Novel retinal vascular phenotypes for the potential assessment of long-term risk in living kidney donors

Novel retinal vascular phenotypes for the potential assessment of long-term risk in living kidney donors

OPEN

Ylenia Giarratano[1], Dan Pugh[2,3], Tariq E. Farrah[2,3], Gabriel C. Oniscu[4], Thomas J. MacGillivray[5], Baljean Dhillon[5,6], Neeraj Dhaun[2,3,4,8] and Miguel O. Bernabeu[1,7,8]

[1]Centre for Medical Informatics, Usher Institute, University of Edinburgh, Edinburgh, UK; [2]University/BHF Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK; [3]Department of Renal Medicine, Royal Infirmary of Edinburgh, Edinburgh, UK; [4]Edinburgh Transplant Centre, Royal Infirmary of Edinburgh, Edinburgh, UK; [5]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK; [6]Princess Alexandra Eye Pavilion, Edinburgh, UK; and [7]The Bayes Centre, University of Edinburgh, Edinburgh, UK

Kidney International (2022) **102,** 661–665; https://doi.org/10.1016/j.kint.2022.06.019

KEYWORDS: cardiovascular disease; chronic kidney disease; kidney donation

Correspondence: *Neeraj Dhaun, University/BHF Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, Midlothian EH16 4TJ, UK. E-mail: bean.dhaun@ed.ac.uk; or Miguel O. Bernabeu, Bayes Centre/Usher Institute, University of Edinburgh, Edinburgh EH16 4UX, UK. E-mail: miguel.bernabeu@ed.ac.uk*

[8]ND and MOB contributed equally.

Received 17 May 2022; accepted 16 June 2022; published online 8 July 2022

Chronic kidney disease (CKD) has a global prevalence of ≈10% and is independently associated with incident cardiovascular disease (CVD).[1] The risk of CVD increases as estimated glomerular filtration rate (eGFR) declines and is highest in those with kidney failure.[1] Kidney transplantation prolongs survival of patients with kidney failure, and transplantation of a kidney from a living donor is considered the optimal form of renal replacement therapy. However, kidney donation may be associated with longer-term risks of developing both CKD and CVD.[S1,2] Currently, we are unable to identify kidney donors most at risk of these complications.

The kidney and eye are remarkably similar, and diseases in the 2 organs may manifest via common pathways.[3] Transparency of the ocular media allows direct visualization and imaging of the chorioretinal microvasculature that may be affected in systemic diseases, such as CKD. Optical coherence tomography angiography (OCT-A) allows rapid visualization of the retinal microcirculation, and its potential clinical use has been demonstrated in Alzheimer disease, diabetes mellitus, and hypertension.[4–6] Herein, we compared retinal microvascular phenotypes of patients with CKD, living kidney donors, and healthy volunteers. We hypothesised that living kidney donors, without CKD or overt CVD and following kidney donation, would have retinal microvascular changes similar to patients with CKD, mirroring their higher risk of developing such complications.

We imaged 1 eye (either left or right) of 30 patients with CKD, 30 age- and gender-matched healthy volunteers, and 30 kidney donors at the same time of day using the RTVue-XR Avanti machine (Optovue, Inc.). Subject demographics are shown in Supplementary Table S1. Both the superficial capillary plexus and deep capillary plexus were imaged with a 3×3-mm^2 field of view and used to extract retinal metrics, as previously described[S2] (Supplementary Figure S1A and Supplementary Methods).

First, in a cross-sectional study, we investigated candidate retinal biomarkers that might discriminate patients with CKD from healthy volunteers. We used multiple regression models corrected for age, gender, systolic and diastolic blood

pressure, smoking status, and body mass index to investigate associations between health and CKD and each extracted retinal phenotype. Given the large number of retinal features, false discovery rate was applied to correct P values as a conservative approach to reduce the chance of false-positive results (type I error). In addition, to avoid collinearity among significant retinal phenotypes in downstream analyses, correlated features were identified by applying hierarchical clustering, where the proximity between variables is based on the value of the Pearson correlation coefficient. By selecting a value for the proximity equal to 0.5, we grouped variables containing similar information (Supplementary Figure S1B). For each group, the retinal feature with the smallest P value was included in further analyses. Principal component analysis and k-means clustering were then applied to assess the separability of the 2 groups based on the significant retinal features selected. Finally, the similarity of kidney donors to either healthy volunteers or patients with CKD was investigated by assessing their proximity to each of the clusters previously identified.

