UiT The Arctic University of Norway

Faculty of Health Science

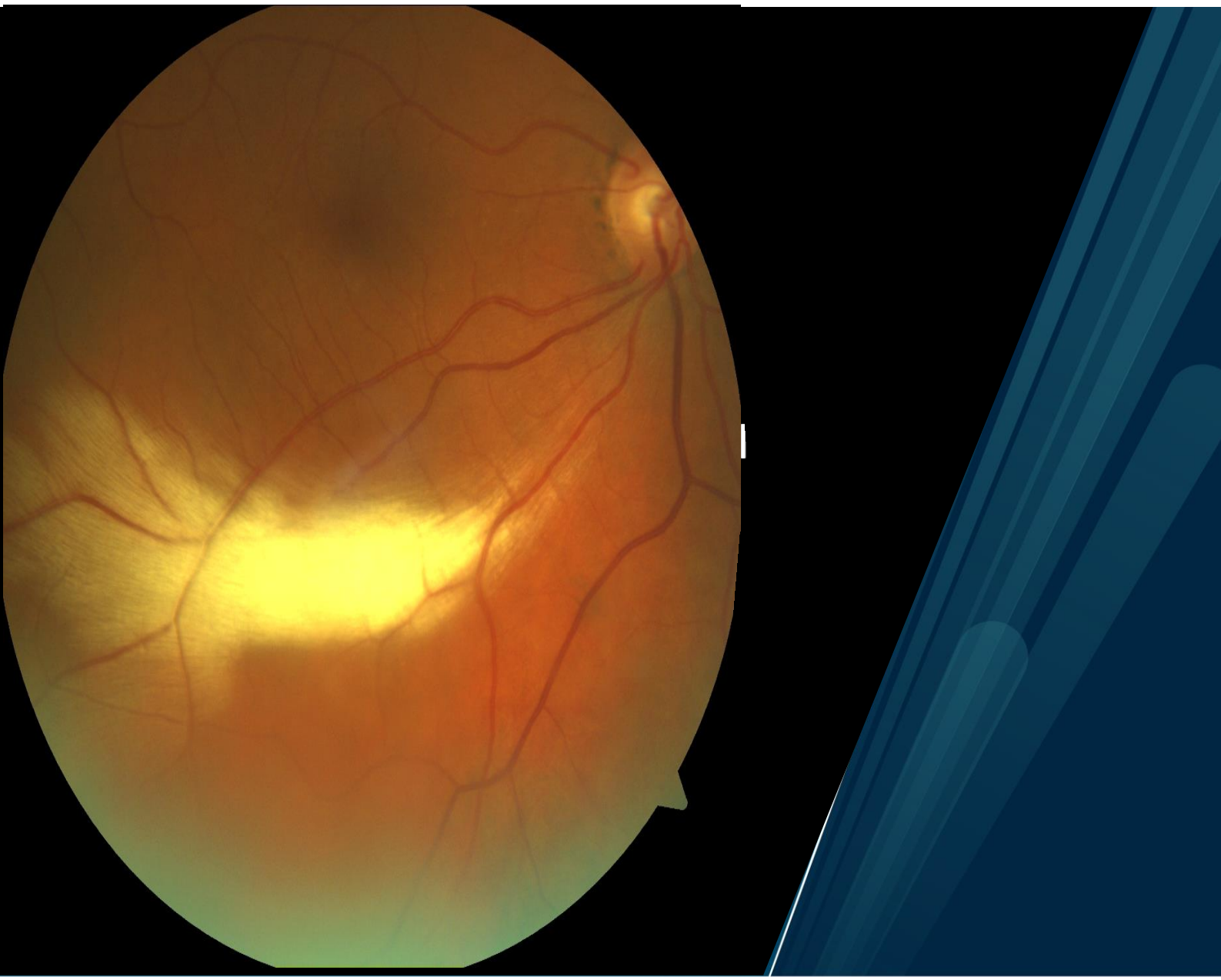
Department of Clinical Medicine

Myelinated Retinal Nerve Fibre: Prevalence and its Association with Ocular and Systemic Parameters in Tromsø.

Dominic Okoro Kalu

Master's thesis in Public Health…HEL3950…November 2020

Supervisor: Geir Bertelsen



Acknowledgement

Man, they post, does not dwell in isolation and can never exist in autarky. Therefore, I would like to recognize specially those who contributed immensely to the success of this work. First, I would like to thank God almighty for sustaining me and my family.

Second, I would like to express my deepest appreciation to my amiable supervisor, Geir Bertelsen for his support, consistent guidance, and meticulous and constructive supervision. I also would like to extend my sincere gratitude to UiT The Arctic University of Norway, Faculty of Health Science, my Professors and fellow students for providing me with a vibrant and memorable learning experience. I can never forget to sincerely appreciate my baby, Peace Onuoha and my friends Sarah Tione and Samuel Ndukuba for their contribution to the success of this project.

Last but certainly not the least, I would like to express my unwavering love to my family for their prayers, encouragement, and all-round support through these years.

P.S: Copyright permission to use figures 1 and 2 was obtained from Springer Nature through RightLinks.

Table of Contents

Abstract

Background: Myelinated retinal nerve fibres (MRNFs) are congenital anomalies of the fundus that occur in about 1% of the population. Clinically, they appear as grey or white well-separated patches with feathery borders along the retinal nerve fibre layer. MRNF has been reported to occur in association with some ocular parameters as well as stroke. However, most cases of MRNF occur in isolation and are asymptomatic.

Purpose: To determine the prevalence, ocular and systemic associations of MRNF in Tromsø.

Methods: The present study included 7396 participants aged 40–84 years from the Tromsø Eye Study, a sub-study of the population-based Tromsø Study in Norway. Data on ocular and systemic parameters were collected from self-report questionnaires, clinical examinations, and laboratory measurements. Retinal images from both eyes were assessed for the presence of MRNF. Logistic regression models were used to assess the association between MRNF and ocular and systemic parameters.

Results: Readable fundus photographs of at least one eye were available in 7396 (88.0%) of 8346 participants of the Tromsø eye study (TES). The study cohort included 3979 (55.8%) females and 3417 (46.2%) males. The mean age in the whole study cohort was 62.9 ± 10.4 years with 62.8 ± 10.4 and 63.1 ± 10.4 years in females and males respectively. The prevalence of MRNF in the study sample was 1.1%. In a multivariable logistic regression analysis, MRNF was associated with male sex (OR, 1.785; 95% CI, 1.138–2.799; P, 0.012) and glycosylated haemoglobin (HbA1c) (OR, 0.559; 95%CL, 0.316 – 0.989; P, 0.046). MRNF was not associated with age, systemic conditions or ocular parameters such as visual acuity, refractive error, or intraocular pressure.

Conclusion: This population-based study is the first to provide data on the prevalence of MRNF in the Nordic population. It is also the first study to report an association between MRNF and HbA1c, although this may be a chance finding and must be explored further. This study tends to affirm the general perception that MRNF is without serious clinical implications, however, this must also be investigated further in future studies.

Key words: Myelinated retinal nerve fibres, prevalence, population-based study, HbA1c.

List of Abbreviations

HbA1c – Glycosylated Haemoglobin

RGC - Retinal Ganglion Cell

BBB – Blood-Brain Barrier

BMI – Body Mass Index

BRB – Blood Retinal Barrier

CNS – Central Nervous System

CVRFs – Cardiovascular Risk Factors

MRNF – Myelinated Retinal Nerve Fibre

OCT – Optical Coherence Tomography

RNFL – Retinal Nerve Fibre Layer

TES – Tromsø Eye Study

VA – Visual Acuity

WHO – World Health Organization

1 Introduction

1.1 Background

1.1.1 The Eye as a window into the brain

Over the years, the concept that 'the eye is a window into the brain', has become increasingly popular and relevant. During normal prenatal development, the retina and the optic nerve emerge from a relatively small part of the central nervous system (CNS) called diencephalon and, therefore, are considered part of the CNS (London et al., 2013). The retina comprises of layers of specific neurons that are interconnected through synapses. When light enters the eye, it is captured by the photoreceptor cells in the retina. These photoreceptor cells then trigger a surge of neuronal signals that travel to the retinal ganglion cells (RGCs). The axons of the RGCs extend to the lateral geniculate nucleus in the thalamus and the superior colliculus in the midbrain, from where visual impulse is further transmitted to the higher visual centres that enable us to see a picture of our world (London et al., 2013).

The connection between the eye and the brain is made possible through the visual pathway. The latter consists of, in addition to the retina and optic nerve, the optic chiasma, optic tract, lateral geniculate nucleus, optic radiations, and visual cortex. As an extension of the CNS, the retina shows characteristics akin to the brain and spinal cord in terms of its anatomical features, functionality, inflammatory response, and immunology (London et al., 2013). There is a close similarity between both the retinal and cerebral micro and macro-vasculatures and, these vascular networks also share identical vascular regulatory processes (Patton et al., 2005). Hence, changes in the retinal vasculature may lead to similar changes in the vascular networks of the brain. The similarity between the blood-brain-barrier (BBB) and blood-retinal barrier (BRB) and “immune privilege” involved in preventing harmful immune system reactions in both the CNS and the eye, also gives credence to their shared origin (Allegri, Rissotto, Herbort, & Murialdo, 2011).

Given the similarities and connections between the eye and the brain, lessons from eye research could be applied to the brain and vice versa (London et al., 2013). Figure 1 represents the connection between the posterior segment of the eye and the brain; depicting the eye as an extension of the CNS.

