How Significant Is a Family History of Primary Open Angle Glaucoma?

Experience from the Glaucoma Inheritance Study in Tasmania

by

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STATEMENT OF AUTHORSHIP

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15 April 2006

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15 April 2006

ABSTRACT

TITLE: How significant is a family history of glaucoma? Experience from the Glaucoma Inheritance Study in Tasmania (GIST).

AIM: To determine the prevalence of a familial glaucoma amongst glaucoma sufferers in Tasmania.

METHODS: With the cooperation of ophthalmologists, optometrists and pharmacists in Tasmania, patients diagnosed with glaucoma and their family members were identified and invited to participate in the study. All patients gave informed consent and a detailed questionnaire was administered. Family history of POAG was noted and pedigrees constructed with the help of a research genealogist. Each participant underwent a detailed examination, including visual acuity, IOP, gonioscopy, disc assessment and visual field testing. A score (termed the GIST score) was given to each patient which denotes the probability of the diagnosis of POAG being present. Subjects were classified as normal, suspect or POAG. Age-matched, unaffected participants in the Twins Eye Study in Tasmania were used as a control group.

RESULTS: A total of 1702 POAG patients were identified. 1014 patients belonged to families in which other members were affected (familial glaucoma). 688 patients did not have a known family history of POAG (sporadic glaucoma). The size of the family groups varied from 2 to 29 affected individuals. The patients in the familial group had higher GIST

scores than those in the sporadic group. Only 24% of the participants in the control group had a family history of glaucoma.

CONCLUSIONS: 59.6% of POAG in Tasmania is familial. This percentage is higher than most previous reports of familial glaucoma and emphasises the importance of genetics in POAG. Patients with familial glaucoma had higher GIST scores, which may reflect an earlier onset and/or higher severity of glaucoma in the familial group. This has important implications for glaucoma screening and for further research in glaucoma genetics.

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PUBLICATIONS

The data in this thesis have not yet been published.

The following papers have been written and are to be submitted for publication:

How significant is a family history of glaucoma? Experience from the Glaucoma Inheritance Study in Tasmania (GIST).

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Familial glaucoma is more severe than sporadic glaucoma.

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS.....10

LIST OF FIGURES 11

LIST OF TABLES 13

BACKGROUND

I INTRODUCTION 14

II. DEFINITION OF GLAUCOMA AND CLASSIFICATION..17

III. GLAUCOMA: A FAMILIAL DISEASE..... 23

IV. EPIDEMIOLOGY OF PRIMARY OPEN ANGLE GLAUCOMA..... 32

V. FAMILY HISTORY IN THE CONTEXT OF EPIDEMIOLOGICAL STUDIES..... .41

VI. THE GENETICS OF PRIMARY OPEN ANGLE GLAUCOMA.....50

VII. BACKGROUND OF THE GLAUCOMA INHERITANCE STUDY IN TASMANIA.....62

METHODS 65

RESULTS 75

DISCUSSION 108

CONCLUSIONS ..... .119

BIBLIOGRAPHY 122

APPENDICES.....131

LIST OF ABBREVIATIONS

ABS	Australia Bureau of Statistics
AD	Autosomal Dominant
AGIS	Advanced Glaucoma Intervention Study
BES	Baltimore Eye Survey
BMES	The Blue Mountains Eye Study
GIST	Glaucoma Inheritance Study in Tasmania
IOP	Intraocular pressure
JOAG	Juvenile Open Angle Glaucoma
Melbourne VIP	The Melbourne Visual Impairment Project
mmHg	millimetres of mercury
MYOC	myocilin
NTG	normal tension glaucoma
OAG	open angle glaucoma
OPTN	optineurin
PDS	Pigment Dispersion Syndrome
POAG	Primary Open Angle Glaucoma
TIGR	Trabecular meshwork inducible glucocorticoid receptor protein

LIST OF FIGURES

Figure 1. Gonioscopic view of normal open angle.....19

Figure 2. Colour photographs of right and left optic discs of a POAG patient showing characteristic glaucomatous changes.....20

Figure 3. Examples of field defects.....21

Figure 4. Tasmanian glaucoma pedigrees published by Dr Bruce Hamilton in 1938.....25

Figure 5. Letter from Dr David Waterworth, a Tasmanian ophthalmologist.....30

Figure 6. Example of the discrepancy in knowledge of family history of POAG.....48

Figure 7. A pedigree of autosomal dominant juvenile glaucoma.....54

Figure 8. Photographs showing a large family from the GIST with pictures of the original matriarch and patriarch.....63

Figure 9. Bar graph showing distribution of cases between the two groups.....79

Figure 10. Bar graph showing gender distributions between the two groups.....105

Figure 11. Gender distribution by relationship in familial group.....106

Figure 12. Bar graph showing age distributions between the two groups.....107

Figure 13. Bar graph showing distribution of relationships amongst familial group.....108

Figure 14. Distribution of GIST scores for familial and sporadic groups.....109

LIST OF TABLES

Table 1. The Prevalence of Primary Open Angle Glaucoma as Reported in Prevalence Studies.....35

Table 2. Glaucoma genes and linkages identified.....58

Table 3. Projections of number of glaucoma cases in Tasmania.....113

BACKGROUND

I. INTRODUCTION

Glaucoma is a progressive disorder of the optic nerves that is characterised by excavation of the optic nerve head and loss of peripheral vision. Occasionally, there is also loss of central vision (Alward, Fingert, Coote et al 1998; Quigley 1998). Glaucoma is a leading cause of irreversible blindness throughout the world. In the developed world, the commonest form is primary open angle glaucoma (POAG) (Leske 1983).

Glaucoma is a treatable condition if detected sufficiently early in its course. Established optic nerve damage is irreparable, but progression to blindness can frequently be halted if the condition is diagnosed before this has occurred, usually by lowering of the intraocular pressure (Migdal and Hitchings 1986; Collaborative Normal-Tension Glaucoma Study Group 1998; Investigators-The Advanced Glaucoma Intervention Study (AGIS) 2000; Kass, Heuer, Higginbotham et al 2002; Leske, Heijl, Hussein et al 2003).

POAG is almost always asymptomatic in the earlier stages of the disease and progressive visual field loss occurs gradually; symptomatic central visual loss occurs in advanced disease. It is for this reason that much of the world's glaucoma remains undetected, even in developed countries. Most of the large prevalence studies conducted to date have found that only approximately 50% of glaucoma cases in the community are diagnosed (Bengtsson 1981;

Collaborative Normal-Tension Glaucoma Study Group 1998; Investigators-The Advanced Glaucoma Intervention Study (AGIS) 2000; Kass, Heuer et al 2002; Leske, Heijl et al 2003).

Blindness and visual impairment are important public health issues with significant socio-economic implications. Early detection and treatment of glaucoma represent important public health challenges throughout the world. No ideal screening method with suitable sensitivity and specificity has yet been identified. Cost-effectiveness of screening programmes for glaucoma is controversial (Coleman 1803; Wormald and Rauf 1995; Boivin, McGregor and Archer 1996; Tuck and Crick 1997).

Since the 19th century, a family history of glaucoma has been known to be a risk factor for developing the condition. How great a role family history plays has been more difficult to elucidate. This can be explained by several factors and will be discussed in more detail in the following chapters. Quantifying family history as a risk factor may allow the development of screening programmes, targeting those individuals known to be at risk.

During the last part of the 20th century, glaucoma emerged as, at least partially, a genetically determined disease. Several glaucoma genes have been identified (Alward, Fingert et al 1998; Craig and Mackey 1999; Rezaie, Child, Hitchings et al 2002), but these only account for a small percentage of glaucoma cases. There remains much yet to be understood about the pathogenesis of the disease. Identifying causative genes may provide

information about underlying disease mechanisms and lead to the development of targeted treatment and improved screening programs.

The Glaucoma Inheritance Study in Tasmania (GIST) is a large-scale study based in Tasmania and other states in Australia. The primary aim has been to recruit large Australian POAG pedigrees to allow identification of POAG genes. This thesis discusses some of the findings of GIST and evaluates the role a positive family history of glaucoma plays in this population (Mackey 2002-2003).

II. DEFINITION AND CLASSIFICATION OF GLAUCOMA

Glaucoma refers to a group of diseases characterised by a progressive optic neuropathy, secondary to loss of retinal ganglion cells, resulting in typical visual field defects.

There are several risk factors for the development of glaucoma, but the pathogenesis of the disease is still, to a large extent, unknown. Elevated intraocular pressure (IOP) is one of the primary risk factors and was until recently, considered part of the diagnosis. However, the early population based prevalence studies performed in the 1960's and 1970's showed a normal IOP in a significant number of glaucoma cases (Hollows and Graham 1966; Leibowitz, Krueger, Maunder et al 1980) and elevated IOP is no longer considered a prerequisite for the diagnosis of glaucoma. Conversely, the majority of individuals with statistically elevated IOP never develop glaucoma (Kass, Heuer et al 2002).

IOP nevertheless plays an important role in the management of the disease as lowering of the IOP has been shown to slow the progression of optic nerve damage and to stabilise the vision(Collaborative Normal-Tension Glaucoma Study Group 1998; Investigators- The Advanced Glaucoma Intervention Study (AGIS) 2000; Heijl, Leske, Bengtsson et al 2002; Leske, Heijl et al 2003).

A number of schemes for the classification of glaucoma have been proposed. They are based on the age of the patient (infantile, juvenile, adult), the site of obstruction to aqueous outflow (pre-trabecular, trabecular, post-trabecular) and aetiology. The most widely used is one that separates open-angle from closed-angle glaucoma. In open angle glaucoma, the drainage angle of the eye is unobstructed when examined gonioscopically. In angle closure, there is obstruction of the trabecular meshwork, usually by peripheral iris, impeding drainage of aqueous from the eye.

