Systemic and Ocular Determinants of Peripapillary Retinal Nerve Fiber Layer Thickness Measurements in the European Eye Epidemiology (E3) Population

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Systemic and ocular determinants of peripapillary retinal nerve fiber layer thickness measurements in the E3 population

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Abstract

***Objective*:** To investigate systemic and ocular determinants of peripapillary retinal nerve fiber layer thickness (pRNFLT) in the European population.

Design: Cross-sectional meta-analysis.

Participants: 16,084 European adults from eight cohort studies (mean age range from 56.9 ± 12.3 to 82.1 ± 4.2 years) of the European Eye Epidemiology (E3) consortium.

***Methods*:** We examined associations with pRNFLT measured by spectral – domain optical coherence tomography in each study using multivariable linear regression and pooled results using random effects meta-analysis.

Main Outcome Measures: Determinants of pRNFLT.

Results: Mean pRNFLT ranged from 86.8 ± 21.4 in the Rotterdam Study I to 104.7 ± 12.5 μm in the Rotterdam Study III. We found the following factors to be associated with reduced pRNFLT: Older age (β=–0.38 μm/year, 95% confidence interval (CI)=–0.57, –0.18), higher intraocular pressure (IOP; β= –0.36μm/mmHg, 95% CI=–0.56, –0.15), visual impairment (β=–5.50μm, 95% CI=–9.37, –1.64) and history of systemic hypertension (β=–0.54μm, 95% CI=–1.01, –0.07) and stroke (β=–1.94μm, 95% CI=–3.17, –0.72). A suggestive, albeit non-significant, association was observed for dementia (β=–3.11μm, 95% CI=–6.22, 0.01). Higher pRNFLT was associated with more hyperopic spherical equivalent (SE; β=1.39μm/diopter, 95% CI=1.19, 1.59) and smoking (β=1.53μm, 95% CI=1.00, 2.06 for current smokers compared to never-smokers).

***Conclusions*:** In addition to previously described determinants such as age and refraction, we found that systemic vascular and neurovascular diseases were associated with reduced pRNFLT. These may be of clinical relevance, especially in glaucoma monitoring of patients with newly occurring vascular co-morbidities.

INTRODUCTION

The assessment of peripapillary retinal nerve fiber layer thickness (pRNFLT) with Spectral – Domain Optical Coherence Tomography (SD-OCT) has become of increasing importance in the evaluation of glaucoma and its progression[1,2]. Although debated, pRNFLT measurements hold promise as a biomarker for neurodegenerative diseases such as Alzheimer's disease (AD) and multiple sclerosis (MS)[3,4].

While pRNFLT measurements have increased insight into the development of diseases, it has been difficult to evaluate which changes fall within the physiological range. Most OCT devices compare pRNFLT measurements against reference databases that are built into the machine analysis software. These data are mostly derived from relatively small sample populations. Whether these databases adequately capture normal anatomical variation across a wide age range remains unclear.

Only few studies investigated ocular and systemic determinants of pRNFLT in the general population[5]. They reported inconsistent results for many ocular and systemic parameters including sex or body-mass-index (BMI)[5,6]. To date, only age[7,8], refraction[9] or axial length (AL)[10] have been consistently associated with measured pRNFLT across studies. In addition, the majority of large-scale studies assessing these associations were performed in (young) Asian populations[6,11–14]. It is unclear whether or not these results can be applied to European, i.e. mostly Caucasian, populations.

The purpose of this study was to assess systemic and ocular determinants of pRNFLT using pooled data from eight European population-based studies.

METHODS

Included studies

The European Eye Epidemiology (E3) consortium is a collaborative network of population-based studies across Europe with the overarching aim of developing and analyzing large pooled datasets to increase understanding of eye disease and vision loss[15]. For this study, we analyzed data on pRNFLT from eight different studies. The included data were cross-

sectional and the right eye was chosen to be the study eye. All studies adhered to the tenets of the Declaration of Helsinki and had local ethical committee approval. All participants gave written informed consent.