Next, we explored how retinal phenotypes might change over time following kidney donation. We examined OCT-A images in 10 kidney donors from predonation up to 15 months after kidney donation. Subject demographics are shown in Supplementary Table S2. We investigated changes in the 2 most common CKD-related vessel morphometrics reported in previous investigations[7,S3]—(i) vessel density in the parafoveal ring and (ii) foveal avascular zone (FAZ) area (Supplementary Figure S1C)—as well as the significant features obtained in our cross-sectional study. We calculated differences in retinal phenotypes compared with predonation measurements. Repeated OCT-A imaging was also performed in 18 healthy volunteers to assess if these retinal measures might change over time in health.

RESULTS

Living kidney donors align phenotypically with CKD

We found 6 retinal features associated with CKD, 2 related to the distribution of shapes of the intercapillary spaces in the temporal and superior regions, and 4 describing the tortuosity and curvature of vessels in the whole image and inferior region. Clustering analysis performed on the principal component analysis (Figure 1a) identified 2 clusters of retinal features that separated those with CKD from healthy volunteers (controls) with accuracy of 78% (Figure 1b). Using the same set of retinal features and methodological approach, we defined where subjects who had previously donated a kidney (donors; n = 30) might sit in relation to controls and patients with CKD. Notably, donors did not have eGFR impairment diagnostic of CKD, or overt CVD. Principal component analysis in Figure 1c shows that donors spanned both control and CKD groups, with most donors falling into the CKD cluster (Figure 1d), contrary to their clinical assessment. In addition, we investigated whether the distribution of donors in the first principal component of the controls-CKD plane (the one with highest variance, principal component 1) correlated with covariates such as age, time since kidney donation, and eGFR. No associations between principal component 1 and these covariates were observed (Supplementary Table S3), confirming that the alignment of donors to either healthy subjects or patients with CKD was not influenced by these recognized risk factors for CKD or CVD.

Retinal vascular phenotypes associated with CKD evolve over time in living kidney donors

We then investigated how the retinal features identified in the cross-sectional study changed over time in healthy controls and in kidney donors before, and following, kidney donation. In health, we found that changes in almost all retinal phenotypes distributed around 0 ($P > 0.05$), suggesting that they did not change over time in this group. In donors, we observed significant temporal changes in 6 of the phenotypes that were stable in controls, including an increase in FAZ area, a decreased asymmetry in the distribution of shapes of the intercapillary spaces (skewness of circularity ratio measure) (Figure 2a and b), an increase of vessel connectivity (clustering coefficient), a decrease in extreme values of vessel curvature (kurtosis of curvature measure), an increase in extreme values of vessel tortuosity (kurtosis of tortuosity metric), and a decreased asymmetry in the distribution of the vessel tortuosity (skewness of tortuosity metric) (Supplementary Figure S2). Finally, as kidney donors lose a degree of kidney function following donation, we assessed if these longitudinal retinal changes were associated with changes in eGFR (Figure 2c and d). Interestingly, from the 6 phenotypes discovered, only the increase in FAZ area showed a moderate degree of correlation ($R = 0.54$; $P = 0.03$), whereas the temporal changes in the remaining 5 could not be explained by changes in eGFR after donation.

DISCUSSION

Following donation, living kidney donors lose overall functional kidney mass but retain near-normal eGFR. It is a recent recognition that living kidney donation has increased longer-term risks of developing CKD and CVD.[S1,2] Identifying those donors most at risk remains a clinical challenge. Using OCT-A imaging, we analyzed retinal microvascular changes between 3 groups (healthy volunteers, CKD, and donors). We found that OCT-A discriminated healthy volunteers from patients with CKD, and interestingly, living kidney donors aligned phenotypically with CKD, despite having near-normal kidney function. On the basis of these findings, we investigated how OCT-A phenotypes that are stable in healthy individuals evolved over time following kidney donation. Our longitudinal analyses suggest that changes in FAZ, intercapillary space shape, vessel connections, tortuosity, and vessel curvature are retinal microvascular characteristics that might, in future, help track the renal and cardiovascular consequences of kidney donation beyond existing clinical

