

# Evaluation of Heredity as a Determinant of Retinal Nerve Fiber Layer Thickness as Measured by Optical Coherence Tomography

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**PURPOSE.** To study to what extent genetic factors determine the retinal nerve fiber layer thickness (RNFLT) in healthy subjects.

**METHODS.** In vivo peripapillary optical coherence tomography (OCT), clinical examination, lens fluorescence, and fundus photography were performed on both eyes of 25 monozygotic and 25 dizygotic same-sex pairs of twins. The cross-sectional study included twins aged from 20 to 45 years recruited from a population-based register. Only healthy eyes were included. Main outcome variables: peripapillary OCT RNFLT, reproducibility, and heritability (the proportion of the total observed variance statistically attributable to genetic factors).

**RESULTS.** The within-pair difference in RNFLT was 4.6% (0.7%–15.2%; median [range]) in monozygotic versus 7.3% (0.2%–20%) in dizygotic twins ( $P = 0.032$ , Mann-Whitney test). The RNFLT heritability was 66%. The RNFLT measurement was found to decrease 3.8  $\mu\text{m}$  per decade ( $P = 0.003$ ). The RNFLT heritability increased to 82%, when corrected for the effect of age and excluding within-pair refractive differences of 2 D or more. The signal-to-noise ratio correlated with lens transmittance ( $r = 0.25$ ,  $P = 0.012$ ), age ( $r = -0.29$ ,  $P = 0.004$ ), and RNFLT ( $r = 0.43$ ,  $P < 0.001$ ). Intra-visit RNFLT reproducibility was 4.2%.

**CONCLUSIONS.** Peripapillary RNFLT in healthy adults, as measured by OCT, was determined predominantly by genetic factors in this study population. Theoretically, these factors may involve variations in the number of ganglion cells and nerve fiber formations early in life and/or in the rate at which these structures are subsequently lost. (*Invest Ophthalmol Vis Sci*. 2003;44:3011–3016) DOI:10.1167/iovs.02-1090

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Open-angle glaucoma is characterized by premature retinal nerve fiber loss which most commonly occurs as a gradual, insidious loss that mimics the normal loss of nerve fibers with increasing age but occurs at a pathologically accelerated rate. Previous studies have demonstrated evidence of genetic involvement in primary open-angle glaucoma (POAG) in adults, as well as in the primary congenital forms of glaucoma. At present at least six major gene loci have been found to segregate with POAG (including high-tension glaucoma, normal-tension glaucoma, and primary juvenile glaucoma).<sup>1</sup> Specific gene loci involved include the loci 1p36 and 2q21 in primary congenital glaucoma (buphthalmia), 1q23-q25/TIGR in primary juvenile glaucoma and adult onset POAG, and the optineurin gene on 10p14 in adult-onset POAG.<sup>2–5</sup> Rare families with adult-onset POAG demonstrate patterns of Mendelian inheritance, that suggest single-gene disease, but multiple gene involvement, is probably involved in the typical patient. Thus, defects in currently identified genes account for only a small fraction of glaucoma cases. There are few data available to assess the overall effect of hereditary factors in the development of glaucoma, but several studies show that a family history of adult-onset POAG is a major risk factor.<sup>6,7</sup>

The objective of the present study was to examine the relative influence of genetic factors in determining the amount of retinal nerve fibers in healthy adults using optical coherence tomography (OCT) to measure peripapillary retinal nerve fiber layer thickness (RNFLT) in healthy adult twins. Theoretically, these factors may be involved in the process of ganglion cell and nerve fiber formation early in life and/or in the rate by which these cells and nerve fibers are subsequently lost to the time of measurement. An additional objective of the present study was to determine the rate of the age-related attenuation of the retinal nerve fiber layer (RNFL) in healthy adult subjects. Furthermore, we have attempted to determine the confounding effect on the OCT measurement of RNFLT by the optical quality of the refractive components of the eye. A future perspective is to assess the rate of RNFL attenuation in adults and the relative influence of genetic and environmental factors on this rate.

