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Received for publication: 10.6.09; Accepted in revised form: 17.12.09

Nephrol Dial Transplant (2010) 25: 2252–2258

doi: 10.1093/ndt/gfq007

Advance Access publication 1 February 2010

## Fractal analysis of the retinal vasculature and chronic kidney disease

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### Abstract

**Background.** Fractal analysis provides a global index of the geometric complexity and optimality of vascular networks. In this study, we investigated the relationship between fractal measurements of the retinal vasculature and chronic kidney disease (CKD).

**Methods.** This was a population-based case-control study which included participants from the Singapore Prospective Study Program. We identified 261 participants with CKD, defined as estimated glomerular filtration rate of <60 mL/min/1.73 m<sup>2</sup>, and 651 controls. The retinal fractal dimension ( $D_f$ ) was quantified from digitized fundus photographs using a computer-based programme.

**Results.** The mean  $D_f$  was  $1.43 \pm 0.048$  in the participants with CKD and  $1.44 \pm 0.042$  in controls ( $P = 0.013$ ). Sub-optimal  $D_f$  in the lowest (first) and highest (fifth) quintiles were associated with an increased prevalence of CKD after adjusting for age, systolic blood pressure, diabetes and other risk factors [odds ratio (OR) 2.10, 95% confidence interval (CI) 1.15, 3.83 and OR 1.84, 95% CI 1.06, 3.17; compared to the fourth quintile, respectively]. This association was present even in participants without diabetes or hypertension.

**Conclusions.** Our study found that an abnormal retinal vascular network is associated with an increased risk of CKD, supporting the hypothesis that deviations from optimal microvascular architecture may be related to kidney damage.

**Keywords:** chronic kidney disease; retinal vascular network

### Introduction

Microvascular disease has been suggested to play a major aetiological role in a large proportion of patients with chronic kidney disease (CKD). For example, microvascular risk factors, such as diabetes and hypertension, are strongly associated with CKD [1–4], and microvascular alterations in the renal circulation have been documented in animal models with CKD [5].

Changes in the retinal microvasculature, which is accessible to direct non-invasive visualization, can be used to probe the state of the systemic microcirculation [6–8]. Retinal microvascular abnormalities, such as retinopathy (microaneurysms, retinal haemorrhages) have been previously shown to be associated with renal impairment in several studies [9–14]. Studies have also reported that changes in the calibre of retinal vessels is related to CKD [15,16]. However, these changes represent specific aspects of the retinal vascular network.

The retinal vasculature can be considered to be a fractal structure because it has a branching pattern that demonstrates the property of self-similarity [17,18]. Fractal analysis provides a global index that quantifies the geometric complexity of the retinal vascular network, summarizing the entire branching pattern, and may be a more sensitive indicator of early microvascular disease compared to overt retinopathy signs [19]. We have reported that fractal analysis of digital retinal images using a novel computer-based programme can be performed reliably and efficiently [19], correlates with biological parameters including age and

blood pressure, and is independently associated with retinopathy signs in persons with type 1 diabetes [20].

In this study, we examined the relationship between retinal vascular fractal dimension ( $D_f$ ) and CKD in a population-based case-control study.

## Materials and methods

### Participants

We conducted a population-based case-control study utilizing data from the Singapore Prospective Study Programme, as previously reported [8,21,22]. In brief, subjects were recruited from one of four previous cross-sectional studies: Thyroid and Heart Study 1982–83 [23], National Health Survey 1992 [24], National University of Singapore Heart Study 1993–1993 [25] or National Health Survey 1998 [26]. Participants, aged 24–95, were randomly sampled from the Singapore population. The study population was selected by the Ministry of Health, Singapore. The details on the recruiting process and the number of the participants who were included have been described previously [8,21,22]. Ethical approval was obtained from the Institutional Review Board.

Of the 7742 eligible participants, 5157 had a clinic examination, and 4137 were offered retinal photography. Of these 4137 participants, retinal photographs were available for 4098 participants, who formed our base population.

