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Is arterial stiffness in the carotid artery associated with choroidal thinning in patients with pseudoxanthoma elasticum or controls?

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ABSTRACT.

Purpose: **Patients with pseudoxanthoma elasticum (PXE) develop calcification of Bruch's membrane (BM) and choroidal thinning, as well as calcification of intracranial arteries, leading to arterial stiffness. We investigated whether arterial stiffness is associated with choroidal thinning in PXE patients, besides the presumed effect of BM calcification.**

Methods: **Cross-sectional study with 75 PXE patients and 40 controls. Macular choroidal thickness was measured using optical coherence tomography scans. Functional magnetic resonance imaging was used to calculate the pulsatility index (PI) of the carotid siphon as a measure of arterial stiffness. Associations between PI and choroidal thickness were investigated using linear mixed effects models adjusted for age and ocular axial length. Furthermore, we investigated choroidal thickness in relation to the presence of retinal pigment epithelium (RPE) atrophy, its topographical distribution and age.**

Results: **Median age was 58 years (IQR 53–66) in PXE patients and 62 years (IQR 56–67) in controls ($p = 0.08$). Pseudoxanthoma elasticum (PXE) patients had a thinner choroid than controls (138 µm versus 248 µm, $p < 0.01$). No association was observed between PI and choroidal thickness in PXE patients ($\beta = -1.6$, 95% CI −59.4 to 54.5) nor in controls ($\beta = -47.6$, 95% CI −129.7 to 31.9). In PXE patients, RPE atrophy was associated with a thinner choroid ($p < 0.01$). Also, the nasal choroid was thinner than the temporal choroid, and choroidal thickness already decreased with age in PXE eyes without RPE atrophy.**

Conclusion: **There was no independent association between measures of arterial stiffness and choroidal thinning in PXE patients and controls. Probably, changes in BM lead to choroidal thinning in PXE.**

Key words: angioid streaks – arterial stiffness – arteriosclerosis – choroidal thickness – internal carotid artery – pseudoxanthoma elasticum – pulsatility index

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Introduction

Pseudoxanthoma elasticum (PXE) is a rare genetic disease leading to ectopic calcification of elastic tissues, including the arteries, skin and Bruch's membrane (BM) in the retina (Plomp et al. 2010). Clinically, this leads to peripheral arterial disease, pseudoxanthomas on the skin and macular degeneration with considerable visual morbidity (Finger et al. 2009). Calcification in the vasculature is predominantly present in the intracranial internal carotid artery (IICA) and the arm and leg arteries (Kranenburg et al. 2017). Calcification in the eyes occurs in BM and is visible as peau d'orange and angioid streaks, which are breaks in the brittle BM (Gliem et al. 2013). In angioid streaks, choroidal neovascularization (CNV) and subsequent scarring may develop. In addition, severe visual loss is caused by macular atrophy and since PXE is a progressive disease, the visual acuity deteriorates with older age (Gliem et al. 2016; Risseeuw et al. 2019).

Furthermore, the choroid is significantly thinner in patients with PXE (Ellabban et al. 2012; Gliem et al. 2014; Dolz-Marco et al. 2016). The cause of the choroidal thinning is unclear. The choroid is a vascular tissue that receives most of the ocular blood flow (Nickla & Wallman 2010). Integrity of the choroid is essential for retinal functioning by exchanging nutrients and oxygen across the retinal pigment epithelium (RPE), and ultimately, choroidal disease can cause degenerative changes in the retina (Nickla & Wallman 2010). Gliem et al. (2014) found that choroidal thinning in PXE patients seems to follow the centrifugal pattern of BM calcification, suggesting that BM calcification affects choroidal thickness. Probably, BM calcification impedes diffusion of factors secreted by the RPE, such as vascular endothelial growth factor (VEGF),

which are essential for maintaining the integrity of the choroid. However, it is plausible that arterial calcification in PXE is associated with choroidal thinning as well, since systemic vascular changes are known to affect the choroidal thickness (Bhutto & Lutty 2012; Ahmad et al. 2017).

In the general population, arterial stiffness increases with age and is associated with end-organ damage in the kidneys and the brain (Mitchell 2018). This might be due to reduced pulse pressure dampening and increased pulsatile load in the microvasculature of these organs (Mitchell 2018). Arterial calcification contributes to arterial stiffness (Tsao et al. 2014), and the progressive arterial calcification in PXE is therefore thought to cause the increased arterial stiffness seen in these patients (Kranenburg et al. 2018). Recently, it was shown that calcification of the IICA is associated with an increased pulsatility index (PI) in the intracranial arteries in PXE patients. The PI is a proxy for carotidal vascular stiffness (de Riva et al. 2012), and contributed to microvascular damage and cognitive decline in PXE patients (Bartstra et al., manuscript in preparation).