## MATERIALS AND METHODS

### Subjects

All eyes ( $n = 100$ ) of 25 monozygotic (MZ) and 25 dizygotic (DZ) pairs of twins were examined in a cross-sectional study including twins aged 20 to 45 years. All twin pairs were same-sex and recruited from a population-based register comprising twins born in Denmark between 1870 and 1996 (The Danish Twin Registry, University of Southern Denmark, Odense, Denmark).<sup>8</sup> Exclusion criteria included cataract and other opacities near the optical axis of the eye, as well as other manifest eye disease, with such findings leading to the exclusion of both twins in a pair.

TABLE 1. Characteristics of Healthy Twins in Relation to Zygosity

	Monozygotic Twins	Dizygotic Twins	P*
Number of twins	50	50	
Female/male (n/n)	24/26	32/18	—
Age (y)	36 (20–45)	35 (20–45)	NS
Refraction (spherical equiv.; D)	0.00 (–5.28–1.50)	–0.56 (–6.38–4.38)	NS
Fasting blood glucose (mmol/L)	4.9 (4.2–5.6)	4.9 (4.2–6.2)	NS
Diastolic blood pressure (mm Hg)	70 (55–81)	69 (51–91)	NS
Body mass index (kg/m <sup>2</sup> )	23.5 (18.9–28.0)	23.8 (17.9–35.3)	NS
Smoking habits (Pack-years)	0.4 (0.0–29.0)	0.0 (0.0–24.0)	NS

Data are presented as the median (range).

\* Mann-Whitney.

## Clinical Examination

All subjects underwent a clinical eye examination, lens fluorometry, fundus photography, and in vivo circular peripapillary OCT. On the same day, the subjects answered a detailed health questionnaire and underwent a comprehensive systemic examination as part of a larger study of cardiovascular risk factors (GEMINAKAR; The Danish Epidemiology Science Centre, The Institute of Preventive Medicine, Copenhagen University Hospital, Copenhagen, Denmark). Visual acuity was determined using a Snellen decimal projection chart. All eyes included had visual acuity of at least 0.9. The distributions of age, sex, refraction, fasting blood glucose, diastolic blood pressure, body mass index, and smoking habits are summarized in Table 1.

Blood pressure, height, weight, blood lipids (total cholesterol, LDL, VLDL, HDL, and triglycerides) were determined before an oral glucose tolerance test using 75 g of glucose after a 12-hour overnight fast, assessing whole capillary blood glucose at 0, 30, and 120 minutes. Zygosity was determined based on genetic markers using nine microsatellite and RFLP markers. Life-long smoking habits were assessed and quantified into pack-years: 1 pack-year equals a consumption of 20 cigarettes a day for 1 year. The subjects answered a questionnaire relating to diabetes or glaucoma in first- or second-degree relatives.

All participants gave their informed consent according to the Declaration of Helsinki. The study was approved by the regional medical ethics committee.

## Optical Coherence Tomography

Thirty minutes after instillation of topical mydriatics, the peripapillary RNFL was examined by OCT (OCT 2000; Zeiss Humphrey, Inc., Dublin, CA, software application ver. A4.1), using a circular scan with a nominal diameter of 3.40 mm.<sup>9–14</sup> Using an internal fixation light, the scan circle was centered on the optic nerve head (ONH). No scaling was made for variation in subject refraction and fundus magnification. All scans were obtained at the maximum power of 750 mW and stored in a digital archive for later processing.

The OCT parameters of interest were the peripapillary RNFLT and the signal-to-noise ratio (S/N-ratio). The two highest intensity scans without artifacts of three scans per eye were used. The RNFLT was calculated for each scan as the mean of the 100 juxtaposed axial A-scans that compose the peripapillary cross-sectional image of the retina. Similarly, the mean RNFLT was calculated for each quadratic sector of the scan. The mean of both eyes, based on two scans per eye, was used when calculating the RNFLT of a subject. The anterior and posterior limits of the RNFL were detected by using the commercial data analysis algorithm supplied by the manufacturer.

