soc Nephrol*. 2004;15:1413-1420.

10. Thomson SC, Vallon V, Blantz RC. Kidney function in early diabetes: the tubular hypothesis of glomerular filtration. *Am J Physiol Renal Physiol*. 2004;286:F8-F15.

11. Liu B, Preisig PA. Compensatory renal hypertrophy is mediated by a cell cycle-dependent mechanism. *Kidney Int*. 2002;62:1650-1658.

12. Thomas MC, Burns WC, Cooper ME. Tubular changes in early diabetic nephropathy. *Adv Chronic Kidney Dis*. 2005;12:177-186.

13. Taal MW, Brenner BM. Adaptation of nephron loss. In: Brenner BM, ed. *Brenner and Rector's The Kidney.* 8th ed. Maryland Heights, MO: W.B. Saunders; 2007:783-788.

14. Manalich R, Reyes L, Herrera M, Melendi C, Fundora I. Relationship between weight at birth and the number and size of renal glomeruli in humans: a histomorphometric study. *Kidney Int*. 2000;58:770-773.

15. Nyengaard JR, Bendtsen TF. Glomerular number and size in relation to age, kidney weight, and body surface in normal man. *Anat Rec*. 1992;232:194-201.

16. Rule AD, Amer H, Cornell LD, et al. The association between age and nephrosclerosis on renal biopsy among healthy adults. *Ann Intern Med*. 2010;152:561-567.

17. Melton LJ III. The threat to medical-records research. *N Engl J Med*. 1997;337:1466-1470.

18. Rule AD, Gussak HM, Pond GR, et al. Measured and estimated GFR in healthy potential kidney donors [published correction appears in *Am J Kidney Dis*. 2004;44(6):1126]. *Am J Kidney Dis*. 2004;43:112-119.

19. Levey AS, Coresh J, Greene T, et al. Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin Chem*. 2007;53:766-772.

20. Textor SC, Taler SJ, Driscoll N, et al. Blood pressure and renal function after kidney donation from hypertensive living donors. *Transplantation*. 2004;78:276-282.

21. Taler SJ, Textor SC, Canzanello VJ, Wilson DJ, Wiesner RH, Krom RA. Loss of nocturnal blood pressure fall after liver transplantation during immunosuppressive therapy. *Am J Hypertens*. 1995;8:598-605.

22. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome: a new world-wide definition: a consensus statement from the International Diabetes Federation. *Diabet Med*. 2006;23:469-480.

23. Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int*. 1999;55:713-723.

24. Rea DJ, Heimbach JK, Grande JP, et al. Glomerular volume and renal histology in obese and non-obese living kidney donors. *Kidney Int*. 2006;70:1636-1641.

25. Young RJ, Hoy WE, Kincaid-Smith P, Seymour AE, Bertram JF. Glomerular size and glomerulosclerosis in Australian aborigines. *Am J Kidney Dis*. 2000;36:481-489.

26. Luyckx VA, Brenner BM. Nephron endowment. In: Brenner BM, ed*: Brenner and Rector's The Kidney.* 8th ed. Maryland Heights, MO: W.B. Saunders:chap 19.

27. Fogo A, Hawkins EP, Berry PL, et al. Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal glomerular sclerosis. *Kidney Int*. 1990;38:115-123.

28. Goumenos DS, Kawar B, El Nahas M, et al. Early histological changes in the kidney of people with morbid obesity. *Nephrol Dial Transplant*. 2009;24:3732-3738.

29. Hoy WE, Bertram JF, Denton RD, Zimanyi M, Samuel T, Hughson MD. Nephron number, glomerular volume, renal disease and hypertension. *Curr Opin Nephrol Hypertens*. 2008;17:258-265.

30. Hsu CY, McCulloch CE, Iribarren C, Darbinian J, Go AS. Body mass index and risk for end-stage renal disease. *Ann Intern Med*. 2006;144:21-28.

31. Iseki K, Ikemiya Y, Kinjo K, Inoue T, Iseki C, Takishita S. Body mass index and the risk of development of end-stage renal disease in a screened cohort. *Kidney Int*. 2004;65:1870-1876.

32. Obermayr RP, Temml C, Gutjahr G, Knechtelsdorfer M, Oberbauer R, Klauser-Braun R. Elevated uric acid increases the risk for kidney disease. *J Am Soc Nephrol*. 2008;19:2407-2413.

33. Weiner DE, Tighiouart H, Elsayed EF, Griffith JL, Salem DN, Levey AS. Uric acid and incident kidney disease in the community. *J Am Soc Nephrol*. 2008;19:1204-1211.

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34. Sanchez-Lozada LG, Tapia E, Santamaria J, et al. Mild hyperuricemia induces vasoconstriction and maintains glomerular hypertension in normal and remnant kidney rats. *Kidney Int*. 2005;67:237-247.

35. Mazzali M, Kanellis J, Han L, et al. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. *Am J Physiol Renal Physiol*. 2002;282:F991-F997.

36. Hughson M, Farris AB III, Douglas-Denton R, Hoy WE, Bertram JF. Glomerular number and size in autopsy kidneys: the relationship to birth weight. *Kidney Int*. 2003;63:2113-2122.

37. Vazquez MA, Jeyarajah DR, Kielar ML, Lu CY. Long-term outcomes of renal transplantation: a result of the original endowment of the donor kidney and the inflammatory response to both alloantigens and injury. *Curr Opin Nephrol Hypertens*. 2000;9:643-648.

38. Keller G, Zimmer G, Mall G, Ritz E, Amann K. Nephron number in patients with primary hypertension. *N Engl J Med*. 2003;348:101-108.

39. Reyes L, Mañalich R. Long-term consequences of low birth weight. *Kidney Int Suppl*. 2005;97:S107-S111.

40. Goyal VK. Changes with age in the human kidney. *Exp Gerontol*. 1982;17:321-331.

41. Abdi R, Slakey D, Kittur D, Racusen LC. Heterogeneity of glomerular size in normal donor kidneys: impact of race. *Am J Kidney Dis*. 1998;32:43-46.

42. McLachlan MS. The ageing kidney. *Lancet*. 1978;2:143-145.

43. Li M, Nicholls KM, Becker GJ. Glomerular size and global glomerulosclerosis in normal Caucasian donor kidneys: effects of aging and gender. *J Nephrol*. 2002;15:614-619.

44. Kriz W, LeHir M. Pathways to nephron loss starting from glomerular diseases: insights from animal models. *Kidney Int*. 2005;67:404-419.

45. Rook M, Bosma RJ, van Son WJ, et al. Nephrectomy elicits impact of age and BMI on renal hemodynamics: lower postdonation reserve capacity in older or overweight kidney donors. *Am J Transplant*. 2008;8:2077-2085.

46. Christensen T, Klebe JG, Bertelsen V, Hansen HE. Changes in renal volume during normal pregnancy. *Acta Obstet Gynecol Scand*. 1989;68:541-543.

47. Ibrahim HN, Foley R, Tan L, et al. Long-term consequences of kidney donation. *N Engl J Med*. 2009;360:459-469.

48. Segev DL, Muzaale AD, Caffo BS, et al. Perioperative mortality and long-term survival following live kidney donation. *JAMA*. 2010;303(10):959-966.

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