The choroidal vasculature is supplied by the ophthalmic artery, which branches from the IICA. Over 80% of its blood flows to the choroid (Bhutto & Lutty 2012). The choroid can regulate perfusion pressure to some degree, but its autoregulation is assumed to be less effective than the autoregulation of other tissues, such as the retina (Nickla & Wallman 2010; Kur et al. 2012). Therefore, increased vascular stiffness in the IICA might contribute to microvascular damage and thinning of the choroid.

The aim of this study was to determine whether arterial stiffness is associated with choroidal thinning in patients with PXE, besides the presumed effect of BM calcification. Also, we aim to investigate whether a possible relationship between arterial stiffness and choroidal thinning is part of a normal ageing process in controls. In addition, we investigated choroidal thickness in relation to age, the presence of RPE degeneration and the presumed spread of BM calcification.

Methods

Participants

This cross-sectional study (METC number 16-622) was conducted in adherence to the tenets of the Declaration of Helsinki. Pseudoxanthoma elasticum (PXE) patients and controls were recruited from the Dutch National Expertise Center for PXE in the University Medical Center in Utrecht, the Netherlands.

Diagnosis of PXE was confirmed when a patient fulfilled at least two of the major diagnostic criteria: (Plomp et al. 2010) skin involvement (yellowish papules/plaques), eye involvement (peau d'orange, angioid streaks) or genetic confirmation (biallelic *ABCC6* gene mutations, first-degree PXE relative). An age-matched control group was recruited from genetically non-related family members or friends of PXE patients, who did not meet the PXE criteria. Participants were included when they underwent the optional ophthalmologic examination. Participants were excluded if they met any of the following criteria: under 18 years old; unable or unwilling to sign informed consent; severe renal impairment (eGFR < 30 ml/min/1.73 m^2); pacemaker or implantable cardiac defibrillator; metallic foreign body in the eye or claustrophobia; an ocular axial length <20.5 mm or >26.5 mm.

All participants underwent an ophthalmological examination including dilated fundus and slit-lamp examination, automatic refractometry, spectral domain optical coherence tomography (SD-OCT) imaging and ultrasound biometry. Best corrected visual acuity (BCVA) was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) charts. The phenotype in the posterior pole was assessed according to previously reported criteria and categorized into four groups to assess the severity of macular degeneration: no RPE atrophy or CNV, CNV lesions, RPE atrophy or mixed (Risseeuw et al. 2019). Age, sex, body mass index (BMI), blood pressure, smoking history and details on other vascular measurements were obtained from medical files if available. Other vascular measurements included intima–media thickness (IMT), ankle-brachial index (ABI) and the severity and volume of IICA calcification. The majority of participants (71.3%) underwent brain magnetic resonance imaging (MRI).

Measurement of choroidal thickness

Enhanced depth imaging SD-OCT was obtained after pupil dilatation (Heidelberg Engineering, Heidelberg, Germany). The 20° × 5° OCT volumes consisted of seven horizontal and seven vertical B-scans through the macular area which were averaged 25 times. All scans were assessed in the Heidelberg Eye Explorer software (Heidelberg Engineering, Heidelberg, Germany) by manually measuring the choroidal thickness, defined as the distance between the outer border of the hyperreflective RPE/BM band and the inner surface of the sclera (Fig. 1). An ETDRS grid (with diameters of 1, 3 and 6 mm) was centred on the foveal dip. Measurements were made subfoveal and at 500 and 1500 μm distance parafoveal in the horizontal and vertical scans, resulting in a total of nine measurement points: nasal, temporal, superior and inferior to the foveal centre. We averaged these measurements to obtain a mean choroidal thickness per eye, which was used in further analysis unless mentioned otherwise. The 20° scans often did not reach 3000 μm eccentricity, since one degree of visual angle equals 288 μm on the retina. Therefore, we did not include measurements at 3000 μm in the measurement protocol. In some cases, the foveal centre could not be identified visually on OCT scans alone as a result of retinal or BM changes and fixation affected by macular pathology. Colour fundus photographs and the average optic disc to fovea distance of 4.7 mm were then used to identify the most plausible foveal dip (van de Put et al. 2013; Jonas et al. 2015). Measurements were performed by two researchers (CL and SR), and all measurements were visually verified by one grader (SR).