The OCT S/N ratio was also calculated automatically by the internal software of the instrument. In brief, raw data are converted into a decibel scale, where 0 dB represents the mean signal intensity + 2 SD in the vitreous. Subsequently, the 10 of the 100 A-scans that have the highest maximum intensity peaks are identified and the average value (in decibels) of these 10 peaks is used to define the S/N ratio. Thus, the S/N ratio is related, among other properties, to the optical quality of

the refractive components of the eye being examined, especially when no retinal disease (e.g., hard exudates) is present.<sup>15,16</sup>

## Lens Transmittance

Lens transmittance was measured with an ocular fluorometer (Fluorotron; Coherent Medical Division, San Jose, CA) fitted with an anterior segment adaptor that concentrates 149 steps of measurement along the optical axis in the anterior segment of the eye giving detailed information on the lens autofluorescence.<sup>17,18</sup> The excitation wavelength was 430 to 490 nm, and detection was at 530 to 630 nm. Lens transmittance was calculated as the square root of the ratio between the posterior and anterior peak in lens fluorescence, using the mean of six scans (three per eye).

## Fundus Photography

Digital gray-scale fundus photographs (20° and 50°, 1024 × 1024 pixels) were recorded in red-free illumination (filter: Wratten 54; Eastman Kodak, Inc., Rochester, NY) using a retinal camera (model TRC-50X; Topcon Corp., Tokyo, Japan) equipped with a digital back piece (MEGAPLUS model 1.4; Eastman Kodak, San Diego, CA) and a PC-based image-management system (Ophthalmic Imaging Systems Inc., Sacramento, CA). The same camera was used to record 50° color fundus photographs on transparency film (Ektachrome Elite 100; Eastman Kodak, Inc.).

Fundus photographs were subjected to visual evaluation by two independent observers to assess RNFL visibility, density, and pattern of distribution, as well as the area and shape of the neuroretinal rim of the optic nerve head (ONH), the vascular status of the ONH, the presence of splinter hemorrhages, and the degree of symmetry of such properties between the subject's two eyes. If glaucoma was suspected, a visual field test was made on a subsequent visit (HFA II, model 750; 81 points full-field screening, stimulus size III, white, threshold related test mode, strategy 3 zones; Zeiss Humphrey) together with applanation tonometry ad modum Goldmann. As mentioned earlier, manifest glaucoma led to the exclusion of the twin pair, as did any manifestation of eye disease other than refractive anomalies at the time of examination.

## Statistical Analysis

The results were analyzed using Student's *t*-test or the Mann-Whitney test (two-sided), linear regression (least square), and correlation analysis (Pearson's correlation coefficient). The proportion of total variation attributable to genetic factors is expressed as heritability ( $h^2$ ), which is twice the difference in interclass correlation ( $r$ ) between MZ and DZ twins<sup>19</sup>

$$h^2 = 2(r_{mz} - r_{dz}) \quad (1)$$

where  $r$  is defined as

$$r = \text{COVAR}_{(\text{twinA}, \text{twinB})} / \sqrt{(\text{VAR}_{(\text{twinA})} \times \text{VAR}_{(\text{twinB})})} \quad (2)$$

covariance and variance.<sup>20</sup> Within a twin pair, the A and B status was randomly chosen. To obtain a symmetric distribution around the identity line, both of the two coordinates A,B and B,A for any one pair of twins was used in the correlation analysis. The 95% confidence interval ( $ci_{95}$ ) of the heritability is calculated as

$$ci_{95} = h^2 \pm 2 \times \sigma_t \quad (3)$$

where  $\sigma_t$  is the SE of the heritability,<sup>21</sup> which is calculated as

$$\sigma_t = \{2[1 + (n - 1)t]^2 \times (1 - t)^2/n(n - 1)(N - 1)\}^{1/2} \quad (4)$$

where  $n$  is the number of offspring per family ( $n = 2$ ) and  $t$  is  $1/2h^2$ .  $N$  is twice the number of families (100) because  $h^2$  is calculated using both of the two coordinates A,B and B,A for any one pair of twins.

The sample size did not allow a more differentiated analysis of the genetic and environmental effects by structural equation modeling.<sup>22</sup> Data analysis was made using R computer software version 1.2.3 (<http://www.r-project.org>).

