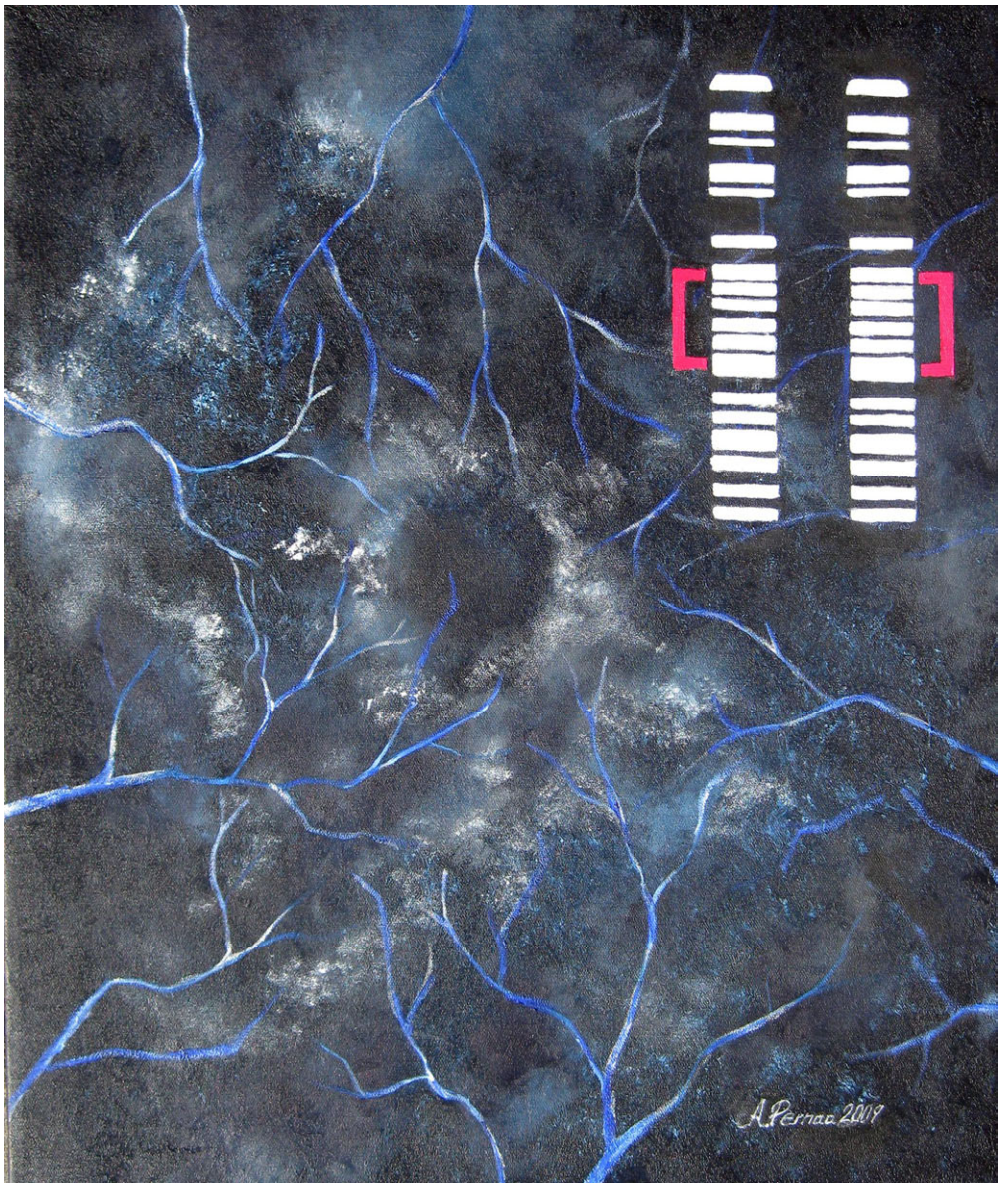
# MOLECULAR GENETICS OF PRIMARY OPEN ANGLE GLAUCOMA AND EXFOLIATION SYNDROME

**Susanna Lemmelä**




UNIVERSITY OF HELSINKI

**Susanna Lemmelä**

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ACADEMIC DISSERTATION

*To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki, in Auditorium XII, Main Building, Unioninkatu 34, Helsinki, on May 29th 2009, at 12 noon.*

Department of Medical Genetics, University of Helsinki, Finland

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**Supervised by**

Docent Irma Järvelä, MD, PhD
Department of Medical Genetics
University of Helsinki
Helsinki, Finland

**Reviewed by**

Docent Anne Remes, MD, PhD
Department of Neurology
University of Oulu
Oulu, Finland

Professor Mansoor Sarfarazi, PhD
Molecular Ophthalmic Genetics Laboratory
University of Conneticut Health Center
Farmington, Conneticut

**Discussed with**

Professor Kari Majamaa, MD, PhD
Institute of Clinical Medicine
University of Oulu
Oulu, Finland

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*”Kaikki on vastausta, tietäisipä vain kysymyksen”*

*”Answers are everywhere, if you just know the question”*

Paul-Eerik Rummo

**To my family**

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## ORIGINAL PUBLICATIONS

This thesis is based on the following original articles on the genetics of glaucoma and exfoliation syndrome, referred to in the text by the Roman numerals I-V.

**I** Forsman.E*, **Lemmelä S***, Varilo T, Kristo P, Forsius H, Sankila EM, Järvelä I (2003) The role of OPTN and TIGR in Finnish glaucoma families: a clinical and molecular genetic study. *Molecular Vision*, 9:217-222.

**II** **Lemmelä S**, Ylisaukko-oja T, Forsman E, Järvelä I (2004) Exclusion of 14 candidate loci for primary open angle glaucoma in Finnish families. *Molecular Vision,* 10: 260-264.

**III** Puska P, **Lemmelä S**, Kristo P, Sankila E-M, Järvelä I (2005) Penetrance and phenotype of the Thr377Met myocilin mutation in a large Finnish family with juvenile- and adult-onset primary open-angle glaucoma. *Ophthalmic Genetics*, 26:17-23.

**IV** **Lemmelä S**, Forsman E, Eriksson A, Forsius H, Järvelä I (2007) Genome-wide scan of exfoliation syndrome. *Investigative Ophthalmology & Visual Science*, 48(9):4136-42.

**V** **Lemmelä S**, Forsman E, Onkamo P, Nurmi H, Laivuori H, Kivelä T, Puska P, Heger M, Eriksson A, Forsius H, Järvelä I (2009) Association of LOXL1 gene to Finnish exfoliation syndrome patients. *Journal of Human Genetics, Apr 3*.

*Equal contribution

Articles I and IV are also included in Eva Forsman's thesis.

Some unpublished data has also been included in the thesis.

## ABBREVIATIONS

| | |
|---|---|
| **α** | proportion of linked families |
| **ACG** | angle-closure glaucoma |
| **ASP** | affected sibpair |
| **bp** | base pair |
| **cM** | centiMorgan |
| **C/D** | cup/disc ratio |
| **CD/CV** | common disease/common variant |
| **DNA** | deoxyribonucleic acid |
| **DZ** | dizygotic |
| **EG** | exfoliation glaucoma |
| **ES** | exfoliation syndrome |
| **$h^2$** | heritability |
| **HGP** | Human Genome Project |
| **HPG** | high pressure glaucoma |
| **HTG** | high tension glaucoma |
| **htSNP** | haplotype tagging SNP |
| **IOP** | intraocular pressure |
| **IBD** | identical by decent |
| **JOAG** | juvenile-onset open angle glaucoma |
| **kb** | kilobase |
| **LC** | liability class |
| **LD** | linkage disequilibrium |
| **LOD** | logarithm of odds |
| **LOH** | loss of heterozygosity |

| | |
|---|---|
| ***LOXL1*** | lysyl oxidase like 1 |
| ***MYOC*** | myocilin |
| **Mb** | megabase |
| **MZ** | monozygotic |
| **NPL** | non-parametric linkage |
| **NTG** | normal tension glaucoma |
| **NPG** | normal pressure glaucoma |
| **OAG** | open angle glaucoma |
| **OR** | odds ratio |
| **OHT** | ocular hypertension |
| ***OPTN*** | optineurin |
| **PAR** | population attributable risk |
| **PCG** | primary congenital glaucoma |
| **PCR** | polymerase chain reaction |
| **POAG** | primary open angle glaucoma |
| **RNA** | ribonucleic acid |
| **RR** | relative risk ratio |
| **SNP** | single-nucleotide polymorphism |
| ***TIGR*** | trabecular meshwork-induced glucocorticoid response |
| **θ** | recombination fraction |
| **VF** | visual field |
| ***WDR36*** | WD40-repeat 36 |
| **XFG** | exfoliation glaucoma |
| **XFM** | exfoliation material |
| **XFS** | exfoliation syndrome |
| $\mathbf{Z_{max}}$ | maximum LOD score |

**ABSTRACT**

Glaucoma is a group of optic neuropathies, characterized by progressive optic nerve degeneration, excavation of the optic disc due to apoptosis of retinal ganglion cells and corresponding visual field defects. Open angle glaucoma (OAG) is a subtype of glaucoma, classified according to the age of onset into juvenile (JOAG) and adult- forms with a cut-off point of 40 years of age. The prevalence of adult onset OAG is 1-2% of the population over 40 years old and higher, even 10%, of over 65-year old individuals, whereas JOAG is rare and especially aggregated in families.

Exfoliation syndrome, age, elevated intraocular pressure (IOP) and genetic predisposition are known risk factors for OAG. Exfoliation syndrome (XFS) is characterized by accumulation of grayish scales of fibrillogranular extracellular material (exfoliation material, XFM) in the anterior segment of the eye. XFS is overall the most common identifiable cause of glaucoma (exfoliation glaucoma, XFG). The prevalence of XFS and XFG varies between different ethnic populations, but the average worldwide prevalence ranges from 5% to 20% of the population older than 60 years. Familial aggregation and twin studies have shown a strong genetic contribution to both OAG and XFS/XFG, but the underlying genetic component is in many respects unknown.

During the last decade several candidate loci and three candidate genes, *myocilin (MYOC)*, *optineurin (OPTN)* and *WD40-repeat 36 (WDR36)*, for OAG have been identified. We investigated the role of the *MYOC* and *OPTN* genes and fourteen candidate loci in eight Finnish glaucoma families. Both candidate genes and loci were excluded in families.

To investigate the genetic basis of glaucoma in a large Finnish family with juvenile and adult -onset OAG, we analysed the *MYOC* gene in family members. We identified glaucoma associated mutation (Thr377Met) in the *MYOC* gene segregating with the disease in the family. This was the first molecular genetic explanation of glaucoma reported in the Finnish population.

In order to identify the genetic susceptibility loci for XFS, we carried out a genome-wide scan in the extended Finnish XFS family. This scan produced promising candidate locus on chromosomal region 18q12.1-21.33 and several additional putative susceptibility loci for XFS.

In the past year, three single nucleotide polymorphisms (SNPs) on the *lysyl oxidase like 1* (*LOXL1*) gene have been repeatedly associated with XFS and XFG in several populations. We performed a case-control and family-based association study and family-based linkage study to evaluate whether SNPs in the *LOXL1* gene contain a risk for XFS, XFG or POAG in the Finnish patients. A significant association between the *LOXL1* gene SNPs and XFS and XFG was confirmed in the Finnish population. However, linkage was not observed for *LOXL1* risk alleles in the Finnish XFS family.

This thesis describes the first molecular genetic studies of OAG and XFS/XFG in the Finnish population. Previously reported candidate genes and loci were not responsible for glaucoma in Finnish families, further confirming the heterogeneous nature of OAG. Identification of a susceptibility mutation, Thr377Met in the *MYOC* gene, has great significance for the glaucoma family and encourages investigating the *MYOC* gene also in other Finnish OAG families. Genome-wide scan of XFS highlighted an interesting candidate region on chromosome 18. This locus provides a solid starting point for the future fine-scale mapping studies, which are needed to identify variants conferring susceptibility to XFS in the region. Three SNPs in the *LOXL1* gene were found to confer risk to XFS and XFG in the Finnish population. However, probably other genetic and environmental factors also are involved in the pathogenesis of XFS and XFG.

# 1 INTRODUCTION

The word "Glaucosis" was first mentioned in Hippocratic writings in ancient Greece, in 400BC, as a blinding disease occurring most commonly in the elderly. It is thought that “glaucosis” probably included various sight-threatening conditions including cataract and keratitis in addition to glaucoma. The first clear recognition of absolute glaucoma came with Rikchard Banister in 1622 but it was not until the beginning of the 19$^{th}$ century that the first excellent description of glaucoma with raised ocular tension was given by the Frenchman Dr Antoine-Pierre Demours (1818) (Sorsby 1963). Over the last 100 years, the concept of glaucoma has been further refined. Dr Drance (1973) provided for the first time a definition of glaucoma as a disease of the optic nerve (an optic neuropathy) caused by numerous risk factors. Currently, glaucoma refers to a group of eye conditions that cause characteristic damage to the optic nerve.

Open angle glaucoma (OAG) is an asymptomatic, progressive optic neuropathy characterized by enlarging optic disc cupping and visual field loss. About one half of OAG patients are unaware of their disease and it is usually discovered during an adult eye evaluation performed for other indications (Sommer et al. 1991; Klein et al. 1992; Dielemans et al. 1994; Weih et al. 2001). Without treatment, OAG can end in irreversible vision loss. Nowadays glaucoma is the second leading cause of blindness worldwide (Resnikoff et al. 2004). Identifying genetic variants that predispose to OAG would facilitate early diagnosis and follow-up for individuals at risk and enable the treatment of glaucoma on time. With appropriate treatment glaucoma can usually be stopped before significant vision loss occurs.

A strong genetic component has been established for OAG suggesting that it is a complex disease, caused by several genetic and environmental factors, each contributing minor effects and probably interacting with each other (Libby et al. 2005; Hewitt et al. 2006a). Molecular genetic studies of OAG during the past decade have yielded some success. Several chromosomal candidate loci have been identified, but only three candidate genes, *myocilin (MYOC)*, *optineurin (OPTN)* and *WD40-repeat 36 (WDR36)*, have been described, accounting together for less than 10% of OAG (Fan et al. 2006a). The *MYOC* gene has been established as a directly glaucoma causative, whereas the roles of *OPTN* and *WDR36* genes are controversial.

Exfoliation syndrome (XFS) was first highlighted in 1917 by the Finnish ophthalmologist John Lindberg, who noted progressive accumulation of white, small deposits of fibrillogranular extracellular material ocular tissues in 50% of patients with open angle glaucoma (Lindberg 1917; Lindberg 1989). In 1925 the Swiss ophthalmologist Alfred Vogt suggested the term '*glaucoma capsulare*' to describe glaucoma occurring in an eye with this syndrome (Vogt 1925). The term *pseudoexfoliation syndrome* (PXS) was introduced in 1954 by Dvorak-Theobald (Dvorak-Theobald 1954) to distinguish it from the true exfoliation seen in glass-blowers, resulting from infrared radiation damage (Cashwell et al. 1989). In 1956 Sunde proposed the term *exfoliation syndrome* (Sunde 1956) and in 1982 Layden (Layden 1982) suggested exfoliation syndrome to be the most appropriate and uncomplicated term considering the rarity of the true exfoliation syndrome. Since exfoliation material is found with and without glaucoma the terms exfoliation syndrome (XFS) and exfoliation glaucoma (XFG) are used in this thesis.

Familial aggregation and twin studies have confirmed a genetic contribution to XFS/XFG (Damji et al. 1998). To date it is generally believed that XFS/XFG is caused by interplay of genetic and environmental factors (Lee 2008). In the past year, a strong association between three single nucleotide polymorphisms (SNPs) on the *lysyl oxidase like 1* (*LOXL1*) gene and XFS/XFG has been reported in several populations (Table 7).

The purpose of this thesis was to investigate the genetic backgrounds underlying OAG and XFS and XFG in the Finnish patients.

# 2 REVIEW OF THE LITERATURE

## 2.1 GLAUCOMA

Glaucoma is a heterogeneous group of progressive optic neuropathies, which is characterized by progressive optic nerve degeneration and excavation of the optic disc due to apoptosis of retinal ganglion cells, and corresponding visual field defects (Bathija et al. 1998; Weinreb and Khaw 2004). Glaucomas may be categorized on the basis of etiology (primary and secondary), the age of onset (congenital, infantile, juvenile and adult), and anatomy of the anterior chamber (open angle and closed angle). In general, glaucomas might be classified in three major categories: primary open angle glaucoma (POAG), primary congenital glaucoma (PCG) and primary angle-closure glaucoma (PACG). In addition a genetically heterogeneous group of developmental disorders known as anterior segment dysgenesis (ASD) have been reported to be associated with increased IOP and glaucoma (Gould and John 2002). These include Rieger's anomaly, Peters' anomaly, iris hypoplasia, aniridia and iridogoniodysgenesis.

### 2.1.1 Prevalence of glaucoma

Glaucoma is of major public health importance worldwide. In 1996 Quigley estimated that approximately 66.8 million individuals would be affected with glaucoma worldwide by year 2000, of which 6.7 would suffer bilateral blindness (Quigley 1996). Several surveys of glaucoma prevalence have been done since then (Mitchell et al. 1996; Bonomi et al. 1998; Weih et al. 2001; Jonasson et al. 2003) and in 2006 Quigley and Broman upgraded the estimations for the years 2010 and 2020 (Quigley and Broman 2006). By their estimation in 2010 there will be 60.5 million people with glaucoma, of whom 45 million will suffer open angle glaucoma (OAG) and 16 million will suffer angle-closure glaucoma (ACG). The number will increase to 79.6 million (58.6 million OAG and 21.0 million ACG) by 2020. It has been estimated that in 2010 over 8.4 million people will be bilaterally blind from glaucoma (4.5 million from OAG and 3.9 million ACG), rising to 11.1 million (5.9 million OAG and 5.3 million ACG) by 2020. According to the World Health Organization's (WHO) bulletin of global visual impairment in the year 2002, cataract is the leading cause of blindness in the world accounting for 48% of blindness worldwide, glaucoma is the second accounting for 12% and age-related macular degeneration (AMD) is third with a

proportion of 9% (Resnikoff et al. 2004). In developed countries AMD is the leading cause of blindness (Taylor and Keeffe 2001).

### 2.1.2 Characteristics of OAG

Open angle glaucoma is most often defined by the presence of two out of the three following characteristics: glaucomatous changes in optic nerve head and/or corresponding visual field defect and/or elevated intra-ocular pressure >22 mmHg. The optic disc is considered as glaucomatous when diffuse damage with cup-to-disc ratios C/D > 0.7, and/or localized thinning of the rim, and/or asymmetry of > 0.2 in C/D between the eyes with equal size discs. In open angle glaucoma (OAG) the anterior chamber angle is open and normally developed. In recent epidemiological studies, the definition is exclusively based on the appearance of the optic nerve head and visual fields. However, there are still differences (Ringvold et al. 1991; Klein et al. 1992; Dielemans et al. 1994; Tielsch et al. 1994; Hirvelä et al. 1995; Mitchell et al. 1996; Bonomi et al. 1998; Weih et al. 2001; Jonasson et al. 2003).

Open angle glaucoma can be classified according to age of onset into two groups: juvenile- and adult-onset forms (Wiggs et al. 1996). Juvenile-onset primary open angle glaucoma (JOAG) refers to patients with chronic open angle glaucoma diagnosed between 10 and 40 years of age. Affected patients are typically present with high IOP, which ultimately requires surgical therapy. Characteristic features include a high incidence of myopia and angle structures of normal appearance. JOAG usually segregate in families and can be inherited as an autosomal dominant trait. Adult-onset primary open angle glaucoma (usually abbreviated as POAG) refers to patients diagnosed over the age of 40 years. Most of the patients in general practice are over 65 years of age. Traditionally POAG has been divided into: eyes having glaucomatous damage and high IOP (high-testion glaucoma HTG / high-pressure glaucoma HPG) (Sommer et al. 1991) and eyes having glaucomatous damage but normal IOP (normal-tension glaucoma NTG/ normal-pressure glaucoma NPG) (Grosskreutz and Netland 1994). Eyes with elevated IOP with a normal optic nerve head and a normal visual field are classified as ocular hypertension (OHT) (Gordon et al. 2002). Open angle glaucoma is symptomless until its later stages, and therefore it is often presented first at an advanced stage when irreversible glaucomatous changes have occurred in the optic disc. Guidelines for glaucoma care have been made by the Finnish Opthalmologic Society with the Finnish Glaucoma Society (Glaukooman käypä hoito -

suositus 2002; Tuulonen et al. 2003) and the European Glaucoma Society (European Glaucoma Society 2003).

Risk factors for open angle glaucoma consist of age, elevated intraocular pressure (IOP), exfoliation syndrome, race, myopia, diabetes, positive family history of glaucoma and decreased perfusion pressure (Tuulonen et al. 2003). IOP is the most common and the only threatable risk factor for glaucoma (Sommer et al. 1991). Exfoliation syndrome (XFS) is a major risk factor and the most common identifiable cause of glaucoma (Ritch et al. 2003).

#### *2.1.2.1 Prevalence of OAG*

The prevalence of OAG varies between ethnic populations, but universally it increases with age. OAG is the most common form of glaucoma among Caucasians and Afro-Americans (McKinnon et al. 2008) whereas angle-closure glaucoma (ACG) is the most prevalent form among Asians (Chew and Aung 2001). Traditionally the prevalence of OAG has been reported as 1-2% in persons over 40 years old and higher, even 10%, of over 65-year old individuals (Mitchell et al. 1996; Bonomi et al. 1998; Tuck and Crick 1998; Wensor et al. 1998; Wolfs et al. 2000; Iwase et al. 2004). The general rough estimate is that the risk of glaucoma doubles with every decade of age (Tuulonen et al. 2003).

Population-based age-dependent OAG prevalence estimates are presented in Table 1. Higher prevalence rates of OAG have been reported in black than in white populations (Tielsch et al. 1991; Leske et al. 1995; Racette et al. 2003; Friedman et al. 2004). In the Barbados Eye Study (West Indies) the prevalence of POAG in 40-84 year old individuals was 7% in blacks, 3.3% in mixed-race, and 0.8% in whites or other participants (Leske et al. 1994) and in the Baltimore Eye Study, the prevalence of OAG was four to five times higher in blacks (1.23% of 40-49 years; 11.26% of $\geq$ 80 years) as compared with whites (0.92% of 40-49 years; 2.16% of $\geq$ 80 years) (Tielsch et al. 1991).

**Table 1. Prevalence (%) of POAG in different age groups in population-based studies.**

| Population-based study | Age groups (%) | | | | | | | | | Prevalence in population (n of POAG / n of all examined) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 40-49 | 50-54 | 55-59 | 60-64 | 65-69 | 70-74 | 75-79 | 80-84 | 85- | |
| Ferndale (Wales)[1] 1966 | | 0.25 | 0.84 | 0.44 | 1.01 | 1.13 | | | | 0.43 (20/4608) |
| Framingham (USA)[2] 1977 | | | 0.48 | 0.92 | 0.92 | 1.70 | 1.94 | 2.92 | | 1.15 (27/2352) |
| Rotterdam Eye Study (Netherlands) [3] 1994 | | | 0.2 | 0.2 | 0.9 | 1.8 | 1.6 | 3.1 | 3.3 | 1.1 (34/ 3062) |
| Baltimore (whites) (USA)[4] 1991 | 0.18 | 0.32 | | 0.77 | | 2.85 | | 1.94 | | 1.1 (32/ 2913) |
| Roscommon (Eire)[5] 1993 | | 0.72 | | 1.76 | | 3.20 | | 3.05 | | 1.88 (41/ 2186) |
| Blue Mountains (Australia)[6] 1996 | | 0.29 | | 1.07 | | 4.17 | | 8.17 | | 2.38 (87/3654) |
| Reykjavik Eye Study (Iceland)[7] 2003 | | 0.6 | | 2.8 | | 8.0 | | 12.8 | | 4.0 (42/1045) |
| Beaver Dam (USA)[8] 1922 | 0.99 | | 1.29 | | 2.65 | | 4.71 | | | 2.11 (104/4926) |

[1](Hollows and Graham 1966), [2](Kahn et al. 1977), [3](Dielemans et al. 1994), [4](Tielsch et al. 1991), [5](Coffey et al. 1993), [6](Mitchell et al. 1996), [7](Jonasson et al. 2003), [8](Klein et al. 1992)

In the population based survey of Finland the prevalence of OAG increased clearly with age and was 5.4% in individuals over the age of 70 (Hirvelä et al. 1995). According to the statistics of the national health insurance refunds for medical expenses compiled by the Social Insurance Institution (KELA) 74 088 individuals had the right to receive free medication for glaucoma in Finland in the year 2007. Of these 7 were under the age of 5 years, 1 181 individuals belonged to age group 5-40 years and 72 900 were 40 years old or older. Both patients with primary glaucoma and secondary glaucoma (caused by trauma etc.) and individuals with ocular hypertension (OHT), (i.e. high IOP (>30 mmHg) with normal optic nerve head and visual fields) are included in KELA's number of individuals with free medication. The number of glaucoma patients receiving free medication increases 3% (approximately 3000 patients) every year and highest proportion the is in age group 65-76 years (Vaahtoranta-Lehtonen et al. 2007).