Assessments and data analyses

Retinal nerve fiber layer thickness was measured as global pRNFLT with different OCT devices, scan modalities (mostly circular scans) and automated segmentation algorithms in the respective studies (see Table 1). pRNFLT outliers were excluded prior to analyses according to Chauvenet's criterion. Briefly, depending on sample size we excluded participants with pRNFLT above or below a certain range of standard deviations from the mean[16]. To investigate determinants of pRNFLT, multivariable linear regression models including the variables of interest were conducted. Factors to be tested for association with pRNFLT were considered in multiple steps. As first and most important step, variables were chosen a priori based on literature and availability in the individual studies. Subsequently, we performed univariable linear regression models of potential factors at study level to assess possible impact on pRNFLT. In the last step the factors of the multivariable models were decided on as a trade-off between priority of the respective factors and the maximum possible population size of the model.

The independent variables of the multivariable linear regression model were age, sex, body-mass-index (BMI), visual impairment as defined by the World Health Organization (WHO) (best corrected visual acuity (BCVA) <0.3 decimal), intraocular pressure (IOP), spherical equivalent (SE), smoking status and history of systemic hypertension, diabetes, stroke and dementia. The multivariable regression model was conducted for each individual study and residuals were then plotted and normal distribution assessed. Since OCT devices were changed within the course of the Rotterdam Study (From 3D-OCT 1000 to 3D-OCT 2000, Topcon Medical Systems, Oakland, NJ, USA), we controlled for the OCT device in the multivariable regression models of the Rotterdam Study II and III. In the TwinsUK Study, we

performed a hierarchical multivariable regression model to control for family dependencies between twins.

Subsequently, random-effects meta-analysis was used to combine effect estimates (beta coefficients) of each individual predictor from the multivariable regression model among studies. A random-effects approach was chosen a priori based on the heterogeneity in the data caused by the different OCT devices[17] and the set-up of the studies. Our analyses were conducted twice, with and without known glaucoma patients.

Not all independent variables of the multivariable regression model were available in every participating study. The multivariable regression models in the respective studies were therefore performed without the missing variables and the study was excluded from the meta-analysis of that respective missing covariate. All analyses were performed with the statistical software RStudio (R version 3.4.1, RStudio Inc., Boston, MA, https://www.rstudio.com/), statistical significance was set at $p < 0.05$.

RESULTS

A total of 16,084 participants from eight population-based studies were included, about one percent pRNFLT outliers per study were excluded (supplemental Table 1b). The mean age of participants ranged from 56.9 ± 12.3 years in the LIFE Study to 82.1 ± 4.2 years in the Alienor Study. Mean global pRNFLT ranged from 86.8 ± 21.4 microns in the Rotterdam Study I to 104.7 ± 12.5 microns in the Rotterdam Study III (Table 1). Further participant characteristics for each study are presented in supplemental Table 1b. The results of the multivariable regression models for each individual study are reported in Table 2. Data on dementia were only available in the Rotterdam Study cohorts and the Alienor Study. Furthermore, in the TwinsUK Study no sufficient data were available on visual impairment, glaucoma, hypertension and smoking status; in the LIFE Study, no data were available on visual impairment, SE and IOP.

In the meta-analyzed multivariable regression model (Table 3 and Figures 1a and 1b), age and IOP were negatively associated with pRNFLT, even after excluding glaucoma patients. A

history of stroke and hypertension were both associated with a reduced pRNFLT. When substituting hypertension with mean systolic blood pressure (in mmHg), no association was found.

A suggestive, but non-significant association with reduced pRNFLT was observed for dementia. Visual impairment as defined by the WHO was associated with reduced pRNFLT in the meta-analysis. We found this association in the Alienor and Rotterdam Study I-III, while there was no association in the Montrachet and Coimbra Study.

