Risk Factors for Open Angle Glaucoma

A Clinical and Molecular Genetic Study

Eva Forsman
Qui amisit oculos, tamen vidit
Quae ad nos pervenerunt, ne sint, effici potest,
Ne fuerint, non potest.

For those who have lost their eyes, but once have seen
We can lose what once belonged to us,
Yet forever, once it was ours.

Seneca de Beneficii

To my family
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**Abbreviations**

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<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ACG</td>
<td>angle closure glaucoma</td>
</tr>
<tr>
<td>AGIS</td>
<td>Advanced Glaucoma Intervention Study</td>
</tr>
<tr>
<td>ALT</td>
<td>argon laser trabeculoplasty</td>
</tr>
<tr>
<td>AMD</td>
<td>age related macular degeneration</td>
</tr>
<tr>
<td>CCT</td>
<td>central corneal thickness</td>
</tr>
<tr>
<td>C/D</td>
<td>cup/disc ratio</td>
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<tr>
<td>CIGTS</td>
<td>Collaborative Initial Glaucoma Treatment Study</td>
</tr>
<tr>
<td>CNTGS</td>
<td>Collaborative Normal Tension Glaucoma Study</td>
</tr>
<tr>
<td>DZ</td>
<td>dizygotic</td>
</tr>
<tr>
<td>EGPS</td>
<td>European Glaucoma Prevention Study</td>
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<tr>
<td>EMGT</td>
<td>Early Manifest Glaucoma Trial</td>
</tr>
<tr>
<td>EG</td>
<td>exfoliation glaucoma</td>
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<tr>
<td>ES</td>
<td>exfoliation syndrome</td>
</tr>
<tr>
<td>GIST</td>
<td>Glaucoma Inheritance Study of Tasmania</td>
</tr>
<tr>
<td>HR</td>
<td>hazard ratio</td>
</tr>
<tr>
<td>IOP</td>
<td>intraocular pressure</td>
</tr>
<tr>
<td>J-POAG</td>
<td>juvenile primary open angle glaucoma</td>
</tr>
<tr>
<td>LOXL1</td>
<td>lysyl oxidase like protein 1</td>
</tr>
<tr>
<td>MZ</td>
<td>monozygotic</td>
</tr>
<tr>
<td>MYOC</td>
<td>myocilin</td>
</tr>
<tr>
<td>NTG</td>
<td>normal tension glaucoma</td>
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<tr>
<td>OAG</td>
<td>open angle glaucoma</td>
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<tr>
<td>OHT</td>
<td>ocular hypertension</td>
</tr>
<tr>
<td>OHTS</td>
<td>Ocular Hypertension Study</td>
</tr>
<tr>
<td>OPTN</td>
<td>optineurin</td>
</tr>
<tr>
<td>OR</td>
<td>odd ratio</td>
</tr>
<tr>
<td>POAG</td>
<td>primary open angle glaucoma</td>
</tr>
<tr>
<td>RCT</td>
<td>randomised clinical trial</td>
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<tr>
<td>RR</td>
<td>relative risk ratio</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>TIGR</td>
<td>trabecular meshwork inducible glucocorticoid response protein</td>
</tr>
<tr>
<td>VA</td>
<td>visual acuity</td>
</tr>
<tr>
<td>VF</td>
<td>visual field</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>WDR 36</td>
<td>WD repeat-40 36</td>
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Original publications

This thesis is based on the following original publications that are referred to in the text by their Roman numerals.


Abstract

Glaucoma, optic neuropathy with excavation in the optic nerve head and corresponding visual field defect, is one of the leading causes for blindness worldwide. However, visual disability can often be avoided or delayed if the disease is diagnosed at an early stage. Therefore, recognising the risk factors for development and progression of glaucoma may prevent further damage. The purpose of the present study was to evaluate factors associated with visual disability caused by glaucoma and the genetic features of two risk factors, exfoliation syndrome (ES) and a positive family history of glaucoma. The present study material consisted of three study groups 1) deceased glaucoma patients from the Ekenäs practice 2) glaucoma families from the Ekenäs region and 3) population based families with and without exfoliation syndrome from Kökar Island.

Study I

For the retrospective study we collected all glaucoma patients who had been treated in a private ophthalmic office in Ekenäs and who had died between 1991 and 2002. The purpose was to find out how many of them really had developed a visual disability during their lifetimes and which factors were associated with blindness caused by glaucoma. A total of 106 patients with open angle glaucoma (OAG) were identified, of these 39 patients had primary open angle glaucoma (POAG), 27 exfoliation glaucoma (EG), and 40 ocular hypertension (OHT) at diagnosis. At the last visit, 17 patients (16%, 95% CI 9-23) were visually impaired. Blindness induced by glaucoma was found in one or both eyes in 16 patients (15%, 95% CI 8-22) and in both eyes in six patients (6%, 95% CI 1-10). The cumulative incidence of glaucoma caused blindness for one eye was 6% (95% CI 2-11) at 5 years, 9% (95%, CI 4-15) at 10 years, and 15% (95% CI 9-23) at 15 years from initialising the treatment. The factors associated with blindness caused by glaucoma were an advanced stage of glaucoma at diagnosis, fluctuation in intraocular pressure during treatment, the presence of exfoliation syndrome, and poor patient compliance.

Studies II and III

The analysis of prevalence and inheritance of exfoliation syndrome was based on a cross-sectional population based study performed in 1960-1962.
on Kökar Island and on the follow-up study in which the same population was followed until 2002. The purpose was: to determine the prevalence of exfoliation syndrome, to analyse how it is inherited, and to localise or to identity a locus/loci or a gene/genes associated with it. A total of 595 subjects (85% of the population) participated in population based study in 1960-1962. The prevalence of ES was 18% among subjects older than 50 years. In total 965 subjects (530 over 50 years) have been examined at least once. The relative risk (RR) of glaucoma (ES vs. no ES) was higher for males 15 (95% CI 6-34) than for females 12 (95% CI 4-32). In families, where at least one of the parents had ES, 16% of the children (9% of males and 27% of females) developed ES. However, in families where both parents were unaffected, ES was found in 18% of the children (8% of males and 24% of females). From this data we concluded, that autosomal dominant inheritance with incomplete penetrance, more reduced in males, would best explain the segregation.

To find the gene or genes associated with exfoliation syndrome, a genome wide scan was performed for 64 members (28 ES affected and 36 controls) belonging to one large pedigree on Kökar Island. A promising result was found: the highest two-point LOD score of 3.45 (θ=0.04) and a multipoint LOD score of 4.2 were found in 18q12.1-21.33. Furthermore, five other chromosomal regions showed a possible linkage with LOD score values of > 1.5.

Study IV

The presence of mutations in glaucoma genes TIGR/MYOC (myocilin) and OPTN (optineurin) in the Finnish glaucoma population was analysed in eight families from the Ekenäs region. An inheritance pattern resembling autosomal dominant mode was detected in all these families. The phenotype was determined by examination or from an ophthalmologist in 102 of 136 family members. Of them 35% had either primary open angle glaucoma or exfoliation glaucoma and 28% were suspected to have glaucoma. In the 11 subjects sequenced for TIGR and OPTN genes no mutations were detected. However, when 4 patients from the extended family on Kökar were analysed, a sequence alteration M98K of OPTN gene, which was reported to be risk-associated factor, was detected in a patient with exfoliation glaucoma and in his three close relatives.
1. Background

Study I

The impulse to conduct the present study occurred when all charts of deceased glaucoma patients were on the author’s desk in order to destroy them. At that time I had worked for 25 years in the same private office in Ekenäs. The charts were my “own patients” whom I had followed and treated in the office from the first glaucoma suspicion to the last visit of their life. I started to wonder how many of them had developed visual disability during their lifetime and what were the factors associated with this. During the follow-up time, the definition of glaucoma had been changed from a disease of intraocular pressure to a disease of the optic nerve head and retinal ganglion cells. Today, open angle glaucoma (OAG) is defined as a slowly progressive atrophy of the optic nerve, characterised by loss of peripheral visual function and an excavated appearance of the optic disc independent of intraocular pressure (IOP). The reason for the large variation in glaucoma definitions is the fact that the aetiology and the mechanisms causing the disease are still unknown.

Though the awareness of glaucoma is relatively low, patients are aware of the fact that glaucoma may lead to blindness and they are afraid of the diagnosis. Their quality of life is affected by this fear especially at the beginning. The lack of symptoms in the early stages of glaucoma makes it difficult for patients to understand the long-term threat to their vision and the need for the regular medication, tests and check-up visits that may contribute to non-compliance in patients. To avoid the late presentation of glaucoma, the recognition of risk factors when glaucomatous changes have not occurred, would be beneficial. It is widely assumed that the key to success in the prevention of visual disability due to glaucoma is early diagnosis and treatment.

Studies II and III

The prevalence of exfoliation syndrome, one of the most important risk factors for glaucoma, has been followed by Prof. Henrik Forsius and Aldur Eriksson since the 1960’s in the Kökar population. In 2000, I was invited to participate in the exfoliation study in Kökar, Åland Islands.
inhabitants of the island and their descendents were aware that they were suspected to have a special feature that was challenging and could be related to glaucoma.

Study IV

Some of my own glaucoma patients started to tell me that they have many relatives with glaucoma and wondered if glaucoma is an inherited disease. This raised my interest, especially as at the same time the first report on a glaucoma gene had been published. In a glaucoma clinic, 50% of glaucoma patients reported to have a positive family history and in a population-based familial aggregation study close relatives to a glaucoma patient had 9 to 10 fold risk for glaucoma compared with the general population. Moreover, a positive family history was a strong risk factor for glaucoma population-based cross sectional studies. The probands and their family members appeared to be as interested as myself to uncover a Finnish glaucoma gene.

The Kökar population and the families in Ekenäs region offered a good starting point for detecting risk factors associated with glaucoma and also for the search for glaucoma genes in Finland. Molecular genetics has become a part of medical research today and now we have much better ways to find genes associated with diseases than some 15–20 years ago. However, detecting a gene and finding its role in the development of a genetically heterogeneous or non-Mendelian disease is still very demanding.
2.  **Aims of the study**

The aims of the present study were:

**Study I**
- To find out how often treated glaucoma patients get visual impairment and blindness during their lifetime in a selected private practice patient population
- To analyse, which factors were associated with the development of visual disability in these glaucoma patients

**Study II**
- To follow the risk for glaucoma in families with exfoliation syndrome in three generations
- To find out whether exfoliation syndrome is inherited and what is the mode of inheritance

**Study III**
- To map a gene/genes associated with exfoliation syndrome

**Study IV**
- To investigate the role of *TIGR* or *OPTN* mutations in glaucoma families in Southern Finland
3. Review of the literature

Glaucoma is defined as progressive loss of neuroretinal rim tissue due to apoptosis of retinal ganglion cells, and consequent excavation of the optic disc with corresponding loss of visual field. The changes are irreversible and can lead to severe visual disability.

We know several risk factors for glaucoma, of them elevated intraocular pressure (IOP) is the most important, and exfoliation syndrome (ES) is the most commonly identifiable cause. However, it is not known why some eyes with risk factors develop glaucoma and others do not. So far, the only treatable risk factor is IOP that is lowered by medication, lasertherapy or surgery. This is not always enough and the disease may progress to its end stage, blindness. The goal of the treatment is to prevent, or at least to delay, the process so that the patient maintains functional vision and a good quality of life. To harmonise and to improve the general standard of glaucoma care, The Finnish Ophtalmologic Society with the Finnish Glaucoma Society have made their own guidelines, which were the first evidence-based guidelines. European Glaucoma Society has produced consensus-based guidelines for diagnosis and management of glaucoma.

3.1 Visual disability induced by glaucoma

3.1.1. Definitions
World Health Organisation (WHO) defines a person as visually impaired if the best corrected visual acuity (VA) is less than 0.3 in the better eye and as blind if VA in the better eye is less than 0.05 or visual field is constricted with a diameter less than 20 degrees. A person is visually disabled if he is unable to perform certain tasks because of visual impairment. A person who has visual disability may have either low vision or blindness (Finnish Federation of the Visually Impaired).

3.1.2. Worldwide
In epidemiological studies, the main problem has been the lack of common diagnostic criteria for glaucoma which makes it difficult to compare results of different studies. In 1996, Quigley estimated that by the year 2000 the number of people with glaucoma worldwide would be nearly 66.8 million, of them 6.7 million would be suffering from bilateral blindness. Since then,
several surveys on glaucoma prevalence have been published 24–27. Based
on these studies Quigley et al. (2006) 28 calculated new estimates for the
years 2010 and 2020. In the analysed studies, the definition of open-angle

glaucoma was based on optic disc appearance and visual field damage, and
the diagnosis was independent on IOP. Worldwide the number of people
with OAG in 2010 will be 45 millions and those with angle-closure glaucoma
(ACG) 16 millions, resulting in a total of 60 millions according to Quigley.
The estimated number of bilateral blindness from OAG is 4.5 millions and
from ACG 3.9 millions, resulting in total of 8.4 millions, in 2010.

The World Health Organisation (WHO) has estimated global causes
for blindness using prevalence studies of blindness. In 1990 29, glaucoma
(15%) was the third major cause of blindness after cataract (46%) and
trachoma (16%). A new set of global data on visual impairment in the year
2002 was presented by Resnikoff et al. (2004) 30. In that report, cataract is
still the leading cause with a proportion of 48% of blindness in the world,
glaucoma the second with a proportion of 12% (4.4 million people) and
age-related macular degeneration (AMD) third with a proportion of 9%.
In industrialised countries AMD is the primary cause of blindness 31. In
third world countries, trachoma as the cause of blindness has dramatically
declined being nowadays only 4% due to better hygienic and health care.

The considerable difference between the estimation (8.4 million) by
Quigley et al. 28 and the collected epidemiologic data (4.4 million) by
Resnikoff et al. 30 may depend on different approaches and methodologies
(prevalence of glaucoma vs. prevalence of blindness). Studies on blindness
tend to assign the “most treatable” disease as the primary cause of blindness
resulting in an underestimation of glaucoma as a cause of blindness in favour
of more “treatable” diseases such as cataracts 32.

Three population-based studies have been published using the WHO
criteria for visual impairment. In the Rotterdam Eye Study 33, the prevalence
of visual impairment increased with age to 12% among subjects aged 85 years
or older. The main cause was cataract, the proportion of glaucoma as the
cause was not reported. In the Australian Visual Impairment Project 34, visual
impairment due to glaucoma had a prevalence of 0.7% among 60-year-olds
and rose to 4% among those older than 90 years. In the Copenhagen City
Eye Study 35, 944 cooperative persons (97%) of randomly selected residents
aged from 60 to 80 underwent an ophthalmologic examination to assess the
cause-specific prevalence of visual impairment. Glaucoma combined with
cataract was the cause in 11% of the cases when visual acuity (VA) was less
than 0.3 in the better eye.

In population-based studies for glaucoma, the criteria for blindness have
been defined both according to WHO criteria (VA in the better eye < 0.05)
and to US criteria (VA in the better eye <0.1) and diameter of visual field
20°. Therefore the reported proportion has varied between 4% and 12%. 36–39
In retrospective studies, where glaucoma caused blindness have
been analysed among glaucoma patients the percentage of blindness has been higher. Kaplan-Meir cumulative probability for bilateral blindness was estimated to be 9% and in one eye 27% at 20 years in a study of 295 treated glaucoma patients (both glaucoma and ocular hypertension) 40. In a clinic-based study the estimates were for one eye 15% and for both eyes 6% at 15 years 41.

3.1.3. Finland
The definition for blindness before 1935 was: not being able to see anything at all or being able to see only daylight. In 1865, the prevalence of blindness was very high at 29/10000 and the most common cause was trachoma 42. In the first scientific study in 1899, trachoma was the leading cause for blindness (31%) and glaucoma was the second (12%) 43. In 1935, Grönholm 44 collected information directly from the blind people and their relatives without from ophthalmologists. He found a remarkable reduction in the number of blind people: 7/10000 inhabitants. In the same year Vannas reported that in the Department of Ophthalmology at Helsinki University, glaucoma was the cause of blindness in 23% of the cases 45. This time the criterion for blindness was a visual acuity counting fingers at 1 m or worse. 1963, Vannas & Raivio 46 reported the number of blind people to be 8/10000. The proportion of glaucoma caused blindness was 9.4%. In their report the definition of blindness was a visual acuity of 0.1 in the better eye, which is comparable to the definition used today in the USA. In the latest published report of habitual visual acuity from Finland, the prevalence of visual impairment was 1.6% and blindness was 0.5% in a nationwide population-based survey (The Health 2000) of 8000 subjects 47. When the results were applied to the Finnish population there were a total of 48 000 visually impaired persons and 15 000 blind persons in 2000.

In a population-based study from Northern Finland 48, blindness was present in 1.9% (9/476) of the population over 70 years of age. Glaucoma-related visual impairment (VA ≤ 0.25) was found in 1.5% of the whole population and 12% of persons with visual impairment. Glaucoma was the main cause to blindness in 7/60 glaucoma patients (12%).

In 1982, the National Research and Development Centre for Welfare and Health in Finland (Stakes) founded the Finnish Register of Visual Impairment, to which ophthalmologists and eye departments are obliged to send information about their patients with visual impairment and blindness. Today, the register includes statistics on about 16 000 living persons who are visually impaired, of them about 3500 are blind according to WHO criteria (visual acuity ≤ 0.05 or visual field diameter ≤ 20°) (personal communication to the register Oct 2007). Glaucoma is the cause of blindness in 7% of all the individuals, the percentage for people over 65 years of age is 9% (approximately 1000 individuals). This is much less than it would be expected when the estimate for the proportion of blindness caused by glaucoma...
Quigley proposed is applied to the Finnish calculations. Currently in Finland (31.12.2006), 72,000 persons (57,000 over 65 years of age) have the right to receive free medication for glaucoma according to the statistics of the national health insurance refunds of medical expenses compiled by the Social Insurance Institution (KELA).

3.1.4. Prevention of blindness induced by glaucoma
In 1999, WHO and a group of non-governmental organisations started a project VISION 2020, which is a global initiative for the elimination of avoidable blindness by the year 2020. The goal is to stop a further increase of the global burden of preventable or treatable blindness. Cataract, trachoma, onchocerciasis, childhood blindness, and refractive errors with low-vision services are the main global priorities among all eye diseases. Glaucoma is also included in this list of avoidable/treatable blindness, but the VISION 2020 indicates different ophthalmological conditions in developed countries in which the five principally targeted diseases are not public health problems. Population-based data from Australia showed that age-specific causes for bilateral visual impairment were refractive errors, AMD, cataract, diabetic retinopathy and glaucoma. Prevention of blindness due to glaucoma is a complex issue, since its success is related to access to detection and treatment, the stage of disease at diagnosis, life expectancy and compliance with treatment.