### Retinal photography and fractal analysis

Digital fundus photography was performed with a 45-degree digital retinal camera (Canon CR-DGi with a 10D SLR back, Canon, Japan) after pupil dilatation. Early Treatment for Diabetic Retinopathy Study (ETDRS) standard fields 1 and 2 retinal images of each eye were centred at the optic disc and fovea, respectively. Retinal photographs were considered ungradeable if there were less than two traceable retinal vessels present [19,20].

Fractal analysis was performed from the ETDRS field 1 fundus photographs which were centred on the optic disc. Retinal images from the right eye were analysed, unless they were ungradeable, in which case the left eye retinal images were used. A trained grader, masked to participants' characteristics, used a computer-based programme [International Retinal Imaging Software (IRIS-Fractal)] for fractal analysis of the photographs based on a standardized protocol described in an earlier trial [19]. The  $D_f$  of the retinal vasculature was measured within a predefined circular area centred on the optic disc of 3.5 disc radii. After all the retinal vessels within this region were automatically traced by IRIS-Fractal, the grader compared the tracing with the photograph and deleted artefacts which were mistakenly identified as vessels, such as peripapillary atrophy, retinal pigment abnormalities, choroidal vessels and reflection from the nerve fibre layer. Subsequently, fractal analysis was performed by the programme, and the fractal dimension was calculated using the box-counting approach [18]. The intragrader intraclass correlation coefficient of IRIS-Fractal measurements ranged from 0.93 to 0.95, demonstrating high reproducibility [19].

### Definition of cases and controls

We defined CKD based on kidney function as estimated glomerular filtration rate (eGFR) of  $<60$  mL/min/1.73 m<sup>2</sup>, which represents stage 3 and above of CKD as defined by US National Kidney Foundation Kidney Disease Outcome Quality Initiative [27]. The eGFR was calculated from the serum creatinine concentration by using the Modification of Diet in Renal Disease Study equation [28]. This was defined as:  $eGFR = 186.3 \times [\text{serum creatinine (milligram per decilitre)}]^{-1.154} \times \text{age}^{-0.203} \times (0.742 \text{ for women})$ . The serum creatinine level was reported as micromole per litre, and its measurement was performed at the National University Hospital Reference Laboratory.

Among the 4098 participants with retinal photography, 261 fulfilled the criteria of CKD. From the remaining 3837 participants free of CKD, we identified two to three controls per case (261 cases and 651 controls) frequency matched on 5-year age interval and gender. We had difficulty finding controls for cases aged  $\geq 65$  years even after ignoring gender. We matched 651 controls for 261 cases. Of the total 912 cases and controls, participants with ungradeable photographs of both eyes ( $n = 20$ ) and those with missing information on important covariates such as blood pressure

(BP), plasma glucose, age and ethnicity ( $n = 8$ ) were excluded from the study. The remaining 884 (97.0%) participants were included in the current analysis, including 251 participants with CKD and 633 controls.