Women had a thicker pRNFLT than men in the meta-analysis. However, when correcting for AL rather than SE in the five studies with data on AL, this association disappeared. SE was positively associated with pRNFLT, even after excluding highly myopic (< -6 diopters) and highly hyperopic eyes (> +4 diopters) as well as eyes with pseudophakia (supplemental Figures A and B). Longer AL was associated with reduced pRNFLT in our sensitivity analyses (beta=–3.48μm per mm longer AL, 95% CI=–4.18, –2.77) (supplemental Figure C). Both, former and current smoking were associated with thicker pRNFLT, but prevalence and associations differed considerably between studies. To assess the influence of education on smoking, we corrected for education and the associations persisted. After excluding data from the LIFE Study, which is the largest study with the highest proportion of smokers (data weighted >60% in the meta-analysis), the association remained significant for current but not for former smoking (supplemental Figures D-G). For BMI, we found a small but significant association with increased pRNFLT after excluding glaucoma patients. All associations except for former smoking held true after excluding the 619 known glaucoma patients (Table 3). Furthermore, we detected no relevant changes of associations when performing the multivariable regression analyses stratified by sex or when excluding the LIFE study cohort being the largest single study (results not reported).

DISCUSSION

Our study confirms the previously reported associations of age and SE with pRNFLT and identifies several additional factors associated with pRNFLT, namely IOP (even in individuals

without a history of glaucoma), stroke, hypertension and smoking. Furthermore, we found a trend of reduced pRNFLT in participants with dementia. Our results suggest that a number of ocular as well as systemic factors need to be considered when assessing pRNFLT. To date, none of this has for example been implemented as potentially influencing factors in reference databases for OCT devices or any algorithms assessing pRNFLT change.

First publications on determinants of OCT – based pRNFLT measurements reported older age and greater AL to be associated with thinner pRNFLT[18,19]. Budenz and coworkers investigated determinants of pRNFLT in 328 normal subjects aged 18 to 85 years using time domain – optical coherence tomography (TD–OCT) and described a decrease of 2.0 microns pRNFLT per decade and a decrease of 2.2 microns per millimeter AL[19]. These estimates are smaller but still compare to our results (decrease of 3.8 microns pRNFLT on average per decade and 3.48 microns per millimeter AL). A subsequent study evaluated determinants of pRNFLT in 542 healthy adults aged 40 to 80 years using SD – OCT (Cirrus HD-OCT; Carl Zeiss Meditec, Inc., Dublin, CA) and confirmed the associations of pRNFLT with age and AL[11].

Subsequently, larger population studies mostly from Asia were conducted to investigate further determinants of pRNFLT. We have affirmed results from the Beijing Eye Study in 2548 participants considering the influence of age and refractive error. That study also showed a higher pRNFLT of 2.9 microns in women[14], in keeping with our results of women having a higher pRNFLT of 2.2 microns. Similar to our models, the Beijing Eye Study corrected for refractive error instead of actual AL. Interestingly, after correcting for AL in our analyses, sex was no longer associated with pRNFLT. Based on this, we hypothesize that AL, which is on average shorter in women, confounds the effect of sex on pRNFLT. In general, SE is a good proxy for AL and we found a strong association of higher SE with thicker pRNFLT, even in both our sensitivity analyses, which eliminated subjects with high refractive errors. The underlying mechanisms of the association of longer AL and thinner pRNFLT are arguable[20]. Frequently suggested mechanisms are either a stretching due to a longer eye bulb or artificially decreased measurements due to magnification[21,22]. However,

irrespective of the causal mechanism, the clinical relevance of adjusting for refraction or AL in OCT – imaging seems obvious.

Higher IOP was associated with reduced pRNFLT in our analyses even after excluding known glaucoma patients. However, since glaucoma was self-reported in some of the participating studies, not all actual glaucoma patients might have been excluded in our analyses. Visual impairment (BCVA < 0.3 decimal) as a proxy for any ocular pathology was associated with thinner pRNFLT in the Alienor Study and all of the Rotterdam Studies. The Coimbra and Montrachet Study were likely underpowered to find an effect, because of very few cases with reduced BCVA in these studies.

Previous studies reported contradictory results on the impact of hypertension and blood pressure on pRNFLT[9,23,24]. Our results show reduced pRNFLT in hypertensive patients, but no association of pRNFLT with actual systolic blood pressure. Blood pressure measurements, however, are known to vary with method and associations with systolic blood pressure may have been masked by any use of antihypertensive medication. In contrast to hypertension, most studies investigating the effect of diabetes on pRNFLT report diabetic patients to have thinner pRNFLT[25,26]. This is in not agreement with our results that do not show an association of reduced pRNFLT in diabetic patients. Nether the less, we hypothesize that microvascular pathology and ischemia due to hypertension and/or diabetes may be a cause for reduced pRNFLT, as it has been suggested previously[25].