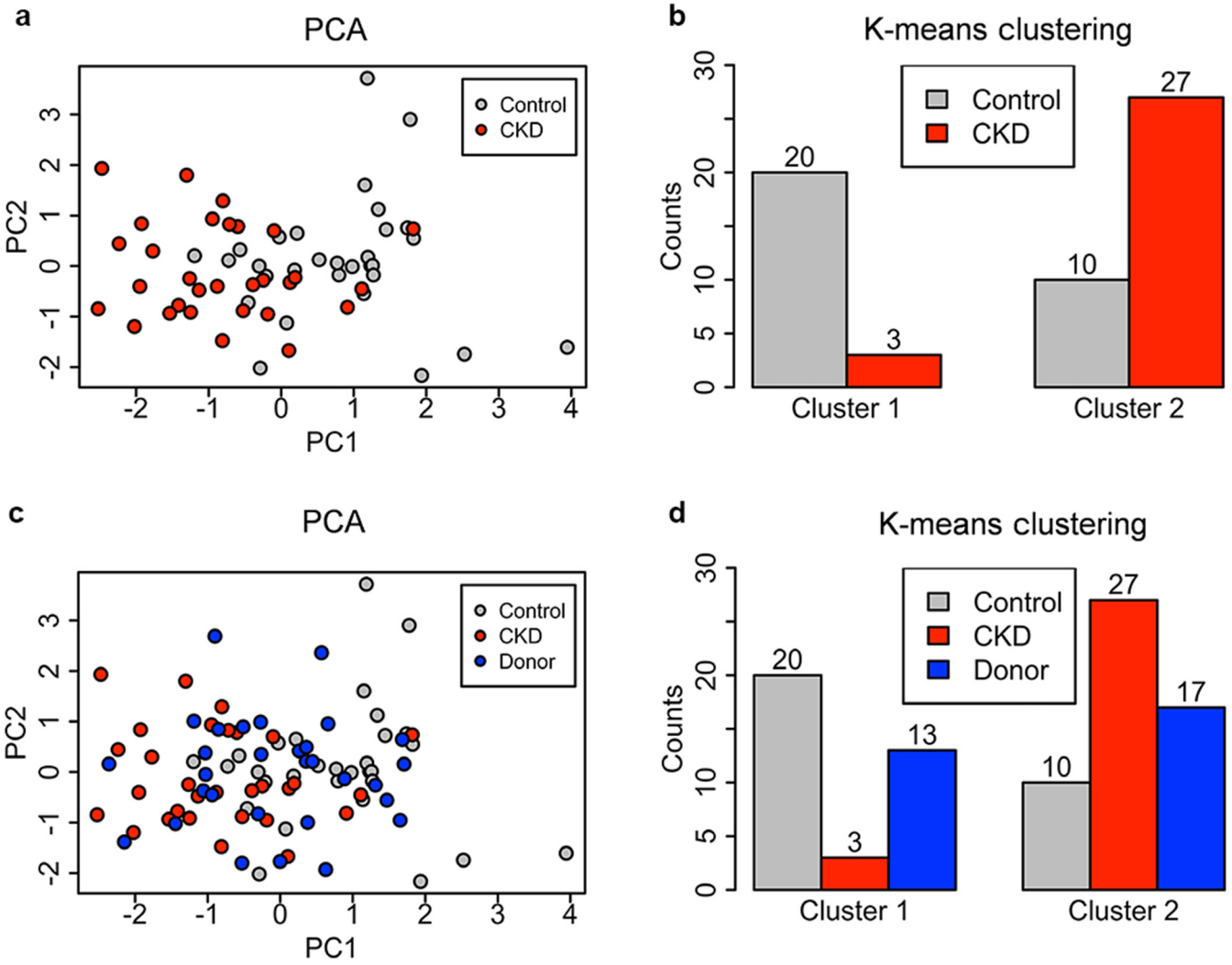

Figure 1 | (a) Principal component analysis (PCA) of chronic kidney disease (CKD) and control groups. (**b**) Histogram of number of CKD and control participants in each cluster. (**c**) PCA of control, CKD, and kidney donor groups. (**d**) Histogram of number of CKD, control, and kidney donor participants in each cluster. PC, principal component.