3.1.5. Screening of glaucoma
The data suggest that only about half of all cases with POAG are detected, and one out of five cases with newly confirmed disease have already advanced visual field loss. The intention of screening is to detect the disease in a population defined healthy or unsuspected to have the disease thus enabling earlier diagnosis and management. Very little is known about the primary prevention of glaucoma; however, there are effective methods for medical and surgical treatments if the disease is diagnosed in its early stage.

The major problems in population screening are the lack of a uniform case definition and suitable simple diagnostic screening tests for glaucoma. An abnormally high IOP level is not an effective diagnostic criterion for OAG, since 50% of those affected have IOPs within the normal range. In addition to measuring IOP, the screening requires either assessment of optic disc appearance or a functional test for peripheral visual function. Thus in a population, specificity and sensitivity are low resulting in many false positives and negatives and a need for secondary evaluations. The situation is different in high risk populations (e.g. first grade relatives) if the genes and mutations causing elevated risk for glaucoma are known. This could create an excellent tool for screening at-risk individuals in the affected families and possibly even at the population level. The goal for
glaucoma screening should be at least to identify those individuals whose risk for glaucoma requires a complete examination. On the other hand, the north London trial showed that 75% of cases with “definite” glaucoma can be detected using a simple combination of existing tests i.e. suprathreshold perimetry, tonometry and slit lamp examination of the anterior eye and optic nerve head.\(^{56}\)

Because cost-effectiveness in glaucoma screening with present methods is questionable nationwide screening programs do not exist\(^{54,57}\), the detection of glaucoma in its early stage would be easier if we were able to recognise specific risk factors including genetic factors and then concentrate our efforts on specific groups at risk. Identification of such risk factors would allow detection of subpopulation to which targeted screening with advanced methods could be applied in order to detect glaucoma at the early stage and to start the treatment prior to loss of visual function.

### 3.2. Risk factors for glaucoma

Most often glaucoma has been defined by the presence of two out of the three following characteristics: glaucomatous change in the optic nerve head, and/or corresponding visual field defect, and/or elevated IOP. In recent epidemiological studies, the definition is exclusively based on the appearance of the optic nerve head and visual fields. Nevertheless, there are still differences. The definitions of glaucoma in population-based studies and inclusion criteria for randomised clinical trials (RCT) referred in this review are shown in Table 1 and Table 2.

A risk factor increases the (statistic likelihood)/probability of a certain event and withdrawal decreases. Risk factors are those characteristics which are associated with the development of the disease and prognostic factors refer to the possible outcome of the disease. In the glaucoma literature both are called risk factors. Clinically these are useful only if we can do something positive to lower the risk. Risk factors are evaluated by comparing the risk of those exposed to the potential risk factor to those not exposed. The best approach for analysing risk factors is the results of population-based cross-sectional studies and prospective, controlled, randomised clinical trials (RCT), where two similar patient groups are compared to each other. Often, however, we have to rely on case-control studies that do not have the same power as RCT. The risk factors for developing and progressive glaucoma need not to be the same. The presence of a risk factor is a contributing factor and does not mean that all carriers will develop changes. A causal relationship can only be determined using an intervention-study where a risk factor is eliminated (e.g. IOP is lowered) and the result is a better prognosis.
<table>
<thead>
<tr>
<th>Study</th>
<th>Population-based study</th>
<th>Optic disc</th>
<th>Visual field test in screening</th>
<th>IOP</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaver Dam Eye Study¹ (4926)</td>
<td></td>
<td>C/D ≥ 0.8</td>
<td>Henson CFS 2000</td>
<td>&gt; 22mmHg</td>
<td>2.1% → 4.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>asymmetry&gt; 0.2</td>
<td></td>
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<tr>
<td>Baltimore Eye Survey² (5308);</td>
<td></td>
<td>C/D 0.7</td>
<td>Humphrey</td>
<td>&gt;22mmHg</td>
<td>white 0.9%-2.6%</td>
</tr>
<tr>
<td>2913 white, 2395 black.</td>
<td></td>
<td>“best clinical judgment”</td>
<td>-120 3 zone†</td>
<td></td>
<td>black 1.2%-11.3%</td>
</tr>
<tr>
<td>The Rotterdam Study¹ (3062)</td>
<td></td>
<td>C/D 0.5</td>
<td>Goldmann</td>
<td>≥ 21 mmHg</td>
<td>1.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or vertical asymmetry&gt;0.2</td>
<td></td>
<td></td>
<td>0.2% → 3.3%</td>
</tr>
<tr>
<td>Blue Mountains Eye Study³ (3654)</td>
<td></td>
<td>C/D ≥ 0.7</td>
<td>Humphrey-76</td>
<td></td>
<td>3.0%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>suprathreshold defect§</td>
<td></td>
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<tr>
<td>Visual Impairment Project⁴ (4744)</td>
<td></td>
<td>C/D ≥ 0.7</td>
<td>Humphrey fastpac 24-2</td>
<td></td>
<td>1.8%</td>
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<tr>
<td></td>
<td></td>
<td>asymmetry 0.3</td>
<td></td>
<td></td>
<td>all OAG 3.4%</td>
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<tr>
<td>Egna Neumarkt Study⁴ (4297)</td>
<td></td>
<td>C/D ≥ 0.7</td>
<td>Humphrey Armaly full-field screen</td>
<td>&gt; 22mmHg</td>
<td>POAG 1.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vertical asymmetry&gt;0.2</td>
<td></td>
<td></td>
<td>NTG 0.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Focal change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crete Glaucoma Study⁵ (1107)</td>
<td></td>
<td>C/D &gt;0.5</td>
<td>Henson CFA 3000</td>
<td>≥ 21 mmHg</td>
<td>2.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vertical asymmetry&gt;0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Focal change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reykjavik Eye Study⁶ (1045 over 50 years of age)</td>
<td></td>
<td>C/D ≥0.7</td>
<td>Octopus G1X</td>
<td></td>
<td>0.6% → 4.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Focal change asymmetry&gt;0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle-Norway eye screening study⁷ (1941 over 64 years)</td>
<td></td>
<td>C/D ≥0.8</td>
<td>H-Armaly full-field, quantify</td>
<td>&gt;25 mmHg</td>
<td>8.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oulu, Finland⁸ (500 over 70 years of age)</td>
<td></td>
<td>Appearance of neuroretinal rim, asymmetry</td>
<td></td>
<td></td>
<td>12%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NFL wedge defect§</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Glaucomatous C/D ≥0.7, notching, asymmetry between C/D 0.3 ** C/D ≥0.7, notching, asymmetry 0.2, § VF controlled with Humphrey 30-2 †control Goldmann, §§ evaluation based on black/white 45 photos and normal fundus photographs, *Category 1 diagnosis §§§ only glaucoma suspects n=136)

### Table 2 Inclusion criteria in randomised clinical trials

<table>
<thead>
<tr>
<th>Inclusion criteria:</th>
<th>Optic disc</th>
<th>Visual field</th>
<th>IOP</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMGT(^1) (255)</td>
<td>Optic disc</td>
<td>Visual field</td>
<td>IOP</td>
<td>Other</td>
</tr>
<tr>
<td>treatment: 129</td>
<td>not advanced</td>
<td>not advanced</td>
<td>IOP&lt;35mmHg</td>
<td>new diagnosis</td>
</tr>
<tr>
<td>control: 126</td>
<td>MD &lt; 16 dB</td>
<td>VF loss</td>
<td>mean&lt;30mmHg</td>
<td>age 50–80</td>
</tr>
<tr>
<td>AGIS (^2) (591)</td>
<td>glaucomatous</td>
<td>H-24-2</td>
<td>elevated</td>
<td>not controlled</td>
</tr>
<tr>
<td>(249 white, 332 black)</td>
<td>VF loss</td>
<td>H-24-2</td>
<td>by medication</td>
<td>age 35–80</td>
</tr>
<tr>
<td>ATT (^3) 380 eyes</td>
<td>defects in</td>
<td>&gt; 27 mmHg</td>
<td>age 35–80</td>
<td>new diagnosis</td>
</tr>
<tr>
<td>TAT (^4) 367 eyes</td>
<td>3 points</td>
<td>≥ 20 mmHg</td>
<td>20–26 mmHg</td>
<td></td>
</tr>
<tr>
<td>CIGTS (^5) (607)</td>
<td>normal</td>
<td>defects in</td>
<td>&gt; 27 mmHg</td>
<td></td>
</tr>
<tr>
<td>surgery: 300</td>
<td>susp. change</td>
<td>2 points</td>
<td>normal</td>
<td></td>
</tr>
<tr>
<td>medicine: 307</td>
<td>normal</td>
<td>normal</td>
<td>&lt; 24 mmHg</td>
<td></td>
</tr>
<tr>
<td>CNTGS(^6) (607)</td>
<td>glaucomatous</td>
<td>glaucomatous</td>
<td>&lt; 24 mmHg</td>
<td></td>
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<tr>
<td>treatment: 61 eyes</td>
<td>glaucomatous</td>
<td>glaucomatous</td>
<td>&lt; 24 mmHg</td>
<td></td>
</tr>
<tr>
<td>control: 79 eyes</td>
<td>normal</td>
<td>normal</td>
<td>22–29 mmHg</td>
<td>age 30–80</td>
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<tr>
<td>OHTS(^7) (1636)</td>
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<td>normal</td>
<td>22–29 mmHg</td>
<td>age 30–80</td>
</tr>
<tr>
<td>Treatment: 817</td>
<td>normal</td>
<td>normal</td>
<td>24–32 mmHg</td>
<td>age 40–80</td>
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<td>No treatment: 819</td>
<td>normal</td>
<td>normal</td>
<td>21–32 mmHg</td>
<td>refr ± 5 D</td>
</tr>
<tr>
<td>EGPS(^8) (1081)</td>
<td>normal</td>
<td>2 normal VF</td>
<td>age 30–80</td>
<td></td>
</tr>
<tr>
<td>treatment: 538</td>
<td>normal</td>
<td>normal</td>
<td>22–29 mmHg</td>
<td></td>
</tr>
<tr>
<td>placebo: 543</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IOP = intraocular pressure, MD = Mean deviation, H-24-2 = Humphrey 24-2 VF visual field
\(^2\)AGIS investigators \(1996\)
\(^3\)ATT = argon laser trabeculoplasty-trabeculectomy
\(^4\)TAT trabeculectomy-argon laser trabeculoplasty-trabeculectomy

### References

#### 3.2.1. Age

#### 3.2.1.1. Age in developing glaucoma

Open angle glaucoma is classified according to age of onset into two groups: juvenile and adult form with a cut point 40 years of age. Since most glaucoma patients in general practice are over 65 years of age, age is one of the most predominant risk factors.

In the Beaver Dam Eye Study \(^9\) in Wisconsin, the prevalence of definite open-angle glaucoma was 2% in the population of 4926 subjects. The prevalence increased with age from 0.9% among people between 43 and 54 years of age to 4.7% among those of 75 years or older. The odds ratio (OR) increased by 1.7 (95%, CI: 1.45–2.09) for every decade. In the Rotterdam Eye Study \(^10\), the overall prevalence of POAG was 1.1% (95%, CI: 1.09–1.11). Age-specific prevalence figures increased from 0.2% (95%, CI: 0.16–0.24) in the age group of 55 to 59 years to 3.3% (95%, CI: 2.57–4.04) in the age group of 80 years or above.
group of 85 to 89 years. In the Blue-Mountains Eye Study in Australia, the prevalence of open-angle glaucoma (OAG) was 0.4% for people less than 60 years of age and rose to 11% for people 80 years or older. An exponential rise in prevalence was found with increasing age. Tuck used eight population surveys for meta-analysis and found that the prevalence of glaucoma for 40-89 year old mainly white Caucasian people was 1.2%, rising from 0.2% for those in their 40s to 4.3% for those in their 80s. Of all cases analysed, 7% were younger than 55% were aged from 55-74, and 49% were older than 74. In the Reykjavik Eye Study, the prevalence of OAG was 4% (42/1045) (95%, CI: 2.8-5.2) for those 50 and older and increased with age (OR=1.10/year, 95%, CI: 1.07-1.13, P=0.000).

In a randomised clinical trial Ocular Hypertension Study (OHTS), 1636 persons with IOP between 24 mmHg and 32 mmHg in one and between 21 mmHg and 32 mmHg in the other eye were randomised to either observation or treatment with topical hypotensive medication. Older age was one of the baseline factors that predicted the development of POAG (Hazard ratio 1.22, 95%, CI: 1.01-1.49).

In contrast to numerous reports on the prevalence of glaucoma, there are only few population-based studies that have presented incidence data. The oldest one is a Scandinavian study in which the incidence of manifest glaucoma was determined by repeated automated perimetry. The incidence of glaucoma was estimated as 0.24% per year and was independent of age, whereas in other later studies incidence has been closely related to age. Two originally prevalence studies on white populations (the Melbourne Visual Impairment Project VIP and the Rotterdam Study) have been continued resulting in a 5-year incidence rate for glaucoma. The incidence increased significantly with age. In Melbourne, the incidence of definite glaucoma was 0% for participants aged 40 to 49 and 4.1% for participants aged 80 or older. In Rotterdam, the incidence rate was 0.9% for the age group from 55 to 59 and it rose 2.7% for the age group of 80 years or older.

In Finland, an approximate incidence of new glaucoma patients can be assumed from the statistics of the Social Insurance Institution (KELA). The number of glaucoma patients who receive free medication increases yearly by about 3% (3000), the highest incidence being in the age group of 65-75 years. This Social Insurance Institution provided number includes patients with glaucoma but also those patients receiving IOP lowering medication because of ocular hypertension (OHT), in which cases patients have IOPs over 30 mmHg without any glaucomatous changes in the optic nerve head or visual fields.

### 3.2.1.2 Age in progressive glaucoma

Older age as a risk factor for progressive/glaucoma/ disease has not been as obvious as for developing glaucoma, because most patients with glaucoma are already old at presentation. However, when patient groups
are compared with each other, higher age is an independent risk factor for progression. In both randomised clinical trials, the Early Management Glaucoma Trial (EMGT) and the Advanced Glaucoma Intervention Study (AGIS), one of the predictive baseline factors for progression was older age\textsuperscript{10,62}. In the retrospective studies where risk factors for blindness induced by glaucoma were analysed, older age has also been found to be a risk factor for glaucoma caused blindness\textsuperscript{40,63,64}.

\textit{In summary, there is a high level of evidence that for every decade age doubles the risk for glaucoma and also increases the risk for progression of glaucoma}\textsuperscript{14}.

### 3.2.2. Intraocular pressure IOP

#### 3.2.2.1. IOP in developing glaucoma

Elevated intraocular pressure (IOP) has always played an important role in the diagnosis and management of glaucoma. With increasing knowledge on pathogenetic changes in the retina and optic disc, the role of IOP has been altered from a disease to a major risk factor for developing glaucoma. However, the importance of IOP in glaucoma care has not decreased because it is still the only treatable risk factor.

In the Baltimore Eye Survey\textsuperscript{16} the prevalence of POAG increased with screening IOP. Relative risk was 1.0 with IOP $\leq$ 15 mmHg, 13 with IOP from 22 to 29 mmHg, and 40 with IOP $\geq$ 35 mmHg. However, more than half of all glaucomatous eyes had a screening IOP below 21 mm Hg, whether these eyes were receiving treatment or not. In Blue Mountains Eye Study, the prevalence of glaucoma increased with IOP greater than 23 mmHg\textsuperscript{26}.

The probability of having glaucoma at different IOP levels was analysed in the Middle Norway eye-screening study\textsuperscript{65} that included 3489 normal eyes and 237 eyes with glaucoma. The IOP frequency distribution curve of the healthy eyes had its maximum at 16-17 mmHg, whereas among the glaucomatous eyes it was 26 mmHg. The curves crossed at 27-28 mmHg, indicating that at this IOP level the number of eyes with and without glaucomatous damage was equal.

Eyes with elevated IOP but normal discs and visual fields are classified as ocular hypertensive. The challenging questions have been: Do these eyes convert to glaucoma? If they do, how fast do they do so? When should medication be started? Does the treatment prevent conversion? The purpose of the Ocular Hypertension Treatment Study (OHTS)\textsuperscript{19} was designed to evaluate if hypotensive treatment of elevated IOP delays or prevents the onset of POAG. At 60 months, the cumulative probability of developing POAG was 4.4% in the medication group and 9.5% in the observation group (HR, 0.40; CI: 95%, 0.27-0.59; P < .0001). Higher intraocular pressure was one of the baseline factors that predicted the development of POAG\textsuperscript{7}.
Surprisingly, the European Glaucoma Prevention Study (EGPS) on OHT patients failed to detect a statistically significant protective effect of IOP lowering medication (dorzolamide) compared to placebo treated because a small treatment difference between the two groups 66, but in both study arms a third of the subjects were lost from the follow-up.

The results of the OHTS study suggest that initiating IOP lowering treatment is beneficial in some OHT patients, but it is uncertain whether it is also beneficial in the treatment of mildly elevated IOP is. Maier et al. (2005) 67 performed a meta-analysis of the effectiveness of pressure lowering treatment to delay the development of glaucoma in ocular hypertension. The results from 5 included randomised trials showed a beneficial treatment effect (HR 0.56, 95%, CI: 0.39-0.81) suggesting that the treatment may prevent the onset of glaucoma.

Normally, IOP fluctuates during the day 68, but the fluctuation is larger in glaucomatous eyes than in normal eyes 69. Davanger postulated that the same alteration in calibre of the pores in the aqueous outflow paths will lead to a greater change in IOP at a high pressure level than at a low level.70 In the prospective Malmö Ocular Hypertension Study 71, all measurements of IOP during the follow-up of 8.5 years (mean) were analysed applying multiple Cox regression analysis. The level of IOP was significantly associated with the development of glaucoma (95% CI, 1.09-1.38). For the analysis of the risk of the fluctuation, IOP variation was divided into two categories: IOP fluctuation range <5 mmHg and ≥5 mmHg, but the fluctuation did not correlate with increased risk for glaucoma.