Both, former and current smoking were associated with thicker pRNFLT in our meta-analysis, even in several sensitivity analyses including correction for educational level. This association does not seem biologically plausible given the observed pRNFLT decrease in metabolic diseases. Potential biologic explanations could be reduced axonal flow or axonal swelling in the course of axonal degeneration due to intake of neurotoxins and cytotoxins from cigarette smoke. However, our results are in contrast with findings of earlier studies[27,28], which reported reduced pRNFLT in smokers. Suggested mechanisms leading to decreased pRNFLT were toxic damage through free radicals, increased IOP and reduced perfusion[27–29].

We controlled for IOP as well as hypertension and diabetes, which all may influence

perfusion. It is therefore unclear what might explain this association. Current smokers were on average younger in our participating studies compared to never and former smokers. Hence, even though we controlled for age in our models, we cannot entirely rule out residual confounding. Additionally, the E3 studies are not representative studies of European populations and smoking percentages therefore do not reflect actual percentages. There was heterogeneity between studies considering smoking prevalence and oppositional effects of former smoking in some studies. After excluding the LIFE Study, which was dominantly weighted in the smoking meta-analysis, the Rotterdam Study III showed to be weighted strongest for current smoking. When excluding also the Rotterdam Study III, the impact of smoking is weakened but holds true. Still, the associations seem to be particularly driven by the large studies. This is also underlined by increasing heterogeneity for former and current smoking in the meta-analysis after excluding the LIFE Study. Moreover, there is no information on the time interval between cessation of smoking and OCT – imaging for the former smokers, which may have an impact, as well. Further studies are needed to confirm or refute our observation, which may well be a chance finding.

Past studies have reported stroke patients to have thinner pRNFLT, which was hypothesized to be caused by transneuronal retrograde degeneration[30,31]. Our data confirm the association of stroke and decreased pRNFLT. Additionally, in dementia patients we found a trend of reduced pRNFLT. Again, this is in accordance to various previous studies, which report dementia patients to have reduced pRNFLT[4,32]. Thus far, the underlying mechanisms remain unclear. Loss of peripapillary RNFL is a hallmark of glaucoma and longitudinal pRNFLT evaluation is a crucial part of glaucoma management. In our meta-analysis, all associations persisted after excluding known glaucoma patients except for former smoking. This indicates that the detected determinants are independent of the presence of glaucoma.

As described previously, structural decline of pRNFLT occurs before functional loss in perimetry in glaucoma patients. An earlier study reported the difference in pRNFLT between glaucomatous and healthy eyes eight years before the onset of visual field impairment to be around 5 µm[33]. This is in the range of some associations found in our study and underlines

the potential impact on the interpretation of pRNFLT. Our results have two main clinical implications. Firstly, the normative databases built into the devices should reflect our results, when presenting normal values for pRNFLT. Also, presence of vascular disease including a history of stroke should be considered when defining normative datasets or when clinically evaluating pRNFLT. As discussed above, the magnitude of impact of the respective determinants may have clinical relevance, especially in the presence of more than one factor reducing pRNFLT. Secondly, in glaucoma or other patients followed up with pRNFLT measurements, an incident stroke or dementia may cause a decrease in pRNFLT, which would not primarily be due to glaucoma or other ocular disease progression. For example, this may simulate an aggravation of glaucoma and needs to be considered by the clinician when tailoring the glaucoma management.