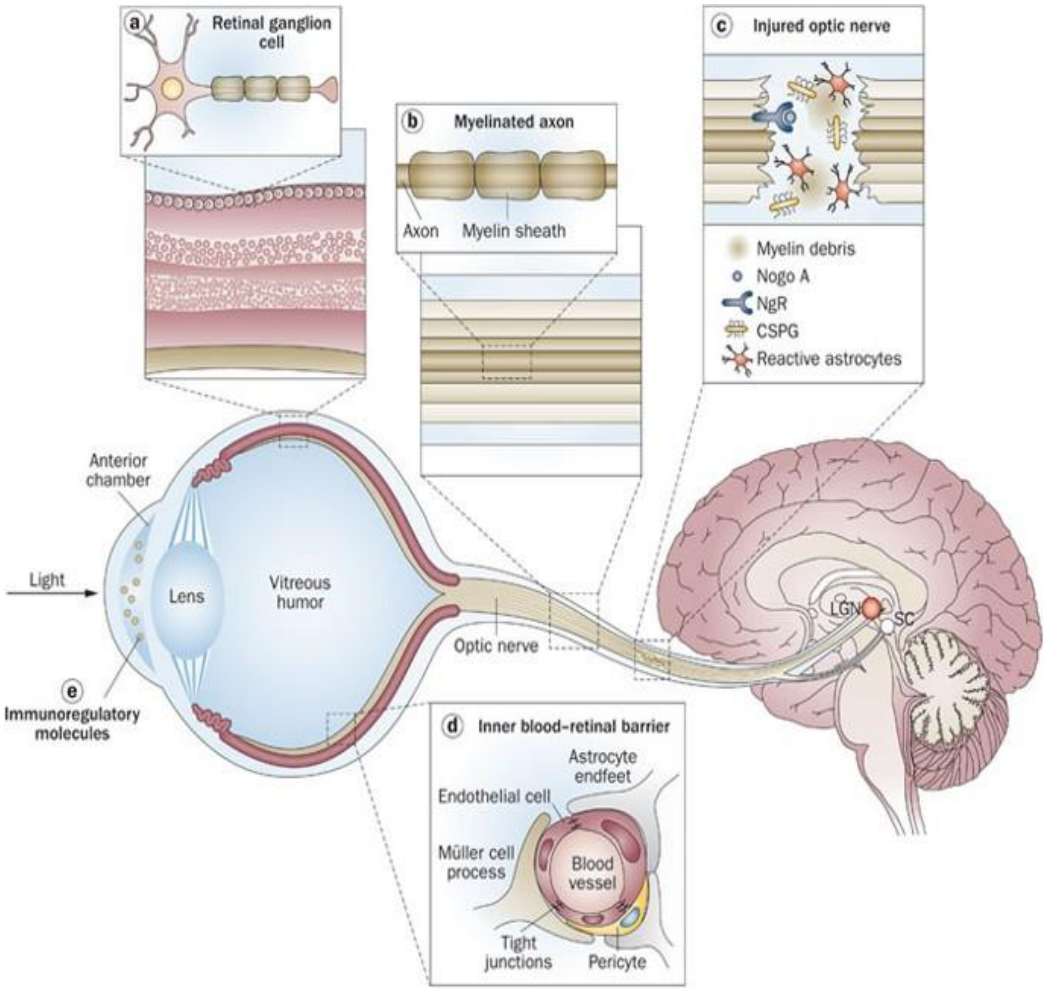


Figure 1: The Eye as an extension of the CNS.

a) *The layers of the retina consist of diverse types of neuron including retinal ganglion cells, which share a similar structure with other CNS neurons;*

b) *The axons of these cells are myelinated by oligodendrocytes just behind the globe, giving rise to the optic nerve, which stretches to the Lateral Geniculate Nucleus (LGN) and Superior Colliculus (SC) of the brain;*

c) *Similar to CNS neurons, an insult to the optic nerve creates an environment that both threatens the survival of neurons as well as hinders the regeneration of injured axons;*

d) *Like the CNS, the eye shares a special connection with the immune system that involves both the inner blood-retinal barrier, the retinal counterpart of the CNS's blood-brain barrier and*

e) *The extremely important immunoregulatory molecules.*

***Note**: Adapted from (London et al., 2013).*

***Abbreviations**: CSPG, chondroitin sulphate proteoglycan; LGN, lateral geniculate nucleus; NgR, Nogo receptor; SC, superior colliculus.*

1.1.2 Ocular manifestations of CNS disorders

Besides having common embryogenic developmental pathway, the posterior segment of the eye and the CNS also have shared vascular pathogenesis (Dhillon & Dhillon, 2008). Several diseases of the CNS have been shown to manifest in the retina (Allegri et al., 2011; London et al., 2013). For instance, previous prospective studies have suggested that abnormal retinal signs such as narrowing of arterioles, arteriovenous nicking and retinal haemorrhages could potentially predict the risk of clinical stroke events (Cheung et al., 2010), ischemic brain changes (T. Y. Wong et al., 2002), as well as stroke mortality (Tien Yin Wong et al., 2001). More so, retinal arteriovenous nicking and hypertensive ocular fundus abnormalities have been reported in association with increased risk of incident brain infarction (Tien Yin Wong et al., 2001; T. Y. Wong et al., 2002). In addition, studies conducted with animal models have linked cerebral infarction to conditions like reactive gliosis, thinning of the retinal nerve fibre layer, as well as optic neuropathy (Kalesnykas, Tuulos, Uusitalo, & Jolkkonen, 2008).

Compared to healthy controls, patients with Alzheimer disease have been reported to show certain abnormal retinal signs such as narrow retinal veins (Berisha, Feke, Trempe, McMeel, & Schepens, 2007), optic nerve fibre loss (Danesh-Meyer, Birch, Ku, Carroll, & Gamble, 2006), thinning of retinal nerve fibre layers (RNFLs) (Parisi et al., 2001), as well as decreased retinal blood flow and extensive retinal ganglion cell loss (Berisha et al., 2007; Blanks, Torigoe, Hinton, & Blanks, 1996). Similarly, substantial retinal involvements have been observed in patients with Parkinson's disorder (Guo, Normando, Shah, De Groef, & Cordeiro, 2018; Inzelberg, Ramirez, Nisipeanu, & Ophir, 2004; Moschos et al., 2011), Lymphoma (Buggage, Chan, & Nussenblatt, 2001), and Multiple sclerosis (Jennifer B. Fisher et al., 2006).

Importantly, many changes in the eye have been identified and described through ophthalmological evaluation in patients with CNS diseases (London et al., 2013). Even though some ocular manifestations are not specific to one particular disease, their presence, however, emphasizes the strong link the eye shares with the brain. Ocular manifestations of major CNS disorders are illustrated in Figure 2.

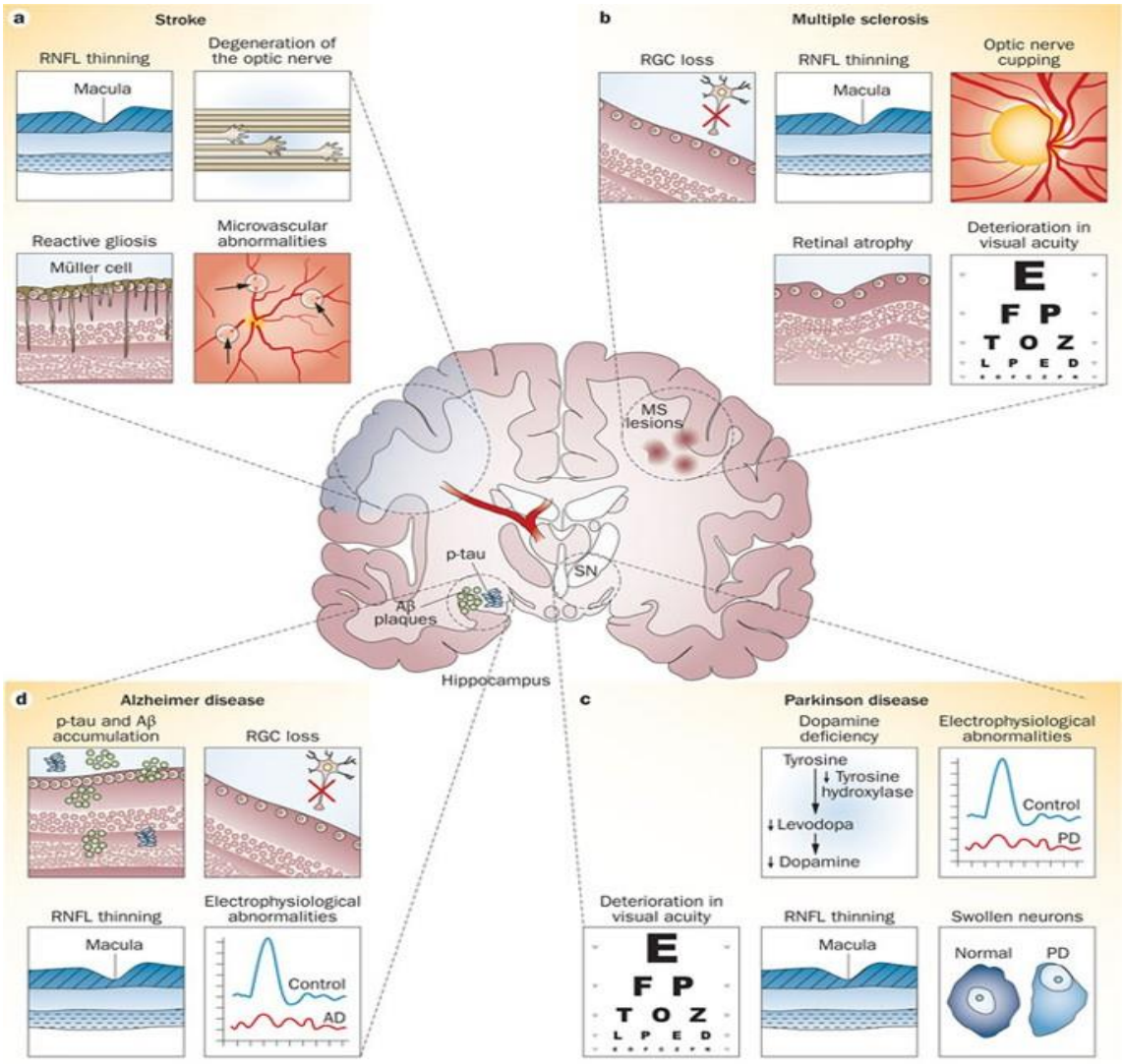


Figure 2: Ocular manifestations of CNS disorders.

a) *Retinal Nerve Fiber Layer thinning, optic nerve degeneration, reactive cell gliosis and abnormal retinal microvasculature are some of the ocular changes seen in stroke conditions;*

b) *Cupping of the optic nerve, RGC loss, retinal atrophy and decreased visual acuity are commonly observed in the eye of patients with Multiple Sclerosis;*

c) *Ocular changes seen in Parkinson's Disorder include retinal dopamine deficiency, swollen neurons, RNFL thinning, electrophysiological abnormalities as well as deterioration of visual acuity;*

d) *In patients with Alzheimer Disease, retinal changes manifest as RCG loss, RNFL thinning, and abnormal accumulation of Aβ and p-tau, reminiscent of the disease phenotype in the brain.*