## RESULTS

### Retinal Nerve Fiber Layer Thickness

The mean RNFLT, using the full-circle peripapillary OCT, in the study population ( $n = 100$ ) was  $104.4 \pm 9.9 \mu\text{m}$ . We found no significant difference between the mean RNFLT in MZ twins ( $105.6 \pm 10.1 \mu\text{m}$ ; mean  $\pm$  SD) and that in DZ twins ( $103.1 \pm 9.7 \mu\text{m}$ ;  $P = 0.21$ ,  $t$ -test). Furthermore, there was no difference in RNFLT between women and men ( $P = 0.70$ ,  $t$ -test). For the superior, nasal, inferior, and temporal quadrants the thicknesses were  $126.1 \pm 13.3$ ,  $79.6 \pm 13.5$ ,  $129.5 \pm 14.0$ , and  $80.1 \pm 12.9 \mu\text{m}$ , respectively. The RNFLT did not differ between the twin A and B groups ( $P = 0.82$ ,  $t$ -test), thus justifying the pooling of all 100 subjects in the analysis. All mention of RNFLT in the text that follows relates exclusively to the mean thickness of the full circle.

The RNFLT was found to decrease with decreasing OCT S/N ratios ( $r = 0.43$ ,  $P < 0.001$ ) and with increasing age ( $r = -0.30$ ,  $P = 0.003$ ; Fig. 1). Thus, the RNFLT was found to decrease  $3.8 \mu\text{m}$  per decade, from  $110 \mu\text{m}$  at age 20 to  $100 \mu\text{m}$  at age 45. The RNFLT decreased slightly with decreasing lens transmittance ( $r = 0.25$ ,  $P = 0.014$ ) and with increasingly negative refraction ( $r = 0.20$ ,  $P = 0.047$ ). For the total study population, the OCT S/N ratio was (mean  $\pm$  SD, [range])  $55.4 \pm 2.2 \text{ dB}$  (50.8–59.5) and lens transmittance was  $0.91 \pm 0.04$  (0.76–0.99).

OCT S/N ratio, age, lens transmittance, and refraction explained 18%, 9%, 6%, and 4% of the total variation in RNFLT, respectively. Among these parameters, the OCT S/N ratio and lens transmittance were significantly interrelated ( $r = 0.25$ ,  $P = 0.012$ ), and both parameters correlated negatively with increasing age ( $r = -0.29$ ,  $P = 0.004$ ;  $r = -0.36$ ,  $P < 0.001$ ). Age explained 8% and 13% of the total variation in OCT S/N ratio and lens transmittance, respectively.

Consequently, the relation between the RNFLT as measured by OCT and age may reflect both the age-related loss of nerve fibers and the age-related decrease in OCT S/N ratio, and a correction of the RNFLT for the effect of OCT S/N, using residuals from the RNFLT as a function of OCT S/N ratio, resulted in a decrease of the age-related RNFLT loss to  $2.2 \mu\text{m}$  per decade ( $P = 0.056$ ).

Body mass index, blood lipids, fasting blood glucose, blood glucose at 30 and 120 minutes after glucose tolerance testing, diastolic blood pressure, and smoking habits did not correlate significantly with the RNFLT.