The strengths of this study consist of the large pooled sample combining data of eight studies from five European countries. To our knowledge, this study represents the largest European study on determinants of pRNFLT thus far. As mentioned, previous population studies reporting data on associations with pRNFLT were conducted in mostly Asian populations and results cannot directly be transferred to European individuals. The associations of this study were assessed in meta-analyses of all participating populations, thus they are not limited to one single population only. This reduces the possibility that an association was solely due to chance within one population and increases generalizability. However, several limitations of our study need to be considered. The use of different OCT–devices between studies may have increased variability and prohibited direct pooling of pRNFLT data. To overcome this lack of direct comparability we performed the analysis separately within studies and then pooled studies' effect estimates using random-effect meta-analysis. Furthermore, we found no interactions between type of device and any predictor variable in additional sensitivity analyses in the Rotterdam Study II and III, which had a device upgrade within course of the study. However, residual influence of different OCT devices cannot be entirely excluded. As expected when combining different large- scale population studies, we observed between study heterogeneity for the independent variables

and their influence on pRNFLT. The degree of heterogeneity of the respective covariates was assessed using the I2 – statistics and ranged from 0% to 97% (see Table 3). As described, this heterogeneity between studies was addressed by using random effect meta-analysis[17]. In accordance with previous literature, the relationship between pRNFLT and age was linear in our sample. Having no data for children and young adults, we do not know whether the relationship between pRNFLT and age is strictly linear throughout life but would assume so based on our data. Thus, we investigated associations using multivariable linear regression modeling. Based on this, any non-linear relationships may have been underrepresented. Quality control was performed within each study differently (supplemental Table 2). Some studies performed manual (re)-segmentation, excluded OCT images below a certain scan quality and scans with artifacts, while others included all scans with sufficient quality as evaluated by the performing technician. As sensitivity analysis we excluded participants with an image quality value below 45 (as recommended by the manufacturer) in the Rotterdam Studies I-III. We found no relevant changes of direction in any association, but the confidence intervals became broader due to a reduced sample size (supplemental Table 3). Hence, even though the lack of centralized quality control is a limitation to our analyses, the impact of poor quality scans seems to be low as indicated by our supplemental sensitivity analyses. Within each study, the number of participants in which OCT imaging could not be performed or in which the images were of low quality and thus unusable is a small proportion only (supplemental Table 2). For example, in the Rotterdam Study I-III the number of participants with no or insufficient OCT data was 10%, 6% and 15%, respectively. These subjects were older and more likely to have stroke (RS I), dementia (RS II and III) and hypertension (RS III) than the included participants. This indicates that our associations may be underestimations of the true effect. Several independent variables were not available in some studies. Therefore not all multivariable models could be corrected for all variables. However, no relevant differences of associations were detectable, when comparing studies with and studies without any missing data. Hence, the absence of certain variables in some studies did not relevantly alter the associations of the available data. Methods of

assessments varied between our studies. This concerns e.g. the best-corrected visual acuity, which was sometimes measured subjectively and sometimes by autorefractor. In addition, information on diseases was assessed differently. While glaucoma was defined based on optic disc evaluation and perimetry in the Alienor Study and Rotterdam Study I-III, it was self-reported in the LIFE Study. Furthermore, we did not distinguish between the various types of dementia, which may have different impact on pRNFLT. These differences contribute again to larger heterogeneity and the relation between self-reported diseases and pRNFLT may have been estimated with less precision. Lastly, our data were cross-sectional only, thus causal deductions from the detected associations are limited and further longitudinal studies are needed.

In conclusion, the current analyses identified important additional determinants of pRNFLT, which should be considered when assessing pRNFLT both clinically and in epidemiological research. The magnitude of changes in pRNFLT by determinant is likely clinically relevant and the biology of pRNFLT thinning is complex, with mechanical pressure, microvascular ischemia and neuronal degeneration being implied. This is reflected in the complexity of factors, which influence pRNFLT and hence need to be considered. In particular, the associations with systemic vascular and neurovascular diseases merit further research.

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Figure legends

Figure 1a: Forest plots of meta-analyzed associations with pRNFLT from multivariable regression models (Age, sex, spherical equivalent, intraocular pressure and visual impairment). The beta-coefficients [95% Confidence Interval] show the influence of each parameter on pRNFLT within the respective study, the percentage represents the mathematically determined weighting of each study within the meta-analysis.

Figure 1b: Forest plots of meta-analyzed associations with pRNFLT from multivariable regression models (Smoking, hypertension, stroke and dementia). The beta-coefficients [95% Confidence Interval] show the influence of each parameter on pRNFLT within the respective study, the percentage represents the mathematically determined weighting of each study within the meta-analysis.