3.2.2.2. IOP in progressive glaucoma

Glaucoma is treated by lowering the IOP with medication (topical eye drops and/or oral acetazolamid). If reduction of IOP is not enough and progression is observed in the optic nerve head and visual fields, further options are surgical procedures. Traditionally a good treatment result has been achieved when IOP is in the statistically normal level i.e. between 10 mmHg and 21 mmHg. Despite that, in some eyes glaucomatous changes in optic discs and visual fields are still progressing. These problems have raised the question of what kind of influence IOP really has on progression, whether there are two different types of glaucoma: IOP responders and nonresponders.

The EMGT study was designed to compare the effect of the lowering of IOP on the progression of newly detected open-angle glaucoma. Half of the eyes were recruited to treatment (laser trabeculoplasty plus topical betaxolol hydrochloride) and half were only followed closely 72. At the 6-year check, progression in visual fields was less frequent in the treatment group (58/129; 45%) than in controls (78/126; 62%) (P =.007) and it occurred significantly later among treated eyes. When the same patients were examined 3 years later, with a median follow-up time of 8 years, 68% had progressed (59% of the treated group and 76% of the control group). The mean IOP was
significantly related for progression with a hazard ratio (HR) of 1.11 (95% CI, 1.06-1.17).

The Advanced Glaucoma Intervention Study (AGIS) analyzed the effect of IOP on progression of visual field defects after ALT or trabeculectomy in eyes with advanced glaucoma. In the predictive analysis (738 eyes), the eyes were divided into three groups based on IOP during the first six month follow-up. The eyes with IOP greater than 17.5 mmHg had only 1 unit more of visual field score deterioration compared with the eyes with IOP less than 14 mm Hg during 6 years follow-up (P = .002). However, in the associated analysis (post-hoc) of 586 eyes if IOP was less than 18 mmHg in all visits during the follow-up, the visual field defect score was close to zero whereas if IOP was less than 18 mmHg in less than 50% of the visits the visual field score worsened. In both analyses low intraocular pressure was associated with decreased progression of visual field defect.

No significant effect of the IOP level on the visual field score was found in the Collaborative Initial Glaucoma Treatment Study (CIGTS), when the eyes were treated initially aggressively either with medicine only or with surgery. The average IOP in the medication group was 17 to 18 mmHg and in the surgery group 14-15 mmHg during the follow-up of five years, which may have been too short to show any difference in progression. Similarly, in a prospective follow-up study on 113 patients with early or moderate glaucoma the mean level of IOP did not differentiate the eyes with progressive visual field loss from those with stable glaucoma. However, the baseline visual field status and peak IOP were associated with progression.

In normal tension glaucoma, in which the initial IOP is within the normal range (10-21 mmHg) but both the optic nerve head and visual fields have glaucomatous changes, the role of IOP has been more controversial. In the Collaborative Normal Tension Glaucoma Study (CNTGS) 145 eyes were randomised in two groups: a treatment group (61 eyes) with IOP lowering of 30% from the initial IOP and a control group (79 eyes) without treatment. The study end point, visual field deterioration, was reached earlier in the control group than in the treatment group suggesting that IOP is also a part of the pathogenic process in normal-tension glaucoma. However, the results were significant only when the analysis was censored for newly developed cataracts, because in the treatment group 35% of 66 eyes developed cataract as an adverse effect for surgery compared to 14% of 79 eyes in the control group. Moreover, the treatment group had a higher IOP at the baseline.

Despite adequate medical therapy, IOP fluctuates in most glaucomatous eyes. The variability as an independent risk factor has been analysed by Asrani et al. (2000). Sixty-four patients (105 eyes) with open-angle glaucoma and documented initial IOP below 25 mm Hg successfully performed home tonometry with a self-tonometer (self-tonometer Zeimer CDS Technology, LLC. West Chicago, Illinois, USA) five times a day for five days. Both the diurnal IOP range and the IOP range over multiple days were
significant risk factors for progression with relative hazards/risk of 5.69 (95% CI, 1.86-17.35) and 5.76 (95% CI, 2.21-14.98), respectively. In both AGIS and EMGT studies, the fluctuation of IOP was analysed using the standard deviation (SD) of measured IOP for all visits. In the AGIS study, the fluctuation of ≥ 3 mmHg was strongly associated with progression (OR 1.31; 95%, CI: 1.12-1.54) when all visits after the initial surgical intervention were included. On the contrary in the EMGT study, the SD of measured IOPs was 2.02 mmHg for eyes with progression and 1.78 mmHg for eyes without progression, and did not increase the risk (HR 1.00; 95%, CI: 0.81-1.24). This calculation included only IOPs until the visit when progression was detected, but when the analysis was conducted including postprogression values, IOP fluctuation was related to progression (HR 1.66, 95%, CI:1.44-1.93). These controversial results have triggered an intensive discussion on the influence of IOP on progression: whether it is dependent or not on the mean level or fluctuation of IOP. The most comparable study for the EMGT would have been the CIGTS which also recruited untreated subjects with mild glaucomatous damage. The main difference between these studies was the target pressure that in the CIGTS was defined as 35% IOP lowering but in the EMGT no target pressure was set and no change in management unless IOP was over 25 mmHg or in the untreated arm over 35 mmHg. In the CIGTS and the EMGT the progression was evaluated as a change in visual fields, but the utmost visual outcome, blindness was also proof of progression. In the Olmsted Study, the probability of blindness was estimated and one of risk factors for glaucoma caused blindness was variability in IOP.

3.2.2.3. IOP and Target pressure
Earlier observations in clinical practice and the results in the CNTGS study have shown that different eyes in different individuals differently tolerate elevated or even “normal” intraocular pressure. Therefore, variation of deterioration, as well as a lack of progression suggest that treatment should be individualized according to the stage of disease and rate of progression. As the first trial, the CNTGS used a predecided 30% decrease from the pre-treatment IOP-level for the treated group of patients. In the CIGTS, a “target pressure” was calculated using IOP and visual field score before treatment was applied. In the OHTS, a reduction of IOP by 20% was effective in delaying or preventing the onset of POAG. In the EMGT, lowering of IOP by 25% from baseline resulted in a 45% decrease in the risk of progression. The target pressure is now defined as an estimate for the mean IOP that should be reached with treatment and that is expected to prevent further glaucomatous damage. In the Finnish EBM guidelines the recommended decrease is at least 25 % from the pretreatment level. The problem with target pressure and other treatment options is that there does not exist any guarantee that the chosen target pressure is optimal for
the selected glaucoma eye, the answer for whether the glaucomatous changes are progressing or not will be found much later by observations.

3.2.2.4. IOP and Central Corneal Thickness CCT
The Goldmann applanation tonometer developed by Goldmann in the early 1950s is regarded as the current gold standard for measuring intraocular pressure. The principle is to measure the force needed to deform the cornea in a standardised manner. It is also based on the main assumption that most of the eyes have approximately the same corneal thickness (520 μm) and therefore deviations could be neglected without losing any accuracy 83. In 1975, Ehlers 84 cannulated 29 otherwise normal eyes undergoing cataract surgery and correlated corneal thickness with errors due to the Goldmann applanation tonometer. They found that if CCT deviated from 520μm the measured IOP values could deviate as much as 7 mmHg per 100μm (thinner cornea - underestimation, thicker cornea - overestimation).

The significance of CCT was recently “rediscovered” in the OHTS when CCT was measured in 1398 of the total of 1618 participants 7. In the multivariate analyses, thinner CCT was found to be one of the most important risk factors for developing glaucoma, HR 1.71 (CI; 1.40-2.09). The risk was highest among participants with the thinnest central corneal measurements in each subgroup divided according to vertical cup-disc ratio. In the EGPS, the HR for a thinner CCT was lower 1.32 (CI; 1.05-1.67), although the technique and the model of pachymeter were the same 85. The EGPS authors speculated that the differences could be due to racial differences: in the EGPS the participants were Caucasians and 25% of participants in the OHTS were African Americans.

The real role of CCT is not yet established; is it an independent risk factor for glaucoma or is it only a value to use for correction in IOP readings? For both OHTS and EGPS the inclusion criterion was elevated IOP, which could have reduced the proportion of eyes with thinner corneas and therefore the selection would not represent normal population in practice. On the contrary, CCT was not found as a risk factor in EMGT which was a population-based study and the inclusion criteria was glaucomatous damage 10,86. Shah et al. (1999) 87 investigated how well CCT correlated with diagnosis in a general ophthalmic clinic. The result was as expected, eyes with NTG had the thinnest corneas followed by eyes with EG. The thickest CCT had eyes with suspicion of glaucoma (OHT).

In a glaucoma clinic, where patients have been referred for tertiary care the thinner cornea correlated with the severity of glaucoma 88. In this analysis the only significant predictor of AGIS score used for visual field defects was CCT. The score improved by 0.31 for every increase of 10μm of corneal thickness. CCT as an independent risk factor for visual field loss was analysed in 98 eyes with preperimetric glaucoma e.g. glaucomatous optic disc neuropathy without visual field loss 89. A thinner cornea predicted
conversion of OHT to glaucoma with HR 1.62 (CI; 1.07-2.45). The mean ± standard deviation of CCT in the eyes which developed visual field loss was 543± 36 μm compared to 565 ± 35 μm of the eyes without a change. The conclusion was to consider CCT when a target pressure is determined for a patient with preperimetric glaucoma. However, there is no accepted model for calculation of the corrected IOP value for the respective corneal thickness value.

In summary, there is strong evidence that IOP is related to high prevalence and progression of glaucoma. Whether the fluctuation is an independent risk factor is still under debate.

3.2.3. Exfoliation syndrome

3.2.3.1. Characteristics of exfoliation syndrome

Pseudoexfoliation syndrome or exfoliation syndrome (ES) was first described by John Lindberg (1917) as “whitish-grey scales which emanated from the outermost part of the pupillary border and occasionally grouped in a more or less dense ring on the anterior capsule of the lens” 90. Besides the deposition on the lens surface in most cases greyish dandruff is seen along the pupillary margin, and as small deposits on the corneal endothelium, iris surface, lens zonules and ciliary processes 91,92. Other clinical features are liberation of pigment after papillary dilatation, pigment deposition on the corneal endothelium as well as in the anterior chamber angle. Loss of pigmentation from the iris also causes a transillumination defect of the iris 93-95.

Clinically, ES is prevalent in both the unilateral and bilateral forms. These may represent different stages of the disease and about 14-41% of the unilateral cases develop into the bilateral involvement 20,96. The probability of exfoliation developing in the opposite eye was found to be 7% in 5 years and 17% in 10 years 97. Puska (2002) followed 63 patients with unilateral ES and observed that 38% had converted to bilateral ES at 10 years 98. The cumulative incidence estimate for conversion of clinically unilateral to bilateral ES 0.11 (95% CI,0.03-0.25) at 5 years, 0.36 (95% CI, 0.15-0.57) at 10 years, and 0.52 (95% CI,0.27-0.93) at 15 years from the day the ES was detected in the 35 eyes 99. Though the clinical impression is a unilateral affection, subtle ultrastructural and immunohistochemical alterations typical of ES were found in the unaffected fellow eye 100,101.

Originally exfoliation or pseudoexfoliation was thought to be limited to the eye, but later the same kind of material has been found in various human tissues, and therefore today, it is considered to be a syndrome involving also the heart, lungs, liver, kidneys etc 102. A possible association between ES and vascular diseases has been suggested, but the reported results have been controversial. Repo et al. 103 found a higher frequency of ES among 62
patients with transient ischemic attacks compared with age-matched controls. Also in the Blue Mountains Eye Study, a positive connection between the exfoliation syndrome and vascular events was observed 104.

Recently, Citirik et al. 105 reported a significantly higher prevalence of ES in 50 patients with coronary artery disease compared with 50 controls with normal coronary angiographic findings. When the same patients were regrouped according to ES, the prevalence of coronary artery disease was more prominent among the ES positives than the ES negatives. However, Shrum et al. (2000) 106 studied the 15-year cumulative mortality from cardiovascular and cerebrovascular diseases in 472 patients with exfoliation. They found no significant difference relative to the general population in the same area. Also the same result, no increase of ES prevalence was found in a study of patients operated for abdominal aortic aneurysm 107.

ES material consists of cross-banded fibrils and filamentous subunits in an amorphous ground substance 91. Despite extensive research, the exact composition has remained unknown. The suggested component has been a complex glycoprotein/proteoglycan structure composed of a protein core surrounded by glycoconjugates also forming the amorphous ground substance 17. The protein components of ES material include both non-collagenous basement membrane components and epitopes of the elastic fibre system 17. Most of the ES material is found beneath the endothelium of Schlemm’s canal in the uveal meshwork 17. The amount of the material in the trabecular meshwork correlates with the presence or absence of glaucoma and correlates inversely with the axon count in the optic nerve, suggesting a direct causative relationship between ES material in the meshwork and glaucoma development 106,107. Histopathologically, EG is well differentiated from chronic OAG 17. Whereas a significant increase in juxtacanalicular plaque material and a decrease in trabecular meshwork cellularity is found in the eyes of chronic OAG, no differences on plaque concentration and cellularity have been found in eyes with EG compared to normal eyes 17. Thus, the challenging question for the future is; why about 65% of eyes with exfoliation remain nonglaucomatous 20.

Exfoliation syndrome is prevalent worldwide but the prevalence varies in different ethnic populations 110,111,112 from almost zero among the Inuits up to 20-25% in Scandinavian countries and Greece 110,113-116 and the prevalence of ES increases with age (Table 3).
Table 3: The prevalence of exfoliation syndrome in population based studies (%)

<table>
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<tr>
<th>Study</th>
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<th></th>
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<th></th>
<th></th>
</tr>
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<td>75–79</td>
<td>80–84</td>
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<td>28</td>
<td>26</td>
<td>30</td>
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<td></td>
</tr>
<tr>
<td>Mid-Norway**</td>
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<td>13</td>
<td>17</td>
<td>23</td>
<td>30</td>
<td>37</td>
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<td>Blue Mountains§</td>
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<td>1</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crete§§</td>
<td>0.8</td>
<td>11</td>
<td>20</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reykjavik‡</td>
<td>3</td>
<td>9</td>
<td>17</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*113, **117 §115 §§114 ‡25

In most studies, the prevalence of ES is higher among females than males: 27% in Oulu, 18% in Norway, 2% in Australia, 12% in Reykjavik compared to 13%, 15%, 1% and 9% in males, respectively. However, the reverse situation has been found in Greece, females 13% and males 21% \(114\). The prevalence varies within a country as well. In France, the overall prevalence over age 70 is about 6%, ranging from 21% in Brest, to 4% in Toulon \(118\). Krause (1988) \(119\) had studied the prevalence of ES in southern (Helsinki), in the middle (Oulu) and in the northern (Kuusamo) part of Finland. In Kuusamo, the participants were randomly selected from a rural commune, but in Helsinki and Oulu they were inmates in an old people’s home (Table 4).

Table 4 Exfoliation syndrome in Finland \(119\)

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Oulu 205</th>
<th>Helsinki 262</th>
<th>Kuusamo 328</th>
</tr>
</thead>
<tbody>
<tr>
<td>60–69 (%)</td>
<td>10</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>70–79 (%)</td>
<td>23</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>&gt; 80 (%)</td>
<td>36</td>
<td>28</td>
<td>47</td>
</tr>
<tr>
<td>Total (%)</td>
<td>14</td>
<td>22</td>
<td>35</td>
</tr>
</tbody>
</table>

3.2.3.2. Exfoliation syndrome (ES) and Intraocular pressure (IOP)

In clinical practice, exfoliation syndrome may or may not to be associated with an elevated IOP. In the population-based study from Oulu, the mean IOP in the exfoliating group was significantly higher than in the non-exfoliation group (17 mmHg vs 15 mmHg, \(p=0.0006\)) \(115\). In Crete, 29% of ES positives had IOPs higher than 21 mmHg compared with 5% of ES-negatives \(114\). In the Blue Mountains Eye Study, eyes with ES had slightly higher IOPs than eyes without ES (17 mmHg vs 16 mmHg) \(115\). The authors analysed the role of IOP in the association between ES and glaucoma in a generalised estimation model by adjusting for IOP (continuously), as well as for other glaucoma risk factors. In this model, the association between glaucoma and ES gave only slightly lower odds (OR 3.7 95%, CI 1.8-7.6) than before IOP adjustment (OR, 4.8). This suggested that the relationship between glaucoma and ES may be relatively independent of its effect on IOP \(115\).
3.2.3.3. Exfoliation syndrome and glaucoma

Exfoliation glaucoma, EG (capsular glaucoma, exfoliative or pseudoexfoliative glaucoma), is defined as open-angle glaucoma with exfoliation syndrome. In the Blue Mountains Eye Study, glaucoma was more prevalent among eyes with ES (14%) than among eyes without ES (2%) (OR 5.0; 95%, CI, 2.6-9.6) 115. In a cohort of 413 subjects of a population based study in Tierp, Central Sweden, from the 72 eyes that were ES positives at baseline, glaucoma developed in 10 cases during the 5 years follow-up 9. The unadjusted relative risk (RR) was 16 (95% CI, 4.3-57) and adjusted RR 10 (95% CI, 2.5-38). Males had a higher risk than females (RR 30 vs 10). In Finland, Klemetti followed originally normotensive eyes with ES and found that 35% of them had developed glaucoma (IOP > 22 mmHg, glaucomatous change in the optic disc and/or visual field defect) during the mean observation time of 5 years (range 1-23 years) 20. In a Finnish cohort of 63 patients, the conversion to exfoliation glaucoma was 32% of in the 56 initially ES-positive eyes 98. 21 of 63 nonexfoliative fellow eyes converted to ES-positives during the 10 years follow-up. Of them 38% (8/21) developed glaucoma. The conversion was associated to the initial IOP. Among screened participants of the EMGT, OHT patients with ES were compared to age-matched OHT patients without ES 9. The presence of ES doubled the conversion rate to glaucoma during the follow-up time of nine years. In a cohort of ES patients in Olmsted, Minnesota, glaucoma medication was started in 16% due to glaucomatous changes or ocular hypertension at the time of initial diagnosis 120. The probability for therapy was 44% at 15 years.

In Scandinavian countries the prevalence of ES among glaucoma patients is significantly higher than in age-matched nonglaucomatous populations 121. About 40% of hospitalised open-angle glaucoma patients in Norway had EG 122. The same kind of result, 42% was found in an analysis of 137 patients with senile open angle glaucoma in Central Finland 123. In a study based on hospital records in southern Finland, the corresponding proportion was 47% 124.