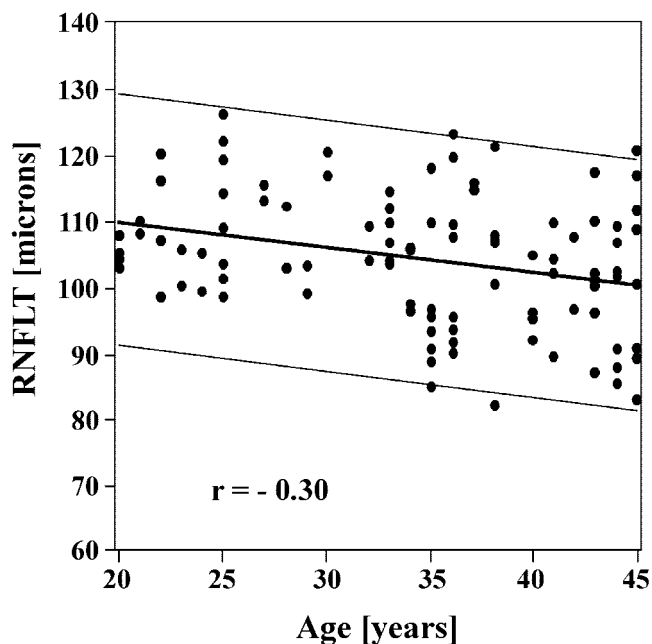


FIGURE 1. Peripapillary RNFLT as measured by OCT in relation to age in healthy subjects ( $n = 100$ ). The RNFLT was found to decrease  $3.8 \mu\text{m}$  per decade ( $r = -0.30$ ,  $P = 0.003$ ). Solid lines: linear regression of the mean in relation to age and outer limits of the 95% prediction interval.

The scan-to-scan intravisit reproducibility of the RNFLT measurement was 4.2%. No significant difference was found between MZ and DZ twins ( $P = 0.83$ , Mann-Whitney test). Reproducibility was calculated as the numeric difference in RNFLT (full-circle mean) between the two scans chosen for analysis relative to the mean of the two scans. Reproducibility decreased significantly with age ( $r = 0.24$ ,  $P = 0.017$ ), with decreasing lens transmittance as determined by lens fluorometry ( $r = -0.31$ ,  $P = 0.002$ ), and with decreasing OCT S/N ratio ( $r = -0.48$ ,  $P < 0.001$ ). These factors explain 6%, 9%, and 23%, respectively, of the total variation in RNFLT reproducibility. The RNFLT reproducibility decreased slightly with decreasing thickness of the retinal nerve fiber layer ( $r = -0.23$ ,  $P = 0.020$ ).

### Family History

Two MZ twin pairs had a family history of glaucoma. Both twins in one of these pairs had RNFLT in the lower part of the normal range but within the 95% predictive interval. No subject was excluded on the basis of family history.

### Systemic Conditions

The medical history of four patients (one MZ, three DZ) included ongoing treatment for hyperthyroidism. None of these subjects had RNFLT outside the 95% predictive interval in their respective age groups.

No other cases of known metabolic disease were found, but in the course of the study, 12 subjects (six DZ and six MZ) were found to have impaired glucose tolerance (fasting blood glucose value  $< 6.1 \text{ mM}$  and a 2-hour glucose tolerance test value  $\geq 7.8 \text{ mM}$ ). One DZ twin had a diabetic fasting blood glucose level of  $6.2 \text{ mM}$ , but this subject's 2-hour glucose tolerance test result of  $8.2 \text{ mM}$  was below the diabetic range.<sup>23</sup> By World Health Organization definitions, this asymptomatic subject was diabetic, but was classified in the study as having borderline diabetes.

TABLE 2. Within-Pair Differences in Peripapillary RNFLT and Associated Ocular Characteristics for MZ and DZ Healthy Twins

	Monozygotic Twin Pairs	Dizygotic Twin Pairs	<i>P</i> ‡
Within-pair absolute numerical difference			
Refraction (spherical equivalent; D)	0.4 (0.0–2.4)	0.7 (0.0–6.1)	0.078
Lens transmittance (%)	3.2 (0.1–6.0)	3.8 (0.3–18.1)	0.35
S/N (dB)*	1.00 (0.00–5.50)	1.00 (0.25–4.25)	0.68
Within-pair relative numerical difference			
RNFLT (%)†	4.6 (0.7–15.2)	7.3 (0.2–20.0)	0.032

*n* = 25 twin pairs for both MZ and DZ twins. Data are expressed as the median (range).

\* OCT S/N ratio.

† As measured by OCT.

‡ Mann-Whitney.

One subject had an isolated systolic blood pressure measurement above 140 mm Hg (147 mm Hg), and two subjects had a diastolic blood pressure equal to or above 90 mm Hg (90 and 91 mm Hg, respectively). No subject was excluded for this or any other systemic condition.