### 2.1.3 Characteristics and prevalence of PCG

Primary congenital glaucoma (PCG) is the most common form of glaucoma in infants, with more than 80% of cases observed within the first year of life (Vasiliou and Gonzalez 2008). It typically manifests at birth or within the first year of life, but may manifest as late as three years of age (Ho and Walton 2004). PCG is characterized by the improper development of the trabecular meshwork in the anterior chamber angle of the eye leading to elevated IOP (>21 mm Hg). The eyes have an isolated maldevelopment of the trabecular meshwork, including the iridotrabecular junction, which is not associated with any other developmental ocular anomalies or ocular diseases that can raise IOP. An increase in IOP can damage the optic nerve and result in vision loss and even blindness in untreated individuals (deLuise and Anderson 1983). Clinical findings in PCG patients typically include epiphora (watery eye), corneal edema, photophobia, and buphthalmolos (enlargement of the globe), which result from increased IOP (Francois 1980). In approximately 75% of cases, primary congenital glaucoma is bilateral (Gencik et al. 1982).

The prevalence of PCG varies across populations from a rate of 1:10 000 newborns in Western countries (Francois 1980), 1:2500 in the middle East (Bejjani et al. 2000) and even 1:1250 in the Romany population in Slovakia (Gencik et al. 1982). Males are more frequently affected than females (65% versus 35%, respectively) (Vasiliou and Gonzalez 2008).

### 2.1.4 Characteristics of XFS and XFG

XFS is an age-related syndrome characterized by abnormal accumulation of white, small deposits of fibrillogranular extracellular material (exfoliation material, XFM) in the anterior segment of the eye, most commonly seen on intraocular tissues such as: the pupillary border and the anterior lens capsule. In most cases dandruffy exfoliation material is seen in lens surface, but also along the pupillary margin, and as small deposits in the iris surface, corneal epithelium, lens zonules and ciliary processes (Tarkkanen 1962; Morrison and Green 1988). Other clinical features are pigment deposition on the corneal endothelium as well as anterior chamber angle and liberation of pigment after papillary dilation (Wishart et al. 1985; Rouhiainen and Terasvirta 1990; Puska 1995).

Clinically, XFS can appear in uni- or bilateral forms. These may represent different stages of the disorder and approximately 14-41% of unilateral XFS cases convert to bilateral XFS (Hansen and Sellevold 1969; Klemetti 1988). The probability of exfoliation developing in the opposite eye was found to be 7% in 5 years and 17% in 10 years (Henry et al. 1987). In a ten-year follow-up study 38% of 63 studied unilateral XFS patients developed bilateral XFS (Puska 2002). Though the clinical impression is a unilateral affection, subtle ultrastructural and immunohistochemical alterations typical of XFS were found in the unaffected fellow eye (Kivela et al. 1997; Hammer et al. 2001).

Despite extensive research, the exact chemical composition of XFM and mechanism behind its production and accumulation remains as yet unknown. Immunohistochemical studies have shown that XFM represents a highly glycosylated, cross-linked and enzymatically resistant glycoprotein-proteoglycan complex bearing epitopes of the basement membrane and the elastic fiber systems and components of elastic microfibrils (Ritch 2001; Ritch et al. 2003). XFM has been shown to consist of fibrillin family members, transforming growth factor beta (TGF-β) associated and regulated proteins, clusterin, and matrix associated glycoproteins in association with chondroitin sulfates, basement membrane proteins such as fibronectin and laminin, and extracellular matrix proteins (Lee 2008). Glycoproteins found in intraocular XFM contain human natural killer (HNK-1) epitopes. It has been speculated that HNK-1 might bind together exfoliation fibres and matrix components that float freely in the aqueous humour and impart XFM adhesive properties that attach it to various tissues along the anterior and posterior chambers (Uusitalo et al. 1993; Kubota et al. 1997b). Antibodies for a carbohydrate epitope,

HNK-1, have been shown to be useful tool for immunohistochemical detection of exfoliation deposits (Uusitalo et al. 1993). The exact origin of XFM remains as yet unknown.

Originally XFS was thought to be solely an eye related condition, but later on similar dandruffy exfoliation material have been found in various extraocular tissues, such as the skin and connective tissue portion of various visceral organs (heart, lungs, kidneys, liver etc) (Streeten et al. 1990; Schlötzer-Schrehardt 1992; Streeten et al. 1992). This suggests that XFS is a systemic disorder with intraocular manifestation. A possible association between XFS and vascular diseases have been studied with controversial results (Repo et al. 1993; Mitchell et al. 1997; Shrum et al. 2000; Hietanen et al. 2002; Citirik et al. 2007; Tarkkanen et al. 2008).

XFS is overall the most common identifiable cause of glaucoma worldwide, causing both open angle glaucoma and angle-closure glaucoma (Ritch 1994). Exfoliation glaucoma (XFG) is most often classified as a high-pressure type of secondary open angle glaucoma that develops as a consequence of XFS. The amount of XFM in trabecular meshwork correlates with the presence or absence of glaucomatous changes in the optic nerve, which suggests a direct causative relationship between XFS and glaucoma development (XFG) (Schlotzer-Schrehardt and Naumann 1995; Gottanka et al. 1997). The clinical course of XFG is more aggressive than that of POAG; i.e. glaucomatous damage progress more rapidly and the prognosis is poorer (Olivius and Thorburn 1978; Konstas et al. 1997). In addition its care is more demanding and surgical intervention is more frequently a necessity. The presence of exfoliation doubles the risk for progressive glaucoma (Leske et al. 2003) and increases the risk for blindness caused by glaucoma (Forsman et al. 2007a).

#### *2.1.4.1 Prevalence of XFS and XFG*

XFS and XFG occur worldwide but their prevalence varies between different populations (Forsius 1979, 1988; Forsius et al. 2002). The prevalence of XFS and XFG increases with age. For a long time XFS and XFG were mistakenly thought of as Scandinavian diseases. Nowadays, it is believed that XFS is a common condition of worldwide significance and underdiagnosis is a cause for the low prevalences of XFS and XFG in certain populations.

The average worldwide prevalence of XFS ranges from 5 to 20% in the general population over the age of 60 years (Ringvold 1999). High prevalence figures have been reported in

Scandinavian populations (Forsius et al. 1974; Ekström 1987; Forsius 1988; Ringvold et al. 1991; Hirvelä et al. 1995), rising from 15 to 25% in subjects over the age of 65 years (Forsius 1979; Ekström 1987; Ringvold et al. 1991; Hirvelä et al. 1995) up to almost 35% in persons over the age of 80 (Jonasson et al. 2003) (Table 2). In a population-based study in Oulu in Finland XFS was observed in one or both eyes in 22% of the individuals aged 70 years or older (Hirvelä et al. 1995) (Table 3). High prevalence ratios have also been reported from Saudi Arabia (9.3%, ≥ 40 years) (Summanen and Tonjum 1988), Central Iran (13.1%; ≥50 years) (Nouri-Mahdavi et al. 1999) and in Crete (16.1% ≥40 years) (Kozobolis et al. 1997) whereas among the Inuits, Japanese, Mongolians and Chinese the prevalence of XFS is remarkably low (Forsius 1988; Forsius et al. 2002; Foster and Seah 2005) (Table 2).

So far, only one incidence study, carried in Olmsted country in Minnesota, has been published. The estimated overall age- and sex-adjusted annual incidence of XFS was 25.9 per 100 000 over a period of 15 years (Karger et al. 2003). The prevalence of XFS increased with age, from 2.8 per 100 000 in 40 to 49 year old individuals to 205.7 per 100 000 in individuals over the age of 79 years ($p<0.001$). The age-adjusted incidence was higher in women than in men (32.7 vs. 16.9 per 10 000, $p<0.001$). Also, in several other studies XFS has been more prevalent in females than in males; the prevalence in females was 27% in Finland (Oulu), 18% in Norway, 2% in Australia and 12% in Iceland (Reykjavik), whereas corresponding numbers for males were 13%, 15%, 1% and 9%, respectively (Ringvold et al. 1991; Hirvelä et al. 1995; Mitchell et al. 1999; Jonasson et al. 2003). However, contrary to that, higher prevalences in males than in females has been reported in Central Iran, (males 18% and females 8%) (Nouri-Mahdavi et al. 1999) and in Greece (males 21% and females 13%) (Kozobolis et al. 1997).

**Table 2. Age-specific prevalence (%) of exfoliation syndrome in population-based studies.**

| study | Age groups (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **40-49** | **50-59** | **60-64** | **65-69** | **70-74** | **75-79** | **80-84** | **85-89** | **>90** | **all** |
| Mid-Norway[1] | - | - | - | 10.7 | 12.8 | 16.6 | 22.6 | 29.6 | 36.7 | 16.6 |
| Central Iran[2#] | 0 | 7.5 | 15.6 | | 22.4 | | 33.3 | | 100 | 13.1 |
| Greece[3] (Crete) | - | 0.8 | 11 | | 20 | | | | 47 | 16.1 |
| Iceland[4] (Reykjavik Eye Study) | - | 2.5 | 8.8 | | 16.7 | | 33.3 | | | 10.3 |
| Australian[5] (Blue Mountains) | 0.2 | | 1.0 | | 3.3 | | 4.4 | | | 1.7 |
| South-Africa[6] (Hlabisa, black) | 2.0 | 4.5 | 13.5 | | 18.9 | | | | | 9.4 (7.7)* |
| South-Africa[6] (Temba, black) | 1.1 | 3.3 | 9.1 | | 16.5 | | | | | 7.8 (6.0)* |

[1](Ringvold et al. 1991), [2](Nouri-Mahdavi et al. 1999), [3](Kozobolis et al. 1997), [4](Jonasson et al. 2003), [5](Mitchell et al. 1999) [6](Rotchford et al. 2003), [#]age groups slightly different from other studies: (40-50 yrs), (51-60 yrs), (61-70 yrs), (71-80 yrs), (81-90 yrs) and (>90 yrs), [*]age adjusted prevalence (%)

**Table 3. Age-specific prevalence rates (%) of exfoliation syndrome in different regions in Finland.**

| Location (n) | Age groups (%) | | | | | |
|---|---|---|---|---|---|---|
| | **60-69** | **70-74** | **75-79** | **80-84** | **>85** | **all ages** |
| Oulu (455)[1] | - | 14 | 28 | 26 | 30 | 22 |
| Oulu (205)[2] | 10 | 23.2 | | 35.7 | | 23.4 |
| Kuusamo (328)[2] | 14.1 | 21.3 | | 47.2 | | 21 |
| Helsinki (262)[2] | 19.1 | 21.6 | | 28.4 | | 23.3 |

[1](Hirvelä et al. 1995), [2](Krause et al. 1988)

The risk for glaucoma is approximately 5-9 times higher in elderly patients with XFS than in those without XFS (Tuulonen et al. 2003). XFS is the most common identifiable cause of OAG worldwide accounting for approximately 20-25% of OAG. OAG in eyes with XFS is defined as exfoliation glaucoma (XFG). A high prevalence of XFG have been reported in Scandinavian populations, probably because of the high prevalence of XFS (Ringvold 1999). In Lindberg's original study XFS was present in 50% of glaucomatous eyes (Lindberg 1917). Similar proportions have been reported in later studies; in central Finland 42% (Krause 1973) and in southern Finland 47% (Valle 1988) of patients with OAG had XFS. In Norway the corresponding proportion was 40% (Aasved 1971).

On the other hand, it has been estimated that about 60 to 70 million people worldwide have XFS and of these 25% have elevated IOP, of whom one-third, 5-6 million people, have glaucoma (Leske et al. 2003; Bengtsson and Heijl 2005; Grodum et al. 2005). In Middle Norway 30% and in Finland (Oulu) 27% of XFS patients had glaucoma, whereas the corresponding numbers in individuals without XFS were 4% and 8%, respectively (Ringvold et al. 1991; Hirvelä et al. 1995).

In a 5-years follow-up study in Central Sweden (Tierp) 17% of the 413 study subjects (aged between 65-74 years) developed XFS and of these 14% developed glaucoma (Ekström 1993). In a 9-years follow-up period in Sweden, Malmö, the glaucoma conversion rate was twice as high in patients with XFS and OH (55%) as in age-matched OH patients without XFS (27.6%) (risk ratio =2.0, $p<0.0001$) (Grodum et al. 2005). During 10 years follow-up period in Finnish cohort of 56 non-glaucomatous patients with unilateral XFS, 32% of initially exfoliative eyes and 38% of initially nonexfoliative fellow eyes converted to XFG, whereas POAG developed in 3.5% of non-exfoliative eyes (Puska 2002). In addition, an association between the initial IOP and conversion to XFG (relative risk = 1.4, P =0.0001) was demonstrated. However, for some unknown reason all patients with XFS do not develop glaucoma (XFG) in their lifetime; about 65% of eyes with exfoliation remain nonglaucomatous (Klemetti 1988).

Only few XFS/XFG prevalence studies outside of Scandinavian have been published. In the Blue Mountains Eye Study in Australia glaucoma was found at almost a seven times higher frequent in eyes with XFS (14 %) than in eyes without XFS (2%) (OR 5.0; 95%

CI, 2.6-9.6) (Mitchell et al. 1999). In the 15-years community-based follow-up study of XFS in all residents of Olmsted County, Minnesota, 16% of XFS cases were placed on therapy at the time of the diagnosis (due to glaucomatous changes or OH) and the probability of remaining patients being placed on therapy in over the next 15 years was 44% (Jeng et al. 2007). In an incidence study carried on the same population, the estimated overall age- and sex-adjusted incidence of XFG was 9.9 per 100 000 population per year (Karger et al. 2003). The incidence increased with age, rising from 0.6 per 100 000 in persons 40-49 years to 114.3 per 100 000 in persons above 79 years ($p<0.001$). The incidence was higher in women than in men.

## 2.2 GENETIC MAPPING STRATEGIES FOR COMPLEX DISORDERS

Genetic mapping studies have been applied to identify genetic loci that include variants, which influence the trait of interest. This is attained by identifying correlation between the genetic marker locus and the studied trait. The highest level of success in genetic mapping has been achieved by mapping monogenic disorders posing Mendelian inheritance pattern, where one mutation has a strong functional effect, which makes the correlation between genotype and phenotype clear cut. In contrast, genetic mapping of common complex disease has resulted only in limited success (Altmuller et al. 2001). In complex diseases several predisposing and preventing genetic variants and environmental factors are involved in the pathogenesis of diseases and those individuals whose liability exceeds a certain threshold will eventually develop the disease. Such interplay between genetic and environmental factors makes the inheritance pattern of the trait less clear and the genetic studies more challenging (Risch 2000; Weiss and Terwilliger 2000; Glatt and Freimer 2002). In addition, in complex disorders any individual genetic variant generally has a relative small effect on the disease risk and hence correlation between a single disease locus and a phenotype is weaker and more difficult to identify than in monogenic disorders.

Complex disease gene mapping strategies fall broadly into two categories: candidate gene studies and genome-wide studies. The selection of the study approach is made based on prior knowledge of the biology of the trait. If hypothesis-based candidate loci or genes are known, the investigations are directed to these regions (candidate gene studies). Candidate gene studies can be done by using association, linkage or resequencing approaches. When no prior knowledge of candidate regions exists, genome-wide approaches are used. Genome-wide studies are divided into two approaches; genome-wide linkage studies and genome-wide association studies.

In genome-wide linkage analysis the only essential information needed is that the trait is inheritable. The first step in genetic linkage analyses is to collect family material of a sufficient size, with DNA from at least two affected subjects per family. In a whole genome linkage scan hundreds (400-1000) highly polymorphic microsatellite markers spread approximately 4-10cM intervals throughout the genome are analyzed in order to identify genomic regions that co-segregate with the trait in familial or siblings material. The positive regions obtained from the genome wide scan are often too wide for candidate gene studies containing possibly several hundreds of genes. By fine scale mapping, using more families and denser marker map, critical regions could be narrowed down. If the linkage remains after narrowing the region, the next step is to analyze regional candidate genes, primarily those biologically relevant candidates for the studied trait. Variations influencing the trait could be identified from candidate regions or genes by SNP association analyses and mutation screening (direct sequencing) (Figure 1).

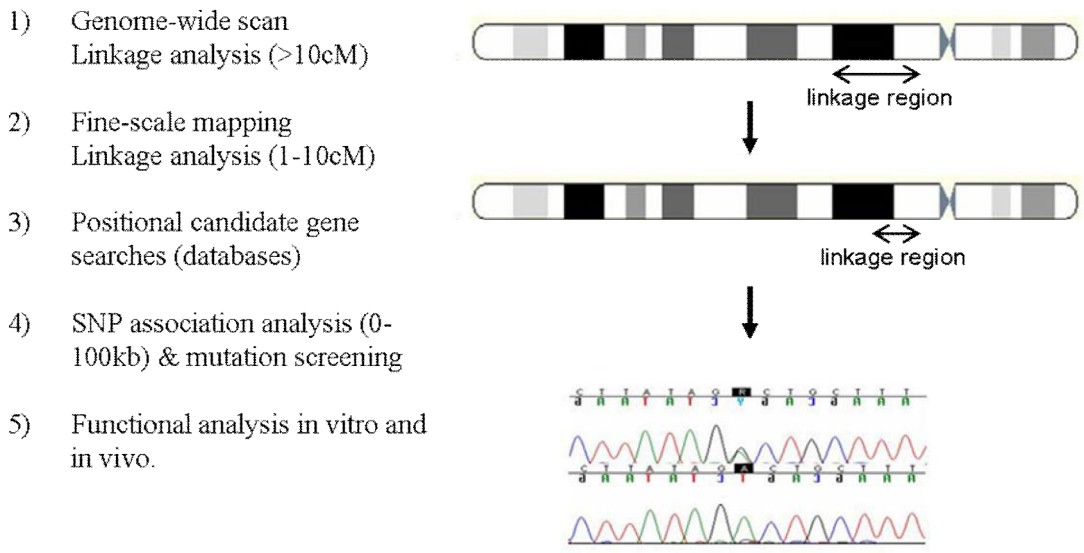


**Figure 1.** Main steps of gene mapping strategy to identify genetic region of interest. A genome-wide scan with hundreds of genetic markers is followed up by analysis of positive regions with denser marker map and larger set samples (fine scale mapping). Positional candidate genes are searched from databases. Direct sequencing of candidate genes or association analysis is performed in order to reveal possible underlying sequence alterations and the validity of mutations are tested by functional analysis.

### 2.2.1 Genetic markers and maps

A genetic marker is specified as a region on the genome where genetic variation between individuals exists. Nowadays the most widely used markers are *microsatellites (short tandem repeats, STR)* and *Single Nucleotide Polymorphisms (SNP)*. Microsatellite markers are the di- tri- tetra- or pentanucleotide repeat sections on the chromosomes of whose lengths vary between individuals, usually being between 30 - 50 nucleotides. Single nucleotide polymorphisms (SNPs) are diallelic variations of a single nucleotide with minor allele frequency more than 1%. SNPs are the most common type of variant in human genome occurring approximately once in 300bp, which makes them well suited to high-resolution genotyping. The human genome is 99.9% identical in all individuals; the remaining 0.1% makes us unique and explains all differences in genetic traits between individuals (e.g. genetic diseases and individual drug response). SNPs constitute approximately 90% of this genetic variation in the population (Kruglyak and Nickerson 2001; Reich et al. 2003; The International HapMap Consortium 2003). The dbSNP

database contains nearly 9 million SNPs, including most of the ~10 million SNPs which are estimated to exist in the human genome (Sachidanandam et al. 2001; The International HapMap Consortium 2003; Hirschhorn and Daly 2005).

Meiotic recombination is an event when the maternal and paternal chromosomes exchange corresponding segments of DNA (cross over) resulting in a chromosome that is a mosaic of the two parental haplotypes. The probability of recombination event is called the *recombination fraction (θ).* In genetic maps the *recombination fraction* is used as a distant measure, whose primarily used unit is the centiMorgan (cM) representing a 1% probability of crossover. The total genetic length of the human genome is approximately 3700 cM. In physical maps distance is measured in base pairs (bp) of DNA. On average, 1cM on genetic map corresponds to about 1 million bp (1 Mb) on a physical map. However this proportionality factor varies approximately from 0 to 9cM per Mb by gender, by chromosomal regions (e.g. recombination hotspots and deserts) and by other factors (Yu et al. 2001).

### 2.2.2 Parametric linkage analysis

In parametric linkage analysis statistical analyses are used to test if a particular allele (genetic marker) segregates with the disease status in a family more commonly than expected by chance. The primary goal is to identify genomic regions that co-segregate with the studied trait within families. In parametric linkage analysis (also called model-based analysis) several parameters, such as inheritance model, recombination fraction, penetrance of the disease allele, disease allele frequency, marker allele frequencies, and phenocopy rate, have to be specified prior to analysis. In complex diseases true values of these parameters are unknown. However it has been proposed that a robust method for identification of linkage in complex disorders, is parametric linkage analysis with both autosomal dominant and recessive models, despite the fact that this model is not entirely correct (Greenberg et al. 1998; Xu et al. 1998; Abreu et al. 1999; Durner et al. 1999). The recombination fraction (θ) is used in linkage analysis as a distance measure between a marker allele and a disease locus. The recombination fraction (θ) increases with the physical distance between two loci, from zero for adjacent loci (complete linkage) to a limiting value of a half meaning that the two loci are unlinked and that loci are far apart

(>50cM) or on different chromosomes. The closer the theta is to zero the stronger is the linkage between loci.

Linkage analysis is based on *maximum-likelihood estimation (MLE)* for which specifying and testing of hypothesis is essential. The null-hypothesis in linkage analysis means that the marker locus (or loci) is not linked to the trait locus ($H_0$: $\theta = 0.5$). An alternative hypothesis is that linkage between a marker locus (or loci) and trait locus does exist ($H_1$: $\theta \neq 0.5$). The goal of MLE is to find such a parameter value, in the linkage analysis $\theta$, which maximizes the value of likelihood function. Hypothesis testing is done by comparing the likelihood function of a given estimated parameter ($L(\theta)$) with likelihood function when $H_0$ holds true ($L(0.5)$) and and examining whether the likelihood functions differ more than expected by chance. Hypothesis testing is based on statistical measurement of the logarithm of odds, (Z = LOD score) (Morton 1955), which is assessed using the following formula:

$$\mathbf{Z(\theta) = \log_{10}[L(\theta)/L(0.5)}$$

In Mendelian disorders traditionally lods of -2.0 is considered convincing evidence against linkage and lods of +3.0 convincing evidence in favour of linkage (Morton 1955). A lod score of +3.0 corresponds to a conventional p-value of 0.0001 (Xu et al. 1998; Ott 1999), which means that two loci are 1000 times more likely to be linked than not linked. More restrictive criteria are needed for complex diseases, multipoint methods and dense marker maps. Lander and Kruglyak (1995) suggested a guideline of a lods of 3.3 or for the nonparametric statistic NPL of 3.6 (Lander and Kruglyak 1995). Multipoint linkage analysis, in which two or more marker loci are used simultaneously, can be used to increase the power to localize the disease gene.

### 2.2.3 Non-parametric linkage analysis

The paramertic linkage method is powerful especially in monogenic disorders, where the required parameters are known, but in complex disorders, the parameters are usually unknown. This dilemma can be circumvented by using non-parametric linkage analysis (also called model free analysis or relative pair method), in which specification of inheritance model is not needed. Instead non-parametric linkage analysis is based on allele

sharing in affected relatives. Alleles of genotyped relatives are distinguished according to whether they are identical by descend (IBD) or identical by state (IBS). Alleles IBD are copies of the same ancestral allele, whereas alleles IBS are the same alleles but their ancestral origin allele is different. The simplest model of non-parametric linkage analysis is affected sibpair analysis (ASP-analysis), in which allele sharing is followed in large sample size of sibpairs. Affected sibpairs are more likely to share marker alleles IBD in the neighbourhood of the disease locus than random pair of sibs. The expected allele sharing in full sibs, when linkage does not exist and thus $H_0$ holds true, is 0, 1, 2 alleles shared with probabilities 0.25, 0.50, 0.25, respectively. When a marker locus is linked to the disease locus affected sibpairs should share more alleles IBD than expected by chance. Statistical tests such as $\chi^2$ or means test are used to test whether allele sharing in IBD differs from that expected. Extensions of ASP that allow missing data (missing information of marker alleles) or take all affected siblings of the family to analysis (not just sibpairs) or take all affected relatives of the family to analysis (extended relative pair analysis, ERPA) have been developed.

### 2.2.4 Association analysis

Association analysis is utilized to identify variations that associate with a trait. The basic idea is simply to measure whether or not a certain allele of particular locus is found in affected individuals with a significantly different frequency than in unaffected individuals in study sample set. Association analysis can either be based on case-control samples or family based samples (Laird and Lange 2006). Association analysis can be divided into two approaches; *direct association* when functional variant is studied directly and *indirect association* (LD-mapping) when genetic marker is in linkage disequilibrium (LD) with functional variant. LD means non-random association between alleles of different loci. In LD-mapping association between genetic marker and studied trait exists if a marker locus and disease alleles are not separated by recombination events and thus segregate together more frequently than expected by chance. Statistical analyses are used to test the association and to observe possible LD. LD- mapping has been claimed to be more suitable and powerful than linkage-based methods for fine-scale mapping purposes and for mapping of common complex traits, which are caused by several susceptibility genes with only modest impact size (Risch and Merikangas 1996; Hirschhorn and Daly 2005; Newton-Cheh and Hirschhorn 2005; Wang et al. 2005).