The primary glaucomas, by definition, are not associated with known ocular or systemic disorders that cause increased resistance to aqueous outflow or cause optic neuropathy.

Primary open angle glaucoma (POAG) is diagnosed in the presence of glaucomatous optic disc damage, which may take the form of changes in the optic disc appearance and/or the presence of abnormalities in the visual field. The drainage angle is open and of normal appearance on gonioscopy (Figure 1). It has an adult onset, in there are no secondary causes of glaucoma present (Thomas 1994).

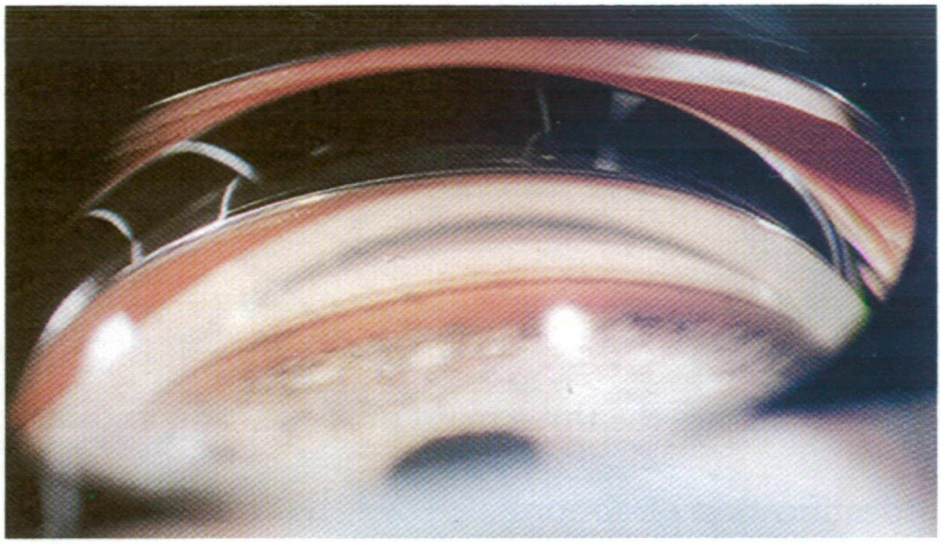


Figure 1. Gonioscopic view of normal open angle (Kanski JJ)

Changes that may be seen in the optic disc may include the following:

- Enlargement of the optic cup
- Asymmetry of the cup when compared with the fellow eye
- Narrowing of the neuroretinal rim
- Vertical elongation of the cup
- Regional pallor
- Presence of a disc haemorrhage
- A defect in the nerve fibre layer
- Exposure of the lamina cribrosa
- Nasal displacement of disc vessels
- Baring of circumlinear vessels
- Peripapillary atrophy.

Differentiating physiological cupping from acquired glaucomatous cupping of the optic disc can be difficult, especially in early disease. The size of the optic

disc needs to be taken into account when assessing cup size (Jonas, Budde and Panda-Jonas 1999). Figure 2 shows characteristic optic disc changes seen in glaucoma.

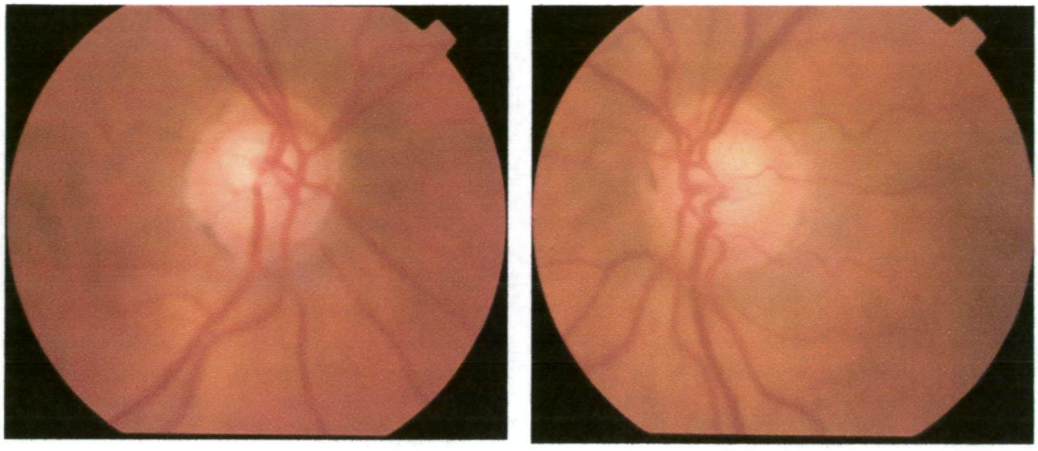
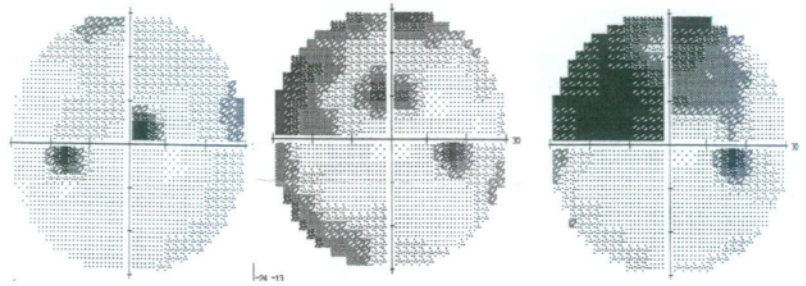


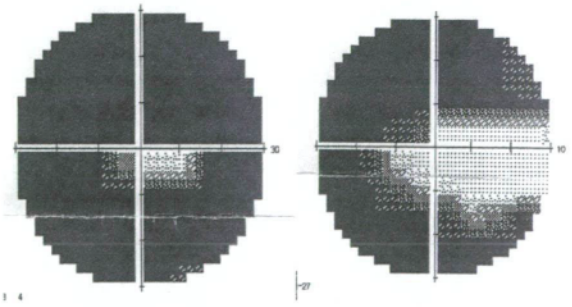
Figure 2. Colour photographs of right and left optic discs of a POAG patient showing characteristic glaucomatous changes

The visual field defects that occur in glaucoma correspond with nerve fibre bundle damage. These may include the following:

- Paracentral scotoma
- Arcuate scotoma
- Nasal step
- Temporal wedge



a. b. c.



d. e.

Figure 3. Examples of field defects.a) Paracentral defect, b) Superior and inferior arcuate defects, c)superior arcuate defect, d) advance field loss showing small central island of vision remaining when 30 degree field tested, e) the same eye tested in (d) showing central 10 degrees of field tested

In advanced glaucoma there may be extensive loss of peripheral vision, as shown in Figure 3d.

The term "glaucoma suspect" refers to patients with findings suggestive of POAG but without definite evidence of established disease.

There is still no universally accepted definition of glaucoma and the definitions used in different population-based studies have varied. Foster et al (Foster, Buhrmann, Quigley et al 2002) propose a scheme for diagnosis of glaucoma in population-based prevalence surveys. Cases are diagnosed on grounds of both structural and functional evidence of glaucomatous optic neuropathy.

Leske published a review article on POAG epidemiology in1979 in which she states: "When all these findings (elevated IOP, characteristic optic disc changes and visual field loss) are present, the diagnosis is not questioned, but when one or two elements of the triad are missing, there is no general agreement as to diagnosis" (Leske and Rosenthal 1979). The Framingham Eye Study explored the effect of different definitions of glaucoma on the prevalence of the condition and on associated risk factors (Kahn and Milton 1980). The authors concluded that the prevalence rates and strength of association could differ according to the definitions used. This was also addressed by Wolfs et al in the Rotterdam Eye Study, in which similar conclusions were reached (Wolfs, Borger, Ramrattan et al 2000).

III. GLAUCOMA: A FAMILIAL DISEASE

Whilst the pathogenesis of primary open-angle glaucoma remains elusive, it has long been recognised that a positive family history is a risk factor for the disease.

As far as we know, Benedict, in 1842, was the first to call attention to the familial tendencies of the disease. He reported glaucoma in "two dark-eyed daughters of an extremely gouty old general"(Benedict 1842).

Ärlt sketchily described 3 families in 1860. In 1880, Schenkl stated "there is hardly an ophthalmologist who has not met with several hereditary cases"(Schenkl 1880). Bowman added another family tree in 1865 and referred to glaucoma as "the most subtle of the hereditary affectations of the eye"(Bowman 1866).

Von Graefe discussed the heredity of glaucoma in 1869. He emphasized the importance of inheritance in the aetiology of glaucoma and referred to families in which the disease had occurred in three or four generations but did not actually report pedigrees (Graefe 1869).

It was only in the 20th century that the heredity of glaucoma began to be studied in more detail. Nettleship stressed the need for collection of more data relating to the inheritance of glaucoma in 1906 (Nettleship 1906).

In 1932, Julia Bell reviewed the body of knowledge regarding the heredity of glaucoma. She had traced glaucoma into 2 generations in 48 of 68 pedigrees she had collected. She wrote: "It is true that glaucoma is for the most part a disease of declining years and the difficulty in tracing any evidence of heredity is enhanced by the death of many individuals who might have developed the disease had they lived longer."

She continues:"... the age of onset in the parents closely determines the period of life at which predisposed offspring will show signs of disease; members of a sibship evidently tend to become liable at the same age"(Bell 1932).

In 1939, Biro stated: "It is essential that there can be no doubt about the fact of heredity of glaucoma any longer and that heredity is an aetiological factor in glaucoma." and suggested that "healthy and affected members of families with hereditary glaucoma should be examined from every, and not only the ophthalmological point of view. The comparison of results of these examinations with those of persons suffering from non hereditary glaucoma will carry us a great step further towards the solution of the pathogenesis of glaucoma"(Biro 1939).