Note: *Adapted from (London, Benhar, & Schwartz, 2013).*

Abbreviations*: Aβ, amyloid-β; AD, Alzheimer disease; MS, multiple sclerosis; PD, Parkinson disease; p-tau, phosphorylated tau; RGC, retinal ganglion cell; RNFL, retinal nerve fibre layer; SN, substantia nigra.*

1.1.3 Retinal Nerve Fiber Layer and the process of Myelination

The retinal nerve fibre layer (RNFL) is one of the ten layers of the human retina. It is situated anterior to the ganglion cell layer and posterior to the inner limiting membrane, and shelters the muller cells that cover the retinal vascular plexuses and nerve fibres. The RNFL comprises of the ganglion cell axons, neuroglia cells, and astrocytes. The ganglion cell axons run parallel to the surface of the retina, collect in a bundle at the optic disc and emerge from the back of the eye to form the optic nerve. The Neuroglia cells form myelin and provide support and protection for neurons. A dense collection of astrocytes at the lamina cribosa provides a barrier preventing oligodendrocytes from migrating to the retina. Within the retina, the nerve fibres are typically not myelinated and exit the eye through the lamina cribosa as the optic nerve. Upon leaving the eye, the axons of the ganglion cells become myelinated (Forrester, Dick, McMenamin, Roberts, & Pearlman, 2015). In a healthy human eye, the thickness of RNFL ranges from about 10 μm (around the fovea) to about 400 μm (margin of the optic disc) (Varma, Skaf, & Barron, 1996). However, the thickness of RNFL has been reported to decrease with age (Varma et al., 1996), and in conditions such as glaucoma (Sugita et al., 2015), Retinitis pigmentosa (Walia, Fishman, Edward, & Lindeman, 2007), Alzheimer disease (Parisi et al., 2001), and multiple sclerosis (Jennifer B Fisher et al., 2006).

Myelination is a process in which axons become ensheathed by highly specialized myelin. It is the final stage involved in the development of the brain. Through this process, myelinated axons are capacitated to transmit electrical impulses at a faster speed than unmyelinated ones, thereby facilitating neuronal activity and communication. Different regions of the brain show different timings of myelination (Tierney & Nelson III, 2009, p. 9). For instance, myelination begins at the sensory (e.g. visual system) and motor regions during the third trimester and continues until about the preschool age. By contrast, myelination is not complete until adolescence or early adulthood in areas involved in higher cognitive functions. As a developmental anomaly, myelination occasionally spreads to the anterior part of the lamina cribosa along the nerve fibres of the optic disc and the retina. The myelination of the retinal may be continuous with or completely detached from the optic disc (Straatsma, Foos, Heckenlively, & Taylor, 1981).

1.1.4 Myelinated Retinal Nerve Fibers

Myelinated (or medullated) retinal nerve fibres (MRNFs) are non-inflammatory retinal lesions that occur when there is abnormal myelination of the nerve fibres of the retina. In the normal eye, the retinal nerve

fibre layer is transparent and unmyelinated, thus, allowing retinal blood vessels to be easily visualized. But in MRNF, the underlying retinal blood vessels and disc are obscured. MRNF lesions are diagnosable clinically, and are recognized on fundus examination as grey or white well-separated patches with feathery borders along the retinal nerve fibre layer (Osaguona & Uhumwangho, 2014). They have been reported to occasionally occur in other parts of the retina as well as in the fovea (Osaguona & Uhumwangho, 2014). In the mid-eighteenth century, a German pathologist named Rudolf Virchow first described the pathologic features of MRNF as follows:

"*Retina was white, very thick and wrinkled. Macula was normal and near the optic disc, though more deeply situated, were thick, opaque, chalk-white spots, which spread around the disc in the shape of a star, so that when I wanted to draw the line between the disc and macula on each side of the two had the same divergence. In the other eye, I found, without much surprise, in the same place, the ring around the disc with a width of 2–2, 5 mm, regressing towards the outside"* (Grzybowski & Winiarczyk, 2015).

Figure 3 shows a left eye with extensive myelination of the retinal nerve fibre layer.

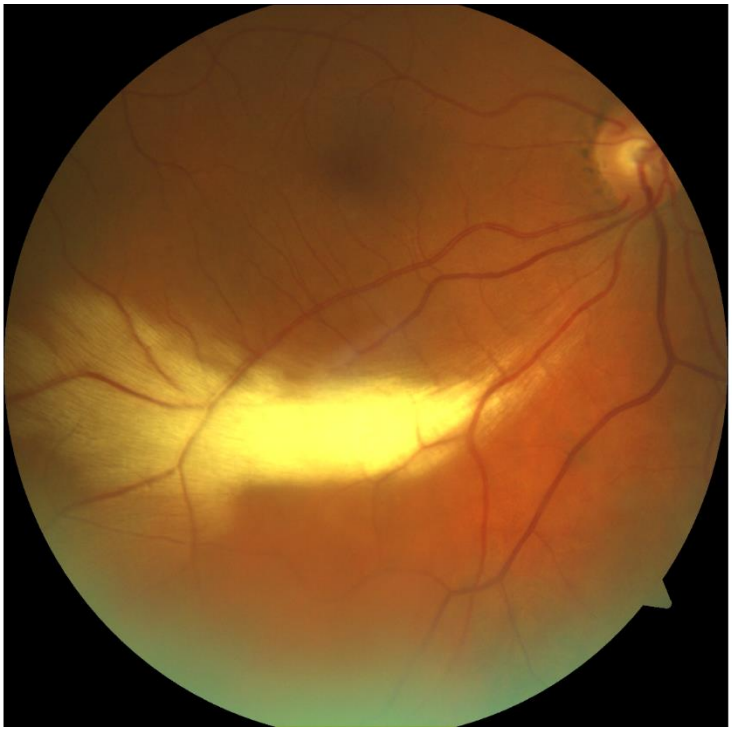


Figure 3: Fundus photograph showing a left eye with extensive myelinated retinal nerve fibre.

Clinically, most cases of MRNF represent benign clinical findings (Rao, Turkoglu, Say, & Shields, 2019). Some patients with MRNF may experience no troubling symptoms; however, visual functions have been reported to be significantly impacted especially in patients with axial myopia, amblyopia, and strabismus in the affected eye (Tarabishy, Alexandrou, & Traboulsi, 2007). MRNFs are mostly unilateral, although bilateral cases of the lesions have also been observed in some of the affected patients (Straatsma, Heckenlively, Foos, & Shahinian, 1979).

1.1.5 Epidemiology and Pathogenesis of MRNF

Previous studies have reported varying results on the prevalence of MRNF. In a study of 3,968 autopsy cases, Straatsma and colleagues found MRNF to be present in 0.98% of the subjects and in 0.54% of eyes examined, with bilateral involvement in about 8% of the study sample (Straatsma et al., 1979). A population-based study in India found MRNF to be present in approximately 1% of the Indian adults examined (Nangia et al., 2014). The Beijing Eye Study reported a 0.7% prevalence of MRNF among elderly Chinese in Northern China (You, Xu, & Jonas, 2007). The lesions have also been reported in 0.57% of 5789 Japanese (Kodama, Hayasaka, & Setogawa, 1990), as well as in 0.4% of 12,960 Caucasian cohorts in Western

Germany (Elbaz et al., 2016). While some studies have reported male predominance (Tarabishy et al., 2007); Straatsma and colleagues, however, reported no statistically significant gender-difference in MRNF prevalence (Straatsma et al., 1979). Although familiar cases of MRNF rarely occur, a few have been reported to occur both in isolation and in combination with ocular and systemic abnormalities (Ramkumar, Verma, Ferreyra, & Robbins, 2018).

The pathogenesis of MRNF has not been sufficiently described, however, Oligodendrocytes have been implicated (Elbaz et al., 2016). Oligodendrocytes are the major supporting cells of the CNS responsible for medullating axons in order to accelerate the conduction of electric signals along the nerves (Elbaz et al., 2016). Before birth, the process of optic nerve myelination starts at the lateral geniculate body, and continues anteriorly towards the eye to terminate at the lamina cribosa – widely regarded as a major impediment against myelination (M. S. Lee & Gonzalez, 1998; Tarabishy et al., 2007). Thus, under a normal circumstance, the process of myelination of the optic nerve stops at the lamina cribosa and the retinal nerve fibres remain unmyelinated.

Nevertheless, in MRNF, the retinal nerve fibres are also ensheathed with myelin. In a histological study of MRNF samples, Straatsma and colleagues found oligodendrocyte-like cells in the retina, while at the same time reporting an intact-looking lamina cribosa (Straatsma et al., 1981). There could be two possible explanations for this: it is either the progenitors of oligodendrocytes moved to the retina before the barrier function of lamina cribosa was fully developed, or they moved at a time when the functional integrity of lamina cribosa was temporarily compromised (Tarabishy et al., 2007). The myelination of the retinal nerve fibre has also been speculated to result from medullated glial cells of the immune system that were activated to the retina in the uterus (Tarabishy et al., 2007). Some studies have suggested that the development of the acquired form of MRNF could be as a result of possible optic nerve injury caused by optic nerve head drusen or increased intracranial pressure (Prakalapakorn & Buckley, 2012). Besides, there is a reported case of MRNF resulting from an optic pathway glioma, which could have led to the disruption of the lamina cribosa (Eneh, Schweitzer, & Sharma, 2010).

MRNFs are usually regarded as congenital and stable lesions, but a few cases of acquired and progressive lesions in both childhood and adulthood or after surgical procedures like optic nerve decompression have been reported (Prakalapakorn & Buckley, 2012; Rosen, Barry, & Constable, 1999; Shelton, Digre,

Gilman, Warner, & Katz, 2013). The lessions have also been reported to partially or totally regress in conditions such as multiple sclerosis (Sharpe & Sanders, 1975), glaucoma (Sowka & Nadeau, 2013), and post intraocular surgery (Williams & Fekrat, 2006). Furthermore, MRNF has been reported to occur in association with oculo-visual parameters such as visual field defects, myopia, amblyopia, and strabismus (M. S. Lee & Gonzalez, 1998), as well as systemic conditions like the Gorlin syndrome (De Jong et al., 1985).