Choroidal thickness measurements are known to be highly reproducible in eyes without pathology (Malamas et al. 2019). However, these data have not been reported yet for PXE eyes; therefore, we assessed the agreement and reliability of choroidal thickness measurements in PXE eyes. Two researchers (CL and SR) measured the subfoveal choroidal thickness in a random subset of 20 PXE eyes. The mean difference was 12 μm (95% CI 3–21 μm) and 95% limits of agreement were −25 μm (95% CI −41 to −10 μm) and 50 μm (95% CI 34–66 μm) for the lower and upper limit, respectively. The intraclass correlation coefficient was 0.98 (95% CI 0.91–0.99)

based on an average measure, absolute-agreement two-way model (Koo & Li 2016).

MRI acquisition and processing

Time-resolved 2D phase contrast (2Dpc) scanning was performed on a 3 Tesla MRI (Ingenia CX, Philips, the Netherlands). The 2Dpc acquisitions were planned at the IICA segment C4, C6 and at the origin of the middle cerebral artery (MCA) (Bouthillier et al. 1996). An in-house developed Matlab script (Mathworks Inc., Natick, MA, USA) was used to quantify the blood flow in these locations. The PI was derived from the blood flow time curves and was calculated using the following formula:

$$\mathrm{PI} = \frac{V_{\max} - V_{\min}}{V_{\mathrm{mean}}}$$

with $V_{\max}$ being the peak systolic flow, $V_{\min}$ the lowest diastolic flow and V_{mean} the mean blood flow during one cardiac cycle. The mean of the PI in C4 and MCA was used for further analysis. The PI at segment C6 was hard to measure due to imaging artefacts and therefore not included in the averaged PI for further analysis.

For further insight into the vascular status of PXE patients and its potential effect on choroidal thickness, measurements of carotid IMT using ultrasound, the ABI and siphon calcification on CT scan (both the absolute mass and a severity classification) were collected. These parameters were not available from controls. Further details on these measurements can be found in the Appendix S1.

Data analysis

Best corrected visual acuity was converted to the logarithm of the minimum angle of resolution (logMAR) for statistical purposes. Continuous variables are presented as mean ± standard deviation (SD) or as median [interquartile range (IQR)], depending on their distribution. Categorical variables are presented as numbers (%). To test differences between PXE patients and controls, we used the Chi-square test for categorical variables, the two-sample *t*-test for normal distributions and the Mann–Whitney *U*-test for non-normal distributions. ANOVA test was used to test differences between the severity of macular degeneration. A p-value of <0.05 was considered statistically significant.

Missing values on baseline characteristics were considered 'missing at random' and imputed using multiple imputation for further association analyses (Donders et al. 2006). The highest proportion of missing values was 17%; therefore, we used $m = 20$ imputed and combined data sets for further multivariable analyses.

We used a linear mixed effects model to analyse the association between PI and choroidal thickness, since this allows for the use of correlated data of two eyes of a

