Glaucoma phenotype in a large Swiss pedigree with the myocilin Gly367Arg mutation

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Abstract

Aims To characterize genotype, phenotype, and age-related penetrance in a Swiss pedigree with juvenile open-angle glaucoma (JOAG).

Methods In a large Swiss family with history of glaucoma and 82 living members of four generations, we conducted molecular analysis and a detailed phenotype characterization in 52 family members. Mutation analysis was carried out using single-strand conformation polymorphism and DNA sequence analyses of the suspected candidate gene, myocilin (MYOC).

Results We detected a Gly367Arg mutation in the MYOC gene of 13 family members. Nine of them (69.2%) had glaucoma: mean IOP 35.3 mm Hg, range 24–50 mm Hg; mean age at diagnosis 34.9 years, range 28–51 years. Two mutation carriers were glaucoma suspects, one (age 15) was unaffected, and one (age 16) not available for clinical examinations. Age-related glaucoma penetrance was 50% at 30 and 78% at 40. Untreated IOP resulted in rapid disease progression, whereas good IOP control, usually only by means of filtration surgery, could stabilize the disease. None of the wild-type members had glaucoma.

Conclusions This Swiss family is the largest reported Gly367Arg pedigree to date. The exact genotype and phenotype characterization allowed a reliable risk and prognosis assessment and targeted eye-care planning for the family. The study demonstrates the importance of genetic investigations in glaucoma families, carrying the potential of long-term socio-economic benefits.

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Introduction

Primary open-angle glaucoma (POAG) is a major cause of visual impairment and blindness worldwide with a prevalence of approximately 2% in individuals over 40 years of age.^{1–3} Most glaucoma cases seem to follow complex inheritance and are likely caused by multiple interacting genes, of which some may display incomplete penetrance or variable expressivity.⁴ Monogenic genotypes exhibiting classic Mendelian inheritance are relatively rare and may be associated with POAG, juvenile open-angle glaucoma (JOAG), congenital glaucoma, and the anterior segment dysgenesis syndromes. Linkage studies of such pedigrees have led to the identification of several genes harbouring various disease-causing mutations, the most prominent members being myocilin (MYOC/TIGR OMIM = *601652), cytochrome P450 1B1 (CYP1B1, OMIM = *601771), optineurin (OPTN OMIM = *602432), and the recently linked WD repeat-containing gene (WDR36, OMIM = *609669), coding for a protein which is involved in T-cell activation.⁵

Mutations in the MYOC gene are responsible for approximately 3% of POAG with a prevalence ranging from 2.8 to 4.3% in different population groups.^{6,7} MYOC is localized on chromosome 1q21–31, which was mapped 1993 by linkage analysis in families with JOAG.⁸ Stone *et al*⁹ identified the gene in 1997 as the TIGR gene (trabecular meshwork-induced glucocorticoid response protein). Some of these mutations have been found to descend from a single common ancestor, for example, GLN368STOP,^{7–10} others have arisen independently in multiple individuals. Regarding the phenotype, some MYOC mutations cause an early onset, severe glaucoma, others account for a less typical, late-onset disease,¹¹ and some are associated with a quite variable phenotype (eg, Lys423Glu).^{12,13}

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In this study, we present a four-generation pedigree with autosomal-dominant JOAG living in the area of Bern, Switzerland. The primary aims were to identify the mutation and all mutation carriers, and to characterize disease phenotype and age-related penetrance. A secondary aim was to define an adequate plan to manage the mutation carriers and to counsel the other family members.

Patients and methods

Patients

The study was conducted in accordance with the Declaration of Helsinki. An attempt was made to contact all living family members and their relatives by marriage and invite them to participate. If available, clinical data were completed with the records of private ophthalmologists. This study was approved by the ethical committee of the Kanton Bern (KEK, No. 238/03), and informed consent regarding both the clinical and the molecular analysis was obtained from all participants.

Clinical work up

This study was conducted between March and November 2004. All participants underwent the following examination protocol: best-corrected visual acuity, goldmann applanation tonometry, gonioscopy, standard automated perimetry (Octopus perimeter), colour optic disc photography, optic nerve head analysis with Scanning Laser Ophthalmoscopy (HRT II), retinal nerve fiber layer (RNFL) analysis with Scanning Laser Polarimetry (GDx VCC), and ultrasound pachymetry. A person was classified as having glaucoma if at least two of the following criteria were met: untreated IOP >21 mm Hg, glaucomatous optic disc damage and/or RNFL loss, and glaucomatous changes in the visual field (VF). A patient was designated a glaucoma suspect in this study when having normal IOP but glaucomatous looking optic disc and normal or borderline VF.