3.2.3.4. Exfoliation syndrome in progressive glaucoma

Compared to POAG, the clinical course of EG is more aggressive i.e. glaucomatous damage which progresses more rapidly and the prognosis is poorer 125,126. In the EMGT, patients with newly diagnosed open-angle glaucoma who had reproducible visual field defects were followed for 6 years (median) in two groups: treatment vs no treatment 72. Progression was defined as a loss of 2 dB in mean deviation in the Humphrey full threshold field test C-30-2 10. In the analysis of risk factors, ES was found as an independent risk factor that doubled the risk for progression (95%, CI: 1.31-3.74).

In a prospective study, Konstas (1997) 127 compared diurnal curves of newly diagnosed untreated EG eyes and POAG eyes. The mean range of IOP as well as both maximum and minimum IOPs were significantly higher in EG eyes than in POAG eyes. In another study, a significant relationship
between IOP and visual field mean deviation (MD) was found in untreated eyes with ES compared to eyes without ES. Similarly, in EG eyes Bergeå et al. reported a correlation between progression of visual field defects and both IOP range and mean IOP. Harju (2000) studied 114 eyes with ES combined either with glaucoma or OHT and found that the weighted mean IOP was associated with progression, while the single maximum IOP was not.

In summary, the evidence that the presence of exfoliation syndrome is a risk factor for developing and progression of glaucoma is relatively high level. The risk for glaucoma increases 5 to 10 fold.

3.2.4. Genetic risk factors for glaucoma

3.2.4.1. Positive family history of glaucoma

3.2.4.1.1. Family history of glaucoma

A familial association with open angle glaucoma has been discussed ever since von Graefe described a family with hereditary glaucoma in 1869. Several studies on different types of glaucoma have indicated an increased probability of having glaucoma if a family member is affected. In a clinic based study, 50% of glaucoma patients (n=150; white and black) and in 43% of OHT patients had a positive family history.

3.2.4.1.2. Family history of glaucoma in population studies

In population-based studies, data on positive family history of glaucoma has been self-reported. In the Baltimore Eye Survey, positive family history was reported in 16% of cases with POAG compared to 7% of control subjects. Also, age-adjusted association with glaucoma was higher in siblings, (odds ratio (OR) 3.69 (95% CI, 2.10-6.48) than in parents (OR 2.17 (95% CI, 1.07-4.41) or in children OR 1.12 (95% CI, 0.26-4.86). A selection bias was suspected when OR was doubled for those who were aware of their glaucoma diagnosis compared with those who first received their diagnosis during the study examination. All ORs have been slightly higher in blacks than in whites. In the Barbados Eye Study (BES) (only black individuals) positive family history was reported by 18% of glaucoma patients and the OR was twice as high among males than among females (7.88 vs 2.48). In the analysis of the self-reported family history, siblings had slightly higher OR 5.7 (95% CI, 3.5-9.1) than parents 4.6 (mother 4.6, 95% CI, 2.8-7.6 and father 4.7, 95% CI, 2.4-9.2). The same selection bias as in the Baltimore Eye Survey was also found in Barbados, participants with OAG were over
four times more aware of a parental glaucoma history than participants without OAG. In a population based study in Australia (Visual Impairment Project) a total of 4744 subjects participated in interviews and examinations for detecting different eye conditions. In multivariate logistic regression analysis, participants with a family history of glaucoma were three times more likely to have possible, probable or definite glaucoma. This was also the only characteristic other than age that remained significantly associated with increased risk of definite glaucoma (OR 3.5, CI 1.9-6.7). However, the awareness of diseases of other family members is not always reliable. Within the Tasmanian project GIST, 41 subjects previously diagnosed with POAG coming from five large POAG pedigrees were asked whether any of their relatives had glaucoma. Nearly a third (27%) of them was unaware of their family history of glaucoma.

3.2.4.1.3. Family history and disease severity

There are only a few studies in which the association between positive family history of glaucoma and the stage of glaucoma at diagnosis has been analysed. In randomised trials a positive family history has not been a risk factor for progression. Subjects who have family members with glaucoma tend to come earlier for screening. Whether the severity of glaucoma at diagnosis is affected by the patients’ knowledge about relatives having glaucoma, was investigated in a cohort of 292 patients. They found that family history of glaucoma was not associated with better or worse visual field defect at diagnosis, but subjects with a family history were diagnosed earlier than those without (58 years vs 63 years of age).

In the Glaucoma Inheritance Study of Tasmania (GIST), a “GIST score” was developed to facilitate linkage analysis in POAG pedigrees, not to be used in clinical practice. It was a numeric value between zero and one where zero was clinical certainty of the absence of the disease and one was the definite diagnosis of POAG. The first part of score was based on the clinical examination (the findings of optic disc assessment, visual field deficit, and elevated IOP) giving a “raw” which was then translated into the GIST score incorporating also family history. Familial glaucoma was defined as the presence of a fourth-degree (i.e. second cousins or great-great grandparents) or closer relative affected by POAG. The accuracy of diagnosis was controlled by clinical examination of relatives or searching for the data from the clinical data-base. The study population of 1700 glaucoma patients (60% with familial and 40% with sporadic glaucoma) was divided into groups of mild, moderate, severe and very severe disease according to the GIST severity score. These scores were significantly skewed toward higher disease severity in the familial group than in the sporadic group (p<0.001), also without including data on IOP. Subjects in the familial group were younger at diagnosis than in the sporadic group (61 years vs. 64 years).
In summary, there is evidence that a positive family history is an important risk factor for developing glaucoma. In population-based studies, the risk increases 3-4 fold, and 10 fold for close relatives. The risk of a positive family history for progression has not been much studied.

3.2.4.2. Genetics of glaucoma

3.2.4.2.1. Genetic predisposition

The fact that genetics plays an important role in the pathogenesis of glaucoma, is supported by the observations of varying prevalence of POAG in different ethnic populations, e.g. African Americans vs. Caucasians. Further, close relatives of glaucoma patients have an increased prevalence of glaucoma, ranging from 3% to 14%, compared with the general population 1% to 2%. In most cases of juvenile type of glaucoma, the pattern of inheritance in the families has resembled autosomal dominant. In the late onset of POAG, the previous generations have often deceased making it difficult to estimate the mode of inheritance.

The weakness in population-based studies has been that they rely on family history and family members of the proband have not been examined ophthalmologically. Therefore, Wolfs et al. investigated whether the occurrence of glaucoma was higher among first degree relatives of glaucoma patients than among first degree relatives of general population. First degree relatives (siblings, offspring) of glaucoma patients (probands n=48) and their control group with age- and sex-matched randomly selected subjects (n=155) from the population-based Rotterdam Study were invited to a comprehensive ophthalmological examination. The overall response rate for all relatives was 82%. The prevalence of glaucoma (VF defect with cup/disc (C/D) ratio of ≥0.7 or asymmetry in C/D ratio of ≥ 0.3) among the siblings of the glaucoma patients was 10% (n=6) compared with 0.7% (=1) among the siblings of the controls. Only one offspring of a glaucoma patient had glaucoma, but the mean age for all offspring was only 42 years. The lifetime absolute risk of glaucoma at age 80 years was estimated to be 22% for relatives of patients with glaucoma compared with 2% for relatives of the controls (risk ratio 9.2, 95%, CI: 1.2-73.9). In addition, the attributable proportion among the genetically exposed was estimated as 89%, indicating that 89% of the familial occurrence is genetically determined. The population-attributable risk of glaucoma was low, only 16%, indicating that non-genetic factors determine the overall occurrence of glaucoma. The same study population was investigated again five years later with a newer definition of definite glaucoma. A family score was created to describe whether a subject had glaucoma or not and whether her/his relatives had glaucoma or not with regard to the age-and sex-specific prevalence of glaucoma in the Rotterdam
Eye Study. The logistic regression analysis showed that an increase in family score by 1 unit was associated with a significantly increased risk of OAG. The positive family score value indicated that more cases with glaucoma in the family were detected than expected compared to the prevalence of glaucoma in the population.

3.2.4.2.2. Twin studies

For determining the relative influence of genetic and environmental factors, the most often used method is twin study where monozygotic (MZ) twins are compared with dizygotic (DZ) ones. The greater similarity of a trait or a disease in MZ twins than DZ twins points to a genetic influence. Similarity may be estimated by concordance or intraclass correlation. In a Finnish twin study, 3 of 29 MZ and 3 of 79 DZ were concordant for OAG. The heritability, the percentage of population variation in a trait that is due genes, was 10%, for POAG and 13% for combined POAG and EG. The remaining proportion, 87% remains to be explained by non-genetic factors. This indicates that glaucoma may be a multifactorial disease. The number of twins with exfoliation glaucoma was too low to allow a separate calculation for exfoliation. In contrast, in an Icelandic twin study of 50 MZ twin pairs and their spouses, the concordance for OAG was high, 98%, among twins compared with a surprisingly high 70% concordance found in twin/spouse pairs.

3.2.4.2.3. Inheritance

In family studies, the proposed mode of inheritance has varied. Both autosomal dominant and recessive pattern have been reported, but mostly the inheritance has not followed a Mendelian model of single-gene disease. On the other hand, the pattern of inheritance may have been complicated by reduced or age-dependent penetrance, or new mutations resulting in an atypical pedigree. In the population-based Barbados Eye Study, 1048 families with a family history of glaucoma were analysed by the Statistical Analysis for Genetic Epidemiology (S.A.G.E.). This method is a logistic regression procedure that incorporates family structure and is used to test hypotheses about transmission of traits to be correlated in relatives. In this population, the results of S.A.G.E. analysis were consistent with a major gene with a dominant OAG allele. However, no single Mendelian mode of inheritance (i.e. autosomal dominant) can adequately describe POAG as a whole.

Because of large variations in disease expression, great differences in prevalence, and higher recurrence risk for close relatives, glaucoma is today considered to be a polygenic (multiple contributing genes) or multifactorial disease where both genetic and environmental factors affect
The individual genes underlying a multifactorial trait follow the Mendelian principles of segregation, but many of them act together to influence the trait. Those who have more disease-causing genes and environmental factors are more likely to develop the disease. Also, other individual signs of POAG maybe heritable, including cup-to-disc ratio, IOP, the steroid response and aqueous outflow facility. 

### 3.2.4.2.4. Heritability of IOP

Siblings of glaucoma patients have been reported to have higher IOP. Their lifetime risk of elevated IOP was 43% compared to 7% among relatives of controls (risk ratio 6.3, 95% CI 2.1-19.2) in the population-based family aggregation study. Already in 1967, Armaly found a highly significant correlation for IOP between siblings and concluded that the mode of inheritance was polygenic and multifactorial. A classical twin study of 61 MZ and 32 DZ pairs also suggested that hereditary factors may play a role in IOP. In the Beaver Dam Study, families with two or more siblings attending the study were identified during the first follow-up visit, and later the families were extended to include also the cousins. Multivariate family correlations of IOP using FCOR in the SAGE software was between sibling pairs (n=1136), parent-child pairs (n=514) and cousin pairs (n=1807) were 0.17, 0.18, and 0.12, respectively, and these were statistically significant whereas the spouse pair correlation was not. Heritability for IOP, a ratio of total genetic variance to total phenotypic variance, was estimated to be 36% when it was adjusted for age and sex. A complex segregations analysis was performed for 2337 subjects of 620 extended pedigrees from the same Beaver Dam Study material. A modest positive correlation was found between both parent-offspring (0.13) and between siblings (0.15). In this analysis, the heritability for IOP was 30%, lower than in the previous report. The correlations and heritability declined after inclusion IOP treatment and systolic blood pressure. The authors suggested some environmental factors influence both IOP and blood pressure. A genome-wide linkage analysis was performed using 486 pedigrees from the Beaver Dam Study. Seven regions of interest were identified on chromosomes 2, 5, 6, 7, 12, 15, 19.

### 3.2.4.2.5. Heritability of cup-to-disc parameters

In addition to elevated IOP, some of above mentioned studies have also investigated a possible genetic contribution and heredity of specific characteristics of an optic disc. Armaly carefully analysed C/D ratios in first-degree relatives of three different groups (2 of healthy controls and one of siblings to glaucoma patients). He found that in 92% the difference in C/D ratio between the eyes of an individual did not exceed 0.1. Also C/D in an individual was related to that of his parents and siblings. Like in his
analysis of IOP in first-degree relatives, the proposed mode of inheritance was polygenic and/or multifactorial. In the Rotterdam Eye Study, the siblings of glaucoma patients had higher and greater lifetime risk for enlarged cup-disc ratio than the relatives of subjects without glaucoma. Also, Klein investigated a possible heredity of disc parameters among the same family members as described above. The correlation of vertical cup-to-disc ratios was 0.25 in siblings, 0.24 in parents and offspring, 0.14 in avunculars, 0.04 in cousins, and 0.01 in spouses. The heritability was 0.48, but the multivariate family correlations were even higher separately for vertical diameter of optic disc and cup, 0.55 and 0.57 respectively.

3.2.4.2.6. Heredity of exfoliation syndrome

Already in 1930, Vogt suggested that exfoliation syndrome is inherited. In a Finnish study, exfoliation syndrome was found in 7% of relatives of an ES-positive patient compared with 3% in relatives of POAG patients. The respective proportion of exfoliation syndrome of an ES-positive patient was 10% among Norwegian patients. In an Icelandic twin study, 5 of 8 monozygotic twins with exfoliation syndrome were concordant for ES with their co-twin but not with their spouses, supporting the role of genetics in the aetiology underlying ES.

Tarkkanen in his thesis presented eight families with both POAG and ES or EG in two generations. Families were extended to include more members and generations. Based on their pedigrees he proposed that exfoliation was inherited in an autosomal dominant mode. In Nova Scotia, where the population originates mostly from Scotland, patients affected with ES and their relatives were invited to participate in a genetic study. Four hundred sixty-seven of a total 782 examined subjects were definitely ES positives. A well documented paternal transmission of the trait was detected among 30 multiplex families. This observation also supported the suggestion of an autosomal dominant inheritance. In two other studies, one from Canada and one from Iceland, none of fathers was the transmitter of the trait and therefore mitochondrial inheritance, X linked inheritance or autosomal inheritance with genomic imprinting was suggested.

3.2.4.3. Molecular genetics of glaucoma

The development of modern techniques in molecular genetics has made it possible to study the genetic basis of POAG, and thus possibly to find genes associated with glaucoma risk which could help us discover the disease mechanisms in POAG pathogenesis. Because there have not been any clues of the disease-causing gene(s), the most valuable method has been linkage analysis. Its principle is based on the fact that genes located near one another on the same chromosome are transmitted together and are less likely to be separated by the process of recombination during meiosis than those
which lie far apart. The relative position of the disease gene can be established with the help of genetic markers with known chromosomal locations in affected pedigrees. The probability of linkage is expressed as the logarithm of the odds in favour of linkage. In multifactorial diseases a lod score of +3.3 or higher is accepted as proof of linkage, while a lod score of -2.0 or lower is strong evidence against linkage. The main advantage of linkage analysis is that it can be used without prior knowledge of the underlying pathogenesis. Large families where the disease trait is inherited in a manner resembling the Mendelian mode of inheritance are needed for linkage analysis. However, such POAG families with several generations are difficult to find because of the late onset of glaucoma, and therefore the first genetic investigations have used juvenile open angle glaucoma (J-POAG) as a model.

The genetic evidence of heredity of juvenile-onset glaucoma was confirmed when Sheffield et al. mapped the location of a gene which causes J-POAG to the region 1q21-q31 using linkage analysis of a single family. This locus was named GLC1A, where "GLC" is for glaucoma, "1" for primary open angle glaucoma, and "A" for the first linkage for this disease. Subsequently, also other researchers reported additional families in which J-POAG was mapped in the same locus. Finally, the gene responsible for 1q linked glaucoma was identified by Stone et al. and was named trabecular meshwork induced glucocorticoid response protein (TIGR) or myocilin (MYOC). Later on seven other loci (GLC1B-H), associated with POAG have been mapped and two disease-causing genes, optineurin (OPTN) and WD40-repeat 36 (WDR36), have been identified.

3.2.4.3.1. Trabecular meshwork induced glucocorticoid response gene TIGR /MYOC

In adult-onset open angle glaucoma, three different mutations in TIGR/MYOC gene were detected in 4% of glaucoma patients with at least one first degree relative with glaucoma, 3% in unselected glaucoma patients and 0.3% in the general population suggesting that this gene is in addition to J-POAG also associated with a portion of all open angle glaucoma cases. Since then many myocilin mutations (more than 50) have been identified, both disease-causing and variations of sequence, which apparently do not cause disease and are just polymorphisms. The statistically higher prevalence of some mutations among glaucoma patients than among controls has suggested an association with glaucoma.

In three studies, the most common mutation GLN368STOP of TIGR was detected in patients with adult-onset glaucoma. The reported age at diagnosis was between 56 and 62 years, and the reported IOPs were elevated between 29 and 31mmHg. Among 1703 screened patients in different ethnic groups, this sequence variation was detected in 27 glaucoma probands.
GLN368STOP mutation was considered to be an important phenotypic modifier in the Tasmanian study GIST, where 29 individuals of 8 pedigrees carrying the mutation were identified among 1730 consecutive POAG patients. The age at diagnosis was variable as well as the severity of glaucoma which ranged from OHT to glaucoma caused legal blindness. Age-related penetrance for OHT or POAG was 72% at age 40 years and 82% at age 65 years for Gln368STOP carriers. In a case-control study, the clinical features of 66 glaucoma patients with myocilin mutation were compared to 105 matched (based on visual fields) glaucoma patients without the mutation. OAG patients with myocilin were diagnosed younger and had higher IOPs than the controls but otherwise no differences were found.

However, the patients with mutations in the MYOC gene often have an early-onset of glaucoma with high IOP, but there is a large variability of phenotypes. In 20 of 23 studies IOP have varied 29 and 62 mmHg except in one study 2 patients had 16mmHg, and age at diagnosis was between 11 and 38 years. In a six-generation Finnish family with J-POAG and POAG, 20 subjects out of 51 examined family members were carriers of mutation Thr377Met. Glaucoma was found in nine individuals (45%) and OHT in two (10%). The mean age at diagnosis was 34 (range 14–66 years). To date, about 20% of patients with early-onset glaucoma and 3%–5% of patients with adult-onset glaucoma have defects in the myocilin gene.