William Stokes published a glaucoma pedigree with five affected generations in 1940. The type of glaucoma reported was a severe, blinding form of the disease with affected family members progressing to blindness in early

adulthood. He noted the glaucoma in this family to have been inherited in an autosomal dominant pattern (Stokes 1940).

The first reports of family studies appeared in 1949. Until then, despite the fact that it was often stressed that heredity was an important factor in the pathogenesis of glaucoma, only approximately 90 pedigrees of families with glaucoma had been reported in the literature including 9 from Tasmania, identified by ophthalmologist Dr Bruce Hamilton in 1938 (Hamilton 1938). (Figure 4).

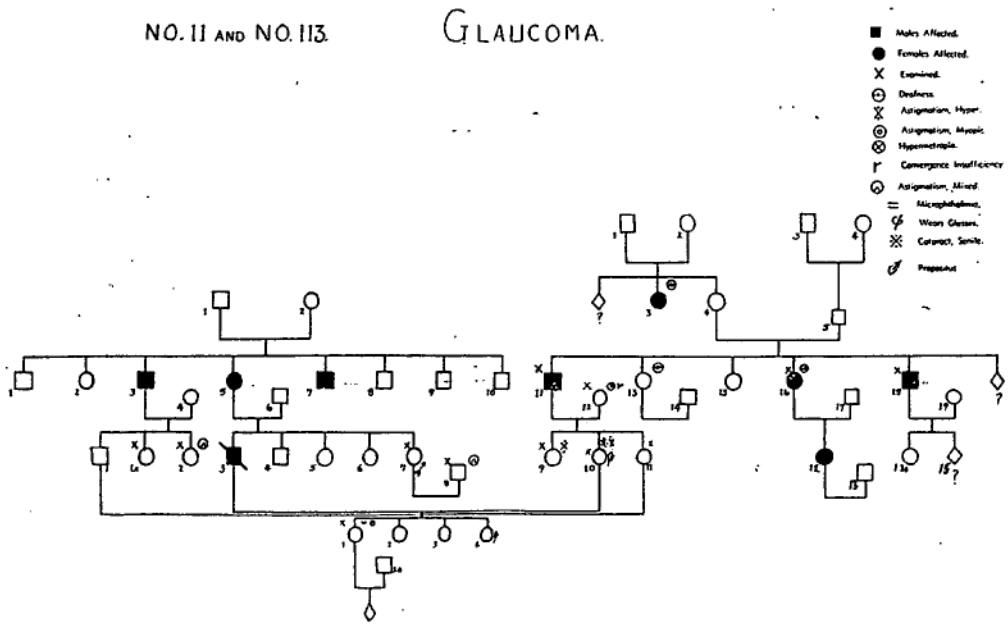


Figure 4. Tasmanian glaucoma pedigrees published by Dr Bruce Hamilton in 1938.

These family trees had been presented as isolated examples, without relation to glaucoma as a whole, thus creating the impression that hereditary glaucoma was rare and that it differed from the non-hereditary form.

Posner and Schlossmann reported in 1949 on a study they performed of glaucoma patients. Of 373 cases of primary glaucoma, 51(13.7%) had one or more relatives with the disease. They postulated that the hereditary group of glaucomas could be used as a starting point for the investigation of some of the more elusive problems in glaucoma, such as the pre-glaucomatous state, the possibility of predicting the severity of the disease and the relation between glaucoma and certain constitutional diseases. They reported 30 pedigrees of glaucoma families. However, not all the pedigrees were of POAG, or chronic simple glaucoma as it was referred to in this paper. All types of glaucoma had been included, including examples of what is now termed angle closure glaucoma. They concluded: "It was previously shown that some persons are genetically glaucomatous, but show no clinical manifestations of the disease. They may have glaucoma at some future time of life, either spontaneously or as a result of a provocative factor, such as instillation of atropine, cataract or emotional disturbances. From this point of view, their normal state may be regarded as a pre-clinical stage. It would be interesting to find tests which would detect patients who are potentially glaucomatous while they are still clinically normal.

"For a better understanding of the mode of transmission of glaucoma, it is essential to have a large series of good pedigrees. In most families, the disease follows a similar course in the various affected members. The genetic approach may be an aid in the early recognition of glaucoma and in the study

of the pre-clinical and mild phases of the disease" (Posner and Schlossman 1949).

Other similar studies were conducted over the next decade and found between 13 – 25% of relatives of glaucoma patients were affected (Waardenberg 1949; Biro 1951; Probert 1952; Kellerman and Posner 1955).

However, at this stage, the classification and definition of glaucoma were not well established. Some of the studies included all types of glaucoma, and the criteria for definition of glaucoma differed in each study. In addition, the criteria for the diagnosis of glaucoma differed from the definitions used today. In previous studies, there was significant emphasis placed on elevated IOP.

In 1960, Becker, Kolker and Roth published a study of relatives of known glaucoma patients. They included only families with chronic simple glaucoma in whom there were at least two known affected members. Siblings, children and parents of the affected individuals were studied. All family members over the age of 15 years were included. Of the 110 relatives examined from 24 unrelated families, 6 individuals (5.45%) met the criteria for recommending treatment. This figure is lower than the studies published until then. However, the criteria for diagnosis were strict, using an IOP of 30 mmHg or greater as the threshold for treatment, a value much higher than would be used today. Additional bias could have been introduced by the fact that they studied many younger family members (46% of the subjects studied

were under the age of 40 years), whilst all the newly diagnosed cases of glaucoma were found in individuals over the age of 40 years. Had they restricted the family members studied to those over the age of 40 years, they would have found approximately 10% of family members affected, which is consistent with previously published data (Becker, Kolker and Roth 1960).

During the following decades, the importance of glaucoma as a preventable cause of blindness was recognised and efforts were made to establish effective methods of screening. Mass screening presents challenges as the diagnosis of glaucoma is based on thorough clinical examination, which is time-consuming and costly. In 1962, Miller and Paterson published a study whose aim was to determine whether thorough screening of a small selected population would be valuable in the early diagnosis of glaucoma simplex. Relatives of known glaucoma cases were examined. The study was divided into two parts: siblings between 40 and 60 years, and offspring between the ages of 15 and 55 years. A glaucoma-free control group was selected, and matched for age and sex. In the sibling group, 8.0% of the subjects had definite glaucoma and 38.0% were regarded as glaucoma suspects. In the control group, only one patient of 50 (i.e. 2.0%) was regarded as a suspect. This was statistically significant.

In the offspring group, 2.7% had glaucoma, with 41.3% regarded as suspects. All the controls were normal. This again highlighted the hereditary nature of glaucoma. The authors recommended that individuals with a positive family history of glaucoma be regularly tested for glaucoma and that siblings of all

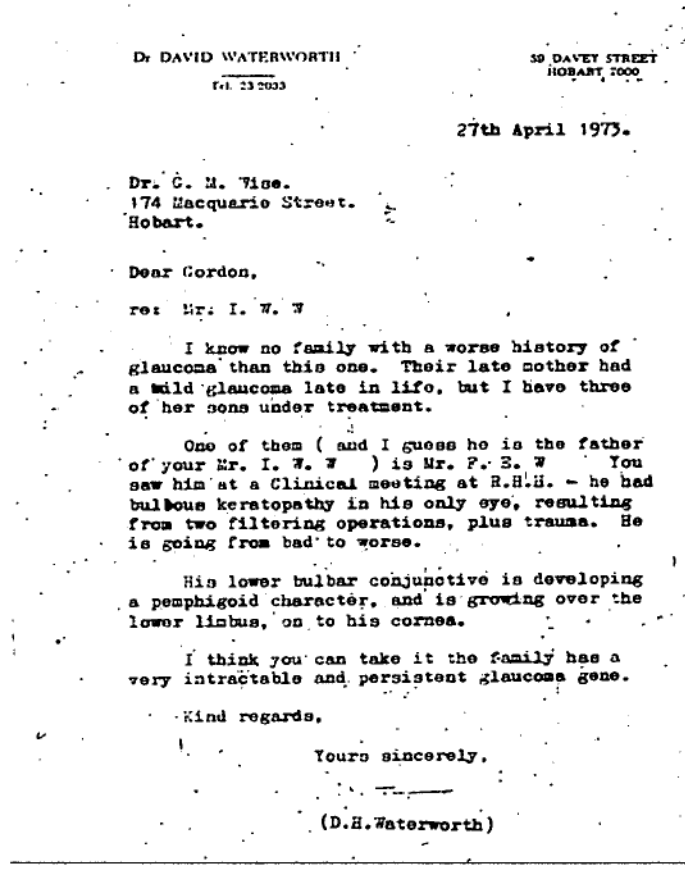
known cases of glaucoma simplex be routinely examined (Miller and Paterson 1962).

In 1966, Francois and Heintz-de Bree reported on a study of the families of 79 randomly selected glaucoma cases from their clinic. The cases were not from families previously known to be affected by glaucoma. 10 families did not meet the study criteria and were excluded. Glaucoma was found to be familial in 26 of the 69 families studied (37.6%), and to be inherited in an autosomal dominant fashion with very high penetrance. Again the criteria for diagnosis of glaucoma included an IOP of greater than 25 mmHg, which differs from our current definitions. This definition may have selected pedigrees with severe disease which is strongly expressed, thus resulting in the high percentage of families affected (Francois and Heintz-De Bree 1966).

Other workers including Leighton (Leighton 1968) and Perkins (Perkins 1974) obtained similar results. Many centres began to run glaucoma family clinics.