### Heritability

The within-pair numerical difference in RNFLT was (median [range]) 4.6% (0.7%–15.2%) in MZ versus 7.3% (0.2%–20.0%) in DZ twins (*P* = 0.032, Mann-Whitney test; Table 2). Interclass correlation (*r*) on RNFLT (crude data) was 0.812 and 0.481 in MZ versus DZ twin pairs (Fig. 2). Thus, the heritability (*h*<sup>2</sup>) was 66% (ci<sub>95</sub> 48%–84%) when based on raw data. After correction for the effect of age, using linear regression residuals from RNFLT analyzed as a function of age, the interclass correlation (*r*) was 0.802 and 0.411 in MZ versus DZ, yielding a heritability of 78% (61%–95%).

The difference in refraction within a pair of twins tended to be larger in DZ than in MZ pairs (*P* = 0.078; Table 2), potentially confounding the analysis on RNFLT heritability because RNFLT was found to correlate with refraction (as mentioned earlier). Consequently, we performed an additional analysis on age-corrected residuals after exclusion of all twin pairs with an intrapair difference in refraction (spherical equivalent) equal to or greater than 2 D. The remaining 24 MZ and 17 DZ twin pairs demonstrated an interclass correlation (*r*) of 0.800 in MZ versus 0.388 in DZ twin pairs, yielding a heritability of 82% (64%–100%).

### DISCUSSION

The present cross-sectional study demonstrates that genetic factors are major determinants of RNFLT, as measured by OCT,

in adults without eye disease. We confirmed the existence of an age-related decrease in RNFLT in the study population, of 3.8 μm per decade, using linear regression analysis (*P* = 0.003).<sup>24,25</sup> A cohort effect cannot be ruled out in this cross-sectional study, but it seems unlikely. A follow-up of the present study should clarify this matter.

The measurement of RNFLT is significantly influenced by the OCT S/N ratio and the lens transmittance. Although, these two parameters were interrelated and correlated significantly with age, the variation in lens transmittance explained only a minor proportion of the total variation in OCT S/N ratio. Consequently, if the OCT S/N ratio truly reflects the optical quality of the eye, then the age-related degradation of the lens is not the only source of optical noise in the eye that interferes with OCT measurement of the RNFLT. A statistical correction of the RNFLT measurement for the effect of the age-related decrease in OCT S/N ratio resulted in a modest reduction in the estimated decline per decade in RNFLT, to a decline of 2.2 μm per decade (*P* = 0.056).

The proportion of the total observed variance in RNFLT in the study population statistically attributable to genetic factors (heritability) was 66%, but increased to 78% after correction for the effect of age. This increase in heritability may reflect the elimination of age-related effects on the optical quality of the refractive components of the eye, where environmental factors may play a considerable role.<sup>22</sup> The design of our study does not permit any conclusion regarding the potential relation between such factors and age-related loss of nerve fibers.

Theoretically, the RNFLT, as measured by OCT, may be influenced by refractive status. The present study was not designed to examine this relationship, but data analysis revealed a significant correlation and a considerable variation in refraction in the study population. This prompted us to assess

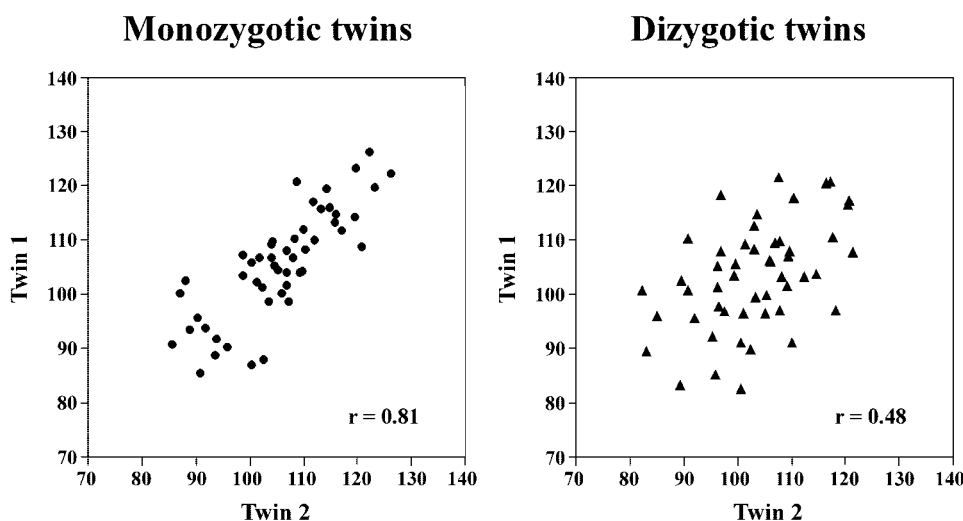


FIGURE 2. Peripapillary RNFLT (in micrometers) in relation to zygosity in healthy twins. The interclass correlation was 0.81 in MZ and 0.48 in DZ twins. Thus, the heritability was 66% (crude data).