Mutational screening analysis

Mutation analysis was performed using a single-strand conformation polymorphism analysis approach.¹⁴ The

Figure 1 Pedigree.

Table 1 Identified living Gly367Arg mutation carriers, $n = 13$

Study ID	Generation	Year of birth	Gender	Age at study (years)	Type of glaucoma	Age at Dx (years)	IOP max/at Dx (mm Hg)	Age at first surgery	C/D	Visual field/damage
2	I	1911	M	93	JOAG	30	NKN	NKN	1.0	Blind
47	II	1928	M	76	JOAG	27	35	31	0.7	Moderate
1	II	1930	F	74	JOAG	28	NKN	47	0.95	Severe
71	II	1939	F	65	JOAG	28	50	30	1.0	Blind
97	II	1939	F	65	POAG	51	26	—	0.85	Mild
50	III	1957	M	47	JOAG	30	24	38	0.75	Normal
51	III	1961	F	43	JOAG	42	30	42	0.9	Severe
55	III	1961	M	43	Suspect	—	16	—	0.7	Borderline
100	III	1964	M	40	JOAG	40	33	—	0.8	Moderate
101	III	1965	F	39	JOAG	38	45	38	0.9	ND
67	IV	1985	M	19	Suspect	—	21	—	0.45	Normal
68	IV	1989	M	15	Normal	—	19	—	0.3	Normal
135	IV	1988	M	16	NA	—	NA	NA	NA	NA

Abbreviations: F, female; M, male; NKN, not known; ND, not determined (patient mentally retarded, no perimetry possible); NA, patient was not available for the clinical examination.

first 25 blood samples were screened for the entire coding sequences of MYOC (1q23–25). After the unequivocal identification of the mutation, the remainder were analysed for exon 3 only.

Calculation of penetrance

The age-related penetrance of the mutation was calculated as the ratio of the number of mutation carriers with the disease to the total number of mutation carriers at age of 30 and 40 years, respectively.

Statistical analysis

Descriptive statistics was performed using GraphPad Prism, Version 4.03 (GraphPad Software, San Diego, CA, USA). All data are expressed as median $\pm$ SD. Clinical parameters were compared between the groups using the unpaired *t*-test.

Results

The pedigree consisted of 82 living members over four generations (Figure 1). The family is of caucasian origin and all members remained in Switzerland. The first known affected ancestor (1873–1949) was reported to be blind and had 11 children, six of whom had glaucoma. All but one member of this first generation were already deceased. Sixty-six family members (29 female, 37 male) gave blood for molecular analysis; of whom 52 (21 female, 31 male) participated also in the clinical investigation.

Molecular analysis (genotype)

A glycine–arginin exchange at the first position of codon 367 in exon 3 (Gly367Arg = G367R) of the MYOC gene was found in 13 family members (eight males, five females); mean age 48.9 ± 24.5 years (range, 15–93 years). One 50-year-old family member (ID 90), showed a base change at position 398 ($\rightarrow$ K398R). This sequence variant

Figure 2 (a–c) Patient 50, mutation-carrier, now 47 years old; diagnosed and treated at the age of 30 in an early stage of the disease. (a) Optic nerve head photographs showing cupping asymmetry but a well-preserved neuroretinal rim. (b) Standard automated perimetry (Octopus 101): normal VFs. (c) GDx VCC: nerve fibre thickness is in the normal range.

Figure 2 (continued)

is known to represent a benign SNP.¹⁵ Accordingly, the ocular phenotype in this subject was completely normal.

Clinical examination (phenotype)

Mutation carriers

Among the 13 mutation carriers, eight had known open-angle glaucoma (ID 1, 2, 47, 50, 51, 71, 97, and 101) with two of them (ID 51 and 101) only diagnosed in 2003. One member was previously known to be a glaucoma suspect (ID 55). Of the three other members who had their first

glaucoma screening during this study, one had already a moderately advanced glaucoma (ID 100 and age 40), one was a strong glaucoma suspect (ID 67 and age 19), and one was not affected yet (ID 68 and age 15). A 16-year-old mutation carrier (ID 135) was not available for the clinical examination. The age-related penetrance (age at diagnosis) of the Gly367Arg mutation was 50% at 30 years (5/10) and 78% at 40 years (7/9). All mutation carriers had open, unremarkable anterior chamber angles. The mean age at diagnosis was 34.9 ± 8.3 years (range, 28–51 years). Maximum IOP at diagnosis was

34.7 ± 9.6 mm Hg (24–50 mm Hg). Seven of the nine glaucoma-affected mutation carriers (12 eyes) had a history of filtration surgery, with a mean age at first surgery of 37.9 ± 6.6 years (30–47 years) corresponding to a mean latency of 5.75 ± 7.04 years (0.5–19 years) after diagnosis. Although some of the eyes needed several operations, IOP was, in general, well-controlled long term. The glaucoma relevant characteristics of all the mutation carriers are outlined in Table 1.