Myocilin protein is expressed in many ocular tissues relevant to glaucoma, including aqueous humor, trabecular meshwork, ciliary body and retinal ganglion cells but also in most tissues throughout the body. Because glucocorticoids induce myocilin in trabecular meshwork cells and cause steroid-induced glaucoma, increased myocilin production was suggested to increase resistance to aqueous outflow and thus raise IOP. However, myocilin mutations were not found in human steroid glaucoma patients. Despite extensive investigations of the gene the normal role and the mechanisms by which mutations of the encoding MYOC/ TIGR gene cause glaucoma are unknown.

3.2.4.3.2. Optineurin OPTN

Optineurin (optic-neuropathy-inducing protein, OPTN) chromosome 10p14 was the second identified gene with an association to adult-onset glaucoma. Three mutations in OPTN were found in 17% of 54 families with autosomal dominant POAG, predominantly normal tension glaucoma. The most common disease-causing alteration was E50K (Glu50→Lys) that was detected in 14% of 52 families. Clinical features which characterised E50K carriers were younger age, more advanced optic disc cupping and smaller rim area at diagnosis compared with NTG patients without the mutation. However, in a large study of 1048 glaucoma patients and 251 controls only one patient with NTG was detected with E50K.
A risk-associated alteration M98K (Met98Lys) of \textit{OPTN} was found in 14\% of a group of 169 patients with familial or sporadic glaucoma and in 2\% of normal controls\textsuperscript{168}. The M98K mutation was associated with Japanese patients with NTG but not with Caucasians patients\textsuperscript{183}. Wiggs et al.\textsuperscript{184} tried to determine whether mutations in the \textit{OPTN} contribute to susceptibility to POAG and found the M98K was in 9\% of 86 probands with glaucoma and 10\% of 80 age-matched controls, no other DNA changes were detected. Also, among 53 Italian high-tension POAG patients (IOP> 21 mmHg) with glaucomatous changes in the optic disc and visual field defects one M98K alteration was found supporting that \textit{OPTN} contributes only to a portion of NTG\textsuperscript{185}.

Optineurin is a 577 amino acid protein and it is localised in all ocular tissues, its expression in neuronal and glial cells of the retina and optic nerve indicates that it could affect retinal ganglion cell survival. \textit{OPTN} probably interacts with proteins that regulate apoptosis\textsuperscript{179}. More studies are needed to clarify the role and the function of \textit{OPTN}.

3.2.4.3.3. WD40-repeat 36 (WDR36)

Recently, the third glaucoma gene \textit{WDR36} (WD40-repeat 36) was mapped in a new POAG locus GLC1G in chromosome 5q22.1\textsuperscript{169}. In total, four different mutations were found in 17 (5.02-6.92\%) unrelated POAG patients, of them 11 had high-pressure and 6 low pressure glaucoma. However, the results of a case-control study from Australia including POAG patients from GIST showed that variants in \textit{WDR36} are neutral\textsuperscript{186}. In the United States, the prevalence of \textit{WDR36} mutations was evaluated by sequencing all 23 coding exons and flanking introns in 118 probands with POAG, 6 with J-POAG, and 108 control subjects\textsuperscript{187}. Mutations that were originally reported as disease-causing or “potential disease-susceptibility” mutations were found to be equally distributed among POAG probands and controls, and were not segregated consistently with the disease. The conclusion was that \textit{WDR36} sequence variants were associated with a more severe disease form suggesting that \textit{WDR36} may be a glaucoma modifier gene. In the latest study by Fingert et al.\textsuperscript{188} the most common disease-causing alteration Asp658Gly\textsuperscript{169} was not found at all whereas other alterations were detected only in patients without statistical association. The difference could be dependent on ethnic background, phenotypic stratification, or matching of controls. Further studies will determine the role of \textit{WDR36} as a candidate gene in glaucoma.

\textit{WDR36} encodes for a T-cell activation protein with minimum of eight WD40 repeats. The gene contains 23 exons and encodes for a 951 amino acid protein\textsuperscript{169}. It is expressed in all ocular tissues as well as other tissues outside the eyes.
3.2.4.3.4. **LOXL1** (lysyl oxidase like protein 1)

Molecular genetic research on exfoliation syndrome has been challenging. Earlier, Wiggs et al. have suggested chromosome 2p16 as a possible locus for exfoliation syndrome but the results have remained unconfirmed \(^{189}\). Recently in Icelandic and Swedish glaucoma patients, a common sequence variation **LOXL1** (lysyl oxidase like protein 1) in 15q24.1 was found to be strongly associated to exfoliation glaucoma probably through ES \(^{190}\). About 25% of the general population was homozygous for the highest risk haplotype G-G and their risk to have exfoliation glaucoma is over 100 times more than those who were carrier of low-risk haplotypes. The estimated population attributable risk was 99%. The product of **LOXL1** gene modifies elastin fibres that are a major component of exfoliation material \(^{191}\).

*In summary, open angle glaucoma seems to be inherited as an autosomal dominant trait with incomplete penetrance. Three genes TIGR/ MYOC, OPTN and WDR36 are associated with POAG and recently a gene LOXL1 for exfoliation glaucoma through exfoliation syndrome has been identified.*

3.2.5. **Myopia**

3.2.5.1. **Myopia in developing glaucoma**

Myopia has long been assumed to be associated with glaucoma. One of the first studies was a glaucoma-clinic based study by Perkins & Phelps (1982) \(^{192}\). They compared the frequency of myopia among 245 eyes with POAG with that of a normal population using statistics of National Health Service, and found that myopia of -1.0 to -5.0 D was two to six times more frequent among the glaucoma patients than among the normal subjects. In population-based studies of Beaver-Dam \(^{193}\), Blue Mountains \(^{194}\) and EMGT \(^{195}\) myopia has strongly been associated with the prevalence of open-angle glaucoma, the higher the myopia the greater the risk. In a Chinese population-based study (The Beijing Eye Study) \(^{196}\) the refraction was stratified in groups of high (-8 D), marked (-6 to -8 D), moderate (-3 to -6 D), and low myopia (-0.5 to -3 D), as well as emmetropia (-0.5 to < + 2 D) and hyperopia (≥ +2 D). The frequency of glaucoma was significantly higher in the combined group of marked and high myopia compared to the combined group of the remaining eyes OR 4.13 (CI 2.62-6.49). Interestingly, in the EMGT, the overrepresentation of glaucoma among myopic eyes declined with increasing IOP and no relationship was found in eyes with IOP ≥ 30 mmHg \(^{195}\).

3.2.5.2. **Myopia in progressive glaucoma**

So far, myopia as a contributing risk factor for the progression of glaucoma is not considered in any publications.
3.2.6. Decreased perfusion pressure

3.2.6.1. Decreased perfusion pressure in developing glaucoma
The blood flow in the optic nerve head can be disturbed in many ways: elevated IOP, decreased perfusion pressure (blood pressure minus IOP), decreased capillary diameter, and increased blood viscosity\textsuperscript{197}. Systemic hypertension, atherosclerosis, vasospasm and acute systemic hypotension have been reported to be possible factors that affect the risk of POAG\textsuperscript{198,199}. The positive correlation between blood pressure and POAG was found in three population-based studies\textsuperscript{36,200,201}. However, a rise of 10mmHg in systolic blood pressure increases intraocular pressure by 0.24 mmHg, which is not considered clinically significant\textsuperscript{200}. On the contrary, low diastolic perfusion pressure was strongly associated with ageing in POAG\textsuperscript{200,201}. In Baltimore, OR was 6.22 (CI 2.15-17.94) if the perfusion pressure was below 30 mmHg.

Recently, in an Italian survey the cardiovascular risk factors were analysed in 2879 POAG patients and 973 age-matched controls\textsuperscript{202}. POAG patients had higher systolic and diastolic blood pressures and their estimated cardiovascular risk was higher than in the controls, OR 1.38.

New methods have been developed for studying ocular blood flow such as Color Doppler imaging and Scanning laser Doppler flowmetry, with these methods a pulsatile component (pulsatile ocular blood flow, POBF) can be measured. This leads to pulsations of the volume and pressure in the eye. In a prospective study\textsuperscript{203}, eyes with untreated OHT or POAG and with IOP higher than 25 mmHg had significantly lower POBF compared to eyes which had an IOP below 25 mmHg. There was an inverse correlation between POBF and IOP suggesting that IOP is one determinant in POAG and OHT. On the contrary, Aydin\textsuperscript{204} found a significant correlation between pulsatile ocular blood flow and IOP in a group of glaucoma patients and normal participants, but there was no correlation between POBF and visual field indices or nerve fibre layer thickness.

3.2.6.2 Decreased perfusion pressure in progressive glaucoma
The influence of ocular blood flow on the progression of glaucoma has been studied in only one study with 44 POAG patients\textsuperscript{205}. The progression in the visual fields was associated with low diastolic velocity and a high resistivity index in the ophthalmic artery. The reduction in blood pressure during the night, nocturnal dip, is a physiological phenomenon. Tokunaga et al.\textsuperscript{206} studied the relation between nocturnal dip and the progression of visual field defects in patients with NTG or high tension POAG. The patients were grouped according to dip into three groups: normal dipper (physiologic dip of 10-20%), nondipper (dip of less than 10%), and extreme dipper (dip of more than 20%). Both in the nondippers and in the extreme dippers visual field defects progressed.
3.2.7. **Summary of risk factors**

Risk factors for progression of glaucoma in randomised controlled clinical trials are presented in Table 5. An elevated IOP is the only risk factor that alone can cause glaucomatous changes in the eye; if the level of IOP is remarkably high (> 30 mmHg) or if the eye is susceptible even at lower IOP levels. Slightly increased or “normal” IOP as well as other risk factors have a more or less contributing effect on either the development or progression of glaucoma, but a satisfactory pathogenetic/etiological explanation why “just this eye” converts to a glaucomatous eye is still unknown.

<table>
<thead>
<tr>
<th>Table 5. The risk factors in randomised clinical trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Progression of glaucoma</strong></td>
</tr>
<tr>
<td><strong>EMGT</strong></td>
</tr>
<tr>
<td>HR (CI)</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>(1.04–2.09)</td>
</tr>
<tr>
<td>IOP (mean)</td>
</tr>
<tr>
<td>(1.18–2.48)</td>
</tr>
<tr>
<td>IOP fluctuation (SD-IOP)</td>
</tr>
<tr>
<td>0.81–1.24</td>
</tr>
<tr>
<td>Exfoliation syndrome</td>
</tr>
<tr>
<td>(1.31–3.74)</td>
</tr>
<tr>
<td>Field defect at diagnosis</td>
</tr>
<tr>
<td>(1.10–2.28)</td>
</tr>
<tr>
<td>Optic damage at diagnosis</td>
</tr>
<tr>
<td>1.32</td>
</tr>
<tr>
<td>(1.19–1.47)</td>
</tr>
<tr>
<td>Vascular dysregulation</td>
</tr>
<tr>
<td>1.00</td>
</tr>
<tr>
<td>(0.87–3.72)</td>
</tr>
<tr>
<td>CCT (thinner)</td>
</tr>
<tr>
<td>(1.40–2.09)</td>
</tr>
<tr>
<td>Family history</td>
</tr>
<tr>
<td>(0.84–1.77)</td>
</tr>
</tbody>
</table>


‡‡ Pattern Standard Deviation PSD ‡‡‡Low blood pressure
4. Patients and methods

This study is based on three different patient materials. For the retrospective study of visual disability (I), permission to collect data on the diagnoses and causes of death of the glaucoma patients was granted by Statistics Finland. The study protocol for clinical and molecular genetic investigations of exfoliation syndrome in Kókar (II and III) was approved by the Ethics Committee of the Åland Central Hospital. The study of TIGR and OPTN genes in glaucoma families (IV) was also a clinical and molecular genetic study and was approved by the Ethics Committee of the Helsinki University Eye Hospital, and the use of old clinical material was permitted by the Ministry of Social Affairs and Health. All examined subjects for Studies II, III and IV gave their written informed consent.

4.1. Patients

4.1.1. Visual disability, a retrospective study (I)

The patients for the study were drawn from the database of my own private practice office in Ekenäs where I had been working since 1978. The patient compliance with visual field testing was poor at the beginning, because of the long journey to Helsinki and therefore a Fiedmann Analyzer was purchased in 1980 for the office. Since 1989, a Humphrey Field Analyzer I and Canon Fundus Camera CF-60U 60° have been used. At the same time in 1989 patient records of all glaucoma patients and glaucoma suspects were redesigned and separated from the other charts. During subsequent years, a database on glaucoma patients was created and included 643 patients when it was reviewed for the study in 2002 (Figure 1). Of these 118 had deceased between 1991 and 2002. The primary inclusion criteria for the study of visual disability (I) were: a treated open angle glaucoma or ocular hypertension (OHT), and treatment time of at least 2 years unless blindness had occurred earlier. The follow-up of two years was chosen to allow the possibility to detect a progression in glaucoma during the follow-up. Visual disability induced by glaucoma were analysed patient wise, but when associations between risk factors and blindness were calculated, only one eye of each patient was chosen as the "study eye". This eye was the eye with more advanced glaucoma at diagnosis or, when both eyes were equally affected the right eye.

A total of 106 patients who fulfilled the inclusion criteria were identified (Figure 1). Sixty-seven of these were females and 39 males. At diagnosis,
Figure 1. The study design in Ekenäs.
39 patients had primary open angle glaucoma (POAG), 27 exfoliation glaucoma (EG) and 40 OHT. The mean age was 72 years (SD 8.4; range 47–88). Glaucoma was bilateral in 64 patients (60%). The mean treatment time from the initialisation of treatment to the last follow-up visit was 10 years (SD 5.8; range 1–23). The mean interval between the last visit to the office and the date of death was 8 months (SD 9.7 months). The mean age at death was 82 years (SD 7.1)

4.1.2. Exfoliation Syndrome; prevalence and inheritance; population-based cross-sectional and longitudinal study (II)

H. Forsius and A. Eriksson conducted a population-based study between 1960 and 1962 on the Kökar Island. In that study, all inhabitants over 10 years of age were invited for a clinical examination. A total of 595 subjects (85% of the population) were examined, 247 of them were over 50 years old (89 males/158 females) (Figure 2). The mean age of all the subjects was 64 years. During the subsequent decades, follow-up studies of the same population, their relatives, and those who had not participated in the first examination were organised. Studies were performed five times between 1975 and 1995. In 2000, the author was invited to join the team for the sixth follow-up study of the population. This latest expedition to Kökar was arranged in 2001–02 when 183 Kökar inhabitants or descendents participated in the study (II and III) in 65 of them this was their first examination. At the same time, they also gave a blood sample for molecular genetic investigations. In all 7 examinations, altogether 965 subjects were examined at least once. At the last examination 530 (237 males/293 females) were over 50 years of age with a mean age of 63 (Figure 2). The population was followed over 40 years in three generations.

4.1.3. Genome wide scan of exfoliation syndrome (III)

The patients for the study III were chosen from the 183 participants who attended the last examination on the Kökar islands. These were all 28 ES affected subjects, (of them 9 had glaucoma), and as the control group 36 unaffected relatives: 22 first degree and second degree relatives (siblings, uncles, aunts, cousins) (mean age 77, range 63–90), 10 more distant relatives (mean age 80, range 70–94), and four offspring (mean age 58, range 51–65). None of the controls had POAG. Altogether 64 samples were analysed.

4.1.4. The role of TIGR and OPTN genes in Finnish glaucoma families (IV)

A possible existence /prevalence of mutations /polymorphisms in the two first identified glaucoma genes TIGR and OPTN was studied primarily in Study IV and additionally in four patients included in Study III.

The eligibility for Study IV was family history of glaucoma. During the practice in the office, all glaucoma patients had answered inquires on
Figure 2. The study population in Kökar.
ES exfoliation syndrome; +, affected, -, unaffected;
EG; exfoliation glaucoma; POAG, primary open angle glaucoma.
whether they had any knowledge of family members with glaucoma. The inclusion criterion was at least three glaucoma patients in the same family (including grandparents, aunts, uncles and cousins). Nine probands were identified. First the pedigrees were constructed with the probands’ help, but later after the genealogical investigations using The National Archives of Finland, two pedigrees were linked together into one large pedigree resulting in eight pedigrees. Family members over 40 years of age were invited to a comprehensive ophthalmological examination. The clinical status was determined in 102 subjects after an examination. In 23 cases, the diagnosis of glaucoma was confirmed from the files of their responsible ophthalmologist or from the hospital records after receiving their permission. In 11 subjects data on blindness and glaucoma remained as a reported history. Thus the phenotype was determined altogether for 136 family members. Of these 107 subjects gave a blood sample for molecular genetic analysis. Sequencing of *TIGR* and *OPTN* genes was performed on 11 subjects of the 8 pedigrees (8 POAG, 2 EG and 1 ES).

From the Kökar population (II and III), four glaucoma patients, one with POAG and three with EG; were chosen for sequencing of *TIGR* and *OPTN* genes as a comparison to the glaucoma patients from the Ekenäs region (IV). Surprisingly, an alteration in the *OPTN* gene was detected in one patient with exfoliation glaucoma. Subsequently, this exon was sequenced in 70 family members of this proband. Of these 18 had ES, 6 had EG, 4 had POAG and the remaining 42 subjects were healthy relatives.

4.2. Methods

4.2.1 Clinical examination

Clinical examination (I, II, IV) included refraction with the Welch-Allyn refractometer, the best corrected visual acuity with subjectively adjusted refraction values at five metres, IOP measurement with Goldmann applanation tonometer, slit-lamp biomicroscopy with a Haag Streit biomicroscope (Bern, Switzerland). Gonioscopy was performed with Goldman gonioscope-lens before dilatation (I and IV). For pupillary dilatation, tropicamide 5mg/ml, (Santen, Tampere, Finland) was instilled. All optic discs were evaluated after dilatation stereoscopic using a Volk 78D, Volk Super Field NC lens (Keeler, Windsor. UK) or Goldmann 3-mirror lens. The description of the optic disc included drawings and C/D values vertically and horizontally, but also in words as excavated, totally excavated, pale, notching, and disc haemorrhage. Stereo photographs (two separate slides in different angles) taken during the follow-up were used for the disc evaluation if possible (I) and in Study IV for all examined subjects.
4.2.2. Definitions

4.2.2.1. Exfoliation syndrome
Exfoliation syndrome was defined after dilatation by the presence of a greyish central disc with/without focal breaks, with or without a peripheral band on the anterior lens capsule, and/or fibrillary material on the pupillary ruff, and recorded without grading. A subject was defined as ES-positive, if ES was detected at least in one eye. Suspect changes such as Krukenberg’s spindle and pigmentation of cornea endothelium, diffuse haze on the anterior capsule, were noted as ES negative.