Although the associations between MRNF and systemic parameters have not been fully investigated, however, the Gutenberg Health Study reported a positive association between MRNF and history of stroke in a Caucasian cohort in Western Germany (Elbaz et al., 2016). To the best of our knowledge, there is currently no published study describing the prevalence and associations of MRNF in the Nordic population.

1.2 Statement of Problem

As previously stated, the eye and in particular the retina is considered a window into the brain, and several studies have shown associations between retinal markers and systemic diseases (London et al., 2013). Although one previous study has reported an association between MRNF and a history of stroke (Elbaz et al., 2016), the clinical significance of MRNF is still unclear. The prevalence of MRNF has been reported in some regions, but to our current knowledge, no data exist from the Nordic populations. It is therefore important to investigate the prevalence of MRNF and its associations with ocular and systemic parameters with special focus on cardiovascular diseases and stroke. This study also seeks to yield data to a sparse data base on MRNF.

1.2.1 Objectives of the Study

The objectives of the present study included:

- To determine the prevalence of MRNF in the Tromsø population.
- To explore the relationship between MRNF and Sex and Age.
- To identify ocular and visual parameters associated with MRNF.
- To investigate systemic parameters associated with MRNF with special focus on cardiovascular diseases and stroke.

1.2.2 Significance of the study

The human eye provides a non-invasive means of detecting many systemic conditions (London et al., 2013), therefore data from this present study would be utilized to clarify the clinical impact of random detection of MRNF in clinical routines. Generally, the awareness about MRNF and its potential associations would be provided by this study to the public.

2 Materials and Methods

2.1 The Tromsø Study

The Tromsø Study is a population-based prospective study in the municipality of Tromsø, Norway (Eggen, Mathiesen, Wilsgaard, Jacobsen, & Njølstad, 2013; Jacobsen, Eggen, Mathiesen, Wilsgaard, & Njølstad, 2012). The study design includes repeated population health surveys to which total birth cohorts and random samples are invited. The study started as a cardiovascular study of 6595 men in 1974 and was later expanded to include many other chronic diseases (Eggen et al., 2013). Women were included from the second survey in 1979/80, and new surveys subsequently took place in 1986/87, 1994/95, 2001, 2007/08 and 2015/16. At each screening survey, a standard set of data was collected for all participants. Information was covered by questionnaires, measurements of blood pressure, height, weight, and blood analyses, amongst them serum lipids. Since the inception of the study, a total of 45473 subjects have participated in at least one of the seven surveys, while 18510 have participated on three or more occasions. To be eligible for the Tromsø Study, participants must be residents in the municipality of Tromsø based on the official population registry. The large number of variables collected in these surveys are described in more details on http://tromsoundersokelsen.uit.no/tromso/. Blood samples from each survey and DNA samples from the third survey and thereafter are securely stored in a bio-bank.

2.1.1 Tromsø 6 & 7 Surveys

The sixth (2007–2008) and the seventh (2015-2016) surveys comprised of two separate visits each. All participants were invited to a first visit where they filled a questionnaire and underwent physical examinations that involved measurement of blood pressure, height, weight, and waist-to-hip ratio. Other tests conducted include blood sampling, bone mineral density and pain threshold tests. In each of the surveys, a large subgroup was invited to a second visit a few weeks after the first visit, where special investigations were performed. Eye examination was one of the special investigations conducted during the second visits of both Tromsø 6 and Tromsø 7 surveys and, thus, laid the foundation for the Tromsø Eye Studies 1 and 2, respectively. In addition to the eye examination, each of these second visits included a second questionnaire, blood sampling, cognitive tests, ultrasound of the carotid artery, 12-lead electrocardiogram, echocardiography, spirometry and bone mineral densitometry.

2.1.2 Study Sample

The sample for this present study is based on data from Tromsø Eye Study 2, which is a sub-study of the Tromsø 7 survey and a follow-up of the Tromsø Eye study 1 (TES 1). All inhabitants in the municipality of Tromsø aged 40 and older were invited to the first visit of Tromsø 7 survey (n=32591) and of those, 21083 (64.7%) participated. A predefined random selection of 20% aged 40-59 years and 50% aged 60-85 years (n=10150) supplemented with those who participated in the Tromsø Eye Study 1, but not included in the random selection (n=3154) were invited to the second visit of Tromsø 7, where Tromsø Eye Study 2 was conducted. Participation in the first visit of Tromsø 7 survey was a prerequisite to be invited to the second visit. In total, 13304 subjects aged 40 years and above were invited to participate in the Tromsø Eye Study 2. Of those invited, 8346 (63%) subjects attended. However, retinal photographs were not available for 950 participants due to lack of consent or technical and logistic reasons, leaving a total of 7396 participants with valid retinal images. Figure 4 describes the participation flowchart.

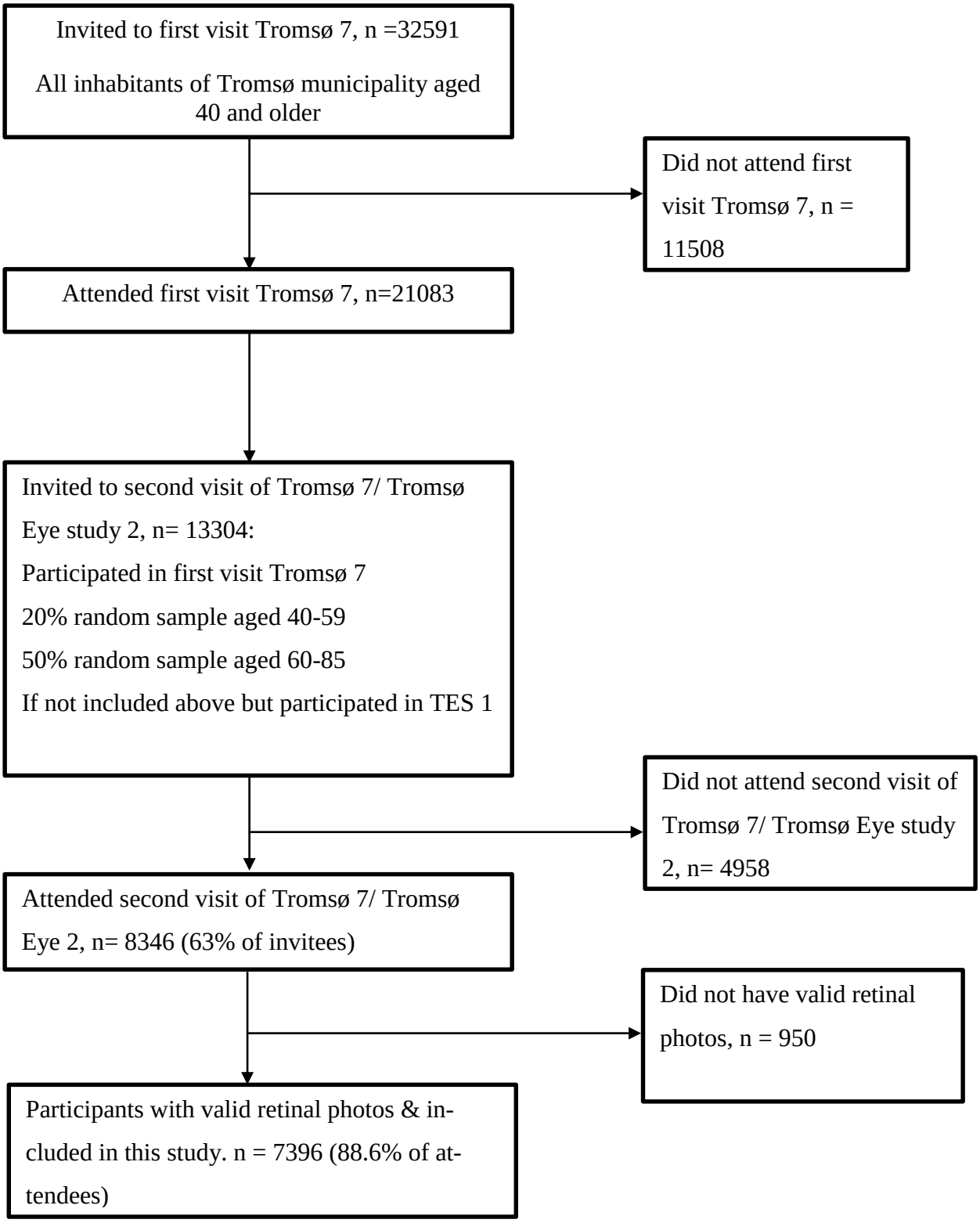


Figure 4: Flow diagram of the study sample. The Tromsø Eye study, 2015-16.

2.1.3 Eye Examinations

In addition to the information gathered through questionnaires and a standardized medical examination, all the subjects included in this study underwent detailed eye examinations that included comprehensive ocular history. Auto-refraction and visual acuity (VA) assessments were performed with Nidek ARK 560a autorefractor (Nidek CO., LTD. Japan). Visual acuity was categorized into best eye and worst eye acuities. Visual impairment was classified based on the International Classification of Disease 11th edition (World Health Organization, 2018). Briefly, visual impairment was defined as no impairment (distant VA ≤ Log MAR 0.3), mild (worse than log MAR 0.3), moderate (worse than Log MAR 0.5), severe (worse than Log MAR 1.0) and blindness (worse than log MAR 1.3). The mean spherical equivalent was computed in diopters (D) as spherical power plus half the cylindrical power and recorded as the mean value of the left and right eye. Intraocular pressure was measured with I-care tonometer (model TA01i; Helsinki, Finland) on both eyes. Mydriasis was obtained by application of one drop of Tropicamide 0.5 % (Chauvin Pharmaceuticals Ltd. Kingston upon Thames, Surrey, England) on both eyes. Retinal photography was performed with a Visucam 500 (Carl Zeiss Meditec (CZM)) digital retinal camera 10-45 minutes after the application of Tropicamide. Five field's 45-degree colour retinal photographs with resolution 2196x1958 pixels were taken using the camera pre-set internal fixation. A sixth image, 30 degrees (resolution 1620x1444 pixels) was taken centred on the macula using the "small pupil" setting. Optical coherence tomography (OCT) of both eyes was performed using Cirrus HD-OCT model 4000 (CZM). The standard 512 x 128 macular cube and optic disc cube scan protocol were used and performed according to the instrument manual. Table 1 shows a summary of eye examinations performed.