markers. Among these features, changes in FAZ area have been reported previously in studies including patients with CKD,[7] whereas vessel tortuosity has been associated with diabetes and hypertension, common risk factors for both CKD and CVD.[S4]

Previous work has shown that retinal microvascular injury is associated with CKD independently of age, gender, race, hypertension, and diabetes.[8] This suggests shared mechanisms of injury between the eye and the kidney. It is well recognized that alterations to the kidney's microvasculature—resulting in compromised blood flow—contribute to the development of CKD.[9] These can be a result of systemic inflammation, an imbalance in angiogenic growth factors, and/or dysfunction of the endothelium. They can promote vessel rarefaction, which will worsen the existing hemodynamic dysfunction, leading to renal ischemia, the gradual accrual of scarring, and progression to kidney failure. These systemic mechanisms may also account for injury within the eye in patients with CKD. Indeed, they may also be responsible, in part, for the retinal microvascular changes we are seeing in living kidney donors.

We recognize that the small sample size is a limitation of our study. However, our 3 groups were matched in terms of age and sex, and no family members of individuals with CKD were included in the kidney donor group. In terms of strengths and novelty, we have developed a new method to analyze OCT-A images and extract retinal microvascular phenotypes and, despite the preliminary nature of the longitudinal study, we have shown and quantified, for the first time, changes in the retinal microvasculature over time in living kidney donors.

In conclusion, this study represents a step forward in characterizing the renal and cardiovascular consequences of living kidney donation, and our findings may contribute to refining risk assessment of these patients. Future work should validate the generalizability of these findings in larger longitudinal cohorts of kidney donors and so help translate this knowledge into clinical practice.

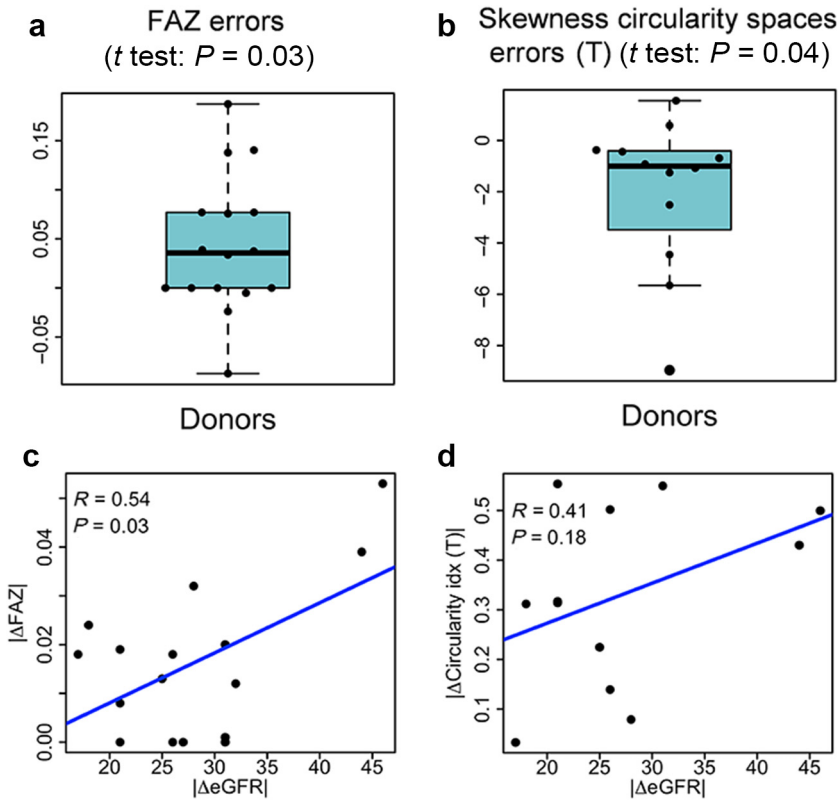

Figure 2 | (a,b) Box plots of signed relative errors in measurements between baseline and follow-up visits in kidney donors. (**c,d**) Pearson correlations between differences in retinal phenotypes and differences in estimated glomerular filtration rate (eGFR) values (both over the same period). FAZ, foveal avascular zone.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