### Measurement of other variables

Prior to retinal photography, all participants included in this study were interviewed at their homes regarding their medical history, demographic and lifestyle (including alcohol intake and smoking) factors. The age of each participant was recorded at the time of health screening, when retinal photography was performed. Systolic and diastolic BP were evaluated with a digital automatic BP monitor (Dinamap model Pro 100V2; Criticon, Norderstedt, Germany), after the participants were seated for 5 min with legs uncrossed. The average of the two closest BP readings out of a total of three measurements was taken as each participant's BP. Hypertension was defined as systolic BP  $\geq 140$  mm Hg, diastolic BP  $\geq 90$  mm Hg or self-reported physician-diagnosed hypertension. Diabetes mellitus was defined as fasting plasma glucose of  $\geq 7$  mmol/L, self-reported physician-diagnosed diabetes and the use of diabetic medication. The body mass index (BMI) was calculated as body weight in kilograms divided by the square of body height in metres measured using a wall-mounted measuring tape. Being overweight was defined as BMI of 25 to 29.9 kg/m<sup>2</sup>, while obesity was defined as BMI  $\geq 30$  kg/m<sup>2</sup>. Current smokers were identified by those who were smoking every day or on some days. Alcohol consumption was categorized into drinkers (irrespective of quantity) and non-drinkers. Biochemical analysis of fasting venous blood samples was performed on the day of health screening at the National University Hospital Reference Laboratory for serum creatinine, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, triglycerides and fasting glucose. Microalbuminuria was defined as a urinary:creatinine ratio (ACR) of  $\geq 17$   $\mu\text{g/g}$  for men and  $\geq 25$   $\mu\text{g/g}$  for women. Proteinuria (macroalbuminuria) was defined as ACR  $\geq 250$   $\mu\text{g/mg}$  for men and  $\geq 355$   $\mu\text{g/mg}$  for women [27]. Trained graders at the Centre for Eye Research Australia, University of Melbourne, masked to participant characteristics, evaluated the retinal photographs for the presence of retinopathy and laser treatment of retinopathy. Any retinopathy was defined as a severity score of level 15 and above according to a scale graded using the Early Treatment Diabetic Retinopathy Study adaptation of the modified Airlie House Classification System corresponding to the presence of any of the following lesions: microaneurysms, haemorrhages, cotton wool spots, intraretinal microvascular abnormalities, hard exudates, venous beading and new vessels [29,30]. Laser treatment of retinopathy was defined as present when laser scars were detected in the retinal photographs.

### Statistical analysis

We used combined data from right and left eye (left eye measurements were taken when right was ungradeable) for our analysis.  $D_f$  was categorized into quintiles and also analysed as a continuous variable (per standard deviation change). We examined the association between  $D_f$  and CKD using unconditional logistic regression analyses in two multivariate models. In the first model, adjustment was made for age (years), ethnicity (Chinese, Malay, Indian) and gender (female, male). In the second model, adjustment was made for variables in the first model, as well as other covariates known to be associated with CKD, including diabetes status (absent, present), systolic BP (millimetres of mercury), total cholesterol (micromole per litre), HDL cholesterol (micromole per litre), BMI (kilogram per square metre), current smoking (absent, present), alcohol consumption (absent, present), the presence of retinopathy and laser treatment of retinopathy. In subgroup analyses, we examined the association between  $D_f$  and CKD in subjects without diabetes mellitus or hypertension. The test for trend was carried out in the same multivariate regression model including quintiles of  $D_f$  as an ordinal variable. The Pearson correlation between eGFR and microalbuminuria was calculated.  $P < 0.05$  was considered to be statistically significant. All statistical analyses were undertaken using SPSS version 17.0 (SPSS Inc, Chicago, Ill).

## Results

Baseline characteristics of the participants are summarized in Table 1. Due to the difficulty in finding controls

**Table 1.** Demographic and retinal vascular characteristics by cases and controls

Characteristic	All ( <i>n</i> = 884)	Cases ( <i>n</i> = 251)	Controls ( <i>n</i> = 633)	<i>P</i>
Female gender (%)	44.7	44.2	44.9	0.862
Age (years) mean (SD)	60.4 (11.18)	62.1 (11.71)	59.7 (10.90)	0.003
Ethnicity (%)				
Chinese	57.9	45.0	63.0	<0.001
Malay	21.8	27.1	19.7	
Indian	20.2	27.9	17.2	
Diabetes (%)	12.9	27.5	7.1	<0.001
Ever smoker (%)	21.0	16.7	22.7	0.048
Current smoker (%)	10.4	7.2	11.7	0.047
Ever drinker (%)	29.8	22.7	32.5	0.004
Overweight (%)	34.3	41.8	31.3	0.003
Obesity (%)	7.4	10.0	6.3	0.061
Retinopathy (%)	12.9	19.0	10.5	0.001
Treatment (%)	1.2	3.0	0.5	0.003
BMI (kg/m <sup>2</sup> ) mean (SD)	24.2 (6.71)	24.8 (3.85)	24.0 (7.54)	0.089
SBP (mm Hg) mean (SD)	140.7 (23.91)	150.2 (26.26)	136.9 (21.80)	<0.001
HDL cholesterol mean (SD)	1.38 (0.3445)	1.33 (0.3193)	1.40 (0.3522)	0.005
Total cholesterol mean (SD)	5.31 (0.9666)	5.40 (1.0385)	5.28 (0.9350)	0.086
Creatinine	1.04 (0.4215)	1.42 (0.5770)	0.89 (0.1934)	<0.001
eGFR	75.6 (22.05)	51.1 (9.48)	85.3 (17.63)	<0.001
Proteinuria	766.0 (652.6)	826.1 (719.1)	537.8 (198.9)	<0.001
Microalbuminuria	96.3 (72.8)	115.7 (78.1)	79.1 (63.6)	0.001
Refined fractal dim mean (SD)	1.44 (0.0442)	1.43 (0.0479)	1.44 (0.0424)	0.013

BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; SBP, systolic blood pressure.

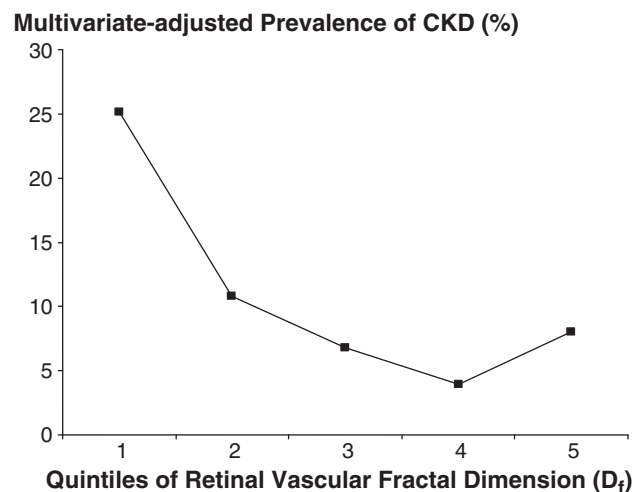
in the older age group, cases were older than controls. Compared to controls, cases were more likely to have higher prevalence of diabetes, to be overweight, to have higher levels of systolic BP, lower levels of HDL cholesterol and less likely to be Chinese and drinkers ( $P < 0.05$ ). The Pearson correlation between eGFR and microalbuminuria was  $-0.19$  ( $P < 0.001$ ).

The mean  $D_f$  was  $1.43 \pm 0.048$  in the participants with CKD and  $1.44 \pm 0.042$  in controls ( $P = 0.013$ ). Multivariate-adjusted (including age, ethnicity, gender, diabetes status, systolic BP, BMI, total cholesterol, HDL cholesterol, current smoking and alcohol consumption) curves for this cohort suggested a U-shaped relationship between  $D_f$  and CKD where the lowest prevalence of CKD was associated with the fourth  $D_f$  quintile, and the lowest (first) and highest (fifth) quintiles were associated with an increased prevalence of CKD (Figure 1). Hence, we compared the odds of CKD in the lowest and highest (first and fifth) quintiles to the fourth quintile. Table 2 shows that after adjustment for age, gender and ethnicity, the first and fifth quintiles were significantly associated with increased odds of CKD compared to the fourth quintile [odds ratio (OR) 2.47, 95% confidence interval (CI) 1.45, 4.21 and OR 1.74, 95% CI 1.04, 2.92, respectively). This association persisted after additional adjustment for diabetes, systolic BP, BMI, alcohol consumption, current smoking status, total cholesterol, HDL cholesterol, presence of retinopathy and laser treatment of retinopathy, with participants at the lowest and highest  $D_f$  quintiles having 110% and 84% higher odds of CKD, respectively, compared to those in the fourth quintile (OR 2.10, 95% CI 1.15, 3.83 and OR 1.84, 95% CI 1.06, 3.17, respectively). The odds of CKD were not significantly increased in the lowest (first) quintile compared to the rest of the quintiles (second to fifth).