Paterson studied family members of glaucoma patients in 1961 and found 8% of family members were affected as compared with 0.7% in the general population. This increased to 10% when the subjects of were followed up 9 years later. This figure may have been higher if all subjects had been re-examined; 11 of the 27 subjects (40%) who had previously been classified as normal did not attend for a follow-up examination (Paterson 1970). All these studies confirmed what many ophthalmologists had suspected for years: that a

positive family history of POAG was risk factor for developing the disease. In the course of the GIST, the following letter was found, written in 1973, which illustrated this elegantly (Figure 5). This pedigree was not published until 2001, after the identification of the GLC1A Gln368 STOP mutation (Craig, Baird, Healey et al 2001).



Dr DAVID WATERWORTH
Tel 23 2033

59 DAVEY STREET
HOBART, 7000

27th April 1973.

Dr. C. M. Wise.
174 Macquarie Street.
Hobart.

Dear Gordon,

re: Mr. I. W. W

I know no family with a worse history of glaucoma than this one. Their late mother had a mild glaucoma late in life, but I have three of her sons under treatment.

One of them (and I guess he is the father of your Mr. I. W. W) is Mr. P. S. W You saw him at a Clinical meeting at R.H.H. - he had bullous keratopathy in his only eye, resulting from two filtering operations, plus trauma. He is going from bad to worse.

His lower bulbar conjunctive is developing a pemphigoid character, and is growing over the lower limbus, on to his cornea.

I think you can take it the family has a very intractable and persistent glaucoma gene.

Kind regards,

Yours sincerely,

(D.H.Waterworth)

Figure 5. Letter from Dr David Waterworth, a Tasmanian ophthalmologist

All the above studies differ significantly in methods and in criteria for the diagnosis of glaucoma, resulting in different conclusions regarding the inheritance. There was a great emphasis placed on elevated IOP as part of the definition of glaucoma. This may have resulted in a significant number of cases not being detected. It was only in 1966 with the publication of the Ferndale Eye Study by Hollows and Graham that it was realised that elevated

IOP was not present in all patients with glaucoma. This caused a change in the approach to the definition of glaucoma. Visual fields were tested by means of either a Bjerrum screen or Goldmann field test. These methods of visual field testing are considerably less sensitive than the computerised tests used today. The numbers of individuals in the studies were small and as most studies were clinic-based, which may have opened the possibility of selection bias related to family history and severity of disease.

Until this stage, there was little known about the true burden of glaucoma in the general population and thus what role a positive family history of the disease played.

During the following decades, large, population-based studies were conducted in several countries, which shed more light on the epidemiology and risk factors of the disease.

IV. EPIDEMIOLOGY OF PRIMARY OPEN ANGLE GLAUCOMA

Epidemiology is the study of the distribution and determinants of diseases in human populations. It is based on the fact that disease is not randomly distributed throughout a population but rather that the frequency varies in different sub-groups. This knowledge can be used to identify features that cause disease. Such information is central to the design and implementation of intervention strategies necessary for disease prevention and treatment (Wilson 1990).

It is estimated that 67.7 million people were affected by glaucoma worldwide in the year 2000 (Quigley 1996). Primary open angle glaucoma is the commonest form of glaucoma in the western world (Leske 1983). It would not have been possible to make this estimate without well-designed, rigorously conducted population based studies. These confirmed the importance of glaucoma as a leading cause of preventable blindness throughout the world.

The purpose of epidemiological studies of POAG is to establish the relative importance of disease in the population and to identify groups with high and low rates of the disease. Thus, potential risk factors can be identified and hypotheses formulated. Identification of risk factors has far-reaching preventive and therapeutic implications. Some characteristics that predict future glaucoma may be both causal and changeable and may therefore lend themselves to intervention and disease prevention strategies. Others, such as

age, sex, ethnicity and family history are not subject to change, but are often major determinants of risk and may be used to identify individuals for whom close medical supervision is indicated. Furthermore, factors that affect glaucoma risk may also predict the rate of progression of the disease.

There are several difficulties with the design and interpretation of glaucoma studies. The first is that there is no universally agreed-upon definition of glaucoma, although recently efforts have been made to devise a standardised scheme for definition and diagnosis of disease (Foster, Buhrmann et al. 2002). The definition of glaucoma has been discussed in more detail in a previous section.

In many of the earlier studies of glaucoma epidemiology, methodological shortcomings in study design are likely to have resulted in biased estimates. Many of the initial prevalence studies involved populations that were self-selected or comprised a small, non-representative segment of the total population. Some were based on retrospective chart reviews or blindness registries for a given locality.

Incomplete case-finding can also bias results. For instance, studies that used visual field defects as a diagnostic criterion, but subjected only a proportion of study participants to perimetry, could under-estimate the disease prevalence (Wilson 1994).

Glaucoma is a disease with a low incidence, which necessitates large cohorts and long follow-up periods to obtain a sufficient number of events to ensure valid estimates of incidence. True population-based study designs have often been sacrificed in favour of targeting specific high-risk populations of ocular hypertensives or glaucoma relatives.

PREVALENCE OF GLAUCOMA

A well-designed prevalence study should have the following characteristics:

- There should be a well-defined population to which the prevalence estimate corresponds.
- Every effort should be made to examine all the defined population or a specified sample of the defined population.
- The proportion of the population that was actually examined should be reported.
- If sampling is used, individuals sampled should be representative of the population with no sub-group systematically excluded from the examination.
- Diagnostic criteria for glaucoma should be specified and consistently applied.

In the last few decades, numerous glaucoma prevalence studies have been conducted which meet these criteria (Table 1).

Table 1. The Prevalence of Primary Open Angle Glaucoma as Reported in Prevalence Studies

Study Location	Age group(years)	Response (%)	Prevalence (%)
Sweden (Bengtsson 1981)	55-69	77	0.93
Ireland (Coffey, Reidy, Wormald et al 1993)	50+	99.5	1.7
Beaver Dam (Klein, Klein, Sponsel et al 1992)	43-84	83.1	2.1
Baltimore (Tielsch, Katz, Singh et al 1991)	40+	79.2	3.0
Wales (Hollows and Graham 1966)	40-75	91.9	0.4
South Africa (Salmon, Mermoud, Ivey et al 1993)	40+	82.7	1.5
Rotterdam (Dielemans, Vingerling, Wolfs et al 1994)	55+	71.0	1.0
Casteldaccia (Giuffre, Giammanco, Dardanoni et al 1995)	40+	67.3	1.2
Barbados (Leske, Connell, Schachat et al 1994)	40-84	83.5	6.1
Blue Mountains (Mitchell, Smith, Attebo et al 1996)	49+	87.9	3.0
Melbourne (definite cases)(Wensor, McCarty, Stanislavsky et al 1998)	40-98	83	1.7
Melbourne (definite and probable glaucoma) (Wensor, McCarty et al 1998)	40-98	83	2.2

Major methodological differences between studies limit direct comparison of the results.

THE EPIDEMIOLOGY OF POAG IN AUSTRALIA

Two major population-based studies of glaucoma epidemiology have been conducted in Australia: the Blue Mountains Eye Study in New South Wales (Mitchell, Smith et al 1996) and the Melbourne Visual Impairment Project in Victoria (Wensor, McCarty et al 1998).

The Blue Mountains Eye Study

The Blue Mountains Eye Study (BMES) is a population based survey of vision and eye diseases in the Blue Mountains region, west of Sydney (Mitchell, Smith et al. 1996). This urban area has a stable and homogeneous

population, representative of Australia for income measures and other measures of socio-economic status (Mitchell, Smith, Attebo et al 1995). The study participants were residents aged 49 years or older and were identified by means of a door-to-door census of the study region. Of the 4433 eligible persons, 3654 (82.4%) participated in the study from January 1992 to January 1994. After the potential participants who had died or had moved away from the area were excluded, the response rate was 87.9%, which compares favourably with most population-based glaucoma surveys (Table1).

All participants underwent a detailed interview and eye examination, which included subjective refraction, visual field testing, applanation tonometry and stereo disc photography. The visual field testing component of the examination was conducted in two phases. In the first phase, subjects underwent a 30° suprathreshold visual field screening test (Humphrey 76-point test). In the second phase a subset of participants underwent a full-threshold Humphrey 30-2 test.

Diagnosis of glaucoma:

Open-angle glaucoma was diagnosed if typical glaucomatous visual field loss on the Humphrey 30-2 was present, combined with matching optic rim thinning and an enlarged cup-disc ratio (≥ 0.7) or cup-disc asymmetry between the two eyes of ≥ 0.3. The diagnosis of open-angle glaucoma was excluded if gonioscopy showed signs of angle closure, rubeosis or secondary glaucoma, other than pseudoexfoliation. IOP was not one of the criteria used in the definition of glaucoma.

There were 3654 participants in the study. Definite or probable glaucoma was diagnosed in 108 participants, a prevalence of 3.0% (CI, 2.5-3.6). An exponential increase in prevalence was found for increasing 10-year age groups. The prevalence of glaucoma was 0.4% for people younger than 60 years of age, 1.3% for people 60 to 69 years of age, 4.7% for people 70 to 79 years of age and 11.4% for people aged 80 years or older. Women had a higher prevalence of glaucoma for each age group but this was of borderline significance after adjusting for age using logistic regression.

51% of glaucoma cases detected were previously undiagnosed. This figure is remarkably similar to that found in Rotterdam (53%) (Dielemans, Vingerling et al 1994), Roscommon (49%) (Coffey, Reidy et al 1993) and Baltimore (50% amongst whites) (Sommer, Tielsch, Katz et al 1991).

The Blue Mountains Eye Study also found that 75% of previously undiagnosed glaucoma cases had a presenting IOP less than 22 mmHg, which emphasizes the low yield likely from glaucoma screening that includes only a single IOP measurement. Nevertheless, the prevalence of glaucoma increased dramatically in patients with elevated IOP with nearly 40% of patients with an IOP of $\geq$ 28 mmHg having glaucoma, the rise being highly significant ($p<0.0001$). This emphasises the importance of elevated IOP as a risk factor for the disease.