Table 1: Ocular Examinations: The Tromsø Eye Study 2015-16.

Examination type/Method/Tool	**Manufacturer/Specifications**
Visual acuity and Auto-refraction	Nidek ARK 560a autorefractor [a]
Tonometry	I-care Tonometer [b]
Retinal photography	Visucam 500 [c] 45^{o} field fundus 30^{o} field optic and macular area
Mydriasis	One drop of tropicamide 0.5% [d]
Optical Coherence Tomography (OCT)	Cirrus HD-OCT Model 4000 [e]

[a] *Nidek 20 Co., LTD*
[b] *Model TA01i, Helsinki, Finland*
[c] *Carl Zeiss Meditec (CZM), Jena, Germany*
[d] *Chauvin Pharmaceutical LTD, Kingston upon Thames, Surrey, England*
[e] *Carl Zeiss Meditec (CZM), Jena, Germany*

Retinal photography and OCT scans were performed by trained technicians. Images and OCT-scans were stored using Forum software (CZM). Grading of retinal images was done using high quality 24" LCD monitors (Eizo ColorEdge CG241W/CG242W). All the valid retinal images were assessed for the presence of MRNF by a certified Ophthalmologist. The detection of the condition from the retinal photographs was based on its normal funduscopic appearance (see Figure 3). Eye conditions such as glaucoma and retinal detachment were self-reported. Branch retinal vein occlusion (BRVO), central retinal vein occlusion CRVO), and arterial occlusion were diagnosed based on fundus image grading. All fundus images were graded for retinopathy based on 'The International Clinical Diabetic Retinopathy and Diabetic Macular Oedema Disease Severity Scales' (Wilkinson et al., 2003).

2.1.4 Assessment and definitions of Systemic conditions and risk factors

Systemic diseases assessed include self-reported heart attack, angina pectoris, atrial fibrillation, cerebral stroke, and heart failure. Cardiovascular risk factors (CVRFs) assessed were defined as follows: Smoking was dichotomized into non-smokers (never smokers) and smokers (former smokers and current smokers). Hypertension was diagnosed if antihypertensive drugs were taken or mean systolic blood pressure of 140

mmHg or more in the second and third standardized measurement or mean diastolic blood pressure of 90 mmHg or more in the second and third standardized measurement. Diabetes diagnosis applied to individuals with self-reported diabetes, taking anti-diabetic medications or glycosylated haemoglobin (HbA1c) level of more than 48mmol/mol (6.5%). Obesity was defined as a body mass index (BMI) of 30 kg/m^2 or more. Dyslipidemia diagnosis applied to individuals taking lipid-lowering drugs or a low-density lipoprotein-to-high density lipoprotein ratio of 3.5 or more.

2.1.5 Ethics

The Tromsø Study and The Tromsø Eye Study were approved by the Regional Committee for Medical and Health Research Ethics and the Data Inspectorate. In line with the tenets of the Helsinki Declaration for research involving humans, all participants gave written informed consent. This present study was also approved by "Norwegian centre for research data" no 660960, and regional ethics committee no 68904.

2.1.6 Statistical Analysis

All data were checked for completeness and accuracy, and missing and duplicate values were addressed. This study utilized the Predictive Analytics Software Statistics version 26 (SPSS, Inc, Chicago, IL) and Stata (Version 16.0, Stata Corp., College Station, TX) for all statistical analyses. Independent t-test was used to compare means for classical ocular features and systemic parameters. The Pearson Chi-square test or Fisher's exact test was used for comparing proportions. P-value <0.05 was considered significant. The results of prevalence were presented as relative numbers in per cent and weighted to the local Tromsø population, Europe standard population, and the WHO standard population.

2.1.7 Logistic regression model building

Ocular and systemic associations with MRNF were analyzed using multivariable logistic regression model (Odd Ratio, Confidence interval, and P-values). Variables with $p<0.25$ in the univariable analyses combined with clinically relevant variables (stroke, age, and sex) were included in a multivariable logistic model. This was followed by several multivariable logistic regression models, eliminating the variable with the highest P-value per time. The log-likelihood ratio test was used to identify if the reduced model fits as well as or significantly better than the preceding model. In each succeeding model, the coefficients of the remaining predictors were compared to the coefficients in the preceding model. If the change in the

coefficients ($\Delta\beta$) was more than 20%, the excluded predictors were adjudged to have provided important adjustment of the effect of remaining variables. Such variables were therefore added back to the model. This process of elimination and addition of variables and model fitting and refitting continued until all variables excluded were both clinically and statistically insignificant. The final model included only variables that were either clinically relevant or statistically significant at less than 5%.

3 Results

Readable fundus photographs of at least one eye were available in 7396 (88.0%) of 8346 participants of the Tromsø eye study 2 (TES2). Table 2 shows characteristics of the study population stratified by sex. The study cohort included 3979 (55.8%) females and 3417 (46.2%) males. The mean age in the whole study cohort was 62.9 ± 10.4 years with 62.8 ± 10.4 and 63.1 ± 10.4 years in females and males respectively (Table 2). In total, MRNF lesions were present in 91 eyes of 81 (1.1%) subjects, out of which 33 (40.7%) were females and 48 (59.3%) were males. Unweighted prevalence of MRNF was 1.1%. The prevalence weighted to the Tromsø population, the European standard population and the WHO standard population was calculated as 1.08%, 1.05%, and 1.04% respectively.

Table 2: Characteristics of Study Participants. N =7396. Tromsø Eye Study 2015/2016.

Variable	All subjects	Males	Females	P-value
Sex	7396	3,417 (46.2)	3,979 (53.8)	
Age (years)	62.9 ± 10.4	63.1 ± 10.4	62.8 ± 10.4	0.185*
BMI (kg/m2)	27.3 ± 4.4	27.8 ± 3.9	26.9 ± 4.7	**<0.001****
Obesity n, (%)	1,753 (23.8)	842 (24.7)	911 (23.0)	0.091*
LDL (mmol/l)	3.6 ± 1.0	3.5 ± 1.0	3.7 ± 1.0	**<0.001****
HDL (mmol/l)	1.6 ± 0.5	1.4 ± 0.4	1.8 ± 0.5	**<0.001****
Triglyceride (mmol/l)	1.5 ± 0.9	1.6 ± 0.9	1.4 ± 0.7	**<0.001****
Total Chol (mmol/l)	5.5 ± 1.1	5.3 ± 1.1	5.7 ± 1.1	**<0.001****
Blood glucose (mmol/l)	5.5 ± 1.4	5.7 ± 1.5	5.4 ± 1.3	**<0.001****
HbA1c (%)	5.8 ± 0.6	5.8 ± 0.6	5.7 ± 0.5	**<0.001****
Mean SP (mmHg)	133.2 ± 20.1	134.6 ± 18.3	132.1 ± 21.5	**<0.001****
Mean DP(mmHg)	75.5 ± 9.9	77.9 ± 9.6	73.4 ± 9.6	**<0.001****
Oxygen saturation	97.8 ± 1.3	97. 6 ± 1.4	98.0 ± 1.3	**<0.001****
Serum CRP (mg/l)	2.2 ± 6.1	2.3 ± 6.8	2.1 ± 5.3	0.160**
Smoking n, (%)	4481 (61.2)	2120 (62.6)	2361 (60.0)	**0.024***
Hypertension n, (%)	3787 (51.7)	1873 (55.4)	1914 (48.6)	**<0.001***
Diabetes; n, (%)	524 (7.3)	281 (8.5)	243 (6.4)	**0.001***
Heart attack; n, (%)	358 (5.0)	263 (8.0)	94 (2.5)	**<0.001***
Heart failure; n, (%)	195 (2.7)	141 (4.3)	54 (1.4)	**<0.001***
Atrial Fibrillation; n, (%)	558 (7.9)	344 (10.4)	214 (5.7)	**<0.001***
Angina pectoris; n, (%)	229 (3.2)	143 (4.3)	86 (2.3)	**<0.001***
Stroke; n, (%)	242 (3.4)	147 (4.4)	94 (2.5)	**<0.001***
Migraine; n, (%)	1,024 (14.4)	246 (7.4)	778 (20.5)	**<0.001***
Dyslipidemia; n, (%)	1,161 (15.7)	746 (21.9)	415 (10.5)	**<0.001***
Ocular Parameters				
Mean IOP (mmHg)	13.9 ± 3.4	13.9 ± 3.5	13.8 ± 3.4	0.929**
Mean SE (diopters)	-0.007 ± 2.2	0.005 ± 2.1	-0.018 ± 2.3	0.658**
Glaucoma; n, (%)	201 (2.7)	83 (2.4)	118 (2.9)	0.157*
Retinopathy; n, (%)	924 (13.3)	461 (14.5)	463 (12.3)	**0.008***
BRVO; n, (%)	75 (1.0)	39 (1.1)	36 (1.0)	0.311*
CRVO; n, (%)	12 (0.2)	6 (0.2)	6 (0.2)	0.791*
Arterial occlusion; n, (%)	14 (0.2)	6 (0.2)	8 (0.2)	0.802*
Retinal detachment n, (%)	119 (1.6)	60 (1.8)	59 (1.5)	0.354*

**Chi-Square test, **T-test*

Values are mean ± SD for continuous variables and n (%) for proportions. SE= Spherical equivalent. IOP = Intraocular pressure. BRVO = Branch Retinal Vein Occlusion. CRVO = Central Retinal Vein Occlusion. SP = Systolic Pressure. DP = Diastolic Pressure. Boldface indicates significance at P<0.05

Table 3 below illustrates the classification of visual impairment, while Table 4 shows the age and gender-specific prevalence of MRNF in the study sample.

Table 3: Grouping of Visual Acuity based on the International Classification of Visual Impairment. N =7330

Categories	**Best eye VA**	**Worst eye VA**
Severe VI	6 (0.1)	75 (1.0)
Moderate VI	6 (0.1)	79 (1.1)
Mild VI	226 (3.1)	824 (11.2)
No VI	7090 (96.7)	6352 (86.7)

VI = Visual Impairment. VA = Visual Acuity. Values are n (%)

Table 4: Prevalence of MRNF by age and gender. The Tromsø Eye Study 2015/2016.