In subgroup analyses, the extreme  $D_f$  quintiles (first and fifth quintiles) were also associated with an increased odds of CKD in participants without diabetes mellitus (OR 2.07, 95% CI 1.05, 4.07 and OR 1.89, 95% CI 1.06, 3.39, respectively). This association was stronger in participants without hypertension (OR 3.19, 95% CI 1.28, 7.94 and OR 3.17, 95% CI 1.56, 6.47, respectively, Table 3).

## Discussion

In this population-based study, we examined the association between retinal fractals and CKD and showed that suboptimal retinal  $D_f$  (extreme lowest and highest quin-



**Fig. 1.** Multivariate-adjusted prevalence of chronic kidney disease cases.

**Table 2.** Adjusted odds ratios of chronic kidney disease in relation to retinal fractal dimension

Retinal fractal dimension	Cases (n = 251)	Controls (n = 633)	Model 1 <sup>a</sup>	P	Model 2 <sup>b</sup>	P
			Odds ratio (95% confidence interval)		Odds ratio (95% confidence interval)	
Quintile 1 ( $\leq 1.4183$ )	70 (27.9)	107 (16.9)	2.47 (1.45, 4.21)	0.001	2.10 (1.15, 3.83)	0.016
Quintile 2 (1.4184–1.4407)	50 (19.9)	126 (19.9)	1.55 (0.92, 2.61)	0.100	1.46 (0.83, 2.56)	0.186
Quintile 3 (1.4408–1.4540)	46 (18.3)	131 (20.7)	1.46 (0.88, 2.44)	0.148	1.38 (0.80, 2.38)	0.242
Quintile 4 (1.4541–1.4661)	34 (13.5)	144 (22.7)	1.00		1.00	
Quintile 5 ( $\geq 1.4662$ )	51 (20.3)	125 (19.7)	1.74 (1.04, 2.92)	0.025	1.84 (1.06, 3.17)	0.029
P for trend			0.069		0.439	
Per SD increase (0.0442)			0.90 (0.76, 1.05)	0.182	0.97 (0.78, 1.20)	0.782

<sup>a</sup>Model 1 odd ratios adjusted for age, gender and ethnicity.<sup>b</sup>Model 2 odds ratios adjusted for age, gender, ethnicity, diabetes, systolic blood pressure, body mass index, alcohol consumption, current smoker, total cholesterol, high-density lipoprotein cholesterol, presence of DR and presence of treatment.**Table 3.** Adjusted odds ratios of chronic kidney disease in subjects without diabetes mellitus or hypertension in relation to retinal fractal dimension

Retinal fractal dimension	Cases (n = 182)	Controls (n = 588)	Model 1 <sup>a</sup>	P	Model 2 <sup>b</sup>	P
			Odds ratio (95% confidence interval)		Odds ratio (95% confidence interval)	
<i>Subjects without diabetes mellitus</i>						
Quintile 1 (≤1.4183)	32 (17.6)	120 (20.4)	2.28 (1.23, 4.21)	0.009	2.07 (1.05, 4.07)	0.036
Quintile 2 (1.4184–1.4407)	26 (14.3)	139 (23.6)	1.60 (0.89, 2.88)	0.114	1.57 (0.86, 2.87)	0.147
Quintile 3 (1.4408–1.4540)	44 (24.2)	91 (15.5)	1.38 (0.77, 2.47)	0.280	1.38 (0.76, 2.50)	0.289
Quintile 4 (1.4541–1.4661)	38 (20.9)	115 (19.6)	1.00		1.00	
Quintile 5 (≥1.4662)	42 (23.1)	123 (20.9)	1.82 (1.03, 3.20)	0.038	1.89 (1.06, 3.39)	0.032
	Cases (n = 114)	Controls (n = 461)	Odds ratio (95% confidence interval)	P	Odds ratio (95% confidence interval)	P
<i>Subjects without hypertension</i>						
Quintile 1 (≤1.4183)	19 (16.7)	58 (12.6)	2.43 (1.07, 5.54)	0.035	3.19 (1.28, 7.94)	0.013
Quintile 2 (1.4184–1.4407)	17 (14.9)	85 (18.4)	1.27 (0.59, 2.77)	0.540	1.22 (0.54, 2.79)	0.634
Quintile 3 (1.4408–1.4540)	24 (21.1)	99 (21.5)	1.71 (0.84, 3.47)	0.139	1.68 (0.80, 3.54)	0.171
Quintile 4 (1.4541–1.4661)	16 (14.0)	117 (25.4)	1.00		1.00	
Quintile 5 (≥1.4662)	38 (33.3)	102 (22.1)	2.69 (1.37, 5.28)	0.004	3.17 (1.56, 6.47)	0.001