4.2.2.2. Visual Fields (I, IV)
Initially visual fields were measured with the Friedmann Analyzer (Haag-Streit, Bern, Switzerland) and the Goldmann perimeter (Haag-Streit) and after 1989 with the 30-2 or 24-2 full-threshold or SITA programs of the Humphrey Field Analyzer I or II (Humphery Field Analyzer II Model 745 (Carl Zeiss) Humphrey-Zeiss Systems, Dublin, California). The visual field test was repeated if the result was unreliable or was in conflict with the findings in the optic disc.

4.2.2.3. Diagnostic criteria for glaucoma
The diagnostic criteria for glaucoma were two of the three characters: 1) IOP > 21 mmHg (I) and IOP > 22 mmHg (IV), 2) presence of glaucomatous changes in the optic nerve head, and 3) glaucomatous visual field defect.

The disc changes were graded as glaucomatous when one of the following was detected: localized thinning of the rim, diffuse damage with cup-to-disc ratios C/D > 0.7 (I) and > 0.6 (IV), or an asymmetry of ≥ 0.2 in C/D between the eyes with equal size discs. A suspicious change was noted if C/D was 0.5 to 0.6 (II).

Visual field defects were graded according to definitions by Hodapp et al. Visual field defects were graded according to definitions by Hodapp et al. 207. The minimum criteria for a glaucomatous defect was, when a cluster of three or more nonedge points were depressed on the pattern deviation plot at a p < 5% level and at least one of them was depressed at a p < 1% level. Early defect was: mean deviation (MD) is less than -6 dB, on the pattern deviation plot fewer than 25% of the points are depressed below the 5% level and fewer than 10 points are depressed below the 1% level, and none in the central 5° is less than 15 dB. Moderate defect was: MD is less than -12 dB, on the pattern deviation plot fewer than 50% of the points are depressed below the 5% level and fewer than 20 points below 1% level, none of the points in the central 5° has sensitivity of 0 dB, and only one hemifield may have a point with sensitivity less than 15 dB within 5° of fixation. Severe defect was any of the following findings: MD is greater than -12 dB, on the pattern deviation plot more than 50% of the points are depressed below 5% level and more than 20 points below 1% level, any point in the central 5° has
a sensitivity of 0 dB or there are points less than 15 dB within the central 5° in both hemifields. “Borderline” Glaucoma Hemifield Test results were graded as normal. The older visual fields (before 1990) were tested with a Friedmann Analyser (threshold related points: normal, relative defect or not seen) and these were modified to correspond to visual fields by a Humphrey Analyser.

4.2.2.4. Staging of glaucoma (I)
A real analysis of progression was impossible to do, because stereo photographs of optic discs were available only for 51 patients (I) and different types of visual field tests during the follow-up were used (at diagnosis 104 /106, a follow-up visual field was missing in 19 patients: 10 with visual disability, 9 due to short follow-up time or old age with healthy discs). Therefore a more robust method was chosen: the stage of glaucoma was classified in 5 categories. (Table 6)

<table>
<thead>
<tr>
<th>Stage of glaucoma</th>
<th>Disc AND Visual field*</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHT</td>
<td>Normal</td>
</tr>
<tr>
<td>Stage 1 (early)</td>
<td>Glaucomatous change** or Normal or Early defect MD &lt; -6dB</td>
</tr>
<tr>
<td>Stage 2 (moderate)</td>
<td>Glaucomatous change**</td>
</tr>
<tr>
<td>Stage 3 (advanced)</td>
<td>C/D 0.8–0.9</td>
</tr>
<tr>
<td>Stage 4 (blind)</td>
<td>C/D 1.0, pale</td>
</tr>
</tbody>
</table>

* Modification of the grading system of glaucomatous visual field defects defined by Hodapp et al.207 **Glaucomatous change= diffuse damage with C/D > 0.7 or localised thinning of the rim (notching) or C/D asymmetry between the eyes 0.2 or greater

4.2.2.5 Visual disability (I)
The World Health Organisation (WHO) defines a person as visually impaired if the best corrected visual acuity (VA) is less than 0.3 in the better eye and as blind if VA in the better eye is less than 0.05 or the visual field is constricted with a diameter less than 20 degrees. The US definitions for legal blindness is 0.1 or 20/200, visual field criterion is the same. A person is visually disabled if he is unable to perform certain tasks because of visual impairment. A person who has visual disability may have either low vision or blindness.

4.2.3. Genealogical studies (II and IV)
The information regarding the names and birth dates and places of birth of parents, grandparents and great grand parents was collected from all participants. In the Kökar population the same questionnaire was used throughout the whole study (II). The genealogical study was performed as described by Varilo et al. 208. The names, dates, and places of birth of the
patients’ parents were used to trace ancestors back to the middle of the 1800s from local church and civil registers. Microfilm and microfiche copies of the church records in the Finnish National Archives were used for all earlier periods. The collected data were used to construct the pedigrees with the CorelDRAW 11 program.

4.2.4. Laboratory procedures

4.2.4.1. DNA extraction and polymerase chain reaction (PCR) (III and IV)
Genomic DNA was extracted from peripheral blood samples using Puregene DNA whole blood kit (Gentra Systems, Minneapolis, USA) (study III and IV). PCR reactions were designed based on primer sequences for individual exons. For sequencing Big Dye Terminator kit (version 3) by Applied Biosystems (ABI, Foster City, CA, USA) was used. Mutations were first checked manually and verified by BLAST-program. All possible mutations were verified by new PCR and new sequence analysis from both strands. A detailed description of the procedures is described in the original articles 209,210.

4.2.4.2. Genome wide scan (III)
The screening was performed with the genome wide map of 1000 microsatellite markers of intermarker interval ~3 cM according to the deCode marker map 211. Genotypes were constructed with standard procedures 211. For genotyping error elimination, the PEDCHECK program was utilized 212,213.

4.2.5. Statistical methods
For Study I, the data were analyzed with the SPSS software package (version SPSS 13.0 for Windows, SPSS, Chicago, Ill, USA). One way ANOVA was used to study the association,

The homogeneity of variances was tested with the Levene test and Tukey was used to adjust the multiple comparisons. Fisher’s exact test was used to compare proportions. Kruskal-Wallis test with Bonferroni correction (StatXact-3 software, version 3.02, Cytel Software, Cambridge, MA, USA) was used to analyze the stage of glaucoma between groups. The cumulative incidence and Kaplan-Meyer data were analyzed with the Stata statistical software (Release 7.0, Stata Co, College Station, TX, USA) with the stcompat automatic do-file and R statistical software (version 1.4.0, The R Foundation for Statistical Computing, Vienna, Austria) with the –cmprsk- library.

Cumulative incidence was chosen to analyze time to blindness caused by glaucoma because Kaplan-Meier analysis, originally developed to assess mortality, assumes that all patients censored from analysis are still at risk from the event of interest. In this analysis, a patient who failed from a competing risk and who is therefore not capable of a later failure is not redistributed among all patients known to be at risk of failure 214.
For Study II the genetic contribution to ES was investigated by a segregation analysis conducted on the nuclear families contained within the larger pedigree, where the parents had a known ES status. The heritability of the IOP quantitative trait was estimated using the SOLAR software. To further interpret the result of this analysis, correlations were estimated separately using the FCOR subprogram of the Statistical Analysis for Genetic Epidemiology (S.A.G.E.) software package. The statistical programs used in Study III are shown in Table 7.

<table>
<thead>
<tr>
<th>Program</th>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLINK of LINKAGE</td>
<td>two-point linkage analyses</td>
<td>218,219</td>
</tr>
<tr>
<td>package</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HOMOG 3.35</td>
<td>heterogeneity and proportion</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>of linkage</td>
<td></td>
</tr>
<tr>
<td>ANALYZE</td>
<td>MLINK and HOMOG</td>
<td>221</td>
</tr>
<tr>
<td>DOWNFREQ 2.1</td>
<td>allele frequencies</td>
<td>222</td>
</tr>
<tr>
<td>Vitesse</td>
<td>multipoint analysis</td>
<td>213,223</td>
</tr>
</tbody>
</table>

Table 7 The following statistical programs were used (III)
5. Results and discussions

5.1. Visual disability (I)

The purpose of the study was to find out how many of the treated glaucoma patients really had visual problems, and which risk factors were associated with glaucoma caused blindness. Visual impairment and blindness were analysed patient wise, but the risk factors were evaluated only in the study eyes.

5.1.1. Patients

5.1.1.1. Visual impairment

At diagnosis, two patients were visually impaired because of cataract (visual acuity less than 0.3 or 20/60 in the better eye) and at the last follow-up visit 17 patients (16%, 95 % CI 9-23). Six patients (35% of 17) were bilaterally impaired because of glaucoma. (Table 8)

<table>
<thead>
<tr>
<th>N</th>
<th>study eye</th>
<th>fellow eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>glaucoma</td>
<td>glaucoma</td>
</tr>
<tr>
<td>1</td>
<td>glaucoma</td>
<td>AMD</td>
</tr>
<tr>
<td>1</td>
<td>glaucoma and circulation</td>
<td>vascular event</td>
</tr>
<tr>
<td>1</td>
<td>glaucoma and thrombosis</td>
<td>amblyopy</td>
</tr>
<tr>
<td>2</td>
<td>AMD</td>
<td>AMD</td>
</tr>
<tr>
<td>1</td>
<td>AMD</td>
<td>glaucoma</td>
</tr>
<tr>
<td>1</td>
<td>cataract</td>
<td>AMD</td>
</tr>
<tr>
<td>1</td>
<td>cataract</td>
<td>cataract</td>
</tr>
<tr>
<td>1</td>
<td>Transient ischemic attack</td>
<td>Transient ischemic attack</td>
</tr>
<tr>
<td>1</td>
<td>diabetic retinopathy</td>
<td>diabetic retinopathy</td>
</tr>
<tr>
<td>1</td>
<td>glaucoma and thrombosis</td>
<td>secondary cataract</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.1.1.2. Blindness in glaucoma patients

When both criteria for blindness, WHO and US, were applied three patients were unilaterally blind at diagnosis. The causes were glaucoma, uveitis and trauma, one of each. At the last visit, 34 patients (32%) had blindness at
least in one eye, of them 21 (20%) were unilaterally and 13 (12%) bilaterally blind (Table 9).

Table 9. Visual disability of 106 patients at their last visit

<table>
<thead>
<tr>
<th>Visually impaired</th>
<th>Blindness</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OHT at dg (n=40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ES (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glaucoma at dg (n=66)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>POAG (39)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EG (27)</td>
<td></td>
</tr>
<tr>
<td>unilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>all reasons</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>glaucoma</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>bilateral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>all reasons</td>
<td>17</td>
<td>3†</td>
</tr>
<tr>
<td>glaucoma</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Ocular Hypertension (OHT), Exfoliation syndrome (ES), Primary open angle glaucoma (POAG), Exfoliation glaucoma (EG)

* one developed ES
† in one patient one eye was blind because of glaucoma and the other eye because of other reasons
‡ In three patients one eye was blind because of glaucoma and the other eye because of other reasons
!! See Table 10

Glaucoma was the cause of blindness in both eyes in 6 patients (6%, 95% CI 1–10) and in one or both eyes in 16 patients (in 6 patients bilaterally, in 7 patients unilaterally, and in one eye of 3 bilaterally blind patients) (15%, 95% CI 8–22). (Table10)

Table 10. The causes to bilateral blindness in 13/106 patients (unpublished)

<table>
<thead>
<tr>
<th>N</th>
<th>study eye</th>
<th>fellow eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>glaucoma</td>
<td>glaucoma</td>
</tr>
<tr>
<td>1</td>
<td>glaucoma + thrombosis</td>
<td>amblyopy and cataract</td>
</tr>
<tr>
<td>1</td>
<td>glaucoma+ vascular problems</td>
<td>vascular problems</td>
</tr>
<tr>
<td>1</td>
<td>AMD and glaucoma</td>
<td>glaucoma and AMD</td>
</tr>
<tr>
<td>2</td>
<td>AMD</td>
<td>AMD</td>
</tr>
<tr>
<td>1</td>
<td>vascular problems</td>
<td>vascular problems</td>
</tr>
<tr>
<td>1</td>
<td>cataract</td>
<td>cataract</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

5.1.1.3. Discussion

Visual disability was found in 38 patients of 106 (36%) which indicates that a great proportion of glaucoma patients have some kind of visual problems in their lifetime. Seventeen (16%) had visual impairment bilaterally and 13 (12%) were bilaterally blind. Visual acuity was decreased to a low-vision level in one eye in 21 patients and bilaterally in 17 patients.
The same type of patients as ours was studied in Norwich, United Kingdom. The authors found that at final visit 14% (17/121) had partial sight and 4 were classified as blind. The mean age at diagnosis, the follow-up time, and the age at death (75 years, 8 years, and 80 years, respectively) were close to our patients (72 years, 10 years, and 82 years, respectively). These small differences and the exclusion of exfoliation glaucoma from the study might have been reasons for their better results.

In my glaucoma population, glaucoma was the cause in half of the cases with handicap. In a Swedish study on glaucoma patients with visual impairment, AMD was the main reason (48%) for blindness and glaucoma only in 20% of cases. This result may have reflected a very high mean age of that patient group (80 to 85 years). Among Finnish people over 70 years of age in Northern Finland, glaucoma was considered the main cause of visual impairment 1.5% of the population and in 12% of all individuals with visual impairment, and 12% of the glaucoma patients. In population-based visual impairment studies, AMD and cataract are the major cause of blindness and glaucoma is responsible for only a small proportion of these.

Bilateral blindness caused by glaucoma was found in 6% of the patients in our study which is comparable to the previously reported results. In all retrospective studies, Chen, Hattenhauer and ours, the proportion of glaucoma caused blindness is less than the proportion (10%) which Quigley has used in his calculations for estimated rates on glaucoma caused blindness worldwide. If we assume that our patients were an average Finnish glaucoma patient, and apply our results (6%) to calculate the proportion of blindness caused by glaucoma in Finland using the number of glaucoma patients KELA has provided, we should have about 4000 blind persons because of glaucoma, much more than are registered in the Finnish Register of Visual Impairment. But the KELA’s register also includes patients with ocular hypertension, therefore the real number of glaucoma patients is uncertain. Because all visually impaired persons are not registered in the Finnish Register for Visual Impairment, and the assumed number of individuals with visual disability is much higher, the results of the ongoing study of causes to visual disability in the Health 2000 project will be utmost important in the future.

5.1.2. Study Eyes
(n=106) (the worse eye with glaucoma at diagnosis or the right eye if equally affected)

5.1.2.1. Stage of glaucoma
During the 10 year follow-up, glaucoma remained stable in 51 eyes of 106 study eyes (48%) and a change of stage was detected in 55 eyes (52%): an increase of one stage in 31 eyes (29%), two stages in 15 eyes (14%), and three stages in 8 eyes (8%) (Figure 3). Age in different stages of glaucoma
at diagnosis was: 69 years among OHT patients, 73 in the group of stage 1, 74 in the group stage 2, and 76 in the group of stage 3.

Figure 3 The change in stage of glaucoma in 106 study eyes.

Age at death was associated with the stage of glaucoma: Patients with OHT (n=20) were youngest, mean age 80 years. Age increased according to the severity of glaucoma. Patients with stage 1 (n=31) had a mean age of 81 years, with stage 2 (n=24) and with stage 3 (n=16) 83 years, and with stage 4 (n=15) 86 years.

Originally 40 eyes had OHT, 50% of them converted to glaucoma in a mean time of 8.6 years (range 2-22 years). One of these eyes went blind because of haemorrhagic glaucoma. Factors which may have contributed to the conversion are shown in Table 11 (unpublished)

Table 11. Analysis of progression in 40 patients with OHT (unpublished)

<table>
<thead>
<tr>
<th>40 OHT patients</th>
<th>Stable (n=20)</th>
<th>Change of the stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 stage (n=10)</td>
<td>2 stages (n=6)</td>
</tr>
<tr>
<td>Mean age</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td>Exfoliation</td>
<td>7 (1 male / 6 fem)</td>
<td>2 male</td>
</tr>
<tr>
<td>IOP before*</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>SD-IOP**</td>
<td>3</td>
<td>3.3</td>
</tr>
<tr>
<td>Poor Compliance</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

*weighted mean IOP before the treatment
**standard deviation of IOP during the treatment, reflects fluctuation
5.1.2.2. Discussion
The challenging problem for the clinician is ocular hypertension: to treat or not to treat. In the OHTS 19, 4.4% of the eyes in the medication group developed glaucoma in 5 years. In the Malmö Ocular Hypertension study, 90 subjects were randomized into two groups: treatment with timolol or treatment with placebo 71. During the mean follow-up of 8.5 years (max 17.5 years), altogether 37 (41%) developed glaucoma, the proportions were equal in the treatment and control groups. Our conversion rate was 50% and the mean follow-up was 9.8 years with a maximum of 23 years. The main difference between these studies was mean age at diagnosis: 55 years in the OHTS 7, 65 years in Malmö and 69 years among our patients with OHT. Because of this great difference, the study populations were not comparable. The threshold for treatment has been higher in Ekenäs (i.e. treatment was initialised at higher IOP level), and some patients have been without unnecessary treatment for 14 years compared with the patients in the OHTS. High age, the variability of IOP, and poor compliance seemed to be associated with progression.

5.1.2.3. Blindness in the study eyes
At the last visit before death, 28 of the study eyes (26%, 95% CI 18-35) were blind according to US criteria and 27 eyes according to WHO criteria. Glaucoma had caused blindness in 15 eyes (14%, 95% CI 8-21) according to both criteria. In seven of these 15 eyes the criterion for blindness was constriction of visual field with visual acuity better than 20/200. Other causes for blindness were macular degeneration (4), cataract (4), CRVO (2), cerebral vascular accident (1), and complications due to cataract surgery (2).