	Male		**Female**	
Decade of Age (years)	n	**MRNF**	n	**MRNF**
40-49	482	4 (0.8)	570	4 (0.7)
50-59	539	10 (1.9)	728	7 (1.0)
60-69	1427	19 (1.3)	1580	11 (0.7)
70-79	821	15 (1.8)	949	9 (0.9)
80+	148	0 (0.0)	152	2 (1.3)
Total	3417	48 (1.4)	3979	33 (0.8)

Values are n (%)

Among those manifesting MRNF, 10 subjects (12.3%) had bilateral lesions while 71 (87.7%) had unilateral lesions. Unilateral MRNF was present in 32 right eyes and 39 left eyes. Compared with the study participants without MRNF, subjects with MRNF did not vary significantly in age (63.4 ± 9.7 years versus 62.9 ± 10.4 years; P = 0.698). However, in the univariable logistic regression analysis, this study found a significant association between MRNF and sex (P = 0.019, Odds ratio (OR): 1.704; 95% CI: 1.091 – 2.660) (Table 5).

Table 5: Univariable Regression Analysis: Associations of Demographic, Anthropometric, Ocular, and Systemic Parameters with the Presence of MRNF. The Tromsø Eye Study 2015/2016.

Variables	Beta Coefficient	Odd Ratio	95% CI	P-Value
Age (years)	0.004	1.004	0.983 – 1.026	0.698
Sex, males	0.533	1.704	1.091 – 2.66	**0.019**
BMI (kg/m2)	- 0.024	0.976	0.927 – 1.028	0.360
Obesity (BMI>=30kg/m^2)	- 0.320	0.726	0.414 – 1.276	0.266
LDL (mmol/l)	-0.039	0.961	0.773 – 1.197	0.725
HDL (mmol/l)	- 0.310	0.733	0.458 – 1.173	**0.196**
Triglyceride (mmol/l)	0.027	1.027	0.801 – 1.316	0.834
Total Cholesterol (mmol/l)	-0.090	0.914	0.748 – 1.118	0.381
Blood glucose (mmol/l)	-0.111	0.895	0.725 – 1.104	0.298
HbA1c (%)	- 0.470	0.625	0.368 – 1.062	**0.082**
Mean Systolic pressure (mmHg)	0.004	1.004	0.993 – 1.014	0.519
Mean diastolic pressure (mmHg)	0.014	1.014	0.992 – 1.037	**0.205**
Oxygen saturation	0.037	1.038	0.878 – 1.227	0.661
Serum CRP (mg/l)	-0.032	0.969	0.889 – 1.055	0.463
Hypertension	0.235	1.265	0.810 – 1.975	0.301
Diabetes	-0.165	0.848	0.341 – 2.107	0.722
Smoking	0.002	1.002	0.637 – 1.574	0.995
Heart attack	- 0.149	0.862	0.483 – 1.538	0.615
Heart failure	- 0.085	0.918	0.224 – 3.765	0.906
Atrial Fibrillation	- 0.769	0.463	0.146 – 1.474	**0.193**
Angina pectoris	- 0.264	0.768	0.188 – 3.145	0.714
Stroke	0.205	1.228	0.739 – 2.038	0.428
Migraine	- 0.057	0.945	0.498 – 1.792	0.863
Dyslipidemia	0.277	1.319	0.761 – 2.228	0.324
Ocular Parameters				
Mean IOP (mmHg)	0.000	1.00	0.926 – 1.082	0.998
Best eye visual acuity	0.312	1.367	0.363 – 5.147	0.644
Worst eye visual acuity	0.214	1.239	0.712 – 2.158	0.449
Mean Sph eqivalent (diopters)	- 0.064	0.938	0.855 –1.028	**0.172**
Glaucoma	0.626	1.870	0.678 – 5.161	**0.227**
Retinopathy	0.378	1.459	0.698 – 3.049	0.315
Retinal detachment	-0.278	0.757	0.104 – 5.486	0.783

Boldface indicates significance at P<0.25. CI = Confidence Interval. IOP =Intraocular Pressure

3.1 Ocular Associations

Compared with study participants without MRNF, subjects with MRNF did not vary significantly in mean spherical equivalent (P = 0.172, Odds ratio (OR): 0.938 ; 95% CI: 0.855 –1.028), mean intraocular pressure (P = 0.998, Odds ratio (OR): 1.00; 95% CI: 0.926 – 1.082), presence of glaucoma (P = 0.227, Odds ratio (OR): 1.870; 95% CI: 0.678 – 5.161), retinopathy (P = 0.315, Odds ratio (OR): 1.459; 95% CI: 0.698 – 3.049) and visual acuity (Table 5). There were no cases of MRNF in subjects with BRVO, CRVO and arterial occlusion, hence, they were excluded from the analysis (Table 5).

3.2 Cardiovascular Associations

The present study found no significant association between the presence of MRNF and diabetes (P = 0.722, Odds ratio (OR): 0.848; 95% CI: 0.341 – 2.107), high blood pressure (P = 0.301, Odds ratio (OR): 1.265; 95% CI: 0.810 – 1.975), obesity (P = 0.266, Odds ratio (OR): 0.726; 95% CI: 0.414 – 1.276) and smoking (P = 0.995, Odds ratio (OR): 1.002; 95% CI: 0.637 – 1.574). More so, our study did not report a significant association between the presence of MRNF and other systemic conditions such as atrial fibrillation, angina pectoris, heart attack, heart failure, dyslipidemia or stroke (Table 5). Similarly, lipid profile assessment (Total cholesterol, LDL, HDL and triglycerides), oxygen saturation, serum glucose, serum CRP, HbA1c, body mass index and migraine did not show a significant difference between subjects manifesting MRNF and the rest of the cohort (Tables 5).

3.3 Multivariable Analyses

Variables with P-value < 0.25 in the univariable analysis, together with history of stroke, age, and sex were included in a multivariable logistic regression model (Table 6). The inclusion of clinically relevant variables in our multivariable logistic regression models even when they were not statistically significant is a reflection of the “part experience and common sense” nature of model building strategy. In this model, only sex (male) and HbA1c were significantly associated with the presence of MRNF.

Table 6: Multivariable Logistic Regression Analysis (Dependent Variable: Presence of Myelinated Retinal Nerve Fibers, MRNF) including Age, Sex, stroke and the parameters that showed a P-value <0.25 in the Univariable Analysis. The Tromsø Eye Study 2015/2016.

Variables	Beta Coefficient	Odds Ratio	95% CI	P-Value
Age (years)	0.015	1.015	0.991 – 1.040	0.233
Sex (male)	0.520	1.682	1.006 – 2.281	**0.047**
HBA1C %	-0.700	0.496	0.268 – 0.920	**0.026**
HDL (mmol/l)	- 0.299	0.742	0.432 – 1.273	0.278
Diastolic BP (mmHg)	0.005	1.005	0.981 – 1.029	0.712
Stroke	0.137	1.146	0.635 – 2.069	0.650
Atrial Fibrillation	- 0.830	0.436	0.135 – 1.409	0.165
Mean SE (Diopters)	- 0.071	0.932	0.844 – 1. 028	0.158
Glaucoma	0.861	2.365	0.832 – 6.724	0.106

Boldface indicates significance at P<0.05, CI = Confidence Interval, BP = Blood Pressure, SE = Spherical equivalent.

A final logistic regression model was formed that included age and variables that were statistically significant at less than 5% (Table 7). In this model, male sex (P = 0.012, Odds ratio (OR): 1.785; 95% CI: 1.138 – 2.799) was associated with increased odds for MRNF, while glycosylated hemoglobin (P = 0.046, Odds ratio (OR): 0.559; 95% CI: 0.316 – 0.989) was associated with decreased odds for developing MRNF.

Table 7: *Multivariable Logistic Regression Analysis (Dependent Variable: Presence of Myelinated Retinal Nerve Fibers, MRNF) including age and variables significant at less than 5%. The Tromsø Eye Study 2015/2016.*

Variables	Beta Coefficient	Odds Ratio	95% CI	P- Value
Age (years)	0.012	1.012	0.990 – 1.035	0.286
Sex	0.579	1.785	1.138– 2.799	**0.012**
HBA1C %	- 0.581	0.559	0.316 – 0.989	**0.046**

Boldface indicates significance at P<0.05, CI = Confidence Interval

4 Discussion

To our knowledge, there are few previous reports on the population-based prevalence and associations of MRNF. The present study provides this data for a population sample of subjects who were between the ages of 40 and 84 years. It is the first population-based study in the Nordic region and the second in Europe after the Gutenberg Health Study that provides data on the prevalence, as well as ocular and systemic associations of MRNF in Caucasians.

The results of this study show a higher prevalence of MRNF in individuals with European background than previously reported in the Gutenberg Health Study (Elbaz et al., 2016). More so, the present study found a higher prevalence of MRNF than previously reported in other parts of the world. The Beijing Eye Study found a prevalence of 0.7% in 4,378 participants in China (You et al., 2007). Kodama and colleagues reported a prevalence of 0.6% among Japanese subjects (Kodama et al., 1990). Straatsma documented a prevalence of 0.98% in 3,968 subjects in the United States (Straatsma et al., 1981). The Central India Eye and Medical Study reported a prevalence of approximately 1.0% in 4,485 adult Indians (Nangia et al., 2014), and Cinar and colleagues documented a prevalence of 0.34% in 6,250 subjects in Turkey (Cinar, Zengin, & Kucukerdonmez, 2015). The prevalence of MRNF within the Tromsø Eye Study was 1.1%. The prevalence weighted to the Tromsø population, the European standard population and the WHO standard population was calculated as 1.08%, 1.05%, and 1.04% respectively. While we could not establish a plausible explanation for our study's higher MRNF prevalence, however, we speculate that it could be due to the number of images taken during fundus photography. Our study utilized five different images per eye for the assessment of the presence of MRNF, although it is not clear the number of images the above studies utilized in their assessment.

In agreement with the Gutenberg Health Study (Elbaz et al., 2016), the Central India Eye and Medical Study (Nangia et al., 2014), and the Beijing Eye Study (You et al., 2007), the present study did not report a significant association between MRNF and age. However, myelination of retinal nerve fibre was least common in the fourth and eighth decades of life, followed by the sixth decade, and distributed evenly throughout the other decades (Figure 4). As in the Gutenberg Health Study, our study found unilateral MRNF to occur more in the left than in the right eyes. However,

this eye-difference was not statistically significant. There is conflicting evidence in the literature regarding the association between sex and MRNF. The Gutenberg Health Study (Elbaz et al., 2016) and Straatsma (Straatsma et al., 1981) reported no statistically significant gender difference in MRNF prevalence, while Kodama and associates reported female predominance (Kodama et al., 1990). On the contrary, the present study, as well as the Central India Eye and Medical Study (Nangia et al., 2014), reported a higher prevalence of MRNF lesions in males. We found MRNF in 1.4% of males and 0.8% of females in our study sample. This gender difference was statistically significant, although the reason for this finding is unclear.