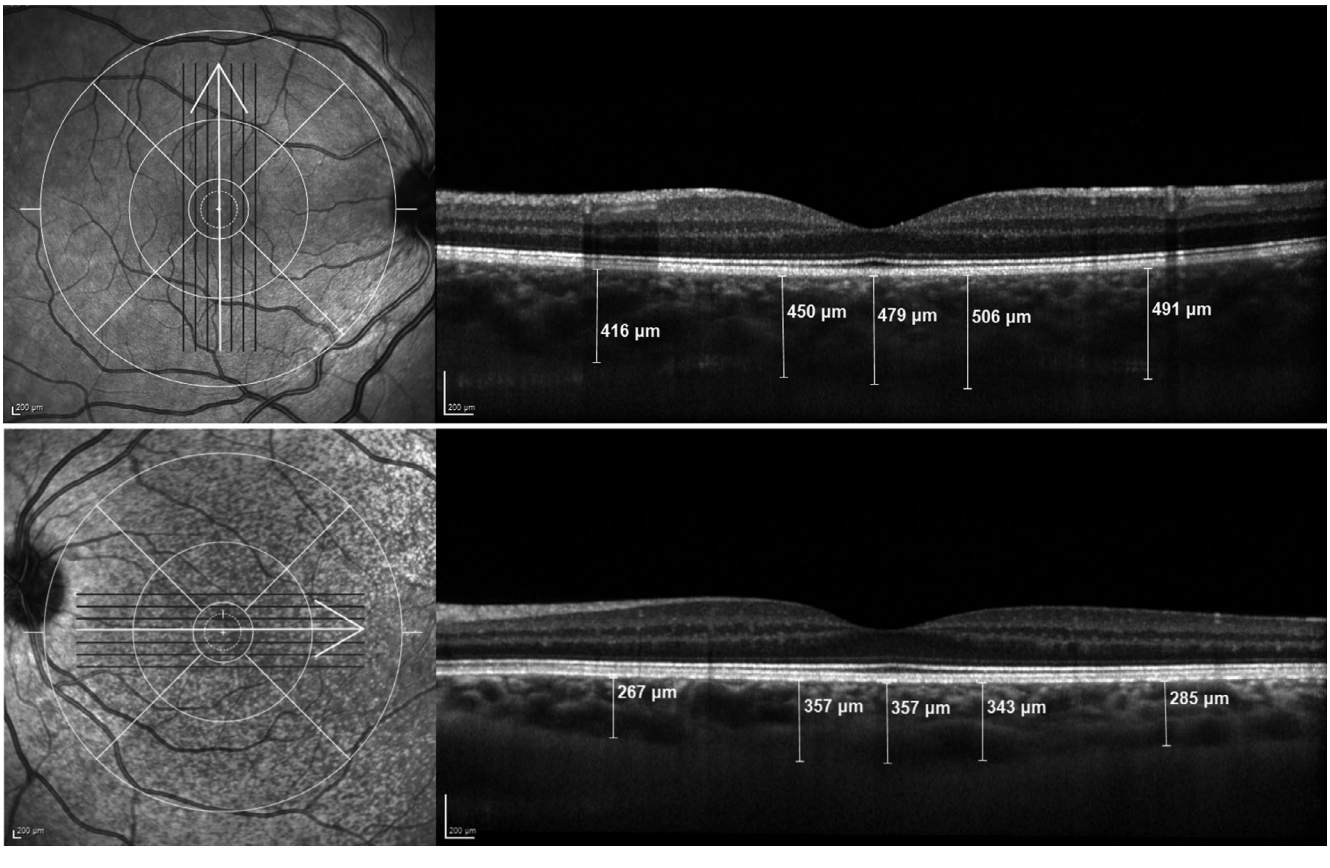

Fig. 1. Choroidal thickness measurements. Choroidal thickness measurements in Heidelberg Eye Explorer. Choroidal thickness was defined as the outer border of the hyperreflective retinal pigment epithelium and Bruch's membrane to the inner surface of the sclera. An ETDRS grid (1, 3, 6 mm) was placed over the foveal dip. Measurements were made subfoveal and at 0.5 and 1.5 mm distance from the fovea in the horizontal and vertical scans. This resulted in nine measurement points. Above: OCT scan of 27-year-old control. Bottom: scan of 28-year-old pseudoxanthoma elasticum (PXE) patient.

participant. In this model, we modelled the individual patient as a random effect to investigate the unilateral effect of the PI on choroidal thickness (Ying et al. 2018). Crude models and models adjusted for age and axial length were constructed, since age is a possible confounder and axial length is known to affect choroidal thickness (Wei et al. 2013). Based on previous research, other confounders might include cardiovascular risk factors (blood pressure, BMI, smoking) (Lau et al. 2018; Gattoussi et al. 2019, Wei et al. 2013). We identified possible confounders based using both rationale and a simulation of the optimal cut-off percentage for the 'change in estimate' (Lee 2014).

We repeated the analysis in subgroups of PXE patients to investigate the effect of RPE integrity on choroidal thinning. Therefore, we stratified for eyes without the presence of RPE atrophy or CNV and eyes with RPE atrophy.

Bruch's membrane calcification follows a centrifugal spread, which means that BM calcification is more severe in the nasal area than the temporal area (Charbel Issa et al. 2010). Therefore, we plotted the topographical distribution of choroidal thickness to investigate the relationship between choroidal thinning and the presumed spread of BM calcification. To study the effect of age on choroidal thickness, we performed a linear regression with age as the determinant and choroidal thickness as the outcome.

R version 3.4.1 was used for statistical analysis. Add-on packages 'mice' (version 3.4.0) and 'lme4' (version 1.1–20) were used for multiple imputation and mixed model regression, respectively.

Results

In total, 75 PXE patients and 40 controls were included. Patient characteristics are presented in Table 1. Median age was 58 years (range 53–66) in PXE patients and 62 years (range 53–67) in controls. Cardiovascular risk factors such as BMI and mean arterial pressure were similar in the two groups, but the use of cholesterol-lowering medication was higher in PXE patients. Visual acuity was worse in PXE patients as compared to controls. Mean choroidal thickness was lower in PXE patients than controls, 138 μm versus 248 μm ($p < 0.01$), respectively.

Choroidal thickness and pulsatility index

No significant differences in PI were found between PXE patients and controls. We analysed the association between mean PI and mean choroidal thickness using a mixed model for PXE patients and controls separately. In the crude models, mean PI was not significantly associated with choroidal thickness in both PXE and controls (details on the models can be found in Table 2). The associations diminished when corrected for age and axial length in both PXE patients ($\beta = -1.6$ μm, 95% CI −59.4 to 54.5, $p = 0.96$) and controls ($\beta = -47.6$ μm, 95% CI −129.7 to 31.9, $p = 0.26$).