The Melbourne Visual Impairment Project

The Melbourne Visual Impairment Project (Melbourne VIP) (Wensor, McCarty et al 1998) is a population based prevalence study of the distribution and determinants of eye disease in Melbourne, Australia. The participants were residents aged 40 or older from 9 parts of randomly selected 1986 Australia Bureau of Statistics Census Collector Districts in Melbourne.

Each participant underwent a standardised interview and clinical assessment, including visual fields and disc photographs. The diagnosis of glaucoma was made by a consensus panel of 6 ophthalmologists, including 2 glaucoma sub-specialists. No specific criteria were used in the diagnosis of glaucoma.The final classification for each individual was decided using all available data for that person, including a past history of glaucoma, IOP elevated >21mmHg in either eye, visual field defects and optic disc changes. Each expert used his or her clinical judgement to classify each case in a masked fashion. Cases that had significant discrepancies between experts' opinions were resolved in open discussion. This approach was used in an attempt to overcome the difficulties in diagnosing glaucoma, especially in a single examination of an individual.

To include institutionalised persons, residents of 13 nursing homes were studied. These nursing homes were randomly selected and were all located within 5km of a test site. Some modifications of the test procedure were

required because of the difficulty of testing elderly institutionalised individuals. The data from the nursing home group was analysed separately from that of the residential group because the data from the two groups were not directly comparable.

In the residential group there was an 83% response rate and 3271 persons were examined. There were 112 (3.4%) with POAG. Of these, 56 (1.7%) were regarded as definite, 16 (0.5%) probable and 40 (1.2%) were possible glaucoma sufferers. Only 28 (50%) of those with definite POAG had been diagnosed previously compared to 6 (38%) of those with probable glaucoma and 8 (22%) of those with possible glaucoma.

In the nursing home group there was a 90.6% response rate. A total of 403 persons participated. There were 27 persons (6.7%) who were considered to have glaucoma. Of these, 9 persons were blind according to World Health Organization guidelines.

The crude rate of POAG was higher in the nursing home group but direct standardization showed no significant difference in glaucoma prevalence between the residential (1.7%) and nursing home (2.36%) populations. The combined adjusted glaucoma rates for the residential and nursing home groups show prevalence rates of 1.7% in males and 1.91% in females older than 40 years of age.

The age specific prevalence of definite POAG in the Melbourne VIP was as follows: 0.1% for those aged 40-49, 0.6% of 50-59 year-olds, 1.9% 0f 60-69 year olds, 5.2% of 70-79 year-olds, 5.5% of 80-89 year-olds and 11.8% of those of 90 years or older.

Both the BMES and Melbourne VIP confirmed the finding that POAG prevalence increases exponentially with advancing age. There were slight differences in the age-specific prevalences, which could also be attributed to differences in the definitions of POAG used and differences in the sample population.

Nevertheless, although the large prevalence studies differ in their methods and in the definition used for POAG, the prevalence of POAG in predominantly Caucasian populations does not differ greatly.

The population of Tasmania is largely Caucasian (Australian Bureau of Statistics 2001) and is likely to be similar to that found in the BMES and Melbourne VIP.

V. FAMILY HISTORY IN THE CONTEXT OF EPIDEMIOLOGICAL STUDIES

Previous studies reported on the role a positive family history plays as a risk factor for the disease, as discussed earlier, but they were not population based and were subject to selection bias problems found with studies using clinic based ascertainment. The Baltimore Eye Survey (Tielsch, Katz, Sommer et al 1994) provided an unbiased source of patients with POAG and controls from a representative sample of black and white residents of east Baltimore, Maryland. This population sample was used to assess the strength of the association between family history and POAG. Participants underwent a standardized examination and interview, which included questions about family history. Two approaches were taken in the analysis of the data. The first approach was to compare the family history of subjects who were diagnosed with POAG with those of subjects without the disease. This method did not control for family size. The second approach analysed the data from a family perspective. A data file was created that included all members of the families of subjects in the Baltimore Eye Survey. Statistical analysis was used to adjust the variance of the regression coefficients to account for correlation between members of the same family.

A total of 16.1% of cases reported a positive family history of glaucoma amongst first-degree relatives vs 7.2% of controls. The strongest association was with siblings and the weakest with children. The small number of positive family histories reported amongst children was likely due to their

young age distribution in a disease whose risk, and therefore discovery, increases significantly later in life.

There was clear evidence that cases' knowledge of their own diagnosis (prior to being diagnosed by the survey examination) was associated with the frequency of a positive family history. Odds ratios were 2 to 3 times higher for cases who knew they had glaucoma than for those whose condition was first diagnosed by the survey team.

These analyses did not account for the different sizes of families for cases and controls. Analysis performed taking this into account showed a similar pattern to that found using the standard case control approach. A total of 2.13% of persons who were first-degree relatives of cases were reported to have a history of glaucoma compared with 0.92% of those who were relatives of controls. A history of glaucoma amongst siblings continued to demonstrate the strongest association with POAG, with lower associations noted for parents and children. Again, the association was stronger among relatives of those index subjects who knew they had glaucoma prior to the study diagnosis compared with relatives of those index subjects whose condition was first diagnosed by the study team.

The Barbados Eye Study (Nemesure, Leske, He et al 1996) was based on a random sample of Barbados-born citizens between 40 and 84 years of age. The self-reported family history of OAG among 4,314 black participants was investigated. All participants underwent a standardised examination including

Humphrey perimetry, fundus photography and ophthalmic measurements. A comprehensive interview, which included questions on family history was conducted. Family members under consideration were father, mother, full and half- brothers and sisters, father's parents, mother's parents and sons and daughters.

Participants with POAG reported a family history of glaucoma more often than those without glaucoma. Differences were most marked for sibling history. In both groups, maternal history was reported twice as frequently as paternal history.

Reports of family history are probably influenced by additional reporting biases due to the participants prior knowledge of their own diagnosis (Tielsch, Katz et al 1994). This was the case in the Baltimore Eye Survey (BES), in which participants with a prior diagnosis of glaucoma reported a sibling history more than twice as often as those with newly detected OAG. Nevertheless, amongst participants with no such prior knowledge, those with newly detected OAG were about 5 times more likely to report sibling history than those without OAG. This finding provides convincing evidence of the association between OAG and sibling history since it is not influenced by reporting biases due to prior diagnosis. A weakness of this study was that a standardized OAG diagnosis was available for the BES participants, but only a reported glaucoma history was available for their relatives. However the study has the strength that unlike most family studies of diseases, it is based

on a cross-sectional sample of families rather than on a sample of families ascertained to have at least one affected individual.

The Melbourne VIP studied the prevalence and investigated predictors of POAG in Victoria (Weih, Nanjan, McCarty et al 2001). This study has been described in detail in the section discussing the epidemiology of glaucoma. In multivariate logistic regression models, participants with a family history of glaucoma were three times more likely to have possible, probable or definite glaucoma. In analysis of only definite glaucoma cases, family history was the only significant risk factor, other than age (OR 3.5; 95% CI, 1.9,6.7).

The study estimated that those with a family history of glaucoma have a threefold increased risk of glaucoma. The authors note that the estimation of family history is likely to be biased by under-reporting. This will be discussed in more detail later in this chapter.

The Melbourne VIP, like the Barbados Eye Study and Baltimore Eye Survey, found a substantial bias in reported family history between those who were diagnosed and undiagnosed at the time of the study. Although not statistically significant, a total of 29% of those with a previous diagnosis of glaucoma compared with 15% who were undiagnosed at the time of the study.

This finding was consistent with results from the BMES in a paper that examined bias in the relationship between self-reported family history of glaucoma and its relationship to the prevalence of glaucoma and ocular

hypertension (Mitchell, Rochtchina, Lee et al 2002). A first-degree family history was given by 15.7% of subjects with glaucoma compared with 8.3% of controls, odds ratio (OR) 3.2 (95% CI 1.8- 5.6), after adjusting for glaucoma risk factors, including IOP. Although recall bias was evident from the finding of increased odds (OR 4.2) among previously diagnosed cases, the relationship also persisted in newly diagnosed cases (OR 2.4).

A limitation of the studies discussed in this chapter so far is that that they relied on a reported family history of glaucoma, without confirming the diagnosis by examining the affected individuals. The body of epidemiological and genetic evidence suggests heredity is an important factor in the development of the disease. However, a more complete knowledge of the actual, rather than the reported familial tendency of POAG would allow a more accurate assessment of the magnitude of this effect.

The Rotterdam Eye Study (Wolfs, Klaver, Ramrattan et al 1998) investigated the familial aggregation of POAG by examining first-degree relatives of glaucoma cases identified through their prevalence study, as well as a matched set of controls. The purpose of this study was to determine whether familial aggregation of glaucoma occurs in the general population and to determine the absolute relative risks for first-degree relatives.

Probands were selected from the population-based Rotterdam Eye Study. First-degree relatives of patients with glaucoma (n=48) and control subjects (n=55) underwent a standardised examination, including perimetry.

The prevalence of glaucoma was 10.4% in siblings of glaucoma patients vs 0.7% in siblings of controls and 1.1% in offspring of patients vs 0% in offspring of controls. Lifetime risk of glaucoma in relatives of patients was 22% vs 2.3% in relatives of controls, yielding a risk ratio of glaucoma of 9.2 (95% confidence interval= 1.2-73.9). The population attributable risk of glaucoma was 16.4%.