The story of the index patient's children (ID 50 and 51) is very illustrative with regard to the course of the Arg367Gly-associated disease. Being aware of the family glaucoma history, they both started regular ophthalmic check-ups early—the brother at 27, his sister at 17. At first examination, the brother was classified as glaucoma suspect (IOP 20/21 mm Hg and C/D 0.6/0.7) and was closely monitored without treatment. At 30, his IOP rose to 24 mm Hg, treatment was initiated, 8 years later trabeculectomy was performed on both eyes, and at 47, during this study, he still showed full VA and normal VF (MD 0.4/1.4 dB²) (Figure 2). His sister had normal findings at 17 (IOP 15/17 mm Hg and C/D 0.2/0.3). Until

the age of 36 years, her IOP increased slightly (19/21 mm Hg), but optic discs and VF remained unremarkable. Then, during a personal crisis, which ended in divorce, the patient stopped seeing her ophthalmologist for 6 years, until she noticed a VF constriction in her right eye. At examination at 42, an IOP of 30 mm Hg, C/D ratio of 0.9, and advanced VF damage (MD 18.2/14.2 dB²) were recorded in both eyes (Figure 3). These two cases allow us to deduce a disease onset at 30 and 36–40 years, respectively, with a rapid and aggressive course afterwards, if left untreated.

Wild-type family members

Of the 53 genetically screened wild-type family members, 40 (age range 13–82 years) could be examined clinically. None had elevated IOP or glaucomatous VF defects. All had open angles. One person had pseudoexfoliation syndrome without glaucoma. Three (age 35, 59, and 81 years) were suspects of normal-tension glaucoma, based on glaucomatous looking optic nerve heads, abnormal results in the GDx, and normal-to-borderline VF. One 48-year-old female had been

Figure 3 (a–c) Patient 51, mutation-carrier, now 43 years old; diagnosed at the age of 42 in an advanced stage of the disease. (a) Fundus photographs showing prominent glaucomatous disk cupping with a C/D ratio of 0.9. (b) Standard automated perimetry (Octopus 101): severe VF damage. (c) GDx VCC: diffuse atrophy of the RNFL.

Figure 3 (continued)

treated for glaucoma with a beta-blocker for 19 years, because of her positive family history, initial IOP of 19 mm Hg, and suspicious optic disc. Based on our clinical and molecular findings, we recommended cessation of treatment.

Comparison between the groups

Comparing mutation-positive and the wild-type family members, IOP, MD, neuroretinal rim area, and RNFL

parameters differed significantly, whereas central corneal thickness and disc area did not (Table 2).

Discussion

The Gly367Arg mutation in the MYOC gene has been described previously in four families of different origin (Table 3),^{16–20} and in one Indian glaucoma patient.²¹ There may be a single common ancestor (founder effect) in the Caucasian pedigrees, as found in the Canadian Gly367Arg family²⁰ and other MYOC mutations.^{7,22,23}

Table 2 Clinical features of mutation carriers and wild-type family members

	Mutation carriers n = 10 ^a (20 eyes)	Wild-type members n = 40 (80eyes)	Unpaired t-test P-value
Age (years)	45.00 ± 21.29	30.50 ± 18.77	0.09 (NS)
IOP at study	16.50 ± 6.50	14.00 ± 3.04	0.0036
CCT (μm)	571.3 ± 34.67	572.80 ± 33.91	0.95 (NS)
MD (dB)	3.00 ± 7.182	1.60 ± 4.95	0.05
Disc area (mm ²)	1.93 ± 0.55	2.00 ± 0.41	0.57 (NS)
Rim area (mm ²)	1.39 ± 0.51	1.54 ± 0.29	0.0038
Linear C/D ratio	0.63 ± 0.17	0.41 ± 0.14	0.0005
NFI	37.00 ± 21.41	19.75 ± 8.41	0.0001
TSNIT SD (μm)	16.88 ± 4.75	23.60 ± 3.51	0.0001
Sup. average (μm)	54.90 ± 8.36	67.39 ± 6.65	0.0001
Inf. average (μm)	52.3.8 ± 8.26	66.01 ± 6.72	0.0001