High genome-wide frequency, low mutation rate and amenability to automation makes single nucleotide polymorphisms (SNPs) well suited and the most commonly used genetic markers in association mapping (Landegren et al. 1998; Wang et al. 1998). A set of SNPs on a single chromatid, which are not being separated by recombination events in history are in LD and are transmitted together in so-called haplotype blocks. All polymorphic sites in haplotype can be identified unambiguously by analyzing only one SNP of the haplotype, called haplotype tagging SNP (tagSNP). The International Hap Map Project has produced new information about tagSNPs and haplotype structures of the entire genome in several populations (from Nigeria, China, Japan, US with Northern and Western European ancestry) (The International HapMap Consortium 2003). Most recently, the International Hap Map Project reported a haplotype map which characterizes over 3.1 million human SNPs from four geographically diverse populations and included 25–35% of the common SNP variation in the populations surveyed (Frazer et al. 2007). The big advantage of tagSNPs is that they enable us to reduce the number of SNPs needed for the whole genome wide association studies making these studies more comprehensive, efficient and less expensive. On the basis of empirical studies, it has been estimated that most of the information about genetic variation represented by the 10 million common SNPs in the population could be provided by genotyping 200,000 to 1,000,000 tagSNPs across the genome (Patil et al. 2001; Gabriel et al. 2002; Carlson et al. 2003; Goldstein et al. 2003). Thus, by using knowledge of the LD present in the genome, a substantial reduction in the amount of genotyping can be obtained with little loss of information.

## 2.3 ESTABLISHING THE GENETIC COMPONENT IN OAG

A genetic predisposition for glaucoma was first suggested as early as 1842 when Benedict reported the presence of glaucoma in two sisters (Benedict 1842). In 1869, von Graefe mentioned hereditary glaucoma (von Graefe 1869) and in 1941 Duke-Elder described familial glaucoma which was inherited in a dominant manner (Duke-Elder 1941). In the following decades a strong genetic component of OAG has been confirmed in several studies (Leighton 1976; Rosenthal and Perkins 1985; Vernon 1991; Charliat et al. 1994; Lichter 1994; Tielsch et al. 1994).

### 2.3.1 Twin and family aggregation studies

Twin and adoption studies have been valuable in determining the influence of genetic and environmental factors for the phenotype. Twin studies compare the concordance of a trait in monodygotic (MZ) and dizygotic (DZ) twin pairs. Higher concordance in MZ twin pairs than in DZ twin pairs indicates a strong genetic effect, whereas equal concordance in MZ and DZ twin pairs or lower concordance in MZ twin pairs indicates that environmental effect is stronger (Allen et al. 1967). The heritability of the trait illustrates which proportion of the total variance of the phenotype is due to genetic factors ($h^2=H_{Genetic}/V_{Phenotype}$) (Strachan and Read 2004). In the Finnish population-based twin study 3 of 29 MZ twin pair and 3 of 79 DZ twin pairs were concordant for OAG. The heritability of OAG was 10.2% and the heritability of OAG and XFG combined was 13%, suggesting a complex model of inheritance (Teikari 1987). A higher concordance was reported in an Icelandic twin study, in which 50 MZ twin pairs (≥55 years) concordance was 98% and their spouses concordance was 70% (Sverrisson 1994; Gottfredsdottir et al. 1999).

Family aggregation studies have been used to determine the genetic component of the trait. A genetic component is involved when the risk of disease is increased in first degree relatives compared to more distant relatives or the general population. In Wolfs and colleagues study, based on population-based Rotterdam Eye study (Netherland) (Dielemans et al. 1994), familial aggregation of POAG was investigated by examining the first-degree relatives (siblings and offspring) of glaucoma cases (n=45) and first degree relatives of their age- and sex-matched randomly selected control individuals (n=135) from the Rotterdam study (Wolfs et al. 1998). The strength of the study was that both first degree relatives of glaucoma patients and controls were ophthalmologically examined. The prevalence of glaucoma was 10.4% in siblings and 1.1% in offspring of patients, compared with 0.7% in siblings and 0% in offspring of the controls. Low prevalence of glaucoma in offspring groups is probably due to their young age (mean age in cases offspring 42.2 years and in controls offspring 48.7 years). The lifetime risk of glaucoma at the age of 80 years was 22.0% in relatives of patients versus 2.3% in relatives of controls, yielding a risk ratio for POAG of 9.2 (95%CI= 1.2-73.9). The population attributable risk of glaucoma was as low as 16.4%, indicating that other, non-genetic, factors determine the overall occurrence of glaucoma to a great extent (Wolfs et al. 1998).

### 2.3.2 Family history of glaucoma in population-based studies

The weakness in population-based studies investigating of family history of glaucoma is that information about positive family history of disease has been self-reported and relatives of patients have not been ophthalmologically examined. Individuals' that are aware of their glaucoma diagnosis are usually more aware of their parental glaucoma history than those who are unaware of their glaucoma diagnosis or are unaffected. However, the awareness of disease of other family members is not always reliable, even though glaucoma diagnosis exists. This was demonstrated in the Glaucoma Inheritance Study in Tasmania (GIST) where 27% of previously diagnosed POAG patients from five families with strong family history of POAG were completely unaware of their family history, mostly in those who had distant relatives affected (McNaught et al. 2000). Similarly in a study including white, black, Hispanic and Asian origin participants, 56% of the first degree relatives of glaucoma patients had never had their eye pressure measured depicting a lack of awareness about the disease (Vegini et al. 2008).

Generally in population-based studies, family members of OAG patient have a 2-4 -fold increased risk of OAG and in first-degree relatives the risk has increased up to 10-fold (Wilson et al. 1987; Charliat et al. 1994; Leske et al. 1996; Wolfs et al. 1998; Tuulonen et al. 2003). In the population-based Baltimore Eye Survey (both black and white ancestral participants) the proportion of individuals reporting a family history of POAG was more than two-fold higher among cases (16%) than among controls (7%). Higher risk for glaucoma was found in siblings (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) of known glaucoma patients (Tielsch et al. 1994). In the Barbados Family Study (black individuals) the frequency of self-reported family history of glaucoma was 4-fold higher in affected (17%) than in unaffected men (4%, OR 7.88, 95% CI 4.07-15.23) and a 3-fold higher in affected (19%) than in unaffected women (7%, OR 2.4, 95% CI 1.46-4.23) (Leske et al. 1995). The risk of glaucoma was higher in siblings (OR 5.7, 95% CI 3.5 - 9.1) than in parents (mother OR 4.6, 95% CI 2.8-7.6; father 4.7, 95% CI 2.4-9.2) (Nemesure et al. 1996). In the population-based study in Australia (Visual Impairment Study in Victoria) interviews and ophthalmic examinations were performed for a random sample of 4744 participants. In multivariate logistic regression models, individuals with a family history of glaucoma had a threefold increased risk of possible, probable or definite glaucoma. Family history

of glaucoma was the only risk factor other than age, which remained significantly associated with increased risk of definite glaucoma (OR, 3.5; 95% CI, 1.9- 6.7) (Weih et al. 2001).

### 2.3.3 Inheritance of OAG

The majority of adult onset OAG families represent complex genetic inheritance caused by several predisposing and preventing genetic and environmental factors, each contributing minor or large effects and probably interacting with each other (Wiggs et al. 1996; Booth et al. 1997; Budde 2000; Libby et al. 2005; Hewitt et al. 2006a; Wiggs 2007). Although adult-onset OAG does not typically exhibit traditional Mendelian inheritance patterns, rare pedigrees in which OAG is clearly inherited as either an autosomal dominant (Posner and Schlossman 1949; Francois 1981) or an autosomal recessive inheritance pattern (Pimentel 1941; Waardenburg 1950; Biro 1951) have been reported. Juvenile-onset OAG (JOAG) is typically inherited as an autosomal dominant model, with penetrance varying from 60% to 100% (Stokes 1940; Crombie and Cullen 1964; Goldwyn et al. 1970; Leydhecker 1979; Francois 1981; Fleck and Cullen 1986; Johnson 1993; Lichter 1994; Wiggs et al. 1995).

## 2.4 OAG ASSOCIATED LOCI

Thus far, at least 22 genetic loci for OAG have been reported, reflecting the complex genetic background of OAG (Table 4) Fourteen of these loci has been designated (GLC1A-GLC1N) by the HUGO Genome Nomenclature Committee. Five of the loci are linked to the juvenile type of open angle glaucoma (GLC1A, GLC1J, GLC1K, GLC1M and GLC1N), whereas the rest are associated with adult onset open angle glaucoma. Most of the loci have been reported only in single, large and usually Caucasian pedigrees and therefore their contribution to OAG has not been established. Only three candidate genes have been identified from these loci; *myocilin* (*MYOC*), *optineurin* (*OPTN*) and *WD40-repeat 36* (*WDR36*), which together account for less than 10% of OAG (Fan et al. 2006a).

**Table 4. Genetic loci for primary open angle glaucoma.**

| Chromosomal location | Locus name | Gene name | Phenotypes | Ethnicity | Original study |
|---|---|---|---|---|---|
| 1q21-q31 | GLC1A | *MYOC* | JOAG | Caucasian | (Sheffield et al. 1993; Stone et al. 1997) |
| 2p14 | - | - | POAG | mainly Caucasian | (Wiggs et al. 2000) |
| 2p16.3-p15 | GLC1H | - | POAG | Caucasian, Afro Caribbean | (Suriyapperuma et al. 2007) |
| 2cen-q13 | GLC1B | - | POAG | Caucasian | (Stoilova et al. 1996) |
| 2q33-q34 | - | - | POAG | African | (Nemesure et al. 2003) |
| 3p21-p22 | GLC1L | - | POAG | Caucasian | (Baird et al. 2005) |
| 3q21-q24 | GLC1C | - | POAG | Caucasian | (Wirtz et al. 1997) |
| 5q22.1 | GLC1G | *WDR36* | POAG | Caucasian | (Monemi et al. 2003; Monemi et al. 2005) |
| 5q22.1-q32 | GLC1M | - | JOAG | Asian | (Fan et al. 2007) |
| 7q35-q36 | GLC1F | - | POAG | Caucasian | (Wirtz et al. 1999) |
| 8q23 | GLC1D | - | POAG | Caucasian | (Trifan et al. 1998) |
| 9q22 | GLC1J | - | JOAG | - | (Wiggs et al. 2004) |
| 10p12-p13 | - | - | POAG | African | (Nemesure et al. 2003) |
| 10p15-p14 | GLC1E | *OPTN* | NTG | Caucasian | (Sarfarazi et al. 1998; Rezaie et al. 2002) |
| 14q11 | - | - | POAG | mainly Caucasian | (Wiggs et al. 2000) |
| 14q21-q22 | - | - | POAG | mainly Caucasian | (Wiggs et al. 2000) |
| 15q11q13 | GLC1I | - | POAG | Caucasian, Afro-American | (Allingham et al. 2005) |
| 15q22-q24 | GLC1N | - | JOAG | Asian | (Wang et al. 2006) |
| 17p13 | - | - | POAG | mainly Caucasian | (Wiggs et al. 2000) |
| 17q25 | - | - | POAG | mainly Caucasian | (Wiggs et al. 2000) |
| 19q12-q14 | - | - | POAG | mainly Caucasian | (Wiggs et al. 2000) |
| 20p12 | GLC1K | - | JOAG | - | (Wiggs et al. 2004) |

## 2.5 OAG SUSCEPTIBILITY GENES

Three candidate genes have been identified so far: *myocilin (MYOC)*, *optineurin (OPTN)* and *WD40- repeat 36 (WDR36)*, which together account for less than 10% of OAG (Fan et al. 2006a).

### 2.5.1 *MYOC*: the first OAG susceptibility gene

The first OAG associated gene, *myocilin* (*MYOC*), was identified from locus GLC1A on chromosomal region 1q21-q31 by Stone and co-workers in 1997 (Stone et al. 1997). They identified three mutations (Tyr430His, Gly357Val, Gln361Stop) in 4% of familial glaucoma patients, in 3% of sporadic glaucoma patients and in 0.3% of general population. Most patients with *MYOC* mutations had juvenile- onset open angle glaucoma (JOAG) but some had adult-onset POAG. The MYOC protein was independently discovered by Kubota et al. (Kubota et al. 1997a), who named the protein myocilin (MYOC) and Nguyen et al. (Nguyen et al. 1998), who named it Trabecular meshwork-induced glucocorticoid response protein (TIGR). Since the original report, more than 70 *MYOC* mutations with slight phenotypic differences have been documented (Wiggs et al. 1998; Fingert et al. 1999; Hewitt et al. 2008a). The *MYOC* gene plays an important role in the pathogenesis of autosomal dominant juvenile-onset glaucoma with high intraocular pressure, but is also involved in a small but significant subset of adult-onset POAG.

#### *2.5.1.1 MYOC structure*

The *MYOC* -gene consists of three exons and encodes a 504 amino acid protein called myocilin (Figure 2). An amino terminal signal sequence, that may target myocilin protein for secretion, is situated on the N-terminal of the myocilin protein (Kubota et al. 1997a). Near the N-terminal is located an alpha helical coiled coil region, which is called the myosin-like domain because it forms a region resembling the myosin tail fibre. This domain contains a leucine zipper motif, which enables the protein to interact with itself or other proteins containing a similar motif. Homology between the myosin-like domain and myosin is relatively low (Kubota et al. 1997a; Ortego et al. 1997). Myocilin contains two hydrophobic regions, a flexible linker region and several potential phosphorylation and glycosylation sites. Near the C-terminal lies an olfactomedin-like domain. Olfactomedin is

a component of the mucus layer that surrounds the chemosensory dendrites of olfactory neurons in frogs. Homologous olfactomedin related glycoproteins have been identified in the neurons of the rat, mouse, and human brain (Danielson et al. 1994; Karavanich and Anholt 1998a; Karavanich and Anholt 1998b; Nagano et al. 1998). The olfactomedin-like domain is primarily a  beta sheet with a disulfide between Cys245-Cys433 (Fautsch and Johnson 2001). Cys433 has been conserved in olfactomedin and the olfactomedin-like proteins through evolution in different organisms (Mukhopadhyay et al. 2002). It has been suggested that both the leucine zipper and the cysteine residue at amino acid 433 are involved in myocilin dimerization and oligomerization (Morissette et al. 1998). Oligomerization mediated by cysteine residues are characteristic of olfactomedin related proteins (Karavanich and Anholt 1998a; Karavanich and Anholt 1998b) and proper dimerization and oligomerisation is probably necessary for the function of normal MYOC protein. The majority of *MYOC* mutations are located in the olfactomedin-like domain in the third exon of the gene (Figure 3). Olfactomedin in frogs has 31-40% amino acid residues in common with MYOC, while the human and rat olfactomedin related glycoproteins have 46-50% amino acid residues in common with MYOC (Adam et al. 1997; Kubota et al. 1997a; Ortego et al. 1997). However, if additional conservative amino acid substitutions are taken into account, the homology with olfactomedin-related glycoproteins is more than 80%. In the C-terminal of MYOC lie three amino acids serine, lysine and methionine (Adam et al. 1997), which have been shown to function as a peroxisome targeting sequence in other proteins (Subramani 1993).

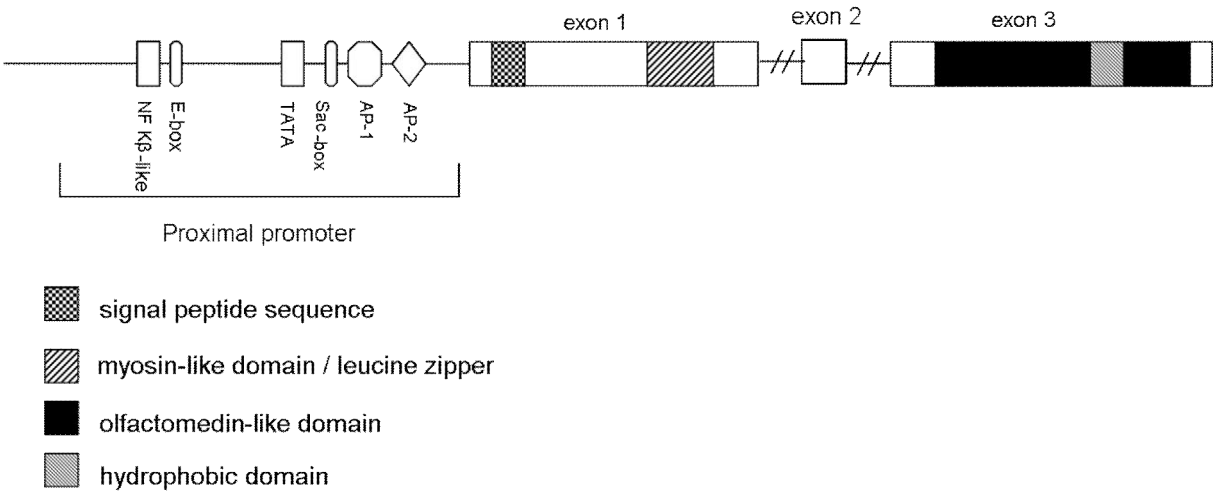


**Figure 2. Putative functional domains of myocilin protein.** Modified from Fingert and colleagues (Fingert et al. 2002).

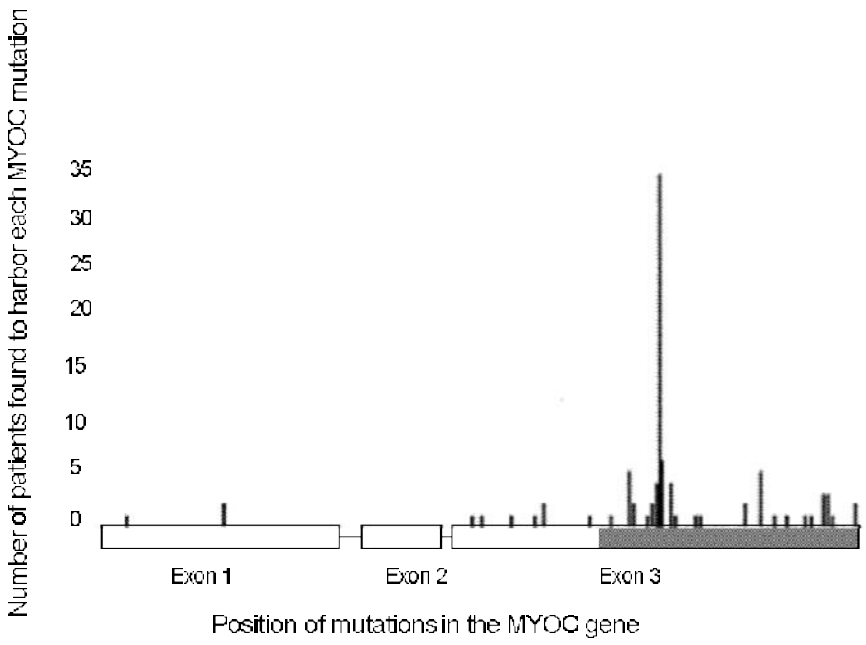


**Figure 3. Position of mutations in the *MYOC* gene.** The position of *MYOC* mutations are shown on the diagram with a vertical line. The height of the vertical line is proportional to the number of unrelated glaucoma patients found to harbor each mutation. The shaded segment of exon 3 is the olfactomedin homology domain. Modified from Fingert and co-workers (Fingert et al. 2002).

#### *2.5.1.2 MYOC mutation frequency and genotype-phenotype correlation*

Since in 1997, when the MYOC gene was reported as a susceptibility gene for glaucoma, more than 180 *MYOC* variants have been documented (Myocilin Allele-Specific Glaucoma Phenotype Database). Roughly 40% of the identified sequence changes are disease causing, of which the majority (~85%) are missense mutations (Hewitt et al. 2008a). It has been approximated that one in 30 unselected patients with OAG have disease-causing mutation(s) in the *MYOC* gene in which case approximately 2-5% of OAG cases have *MYOC* mutation(s) worldwide (Stone et al. 1997; Alward et al. 1998; Wiggs et al. 1998; Challa et al. 2002). Several large population-based studies have confirmed this approximation; according population-specific estimates the *MYOC* gene accounts for 1-4% of unselected POAG patients in different ethnic populations (Stone et al. 1997; Suzuki et al. 1997; Alward et al. 1998; Stoilova et al. 1998; Wiggs et al. 1998; Fingert et al. 1999; Kubota et al. 2000; Lam et al. 2000; Faucher et al. 2002; Pang and

Lam 2002; Pang et al. 2002; Kanagavalli et al. 2003; Gong et al. 2004; Sripriya et al. 2004; Fan et al. 2006b). However, the *MYOC* gene is associated most strongly with familial cases of juvenile-onset open angle glaucoma (JOAG). Prevalences of *MYOC* mutations in JOAG families range from 8% to 36% of affected pedigrees (Adam et al. 1997; Wiggs et al. 1998; Shimizu et al. 2000; Bruttini et al. 2003).

Although patents with *MYOC* mutation usually have early onset glaucoma with high intraocular pressure (IOP), there is a wide variability in the phenotype, depending on the mutation (Hewitt et al. 2008a). The most common *MYOC* mutation, nonsense mutation Gln368Stop, is most frequently found in patients with adult-onset OAG (Allingham et al. 1998; Alward et al. 1998; Angius et al. 2000). This mutation results in a 135 amino acids truncated polypeptide. Individuals with Gln368Stop are diagnosed with glaucoma at an average age of 59 years and maximum IOP of 30 mm Hg, so the IOP elevation is a lower level than with several missense mutations in the *MYOC* gene. Instead, two commonly found *MYOC* mutations Pro370Leu and Tyr437His, are associated with severe, juvenile-onset subtype of open angle glaucoma. Patients with Tyr437His mutation are diagnosed at an average at the age of 20 years and mean maximum IOP of 44 mmHg. Patients with the Gly364Val mutation represent a glaucoma phenotype intermediate to that of the Gln368Stop and Tyr437His phenotypes (Alward et al. 1998; Fingert et al. 2002). In patients with Tyr437His (Johnson et al. 1993), Val426Phe (Mansergh et al. 1998) or 1177GACA>T (Angius et al. 1998) medical therapy has commonly failed to lower IOP and usually these patients have required filtration surgery.

#### *2.5.1.3 Functional consequences of MYOC mutations*

Myocilin is expressed in most ocular tissues; trabecular meshwork (TM), sclera, iris, cornea, lens, ciliary body, retina, optic nerve and aqueous humor, but also in many extraocular tissues (Fingert et al. 2002). However, the normal physiologic function of *MYOC* is unknown. *MYOC* related glaucoma is usually associated with elevated IOP (Fingert et al. 2002) suggesting that *MYOC* is related to IOP elevation, probably facilitating the aqueous humour outflow through the trabecular meshwork (TM) (Johnson 2000). However, several studies have on the contrary suggested that myocilin is not needed for normal aqueous humour outflow (Lam et al. 2000; Kim et al. 2001; Wiggs and Vollrath 2001). Recently, a novel theory for IOP elevation mechanism was presented, in

which glaucoma-causing *MYOC* mutants were suggested to require the *Peroxisomal targeting signal-1 receptor* (*PTS1R*) to elevate intraocular pressure. Mutations in human *MYOC* were shown to induce exposure of a cryptic peroxisomal targeting sequence whose interaction with the *PTS1R* was necessary for IOP elevation (Shepard et al. 2007). However the exact role, if any, that normal *MYOC* plays in IOP homeostasis is still unclear.

Several studies have shown that early truncating mutations or deletion in the *MYOC* -gene are not as pathogenic as some missense mutations (Lam et al. 2000; Wiggs and Vollrath 2001; Pang et al. 2002). For example Gln368Stop mutation leads to a truncated form of the *MYOC* protein, but is associated with an older -onset of POAG and lower level of IOP elevation than several missense mutations (Allingham et al. 1998; Alward et al. 1998; Angius et al. 2000). This suggests that truncated *MYOC* still have some physiological function while missense mutations cause more problematic glaucoma. In a French-Canadian family three siblings heterozygous for Lys423Glu mutation on *MYOC* developed glaucoma, but four siblings with the same mutation in the homozygous form did not have glaucomatous symptoms (Morissette et al. 1998). This dominant heterozygote specific disease phenotype may be due to mutant myocilin's different shape that prevents its interactions with the normal myocilin, but when the mutation is homozygous and the both copies of *MYOC* are mutant, the proteins may interact and glaucoma does not occur (Morissette et al. 1998). In agreement with this, heterozygous or homozygous *MYOC* knockout mice or mice overexpressing wild-type mouse or human *MYOC* do not develop elevated IOP or morphologic changes in the eye (Kim et al. 2001; Gould et al. 2004; Zillig et al. 2005). These findings suggest that the pathogenesis of *MYOC* mutations are not due to loss of function or haplosufficiency, but to a gain of function (Fingert et al. 2002; Gong et al. 2004).