This study had several strengths:

- The patients and controls were selected from the same population-based cohort, minimising selection bias
- The investigators did not rely on history data, but actually examined all first-degree relatives
- The examinations were performed in a masked fashion
- The investigators aimed at full ascertainment and approached all patients with glaucoma in the source population.

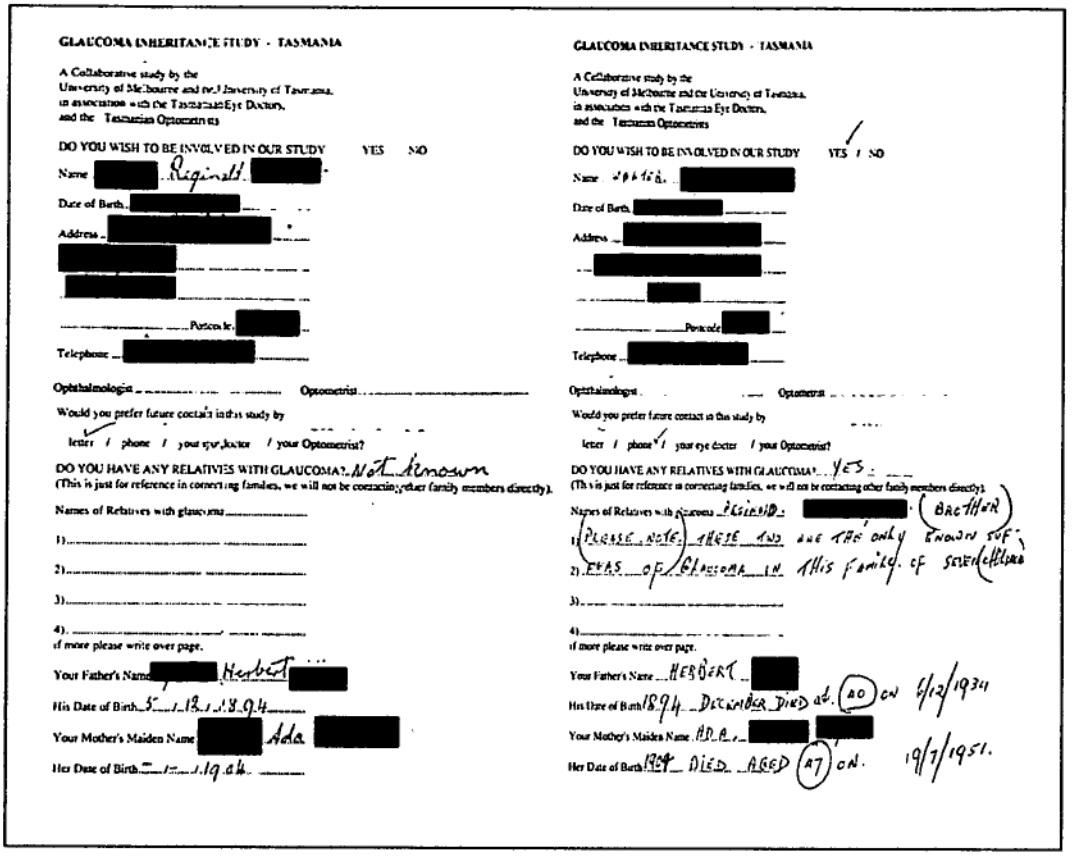
A limitation was the low number of patients, which decreased the statistical power of the study and created wide confidence intervals. However, the strength of the risk associations was strong enough to yield statistical significance. The participation rate in this study was relatively low, at 80% (Dielemans, Vingerling et al 1994), which could also introduce bias.

This study was very thoroughly conducted and provides useful information but could underestimate the genetic component of glaucoma, especially if the children examined were too young to manifest the disease. More extensive

assessment of the glaucoma status in the extended family (uncles, aunts and cousins) may have revealed an even stronger component of familial aggregation.

Inaccuracy in reported family history of disease is well-described (Kee, Tiret, Robo et al 1993; Aitken, Bain, Ward et al 1995). Studies of glaucoma using a self-reported family history may underestimate the true prevalence of glaucoma, which is often an asymptomatic, silent disease. A study was performed as part of the GIST to ascertain the prevalence of previously undiagnosed POAG within 5 large POAG pedigrees and to evaluate the reliability of a reported family history of glaucoma within these pedigrees (McNaught, Allen, Healey et al. 2000). The methods of the GIST are described in more detail in the section of this thesis entitled "Methods".

Of the patients examined for this study, some individuals with POAG had been diagnosed by their ophthalmologist whilst others were diagnosed as a result of their participation in the research project. Family members with a prior diagnosis of POAG were asked to report if they were aware of any relatives with POAG. This reported family history was then compared to the actual pedigree (before the diagnosis of new cases) to calculate agreement. The main outcome measures were the rate of glaucoma in pedigrees and percentage of previously diagnosed glaucoma cases who were aware of the positive family history of POAG. Figure 6 shows an example of responses to the initial questionnaire demonstrating the discrepancies in known family history.



GLAUCOMA INHERITANCE STUDY - TASMANIA

A Collaborative study by the
University of Melbourne and the University of Tasmania,
in association with the Tasmanian Eye Doctors,
and the Tasmanian Optometrists

DO YOU WISH TO BE INVOLVED IN OUR STUDY YES NO

Name [redacted] Reginald [redacted]

Date of Birth [redacted]

Address [redacted]

[redacted] Postcode [redacted]

Telephone [redacted]

Ophthalmologist ..... Optometrist .....

Would you prefer future contact in this study by

letter / phone / your eye doctor / your Optometrist?

DO YOU HAVE ANY RELATIVES WITH GLAUCOMA? Not known
(This is just for reference in connecting families, we will not be contacting other family members directly).

Names of Relatives with glaucoma .....

1) .....

2) .....

3) .....

4) .....

if more please write over page.

Your Father's Name [redacted] Herbert [redacted]

His Date of Birth 5/.../8.9.04

Your Mother's Maiden Name [redacted] Ada [redacted]

Her Date of Birth ?/.../1904

GLAUCOMA INHERITANCE STUDY - TASMANIA

A Collaborative study by the
University of Melbourne and the University of Tasmania,
in association with the Tasmanian Eye Doctors,
and the Tasmanian Optometrists

DO YOU WISH TO BE INVOLVED IN OUR STUDY YES / NO

Name [redacted]

Date of Birth [redacted]

Address [redacted]

[redacted] Postcode [redacted]

Telephone [redacted]

Ophthalmologist ..... Optometrist .....

Would you prefer future contact in this study by

letter / phone / your eye doctor / your Optometrist?

DO YOU HAVE ANY RELATIVES WITH GLAUCOMA? YES
(This is just for reference in connecting families, we will not be contacting other family members directly).

Names of Relatives with glaucoma REGINALD [redacted] (BROTHER)

1) (PLEASE NOTE: THESE TWO ARE THE ONLY KNOWN SUF-
2) FERS OF GLAUCOMA IN THIS FAMILY OF SEVEN CHILDREN

3) .....

4) .....

if more please write over page.

Your Father's Name HERBERT [redacted]

His Date of Birth 1894 DECEMBER DIED ad. (80) ON 6/12/1931

Your Mother's Maiden Name M.A. [redacted]

Her Date of Birth 1904 DIED AGED (47) ON. 19/7/1951.

Figure 6. Example of the discrepancy in knowledge of family history of POAG. Two brothers filled in the initial GIST forms: Walter was aware of a family history, but although Reginald had glaucoma, he was not aware of any affected relatives.

A total of 442 subjects from 5 pedigrees were examined. Of these, 47(11%) were previously diagnosed with POAG and 8 (2%) were previously diagnosed glaucoma suspects. As a direct result of the GIST examination 30 cases (7%) of POAG and 41 suspects (9%)were newly diagnosed.

Of the 47 previously diagnosed POAG cases, 41 were questioned about their prior knowledge of any family history and 11 (27%) were unaware of their family history of POAG. The accuracy of reporting was highest for first-degree relatives and lower for second-degree relatives. One of the pedigrees

participating in this study is a rural Victorian family found to have a GLC1A mutation (THR377MET) (Alward, Fingert et al. 1998). All 8 POAG patients questioned reported at least one affected relative. In this pedigree, the prevalence of glaucoma is high and the mean age of diagnosis is low (fourth decade of life). In contrast, another family, who has normal tension glaucoma that was usually diagnosed n the sixth decade of life had a lower reported positive family history. The overall inaccuracy of the family history knowledge shown by the combined pedigrees would be higher if the Victorian family's result were excluded from the analysis.

No previous study has examined entire glaucoma families in such detail, including both affected and unaffected family members. Even within these large extended pedigrees, an accurately reported family history will underestimate the true prevalence of the disease, as a percentage of those thought to be unaffected are, in fact, affected.

VI. THE GENETICS OF PRIMARY OPEN ANGLE GLAUCOMA

Although a positive family history of POAG has been recognised as a risk factor for centuries, it was only in the last decade that the genetics of glaucoma could be studied in more detail and causative genes identified. Medical genetics was revolutionised during the 1980's by the application of genetic mapping to locate the genes responsible for simple Mendelian diseases. Genetic mapping involves comparing the inheritance pattern of a trait with the inheritance pattern of chromosomal regions and allows one to identify the location of the gene is without knowing what it is (Lander and Schork 1994).

This approach has been used for decades by experimental geneticists, but has only recently begun to be studied in humans. The study of human traits was limited by a lack of abundant supply of genetic markers with which to study inheritance and the inability to arrange human crosses to suit experimental purposes. However, Botstein and colleagues recognised that naturally occurring DNA sequence variation provided a virtually unlimited supply of genetic markers (Botstein, White, Skolnick et al 1980). With highly polymorphic genetic markers, one could trace inheritance in existing human pedigrees as if one had set up the crosses in the laboratory. This led to the study of rare human diseases having simple Mendelian inheritance, with hundreds of diseases having been genetically mapped in this fashion and dozens positionally cloned.