Abbreviations: CCT, central corneal thickness; MD, mean defect in the automated perimetry (Octopus); NFI, nerve fibre indicator in the GDx VCC analysis of the retinal nerve fibre layer; TSNIT SD, standard deviation of the TSNIT curve in the GDx VCC analysis of the retinal nerve fibre layer; Sup. average/Inf. average, average thickness in the superior and inferior quadrant, respectively, in the GDx VCC analysis.

All data are expressed as median ± SD.

^aFor this part of the study, the group of mutation carriers comprised seven glaucoma patients, two suspects, and one normal. The other three identified mutation carriers (one blind, 93-year-old patient, one mentally retarded, and one that was not available for the clinical examination; ID 2, 101, and 135) did not contribute to data collection, except for the parameter 'age'. Patient ID 71 was not able to comply with perimetry, HRT and GDx; and patient ID 1 satisfied the HRT and GDx scans with one eye only.

Table 3 Reported pedigrees with the Gly367Arg mutation

Study year	Authors and origin	Generations	Living family members	Tested	Carriers	Affected	Mean age at Dx (years)
1998	Michels-Rautenstrauss <i>et al</i> , ¹⁸ Germany	2	2	2	2	2	~20
1998	Mansergh <i>et al</i> , ¹⁹ Ireland	3	25	23	5	5	~37
2000	Taniguchi <i>et al</i> , ¹⁷ Japan	3	22	13	8	4	36.7
2002	Faucher <i>et al</i> , ²⁰ Canada	3	6	6	6	6	34.0
2006	Iliev <i>et al</i> (this study), Switzerland	4	82	66	13	9	34.6

The family presented here is the largest with confirmed Gly367Arg mutation and allowed us to study the phenotype in more detail, especially with regard to disease onset and responsiveness to therapy. Furthermore, the large pedigree enabled us to exclude a pleiotropic effect of the mutated protein influencing disease-defining parameters, such as central corneal thickness and disc area, affecting IOP measurement or C/D ratio, respectively.

The glaucoma phenotype can be summarized as follows: it is a late-onset, high-pressure JOAG with an aggressive course without treatment, poor responsiveness to topical medication, and, in general, good responsiveness to filtration surgery. Wild-type family members do not differ from the general population with respect to glaucoma prevalence. The age-related penetrance of 50% at 30 and nearly 80% at 40 is comparable to other MYOC mutations, for example, Cys433Arg and Asn480Lys.^{24,25} So far, all reported MYOC mutations have incomplete penetrance at 30, with Thr377Met resulting in the highest²⁶ and Gln368STOP in the lowest^{22,27} age of onset.

In vitro experiments¹⁵ have supported a widely accepted pathogenic concept, that aggregation of mutant and wild-type MYOC (heteromultimerization) in the trabecular meshwork may lead to intra- and extracellular accumulation of protein complexes, which might affect outflow structures. However, MYOC has also been shown to be expressed in other tissues such as the retrolaminar region of the optic nerve, the perivascular connective tissue, and in the meningeal sheath surrounding the optic nerve.²⁸ Therefore, retinal ganglion cells or optic nerve fibers could be directly affected by the mutant protein in terms of an IOP-independent neuropathy. In our family, however, we could not find any evidence of IOP-independent glaucomatous features. All our glaucoma patients had high IOP levels, and IOP control could reliably slow down disease progression.

Based on the study results, we suggest the following ophthalmic-care approach for descendants of this family: Molecular analysis at the age of 16–18; wild-type members can be released from further screening; mutation carriers should undergo a baseline ophthalmic examination with tonometry, automated perimetry, and

optic disc photography. After the age of 20, mutation carriers should undergo yearly examinations and treatment should be strongly recommended if IOP exceeds 21 mm Hg. Filtration surgery should be considered an early option.

In conclusion, this study exemplifies that genetic screening should be offered to all glaucoma families to identify members at risk and to initiate treatment at an early stage of the disorder, before irreversible loss of vision has occurred. The story of the two siblings (ID 50 and 51) underlines the importance of psychosocial factors. Not irrelevant in a socio-economic aspect, wild-type family members can be released from frequent ophthalmologic examinations and be instructed that their risk of glaucoma does not differ from that of the general population.

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