At the cellular level wild type myocilin secretes from the trabecular meshwork (TM), and is found in the aqueous humor, whereas mutant myocilin misfolds and does not secrete from TM cells (Jacobson et al. 2001; Joe et al. 2003). Inhibition of *MYOC* secretion into the aqueous humor was also demonstrated in transgenic mice carrying the Tyr423His mutation (corresponding to the Tyr437His mutation in the human *MYOC* gene) (Gould et al. 2006; Senatorov et al. 2006) and in transgenic mouse carrying the human *MYOC* gene with Tyr437His point mutation (Zillig et al. 2005). In addition to stopping the secretion of

myocilin, mutant *MYOC* has also a negative effect on wild-type myocilin secretion (Jacobson et al. 2001). Misfolding of *MYOC* prevents its normal interactions (multimerization) and results in an increased production of monomeric forms, which aggregate in the endopalsmic reticulum (ER) of TM cells (Jacobson et al. 2001; Joe et al. 2003). Mutant MYOC also forms heterodimers and heteromultimers with wild-type MYOC and these complexes remain sequestered intracellularly (Gobeil et al. 2004). Mutations such as Gly364Val, Gln368Stop, Lys423Glu, Tyr437His and Ile477Asn, have been reported to stop the secretion of myocilin and found as aggregates in the endoplasmic reticulum of TM cells (Joe et al., 2003)

Interestingly, transgenic mouse carrying the full-length human *MYOC* gene with Tyr437His point mutation showed changes similar to those observed in human OAG (Zhou et al. 2008). Likewise, transgenic mice with Tyr423His mutation (corresponding Tyr437His mutation in human *MYOC* gene) demonstrated similar pathogenical changes observed in the eyes of glaucoma patients (Senatorov et al. 2006). The retinal damage and the degree of IOP elevation produced by expression of the mutant human myocilin were comparable to that obtained with the mutant mouse myocilin. However, contrary results were obtained in a study where transgenic mouse carrying mouse $MYOC^{Tyr423His}$ did not develop high IOP or glaucoma (Gould et al. 2006). Clarification of the exact mechanism whereby *MYOC* mutations cause POAG will rely on further investigation of signalling pathways of the *MYOC* gene.

### 2.5.2 The *OPTN* gene on GLC1E locus

The second OAG associated gene, *optineurin (OPTN*) located to the GLC1E locus on chromosome 10p14-15 (Sarfarazi et al. 1998) was first reported in 2002 by Rezaie and colleagues (Rezaie et al. 2002). Optineurin was previously known as *NEMO-related protein* (*NRP*) (Schwamborn et al. 2000) or *FIP2* (Li et al. 1998). Rezaie and co-workers identified three putative disease-causing alterations (E50K, c.691_692insAG and R545Q) in the *OPTN* gene in 16.7% of Caucasian families with autosomal dominant adult-onset POAG (Rezaie et al. 2002). In addition a risk-associated alteration (M98K) was found in 12% of sporadic POAG cases. The majority of both familial and sporadic patients had normal IOP (less than 22 mmHg).

### *2.5.2.1 OPTN structure*

The *OPTN* gene contains 16 exons of which the three first are noncoding and the remaining 13 code for a protein about 66 kDa with 577 amino acids (Figure 4). The optineurin has at least three transcripts, which are expressed both in ocular tissues such as trabecular meshwork, nonpigmented ciliary epithelium, retina, Schlemm's canal and also in the aqueous humor (Rezaie et al. 2002; Sarfarazi and Rezaie 2003) and non-ocular tissues such as the heart, brain, liver, skeletal muscle, kidney, placenta, and pancreas (Li et al. 1998).

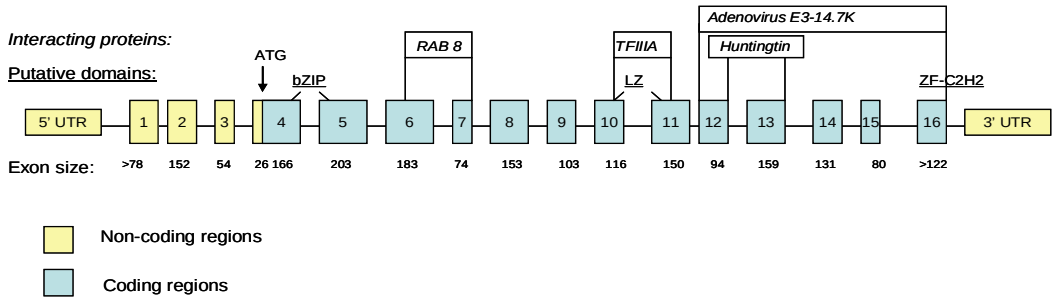


**Figure 4. Schematic representation of the *OPTN* gene.** Putative functional motifs; basic leucine zipper transcription factor (bZIP), leucine zipper (LZ) and zinc finger C2H2-type (ZF-C2H2) as well as 4 known protein interaction regions are shown above the exon line. The sizes of each 3 non-coding and 13 coding-exon are indicated below the exon line. Modified from Rezaie and colleagues (Rezaie et al. 2002).

### *2.5.2.2 OPTN variants in OAG*

Since the original study, a large number of studies have been undertaken in order to identify defects in the *OPTN* gene predisposing to OAG (Alward et al. 2003; Aung et al. 2003; Leung et al. 2003; Melki et al. 2003a; Wiggs et al. 2003; Baird et al. 2004; Funayama et al. 2004; Fuse et al. 2004; Toda et al. 2004; Willoughby et al. 2004; Weisschuh et al. 2005). The results have been conflicting and the role of *OPTN* mutations in the etiology of POAG is still controversial (Walter et al. 2002; Alward et al. 2003; Tang et al. 2003; Wiggs et al. 2003; Fuse et al. 2004; Ariani et al. 2006). Combining the results from all the published studies, mutations in the *OPTN* gene seem to be very rare cause of

glaucoma, constituting only approximately 0.1% of unselected POAG cases worldwide (Alward et al. 2003; Aung et al. 2003; Wiggs et al. 2003). However, in Asian populations, especially in Japan, the prevalence of NTG is relatively high (Shiose et al. 1991) and thus the *OPTN* gene has been studied extensively in China, Japan and India. In the Chinese population *OPTN* mutations accounted for 1.6% of sporadic OAG cases of whom 60% had high tension glaucoma (HTG) (Leung et al. 2003). The mutation pattern and allele frequencies are different China than in Caucasian populations (Lam et al. 2000; Pang et al. 2002; Rezaie et al. 2002; Leung et al. 2003). In the Japanese the prevalence of *OPTN* mutations was slightly higher than in Caucasian populations (Alward et al. 2003; Tang et al. 2003; Fuse et al. 2004), whereas in the Indian population *OPTN* sequence changes have been reported to play a minor role in glaucoma (Mukhopadhyay et al. 2005; Sripriya et al. 2006).

The most common disease causing variant in the *OPTN* gene, Glu50Lys, was initially identified in 13.5% of Caucasian NTG families (Rezaie et al. 2002). In the following studies it was found in 0.1-0.6% of OAG (Alward et al. 2003; Aung et al. 2003; Hauser et al. 2006b; Ayala-Lugo et al. 2007) and 1.5-2.9% of NTG patients in Caucasian and Hispanic populations (Aung et al. 2003; Hauser et al. 2006b; Ayala-Lugo et al. 2007). The Glu50Lys has been suggested to be a private mutation to the Caucasian and Hispanic populations, because it has not been found in several other studies, including reports of 237 POAG cases with Chinese ancestry or 961 POAG cases of Japanese ancestry (Alward et al. 2003; Leung et al. 2003; Tang et al. 2003; Chen et al. 2004; Funayama et al. 2004; Fuse et al. 2004; Toda et al. 2004; Umeda et al. 2004).

The clinical importance of other *OPTN* variants remains controversial. Especially findings of Met98Lys are contradictory. In the original study, the Met98Lys, variant was defined as a risk associated variation since it was found at significantly higher frequency in the OAG group (13.6%) than in the control group (2.1%) (Rezaie et al. 2002). In the following studies evidence for and against association have been represented (Ayala-Lugo et al. 2007). In the combined analysis of more than a dozen studies the Met98Lys was found in Asian and African populations at more than twice the frequency seen in Caucasian and Hispanic populations (Ayala-Lugo et al. 2007). Despite the fact that there have been several investigations it is still unclear whether Met98Lys is a risk associated allele for glaucoma or not (Craig et al. 2006; Ayala-Lugo et al. 2007).

The Arg545Gln mutation was initially reported as a disease causing mutation (Rezaie et al. 2002), but later work confirmed it to be a polymorphism. Since the original study an Arg545Gln variant has been found only in Asian populations (Chinese, Japanese, Korean, Filipino, Indian) (Alward et al. 2003; Leung et al. 2003; Tang et al. 2003; Chen et al. 2004; Funayama et al. 2004; Fuse et al. 2004; Umeda et al. 2004; Fan et al. 2005; Ayala-Lugo et al. 2007) and in mixed ancestry population (Willoughby et al. 2004) with similar allele frequencies in cases and controls. However, it should be noted that in one Indian study Arg545Gln was reported preferentially in glaucoma cases (Mukhopadhyay et al. 2005). The Arg545Gln variant has been suggested to be a private non-disease causing polymorphism of Asian populations (Ayala-Lugo et al. 2007).

The c.691_692insAG is an extremely rare *OPTN* variation seen so far only in two Caucasian OAG cases but not in controls (Rezaie et al. 2002; Ayala-Lugo et al. 2007). This frameshift mutation might be causative, although no conclusion could be drawn because of its rare occurrence.

#### *2.5.2.3 OPTN function*

It has been hypothesized that *OPTN* has a neuroprotective role in the eye and optic nerve, but when defective, it produce typical normal- and high-pressure glaucoma symptoms, such as optic neuropathy and progressing visual filed loss (Rezaie et al. 2002). Optineurin does not show any enzymatic activity and hence its functions are likely to be mediated by interaction with other cellular proteins. Optineurin interacts with proteins that link it to the regulation of cellular morphogenesis and membrane trafficking (RAB8) (Hattula and Peranen 2000), transcription activation (Transcription factor IIIA) (Moreland et al. 2000), vesicular trafficking (Huntingtin) (Faber et al. 1998), inhibition of signalling by metabotropic glutamate receptor (mGluR1a) (Anborgh et al. 2005) and Golgi ribbon formation and exocytosis (myosin VI) (Sahlender et al. 2005). Optineurin probably functions through the tissue necrosis factor α (TNFα) and Fas ligand signalling pathway (Sarfarazi and Rezaie 2003). The TNFα signalling pathway has been proposed to be involved in retinal ganglion cells apoptosis in patients with NTG or POAG (Yan et al. 2000; Yuan and Neufeld 2000). Normal optineurin probably protects the optic nerve from TNFα mediated apoptosis in a direct or indirect way, but when defective it might decrease

the threshold of ganglion cell apoptosis in patients with glaucoma (Wiggs 2007). In a recent study the physiological role of optineurin in TNFα signalling pathway was investigated in more detail (Zhu et al. 2007). It was shown that optineurin inhibits TNFα-induced NF-κB activation by binding poly- ubiquitinated rev-interacting protein (RIP) and competitively inhibiting the binding of NF-κB essential modulator (NEMO) to the RIP. NEMO is a component of a kinase complex that activates NF-κB and it has 53% amino acid similarity with optineurin. When optineurin is silenced by miRNA markedly enhanced TNFα –induced NF- κB activity is observed (Zhu et al. 2007). However, the precise functional role(s) of optineurin in the normal and glaucomatous eye are unclear.

### 2.5.3 The *WDR36* gene on GLC1G locus

The third OAG associated gene, *WD40-repeat 36 gene* (*WDR36*), on chromosomal region 5q22.1 (GLC1G) (Monemi et al. 2003; Kramer et al. 2004; Samples et al. 2004) was first reported in the year 2005 by Monemi and colleagues (Monemi et al. 2005). A coding variation, D658G, in the *WDR36* gene segregated with the disease in all affected and none of the unaffected family members in a large glaucoma family. Further analysis revealed a total of four mutations (N355S, A449T, R529Q, D658G), defined as 'predicted disease causing mutations', present in 5% of POAG families (11 unrelated HTG and 6 NTG subjects) and none of the 200 control chromosomes. In addition three variations (L25P, A163V, Y216P), defined as 'potential disease susceptibility mutations', were observed in 12% of POAG families and 2% of control chromosomes (Monemi et al. 2005).

#### *2.5.3.1 WDR36 structure and function*

The *WDR36* gene comprises 23 exons and encodes 951 amino acids protein with WD40 repeats and three other known motifs (Mao et al. 2004). The WD repeat-containing proteins comprise a large family implicated in a variety of functions ranging from transcription regulation and signal transduction to cell cycle control and apoptosis. A common function for all WD-repeat proteins is that they coordinate multiprotein complex assemblies where the repeating units serve as a rigid scaffold for protein interactions.

The *WDR36* gene is proposed to contain five (Monemi et al. 2005) to eight (Mao et al. 2004) WD40 repeat, which are tandem repeats of approximately 40 residues, containing a

central tryptophan (W) –aspartic acid (D) dipeptide. Sequence variations in the WD40 repeat domains of protein might interfere with its interactions with other proteins. It has been reported that *WDR36* is highly coregulated with interleukin 2 (IL2) and may be involved in T cell activation in response to IL2 (Mao et al. 2004). Previous studies have suggested that some glaucoma patients may have alterations in cellular immunity that are IL-2 dependent (Yang et al. 2001) and the T-cell responses may influence glaucomatous optic nerve degradation in humans (Bakalash et al. 2005) and in mouse glaucoma model (Mo et al. 2003). However, the precise functional role of the *WDR36* in normal and glaucomatous eye remains to be clarified.

*WDR36* have been identified expressed in ocular tissues (such as lens, iris, ciliary muscles, sclera, ciliary body, trabecular meshwork, retina and optic nerve) and in non-ocular tissues (such as heart, placenta, skeletal muscle, liver, kidney and pancreas) (Monemi et al. 2005).

#### *2.5.3.2 WDR36 variants in OAG*

Since the original study, *WDR36* gene studies have produced conflicting results and the role of *WDR36* mutations in OAG etiology remains controversial (Hauser et al. 2006a; Hewitt et al. 2006b; Kramer et al. 2006; Miyazawa et al. 2007; Weisschuh et al. 2007; Pasutto et al. 2008a). Originally described disease-causing mutations have since been found with an equal low frequency in control individuals as in patients with POAG, indicating that *WDR36* gene might not be causative for POAG in all populations (Hauser et al. 2006a; Hewitt et al. 2006b; Fingert et al. 2007b; Pasutto et al. 2008a). However, in some studies the frequency of *WDR36* variants has been higher in glaucoma patients compared with controls, suggesting that *WDR36* is a minor disease-causing gene in glaucoma in certain populations (Miyazawa et al. 2007; Weisschuh et al. 2007; Pasutto et al. 2008a). Hauser and colleagues suggested *WDR36* to be a glaucoma modifier gene since they found that sequence variations in the *WDR36* gene did not consistently segregate with the disease but individuals with variants had more severe glaucoma than those without them (Hauser et al. 2006a). Taken together, the current genetic data suggests that *WDR36* might act as a POAG modifier gene and/or causative gene for POAG in certain populations.

We screened the *WDR36* gene for mutations in Finnish subjects by sequencing its 23 exons and the respective exon-intron boundaries from the genomic DNA of 21 POAG, 8 XFG and 1 XFS patients. Two non-synonymous (D658G, I264V), two synonymous (V714V, V727V) and one intronic (IVS5+30C>T) sequence alterations were identified (unpublished data). Heterozygous D658G alteration was found in one POAG patient (age: 52 years), but none of the 198 control cases. In the further studies D658G was found in three close relatives of the index case, one glaucoma suspect (age: 62 years) and two unaffected (ages: 73 and 67 years). In the original study D658G variant was used to refine the critical region for the GLC1G gene and was defined as 'predicted disease-causing mutation' (Monemi et al. 2005). Subsequently, Weisschuh and colleagues found D658G in one NTG patient, but none of the 50 controls German study group (Weisschuh et al. 2007). However, later on it has been found with an equal low frequency in the normal population as in the POAG cases and defined as neutral polymorphism (Hauser et al. 2006a; Hewitt et al. 2006b; Fingert et al. 2007b; Pasutto et al. 2008a; Raymond et al., 2008). All the other variants we identified had previously been found with equal frequencies in cases and controls and categorized as a neutral polymorphisms (Monemi et al. 2005; Hauser et al. 2006a; Hewitt et al. 2006b; Pasutto et al. 2008a).

### 2.5.4 Other candidate gene studies

In addition to the aforementioned candidate genes, at least 16 OAG-associated genes have been reported (Table 5). Most them have been identified only in a single study, but some of the genes have appeared in several association studies. However due to the conflicting results the roles of these genes in glaucoma pathogenesis are still controversial.

One interesting gene is *cytochrome P4501B1* (*CYP1B1*). *CYP1B1* is a major cause of the primary congenital glaucoma (PCG), but mutations in *CYP1B1* have also been reported in JOAG patients (Stoilov et al. 2001; Nebert and Russell 2002; Chakrabarti et al. 2005; Acharya et al. 2006; Lopez-Garrido et al. 2006; Bayat et al. 2008). In certain pedigrees, both PCG and JOAG segregate, indicating that these two forms of glaucoma may also have a common or overlapping *CYP1B1*-mediated pathophysiological mechanisms (Panicker et al. 2002; Soley et al. 2003). In a Canadian JOAG family both variant R368H of the *CYP1B1* -gene and variant G399V of the *MYOC* -gene segregated with the disease indicating that mutation in the *CYP1B1* gene might behave as a modifier of the *MYOC*

gene (Vincent et al. 2002). This is an unequivocal piece of evidence showing the interaction between *MYOC* and another gene in causing POAG and might also serve as one of the mechanisms for incomplete penetrance. Subsequent studies have indicated that *CYP1B1* mutations are significant risk factors for JOAG also in patients who do not carry *MYOC* mutations (Melki et al. 2004; Acharya et al. 2006; Lopez-Garrido et al. 2006). Furthermore, mutations in *CYP1B1* have been proposed as potential factors of severity in POAG patients (Melki et al. 2005).

| **Gene symbol** | **Gene name** | **Chromosomal location** | **Reference** |
|---|---|---|---|
| *AGTR2* | *Angiotensin II receptor, type 2* | Xq22-q23 | (Hashizume et al. 2005) |
| *APOE* | *Apolipoprotein E* | 19q13.2 | (Copin et al. 2002; Vickers et al. 2002) |
| *CDKN1A* | *Cyclin-dependent kinase inhibitor 1A* | 6p21.2 | (Tsai et al. 2004) |
| *CYP1B1* | *Cytochrome P450, subfamily I, polypeptide I* | 2p22-p21 | (Vincent et al. 2002) |
| *EDNRA* | *Endothelin receptor, type A* | 4q31.2 | (Ishikawa et al. 2005) |
| *GSTM1* | *Glutathione S-transferase, mu-1* | 1p13.3 | (Juronen et al. 2000) |
| *IGF2* | *Insulin-like growth factor II* | 11p15.5 | (Tsai et al. 2003) |
| *IL1B* | *Interleukin 1-beta* | 2q14 | (Lin et al. 2003b) |
| *MTHFR* | *5,10-methylenetetrahydrofolate reductase* | 1p36.3 | (Junemann et al. 2005) |
| *NOS3* | *Nitric oxide synthase 3* | 7q36 | (Tunny et al. 1998) |
| *NPPA* | *Atrial natriuretic peptide* | 1p36.2 | (Tunny et al. 1996) |
| *OCLM* | *Oculomedin* | 1q31.1 | (Fujiwara et al. 2003) |
| *OPA1* | *Optic atrophy 1* | 3q28-q29 | (Aung et al. 2002) |
| *TAP1* | *Transporter, ATP-binding cassette, major histocompatibility complex, 1* | 6p21.3 | (Lin et al. 2004) |
| *TNF* | *Tumor necrosis factor α308* | 6p21.3 | (Lin et al. 2003a) |
| *TP53* | *Tumor protein p53* | 17p13.1 | (Lin et al. 2002) |

**Table 5. OAG associated genes reported in previous studies.** Table is modified from Fan and collegues 2006 (Fan et al. 2006a).

## 2.6 GENETICS OF PCG

Primary congenital glaucoma is largely an inherited condition and the inheritance is primary autosomal recessive with variable penetrance (Stoilov et al. 1997; Sarfarazi and Stoilov 2000). Ninety percent of cases are sporadic and pseudodominant transmission has been demonstrated in some families (Stoilov et al. 1997). Three loci responsible for autosomal recessive forms of congenital glaucoma have been located in the human genome; GLC3A at chromosome locus 2p12 (Sarfarazi et al. 1995), GLC3B at chromosome locus 1p36 (Akarsu et al. 1996) and GLC3C at chromosome locus 14q24.3-q31.1 (Stoilov 2002) (Table 6). The first PCG causing gene *Cytochrome P4501B1* (CYP1B1) was identified from the GLC3A locus in 1997 (Stoilov et al. 1997). To date, no specific genes have been yet linked to the GLC3B and GLC3C loci.

| Locus | Chromosomal location | Gene | Phenotype | Reference |
|---|---|---|---|---|
| GLC3A | 2p21 | *CYP1B1* | PCG | (Sarfarazi et al. 1995; Stoilov et al. 1997) |
| GLC3B | 1p36 | | PCG | (Akarsu et al. 1996) |
| GLC3C | 14q24.3-q31.1 | | PCG | (Stoilov 2002) |

**Table 6. Genetic loci for primary congenital glaucoma.**

### 2.6.1 The CYP1B1 gene on GLC3A locus

Mutations in *CYP1B1*, the gene encoding cytochrome P4501B1, are the predominant cause of PCG (Stoilov et al. 1997). The *CYP1B1* mutations have been reported in 94-100% of familial (Bejjani et al. 1998; Stoilov et al. 1998; Plasilova et al. 1999) and in 20-50% of sporadic PCG cases (Mashima et al. 2001; Stoilov et al. 2002). The *CYP1B1* – gene has also been identified as a modifier gene in POAG, and on rare occasions, as a causative gene in JOAG as well as in several anterior segment dysgenesis (ASD) disorders (Vasiliou and Gonzalez 2008). At least 82 mutations have been reported in PCG, Peters Anomaly (PA), Riegers anomaly (RA) and POAG patients. These include missense and nonsense mutations, small deletions, insertions and/or duplications and silent mutations accounting for 56%, 12%, 20%, 10% and 2% of the mutations, respectively (Vasiliou and Gonzalez 2008). These mutations have been reported throughout the gene.

The human *CYP1B1* -gene consist of three exons and codes for a 543-amino acid-long protein that contains three regions; a membrane-bound N-terminal region, a so-called hinge, and a cytosolic globular domain. The *CYP1B1* gene is expressed in several tissues, including the eye, as well as in the nucleus of several cell types, including the tubule cells of the kidney and the secretory cells of the breast (Muskhelishvili et al. 2001).

Although the role of *CYP1B1* in PCG is not well understood, the protein is probably responsible for the metabolism of compounds that are critical for the developing eye (Stoilov et al. 2001). The *CYP1B1* -null mice exhibit abnormalities of the ocular drainage structure similar to those reported for human PCG patients (Libby et al. 2003).

## 2.7 ESTABLISHING THE GENETIC COMPONENT IN XFS AND XFG

### 2.7.1 Twin and family studies

In a Finnish twin study a large number of monozygotic (MZ) and dizygotic (DZ) twins with chronic OAG and XFG were studied (Teikari 1987). The sample size was large enough for determining the heritability of chronic OAG, but insufficient twin pairs made it impossible to determine the heritability of XFS and XFG by classic twin analysis. However, the heritability of OAG and XFG combined was 13%. In a study examining MZ twins over the age of 55 through the Iceland twin registry, eight MZ twins with XFS were found; five of them were concordant and three were disconcordant for the XFS (Sverrisson 1994; Gottfredsdottir et al. 1999). Spouses of the affected twins were found to be free of XFS. The disconcordant twin pairs were in their fifties and thus still may develop XFS later in life.

The familial occurrence of XFS was reported already in 1930 by Vogt (Vogt 1930). In the following decades familiar aggregation and increased frequency of XFS in relatives of affected individuals have been reported in several studies (Gifford 1957; Tarkkanen 1962; Tarkkanen et al. 1965; Pohjanpelto and Hurskainen 1972; Aasved 1975; Damji et al. 1999; Gottfredsdottir et al. 1999; Allingham et al. 2001; Orr et al. 2001). In Norway a tenfold higher prevalence of XFS was reported in relatives (children, siblings, nieces/nephews, paternal and maternal cousins) of the affected individual over the age of

40 years (9.4%) belonging to 25 XFS families (Aasved 1975) than in the general population (1%) (Aasved 1971). In Finland the frequency of XFS was reported to be 8% in relatives (siblings and children) over the age of 40 and raised to 14% in relatives over the age of 60 years (Pohjanpelto and Hurskainen 1972).