<sup>a</sup>Model 1 odd ratios adjusted for age, gender and ethnicity.

tiles) was associated with increased odds of CKD. This association was independent of age, gender, ethnicity, diabetes, BP and other vascular risk factors, and was seen in individuals without diabetes or hypertension.

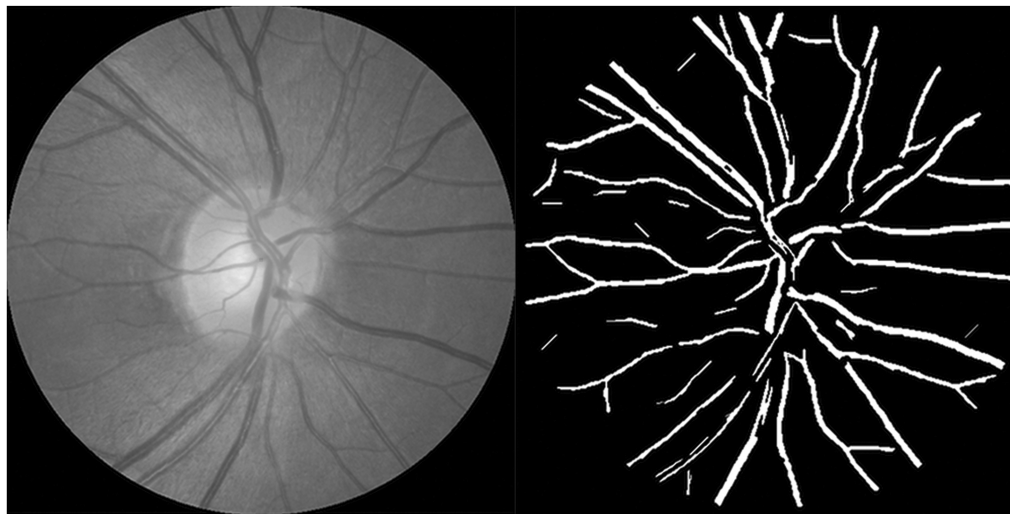
Our results provide new data on the association between geometric complexity and optimality of vascular networks and the risk of CKD. Few studies are available for direct comparison. Retinal vascular fractals have been inversely correlated with age and systolic BP [19], and positively correlated with early retinopathy signs in young individuals with type 1 diabetes [20]. Our results indicate a U-shaped relationship between  $D_f$  and CKD, in which suboptimal  $D_f$  in the lowest and highest quintiles were associated with an increased prevalence of CKD. This suggests that the vascular network has a theoretical optimum which is the most efficient in terms of energy expenditure [31,32], but additional studies are necessary to verify this hypothesis. We propose that deviations from the optimal state could result in haemodynamic

stresses both locally and globally, resulting in disease condition [31].

Our data build on previous work associating retinal vasculature with renal dysfunction [9–15,33–35]. In a recent analysis, retinal arteriolar narrowing was found to be associated with CKD in the same study population [16]. Our results demonstrate that suboptimal  $D_f$  is another useful indicator for CKD. The association between suboptimal  $D_f$  and CKD (OR = 1.99 for first quintile, OR = 1.92 for fifth quintile) may be stronger than that of retinal arteriolar narrowing (OR = 1.68), but further studies are needed to substantiate this hypothesis. Not surprisingly,  $D_f$  was more significant as an independent marker of CKD in individuals without hypertension, which is already known to be strongly associated with CKD [1–4].