In patients with PXE, age was the most important determinant of choroidal thinning ($\beta = -4.8$ μm, 95% CI −6.5 to −3.0, $p < 0.01$), whereas in the control group, this was axial length ($\beta = -20.7$ μm, 95% CI −37.7 to −3.6, $p = 0.02$). Other possible confounding variables, such as BMI and mean arterial pressure, were not associated with choroidal thickness (data not shown) and did not improve the models.

Association between choroidal thickness and retinal pigment epithelium atrophy

Of all PXE eyes, 34 (22.8%) had no RPE atrophy or CNV, 39 (26.2%) had CNV, 11 (7.4%) had RPE atrophy and 65 (43.6%) had a combination of CNV and RPE atrophy. RPE atrophy and CNV were associated with choroidal

Table 1. Patient characteristics of controls and pseudoxanthoma elasticum (PXE) patients.

	Number*	Controls $n = 40$	Number*	PXE patients $n = 75$	p-Value
Patient data					
Age in years		62 [56 to 67]		58 [53 to 66]	0.08
Gender (female)		18 (45%)		39 (52%)	0.60
BMI	34	26.4 [23.6 to 28.0]		25.6 [23.2 to 27.7]	0.48
Mean arterial pressure		93 [85 to 100]		96 [90 to 105]	0.08
Smoking status: never	20	10 (50%)		38 (51%)	>0.99
Use of antihypertensive drugs	39	10 (26%)		23 (31%)	0.73
Use of anti-diabetic drugs		0		3 (4%)	0.52
Use of cholesterol-lowering drugs		8 (20%)		43 (57%)	<0.01
Carotid stiffness†					
Pulsatility index C4 (proximal)	34	1.02 [0.92 to 1.21]	48	1.11 [1.00 to 1.25]	0.20
Pulsatility index MCA (distal)	34	0.90 [0.80 to 1.03]	48	1.00 [0.87 to 1.14]	0.11
Mean pulsatility index	34	0.97 [0.86 to 1.10]	48	1.05 [0.95 to 1.20]	0.10
Eye data†					
Visual acuity (logMar)	37	0.00 [−0.08 to 0.06]	74	0.70 [0.06 to 1.13]	<0.01
Axial length (mm)	37	24.1 (SD 1.1)	64	24.1 (SD 0.9)	0.77
Spherical refractive error (D)	38	−0.12 [−2.44 to 1.47]	73	−0.38 [−1.50 to 0.38]	0.27
Mean choroidal thickness (μm)	39	248 [214 to 295]	74	138 [88 to 179]	<0.01

Values are presented as number (%), mean (standard deviation) or median [interquartile range]. p-Values were based on Chi-square test for categorical variables, the two-sample *t*-test for normal distributions and the Mann–Whitney *U*-test for non-normal distributions.
BMI = body mass index, D = dioptres, logMAR = logarithm of the minimum angle of resolution, MCA = medial cerebral artery.
* In case of missing data, the number patients with available data is presented.
† Pulsatility index and eye data were measured on both the left and right side. The presented data are the averaged data of the left and right side.

Table 2. Crude and adjusted associations of pulsatility index as a proxy for carotidal vascular stiffness and mean choroidal thickness.

	Crude				Adjusted for age and axial length			
	Estimate	95% CI		p-Value	Estimate	95% CI		p-Value
PXE								
Mean pulsatility index	−53.2	−118.6	6.6	0.06	−1.6	−59.4	54.4	0.96
Age in years					−4.8	−6.5	−3.0	<0.01
Axial length in mm					3.4	−11.4	18.3	0.65
Controls								
Mean pulsatility index	−68.6	−147.3	8.4	0.08	−47.6	−129.7	31.9	0.26
Age in years					−0.8	−3.9	2.3	0.64
Axial length in mm					−20.7	−37.7	−3.6	0.02

All models are mixed model linear regression models. Herein, determinants are modelled as fixed effects and different measurements per person (right or left side) are modelled as random effects.
CI = confidence interval, PXE = Pseudoxanthoma Elasticum.

thinning: eyes without CNV and RPE atrophy had a mean choroidal thickness of 206 μm (SD 65), compared to 163 μm (SD 68) in eyes with CNV or scarring, and 103 μm (SD 63) in eyes with RPE atrophy with or without CNV ($p < 0.01$). Further details are presented in Table 3.