### 2.7.2 Inheritance of XFS and XFG

To date it is generally believed that XFS/XFG is a complex trait caused by an interplay of genetic and environmental factors (Jerndal and Svedbergh 1978; Damji et al. 1998; Zenkel et al. 2005; Lee 2008). However, several Mendelian inheritance models have been suggested (Damji et al. 1998; Orr et al. 2001) of which autosomal dominant model with reduced penetrance has been reported most frequently (Tarkkanen 1962; Tarkkanen et al. 1965; Aasved 1975; Forsius 1993; Ceisler 1994; Sotirova 1999; Orr et al. 2001; Hardie et al. 2005; Forsman et al. 2007b). Autosomal recessive transmission (Andersen et al. 1997) or maternal inheritance, such as mitochondrial inheritance, X-linked transmission or autosomal inheritance with genomic imprinting (Andersen et al. 1997; Damji et al. 1998; Damji et al. 1999; Allingham et al. 2001) have been proposed as well. One well-documented (Orr et al. 2001) and one incompletely documented (Gifford 1957) paternal transmissions have been described. Environmental factors suggested to influence XFS are untraviolet light exposure, autoimmunity, slow virus infection and trauma (Damji et al. 1998).

## 2.8 MOLECULAR GENETIC STUDIES OF XFS AND XFG

Our understanding of the field of molecular genetics of XFS and XFG has been advanced during the last year by cutting edge molecular genetic approaches studying this disorder. Only a few molecular genetics studies of XFS and XFG were published earlier. Loss of heterozygosity (LOH) was reported on loci 13q12.11, 7q21.3, 7q21.11 and 7p13, in XFS specimens of the iris and anterior capsule suggesting that a genetic instability at these regions could be associated with XFS (Kozobolis et al. 1999; Zalewska et al. 2003). In addition, Sotirova and colleagues reported the linkage to XFS on loci 2p14-2Cen and 2q35-q36 (Sotirova et al 1999) and Wiggs and co-authors suggested 2p16 as a potential locus for XFS (Wiggs et al. 1999). However, these results have remained unconfirmed.

Gene expression differences in anterior segment tissues (iris, ciliary processes, lens epithelium) of eyes with XFG have been investigated using three XFS-associated open angle or closed-angle glaucomas and three age-matched glaucomatous control eyes without XFS. In total, 23 genes with differential expression pattern were identified (Zenkel et al. 2005). Both upregulated genes, such as; *latent transforming growth factor binding proteins* (*LTBP-1* and *-2*), *cross-linking enzyme transglutaminase-2* (*TGase-2)*, *tissue inhibitor of matrix metalloproteinase-2* (*TIMP-2)*, *A-kinase anchor protein-2* (*AKAP-2)*, *apolipoprotein D*, *the adenosine receptor-A3* (*AdoR-A3)* and *fibrillin-1* and downregulated genes, such as; *tissue inhibitor of matrix metalloproteinase-1* (*TIMP-1*), *clusterin*, *microsomal glutathione-S-transferase-1* (*mGST-1)* and *serum amyloid A1*, were found. Most of these genes were related to extracellular matrix metabolism and cellular stress. Some overlapping proteins with the gene expression data were identified in differential proteomic analysis of anterior lens capsules from cataract surgery patients with and without XFS (Ovodenko et al. 2007). This proteomic screen suggests that the lens capsules from XFS patients contains extracellular matrix and basement membrane structural and metabolic proteins such as fibulin-2, versican, syndecan-3, laminin, fibronectin and fibrillin-1.

### 2.8.1 The *LOXL1* gene on chromosome 15

Recently, Thorleifsson and colleagues reported that the *lysyl oxidase-like protein 1* (*LOXL1*) gene on chromosomal region 15q24.1 is associated, possibly through XFS, with XFG in Icelandic and Swedish glaucoma patients (Thorleifsson et al. 2007). In the primary stage of the study a genome-wide association study with 304 250 SNPs was performed for 195 Icelandic glaucoma cases (90 POAG cases, 75 XFG cases and 30 individuals with no precise classification) and 14 474 population controls. The strongest association to glaucoma was observed with allele T of rs2165241, located on the first intron of the *LOXL1* gene. Further investigations revealed that this effect was strongest for XFG patients (OR = 3.40, P = 4.3 x $10^{-12}$). The finding was replicated in Swedish material (200 POAG cases, 199 XFG cases and 198 controls), which resulted in similar association for XFG (OR = 3.78, P = 3.1 x $10^{-17}$) as in Icelandic material. An additional 55 Icelandic XFS cases showed similar association with rs2165241 as XFG cases (OR = 3.18, P = 1.9 x $10^{-8}$). Subsequently, the association was tested in additional SNPs substantially correlated with rs2165241. Two non-synonymous SNPs, rs1048661 (R141L)

and rs3825942 (G153D), on exon 1 of the *LOXL1* gene showed a strong association to XFG in combined Iceland and Swedish material (OR=2.46, $P=2.3x10^{-12}$ for allele G of rs1048661 and OR=20.10, $P=3.0x10^{-21}$ for allele G of rs3825942). These SNPs were in a substantial linkage disequilibrium (D'=1) and three of four possible haplotypes were found in study material (G/G, T/G, G/A). Haplotype G/A had the lowest estimated risk for XFG, whereas, relatively to G/A, the risk haplotypes G/G and T/G yielded OR=27.05 and OR=8.90 respectively. The individuals carrying two copies of G/G haplotype were estimated to have a 700 -fold higher risk than those carrying two copies of G/A haplotype and a 2.47 -fold higher risk than the population average. Two non-synonymous variations were estimated to account jointly for over 99% of all XFG cases. The high-risk haplotype, G/G, was present also in 50% of individuals in the general Swedish and Iceland population (25% in homozygous).

Later on, strong association between *LOXL1* SNPs and exfoliation phenotype was replicated in several Caucasian populations (Fingert et al. 2007a; Aragon-Martin et al. 2008; Challa et al. 2008; Fan et al. 2008; Mossbock et al. 2008; Pasutto et al. 2008b; Yang et al. 2008), in Australian (Hewitt et al. 2008b), in African-Americans (Fan et al. 2008), and in Asian populations from Japan and India (Fuse et al. 2008; Hayashi et al. 2008; Mabuchi et al. 2008; Mori et al. 2008; Ozaki et al. 2008; Ramprasad et al. 2008). The *LOXL1* SNP allelic frequencies in cases and controls and corresponding p-values in different populations are presented in Table 7.

| origin | rs1048661 G (R141L) | | | | rs3825942 G (G153D) | | | rs2165241 T | | | study |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Frq in cases | Frq in controls | p-value | Frq in cases | Frq in controls | p-value | Frq in cases | Frq in controls | p-value | |
| **Icelandic** | **XFS** | 0.79 | 0.65 | $1.3x10^{-3}$ | 0.98 | 0.85 | $8.5x10^{-7}$ | 0.74 | 0.47 | $1.9x10^{-8}$ | (Thorleifsson et al. 2007) |
| | **XFG** | 0.83 | | $1.8x10^{-6}$ | 0.99 | | $4.1x10^{-9}$ | 0.75 | | $4.3x10^{-12}$ | |
| **Swedish** | **XFG** | 0.83 | 0.68 | $2.7x10^{-7}$ | 0.99 | 0.88 | $9.1x10^{-14}$ | 0.81* | 0.54 | $3.1x10^{-17}$ | (Thorleifsson et al. 2007) |
| **Australian** | **XFS** | 0.78 | 0.66 | $8.49x10^{-4}$ | 0.95 | 0.84 | $7.83x10^{-5}$ | - | - | - | (Hewitt et al. 2008b) |
| **Austrian** | **XFG** | 0.84 | 0.67 | $2.55x10^{-7}$ | 0.99 | 0.82 | $5.76x10^{-15}$ | - | - | - | (Mossbock et al. 2008) |
| **German** | **XFS** | 0.79 | 0.64 | $7.08x10^{-7}$ | 0.95 | 0.86 | $3.15x10^{-6}$ | 0.72 | 0.48 | $7.04x10^{-15}$ | (Pasutto et al. 2008b) |
| | **XFG** | 0.84 | | $1.40x10^{-15}$ | 0.95 | | $4.78x10^{-9}$ | 0.77 | | $1.74x10^{-26}$ | |
| **Italian** | **XFS** | 0.84 | 0.69 | 0.0024 | 1.00 | 0.82 | $5.08x10^{-8}$ | 0.80 | 0.515 | $8.79x10^{-7}$ | (Pasutto et al. 2008b) |
| | **XFG** | 0.82 | | 0.0053 | 1.00 | | $1.96x10^{-12}$ | 0.80 | | $5.18x10^{-9}$ | |
| **Iowa** | **XFS/XFG** | 0.82 | 0.60 | 0.000036 | 0.99 | 0.88 | 0.0003 | - | - | - | (Fingert et al. 2007a) |
| **USA, Caucasian** | **XFG** | 0.79 | 0.67 | 0.02 | 0.94 | 0.84 | 0.02 | 0.67 | 0.49 | 0.001 | (Challa et al. 2008) |
| **USA, Caucasian, African-American** | **XFS** | 0.80 | 0.72 | 0.12 | 0.98 | 0.80 | $2.7x10^{-7}$ | 0.75 | 0.46 | $1.0x10^{-6}$ | (Fan et al. 2008) |
| | **XFG** | 0.84 | | 0.0031 | 0.99 | | $1.3x10^{-13}$ | 0.76 | | $1.5x10^{-10}$ | |
| **USA, European (Caucasian)** | **XFS** | 0.83 | 0.70 | $5.44x10^{-4}$ | 0.92 | 0.80 | $1.01x10^{-4}$ | 0.73 | 0.45 | $2.30x10^{-11}$ | (Aragon-Martin et al. 2008) |
| | **XFG** | 0.85 | | $2.53x10^{-6}$ | 0.99 | | $5.59x10^{-13}$ | 0.75 | | $4.17x10^{-17}$ | |
| **Indian** | **XFS/XFG** | 0.72 | 0.63 | 0.16 | 0.92 | 0.74 | 0.0001 | - | - | - | (Ramprasad et al. 2008) |
| **Japanese** | **XFS** | 0.025 | 0.49 | $1.5x10^{-8}$ | 1.00 | 0.88 | 0.027 | - | - | - | (Fuse et al. 2008) |
| | **XFG** | 0.042 | | $1.7x10^{-12}$ | 1.00 | | $5.2x10^{-3}$ | | | | |
| **Japanese** | **XFS** | 0.02 | 0.46 | $1.7x10^{-11}$ | 1.000 | 0.86 | $1.3x10^{-3}$ | - | - | - | (Hayashi et al. 2008) |
| | **XFG** | 0.00 | | $1.1x10^{-10}$ | 1.000 | | $3.0x10^{-3}$ | | | | |
| **Japanese** | **XFS/XFG** | 0.60 | 0.45 | <0.0001 | 0.99 | 0.85 | <0.0001 | - | - | - | (Mabuchi et al. 2008) |
| **Japanese** | **XFS** | 0.07 | 0.50 | $3.39 x10^{-28}$ | 0.99 | 0.86 | $1.49 x 10^{-7}$ | 0.02 | 0.10 | $5.33 x 10^{-4}$ | (Ozaki et al. 2008) |
| | **XFG** | 0.04 | | $1.44 x 10^{-34}$ | 0.99 | | $1.40 x 10^{-7}$ | 0.009 | | $4.76 x 10^{-6}$ | |

**Table 7.** The *LOXL1* gene risk allele frequencies for three SNPs and their corresponding p values in different populations. Frq. = frequency; XFS/XFG = unclassified XFS patients with or without glaucoma.

#### *2.8.1.1 LOXL1 structure and function*

The product of the *LOXL1* gene modifies elastin fibers that are major components of the intraocular lesions in XFG (Figure 5). The LOXL1 protein is a member of the lysyl oxidase family of proteins which catalyzes the oxidative deamination of lysine residues of tropoelastin that leads to the cross-linking of these residues and consequential formation of elastin polymer fibers (Liu et al. 2004; Lucero and Kagan 2006). The lysyl oxidase family of proteins has five members: LOX protein and four LOX-like proteins (LOXL1-LOXL4). All of these have seven exons of which exons 2-7 are highly homologous and encodes the C-terminal catalytic domain of these proteins. The most sequence differences between genes resides on the first exon, which encodes the N-terminal pro-peptide of the protein, that binds to tropoelastine and fibulin-5, and directs the deposition of the enzyme onto elastic fibers (Liu et al. 2004; Thomassin et al. 2005), but is subsequently cleaved off for catalytic activation of the enzyme. Two non-synonymous SNPs, rs1048661 (Arg141Leu) and rs3825942 (Gly153Asp) are located on the first exon of the LOXL1 gene and thus lie on this pro-peptide of the protein (Figure 6). These sequence variants might change the role of pro-peptide either by modifying pro-peptide cleavage or disturbing interactions with substrates, like tropoelastin and fibulin-5.

The *LOXL1* gene is expressed in several ocular tissues (e.g. lamina cribrosa, lens epithelium, ciliary muscle, cornea, trabecular meshwork and optic nerve head astrocytes) (Urban et al. 2007) and extraocular tissues (e.g. lung, kidney, liver, heart, and muscle tissue) (Kenyon et al. 1993; Kim et al. 1995) of which all are known to be affected by accumulations of XFS material (Schlötzer-Schrehardt et al. 1992). Mice genetically knocked out of the LOXL1 protein display diffuse connective tissue associated changes including loose skin, vascular abnormalities and emphysematous changes with large alveolar spaces (Liu et al. 2004) as well as pelvic floor and urinary tract disorders associated with increased pelvic floor laxity (Liu et al. 2006) secondary to failed elastic fiber homeostasis. LOXL1 expression appears to diminish with age (Liu et al. 2006), which is interesting since XFS/XFG is an age-related disease.

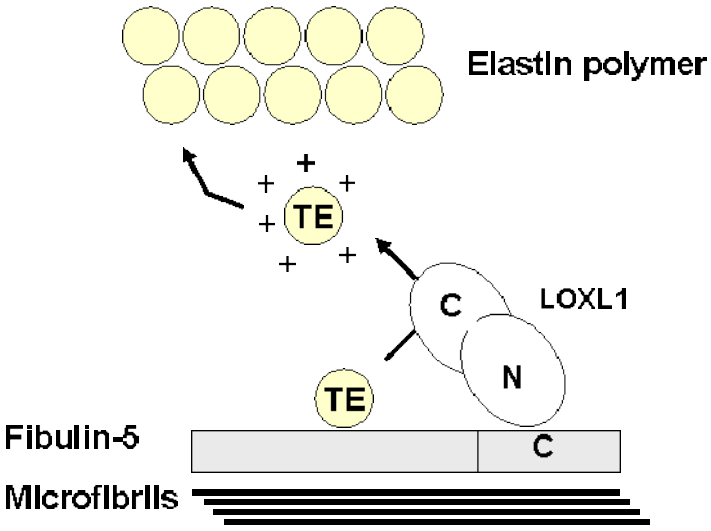


**Figure 5. A simplified model for the role of LOXL1 in elastogenesis.** Fibulin-5 binds both tropoelastin (TE) and LOXL1, thus bringing substrate (TE) and enzyme (LOXL1) into juxtaposition for polymer formation. LOXL1 converts TE into lysyl-deaminated form. 'Activated' TE associates with one another or deposits onto the existing polymer, followed by spontaneous covalent cross-linking. Fibrillin containing microfibrils act as scaffolds in this process guiding the cross-linking process and elastin deposition. TE = tropoelastin. Modified from Liu and colleagues 2004 (Liu et al. 2004).

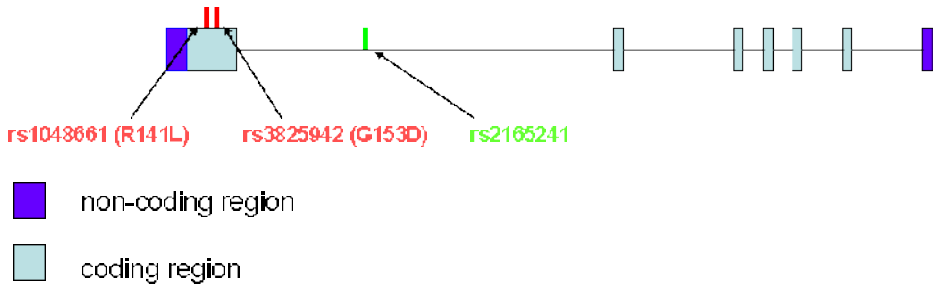


**Figure 6. The schematic representation the LOXL1 gene.** The positions of the two exonic SNPs (rs1048661, rs3825942) and one intronic SNP (rs2165241) are shown by arrows.

### 2.8.2 Other candidate gene studies

Numerous functional candidate genes such as *Oculomedin* (*OCLM*) (Jansson et al. 2003a), *adenosine A3 receptor* (*ADORA3*) (Schlotzer-Schrehardt et al. 2005), *glutathione S-transferases (GSTs)* (Yilmaz et al. 2005b), *5,10-methylenetetrahydrofolate reductase* (*MTHFR*) (Junemann et al. 2005; Turacli et al. 2005), *apolipoprotein E (ApoE)* (Yilmaz et al. 2005a), *matrix metalloproteinases (MMPs)* and *tissue inhibitor of metalloproteinases (TIMPs)* (Schlotzer-Schrehardt et al. 2003; Ho et al. 2005; Zenkel et al. 2005), *clusterin* (Zenkel et al. 2005; Zenkel et al. 2006; Burdon et al. 2008), and genes previously associated with OAG (*MYOC*, *OPTN*) (Jansson et al. 2003b; Abu-Amero et al. 2008) have been investigated in XFS patients. However the role of these genes in XFS/XFG has remained uncertain (Sjöstrand et al. 2002; Jansson et al. 2003b; Jansson et al. 2003a).

# 3 AIMS OF THE PRESENT STUDY

I To investigate the role of two glaucoma candidate genes, *MYOC* and *OPTN*, in glaucoma families originating from Southern Finland.

II To analyse 14 genetic candidate loci for POAG in Finnish glaucoma families using the linkage analysis approach.

III To analyse the role of the *MYOC* gene in a Finnish family with JOAG and POAG.

IV To perform a genome-wide scan in a Finnish family with XFS, in order to identify susceptibility loci for XFS.

V To investigate whether three single nucleotide polymorphisms in the *LOXL1* gene contain risk for Finnish XFS, XFG or POAG.

# 4 SUBJECTS AND METHODS

## 4.1 Patients and families

The family material was the same in Studies I and II from the Tammisaari region. The family material used in Study III was collected via the Department of Ophthalmology of Helsinki University Hospital. The families and family members included in Studies IV and V were identified from the population-based study of Kökar. Sporadic glaucoma patients and unaffected individuals in Study V were collected from a private practice in Tammisaari (129 XFS/XFG, 64 POAG, 26 controls) and from the Eye Department of Helsinki University (12 XFS/XFG, 7 POAG). Anonymous, unexamined blood-donors from the Red Cross were used as population-based controls (Figure 7).

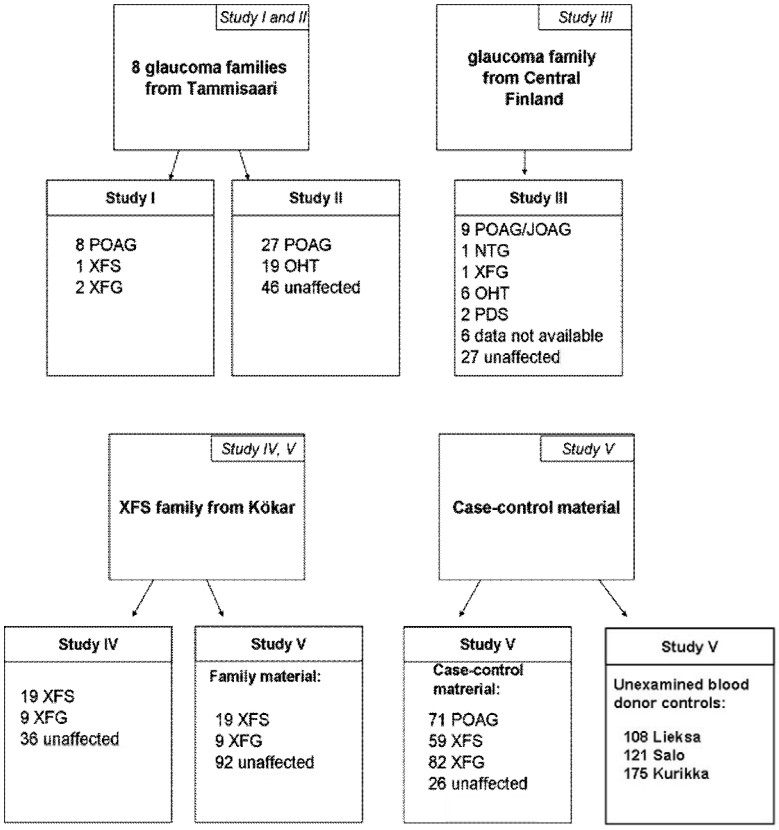


**Figure 7. Number of patients and controls used in studies I-V.** POAG = adult onset primary open angle glaucoma, JOAG = juvenile-onset primary open angle glaucoma, XFS = exfoliation syndrome, XFG = exfoliation glaucoma, OHT = ocular hypertension, PDS = pigment dispersion syndrome, unaffected = ophthalmologically examined individuals with no evidence of OAG or XFS/XFG.

A comprehensive ophthtalmologic examination with dilatation was performed for all participants except the anonymous blood-donors. The familial and sporadic controls had no signs of glaucoma or exfoliation syndrome.

The family material in Study I included eight unrelated families from Tammisaari region, three of them having POAG as the only diagnosis and five had both POAG and XFG or XFS (Figure 8). In all families, the inheritance of the disease resembled that of the autosomal dominant trait. All family members over the age of 40 were invited to comprehensive ophthalmological examination. The phenotype was determined in a total of 136 family members and of these glaucoma was diagnosed in a total 53 (44 had POAG, 7 had XFG and 2 had other types of glaucoma). Of these 136 family members eleven subjects, 8 POAG-, 2 XFG- and 1 XFS- patient, representing different families, were chosen for molecular genetic studies (Study I).

The subjects for Study II were selected from the eight Finnish glaucoma families described in Study I. In total 92 samples were analysed; of these 27 were classified as POAG patients with narrow diagnostic category (liability class 1, LC1), 19 individuals having ocular hypertension or glaucoma suspicion were classified as affected with broad phenotypic classification (liability class 2, LC2) and 46 family members were categorized as unaffected. Family members classified to the unaffected category were ophthtlamologically examined. XFG was diagnosed in three patients and these individuals were included in unaffected category.

The family material used in Study III consist of 52 family members of a six-generation family originating from Central Finland in which JOAG/POAG segregate in a fashion resembling autosomal dominant inheritance. Extensive clinical heterogeneity was present in this family. Of 52 family members 10 had juvenile or adult onset OAG (one of whom had NTG), 1 had XFG, 2 had pigment dispersion syndrome (PDS), 6 had ocular hypertension (OHT), 27 were unaffected relatives and data was not available for 6 family members. The unaffected family members were ophthlamologically examined and were found to be without POAG, JOAG or OHT. The youngest glaucoma patient was diagnosed at the age of 14 years and oldest at the age of 66 years (mean 34.3 years).

The family material of Study IV and V was based on a population-based study performed 1960-62 by Professors Henrik Forsius and Aldur Eriksson on Kökar Island in the southwestern Finnish archipelago. In total 595 individuals (85% of population) were examined (mean age 64 years), of which 247 were over 50 years old (89 males/158 females). Later the same population and earlier unexamined relatives were invited to several follow-up-studies. The latest, seventh, was arranged in 2001-02, when ophthalmologist Eva Forsman joined the study team. By that time, in total 530 individuals (237 males/293 females) over the age of 50 years (mean age of 63 years) were examined at least once (76 XFS, 24 XFG, 12 POAG patients) (Forsman et al. 2007b). In addition to these, two women younger than 50 years were affected (one with XFS, and one with XFG). Seventy-five of these 78 XFS positive individuals were linked to an extended pedigree with 332 examined subjects, in which XFS segregated in a fashion resembling autosomal dominant inheritance. XFS was present in 22% of the examined relatives within the family, compared to 14.5% in the whole island population. Over 40 years, the 3 most recent generations were ophthalmologically examined several times.

The subjects for Study IV were selected from the 183 Kökar inhabitants or descendants (28 XFS, of these, 9 had XFG, 7 POAG patients and 148 unaffected family members) who had participated in the latest follow-up examination in Kökar Island. In all, sixty-four subjects from the family were analyzed; of these 28 had XFS (mean age 70, range 59 - 90), of whom 9 had XFG, and 36 were ophthalmologically investigated family members with no evidence of XFS/XFG (mean age 75.3, range 51 - 94). The control group of 36 unaffected relatives included 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). Furthermore, four glaucoma patients (1 with POAG and 3 with XFG) and 70 additional relatives (18 XFS, 6 XFG, 4 POAG patients and 42 were unaffected relatives) from the Kökar population, were selected for the OAG candidate gene study.

The study material for Study V consisted of sporadic case-control material of 238 subjects from Tammisaari region and family material of 120 family members from the extended family from the Kökar Island (Study IV). The case-control material contained 59 patients with XFS, 82 with XFG, 71 with POAG and 26 ophthalmologically examined individuals with no evidence of ocular disease (mean age 86 years, range: 81-93 years). A total of 404

unexamined Finnish blood donors from Salo (121), Lieksa (108) and Kurikka (175) parishes were used as anonymous population-based controls. The family material consisted of 19 XFS and 9 XFG patients along with 92 ophthalmologically examined unaffected relatives (mean age 63; range 32-92). The linkage analysis was performed for 64 family members; of these, 28 were XFS/XFG affected (mean age 70; range 59-90) and 36 were unaffected (mean age 75.3; range 51–94) (Study IV).