In the subjects within the Tromsø Eye study, the prevalence of MRNF was not significantly associated with ocular parameters such as refractive error, best-corrected visual acuity, and intraocular pressure. In addition, our study did not report a significant association between MRNF and other ocular conditions such as retinopathy, retinal detachment, and glaucoma. The finding of this study is in agreement with the results of previous population-based studies (Cinar et al., 2015; Elbaz et al., 2016; Nangia et al., 2014; You et al., 2007).

Several oculo-visual parameters have been reported in conjunction with MRNF mostly by case reports and series. Extensive unilateral as well as bilateral (rarely) cases of MRNF has been associated with mild hyperopia, emmetropia or myopia in the affected eye (Straatsma et al., 1979). Lee and associate reported an association between the presence of MRNF and anisometropic amblyopia (J. C. Lee & Salchow, 2008). However, they also noted that the association was more of a coincidence than causally related because, their patient followed a course of treatment peculiar to anisometropic amblyopia rather than amblyopia associated with MRNF. More so, Osauguona and associate (Osaguona & Uhumwangho, 2014), Straatsma, (Straatsma et al., 1979) and Tarabishy (Tarabishy et al., 2007) have reported on a syndrome of anisometropic myopia, amblyopia, strabismus and homolateral extensive MRNF. In addition, ocular abnormalities such as coloboma, keratoconus, and polycoria have been reported concurrently with MRNF (Straatsma et al., 1979). Nonetheless, these findings are neither shared by the present study nor by other previous population-based studies. There is a reasonable chance that the associations reported in the literature

above may have been potentially impacted by coexisting organic or structural pathology. For instance, Tarabishy and associates (Tarabishy et al., 2007) also reported the presence of optic nerve dysplasia in some of the patients examined.

The effect of MRNF on visual function is highly contestable, and, it is influenced by the location, area of the lesion, as well as by coexisting visual pathology. In some cases, visual function may be affected if the MRNF lesions are widespread or if there is a macula involvement. Interestingly, several cases of retinal nerve fibre myelination neither show visual deficits nor significant refractive anomalies. In MRNF, visual function and prognosis rely on the severity of ocular complications or associated conditions. The query as to whether retinal myelination causes visual deficits or vice versa remains debatable. Ruttum and poll, in their study, suggested that anisometropia could be a more powerful influence on the relative visual acuity of each of the eyes than the presence of MRNF (Ruttum & Poll, 2006).

4.1 Systemic parameters and MRNF

The present study is the first to report a significant association between MRNF and glycosylated haemoglobin (HbA1c). This unexpected finding was observed in the multivariable logistic models. Although previous studies have reported associations of HbA1c with ocular signs such as macular oedema (Chou, Wu, Kuo, Lai, & Kuo, 2009) and average thickness of retinal nerve fibre layer (Shi et al., 2018), however, there is no known pathophysiological connection between the absence of MRNF and HbA1c. Thus, the significant association between MRNF and HbA1c reported in our study could be a chance finding as we could not establish a plausible explanation for it.

Conversely, our study did not find a significant association between MRNF and cardiovascular risk factors such as systolic BP, diastolic BP, blood glucose level, serum lipids, diabetes, smoking, and hypertension. Similarly, we did not establish a significant relationship between MRNF and other systemic conditions such as atrial fibrillation, dyslipidemia, heart attack, and heart failure. The result of this study is consistent with the outcome of previous evidence (Elbaz et al., 2016).

4.2 Myelinated Retinal Nerve Fibre and Stroke

The Gutenberg Health Study reported a positive association between MRNF and a history of stroke (Elbaz et al., 2016). This finding is neither shared by our study nor by other previous studies. The reason for the disparity in findings is not entirely clear. However, there are two things to deduce from the report of the Gutenberg Health Study. First, the statistical power of the result was low due to the low prevalence of MRNF reported in the study. Second, the confidence interval around the effect estimate was wide (2.86, 16.09) which might have impacted the precision of the result thus, reducing the confidence on the reliability of the finding.

Indeed, stroke patients demonstrate microvascular changes in the retina. For example, previous studies have reported on the stroke-induced retinal damage and microvascular changes such as increased retinal vascular tortuosity, reduced dimension of retinal fraction, retinal haemorrhage, arteriolar narrowing and increased venular diameter (Kumar, 2017). Several studies have also reported associations between stroke and certain retinal signs such as retinal vein occlusion, age-related macular degeneration, diabetic and hypertensive ocular signs (Baker, Hand, Wang, & Wong, 2008). Besides, a study conducted with animal model showed that cerebral ischemia resulted in retinal and optic nerve degeneration in rats (Stevens, Fortin, & Pappas, 2002). However, as this present study did not report a statistically significant association between MRNF and history of cerebral stroke, we, therefore, could not comment on any potential clinical relevance of MRNF in patients presenting with stroke.

Retinal imaging has been widely considered to be a very powerful tool in understanding and predicting stroke (Kumar, 2017). And to understand the impact of systemic parameters on vision, it is important to recall that the retinal and cerebral small vessels share similar embryological origins, anatomical features and pathophysiological properties (London et al., 2013). Hence, it would be logical to observe a more in-depth study of the actions of the myelin-producing cells as regards systemic diseases in general, as well as cardiovascular and cerebrovascular conditions in particular. This may be useful in order to fully comprehend the clinical implications of random detection of MRNF during normal clinical routines.

Associations between acute retinal events and acute systemic parameters have both clinical and epidemiological implications with regards to optimizing clinical care to reduce the ample morbidity and mortality of stroke. Vision loss precipitated by cerebral infarction often results in reduced quality of life and can lead to loss of independence and depression (Hepworth & Rowe, 2016). Due to the disparity between our study and the report of the Gutenberg Health Study regarding the association of MRNF with history of stroke, there is need for further studies to confirm the role of MRNF in stroke patients. These further studies are recommended in diverse populations and with bigger sample size. This is crucial before considering whether or not to include MRNF in prediction models containing stroke risk factors.

4.3 Strengths and limitations of the study

The strengths of this present study include the population-based design and sizeable sample size, relatively high response rate and high percentage of gradable retinal images. Furthermore, a detailed ophthalmic examination was undertaken allowing adjustment for important covariates in the analyses. However, there are limitations to this study. First, as this was a cross-sectional study, we could not establish any cause-and-effect relationship. Second, there is a legitimate concern for selection bias as some of the study participants were selected through predetermination. Finally, as much as we are inclined to believe that our study includes a representative survey of the general Norwegian population, however, the generalizability of our data beyond the Tromsø municipality and the examined age group is not certain.

5 Conclusion

In conclusion, the present study provides prevalence estimates of MRNF that are different from previous epidemiological studies in Caucasian and other populations. To the best of our knowledge, it is the first study to provide prevalence estimates of MRNF and its associations in the Nordic population. Importantly, this is also the first population-based study to report an association between MRNF and HbA1c, although this may be a chance finding and must be explored further. The finding of this study is largely consistent with previous studies. Despite many reports

on retinal abnormalities associated with systemic diseases, and some on MRNF, the general perception that MRNF is without serious clinical implication is probably true, although this must be investigated further in future studies.

References

Allegri, P., Rissotto, R., Herbort, C. P., & Murialdo, U. (2011). CNS diseases and uveitis. *Journal of ophthalmic & vision research, 6*(4), 284-308.

Baker, M. L., Hand, P. J., Wang, J. J., & Wong, T. Y. (2008). Retinal Signs and Stroke. *Stroke, 39*(4), 1371-1379.

Berisha, F., Feke, G. T., Trempe, C. L., McMeel, J. W., & Schepens, C. L. (2007). Retinal Abnormalities in Early Alzheimer's Disease. *Investigative Ophthalmology & Visual Science, 48*(5), 2285-2289.

Blanks, J. C., Torigoe, Y., Hinton, D. R., & Blanks, R. H. I. (1996). Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. *Neurobiology of Aging, 17*(3), 377-384.

Buggage, R. R., Chan, C.-C., & Nussenblatt, R. B. (2001). Ocular manifestations of central nervous system lymphoma. *Current Opinion in Oncology, 13*(3), 137-142.

Cheung, N., Mosley, T., Islam, A., Kawasaki, R., Sharrett, A. R., Klein, R., . . . Wong, T. Y. (2010). Retinal microvascular abnormalities and subclinical magnetic resonance imaging brain infarct: a prospective study. *Brain, 133*(Pt 7), 1987-1993.

Chou, T. H., Wu, P. C., Kuo, J. Z. C., Lai, C. H., & Kuo, C. N. (2009). Relationship of diabetic macular oedema with glycosylated haemoglobin. *Eye, 23*(6), 1360-1363.

Cinar, E., Zengin, M. O., & Kucukerdonmez, C. (2015). Prevalence and clinical characteristics of myelinated retinal nerve fibres: a cross-sectional study of Turkish individuals between 8 and 75 years. *Acta Ophthalmol, 93*(7), e599-600.

Danesh-Meyer, H. V., Birch, H., Ku, J. Y., Carroll, S., & Gamble, G. (2006). Reduction of optic nerve fibers in patients with Alzheimer disease identified by laser imaging. *Neurology, 67*(10), 1852-1854.

De Jong, P. T., Bistervels, B., Cosgrove, J., de Grip, G., Leys, A., & Goffin, M. (1985). Medullated nerve fibers: a sign of multiple basal cell nevi (Gorlin's) syndrome. *Archives of ophthalmology, 103*(12), 1833-1836.

Dhillon, B., & Dhillon, N. (2008). The retina as a window to the brain. *Arch Neurol, 65*(11), 1547-1548; author reply 1548.

Eggen, A. E., Mathiesen, E. B., Wilsgaard, T., Jacobsen, B. K., & Njølstad, I. (2013). The sixth survey of the Tromsø study (Tromsø 6) in 2007–08: collaborative research in the interface between clinical medicine and epidemiology: study objectives, design, data collection procedures, and attendance in a multipurpose population-based health survey. *Scandinavian journal of public health, 41*(1), 65-80.