To perform the cumulative incidence analysis, the study eyes were regrouped according the causes for visual outcome: glaucoma caused blindness, competing causes (other reasons) for blindness and no blindness. The cumulative incidence of blindness because of glaucoma was 6% (95% CI 2-11) at 5 years, 9% (95%, CI 4-15) at 10 years, and 15% (95% CI 9-23) at 15 years compared to the Kaplan-Meyer estimates 7% (95% CI 3-14), 11% (95% CI 6-21), and 28% (95% CI 7-45) respectively. The corresponding incidences for blindness caused by competing causes were 3% (95% CI 1-8), 9% (95% CI 4-15), and 11% (95% CI 6-18) respectively (Figure 4 A and B). The figure shows that among glaucoma patients, glaucoma is the cause of blindness as often as all other causes combined.
5.1.2.4 Factors associated with blindness in the study eyes (Table 12)
In cumulative incidence analysis, exfoliation syndrome doubled the incidence of blindness caused by glaucoma compared with POAG eyes; 8% (95% CI 2-19) at 5 years, 16% (95% CI 6-29) at 10 years, and 21% (95% CI 10-35) at 15 years vs. 5%, 5%, and 12%, respectively (Figure 4 C and D) as well as Kaplan-Meyer estimates 9%, 24%, and 46% for EG eyes, vs. 5%, 5%, and 21% for POAG eyes. This difference was not seen when blindness was caused by other causes.

High IOP before initialisation of the treatment (pre-IOP), and fluctuation of IOP defined as high standard deviation (SD) of all measured IOPs during the follow-up (SD-IOP) until the end-point blindness was reached, were significantly associated with glaucoma caused blindness, p=0.03 and 0.001 respectively (Table 12 ). Though the mean decrease in IOP from pre-IOP
(mean 33 mmHg, range 22-52) was 33% in the group of glaucoma caused blindness it had not any favourable effect (p=0.10) on the visual outcome. The level of the mean IOP during treatment was nearly the same in all three groups (glaucoma caused blindness, competing causes (other reasons) for blindness and no blindness), and no effect could be seen.

The stage of glaucoma at diagnosis was significantly associated for developing blindness. From the eyes going blind because of glaucoma, 10 of 15 eyes (67%) had moderate or advanced glaucoma at the diagnosis. The corresponding proportion was 2 of 13 eyes (15%) among those going blind because of other causes and 15 of 78 eyes (19%) among those with preserved vision. The more advanced was the glaucomatous damage in the optic discs and visual fields, the more likely was it that glaucoma progressed to blindness (p=0.002 Kruskall-Wallis, exact).

The definition of non-compliance for the study included: not taking prescribed medication, refusal of operations or visual field testing, and forgetting scheduled visits. In total 33% of 106 patients were non-compliant. The compliance was poorer among those with moderate or advanced glaucoma at diagnosis than among those with early glaucoma (p=0.04, Fisher’s exact test).

High age at diagnosis was associated with poor visual outcome (p = 0.04 ANOVA). In both groups of blindness (caused by glaucoma and by other causes), the mean age at diagnosis was the same, but the mean age of the nonblind group was lower. However, pairwise comparisons failed to confirm a statistically significant difference between any two groups.

A positive family history of glaucoma and vascular causes of death did not differ significantly between the three groups (glaucoma caused blindness, competing causes (other reasons) for blindness and no blindness).
Table 12. Analysis of possible factors associated with visual disability in 106 study eyes

<table>
<thead>
<tr>
<th>Factor</th>
<th>Blindness caused by glaucoma (N=15)</th>
<th>Blindness caused by other reasons (N=13)</th>
<th>No blindness (N=78)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, years</td>
<td>75</td>
<td>76</td>
<td>71</td>
<td>0.04†</td>
</tr>
<tr>
<td>Family history (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3 (20)</td>
<td>3 (24)</td>
<td>12 (15)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>12 (80)</td>
<td>10 (76)</td>
<td>66 (85)</td>
<td>0.64§</td>
</tr>
<tr>
<td>Stage of glaucoma at diagnosis * (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHT</td>
<td>1 (6)</td>
<td>4 (30)</td>
<td>35 (45)</td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>4 (27)</td>
<td>7 (54)</td>
<td>28 (36)</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (47)</td>
<td>1 (8)</td>
<td>14 (18)</td>
<td></td>
</tr>
<tr>
<td>Advanced</td>
<td>3 (20)</td>
<td>1 (8)</td>
<td>1 (1)</td>
<td>0.002‡</td>
</tr>
<tr>
<td>Pre-IOP, mmHg</td>
<td>33</td>
<td>30</td>
<td>28</td>
<td>0.03†</td>
</tr>
<tr>
<td>Treatment IOP, mmHg</td>
<td>19</td>
<td>21</td>
<td>19</td>
<td>0.39†</td>
</tr>
<tr>
<td>Decrease in IOP (%)</td>
<td>33</td>
<td>26</td>
<td>23</td>
<td>0.10†</td>
</tr>
<tr>
<td>IOP-SD, mmHg</td>
<td>4.6</td>
<td>3.8</td>
<td>3.2</td>
<td>0.001†</td>
</tr>
<tr>
<td>Compliance (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good (67%)</td>
<td>6 (40)</td>
<td>8 (61)</td>
<td>57 (73)</td>
<td></td>
</tr>
<tr>
<td>Poor (33%)</td>
<td>9 (60)</td>
<td>5 (39)</td>
<td>21 (27)</td>
<td>0.04†</td>
</tr>
<tr>
<td>Vascular cause of death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>8 (53)</td>
<td>7 (54)</td>
<td>39 (50)</td>
<td>1.00§</td>
</tr>
<tr>
<td>Negative</td>
<td>7 (47)</td>
<td>6 (46)</td>
<td>39 (50)</td>
<td></td>
</tr>
</tbody>
</table>

†ANOVA ‡Kruskal-Wallis, exact §Fisher’s exact test *See Table 6

5.1.2.5. Discussion
There are no studies on treatment of glaucoma where the study end point has been visual disability. In the Olmsted Study, the Kaplan-Meyer estimate at 20 years was 9% for glaucoma caused bilateral blindness and 27% for unilateral blindness in patients with OHT or “classical glaucoma” (at least two of the following: IOP ≥ 22, glaucomatous optic disc and/or visual field) corresponding well with our estimate at 20 years in this study. In our study, exfoliation syndrome doubled the risk of getting blind because of glaucoma. This was shown in the curves of cumulative incidence analysis. In clinical practice, exfoliation syndrome has seemed to be associated with worse prognosis, and there are only a few studies evaluating its effect on progression. In the EMGT, the risk for progression was increased by 2.2. At least in Scandinavian countries, where the prevalence of exfoliation glaucoma is highest, it is important to remember this worse prognosis when treating patients with exfoliation glaucoma.

There has been a lot of argument about whether initial IOP before treatment, decrease in IOP from pre-treatment level, mean level of IOP during treatment, and/or high fluctuation of IOP during treatment have any
effect on the progression of glaucoma. So far, IOP is still the only treatable risk factor. In our study, the pre-treatment IOP and high variability of IOP described as standard deviation of all measured IOPs (SD-IOP) until blindness was noted were associated with glaucoma caused blindness. However, mean level of IOP or the decrease in IOP from the pre-treatment level had no effect.

In the AGIS and the EMGT, the fluctuation of IOP was also analysed as standard deviation of measured IOPs as we had. The AGIS investigators found that eyes with SD-IOP of over 3.0 mmHg had a significant visual field progression with time whereas eyes with SD-IOP less than 3.0 mmHg remained stable during the follow-up \(^6\). In the EMGT, SD-IOP was 2.02 mmHg in those who progressed and 1.78 mmHg in those who did not progress \(^7\), but if the analysis was performed in a similar way as in the AGIS, the fluctuation was a risk factor. The authors speculated if the intensive care caused an iatrogenic effect. In all three groups (glaucoma caused blindness, competing causes (other reasons) for blindness and no blindness) of our study SD-IOP was over 3.0 mmHg and in the eyes with glaucoma caused blindness SD-IOP was 4.6 mmHg.

In the CIGTS, the targeted decrease in IOP was 35% from pre-treatment level, and glaucoma progression was found in 10% to 12% of subjects in both study arms (initial medical treatment vs. initial surgical treatment \(^8\)). In our study, 33% decrease in IOP from pre-treatment level was not associated with blindness caused by glaucoma. At first glance our results were controversial: the greatest decrease was in the study eyes which progressed to blindness and lowest in the eyes which did not go blind. This could be explained by the fact that the eyes which had got blind had higher pre-treatment IOP and also more damaged optic discs which were more vulnerable to even to moderate level IOP. Though the decrease in IOP seemed to be adequate it may not have been enough for these eyes. In the AGIS, the visual field score remained stable in the eyes where the mean IOP was 12 mmHg and always under 18 mmHg during the follow-up \(^9\). Therefore the goal should be a decrease of at least 50%, if possible, or to an IOP close 10 mmHg in eyes with moderate or advanced glaucoma.

Poor compliance reflects a difficulty to understand the severity of glaucoma. Approximately 30% to 60% of glaucoma patients do not use their medications as prescribed \(^4,5\). Surprisingly, many patients with an advanced stage of glaucoma at diagnosis were less compliant than those with early glaucoma. In my experience, the patients may obey the instructions they receive with their prescriptions quite well, but they frequently refuse visual field testing or a proposed visit to a tertiary care centre. We may recognise the noncompliant patients as those who need more of our time and patience. We may improve compliance with better patient education \(^22\).
5.2 Exfoliation Syndrome; prevalence and inheritance (II)

The purpose of the study was to determine the prevalence of exfoliation syndrome in an isolated population in both cross-sectional and longitudinal assessment, and then using the collected information of the families of three generations to determine the mode of inheritance of exfoliation syndrome.

Two hundred and forty-seven (247) of all the subjects examined (595) were older than 50 (89 males/158 females). The prevalence of ES among them was 8%, in males 7% and in females 9%. The prevalence increased with age and was 18.4% among 76 individuals over 70 years (males 13% and females 25%). Three males had EG and one male POAG. No female of over 50 years had any type of glaucoma. The odds ratio (OR) of glaucoma (ES vs. no ES) was 40 (95% CI 4-404) for all and for males 82 (95% CI 7-1039). However, two females who were younger than 50 had ES (one with exfoliation glaucoma).

5.2.2. The population studied between 1960 and 2002
Altogether 530 subjects, 237 males and 293 females, were over 50 years of age at their last examination. During this period, a total of 76 subjects (14%) with ES were identified, of them 23 were males (10%) and 53 females (18%). (Table 13)

<table>
<thead>
<tr>
<th>Age in years</th>
<th>50–59</th>
<th>60–69</th>
<th>70–79</th>
<th>≥ 80</th>
<th>mean Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>68</td>
<td>100</td>
<td>4</td>
<td></td>
<td>454</td>
</tr>
<tr>
<td>Male ES– n</td>
<td>86</td>
<td>96</td>
<td>11</td>
<td>5</td>
<td>76</td>
</tr>
<tr>
<td>Female ES– n</td>
<td>72</td>
<td>87</td>
<td>15</td>
<td>8</td>
<td>52</td>
</tr>
<tr>
<td>%</td>
<td>100</td>
<td>96</td>
<td>11</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>ES+ n</td>
<td>4</td>
<td>11</td>
<td>10</td>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>%</td>
<td>0</td>
<td>4</td>
<td>13</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>unilateral ES+</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>bilateral ES+</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Individuals with EG</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 13 Exfoliation Syndrome (ES) and Exfoliation Glaucoma (EG) in the population

ES– unaffected with ES, ES+ at least one eye affected with ES, ES in either eye unilateral, ES in both eyes bilateral
The mean IOP in subjects with ES without glaucoma was 17 mmHg (range 10-27), and among those with glaucoma (EG) 28 mmHg (range 16-52). The mean IOP in unaffected subjects without glaucoma was 17 mmHg (8-29) and among those with glaucoma (POAG) 26 mmHg (range 16-38). In unilateral cases, the affected eye had a mean IOP of 19 mmHg (range 10-47) and the unaffected eye 16 mmHg (range 11-24).

Exfoliation glaucoma was found more often in males (11 subjects of 23 ES positive males, 48%) than in females (13 subjects of 53 ES positive females, 25%) whereas POAG was almost as frequent in males (7 of 214 ES negative males, 3%) as in females (5 of 240 ES negative females, 2%). The relative risk (RR) of glaucoma (ES vs. no ES) was 12 (95% CI 6.2-22.9) for all the subjects, for males it was 15 (95% CI 6.3-34.0) and for females it was 12 (95% CI 4.4-31.6).

5.2.3. Discussion
The prevalence of exfoliation syndrome in the cross-sectional study (18%) was comparable to that (22%) in Oulu, Northern Finland, among subject aged 70 or more. This invalidated our hypothesis that the prevalence would have been higher on the Kókar island than elsewhere in Finland because of its isolation. The other characteristics that were found: the probability of having ES was associated with higher age, ES was more prevalent among females than males, and IOP was higher in eyes with ES, were similar as reported in many studies. The relative risk for glaucoma was considerably increased in ES positive males (15 in males vs. 12 in females). The higher risk for males (RR 30 for males vs. 10 for females) has also been previously detected in a cohort of 72 ES positives. Ringvold et al. had reported a sex difference in proportions of subjects with exfoliation glaucoma in a Mid Norway study (36% in males vs. 26% in females).

5.2.4. Family studies
A pedigree was constructed for all exfoliation affected subjects, including two ES positive females under 50 years of age. Seventy-five of the 78 ES positive subjects were genuine Kókar inhabitants, and their small pedigrees were linked into an extended large pedigree with 332 examined individuals over 50 years of age (Figure 5 on the back page). An aggregation of ES was found among examined family members (22%) compared with the whole population (14.5%). However, the sibling recurrence risk \(K_s\) for siblings over 70 years of age in this extended family was 15% and the sibling recurrence risk ratio \(\lambda_s\) for all population was 0.8 according to the formula suggested by Farbrother et al.

5.2.5. The pattern of transmission
To evaluate the mode of transmission, 110 nuclear families were split into subgroups: unaffected ES negatives, affected ES positives, and unexamined
(Table 14). About one third of the parents in each subgroup had at least one affected offspring except for the subgroup of ES-positive fathers, in which only one had two affected offspring (a daughter aged 59 years and a son aged 64). (Figure 6)

<table>
<thead>
<tr>
<th>Offspring</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
<th>Siblings</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=117</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES-</td>
<td>80</td>
<td>37</td>
<td>116</td>
<td>74</td>
</tr>
<tr>
<td>ES+</td>
<td>106</td>
<td>13</td>
<td>119</td>
<td>21</td>
</tr>
<tr>
<td>ES-</td>
<td>186</td>
<td>50</td>
<td>236</td>
<td>62</td>
</tr>
<tr>
<td>ES+</td>
<td>74</td>
<td>36</td>
<td>110</td>
<td></td>
</tr>
</tbody>
</table>

*at least one of the sibs was affected; unaffected individual ES-, affected individual ES+

Figure 6. Pedigree of the father with exfoliation syndrome (ES) (indicated by an arrow) and his affected offspring. ES is represented by the black upper half of the symbol. The unexamined individuals are represented by a question mark. The age of diagnosis for affected individuals or age at last examination for unaffected is below the symbol. The generations represented by Roman numerals.
All daughters of the other ES affected fathers were unaffected, 4 of 5 were under 60 years of age and a probability of later exfoliation cannot be excluded. The proportion of ES positives among males (11%) was much lower than among females (32%), though the total number of investigated male and female offspring was nearly the same.

### 5.2.6. Segregation ratio

The segregation ratio of ES was estimated in the offspring from the nuclear families (Table 15). These nuclear families were stratified into two classes of parental mating types 1) diagnoses of unaffected by unaffected in the parents and 2) at least one parent diagnosed with ES. When both parents were unaffected, the segregation ratio was 18%, in males 2 affected of 26 (8%) and in females 10 affected of 42 (24%) and when at least one parent was affected the corresponding ratios were very similar 16%, 4/46 (9%) and 9/33 (27%), respectively.

<table>
<thead>
<tr>
<th>Mating type</th>
<th>Nuclear families (n)</th>
<th>Children (n)</th>
<th>Affected Children (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Female Male</td>
<td>Female Male</td>
</tr>
<tr>
<td>U x U</td>
<td>22</td>
<td>22 16  6 3</td>
<td></td>
</tr>
<tr>
<td>N x U</td>
<td>8</td>
<td>4  5 3 0</td>
<td></td>
</tr>
<tr>
<td>A x U</td>
<td>4</td>
<td>1  7 0 0</td>
<td></td>
</tr>
<tr>
<td>U x N</td>
<td>18</td>
<td>16 26 9 4</td>
<td></td>
</tr>
<tr>
<td>N x N</td>
<td>28</td>
<td>42 26 10 2</td>
<td></td>
</tr>
<tr>
<td>A x N</td>
<td>4</td>
<td>3  8 1 1</td>
<td></td>
</tr>
<tr>
<td>U x A</td>
<td>14</td>
<td>17 15 5 2</td>
<td></td>
</tr>
<tr>
<td>N x A</td>
<td>9</td>
<td>10 12 3 1</td>
<td></td>
</tr>
<tr>
<td>A x A</td>
<td>3</td>
<td>2  4 0 0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>117 119 37 13</td>
<td></td>
</tr>
</tbody>
</table>

U : unknown, A : affected with exfoliation syndrome (ES)
N : not affected with ES

According to pattern of transmission and segregation ratios exfoliation syndrome is most likely to be inherited as an autosomal dominant trait with reduced penetrance.

### 5.2.7. Discussion

The strength of our Kökar pedigree is that the ES affected subjects were drawn from a population-based study and then the pedigrees were constructed. In the earlier studies the investigations have been extended to family members after detecting an ES affected individual. Tarkkanen 92,158 had examined families with exfoliation syndrome and he was the first to propose that ES is inherited in an autosomal dominant mode. Lately this has been supported by
Aasved and Orr. Because affected mothers were the only transmitters in large pedigrees from Iceland and Canada, X-linked or mitochondrial inheritance could be other possibilities for inheritance. Our results in the evaluation of offspring (Table 14) and in the segregation analysis (Table 15), were consistent with an autosomal dominant mode of inheritance. The penetrance of ES was remarkably more reduced in males than in females corresponding well with the prevalence of ES in males and females. The recurrence risk for siblings was low, being 15%.

5.2.8. Heritability of IOP
The heritability of IOP was estimated to be 30% when the entire pedigree was analyzed intact using the SOLAR software. Separate pairwise correlations were calculated for the sibling pairs; 52 male-male, 119 male-female, and 75 female-female. Correlations were 0.02, 0.13 and 0.56 respectively, thus genes have a much greater effect on the variance of IOP in females than in males.