### 4.1.1 Ethical aspects

These studies were approved by the Ethical Committee of the Helsinki University Eye Hospital (Studies I, II, III, V) and by the Ethics Committee of the Åland Central Hospital (Studies IV, V). For collecting old clinical information for Study I the permission was given by Ministry of Social Affairs and Health. All studies were conducted in accordance with the Declaration of Helsinki and informed written consent was obtained from all the examined study subjects.

### 4.1.2 Genealogical studies (I, II and IV)

The information regarding the names and birth dates and places of birth of the patients‘ parents, grandparents and great grandparents were collected from all participants and were used to trace their ancestors back to the 1800s from local church and civil registers. The same questionnaire was used throughout the whole Study (IV) in the Kökar population. The genealogical study was performed as described earlier by Varilo and colleagues (Varilo et al. 1996). Microfilm and microfiche copies of the church records in the Finnish National Archives were used for earlier periods. Pedigrees were constructed with the CorelDRAW 11 program and Microsoft Office Power Point 2003 using the collected data.

### 4.1.3 Clinical definitions

Clinical definition of XFS (Studies I, IV and V) was based on the presence of a greyish central disc with/without focal breaks, with/without a peripheral band on the anterior lens capsule, and/or fibrillary material on the pupillary ruff, observed after dilation and recorded without grading. When XFS was detected at least in one eye, the subject was defined as being XFS-positive. Suspect changes such as Krukenberg´s spindle and pigmentation of cornea endothelium, diffuse haze on the anterior capsule, were noted as XFS negative.

The diagnosis of open angle glaucoma (Studies I and II) was based on two of the three characters: 1) IOP > 22 mmHg, 2) presence of glaucomatous changes in the optic nerve head, and 3) glaucomatous visual field defect. The disc was considered as glaucomatous when one of the following was detected: localized thinning of the rim (notching), diffuse damage with cup-to disc ratios C/D > 0.6, or an asymmetry of > 0.2 in C/D between the eyes with equal size discs. Individuals were classified as glaucoma suspect if only one of the diagnostic criteria was fulfilled or if a disc haemorrhage was detected. Visual field defects were graded according to the definitions by Hodapp et al (Hodapp et al. 1993).

In Study III subjects were classified as POAG/JOAG when glaucomatous disc and/or field defect and IOP≥ 22 mmHg was detected. Persons were categorized as NTG when glaucomatous disc and field defect was detected, but IOP ≥22 mmHg was never documented. The optic disc was graded as glaucomatous in the presence of focal or generalized narrowing or disappearance of the neuroretinal rim with increased cupping or pallor of the disc. Ocular hypertension (OHT) was demonstrated when the disc, visual field, and retinal nerve fiber (NFL) were normal and IOP was ≥22 mmHg and subject was classified as normal when the disc, visual field, and NFL were normal and IOP was < 22 mmHg.

## 4.2 Methods

The methods used in the present study are listed in Table 8 and briefly summarized below. Methods are described in more detail in the original publications I to V.

**Table 8. Methods and statistical programs used in the original articles (I-V)**

| Methods | Software | Original article |
|---|---|---|
| **Laboratory procedures** | | |
| DNA extraction | | I, II, III, IV, V |
| Polymerase Chain Reaction (PCR) | | I, II, III, IV, V |
| Agarose gel electrophoresis | | I, II, III, IV, V |
| PCR- sequencing | | I, III, IV, V |
| Electrophoresis | ABI 3100/ABI 377/ABI3730 DNA sequencer | I, II, III, IV, V |
| Primer design | Primer3 program 0.2 / 0.4.0 | I, II, III, IV, V |
| **Analysis methods** | **Analysis programs** | |
| Sequencing | Sequencher 4.0.5 / Sequencer 4.6 | I, III, IV, V |
| Genotyping | Genotyper 2.0 / Genemapper 3.0 | II |
| Genotype error checking | Pedcheck 1.1 | II, IV, V |
| **Statistical methods** | **Statistical programs** | |
| Allele frequency calculations | Downfreq 2.1 | II, IV, V |
| Heterogeneity testing, calculations of proportion of linked families | Homog 3.35 | II, IV, V |
| Linkage analyses | MLINK/LINKAGE | II, IV, V |
| Linkage analyses | Analyze | II, IV, V |
| Multipoint linkage analyses | Vitesse | IV |
| Multipoint linkage analyses | Superlink 1.6 | V |
| Multipoint linkage analyses | Simwalk 2.83 | IV |
| Haplotype construction | SNPHAP 1.2.1 | V |
| Haplotype construction | FBAT 2.0.2c | V |
| Association analysis | FBAT 2.0.2c | V |
| Association analysis | R-program, R package epitools | V |

### 4.2.1 Laboratory methods

#### *4.2.1.1 PCR-sequencing*

Genomic DNA was extracted from peripheral blood with a DNA purification kit (PureGene® Gentrasystems, Minneapolis, MI) or with the standard phenol-chloroform procedure. Primer sequences were designed using the Primer3 program. The DNA of the study subjects was amplified by the polymerase chain reaction (PCR). The polymerase chain reaction conditions were as follows: 10 or 3 min at 95°C followed by 35 cycles of the denaturation step: 40 s at 95°C, annealing step: 40 s at temperature specific for each primer (50-65°C), the elongation step: 1 min at 68 or 72 °C and final extension for 5 min at 72 °C. Sequencing was performed using cycle sequencing with the Big Dye Terminator kit (ABI, Foster City, CA, USA) and reactions were run on an ABI 3100 or ABI3730 capillary sequencer.

#### *4.2.1.2 Genotyping*

Markers in Study II were selected from the Marshfield Medical Research Foundation map and the primer sequences were from the Genome Database or designed by the Primer3 program. Forward primers were labelled at the 5' -end with the fluorescent dye (6-FAM, TET, NED, VIC, PET, HEX). PCR-products were pooled and electrophoresed on an ABI3730 or ABI 377 DNA sequencer (Applera Corporation, Norwalk, CT). Genotypes were assigned using Genotyper 2.0 or Genemapper 3.0 software (Applera Corporation). All microsatellite and SNP markers used in this study (Studies II, IV, V) were checked and corrected for Mendelian errors prior to analysis using the Pedceck program (O'Connell and Weeks 1998).

### 4.2.2 Statistical analyses

#### *4.2.2.1 Linkage analysis*

The two-point linkage analyses were performed under both homogeneity and heterogeneity using the MLINK program of the LINKAGE package (Studies II, IV, V) (Lathrop and Lalouel 1984; Lathrop et al. 1986). The HOMOG 3.35 program was used to

test for heterogeneity and to calculate the proportion of families showing linkage (α) (Ott 1999). Allele frequencies were derived from the data by the DOWNFREQ 2.1 program (Terwilliger 1995).

In Study II, an affected-only approach was taken due to late onset of this disease and lack of reliable penetrance ratios. The non-parametric option of SIMWALK2.83 was employed for multipoint analyses (Sobel and Lange 1996). In studies IV and V clinically unaffected family members (n=36) were scored as unaffected in analyses because their mean age at the time of the last examination was 75.3 years (range, 51–94) and because using an affected-only method would have led to substantial loss of information. However, in Study IV the affected-only analyses were also performed, in order to compare the models. Multipoint analyses were performed using Vitesse (Study IV) (O'Connell and Weeks 1995; O'Connell 2001) or Superlink 1.6 (Study V) program (Fishelson and Geiger 2002; Fishelson et al. 2005).

#### *4.2.2.2 Association analysis*

In Study V, the association was measured by the Pearsons $\chi 2$ –test with Yates' continuity correction (in R-program). The odds ratios (OR) with 95% confidence intervals (CI) and Fisher's exact p-values were estimated using R package epitools. Levins formula was used to estimate population attributable risk (PAR%). The association analysis was performed for case-control material using a total of 404 unexamined Finnish blood donors as anonymous population-based controls. Hardy-Weinberg was tested in cases and controls separately with the chi-square test (with 5% level of significance). In family material the association was measured in whole family material using independent XFS/XFG cases and unaffected relatives picked from the pedigrees. Haplotypes were constructed in case-control material using the SNPHAP 1.2.1 and in family material using the FBAT 2.0.2c program (Laird et al. 2000; Horvath et al. 2001).

# 5 RESULTS AND DISCUSSION

## 5.1 Molecular genetic studies of OAG in Finnish glaucoma families

In 2002 when we started molecular genetic studies of OAG, linkage studies had produced several candidate loci for OAG and first two candidate genes, *MYOC* and *OPTN*, had been identified. The *MYOC* gene was already confirmed glaucoma associated gene but the role of novel candidate gene, *OPTN*, was still unconfirmed. We wanted to investigate *OPTN* and *MYOC* genes and hitherto known candidate loci in Finnish glaucoma patients. Nowadays it is known that the *MYOC* and *OPTN* are not as common cause of glaucoma as originally thought.

### 5.1.1 Exclusion of candidate genes and loci (I, II)

In order to screen OAG candidate genes for mutations in eighth Finnish glaucoma families, all coding regions and respective exon-intron boundaries of the *MYOC* and *OPTN* genes were sequenced from the genomic DNA of eleven individuals; eight POAG-, two XFG- and one XFS patient, representing different families (Figure 8). No disease associated variants in the coding regions or splice sites of the *MYOC* or *OPTN* genes were identified. Instead, two polymorphisms in the *MYO*C gene (Tyr347Tyr, Arg76Lys) and three in the *OPTN* gene (Thr34Thr, Glu163Glu, 553-5C) were detected (Figure 8, Table 9). Synonymous polymorphism Tyr347Tyr in the *MYOC* gene was found in a mother with XFG and her son with POAG and non-synonymous polymorphism Arg76Lys was present in one of eight POAG patients. In the *OPTN* gene, synonymous polymorphism Thr34Thr was detected in 6 of 11 subjects, of whom 4 had POAG, 1 had XFG, and 1 had XFS. Yet, synonymous polymorphism Glu163Glu was identified in one XFG patient and a novel intronic variation 553-5C was detected in 7 of 11 subjects, of whom 5 had POAG, one had XFG, and one had XFS. Even though intronic variation is located near the exon-intron boundary it does not change splice sites or make any splice site-like formation.

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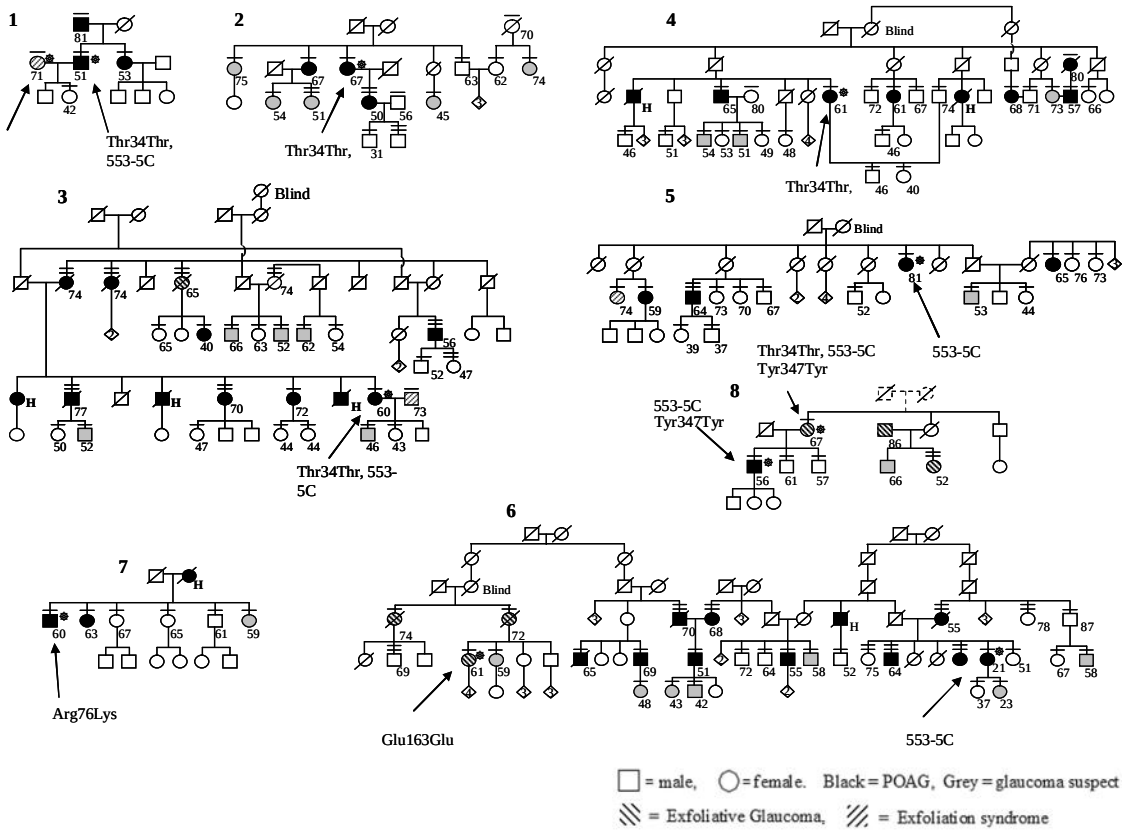


**Figure 8. Pedigrees for Finnish glaucoma families originating from Tammisaari.** Three of the families had POAG as the only diagnosis and five had both POAG and XFG. Disease was inherited resembling autosomal dominant model with reduced penetrance in all families. An asterisk (*) indicates that the *MYOC* and *OPTN* genes were sequenced. Polymorphisms in the *MYOC* or *OPTN* genes are marked in the families by arrows. The age of diagnosis (for affected individuals) or age at examination is presented below the symbol.

| Gene | Polymorphism | Position | POAG | XFG | XFS |
|---|---|---|---|---|---|
| *MYOC* | | | | | |
| | Tyr347Tyr | exon 3/3 | 1 | 1 | |
| | Arg76Lys | exon 1/3 | 1 | | |
| *OPTN* | | | | | |
| | Thr34Thr | exon 4/16 | 4 | 1 | 1 |
| | Glu163Glu | exon 6/16 | | 1 | |
| | 553-5C | intron 6 | 5 | 1 | 1 |

**Table 9. Polymorphisms in the *MYOC* and *OPTN* genes**

Study II was designed to further examine the cause of OAG in the eight glaucoma families, in which susceptibility variants in the *MYOC* and *OPTN* genes were excluded (Study I). Fourteen hitherto reported candidate regions, including loci for the *MYOC* and *OPTN* genes, were analysed in the families (Table 10). Loci for the *MYOC* and *OPTN* genes were analysed in order to exclude intronic and promoter region variants of these genes. In total 92 family members, of whom 27 were classified as POAG patients with narrow diagnostic category (liability class 1, LC1), 19 having ocular hypertension or glaucoma suspicion were classified as affected with broad phenotypic classification (liability class 2, LC2) and 46 diagnosed as unaffected were genotyped using 35 microsatellite markers on 14 candidate regions. Linkage was tested using the affected-only approach, autosomal dominant inheritance model, low phenocopy rate and rare disease allele frequency.

No evidence for linkage was found in any of the analysed loci in two point or multipoint linkage analyses (Table 10). The slightly interesting locus in the analyses was GLC1D on chromosome 8q23, in which two markers, D8S257 and D8S1471, locating 12cM apart, provided slightly positive pair-wise LOD scores of 0.27 (LC1, dominant model) and 1.24 (LC2, dominant model), respectively. However, the surrounding markers did not give any evidence of linkage. Neither non-parametric multipoint analysis on the GLC1D locus yielded increased LOD scores. The increased pair-wise LOD scores were most likely overestimates, probably due to recombinations, which were not shown in the two-point analysis but were revealed in the multipoint analysis.

| Region | Loci | Gene | $Z_{max}$ | LC | Reference |
|---|---|---|---|---|---|
| 1q21–q31 | GLC1A | *MYOC* | 0.625 | LC2 | (Sheffield et al. 1993; Stone et al. 1997). |
| 2cen–q13 | GLC1B | | 0.001 | LC1 | (Stoilova et al. 1996) |
| 2q33-q34 | | | 0.125 | LC2 | (Nemesure et al. 2003) |
| 2p14 | | | 0.002 | LC2 | (Wiggs et al. 2000) |
| 3q21–q24 | GLC1C | | 0.094 | LC2 | (Wirtz et al. 1997) |
| 7q35–q36 | GLC1F | | 0.000 | - | (Wirtz et al. 1999) |
| 8q23 | GLC1D | | 1.243 | LC2 | (Trifan et al. 1998) |
| 10p12-p13 | | | 0.323 | LC2 | (Nemesure et al. 2003) |
| 10p14–p15 | GLC1E | *OPTN* | 0.600 | LC2 | (Sarfarazi et al. 1998; Rezaie et al. 2002) |
| 14q11 | | | 0.000 | - | (Wiggs et al. 2000) |
| 14q21-q22 | | | 0.124 | LC2 | (Wiggs et al. 2000) |
| 17q25 | | | 0.019 | LC2 | (Wiggs et al. 2000) |
| 17p13 | | | 0.111 | LC2 | (Wiggs et al. 2000) |
| 19q12-q14 | | | 0.087 | LC1 | (Wiggs et al. 2000) |

**Table 10.** Highest two-point heterogeneity lod score (LodHet) obtained from each analysed candidate locus. LOD scores are maximized over recombination fractions (θ) ranging from 0 to 0.5. LOD scores with the diagnostic classification (LC) that yielded the highest LOD score are shown.

### 5.1.2 Susceptibility mutation in the *MYOC* gene (III)

Sequencing the coding region and the exon-intron boundaries of the *MYOC* gene resulted in the identification of a cytosine to thymine (C>T) transition at nucleotide 1130 in the third exon of the *MYOC* gene in the family proband (IV-23 in Figure 9). The mutation leads to substitution of the threonine residue with methionine (Thr377Met) in the olfactomedin like domain of the myocilin protein. To examine the segregation of the mutation in the family, the DNA of a total of 52 family members was analysed. The Thr377Met segregated in the right branch of the pedigree, in which 20 of 44 individuals older than 12 years carried the mutation. Of these 9 (45%) were glaucomatous (8 high pressure glaucoma and 1 normal tension glaucoma) and 2 (10%) had ocular hypertension

(OHT), demonstrating a wide clinical intrafamilal heterogeneity. The mean age at diagnosis of glaucoma was 34.3 years (range: 14-66 years) and seven of nine patients had glaucoma before the age of 35 years. No evidence of glaucoma was identified in 9 mutation carriers (ages 12-61, mean 38.3), some of them at the age when disease could have manifested. A sixty-one-year old unaffected family member with Thr377Met mutation was the oldest hitherto reported unaffected mutation carrier. In total 24 family members did not carry Thr377Met and of these one (IV-34) had JOAG, three had OH and one had pigment dispersion syndrome (PDS). None of the eight individuals in the left branch of the family carried the mutation (Figure 9).

The age of onset was the earliest hitherto described with the Thr377Met mutation; the youngest patient was diagnosed at the age of 14 years. The penetrance of Thr377Met mutation in the family was 5% in family members under the age of 15 years, 35% at the ages 16-35 years, 40% at ages 36-50 years and 45% over 50 years.

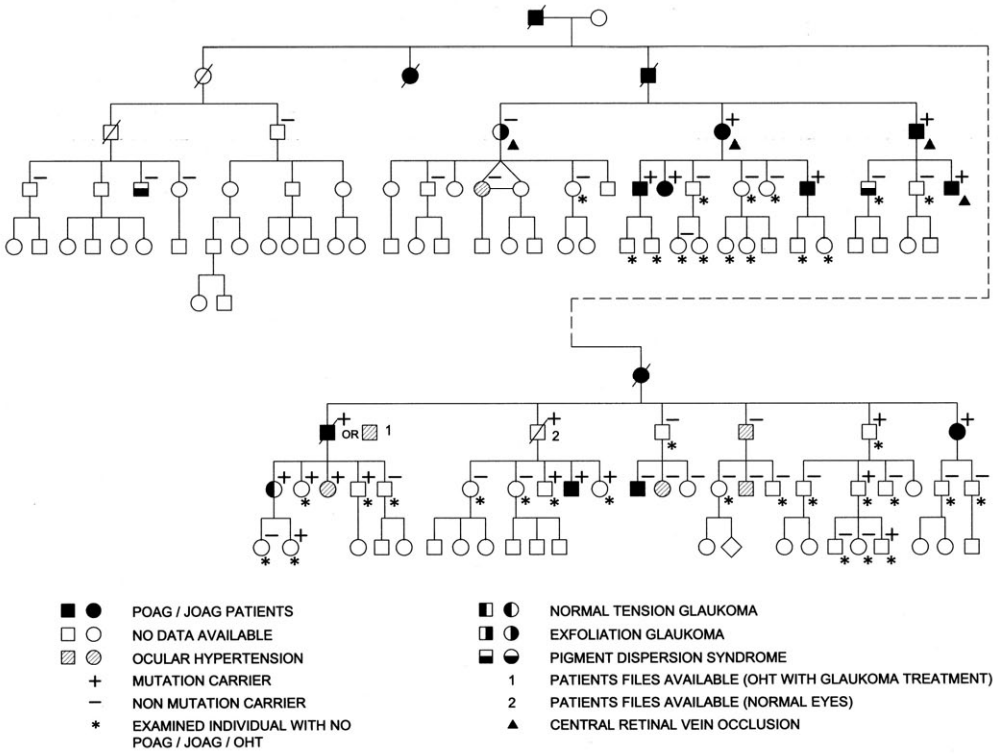


**Figure 9**. **The pedigree of the glaucoma family with Thr377Met mutation in the *MYOC* gene.** The Thr377Met mutation segregates on the right branch of the family.

### 5.1.3 Discussion of genetic basis of glaucoma (I, II, III)

The prevalence of glaucoma in the families included in studies I and II was 35% among examined first and/or second degree relatives, corresponding to previous reports (Shin et al. 1977; Lindberg 1989; Nemesure et al. 1996). The proportion of glaucoma suspects was even 28% among family members considered unaffected. Although the genetic contribution was clear and glaucoma segregated in a fashion resembling autosomal dominant inheritance in all families, the genetic background underlying glaucoma remained unidentified. No functional variants in the *MYOC* and *OPTN* genes were established and linkage was not observed for any of the 14 OAG candidate loci. Preliminary results for the most recently identified candidate gene *WDR36* suggest that neither it has a major role in the glaucoma in the families (unpublished data). These negative results confirm previous results from other populations, where hitherto known candidate genes and loci have been shown to be a rare cause glaucoma (Libby et al. 2005; Fan et al. 2006a). At the same time, the results encourage a search for novel susceptibility genes for glaucoma. These results are suggestive due to the small number of analysed families, but as the first molecular genetic studies of OAG in Finland this was the first step in a new era.

The results of Study III provided the first molecular genetic explanation for glaucoma in Finland. The glaucoma associated mutation Thr377Met in the *MYOC* gene segregated with the disease in the Central Finnish JOAG/POAG family. For this glaucoma family the most important effect of identifying susceptibility mutation is enabling accurate screening of risk individuals before they show any manifestation of the disease and hence facilitating early diagnosis and follow-up for family members at risk. The finding of a causative variant enables the treatment of glaucoma on time, which is important in such a late-onset, insidiously progressing disease.

The finding of the Thr377Met mutation in the glaucoma family confirmed the contribution of the *MYOC* gene to the pathogenesis of glaucoma also in the Finnish population. It has been hypothesized that the Thr377Met has been introduced to Finland from the East, the connection is at least 1,500 years old, from when the Finno-Ugric (Huns) people migrated from Central Asia (Hewitt et al. 2007). Thus far, the Thr377Met mutation has been reported at least in 14 studies, mainly in JOAG or POAG patients with

Caucasian ethnicity, but also in patients originating e.g. from India, Morocco and Macedonia (Wiggs et al. 1996; Allingham et al. 1998; Alward et al. 1998; Wiggs et al. 1998; Fingert et al. 1999; Simm et al. 1999; Shimizu et al. 2000; Wiggs and Vollrath 2001; Vincent et al. 2002; Kanagavalli et al. 2003; Mackey et al. 2003; Melki et al. 2003b; Wilkinson et al. 2003; Petersen et al. 2006) (myocilin allele-specific glaucoma phenotype database). The disease type is similar in patients with the Thr377Met mutation in most of the studies. The varying age of onset, demonstrated in the present study (mean: 34.3 years, range: 14-66), has been detected also in previous studies (mean 39.9 ± 13.1 years) (myocilin allele-specific glaucoma phenotype database). Severe disease type in the family, leading in surgical procedures in 56% glaucomatous mutation carriers correspond previous studies, where c.a. 53% of the cases required trabeculectomy (myocilin allele-specific glaucoma phenotype database). Incomplete penetrance of the Thr377Met mutation has been reported in several studies (Alward et al. 1998; Mackey et al. 2003) (myocilin allele-specific glaucoma phenotype database). In the present study Thr377Met was found in nine non-glaucomatous family members, but was missing in one JOAG patient, indicating that other genetic or environmental factors may contribute to the pathogenesis of OAG.