Most diseases and traits, however, do not follow simple inheritance patterns. The term "complex traits" refers to any phenotype that does not exhibit classic Mendelian recessive or dominant inheritance attributable to a single gene locus. Complexities arise when the simple correspondence between genotype and phenotype breaks down, either because the same gene type can result in different phenotypes (due to the effect of chance, environment or the interactions of other genes) or different genotypes can result in the same phenotype.

It is often impossible to find a genetic marker that shows perfect co-segregation with a complex trait. The reasons for this can be ascribed to a few basic problems.

These include:

- Incomplete penetrance and phenocopy
- Genetic or locus heterogeneity
- Polygenic inheritance
- High frequency of disease-causing alleles

INCOMPLETE PENETRANCE AND PHENOCOPY

Some individuals who inherit a predisposing gene may not manifest the disease (incomplete penetrance), whereas others who do not inherit the gene nonetheless develop the disease as a result of environmental or random causes (phenocopy). Thus the genotype at the given locus may affect the probability of the disease but not fully determine the outcome.

GENETIC OR LOCUS HETEROGENEITY

Mutations in any one of several genes may result in identical phenotypes. This hampers genetic mapping because a chromosomal region may co-segregate with a disease in some families but not in others.

POLYGENIC INHERITANCE

Some traits may require the simultaneous presence of mutations in multiple genes.

HIGH FREQUENCY OF DISEASE-CAUSING ALLELES

Even a simple trait can be difficult to map if disease-causing alleles occur at a high frequency in the population. This becomes an even greater problem if genetic heterogeneity is also present.

All of these difficulties apply to the genetics of POAG and this makes the identification of disease-causing genes challenging.

By narrowing the definition of the disease or restricting the patient population, it is often possible to work with a trait that is more nearly Mendelian in its inheritance pattern and more likely to be homogeneous. This may apply to the clinical phenotype, the age of onset, those with a family history of the condition and those with more severe disease (Lander and Schork 1994).

There are three main approaches to identifying a disease-causing gene :

- Candidate gene approach
- Utilising clues from chromosomal deletions and translocations
- Linkage analysis

Often a combination of these techniques is used (Alward 2000).

The candidate gene approach is useful when there is a known gene whose function makes it a strong suspect.
In POAG, there are too many potential candidate genes including all the genes involved in the development, structure and function of the trabecular meshwork and optic nerve.

By identifying patients who manifest the disease of interest and also have a chromosomal deletion or translocation, it is sometimes possible to show that the disease-causing gene is in or near the break in the chromosome. This technique has been used successfully in identifying genes involved in the developmental glaucomas such as Axenfeld-Rieger anomaly and in congenital glaucoma.

Linkage studies are used in the absence of other clues as to the location and nature of the genes causing a disease. These studies are usually conducted on large families affected with the disease, looking for co-segregation between the disease phenotype and polymorphic genetic markers. This method usually requires large numbers of living affected individuals.

In POAG, the disease is usually of later onset which means that the parents and siblings of affected individuals are often deceased. Children of affected individuals may be too young to have manifest the disease.

In identifying genes which cause POAG a combination of the techniques described above was used. In 1993, Johnson described a family with early onset, severe POAG (Johnson, Drack, Kwitek et al 1993) (Figure 7). Of 59 individuals in 5 generations who were at risk of the disease, 30 were affected.

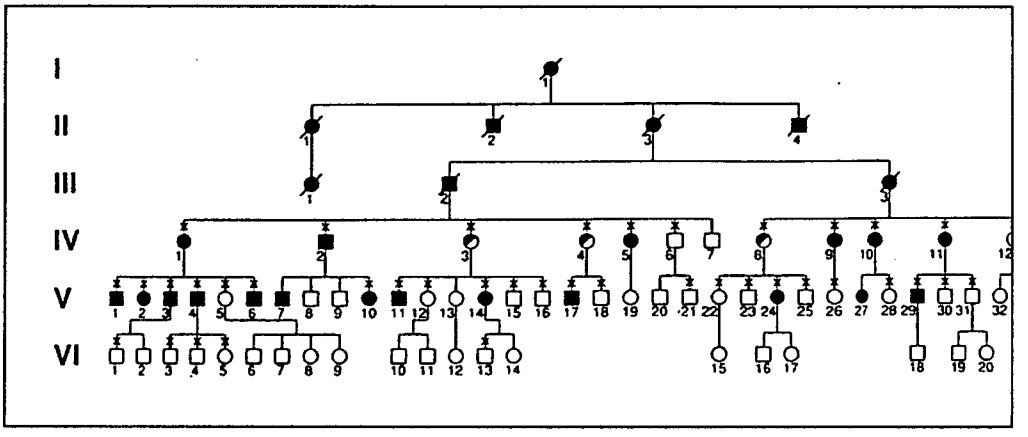


Figure 7. A pedigree of autosomal dominant juvenile glaucoma. Individuals affected by glaucoma are indicated with filled symbols. Half-filled symbols represent ocular hypertension. X = individuals examined by the original authors (Johnson et al, 1993).

The phenotype in this pedigree of juvenile glaucoma resembled that of POAG in that the irido-corneal angle was open and the trabecular meshwork was normal in appearance. It differed from POAG in its early age of onset, autosomal dominant inheritance pattern. This led to the idea that this disease could serve as a model for adult-onset disease and linkage analysis was performed.

Linkage was found on the long arm of chromosome 1 (1q21- 1q31) by Sheffield and colleagues (Sheffield, Stone, Alward et al 1993). After this linkage was reported, it was confirmed in populations in the United States (Richards, Lichter, Boehnke et al 1994; Wiggs, Haines, Paglinauan et al 1994) and around the world (Graff, Urbak, Jerndal et al 1995; Lichter, Richards, Boehnke et al 1997; Morissette, Cote, Anctil et al 1997). The majority of juvenile onset POAG families link to chromosome 1q. A large family with both juvenile-onset and adult-onset POAG was also found to link to chromosome 1q (Morissette, Cote et al 1997).

The locus on chromosome 1 was assigned the name GLC1A. 'GLC' stands for glaucoma, '1' stands for POAG and 'A' stands for the first linkage of the disease. Even though the locus had been identified, within the interval described there were still hundreds of genes and millions of base pairs, making the identification of the gene very difficult. Sunden and colleagues were able to narrow the interval to 3 centimorgans in 1996 (Sunden, Alward, Nichols et al 1996).

At the same time as the family studies were being conducted, another group was working on gene expression of trabecular meshwork cells and their response to dexamethasone (Polansky, Fauss, Chen et al 1997). This was prompted by the fact that POAG can be induced in humans by exposure to corticosteroids. They determined the changes in gene expression of the trabecular meshwork cells exposed to dexamethasone compared to controls. They discovered a protein that was markedly increased when the cells were

exposed to corticosteroids and named the protein trabecular meshwork inducible glucocorticoid receptor protein (TIGR).

The gene producing the TIGR protein was regarded as an attractive candidate gene for glaucoma because its expression in the trabecular meshwork and its response to corticosteroids. Stone et al discovered that the TIGR gene was in the interval containing GLC1A, further increasing the interest in this gene (Stone, Fingert, Alward et al 1997; Alward 2000). The gene was screened for mutations and was found in eight families with juvenile-onset POAG. Mutations have now been found in a large number of patients in populations around the world.

Kubota et al (Kubota, Noda, Wang et al 1997) independently isolated the same protein using a subtraction strategy to isolate genes expressed in the retina and named it "myocilin" (gene symbol MYOC). They showed MYOC expression in the retina to be localised to the connecting cilium of photoreceptor cells.

The HUGO Database Nomenclature Committee has adopted the term "myocilin" for this protein. The physiologic role of MYOC and the mechanisms by which mutations lead to glaucoma have yet to be elucidated. There is some evidence that mutations cause trabecular dysfunction (Lutjen-Drecoll, May, Polansky et al 1998; Nguyen, Chen, Huang et al 1998; Polansky and Nguyen 1998; Wilkinson, van der Straaten, Craig et al 2003) and reduced aqueous outflow

Reports have confirmed that mutations in GLC1A are responsible for 3-5% of adult POAG (Meyer, Bechetoille, Valtot et al 1996; Morissette, Cote et al 1997; Alward, Fingert et al 1998; Fingert, Heon, Liebmann et al 1999). The overall frequency of disease-causing mutations is similar across five populations representing three racial groups (Fingert, Heon et al 1999).

In 2002, Rezaie et al identified a gene responsible for autosomal dominant normal tension glaucoma (NTG) and designated it OPTN (for "optineurin") (Rezaie, Child et al 2002). They had previously mapped an adult-onset POAG locus (GLC1E) to chromosome 10p14-p15. It is thought that optineurin may play a role in neuroprotection in the optic nerve and if defective, visual loss and optic neuropathy typically seen in glaucoma result.
Their initial data suggested that mutations in optineurin could be responsible for 16.7% of hereditary forms of normal-tension glaucoma. However, familial normal tension glaucoma is rare and a later study found that this mutation seems to be responsible for less than 0.1% of all open angle glaucoma (Alward, Kwon, Kawase et al 2003).

Identification of OPTN as an adult-onset glaucoma gene provides an opportunity to study the biochemical pathways that may be involved in the pathogenesis of this group of optic neuropathies and will facilitate a shift of attention from the trabecular meshwork to examining factors affecting retinal and optic nerve head susceptibility to glaucomatous damage.

