SUPPLEMENTARY METHODS

Choriocapillaris measurements

The PLEX® Elite 9000 2.0 (Carl Zeiss Meditec, Dublin, CA) is equipped with a Dual-Speed Swept Source optical coherence tomography (OCT) with 100 and 200kHz speed and a central wavelength of 1040-1060 nm. All the images were captured at 100kHz. The FastTrac™ motion correction software was used during image acquisition to minimize motion artifacts. Three-by-three-mm Angio Cubes were acquired for each patient; only scans with a signal strength >8 were selected. Scans with motion artifacts were discarded, and the exam was repeated until good quality was achieved. The manufacturer’s fully automated layers’ segmentation algorithm was used to select a custom 10-μm choriocapillaris slab starting 21 μm posteriorly to the retinal pigment epithelium (RPE)-fit centerline.[1, 2] Projection artifacts were removed using the embedded manufacturer’s software, and a maximum projection method was used to generate the images. Each slab was imported to the open-source imaging processing software FIJI (http://imagej.nih.gov/ij, version 2.1.0/1.53c, date: 2020-08-02) as an 8-bit image of 1024X1024 pixels; the scale was set in μm using the “Set scale” option (3000 μm = 1024 pixels, 0.341 pixels/μm). Images were binarized using the Phansalkar local threshold with a radius set at 4 pixels (Figure 1.A). All the slabs were processed as binary images (Process→Binary→Make binary); the “Analyze particles” tool was used to calculate the flow deficits (FD) number, the mean FD size, the total FD area, and the FD density. The FD number was the integer count of particles with size comprised between 0 and infinite pixels$^2$ and circularity comprised between 0 and 1; the mean FD size was the mean area of each FD expressed as μm$^2$, calculated as the ratio between the total FD area and the FD number; the FD density was defined as the percentage ratio of the black pixels over the total slab area.

Retinal thickness and temporal thinning index

All study subjects underwent macular spectral domain-OCT (SD-OCT, Spectralis HRA2; Heidelberg Engineering, Heidelberg, Germany). All the images were acquired in the late morning or early afternoon.

The central macular thickness (CMT) was automatically calculated in the one mm-diameter ETDRS map obtained through a 30-line horizontal raster centered on the fovea. The temporal thinning index (TTI) was calculated as [(N1+N2)-(T1+T2)/(N1+N2)]*100, where N1 and N2 were the retinal thickness of the inner and outer nasal sectors and T1 and T2 were the thickness of the inner and outer temporal sectors of a 6 mm-diameter EDTRS map centered on the fovea. Higher values suggest worse temporal thinning.[3, 4]

Choroidal thickness and choroidal vascularity index

The central horizontal scan passing through the bottom of the foveal depression was selected for each eye. All the patients had some foveal depression, even in the case of staircase foveopathy or foveal hypoplasia.[5] The choroidal thickness (CT) was manually measured as the vertical distance between Bruch’s membrane (BM) and the choroid-scleral interface using the Heidelberg Spectralis caliber tool; measurements were performed subfoveally (subfoveal CT), at 500 μm nasally, 1000 μm nasally, 500 μm temporally, and 1000 μm temporally. The measurements were averaged to obtain a single value, the total choroidal thickness (total CT).
The choroidal vascularity index (CVI) was calculated as previously described.[6] All the scans were processed on FIJI; the image scale was adjusted using the 200-µm reference line on the SD-OCT (14 pixels =200 µm, scale 0.07 pixel/µm) and the global calibration was applied to all the imported scans. Two 750-µm white lines parallel to the RPE were drawn starting from the foveal depression. A choroidal area having as boundaries the RPE (upper boundary), the sclero-choroidal junction (lower boundary), and two vertical lines perpendicular to the RPE set at the end of the 750-µm segments (lateral boundaries) was selected; this region of interest (ROI) was saved as the total choroidal area (TCA, mm²). The SD-OCT image was binarized using the Niblack autolocal threshold; the dark pixels corresponding to the choroidal vascular spaces were highlighted and added to the ROI list. The first polygonal selection and the highlighted pixel were combined (using the “AND” option from the ROI manager list) into a third ROI, corresponding to the luminal choroidal area (LCA, mm²) (Figure 1.B). The stromal choroidal area (SCA, mm²) was calculated as the difference between TCA and LCA; the CVI was calculated as the ratio between the LCA and the TCA and expressed as a percentage.

Statistical analysis

Statistical calculations were conducted with the open-source programming language R. The cutoff point for statistical significance was set at p<0.05. Descriptive statistics of continuous variables were reported as mean±standard deviation; qualitative variables were reported as frequency and percentage proportions. The BCVA was converted into LogMAR.

The primary outcome was to compare the choriocapillaris (FD number, mean FD size, total FD area, and FD density) and the choroidal (subfoveal CT, total CT, CVI, LCA, SCA, and TCA) quantitative parameters in AS patients with healthy controls with a focus on AS patients with kidney failure requiring transplant. Univariable linear mixed models were designed where each parameter was the dependent variable, the eye category (kidney transplant yes/kidney transplant no, control) was the main explanatory variable, and the patient identification number was the random factor to correct for potential intra-individual correlations having included both eyes of each participant. Models with the choroidal parameters (subfoveal CT, total CT, CVI, LCA, SCA, TCA) as the dependent variables were corrected for the time of day when the SD-OCT scan was performed to account for diurnal variability.26 This correction was not applied for choriocapillaris FD, as less variation has been shown.27 Tukey-adjusted least square means were compared between the study groups (i.e., between AS patients having a history of kidney transplant, AS patients with no history of kidney transplant, and healthy controls). The degree of correlation between study variables was investigated with Pearson’s test.

The secondary outcome was to explore the demographic and clinical factors associated with the FD density and the CVI in AS patients. This included demographic data (age, gender), refraction (as spherical equivalent), pattern of inheritance (namely X-linked, X-linked carrier, or AR), type of mutation, clinical characteristics (hearing loss, history of kidney transplant), ocular findings (anterior lenticonus, dot maculopathy, foveal hypoplasia, and staircase foveopathy), retinal thickness (CMT and TTI), and choroidal thickness (subfoveal CT and total CT). Two separate linear mixed models were designed for each outcome, with the patient identification number included as the random factor. The degree of collinearity was inspected with cluster analysis, obtained with the varclus function of hmisc package.28 Candidate factors to be included in a multivariable model were selected with a parsimonious approach, using a least absolute shrinkage and selection operator regression (LASSO);29 additional variables were included based on
clinical judgment and literature search. Linear regression estimates, standard error, and 95% confidence interval were provided for each factor included in the final models. The marginal R-squared and the conditional R-squared, providing the variance explained by fixed effects only and the variance explained by both fixed effects and random effects, respectively, were calculated as a goodness-of-fit measure for each linear model.

For both descriptive and predictive statistics, only complete cases were used (missing data are shown in Supplementary Figure 1).

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