According to the myocilin database Thr377Met mutation accounts for approximately 5.6% of diseases causing variants in the *MYOC* gene (myocilin allele-specific glaucoma phenotype database). Both Thr377Met mutation and Tyr347Tyr polymorphism (Study I) lie within the olfactomedin-like domain where majority of glaucoma associated *MYOC* variants are located (Adam et al. 1997; Stone et al. 1997; Suzuki et al. 1997). The olfactomedin-like region shows strong sequence homology to a protein found in the neuro-olfactory epithelium of the nose, called olfactomedin (Snyder et al. 1991; Nguyen et al. 1998). The amino acid Thr377 is the phosphorylation substrate of the casein kinase II (CK2) -site and its phosphorylation is important for normal myocilin function. It has been predicted that the Thr377Met mutation does not make a structural change to the myocilin protein, but alters this target residue of a conserved CK2 motif (Rozsa et al. 1998). The Thr377Met alteration changes the polarity and the putative phosphorylation substrate itself, presumably eliminating phosphorylation of Thr377 at the CK2-site (Rozsa et al. 1998). The Arg76Lys polymorphism (Study I) lies outside of both olfactomedin domain and leucine zipper region and has been classified as non-causative because of its

similar frequencies in both POAG cases and controls (Alward et al. 1998; Fingert et al. 1999).

## 5.2 Molecular genetic studies of XFS and XFG in Finnish patients

During the last year there has been remarkable progress in the field of molecular genetics of XFS and XFG, although the pathogenesis is still poorly understood. In year 2002, when we started molecular genetic studies of XFS and XFG the information of the genetic background underlying XFS and XFG was limited. Chromosomal regions 2p16, 2p14-2cen and 2q35-q36 had been suggested as possible candidate loci for OAG but results had remained unconfirmed (Sotirova et al. 1999; Wiggs et al. 1999). To identify genetic susceptibility loci for XFS we performed the genome wide scan for an extended Finnish XFS family (Figure 10). Shortly after we had published the results of the genome-wide scan, an Icelandic study group reported a strong association between three *LOXL1* gene SNPs and XFS and XFG in the Scandinavian populations. The association was subsequently replicated in several other populations. As we were interested in whether the *LOXL1* gene SNPs confers risk to Finnish XFS, XFG or POAG, we analysed the SNPs in Finnish patient and control cohorts.

### 5.2.1 Genome-wide scan of XFS (IV)

The genome-wide scan of exfoliation syndrome using 1104 microsatellite markers evenly distributed in the genome was performed for 64 family members of an extended XFS family; of these 28 were XFS affected (mean age 70, range 59-90), of whom 9 had XFG, and 36 were unaffected (mean age 75.3, range 51 - 94). Seven markers at chromosomal regions 2q32.3, 5q33.3, 17p13.3, 18q12.1-21.33 and Xp22.2 suggested evidence for linkage ($Z_{max\ dom} > 1.5$) (Table 11). Moreover, seventeen markers (at regions 1q, 4p, 4q, 5p, 7p, 7q, 10p, 10q, 13q, 15q, 16q, 19q, Xp, Xq) exceeded a two-point LOD score of 1.0 (dominant/recessive model). The most promising chromosomal region was located at 18q12.1-21.33, where marker D18S468 produced the highest two-point LOD score of 3.45 (dominant model). Four markers surrounding the best marker exceeded a two-point LOD score of 1.0; D18S1135 ($Z_{max\ dom}$=1.39), D18S450 ($Z_{max\ dom}$ =1.49), D18S64 ($Z_{max\ dom}$ =1.70) and D18S1147 ($Z_{max\ dom}$ =1.68) (Table 11). Four additional markers on the

region produced LOD score > 0.5 and three markers yielded LOD score > 0.2. Combining information from adjacent markers; *D18S1102–D18S468*, *D18S468–D18S1143*, and *D18S1143–D18S450* in the best region produced maximum three point LOD scores of 1.85, 2.28, and 1.73, respectively. Markers D181135 and D18S468, located 5 cM apart, yielded the maximum three-point LOD score of 2.80 and by using markers D18S468 and D18S450, located 8 cM apart, the highest three-point LOD score of 4.33 was obtained. The locus 18q12.1-21.33 extends 30.9 cM or 27.1 Mb, with markers D18S1135 and D18S1147 defining flanking boundaries at 58 - 89cM or 30.4 - 57.6 Mb (UCSC Human Genome Browser). An interesting detail was that one allele on the best marker D18S468 was found more often (37%) in XFS affected than in unaffected (26%) whereas another allele was more often present in XFS unaffected (34%) than in affected (25%) individuals.

| Marker | Location | Position (cM) | $Z_{max}$ | θ-value | α-value |
|---|---|---|---|---|---|
| **D2S117** | 2q32.3 | 199 | 1.725 | 0.06 | 1.00 |
| **D5S2049** | 5q33.3 | 166 | 1.605 | 0.00 | 1.00 |
| **D17S849** | 17p13.3 | 0.07 | 1.911 | 0.04 | 1.00 |
| **D18S1135** | 18q12.1 | 58 | 1.385 | 0.14 | 1.00 |
| **D18S468** | 18q12.3 | 63 | 3.446 | 0.04 | 1.00 |
| **D18S450** | 18q21.1 | 71 | 1.491 | 0.08 | 1.00 |
| **D18S64** | 18q21.32 | 84 | 1.695 | 0.08 | 0.84 |
| **D18S1147** | 18q21.33 | 89 | 1.682 | 0.12 | 0.83 |
| **DXS7108** | Xp22.2 | 20 | 1.519 | 0.12 | 1.00 |

**Table 11. Highest two-point LOD scores.** Markers with $Z_{max\ dom}$>1.5 on the whole genome wide scan and markers with $Z_{max\ dom}$>1.0 on the locus 18q12.1-21.33. The LOD scores are maximised over recombination fractions (θ) ranging from 0 to 0.5. α ranging from 0 to 1.0 indicates the proportion of families showing linkage.

In order to screen glaucoma candidate genes for mutations in the family, we sequenced coding regions and respective exon-intron boundaries of *OPTN* and *MYOC* genes from the genomic DNA of four family members (3 with XFG and 1 with POAG). All three XFG patients had Arg76Lys change, the variation that has previously been reported as a non-disease causing polymorphism (Alward et al. 1998), in the first exon of *MYOC* gene

(Figure 10). One XFG patient had Met98Lys variation in the fifth exon of the *OPTN* gene (Figure 10). The Met98Lys variant was found also in one of two sons and in two nephews of the index case. None of the mutation carriers, except for the index case, had any symptoms of XFS/XFG or POAG at the time of the examination (age, 40–61 years). The variation was not found in the additional 67 family members, of whom 18 had XFS, 6 had XFG, 4 had POAG and 39 were healthy relatives. The Met98Lys was initially defined a risk-associated variant (Rezaie et al. 2002), but due to the following contradictory results its role in OAG is still unclear (Ayala-Lugo et al. 2007).

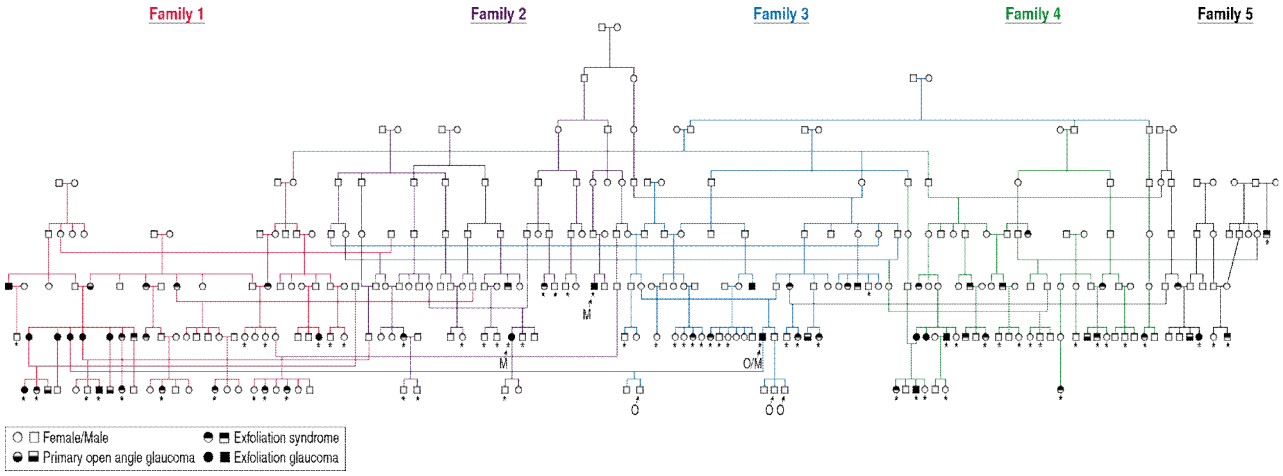


**Figure 10. The pedigree of the Finnish XFS family.** The family was divided into five subfamilies in order to perform linkage analysis. Three last generations have undergone ophthalmological examination. Individuals included in the genome wide scan are marked by asterisks (*). Sequence alterations in *MYOC* (Arg76Lys) and *OPTN* (Met98Lys) genes are shown by arrows and symbols “M” and “O”.

### 5.2.2 Association of the *LOXL1* gene variants to the XFS and XFG (V)

The fifth study was designed to investigate whether three single nucleotide polymorphisms (SNPs) in the *lysyl oxidase-like 1* gene (*LOXL1*) are associated to Finnish XFS, XFG or POAG. Three SNPs, rs1048661 (R141L), rs3825942 (G153D) and rs2165241, were genotyped from the genomic DNA of study subjects from sporadic case-control material and family material from Kökar island (Study IV). All three SNPs were significantly associated with XFS and XFG, but not with POAG, in Finnish patients. The risk allele T of intronic SNP rs2165241 was preferentially present both in the sporadic ($\chi^2$ test, $p=2.62x10^{-13}$) and familial XFS and XFG cases ($\chi^2$ test, $p<0.0001$), compared to the blood donor controls or unaffected family members, respectively (Table 12). Similarly, the risk allele G of exonic SNPs rs1048661 was significantly associated with XFS and XFG both in sporadic cases when compared to blood donor controls ($\chi^2$ test, $p=2.65x10^{-5}$) and in familial cases when compared to unaffected family members ($\chi^2$ test, $p=0.0007$). The highest odds ratio of 6.43 was obtained with the allele G of the coding SNP rs3825942 in sporadic case-control material (Table 12). Interestingly, allele G of rs3825942 was overrepresented in sporadic XFS and XFG cases when compared to the blood donor controls ($\chi^2$ test, $p=2.24x10^{-8}$), but was present with an equally high frequency both in familial XFS/XFG cases and in unaffected family members (Table 12).

Presumably, genotype TT of the intronic SNP rs2165241 showed the strongest association to XFS and XFG ($\chi^2$ test, $p=6.92x10^{-11}$), although association of genotypes GG of rs1048661 and rs3825942 was also remarkable ($\chi^2$ test, $p=5.61x10^{-5}$ and $p=8.88x10^{-8}$, respectively) in sporadic cases when compared to blood donor controls.

The haplotype GG of two coding SNPs, rs1048661 and rs3825942, was the most outstandingly overrepresented in sporadic XFS and XFG cases ($\chi^2$ test $p=9.11x10^{-16}$) but not in POAG cases ($\chi^2$ test, $p=0.98$) compared to the blood donor controls (Table 13). The risk haplotype GG was present in 95% of sporadic XFS/XFG patients (of these 70% were GG homozygous) and in 72% of unexamined blood donor controls (of whom 40% were homozygous). Other two coding locus haplotypes, TG and GA, were underrepresented in XFS/XFG cases compared to blood donor controls (freq. 0.17 versus 0.31, $\chi^2$ test, $p=1.61x10^{-5}$ and freq. 0.03 versus 0.18, $\chi^2$ test, $p=4.32x10^{-9}$, respectively). Interestingly, one XFS patient homozygous for GA haplotype was identified. When haplotypes GG and

TG were compared to GA haplotype odds ratios of 9.37 ($p=2.46x10^{-14}$) and 3.16 (p=0.002) respectively were obtained (Figure 11).

The three-locus haplotype GGT was highly overrepresented both in the sporadic XFS/XFG group ($\chi^2$ test, $p=5.97x10^{-14}$) compared to unexamined blood donor controls and in familial XFS/XFG group compared to unaffected family members ($\chi^2$ test, p=0.0001). (Table 12, Table 13). As expected, association for POAG was not observed (Table 13). Approximately 93% of the sporadic XFS/XFG cases carried the risk haplotype GGT (of these 59% were GGT homozygous), whereas 69% of unexamined blood donors had GGT haplotype (34% homozygous). Both TGC and GAC haplotypes were underrepresented in sporadic XFS/XFG cases compared to blood donor controls (freq. 0.16 versus 0.30, $\chi^2$ test, $p=2.79x10^{-5}$ and freq. 0.02 versus 0.18, $\chi^2$ test, $p=2.19x10^{-10}$, respectively) (Table 13). Again one aforementioned XFS patient was found homozygous for the low risk haplotype GAC. Comparison of GGT and TGC haplotypes relative to GAC haplotype in sporadic XFS/XFG cases yielded odds ratios of 14.91 ($p=1.67x10^{-16}$) and 4.98 (p=0.00018), respectively (Figure 11).

In the sporadic case-control material population attributable risks (PARs) for alleles G of rs1048661 and rs3825942 and T of rs2165241 were of 45%, 82%, 50%, respectively and the corresponding genotype risks were 50%, 79%, and 62%, respectively. The PAR value for the two-locus haplotype GG was 60% and for the three-locus haplotype GGT was 52%.

In the original genome-wide scan of XFS, the area around *LOXL1* showed a suggestive LOD score of 1.19 ($\theta=0.10$, $\alpha=1.00$), with marker D15S1032 at region 15q21.2, locating some 21cM apart from the aforementioned SNPs on the *LOXL1* gene (Study IV). Yet, the nearest markers of the *LOXL1* gene did not show any hint of linkage. Two-point and multipoint linkage were calculated in *LOXL1* SNP genotypes, by adding the familial *LOXL1* SNP genotype data with chromosome 15 microsatellite genotype data from the genome wide scan (Study IV). No significant two- or multipoint LOD scores were obtained with any of three *LOXL1* SNPs, or with the surrounding markers.

| | rs1048661 G (R141L) | | | rs3825942G (G153D) | | | rs2165241 T | | |
|---|---|---|---|---|---|---|---|---|---|
| **Study Groups (n)** | **Frq. (counts)** | **OR (95% CI)** | **p-value** | **Frq. (counts)** | **OR (95% CI)** | **p-value** | **Frq. (counts)** | **OR (95% CI)** | **p-value** |
| **Finnish case-control material** | | | | | | | | | |
| **blood donor controls (404)** | 0.68 (444/650) | | | 0.82 (535/650) | | | 0.47 (296/632) | | |
| **exfoliation combined (141)** | 0.83 (208/252) | 2.19 (1.53-3.18) | $1.47 \times 10^{-5}$ | 0.97 (244/252) | 6.43 (3.29-14.60) | $4.82 \times 10^{-10}$ | 0.73 (205/280) | 3.10 (2.28-4.23) | $7.36 \times 10^{-14}$ |
| **XFS (59)** | 0.82 (82/100) | 2.10 (1.25-3.40) | 0.0048 | 0.94 (94/100) | 3.29 (1.52-8.67) | 0.0020 | 0.72 (85/118) | 2.91 (1.91-4.54) | $4.12 \times 10^{-7}$ |
| **XFG (82)** | 0.83 (126/152) | 2.24 (1.44-3.59) | 0.00032 | 0.99 (150/152) | 14.97 (4.69-98.14) | $2.43 \times 10^{-9}$ | 0.74 (120/162) | 3.23 (2.22-4.79) | $4.12 \times 10^{-10}$ |
| **POAG (71)** | 0.69 (93/134) | 1.05 (0.71-1.59) | 0.84 | 0.79 (107/136) | 0.79 (0.51 -1.27) | 0.33 | 0.46 (64/140) | 0.96 (0.66-1.38) | 0.85 |
| **Finnish family material** | | | | | | | | | |
| **Study Groups** | **Frq.** | **$\chi^2$ test statistic** | **p-value** | **Frq.** | **$\chi^2$ test statistic** | **p-value** | **Frq.** | **$\chi^2$ test statistic** | **p-value** |
| **unaffected** | 0.45 | | | 0.93 | | | 0.40 | | |
| **exfoliation combined** | 0.79 | 11.55 | 0.0007 | 0.98 | 0.49 | | 0.79 | 15.21 | <0.0001 |
| | **Combined haplotype GGT** | | | | | | | | |
| **unaffected** | 0.38 | | | | | | | | |
| **exfoliation combined** | 0.77 | 14.77 | 0.0001 | | | | | | |

**Table 12. Upper part:** Risk allele counts, frequencies, odds ratios with 95% confidence interval and their corresponding p-values at three *LOXL1* SNPs, rs1048661 (R141L), rs3825942 (G153D) and rs2165241, in the sporadic case-control material are presented. Anonymous, unexamined blood donors were used as controls. **Lower part:** Risk allele and haplotype frequencies and allele and haplotype associations in the family material from Kökar island. Association is measured by $\chi^2$ test using independent XFS/XFG cases and unaffected relatives picked from the pedigrees. $\chi^2$ test statistic and p-values were calculated for combined three-locus haplotype GGT compared to all the other haplotypes. Frq=frequency, OR= odds ratio.

| | | | XFS | XFG | exfoliation combined | POAG | blood donor controls |
|---|---|---|---|---|---|---|---|
| **rs1048661** | **rs3825942** | | | | | | |
| **G** | **G** | **freq:** | 0.78 | 0.82 | 0.80 | 0.50 | 0.51 |
| | | **p-value:** | $1.42x10^{-7}$ | $3.67x10^{-13}$ | $1.12x10^{-16}$ | 0.92 | |
| | | **OR (95% CI):** | 3.40 (2.13-5.63) | 4.45 (2.90-7.04) | 3.97 (2.83-5.67) | 0.98 (0.67-1.42) | |
| **T** | **G** | **freq:** | 0.17 | 0.17 | 0.17 | 0.29 | 0.31 |
| | | **p-value:** | 0.0024 | 0.00026 | $9.07x10^{-06}$ | 0.68 | |
| | | **OR (95% CI):** | 0.44 (0.25-0.74) | 0.44 (0.27-0.69) | 0.44 (0.30- 0.64) | 0.90 (0.59-1.35) | |
| **G** | **A** | **freq:** | 0.06 | 0.01 | 0.03 | 0.20 | 0.18 |
| | | **p-value:** | 0.00052 | $7.45x10^{-10}$ | $8.82x10^{-11}$ | 0.62 | |
| | | **OR (95% CI):** | 0.27 (0.10-0.59) | 0.062(0.01-0.20) | 0.14 (0.06-0.28) | 1.14 (0.71-1.81) | |
| **T** | **A** | **freq:** | 0.00 | 0.00 | 0.00 | 0.007 | 0.00 |
| | | **p-value:** | | | | | |
| | | **OR (95% CI):** | | | | | |
| **Combined haplotypes** | | | | | | | |
| **GGT** | | **freq:** | 0.74 | 0.74 | 0.74 | 0.46 | 0.46 |
| | | **p-value:** | $9.02x10^{-8}$ | $2.32x10^{-10}$ | $1.66x10^{-14}$ | 1 | |
| | | **OR (95% CI):** | 3.34 (2.13-5.39) | 3.39 (2.30-5.10) | 3.38 (2.45-4.69) | 0.99 (0.68-1.44) | |
| **TGC** | | **freq:** | 0.17 | 0.16 | 0.16 | 0.29 | 0.30 |
| | | **p-value:** | 0.0034 | 0.00033 | $1.66x10^{-5}$ | 0.84 | |
| | | **OR (95% CI):** | 0.46 (0.26-0.78) | 0.44 (0.27-0.69) | 0.45 (0.31-0.65) | 0.94 (0.62-1.41) | |
| **GAC** | | **freq:** | 0.03 | 0.01 | 0.02 | 0.20 | 0.18 |
| | | **p-value:** | $8.14x10^{-6}$ | $1.32x10^{-9}$ | $4.96x10^{-13}$ | 0.54 | |
| | | **OR (95% CI):** | 0.14 (0.03-0.37) | 0.06 (0.01-0.20) | 0.09 (0.03-0.20) | 1.16 (0.71-1.83) | |
| **GGC** | | **freq:** | 0.04 | 0.08 | 0.06 | 0.04 | 0.05 |
| | | **p-value:** | 1.00 | 0.15 | 0.39 | 1.00 | |
| | | **OR (95% CI):** | 0.83 (0.23-2.21) | 1.75 (0.83-3.52) | 1.35 (0.69-2.57) | 0.97 (0.35-2.28) | |
| **TGT** | | **freq:** | 0.00 | 0.006 | 0.004 | 0.00 | 0.009 |
| | | **p-value:** | 1.00 | 1.00 | 0.67 | | |
| | | **OR (95% CI):** | 1.12 (0.04-7.35) | 0.78 (0.03-5.11) | 0.46 (0.02-3.00) | | |
| **GAT** | | **freq:** | 0.03 | 0.00 | 0.01 | 0.00 | 0.002 |
| | | **p-value:** | 0.02 | | 0.10 | | |
| | | **OR (95% CI):** | 14.27 (1.64-411.64) | | 5.76 (0.67-165.75) | | |
| **TAT** | | **freq:** | 0.00 | 0.00 | 0.00 | 0.007 | 0.00 |
| | | **p-value:** | | | | | |
| | | **OR (95% CI):** | | | | | |

**Table 13.** Two- and three-loci haplotype frequencies formed by *LOXL1* SNPs, rs1048661 (R141L), rs3825942 (G153D) and rs2165241, their odds ratios with 95% confidence interval and corresponding p-values in the sporadic case-control material are provided. Unexamined blood donors were used as population-based controls. The odds ratios and p-values were calculated for each individual haplotype compared to all the other haplotypes. Frq=frequency, OR= odds ratio.

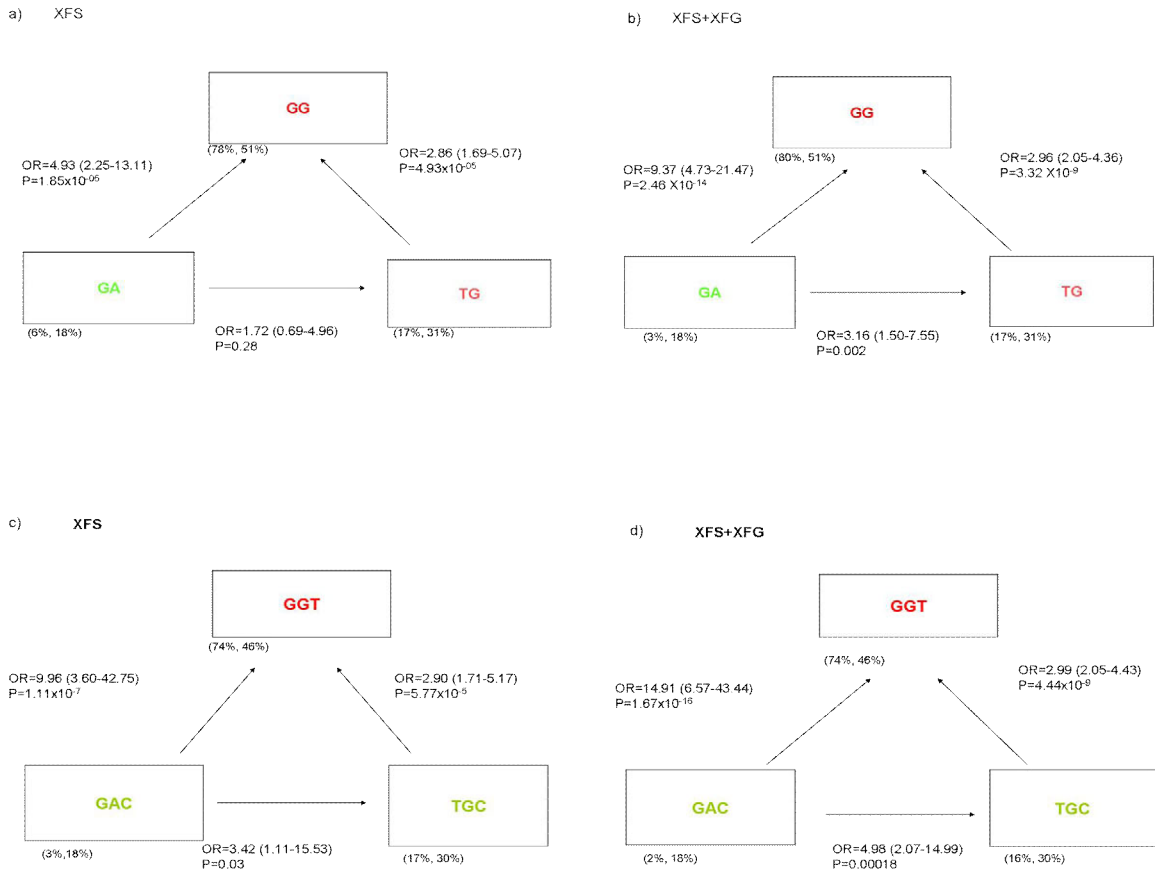


**Figure 11.** The association of two-locus haplotypes GG, TG and GA of (SNPs rs1048661 and rs3825942) to a) XFS b) combined XFS and XFG cohort and three-locus haplotypes GGT, TGC and GAC (SNPs rs1048661, rs3825942 and rs2165241) to c) XFS d) combined XFS and XFG patient material. The figures depict pairwise comparisons between the haplotype box. Haplotype frequencies in sporadic cases and unexamined blood donor controls are shown in parentheses below each haplotype box.