In the stratified analysis, a possible crude effect of PI on choroidal thickness was largest in eyes without RPE atrophy or CNV ($\beta = -137.9$ μm, 95% CI −276.4 to −0.5, $p = 0.05$). However, the adjusted analyses again showed no statistically significant association between mean PI and mean choroidal thickness in both categories.

Distribution of choroidal thickness

In both controls and PXE subgroups, the most nasal measurement on the horizontal scan was thinner than the other four measurements (Fig. 2). Also, in PXE patients without macular degeneration, the choroid was thinner than in the control group on all positions.

The effect of age on mean choroidal thickness is plotted in Fig. 3. In this graph, only controls and PXE patients without RPE atrophy or CNV are shown. In linear regression analysis, age was negatively associated with choroidal thickness in PXE patients ($\beta = -2.7$ μm, 95% CI −5.3 to −0.1, $p = 0.05$), but not in controls ($\beta = -0.4$ μm, CI −3.3 to 2.4, $p = 0.76$).

Other vascular parameters in patients with pseudoxanthoma elasticum

For the other vascular parameters, data were only available in PXE patients. The median IMT was 0.70 mm (IQR 0.63–0.82) and median ABI in rest was 0.97 (IQR 0.73–1.04). Most PXE patients had calcification in the IICA: 15 (20.3%) had no calcification, 17 (23.0%) had mild calcification, 15 (20.3%) had moderate calcification and 27 (36.5%) had severe calcification. The median IICA calcification mass score was 16.6 (IQR 7.7–50.9). Only IMT showed a negative association with mean choroidal thickness in the crude models (Table 4). However, when adjusted for age and axial length, these effects disappeared. Ankle-brachial index (ABI) and siphon calcification were not associated with choroidal thickness.

Discussion

In this study, we found no association of choroidal thinning in PXE patients with arterial stiffening; hence, the diseased BM remains the most likely

Table 3. The association of vascular stiffness with choroidal thickness, stratified for integrity of the retinal pigment epithelium.

				Model details								
				Crude					Adjusted			
RPE integrity	Mean age	Mean choroidal thickness	Mean PI	Determinants	Estimate	95% CI		p-Value	Estimate	95% CI		p-Value
No RPE atrophy or CNV $n = 34$ eyes	49.1 (SD 7.5)	206 (SD 65)	0.91 (SD 0.17)	Mean PI	137.9	276.4	0.5	0.05	82.8	234.1	60.4	0.29
				Axial length (mm)					24.9	0.1	48.3	0.03
				Age (years)					3.2	6.6	0.2	0.08
RPE atrophy with or without CNV* $n = 76$ eyes	62.6 (SD 10.4)	103 (SD 63)	1.19 (SD 0.22)	Mean PI	34.5	28.2	92.0	0.25	34.6	28.0	92.0	0.26
				Axial length (mm)					4.8	30.6	20.7	0.72
				Age (years)					4.7	9.8	0.4	0.09

All models are mixed model linear regression models with determinants as fixed effects and different measurements per person (right or left side) as random effects.
CNV = choroidal neovascularization, CI = confidence interval, PI = pulsatility index, RPE = retinal pigment epithelium.
* Atrophy is defined as macular atrophy with loss of RPE alone, or in combination with CNV.

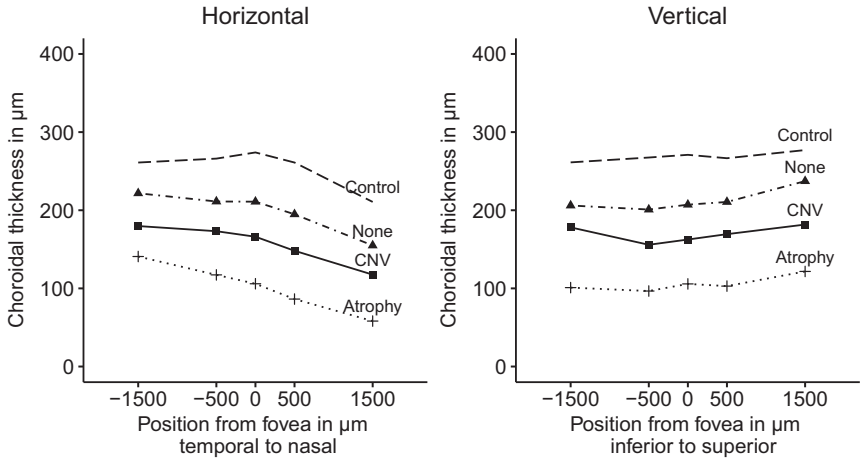

Fig. 2. Topographical distribution of choroidal thickness. Topographical distribution of choroidal thickness in pseudoxanthoma elasticum (PXE) patients and controls. The graphs show the choroidal thickness at each measurement position on horizontal and vertical optical coherence tomography scans. Pseudoxanthoma elasticum (PXE) eyes were categorized into three groups based on the ocular phenotype: no retinal pigment epithelium (RPE) atrophy or choroidal neovascularization (CNV) (none), CNV without RPE atrophy and RPE atrophy with or without CNV.