These findings offer further insights into the pathophysiology of reduced GFR and the development of renal dysfunction. The renal microcirculation is intricately related to kidney function and urine production [36]. A low  $D_f$ ,





(a) shows a Df of 1.4958



(b) shows a Df of 1.4541



(c) shows a Df of 1.4073

**Fig. 2.** Fractal pattern of retinal vessels. **(A)** An eye with a high fractal dimension ( $D_f = 1.4958$ ) and a more complex vascular branching pattern. **(B)** An eye with an intermediate fractal dimension ( $D_f = 1.4541$ ). **(C)** An eye with a low fractal dimension ( $D_f = 1.4073$ ) and a less complex vascular branching pattern (vascular rarefaction). The participants whose eyes are shown in A and B have chronic kidney disease.

which occurs in ageing, hypertension and other vascular diseases [37–39], reflects rarefaction of the microvasculature. This could be associated with a reduction in glomerular volume, which has been described in patients with hypertension and renal disease [40–42]. A high  $D_f$  reflects an increased geometric complexity of the vasculature, and is likely to be associated with a high glomerular volume. This leads to marked glomerular stress [42] and hyperfiltration, which causes renal damage and eventual decline of GFR. Hyperfiltration predicts the risk of diabetic nephropathy in type 1 diabetics [43]. These changes in  $D_f$  may be mediated by the endothelium, which is crucial in ensuring optimality at vascular bifurcations [44]. Additional studies are required to verify our hypotheses. Our study adds to other studies reporting a U-shaped relationship between diastolic BP and progression of CKD in patients with diabetes [45]. A similar U-shaped relationship between BP and mortality in end-stage renal disease patients has also been reported [46,47].

Our results have both potential clinical and academic implications. Firstly, we speculate that retinal image analysis of  $D_f$  can be used as a non-invasive tool to identify individuals at higher risk of CKD (Figure 2). Early treatment in asymptomatic individuals can prevent the progression of CKD [48]. Secondly, our results support the hypothesis that suboptimal microvasculature leads to renal impairment. Further research on the detrimental effects of suboptimal microvascular architecture may enhance our understanding of the mechanisms underlying microvascular disease, and elucidate new targets for the diagnosis and treatment of CKD.

Strengths of our study include the definition of CKD using specific criteria, the selection of controls from the same base population as cases and detailed information on possible confounders, such as BP, diabetes, smoking status, cholesterol levels and BMI. However, some limitations should be considered in the interpretation of our findings. Firstly, this was a cross-sectional study, and did not allow us to infer the temporal relationship between changes in retinal  $D_f$  and CKD. This would be a subject of future research. Secondly, our case-control study included cases as patients with CKD stage 3 and above, defined as  $\text{eGFR} < 60 \text{ mL/min/1.73 m}^2$  [27]. Further studies are needed to investigate possible associations between retinal  $D_f$  and earlier indicators of renal dysfunction, such as microalbuminuria. We also did not have information on whether dialysis patients were included in the study population, hence we were unable to ascertain if the observed difference between the cases and controls were also present in patients without dialysis. Lastly, we could only speculate on the possible mechanisms underlying the association between suboptimal microvascular architecture and CKD. More definitive evidence demonstrating inefficient flow and increased ischaemia in the presence of suboptimal  $D_f$  should be obtained from animal or human tissue models, as well as longitudinal studies.

In summary, our study shows that suboptimal retinal fractals are associated with CKD. Our results support the hypothesis that optimal microvascular architecture is important in the maintenance of kidney health, and deviations

from the ideal design appear to occur in disease states such as CKD.

**Acknowledgements.** This study was funded by National Medical Research Council (NMRC), IRG07nov013, STaR/0003/2008 and the Singapore Bio Imaging Consortium (SBIC) C-011/2006.

**Conflict of interest statement.** None declared.

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Received for publication: 28.8.09; Accepted in revised form: 6.1.10