heritability corrected for the effect of age within a restricted range of variation in refraction. This resulted in an increase in the calculated heritability to 82%. Further stratification of the study population on other characteristics was not possible because of the limited size and variation of parameters in the study population.

The OCT scan is basically determined by the optical properties of the tissue examined and consequently may not reflect the exact dimensions of the retinal layers or the functional capacity of the retinal nerve fibers. There is, however, a striking correlation between the retinal nerve fibers as they are depicted by OCT and the histology of the retina.<sup>11,26,27</sup>

The highly reflecting inner retinal band corresponding to the RNFL appears to be overlaid by a broadened reflection from the anterior surface of the retina. Thus, a high-intensity band can be seen to move down through the retina as it is ablated layer-by-layer by excimer laser radiation, albeit on a histologically fixed tissue preparation.<sup>28</sup> It is not known whether this phenomenon can be reproduced in viable tissue. The effect may be caused by the abrupt increase in refractive index between the vitreous and the retina, notably the specular surface reflection of the retina.

Limitations in the optical resolution may be involved in the ability to resolve the RNFL from the adjacent layers of the retina.<sup>28,29</sup> Consequently, this may lead to problems for the data analysis algorithms defining the thickness of the RNFL by OCT. OCT has been shown systematically to yield lower RNFL values than histologic tissue sections,<sup>30-32</sup> but other potential causes could involve optical artifacts in the living human eye as well as artifacts introduced by histologic tissue preparation. In addition, the exact location of histologic sections in relation to the landmarks of the fundus is notoriously difficult. Furthermore, effects of variations in data analysis algorithms have been demonstrated (Hougaard JL, Sander B, ARVO Abstract 271, 2002). Unfortunately, the fundamental characteristics of the proprietary algorithms used in current instruments have not been documented, but empiric testing demonstrates a high level of agreement with visual evaluation of the RNFL on biomicroscopy or fundus photography and with automated perimetric localization of RNFL defects. Our results are amenable, however, to confirmation by independent methods, and we have found a comparable level of heritability of the optic nerve head rim area as assessed by stereoscopic inspection of fundus photographs in the present study sample (Dejgaard N, Hougaard JL, Larsen M, unpublished data, 2002). In addition, a genetic influence on the cup-to-disc area ratio was found in 17 healthy twin pairs in a study by Teikari and Airaksinen.<sup>33</sup>

The high RNFLT heritability found in the present study implies that major causes of glaucoma may be identifiable by genetic studies. Currently available data suggest that multiple genes are involved, thus supporting the continued use of population-based genetics in the elucidation of the pathogenesis of glaucoma.

It is not surprising to find a lower heritability for glaucoma than for the RNFLT, because glaucoma is acquired only by a small segment of the population in which a critically accelerated retinal nerve fiber loss and a sufficiently long life span are found in combination. Thus, Teikari<sup>34</sup> found a heritability of open-angle glaucoma of 13%, based on concordance rates in MZ and DZ twins.

A future follow-up of the present study should enable estimation not only of RNFLT status at the time of examination but also of the rate of RNFL attenuation in adults and its relationship with genes and the environment. The fundamental question is to what extent variations in RNFLT in adults are evidence of traits established early in life or caused by variations in the rate of nerve fiber loss in adult life.

In summary, we found that the human peripapillary RNFLT in healthy adults, as measured by OCT, from a statistical standpoint, was determined predominantly by genetic factors. We also documented an effect on the measurement of RNFLT by OCT of aging and reduced optical quality of the refractive components of the eye, which should be taken into account in clinical and research applications of this technique.

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