### 5.2.3 Discussion of the genetic basis of XFS and XFG (IV, V)

The ophthalmologically well-characterized and relatively isolated population of Kökar provided a suitable material for the search for genetic susceptibility loci for XFS and XFG. The small population size has made it possible to examine and follow-up nearly all inhabitants of the island during the last 40 years. It can be hypothesized that the Kökar population is characterized by genetic and environmental homogeneity that should make it easier to identify common predisposing alleles that are identical by descent (IBD). Despite the common nature of the XFS and the high prevalence of the syndrome in elderly (over the age of 70 years) in the mainland of Finland (22%) and in Kökar (18.4%), the possibility of a founder effect in the family could not be excluded. Nearly all the examined XFS positive individuals on the island were linked to an extended pedigree, in which XFS segregated in a fashion resembling autosomal dominant inheritance. XFS was present in 22% of examined family members within the family.

The genetic mapping approach was utilized to investigate the genetic background of XFS in the Kökar family (study IV). However, a large and inbred family with complex and late-onset disease was not an easy combination for linkage analysis. Most of the nowadays linkage software packages are not capable of analysing such an extended family as a whole and thus family had to be divided into five subfamilies leading to substantial loss of linkage information. Moreover, the family contained several internal links, meaning that many family members were related to each other through many routes, which made loop structures and provided an extra challenge for linkage analysis. Although detailed phenotypes were available even from the three most recent generations, DNA was mostly available from the first and second generations and only three genotyped parent-offspring pairs existed in the family. It is obvious that with the sample size available it was possible to locate only variants with relatively strong effects.

The most promising region in the genome-wide scan at 18q12.1-21.33 is considerable in size, extending 30.9 cM or 27.1 Mb and containing approximately 150 genes with known or unknown functions (UCSC Human Genome Browser, Ensembl Genome Browser). Most of these genes are also expressed in the eye at some level. Some interesting genes expressed in ocular tissues are listed in Table 14. Selecting candidate genes for XFS/XFG is difficult because of the systemic nature of the disease. XFM is detected also in non-

ocular tissues and the candidate gene might have quite a ubiquitous expression. Genes related to aging might be interesting candidates. Mitochondrial dysfunction is associated with aging and mitochondria are implicated in the pathogenesis of age-related neurodegenerative diseases (including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis) (Lin and Beal 2006; Kanda et al. 2007). The *LOC387715/high temperature requirement factor A-1 (HTRA1)* gene, of which protein is localized to the mitochondrial outer membrane, has been found to be associated with age-related macula degeneration (AMD) (Rivera et al. 2005; Kanda et al. 2007; Fritsche et al. 2008). With this in mind, mitochondrian proteins might be interesting candidates also in XFS/XFG.

Growth factors circulating with the aqueous have suggested to have an important role in the pathogenesis of XFS (Schlotzer-Schrehardt et al. 2001). Increased concentration of TGF-β1 (Koliakos et al. 2001; Schlotzer-Schrehardt et al. 2001) and CTGF (connective tissue growth factor) (Ho et al. 2005) in the aqueous humor of XFS eyes compared to control eyes have been demonstrated in previous studies. However no difference in levels of TGF- β2 between XFS and control eyes has been detected (Koliakos et al. 2001; Schlotzer-Schrehardt et al. 2001). Thus genes interacting with TGF-β1 might be interesting from the XFS/XFG point of view.

This solid linkage finding for chromosome 18 provides an excellent starting point for follow up studies. More genotyped family members, if possible in successive generations, are needed for further fine-scale mapping, in order to narrow the linkage region and to identify positional candidate genes and sequence variants contributing to the susceptibility of XFS/XFG in this locus. Several additional regions showed positive linkage values, suggesting that many underlying genetic variants might be associated with XFS.

| Gene symbol | Gene name | Location | Tissue specificity / Subcellular location | Function |
|---|---|---|---|---|
| RIT2 | GTP-binding protein Rit2 | 18q12.3 | Neuron-specific/ Cell membrane | Binds and exchanges GTP and GDP |
| ATP5A1 | ATP synthase, H+ transporting, mitochondrial F1 | 18q21.1 | Fetal lung, heart, liver, gut, kidney. High expression: fetal brain, retina, spinal cord / Mitochondrion inner membrane. | Catalyzes ATP synthesis |
| SMAD2 | Sma- and Mad-related protein 2 | 18q21.1 | Ubiquitous. High expression: skeletal muscle, heart and placenta / cytoplasm, nucleus. | Transcriptional modulator activated by TGF-β and activin type 1 receptor kinase. May act as a tumor suppressor gene. |
| SMAD 7 | SMAD family member 7 | 18q21.1 | Ubiquitous. High expression: lung, vascular endothelium / cytoplasm, nucleus. | Antagonist of signaling by TGF-β type 1 receptor superfamily members. |
| ACAA2 | Acetyl-coenzyme A acyltransferase 2 | 18q21.1 | - / Mitochondrion | Catalyzes the mitochondrial fatty acid beta-oxidation. |
| ME2 | Malic enzyme 2, NAD(+)-dependent, mitochondrial | 18q21.2 | Ubiquitous / Mitochondrion | Catalyzes the oxidative decarboxylation of malate to pyruvate |
| SMAD4 | Mothers against decapentaplegic homolog 4 | 18q21.2 | Ubiquitous / cytoplasm, nucleus | Common mediator of signal transduction by TGF-β superfamily. May act as a tumor suppressor gene. |
| MEX3C | Ring finger and KH domain containing 2 | 18q21.2 | Ubiquitous. High expression: fetal brain and testis / cytoplasm, nucleus | RNA-binding protein. |
| WDR7 | rabconnectin-3 beta isoform 2 | 18q21.31 | Ubiquitous / - | WDR proteins are involved in a variety of cellular processes, eg. cell cycle progression, signal transduction, apoptosis, and gene regulation. |
| FECH | ferrochelatase isoform b precursor | 18q21.31 | Ubiquitous / Mitochondrion | Catalyzes the ferrous insertion into protoporphyrin IX in the heme synthesis pathway |
| NEDD4L | neural precursor cell expressed, developmentally | 18q21.31 | Ubiquitous. High expression: prostate, pancreas, kidney / cytoplasm | E3 ubiquitin-protein ligase. Inhibits TGF-beta signaling by triggering SMAD2 and TGFR1 ubiquitination and proteasome-dependent degradation. |
| RAX | retina and anterior neural fold homeobox | 18q21.32 | developing eye, weakly expressed in the adult retina / nucleus | Critical role in eye formation by regulating the initial specification of retinal cells and/or their subsequent proliferation. |
| CPLX4 | complexin 4 | 18q21.32 | eye, brain, muscle, pineal gland / membrane, lipid-anchor, cell junction, synapse | Regulates a synaptic vesicle exocytosis. |
| PMAIP1 | Phorbol-12-myristate-13-acetate-induced protein 1 | 18q21.32 | Ubiquitous. High expression: adult T-cell leukemia cell line / Mitochondrion. | Promotes activation of caspases and apoptosis. Promotes mitochondrial membrane changes and efflux of apoptogenic proteins from the mitochondria. |

**Table 14.** Some interesting genes located in the XFS candidate locus on the chromosomal region 18q12.1-21.33.

Results of the study V show that the *LOXL1* gene SNPs, rs1048661 (R141L), rs3825942 (G153D) and rs2165241, are not linked to XFS/XFG in the Kökar family. However, a strong association between *LOXL1* risk alleles and XFS/XFG was demonstrated. The absence of linkage might be explained by the small family material and most importantly by low power of linkage methods to map common variants with low genotypic relative risk (Risch and Merikangas 1996; Risch 2000; Cardon and Bell 2001; Tabor et al. 2002; Hirschhorn and Daly 2005; Laird and Lange 2006). Further studies are needed to investigate the genetic background underlying XFS/XFG in the Kökar family. After identifying possible susceptibility variants on chromosome 18, their role in XFS/XFG and possible interaction with *LOXL1* variants should be investigated by biochemical and functional studies in order to eventually understand the molecular pathology underlying XFS/XFG in the Kökar family.

A strong association of *LOXL1* gene SNPs to XFS and XFG, but not to POAG, was observed in the Finnish patients (study V), which is convergent with previous studies in several Caucasian populations (Scandinavians, European and American populations) (Fingert et al. 2007a; Aragon-Martin et al. 2008; Challa et al. 2008; Fan et al. 2008; Mossbock et al. 2008; Pasutto et al. 2008b; Yang et al. 2008), in the Australian population (Hewitt et al. 2008b), in African-Americans (Fan et al. 2008) and in Asian populations (Japan and India) (Fuse et al. 2008; Hayashi et al. 2008; Mabuchi et al. 2008; Mori et al. 2008; Ozaki et al. 2008; Ramprasad et al. 2008). The strongest allelic association was detected for allele T of intronic SNP rs2165241, but also allele G of coding SNP rs1048661 showed strong association to XFS and XFG both in case-control and family materials. Interestingly, allele G of rs3825942 was strongly associated to XFS and XFG in the case-control material but not in the family material. This was due to the enrichment of the G allele in the Kökar family, both in affected and unaffected family members. The mean age of the unaffected family members was 63 years (n=92, range: 32-92), suggesting that a significant number of them might develop XFS in their later years.

Similar distribution of two- and three-locus haplotypes was detected in Finland as in other Caucasian populations (Thorleifsson et al. 2007; Aragon-Martin et al. 2008; Mossbock et al. 2008; Pasutto et al. 2008b). Two locus-haplotype GG, of coding SNPs rs1048661-rs3825942, conferred the highest risk to XFS and XFG, whereas TG and GA haplotypes were underrepresented in sporadic XFS and XFG cases compared to blood donor controls.

Likewise, when all three SNPs were considered, the risk haplotype GGT was the most significantly associated with XFS and XFG both in the case-control and in the family material and TGC and GAC haplotypes were underrepresented XFS and XFG cases. Two-locus risk haplotype GG increased the risk of XFS and XFG nine-fold, relative to the low-risk haplotype GA, whereas three-locus risk haplotype GGT increased the risk almost fifteen-fold relative to the GAC haplotype. High risk ratios have also been reported in the other Caucasian populations; in Icelandic and Swedish XFG cohorts GG haplotype increased the risk of exfoliation 27-fold and in European and American XFS/XFG patient groups the risk increased almost 8-fold relative to the GA haplotype (Thorleifsson et al. 2007; Aragon-Martin et al. 2008).

The high risk haplotypes, GG and GGT, seems to be common in XFS and XFG patients, but also in general populations. In the present study the two-locus risk haplotype GG was found in 95% of XFS and XFG cases (66% in homozygous) and in 72% of the general population (29% in homozygous). In agreement with this, the GG haplotype was present in 50% of the general Swedish and Icelandic populations (25% in homozygous) and 50% in unaffected inividuals of mainly European decent (Thorleifsson et al. 2007; Aragon-Martin et al. 2008). In the present case-control material three-locus haplotype GGT was found in 93% of XFS and XFG cases and 69% of population based controls. The high prevalence of XFS in Scandinavia (22% of the population >70 years old in Finland) and the fact that controls were unexamined and not age matched and hence some of them might have XFS or develop XFS in their later years, might partly explain the high frequencies of risk haplotypes in the Finnish general population.

We have hypothesized that XFS is a normal phenomenon associated with aging. This theorem is supported by the fact that the disease-associated variants (R141L and G153D) of the *LOXL1* gene are common ancestral wild type alleles and evolutionary conserved across mammalian species (Hewitt et al. 2008b). Added to this, the high prevalence of *LOXL1* risk haplotypes in the general populations and the association of the risk alleles with XFS and XFG in diverse ethnic groups, suggests that XFS is a normal age-related condition. Yet, some elderly persons with risk haplotype do not have exfoliation; of our examined over 80-year old unaffected control individuals (n=26) as many as 42% carried the risk haplotype GG and 35% carried the risk haplotype GGT. The protective mechanism that delays the onset of the XFS in these individuals is unknown.

Taken together, the high prevalence of risk haplotypes in the general Finnish population (69-72%) and in the elderly non-XFS/XFG cohort (35-42%) as well as the identification of one XFS patient homozygous for low risk haplotype GAC, suggest that *LOXL1* does not alone explain the genetic background underlying XFS and XFG, but probably also other predisposing and/or preventing genetic and environmental factors are involved in the pathogenesis of XFS/XFG (Figure 12). In addition based upon the known presence of many proteins that constitute the XFM (Ritch and Schlotzer-Schrehardt 2001; Conway et al. 2004; Schlotzer-Schrehardt and Naumann 2006), extracellular matrix and elastic connective tissue fibers, it is likely that many other genes are associated with the development of XFM and glaucoma in addition to *LOXL1* (Lee 2008). Several genetic loci have been suggested to be associated with XFS in the previous studies (Zenkel et al. 2005; Lemmela et al. 2007; Burdon et al. 2008; Lee 2008). These loci might contain predisposing genetic factors, but also protective factors, which prevent or delay the onset of XFS in those elderly unaffected individuals with risk haplotypes.

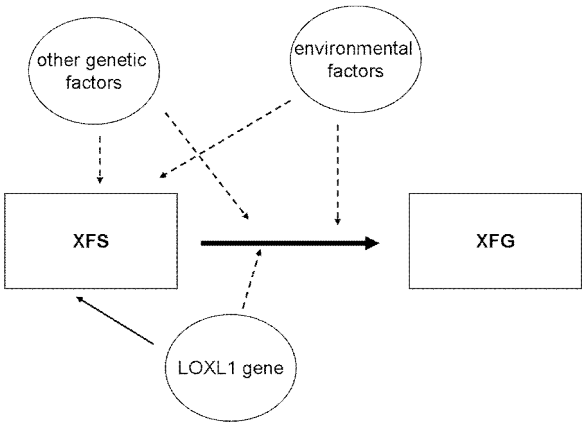


**Figure 12.** XFS and XFG are probably caused by interplay of protective and predisposing genetic and environmental factors. The *LOXL1* gene has been shown to be associated with both XFS and XFG, but whether association to XFG is just a reflection of its association to XFS is unknown. Also the factors that are involved in the conversion of XFS to XFG remain to be clarified.

Although our knowledge of XFS and XFG genetics has increased recently, there are several open questions waiting for answers, such as; why some elderly individuals with *LOXL1* risk haplotype have not developed XFS, why only some individuals with XFS develop XFG and why XFS appears not to have full penetrance in the families.

Recently, deCode genetics began offering genetic testing for XFS risk based upon analysis of three high risk *LOXL1* SNPs. The utility of this genetic test in general population is not known, since the frequency of these SNPs in the worldwide population is unknown and since *LOXL1* risk alleles occur also in elderly individuals without XFS/XFG. Based upon our present knowledge it is likely that many other genes are associated with the development of XFS and XFG in addition to *LOXL1*. Further studies in XFG patients in different populations are necessary to determine the worldwide risk associated with *LOXL1* SNPs and the presence of XFM and development of glaucoma.

### 5.3 Complex genetic nature of glaucoma of exfoliation syndrome

Molecular genetic studies of adult onset OAG and XFS/XFG have been challenging due to the asymptomatic and complex genetic nature of traits and small study materials. The late age of onset makes it difficult to collect multi-generation families with these disorders. Parents of the affected patient are often deceased and their offspring are too young to manifest the disease. Exceptionally, in Kökar XFS family detailed phenotypes where available even from the three most recent generations due to the long follow-up period. The asymptomatic nature of the traits makes the clinical diagnosis difficult. OAG is asymptomatic in its early stage and develops insidiously afterwards and thus diagnosis cannot be made until patients are elderly. XFS and XFG often go unrecognised, or are misdiagnosed, because of the subtlety of the clinical signs. In some populations XFS and XFG might be underdiagnosed because many ophthalmologists still believe that they occur mainly in some areas of Europe. Probably these are the reasons why most XFS/XFG and OAG studies are based on only a few, small or incomplete pedigrees, which make the hypothesis of inheritance model uncertain.

The most common traits and diseases are complex in nature, for which the phenotype is determined by the sum total of, and/or interactions between, multiple genetic and environmental factors (Risch and Merikangas 1996; Hirschhorn and Daly 2005; Newton-

Cheh and Hirschhorn 2005; Wang et al. 2005; Iyengar 2007). Causative genetic variants underlying common diseases have likely arisen before the divergence of peoples because the disorders are widespread globally (Doris 2002). The frequency of these variants is largely unknown, but it has been hypothesized common variants (with frequencies of >1%, SNPs) influence common disease susceptibility (common disease - common variants hypothesis, CD/CV) (Lander 1996; Chakravarti 1999; Lander et al. 2001; Reich and Lander 2001; Doris 2002). Common variants have a low impact on a single individual but a high impact at the population level (Lander 1996; Chakravarti 1999; Reich and Lander 2001; Hirschhorn and Daly 2005; Newton-Cheh and Hirschhorn 2005). Therefore any individual genetic variant generally has a relative small effect on the disease risk, which makes their detection challenging.

To date, there are several examples of common variants contributing to a common disease, most of which increase the risk by two-fold or less in large populations (Altshuler et al. 2000; Rioux et al. 2001; Stefansson et al. 2002; Lohmueller et al. 2003; Ueda et al. 2003). One such example is common age-related eye disease, adult type macular degeneration (AMD) (Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005). Several common variants (e.g. SNPs) are also associated with OAG (Tunny et al. 1996; Suzuki et al. 2000; Colomb et al. 2001; Copin et al. 2002; Lin et al. 2003a; Polansky et al. 2003; Gong et al. 2004; Hewitt et al. 2006a) and three common SNPs on the *LOXL1* gene are strongly associated with XFS and XFG (Table 7).

The CD/CV -hypothesis might not explain the whole genetic background of common diseases. An alternative theorem is that rare mutations at many different loci could cause common diseases and explain their high prevalence (Pritchard 2001). This is evidenced in *MYOC* associated glaucoma, in which a great number of rare mutations in the *MYOC* -gene contribute to a relatively common disease OAG (Gong et al. 2004; Libby et al. 2005; Hewitt et al. 2006a). However, the probable explanation for the high prevalence of common diseases, such as OAG and XFS/XFG, is an interplay between many common and/or many rare variants and environmental risk factors.

Discussion of whether genome-wide linkage studies are accurate approach for detecting genes in complex diseases has been going on lately. The linkage-based methods have a low power to map common susceptibility variants that have modest effects on the disease

(Risch and Merikangas 1996; Risch 2000; Cardon and Bell 2001; Tabor et al. 2002; Hirschhorn and Daly 2005; Laird and Lange 2006). Moreover, collecting a sufficient size of family material for linkage studies is a challenging and time demanding task. Anyhow, genome-wide linkage analysis have been carried out for many common diseases of which some have led to the discovery of variants contributing to susceptibility of diseases, such as type 1 diabetes (Nistico et al. 1996), inflammatory bowel disease (Hugot et al. 2001; Ogura et al. 2001; Rioux et al. 2001; Stoll et al. 2004) and schizophrenia (Stefansson et al. 2002). Identification of single risk allele for AMD in the *complement factor H gene* (*CFH*) was achived through a focused fine SNP association analyses of linkage regions obtained from prior genome-wide linkage studies (Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005). The success story of AMD is encouraging for further analyse of the XFS candidate region on chromosome 18 e.g. by fine-scale SNP association analysis (Study IV). For the most common diseases, however, linkage analysis has attained only limited success (Altmuller et al. 2001; Hirschhorn and Daly 2005; Laird and Lange 2006) and genes discovered by linkage analysis usually explain only a small fraction of the overall heritability of the disease. This is the current situation in OAG research where linkage has been pointed in nearly every chromosome but only three candidate genes, contributing together for less than 10% of OAG, have been identified (Fan et al. 2006a).

Genome-wide association studies (GWAS) have been suggested to be more powerful means of identifying the common variants that underlie complex traits than linkage based methods (Risch and Merikangas 1996; Cardon and Bell 2001; Tabor et al. 2002; Carlson et al. 2004; Hirschhorn and Daly 2005; Newton-Cheh and Hirschhorn 2005; Laird and Lange 2006). Moreover, family material is not required in GWAS and sporadic case-control material is simpler and faster to collect. To date, extensive genome-wide association studies for several complex diseases have been established (Sladek et al. 2007; The Wellcome Trust Case Control Consortium 2007); of which from our point of view one of the most interesting was the genome-wide association study of XFS (Thorleifsson et al. 2007).

# 6 CONCLUDING REMARKS AND FUTURE PROSPECTS

Overall we have little understanding of the genetics of OAG and even less of an understanding of the cell biology underlying it. In the genetic mapping studies of OAG linkage has been pointed in nearly every chromosome, but only three candidate genes have been identified, accounting together for less than tenth of OAG. In the present study two of these candidate genes, *MYOC* and *OPTN,* and 14 additional candidate loci were excluded in eight Finnish glaucoma families, further confirming the heterogeneous nature of OAG. Subsequently, the mutation in the *MYOC* gene was found to segregate with glaucoma in the Finnish JOAG/POAG family, providing the first molecular genetic explanation of glaucoma in the Finnish population. Identification of a susceptibility mutation in the glaucoma family facilitates early diagnosis and follow-up for family members at risk and enables the treatment of glaucoma on time, which is important in such a late-onset, insidiously progressing disease. The *MYOC* gene should also be investigated as a candidate gene for glaucoma in other Finnish families, especially those manifesting juvenile-onset glaucoma.

During the last year there has been remarkable progress in the fields of XFS/XFG genetics. Genome-wide scan of XFS, described in this thesis, highlighted an interesting candidate region on chromosome 18 and produced several additional suggestive regions. The locus on chromosome 18 provides a solid starting point for the future fine-scale mapping studies, which are needed to identify variants conferring susceptibility to XFS in the region. Subsequently, association between three *LOXL1* gene SNPs and XFS/XFG was reported in several populations. In the present study, these SNPs were found to confer risk for XFS and XFG also in the Finnish population. The possible defect in *LOXL1* and the way it contributes to the pathogenesis of XFS and XFG is not fully understood and hence functional studies of the *LOXL1* gene are of special importance.

Since it is likely that combination of common susceptibility variants with small individual effects are responsible for at least some part of XFS/XFG and OAG, large scale genome-wide association studies with extensive sample sets might be useful. It is of great importance in future studies to collect sufficiently large sample sets or combine samples through meta-analysis in order to achieve the relevant statistical power to identify susceptibility variants conferring only modest increases in risk. The correct research

strategy combined with large study materials led to the identification of XFS/XFG risk variants in a recent genome-wide association study. However, pedigree linkage studies and extensive mutation screening of the positional candidates have a good power for detecting uncommon genes with major effects, as was the case for OAG susceptibility genes *MYOC* and *OPTN*. Rapid development of the genotyping and gene-expression technologies as well as bioinformatics are now enabling cheaper, faster and more robust massive high-throughput research. Fully exploiting these possibilities would enhance our understanding of the genetic bases of OAG and XFS/XFG.

Genetics is, however, powerless without sufficient knowledge of clinical aspects of the disease of interest. Different forms and subtypes of glaucoma have been described. Several partially overlapping phenotypes may represent a continuum of the same underlying genetic defect or may have separate genetic background. Confusion still exists whether NTG is a subtype of OAG or a different type of glaucoma. Preliminary studies show that OAG and NTG share at least partially common genetic background. However, the possibility is not discounted that there exist phenotype specific predisposing variants for each phenotype. Breaking down the OAG phenotype into its constitutional anatomical or pathophysiological components and studying these intermediate phenotypes (such as IOP, cup-disc ratio) can be more powerful than simply ascertain whether disease is present or absent.

In the long run, identification of genetic factors behind OAG and XFS/XFG along with biochemical and functional studies will hopefully expand our understanding of the molecular pathology underlying these common eye disorders. With an increased knowledge of the underlying pathology it may be possible to develop novel treatments targeted at the root cause of the disorders as opposed to the currently available rather empirical treatments.

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Susanna Kemnal

## 8 ELECTRONIC DATABASE INFORMATION

dbSNP database, http://www.ncbi.nlm.nih.gov/projects/SNP/

deCODE genetics, http://www.decode.com/

Ensembl Genome Browser, http://www.ensembl.org/

Genome Datasbase, http://www.gdp.org/

HUGO Genome Nomenclature Committee, http//www.gene.ucl.ac.uk/nomenclature/

International Hap Map Project, http://www.hapmap.org/

Marshfield Medical Research Foundation, http://research.marshfieldclinic.org/genetics/

Myocilin allele-specific glaucoma phenotype database, http://www.myocilin.com/

Online Mendelian Inheritance in Man, www.ncbi.nlm.nih.gov/Omim

UCSC Human Genome Browser, http://genome.ucsc.edu/

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