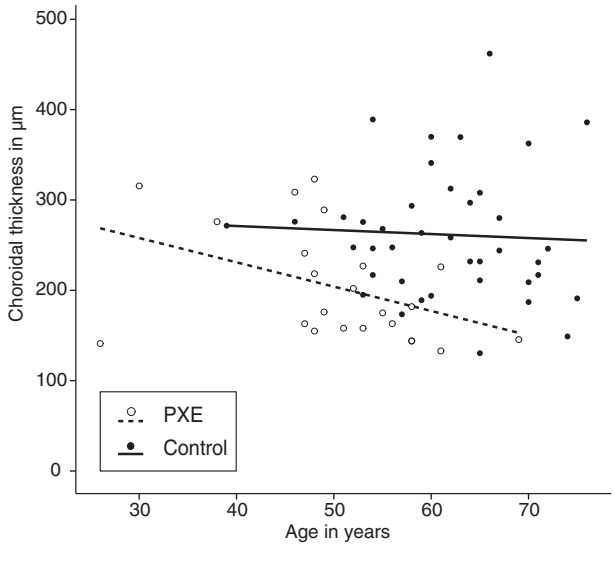

Fig. 3. The effect of age on choroidal thickness. Mean choroidal thickness plotted against age for both controls and pseudoxanthoma elasticum (PXE) patients. For controls, both eyes were averaged. For PXE patients, only eyes without RPE atrophy or choroidal neovascularization (CNV) were included and averaged in case both eyes had no RPE atrophy or CNV.

explanation. This is supported by the strong association between choroidal thickness and the severity of macular degeneration. In addition, another important determinant of choroidal thinning in PXE eyes without RPE atrophy or CNV is age, whereas age is no important determinant of choroidal thinning in controls. This implicates that BM calcification progresses with age in PXE patients, which then progressively impairs the vitality of tissues adjacent to the BM. In this study, we could not demonstrate a relationship between increased arterial stiffness in the IICA and choroidal thickness in patients with PXE. Furthermore, we found no association with choroidal thickness and other vascular parameters in PXE patients. Also, in controls we found no association between the IICA PI and choroidal thickness.

Increased pulsatility is associated with increased microvascular damage in high-flow, low impedance organs such as the kidneys and brain (O'Rourke & Safar 2005). This effect is demonstrated in brain tissue where different indicators of carotid stiffness are associated with impaired cognitive functioning (Jochemsen et al. 2015; Geijselaers et al. 2016) brain atrophy and white matter lesions (Jochemsen et al. 2015) and subcortical infarctions (Mitchell et al. 2011). In the latter study, the pulsatile parameter had the highest predictive value, implicating that an increased pulse pressure is responsible for microvascular damage (Mitchell et al. 2011).

Interestingly, the effect of an increased pulse pressure on the choroid, also a high-flow organ, has not been investigated extensively. Therefore, the association between vascular disease and the choroidal vasculature is less ambiguous. In a study of 43 healthy, young controls, there was no correlation between carotid PI and choroidal thickness (Agladioglu et al. 2015). However, in the same study there was a positive correlation between end-diastolic blood flow and choroidal thinning. Although these analyses were not corrected for age, they might suggest that changes in haemodynamics could affect choroidal thickness. Diseases that cause a reduced ocular blood flow, for example carotid artery stenosis, might also affect choroidal thickness. Three studies investigated this association, and findings were inconsistent. One study found a negative correlation between the extent of stenosis and choroidal thickness (Wang et al. 2017), whereas another study found a positive correlation (Akçay et al. 2016). In another study, the choroid was thinner in patients with carotid artery stenosis, but there was no correlation with the extent of stenosis (Sayin et al. 2015). These findings, together with our results, suggest that there are no reasons to assume that the relationship between IICA haemodynamics and choroidal thinning is an important pathophysiological process.

Our findings support the alternative hypothesis that choroidal thinning in PXE is caused by BM calcification, and subsequently by a diseased RPE. This is supported by the observed distribution of choroidal thinning in PXE patients, following the centrifugal spread of calcification from the optic nerve to the periphery (Charbel Issa et al. 2010). Two previous studies in PXE patients found that the nasal choroid was thinner than the temporal choroid, and this was already detectable in eyes without macular degeneration (Ellabban et al. 2012; Gliem et al. 2014). One of these

Table 4. Correlations between vascular status measurements and mean choroidal thickness in PXE patients.