5.2.9. Discussion
Already in 1967, Armaly had observed that IOP was correlated between siblings and proposed a multifactorial inheritance. Nearly 30 years later, the heritability of IOP was estimated in siblings in the Beaver Dam Study. Their result of 36% or 30% (depending on adjustments used in the analysis) was close to ours of 30%. The genetic contribution to IOP seems to be well confirmed because the studies were performed in different populations (both white) and the approach was different.

5.3. Genome wide scan of exfoliation syndrome (III)

The purpose of the study was to localise or identify gene/genes underlying ES. A genome wide scan was performed in an extended family for 64 subjects, of them 28 ES positives and 36 controls from the same pedigree.

5.3.1. Regions for suggested linkage
Seven markers suggested a linkage (Zmax>1.5) at regions of chromosomes 2q32.3, 5q33.3, 17p13.3, 18q12.1-21.33 and Xp22.2 when autosomal dominant mode of inheritance was assumed. The highest two-point LOD score of 3.45 (θ=0.04) was found in 18q12.1-21.33 (Figure 7). The likelihood for a linkage (higher LOD score, higher likelihood) was even higher when combined information for different markers was used, three-point LOD score of 4.33 (θ= 0.05; 0.08). Clinically interesting was that one allele on the best marker D18S468 was found in 37% of ES affected vs. in 26% of ES negatives. Another allele of the same marker was less frequent (25%) in ES affected than in unaffected subjects (34%).
5.3.2. Discussion

The starting point for detecting genes which, when mutated, cause or predispose to a disease is usually to find the locus on a chromosome where the gene can be expected to reside. This is usually performed with linkage or association studies in families/populations with affected and un-affected individuals. Because of the late age when exfoliation syndrome manifests itself it has been extremely difficult to collect large numbers of families, even only siblings for a normal genetic study. Earlier the region 2p16 has been suggested as a possible locus for exfoliation but the results have remained unconfirmed \(^\text{189}\). Therefore tissues of the anterior segments (iris, ciliary processes, lens epithelium) of eyes have been used in searching for possible genes underlying the syndrome and the causes for glaucoma \(^{129,230}\).

The positional cloning approach is easiest when the disease has monogenic (Mendelian) inheritance and the phenotype is clear cut. In case of ES, the situation is very different. ES is a phenotype that a clinician can detect when the patient is old enough to show that phenotype. Even though genetic factors have an important role in its aetiology and we have suggested autosomal dominant mode of inheritance, with reduced penetrance, based on our Kökar pedigree, other modes of inheritance including multifactorial 

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**Figure 7. Chromosome 18 and the markers. The alleles with and without exfoliation syndrome.**
inheritance cannot be excluded. In addition, the trait is very likely to be genetically heterogeneous, similar to most of the other common traits. To add to the difficulties in this positional cloning approach, the very inbred Kökar population is not easy to analyse as many of the individuals are related to each other through many routes.

In spite of these problems, we managed to find a very promising locus on chromosome 18 and, in addition, evidence for some other possible though less likely loci. Recently, an Icelandic group has identified a common sequence variation in chromosome 15q24.1\textsuperscript{190}. The data suggested that the gene \textit{LOXL1} was associated to exfoliation glaucoma in both Icelandic and Swedish glaucoma patients mainly through exfoliation syndrome. The homozygotic carrier of the risk haplotype was estimated to have 2.5-fold risk for EG compared with general population. In our genome wide scan, 15q21.2 had 2-point LOD score of 1.186. In addition the gene \textit{LOXL1}, there could be other predisposing and preventing genes, as well as exogenic factors, which all together might explain the fact that ES appears not to have full penetrance in the family and only some individuals with ES develop EG.

5.4. The role of TIGR and OPTN in the pathogenesis of glaucoma (III and IV)

The purpose was to search for mutations in \textit{TIGR} or \textit{OPTN} genes in Finnish glaucoma families from two different backgrounds, Ekenäs (IV) and Kökar (III).

\textbf{5.4.1. Clinical investigations in the Ekenäs region}

In all 8 families the disease was inherited resembling the autosomal dominant mode of inheritance. Phenotypes of the 136 family members in the Ekenäs region were determined. (Table 16 and Figure 8) Of these 51 had glaucoma: 44 POAG and 7 EG. Glaucoma was suspected in 22 (28%) of initially healthy relatives. The cause of suspicion was IOP > 22mmHg in 7/22 (32%), asymmetry between the discs in 8/22 (36%), suspicious disc in 6/22 (27%), and disc haemorrhage in 1/22 (5%).
Figure 8. The age of diagnosis (for affected individuals) or age at examination is presented below the symbol. Persons with a bar above their symbol were examined by EF. Persons with a double bar above their symbol had their diagnosis confirmed by hospital records or their own ophthalmologist. A diagnosis based on a history of glaucoma is indicated by an “H”. An asterisk (**) indicates that the TIGR and OPTN genes were sequenced.
### Table 16 Clinical characteristics of 136 family members in eight Finnish glaucoma families

<table>
<thead>
<tr>
<th>136 family members</th>
<th>N</th>
<th>Examined&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Age at diagnosis/ examination years mean (range)</th>
<th>Diagnosis</th>
<th>Age at diagnosis/ confirmed&lt;sup&gt;2&lt;/sup&gt; examination years mean (range)</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blind</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>POAG</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult onset</td>
<td>43</td>
<td>24</td>
<td>62 (40–81)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-POAG</td>
<td>1</td>
<td>1</td>
<td>21</td>
<td>12</td>
<td>65 (50–79)</td>
<td>7</td>
</tr>
<tr>
<td>Suspicion of glaucoma</td>
<td>22</td>
<td>20</td>
<td>54 (23–75)</td>
<td>2</td>
<td>57 (51–66)</td>
<td></td>
</tr>
<tr>
<td>EG</td>
<td>7</td>
<td>4</td>
<td>69 (61–74)</td>
<td>3</td>
<td>68 (52–86)</td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>3</td>
<td>3</td>
<td>73 (71–73)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy relatives</td>
<td>54</td>
<td>49</td>
<td>57 (33–87)</td>
<td>5</td>
<td>57 (31–75)</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>136</td>
<td>102</td>
<td>23</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Examined personally  <sup>2</sup>hospital records or own ophthalmologist

Primary open angle glaucoma = POAG, Juvenile primary open angle glaucoma = J-POAG
Exfoliative glaucoma = EG, Exfoliation Syndrome = ES

### 5.4.2. Molecular genetic investigations

Eleven subjects (8 with POAG, 2 with EG and one with ES) from different families were selected for sequencing (IV). None of them had earlier reported disease causing mutations in TIGR or OPTN genes. Instead, 5 polymorphism were detected, one of them was a novel 553-5C in OPTN (Table 17)

### Table 17. Polymorphisms in TIGR and OPTN (unpublished)

<table>
<thead>
<tr>
<th>TIGR</th>
<th>POAG</th>
<th>EG</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyr347Tyr</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Arg76Lys</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OPTN</th>
<th>POAG</th>
<th>EG</th>
<th>ES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thr34Thr</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glu163Glu</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>553-5C</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

### 5.4.3. Discussion

Reported positive family history of glaucoma was the inclusion criterion to this study. The prevalence of glaucoma was 35% among examined (or confirmed) first and/or second degree relatives which corresponds the previous reports<sup>12,150,190</sup>. The proportion of glaucoma suspects was high, 28%, among those relatives who were considered healthy.

Mutations in the newly detected glaucoma genes, TIGR and OPTN, were not present in the adult-onset glaucoma population originating from
the Swedish speaking costal area in Southern Finland. However later, one mutation of TIGR was detected in a family with J-POAG and POAG from Central Finland. The investigation of our families continued with exclusion of other suggested glaucoma loci, none was detected. Our results were very fair due to small number of families analysed, but as the first molecular genetic study of Finnish glaucoma patients this was the first step into the new era.

5.4.4. Investigations on the Kökar population (III)

This was a pilot study performed at the same time as Study IV. Three patients with EG and one with POAG from Kökar were sequenced for TIGR and OPTN mutations. A sequence alteration M98K (Met98Lys) of OPTN was detected in one male patient with EG. Subsequently, 70 relatives (18 with ES, 6 with EG, 4 with POAG, 42 healthy) were tested for this mutation. The change was found in one of his sons and in two nephews Table 18, who all had healthy eyes.

![Figure 9. The family with exfoliation syndrome and M98K mutation](image)

<table>
<thead>
<tr>
<th>Family members</th>
<th>M98K</th>
<th>Age*</th>
<th>Exfoliation</th>
<th>IOP mmHg</th>
<th>Optic disc</th>
<th>Visual field **</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index</td>
<td>+</td>
<td>69</td>
<td>+</td>
<td>37</td>
<td>+ notching</td>
<td>Excavation</td>
</tr>
<tr>
<td>Son 1</td>
<td>+</td>
<td>61</td>
<td>-</td>
<td>18</td>
<td>18</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Son 2</td>
<td>-</td>
<td>55</td>
<td>-</td>
<td>28</td>
<td>28</td>
<td>Nasal</td>
</tr>
<tr>
<td>Nephew 1</td>
<td>-</td>
<td>46</td>
<td>-</td>
<td>20</td>
<td>16</td>
<td>Normal Normal</td>
</tr>
<tr>
<td>Nephew 2</td>
<td>+</td>
<td>42</td>
<td>-</td>
<td>16</td>
<td>18</td>
<td>Normal Normal</td>
</tr>
<tr>
<td>Nephew 3</td>
<td>+</td>
<td>40</td>
<td>-</td>
<td>16</td>
<td>16</td>
<td>Normal Normal</td>
</tr>
</tbody>
</table>

*age at diagnosis, ** visual fields tested at the Central Hospital of Åland; c/d = cup/disc ratio, ³ Dx right eye, ⁴ Sin left eye
5.4.5. Discussion

The OPTN gene is associated with normal tension glaucoma (NTG). M98K was reported as a risk-associated alteration and was found in 13.6% of the examined families\(^\text{168}\). Our finding was interesting because the index patient had initially high IOP and exfoliation syndrome. At examination, the son and the nephews were young and therefore the answer whether there is any association between M98K and glaucoma will remain to be revealed in the future.

5.4.6. Summary of the Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Purpose and inclusion criteria</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td><strong>Purpose</strong>: to evaluate risk factors associated with visual disability caused by glaucoma</td>
<td>Factors associated with blindness:</td>
</tr>
<tr>
<td></td>
<td><strong>Inclusion</strong>: treated open angle glaucoma at least 2 years, deceased</td>
<td>• Higher age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Fluctuation of IOP (SD-IOP)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Exfoliation syndrome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Advanced stage of glaucoma at diagnosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Poor compliance</td>
</tr>
<tr>
<td>Study II</td>
<td><strong>Purpose</strong>: to analyse characteristics of exfoliation syndrome in an isolated population: prevalence, risk for glaucoma and inheritance</td>
<td>• Prevalence of exfoliation syndrome was not enriched in this population</td>
</tr>
<tr>
<td></td>
<td><strong>Inclusion</strong>: inhabitant of Kōkar Island</td>
<td>• RR (ES vs no ES) for glaucoma was 12 among all</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• RR for glaucoma was 15 among ES-positive males</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Inheritance: autosomal dominant with reduced penetrance</td>
</tr>
<tr>
<td>Study III</td>
<td><strong>Purpose</strong>: genome wide scan of exfoliation syndrome in selected inhabitants of Kōkar island</td>
<td>• Seven regions suggested linkage (Zmax &gt; 1.5)</td>
</tr>
<tr>
<td></td>
<td><strong>Inclusion</strong>: family member of the extended pedigree, all ES-positives and controls oldest possible close relatives</td>
<td>• Promising chromosome 18q12.1-21.33 with two-point Lod score 3.45</td>
</tr>
<tr>
<td>Study IV</td>
<td><strong>Purpose</strong>: to find out possible mutations of TIGR and OPTN genes in glaucoma families from Ekenäs region</td>
<td>• No mutations were found</td>
</tr>
<tr>
<td></td>
<td><strong>Inclusion</strong>: positive family history</td>
<td>• A new polymorphism in OPTN</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Family history was a strong risk factor</td>
</tr>
</tbody>
</table>
6. Conclusions

The daily work with patients challenges ophthalmologists to determine if this patient has a risk to develop glaucoma, or if he/she has glaucoma, what are his/her risks for blindness. For the patient the long follow-up time through his/her lifetime is binding either he/she has glaucoma or is suspected to have. For most patients the diagnosis is unexpected due to the asymptomatic course of glaucoma.

In our study, factors associated with blindness induced by glaucoma were higher age, exfoliation syndrome, fluctuation of IOP, an advanced stage of glaucoma at diagnosis and poor compliance. Of these risk factors, we may try to treat IOP more effectively and may try to improve patient compliance. The goal is to prevent or at least delay visual disability, however, a good care does not prevent progression it only diminishes the risk for progression. To my mind, therefore it is important to have enough time to inform the patient about the disease already at the beginning of treatment.

Exfoliation syndrome contained an increased risk for glaucoma caused blindness and it also remarkably increased the risk for glaucoma (RR 12 for all; RR15 for males), especially in males. Therefore, patients with exfoliation syndrome need aggressive treatment when they have glaucoma and a careful follow-up also if they do not have glaucoma. We found a promising locus for exfoliation syndrome in chromosome 18, but to understand in which way it is associated with exfoliation we need more studies and more patients, if possible in successive generations. The newly identified LOXL1 gene was found to have a strong correlation to exfoliation glaucoma through exfoliation syndrome. The homozygotic carrier of the risk haplotype was associated with 700 times greater risk to have exfoliation glaucoma than those carrying only the low risk haplotype, or about 2.5 times of the population average. For the first time there is a real possibility for genetic tests, but is it worth? Is the LOXL1 gene the major gene or are there more genes to understand better the pathophysiology underlying ES and get an answer to the question: Why do 65% of those affected with ES never develop glaucoma?

In the family study from the Ekenäs region, 51 of the 136 family members had glaucoma and 25 subjects had a suspicion of glaucoma or exfoliation syndrome alone. In clinical practice, it is important to encourage glaucoma patients to tell their close relatives about their own glaucoma. Regular visits to an ophthalmologist may help to detect glaucoma in the early stage before severe damages occurs, and may prevent visual disability caused by glaucoma.
One day, if we know the glaucoma gene in the family or in the population, we will be able to develop a genetic test that would be of great benefit to relatives too.

Because half of the patients are undetected\textsuperscript{16,24,36} a better screening model should be developed. We actually need a randomised screening and follow-up of a defined population to get the real answers for the decision making: is it really worthwhile to screen a population for glaucoma\textsuperscript{34,54}? The second challenge is also what kind of tests we are going to use, a simple disc photo, fast perimetry-programme or a combination of several tests. Is IOP measurement needed for screening? My supervisor, Prof Forsius has proposed that optic discs of all males doing their military service would be systematically documented by photo as a control for the future use when they later visit an ophthalmologist.

In summary, so far we do not have any genetic test to detect individuals who are susceptible for glaucoma and we cannot concentrate our efforts on them. We, ophthalmologists have to improve general awareness of glaucoma in the population and to be positive when we are asked to give lectures to our colleagues with other specialities and to various civil organisations. We also have to learn how we can better recognise risk factors both in healthy persons and in glaucoma patients in order to prevent glaucoma caused blindness.
7. Acknowledgements

I wish to express my deepest gratitude to both supervisors Prof. emer. Henrik Forsius, MD, Folkhälsan Institute of Genetics, and Docent Eija Vesti, MD, Department of Ophthalmology, Helsinki University. They both have encouraged and supported me during the years and shared feelings of success and disappointments. With them I have had the privilege to learn ophthalmic research during six decades from the end of the 1940s up to date 2007. Though times go by, the fundamental approach to science stays.

I am most grateful to Docent Irma Järvelä, MD, Department of Medical Genetics, Helsinki University, whom I met by chance. She was immediately enthusiastic to start working with glaucoma, a new disease for her. I admire her patience to explain again and again how genes are inherited and what molecular genetics is.

I wish to sincerely thank Professor Anja Tuulonen, MD, Oulu and Professor Helena Kääriäinen, MD, National Public Health Institute, Helsinki, for their critical and constructive evaluation of the manuscript.

My deepest gratitude is owed to Anja Olsbo, MD, with whom I share my practice in Ekenäs, for sharing the interest in high quality examinations of our patients. Without good documentation capabilities with Humphrey perimetry and a fundus camera there would not have been any follow-ups to study and analyse. I want to thank Mrs. Beatrice Pulkkinen, who has taken all visual fields and fundus photos with an admirable patience. As well, I thank Mrs Ann-Louise Holmberg for helping me with last minute papers and organising some days free for scientific work.

The practical work in molecular genetics would have never been done without FM Susanna Lemmelä, who started with us as a young student and is just now finishing her own thesis on glaucoma genes. I am proud and grateful for her to be my co-worker.

It has been a real joy to work with the rest of our team at Folkhälsan, Professor emer. Aldur Eriksson who has helped me to find people on Kökar island and Prof. emer. Johan Fellman, who has answered numerous questions about statistics. Through this team of three professors, Forsius, Eriksson and Fellman, I have also discovered a new part of Finland, Åland.

I want to give an especially warm thank you to the former Head of the Department of Ophthalmology Professor Leila Laatikainen, MD, who was the first to help me with scientific writing and applications forms to the ethical committee, and also to Professor Tero Kivelä, MD, the current Head of the Department of Ophthalmology for helping me with statistics and graphics.
I am greatly indebted to Eeva-Marja Sankila, MD, for teaching me how to draw pedigrees and map family members, and Teppo Varilo, MD for encouraging me to make the large pedigree of Kökar inhabitants. Also, many thanks belong to Mrs Helena Dahlblom, Åland, for helping to find all family members on Kökar.

I offer my sincere thanks to Mr Donald Smart, B.sc.M.A., for checking the language in the shortest possible time.

My warm thanks go to Kaija Saarelma, MD, allowing me to use her “thought” on the cover.

Finally, the warmest gratitude goes to my closest people. Without their tireless patience during the years, this work would have been impossible. My husband, Kukka, gave me an enormous amount of support, including with endless valuable long discussions into late nights.

This study was financially supported by grants from the Finska Läkaresällskapet, the Foundation Glaucoma Lux, the Eye Foundation, the Eye and tissue Bank Foundation, the Juselius Foundation, the Mjölbolsta Foundation and the Ålands kulturstiftelse.

Ragnvalds, October 2007

[Signature]
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