Elbaz, H., Peto, T., Butsch, C., Orouji, E., Laubert-Reh, D., Ponto, K. A., . . . Mirshahi, A. (2016). Prevalence and associations of myelinated retinal nerve fibers: results from the population-based Gutenberg Health study. *Retina, 36*(12), 2364-2370.

Eneh, A. A., Schweitzer, K. D., & Sharma, S. (2010). Question: Can you identify this condition? Myelinated retinal nerve fibre layer. *Canadian family physician Medecin de famille canadien, 56*(4), 357.

Fisher, J. B., Jacobs, D. A., Markowitz, C. E., Galetta, S. L., Volpe, N. J., Nano-Schiavi, M. L., . . . Frohman, T. C. (2006). Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. *Ophthalmology, 113*(2), 324-332.

Fisher, J. B., Jacobs, D. A., Markowitz, C. E., Galetta, S. L., Volpe, N. J., Nano-Schiavi, M. L., . . . Balcer, L. J. (2006). Relation of Visual Function to Retinal Nerve Fiber Layer Thickness in Multiple Sclerosis. *Ophthalmology, 113*(2), 324-332.

Forrester, J. V., Dick, A. D., McMenamin, P. G., Roberts, F., & Pearlman, E. (2015). *The eye e-book: basic sciences in practice*: Elsevier Health Sciences.

Grzybowski, A., & Winiarczyk, I. (2015). Myelinated retinal nerve fibers (MRNF)–Dilemmas related to their influence on visual function. *Saudi Journal of Ophthalmology, 29*(1), 85-88.

Guo, L., Normando, E. M., Shah, P. A., De Groef, L., & Cordeiro, M. F. (2018). Oculo-visual abnormalities in Parkinson's disease: Possible value as biomarkers. *Movement Disorders, 33*(9), 1390-1406.

Hepworth, L., & Rowe, F. (2016). Visual Impairment Following Stroke - The Impact on Quality of Life: A Systematic Review. *Ophthalmology Research: An International Journal, 5*, 1-15.

Inzelberg, R., Ramirez, J. A., Nisipeanu, P., & Ophir, A. (2004). Retinal nerve fiber layer thinning in Parkinson disease. *Vision Research, 44*(24), 2793-2797.

Jacobsen, B. K., Eggen, A. E., Mathiesen, E. B., Wilsgaard, T., & Njølstad, I. (2012). Cohort profile: the Tromsø study. *International journal of epidemiology, 41*(4), 961-967.

Kalesnykas, G., Tuulos, T., Uusitalo, H., & Jolkkonen, J. (2008). Neurodegeneration and cellular stress in the retina and optic nerve in rat cerebral ischemia and hypoperfusion models. *Neuroscience, 155*(3), 937-947.

Kodama, T., Hayasaka, S., & Setogawa, T. (1990). Myelinated retinal nerve fibers: prevalence, location and effect on visual acuity. *Ophthalmologica, 200*(2), 77-83.

Kumar, V. (2017). Understanding Retinal Changes after Stroke. *Open J Ophthalmol, 7*(4), 281-292.

Lee, J. C., & Salchow, D. J. (2008). Myelinated retinal nerve fibers associated with hyperopia and amblyopia. *Journal of American Association for Pediatric Ophthalmology and Strabismus, 12*(4), 418-419.

Lee, M. S., & Gonzalez, C. (1998). Unilateral peripapillary myelinated retinal nerve fibers associated with strabismus, amblyopia, and myopia. *American journal of ophthalmology, 125*(4), 554-556.

London, A., Benhar, I., & Schwartz, M. (2013). The retina as a window to the brain-from eye research to CNS disorders. *Nat Rev Neurol, 9*(1), 44-53.

Moschos, M. M., Tagaris, G., Markopoulos, L., Margetis, L., Tsapakis, S., Kanakis, M., & Koutsandrea, C. (2011). Morphologic Changes and Functional Retinal Impairment in Patients with Parkinson Disease without Visual Loss. *European Journal of Ophthalmology, 21*(1), 24-29.

Nangia, V., Jonas, J. B., Khare, A., Bhate, K., Agarwal, S., & Panda-Jonas, S. (2014). Prevalence of myelinated retinal nerve fibres in adult Indians: the Central India Eye and Medical Study. *Acta ophthalmologica, 92*(3), e235-e236.

Osaguona, V. B., & Uhumwangho, O. M. (2014). Syndrome of myelinated retinal nerve fibres, myopia, amblyopia and strabismus in a Nigerian. *Niger Med J, 55*(6), 517-518.

Parisi, V., Restuccia, R., Fattapposta, F., Mina, C., Bucci, M. G., & Pierelli, F. (2001). Morphological and functional retinal impairment in Alzheimer's disease patients. *Clinical Neurophysiology, 112*(10), 1860-1867.

Patton, N., Aslam, T., Macgillivray, T., Pattie, A., Deary, I. J., & Dhillon, B. (2005). Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures. *J Anat, 206*(4), 319-348.

Prakalapakorn, S. G., & Buckley, E. G. (2012). Acquired bilateral myelinated retinal nerve fibers after unilateral optic nerve sheath fenestration in a child with idiopathic intracranial hypertension. *Journal of American Association for Pediatric Ophthalmology and Strabismus, 16*(6), 534-538.

Ramkumar, H. L., Verma, R., Ferreyra, H. A., & Robbins, S. L. (2018). Myelinated Retinal Nerve Fiber Layer (RNFL): A Comprehensive Review. *Int Ophthalmol Clin, 58*(4), 147-156.

Rao, R., Turkoglu, E. B., Say, E. A., & Shields, C. L. (2019). CLINICAL FEATURES, IMAGING, AND NATURAL HISTORY OF MYELINATED RETINAL NERVE FIBER LAYER. *Retina, 39*(6), 1125-1132.

Rosen, B., Barry, C., & Constable, I. J. (1999). Progression of myelinated retinal nerve fibers. *American journal of ophthalmology, 127*(4), 471-473.

Ruttum, M. S., & Poll, J. (2006). Unilateral Retinal Nerve Fiber Myelination With Contralateral Amblyopia. *Archives of ophthalmology, 124*(1), 128-130.

Sharpe, J. A., & Sanders, M. D. (1975). Atrophy of myelinated nerve fibres in the retina in optic neuritis. *British Journal of Ophthalmology, 59*(4), 229-232.

Shelton, J. B., Digre, K. B., Gilman, J., Warner, J. E., & Katz, B. J. (2013). Characteristics of myelinated retinal nerve fiber layer in ophthalmic imaging: findings on autofluorescence, fluorescein angiographic, infrared, optical coherence tomographic, and red-free images. *JAMA ophthalmology, 131*(1), 107-109.

Shi, R., Guo, Z., Wang, F., Li, R., Zhao, L., & Lin, R. (2018). Alterations in retinal nerve fiber layer thickness in early stages of diabetic retinopathy and potential risk factors. *Curr Eye Res, 43*(2), 244-253.

Sowka, J. W., & Nadeau, M. J. (2013). Regression of myelinated retinal nerve fibers in a glaucomatous eye. *Optometry and Vision Science, 90*(7), e218-e220.

Stevens, W. D., Fortin, T., & Pappas, B. A. (2002). Retinal and Optic Nerve Degeneration After Chronic Carotid Ligation. *Stroke, 33*(4), 1107-1112.

Straatsma, B. R., Foos, R. Y., Heckenlively, J. R., & Taylor, G. N. (1981). Myelinated retinal nerve fibers. *American journal of ophthalmology, 91*(1), 25-38.

Straatsma, B. R., Heckenlively, J. R., Foos, R. Y., & Shahinian, J. K. (1979). Myelinated retinal nerve fibers associated with ipsilateral myopia, amblyopia, and strabismus. *American journal of ophthalmology, 88*(3), 506-510.

Sugita, M., Pircher, M., Zotter, S., Baumann, B., Roberts, P., Makihira, T., . . . Hitzenberger, C. K. (2015). Retinal nerve fiber bundle tracing and analysis in human eye by polarization sensitive OCT. *Biomedical optics express, 6*(3), 1030-1054.

Tarabishy, A. B., Alexandrou, T. J., & Traboulsi, E. I. (2007). Syndrome of myelinated retinal nerve fibers, myopia, and amblyopia: a review. *Survey of ophthalmology, 52*(6), 588-596.

Tierney, A. L., & Nelson III, C. A. (2009). Brain development and the role of experience in the early years. *Zero to three, 30*(2), 9.

Varma, R., Skaf, M., & Barron, E. (1996). Retinal Nerve Fiber Layer Thickness in Normal Human Eyes. *Ophthalmology, 103*(12), 2114-2119.

Walia, S., Fishman, G. A., Edward, D. P., & Lindeman, M. (2007). Retinal Nerve Fiber Layer Defects in RP Patients. *Investigative Ophthalmology & Visual Science, 48*(10), 4748-4752.

Wilkinson, C. P., Ferris, F. L., Klein, R. E., Lee, P. P., Agardh, C. D., Davis, M., . . . Verdaguer, J. T. (2003). Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology, 110*(9), 1677-1682.

Williams, A. J., & Fekrat, S. (2006). Disappearance of myelinated retinal nerve fibers after pars plana vitrectomy. *American journal of ophthalmology, 142*(3), 521-523.

Wong, T. Y., Klein, R., Klein, B. E., Tielsch, J. M., Hubbard, L., & Nieto, F. J. (2001). Retinal microvascular abnormalities and their relationship with hypertension, cardiovascular disease, and mortality. *Survey of ophthalmology, 46*(1), 59-80.

Wong, T. Y., Klein, R., Sharrett, A. R., Couper, D. J., Klein, B. E., Liao, D. P., . . . Study, A. I. A. R. i. C. (2002). Cerebral white matter lesions, retinopathy, and incident clinical stroke. *JAMA, 288*(1), 67-74.

World Health Organization. (2018). International classification of diseases for mortality and morbidity statistics (11th Revision.

You, Q., Xu, L., & Jonas, J. B. (2007). Prevalence of myelinated retinal nerve fibres in urban and rural adult Chinese populations: the Beijing Eye Study. *Acta ophthalmologica Scandinavica, 85*(6), 631-632.