	Crude				Adjusted for age and axial length			
	Estimate	95% CI		p-Value	Estimate	95% CI		p-Value
Intima–media thickness	−199.9	−308.6	−91.1	<0.01	−44.3	−156.8	68.2	0.45
Age in years					−4.3	−5.9	−2.6	<0.01
Axial length in mm					−7.0	−19.4	5.7	0.26
Ankle-brachial index	51.9	−30.0	133.8	0.22	−10.2	−78.5	58.2	0.77
Age in years					−4.7	−6.1	−3.2	<0.01
Axial length in mm					−6.6	−18.9	6.2	0.29
Siphon score								
1 versus 0	1.1	−50.6	52.7	0.97	17.4	−24.1	58.6	0.43
2 versus 0	−5.4	−57.8	47.0	0.84	8.5	−33.4	50.3	0.70
3 versus 0	−10.7	−57.7	36.2	0.66	16.5	−21.7	54.6	0.41
Age in years					−4.7	−6.1	−3.3	<0.01
Axial length in mm					−7.3	−19.4	5.7	0.25
Siphon calcification mass	−0.2	−0.5	0.1	0.12	−0.1	−0.4	0.1	0.25
Age in years					−4.6	−6.2	−3.1	<0.01
Axial length in mm					4.3	−10.3	19.0	0.56

All models are mixed model linear regression models. Herein, determinants are modelled as fixed effects, and different measurements per person (right or left side) are modelled as random effects.
CI = confidence interval, PXE = pseudoxanthoma elasticum.

studies also investigated the choroidal vasculature using en-face OCT imaging and found that the vascular density was reduced in PXE patients when compared to controls (Gliem et al. 2014). Furthermore, in line with our findings, these studies found a correlation between the severity of macular degeneration in PXE and choroidal thickness and demonstrated that choroidal thickness is already reduced in relatively young patients without CNV or atrophy (Ellabban et al. 2012; Gliem et al. 2014).

Our findings provide relevant insights into the relationship between the choroid and the RPE. The choroid supplies the retina with oxygen and nutrients and relies on autoregulation, which is the ability of the tissue to regulate the perfusion pressure, to achieve an adequate blood flow (Nickla & Wallman 2010). The RPE disposes its waste products via the choroid and also secretes products (such as VEGF) that regulate the choroid. These physiological processes may be affected by changes in BM, which is located between the choroid and the RPE (Nickla & Wallman 2010). It is likely that BM calcification in PXE impedes the diffusion of RPE products to the choroid. This might impair autoregulation of the choroid and subsequently cause structural changes, such as thinning, of the choroid. This may compromise the supply of oxygen and nutrients to the RPE, which could ultimately contribute to an atrophic RPE and thereby even less production of factors that support the choroid. However, this possible pathophysiological process is speculative. Changes in BM are part of normal ageing and are also associated with other diseases, such as age-related macular degeneration (Booij et al. 2010). To further explore and quantify the effect of BM calcification on choroidal thinning, a biomarker for the severity of BM calcification is warranted. Unfortunately, such an end-point is still lacking.

Strengths of this study include the large number of PXE patients in combination with the detailed and extensive vascular and ophthalmological measurements. To our knowledge, this is the first cohort investigating the association between vascular stiffness parameters and ocular pathology in patients with PXE.

Some limitations need to be addressed as well. There was no significant difference in PI between PXE patients and controls. Unpublished data of our study group did show a higher PI in PXE patients when compared to controls in a slightly larger study population (Bartstra et al., manuscript in preparation). Hence, the difference in sample size might explain why the difference in PI between PXE patients and controls did not reach statistical significance.

Furthermore, we used a combination of the most proximal and the most distal measurements of the IICA (segments C4 and MCA) to obtain the mean vascular stiffness, since the middle measurement (segment C6) had many missing data due to imaging artefacts. However, this location is the most interesting for the purpose of this study, since it is closest to the branching ophthalmic artery and thereby to the choroid.

Choroidal thickness is known to vary within individuals as a result of age, axial length, circadian rhythm and fluid intake (Usui et al. 2012). We corrected for age and axial length, since these appear to have the largest influence on choroidal thickness (Wei et al. 2013). However, no data on the time of choroidal measurement and fluid intake were available.

In summary, PXE patients have a thinner choroid than controls. No independent association between vascular stiffness and mean choroidal thickness could be demonstrated in both PXE patients and controls, suggesting that the observed choroidal thinning in PXE patients is likely to be the result of BM calcification.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Supplementary methods.