Support from National Health Service Lothian Research & Development and Edinburgh Imaging and the Edinburgh Clinical Research Facility at the University of Edinburgh is gratefully acknowledged. YG was supported by a Medical Research Council Precision Medicine Doctoral Training Program scholarship (MR/N013166/1). MOB is supported by grants from the Engineering and Physical Sciences Research Council (EP/R029598/1 and EP/T008806/1), Fondation Leducq (17 CVD 03), the European Union's Horizon 2020 research and innovation programme under grant agreement 801423, and British Heart Foundation/The Alan Turing Institute under a Cardiovascular Data Science Award. TJM, BD, and MOB acknowledge the funders of the SCONe project (https://www.ed.ac.uk/ophthalmology/scone). DP is supported by a Chief Scientist Office Clinical Academic Training Fellowship (CAF/19/01). TEF was supported by a Clinical Research Training Fellowship from the MRC (MR/R017840/1). ND is supported by a Chief Scientist Office Senior Clinical Research Fellowship (SCAF/19/02) and a Kidney Research UK/Stoneygate Trust Project Grant (KS_RP_003_20210111).

AUTHOR CONTRIBUTIONS

MOB, ND, BD, TJM, and YG designed research; DP, TEF, GCO, and YG performed research; YG analyzed data; and MOB, ND, and YG wrote the article.

SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Methods.

Table S1. Overview of demographic data in the cross-sectional study.

Table S2. Overview of estimated glomerular filtration rate (eGFR) values in kidney donors before and after donation.

Table S3. *R* and *P* values obtained using Pearson correlation between the first principal component (PC), PC1, of the donor group and age, estimated glomerular filtration rate (eGFR), and time since donation variables.

Figure S1. (**A**) Image processing pipeline, from original image to vascular network. (**B**) Heat map showing correlations of significant ($P < 0.05$) features obtained by using multivariable linear regression analysis. (**C**) Example of changes in the vasculature in 3 images of the same patient.

Figure S2. Box plots of signed relative errors in 4 retinal measurements between follow-up visit and baseline in donors and Pearson correlations between difference in retinal phenotypes and estimated glomerular filtration rate (eGFR) values.

Supplementary References.

REFERENCES

1. Gansevoort RT, Correa-Rotter R, Hemmelgarn BR, et al. Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention. *Lancet*. 2013;382:339–352.
2. Mjoen G, Hallan S, Hartmann A, et al. Long-term risks for kidney donors. *Kidney Int*. 2014;86:162–167.
3. Farrah TE, Dhillon B, Keane PA, et al. The eye, the kidney, and cardiovascular disease: old concepts, better tools, and new horizons. *Kidney Int*. 2020;98:323–342.

4. Nesper PL, Roberts PK, Onishi AC, et al. Quantifying microvascular abnormalities with increasing severity of diabetic retinopathy using optical coherence tomography angiography. *Invest Ophthalmol Vis Sci*. 2017;58:BIO307–BIO315.
5. Sun C, Ladores C, Hong J, et al. Systemic hypertension associated retinal microvascular changes can be detected with optical coherence tomography angiography. *Sci Rep*. 2020;10:9580.
6. Rifai OM, McGrory S, Robbins CB, et al. The application of optical coherence tomography angiography in Alzheimer's disease: a systematic review. *Alzheimers Dement (Amst)*. 2021;13:e12149.
7. Yeung L, Wu IW, Sun CC, et al. Early retinal microvascular abnormalities in patients with chronic kidney disease. *Microcirculation*. 2019;26:e12555.
8. Wong TY, Coresh J, Klein R, et al. Retinal microvascular abnormalities and renal dysfunction: the Atherosclerosis Risk in Communities study. *J Am Soc Nephrol*. 2004;15:2469–2476.
9. Iliescu R, Fernandez SR, Kelsen S, et al. Role of renal microcirculation in experimental renovascular disease. *Nephrol Dial Transplant*. 2010;25:1079–1087.