A number of groups have collected pedigrees with multiple affected individuals with adult-onset POAG in an attempt to isolate genes that could be relevant to this form of glaucoma. Significant linkage has been established in at least 4 chromosomal regions in addition to 1q and 10p in families with POAG (Stoilova, Child, Trifan et al 1996; Wirtz, Samples, Kramer et al 1997; Trifan, Traboulsi, Stoilova et al 1998; Wirtz, Samples, Rust et al 1999). These are outlined in the Table 2.

Table 2. Glaucoma genes and linkages identified (Craig 1999)

Glaucoma type	Locus	Location	Gene
Primary open-angle glaucoma			
JOAG & adult-onset POAG	GLC1A	1q24.3-q25.2	MYOC/TIGR
POAG (adult onset)	GLC1B	2cen-q13	NYI
POAG (adult onset)	GLC1C	3q21-q24	NYI
POAG (intermediate onset)	GLC1D	8q23	NYI
POAG (adult onset LTG)	GLC1E	10p15-p14	OPTN
POAG	GLC1F	7p35-36	NYI
Primary congenital glaucoma			
	GLC3A	2p21	CYP1B1
	GLC3B	lp36	NYI
Developmental glaucoma			
Rieger syndrome	RIEG1		
AD iris hypoplasia		4q25	PITX2
Iridogoniodysgenesis (IGD) syndrome	IRID2		
Axenfeld-Rieger anomaly			
Iris hypoplasia	RID1	6p25	FKHL7/
Familial glaucoma IGD			FREAC3
Familial glaucoma with GD			
Rieger syndrome	RIEG2	13q14	NYI
Other types			
Nail-patella syndrome	NPS1	9q34	LMX1B
Pseudoexfoliation syndrome		2p16	NYI
Pigment dispersion syndrome (PDS)	GPDS1	7q35-36	NYI
PDS	GPDS2	18q11-21	NYI

NYI= not yet identified

At present, there is no satisfactory method available for screening the population for glaucoma (Wormald and Rauf 1995; Tuck and Crick 1997). Identifying glaucoma genes may improve our ability to detect individuals at risk of developing the disease and commence treatment at an earlier stage of the disease. Understanding the mechanisms of the disease may lead to improved treatment modalities.

Presymptomatic diagnosis of at-risk individuals in pedigrees with GLC1A mutations is already feasible and has been performed with a high degree of patient acceptance in at least one large pedigree (Healey, Craig, Wilkinson et al 2004). Since treatment slows the progression of the disease in many cases, it is beneficial to diagnose patients as early as possible before irreversible damage has occurred. The early stages of the disease are asymptomatic and only half of those with the disease are diagnosed. Genetic testing allows targeting of individuals in a family known to be at risk, facilitating earlier treatment. Family members without the mutation would require less frequent screening, allowing better allocation of finite health resources.

The family studied in this paper was a large pedigree of POAG which has been studied as part of the Glaucoma Inheritance Study in Tasmania since 1994. The family has the MYOC mutation, THR377MET. The 72 participants were offered the results of their DNA testing after a genetic counselling session. The attitudes of affected and unaffected family members to the use of predictive gene testing were determined by the use of a questionnaire. Every participant wished to know the result of the test after the

counselling session and 93% were happy that they had requested the result. 96% stated they would ask for the DNA result if given the initial opportunity again. This study suggests that predictive glaucoma testing in appropriate circumstances is acceptable to patients and their families.

Predictive DNA testing for glaucoma opens the possibility of community management of glaucoma by cascade screening. This would involve: 1) identifying patients with glaucoma; 2) testing affected individuals who wished to be involved for MYOC mutations after counselling (and other glaucoma gene mutations as they are identified); 3) establishing family trees of individuals with suitable mutations and inviting relatives to be DNA tested; 4) identifying new mutation carriers and if individuals are negative for the mutation, providing information and arranging routine population screening; 5) entering mutation positive individuals into a standard clinical screening regimen for high-risk individuals; 6) treating individuals who develop early signs of glaucoma.

Further research is required to determine the best regimen for clinical screening of high-risk individuals. The majority of POAG patients do not yet have an identified genetic cause and therefore predictive gene testing is not an option. With the identification of further genes, the risk and benefit for predictive DNA testing and early treatment will require further study (Mackey and Craig 2003).

When considering the cost-effectiveness of DNA testing in a population it is important to know what proportion of the disease is familial and therefore likely to be predicted by genetic testing.

VII. BACKGROUND OF THE GLAUCOMA INHERITANCE STUDY IN TASMANIA

Population isolates are important tools in the identification of genes for diseases. Captive populations with a high standard of health care (ensuring a higher proportion of affected cases being diagnosed) and comprehensive genealogy records (allowing pedigrees of affected families to be identified) are the most suitable for genetic research. All of these characteristics apply to Tasmania, Australia's island state.

Tasmania has a population of approximately 460,000 (Australian Bureau of Statistics 2001). European settlement began in 1803 and comprehensive genealogical records have been kept since this time, providing one of the best sets of such records in the world. Family link programs are available on the internet. Australia has many active genealogical societies with 1 in 30 Australians having traced their family tree back to the original settlers. Family reunions are popular (Figure 8).

Figure 8. Photographs showing a large family from the GIST with pictures of the original matriarch and patriarch

The Tasmanian population is ethnically more homogeneous than the rest of Australia. 91% of Tasmanians were born in Australia, the majority of these descended from Anglo-Celtic stock, whereas only 78% of mainland Australians were born here. Of the remaining Tasmanians not born in Australia, 5% are from the United Kingdom and 1% from the Netherlands (Australian Bureau of Statistics 2001).

The Glaucoma Inheritance Study in Tasmania (GIST) was designed to utilise these advantages.

The aims of the GIST are to:

- Identify glaucoma genes by linkage and association studies
- Establish the frequency, phenotype and origins of genes
- Investigate the natural history of glaucoma
- Evaluate clinical investigations in glaucoma diagnosis
- Evaluate presymptomatic genetic testing
- Create a population, family and genetic database for investigation of new diagnostic and treatment modalities (Mackey 2002-2003)

METHODS

Written informed consent was obtained from patients involved in the Glaucoma Inheritance Study in Tasmanian (GIST) (Appendix A), which was approved by the relevant ethics committees of the following institutions: The Royal Victorian Eye and Ear Hospital (Melbourne), The University of Tasmania (Hobart), and The Royal Hobart Hospital (Hobart). This study was conducted in accordance with the declaration of Helsinki and subsequent revisions.

The identification of glaucoma cases was approached in two phases to maximise the number of patients detected. In the first phase, families with glaucoma were sought through the distribution of information leaflets placed in pharmacies and ophthalmology and optometry practices. The project was publicised through local newspapers, radio and television.

Patients with glaucoma and their families were invited to participate in the GIST project. The presence of a family history was noted and in addition, genealogical information requesting the names of parents and grandparents (or even more relatives, if possible) was used in conjunction with local genealogical resources, such as Tasmanian Family Link (http://www.pioneers.tased.edu.au). Pedigrees were constructed by the professional research genealogist enlisted as part of the GIST team, using computerised family tree databases. The second phase was to identify all cases of glaucoma being seen by ophthalmologists in Tasmania who had not been identified during the first phase of the project.

The Glaucoma Inheritance Study began examining families with glaucoma in 1995. Initially, the larger families were easily identified and were the focus of the project. It soon became clear, however, that it was going to be more difficult to identify smaller families, especially as many people affected with glaucoma are undiagnosed and knowledge of a family history is unreliable.

In early 1996, a 2-day conference of all GIST team members, including me, was held and other interested contributors were invited, including Prof Paul Mitchell, the principal investigator of the Blue Mountains Eye Study. During discussion of the above problem, he suggested that we establish a Glaucoma Registry for Tasmania. In 1996 I was a full-time research fellow for the study and became responsible for the registry's establishment and development.

The approach to the creation of the registry was carefully considered. Establishing a prospective registry of volunteer patients would have been expensive and time-consuming and would not have served the purpose for which it was intended in the time frame available. At first we considered identifying glaucoma patients through reimbursement data from prescriptions filled for anti-glaucoma medications and from medicare item numbers for glaucoma procedures. I pointed out that whilst this would be an effective way of identifying numbers of patients likely to be affected by glaucoma, we would be unable to determine whether these patients had POAG or another form of glaucoma. Many glaucoma patients are one more than one medication so numbers of prescriptions dispensed would have been misleading. Stable glaucoma patients who had undergone surgery in the past

and were not on medication would not be captured by this approach. In addition, confidentiality issues would have prevented us from identifying patients and being able to contact them to invite them to participate in the study.

In 1996, there were only 12 ophthalmologists working in the state, all of whom were well informed about the GIST and were collaborating with the GIST team. All patients diagnosed with glaucoma in the state would be under the care of one of the ophthalmologists; I thus concluded that the most inclusive way of identifying all glaucoma patients was to access them via each eye clinic. This also overcame difficulties with confidentiality and consent, as the treating ophthalmologist contacted the patients directly, inviting them to participate in the study.

I performed an audit of all clinical notes held in each ophthalmologist's practice (well over 60,000 case histories) and cross-referenced the notes with all the visual field tests performed on Humphrey visual field analysers in each eye clinic(over 10,000 field tests).

Each history was opened and read for characteristic comments, measurements, tests and treatments for glaucoma. If any were identified the history was read in detail. If there was evidence of glaucoma or suggestion of glaucoma the key patient data was transcribed to a standard proforma. Over approximately 1000 hours in 1996 and early 1997, I personally performed this data collection with minimal assistance.

All new glaucoma patients thus identified were contacted by their treating ophthalmologist and invited to participate in the study. The research genealogist, Maree Ring, conducted genealogy. I then reviewed pedigree data.

With the assistance of volunteer ophthalmologists, research fellows, orthoptists, nurses and medical students, large numbers of affected and unaffected members of extended families were examined during study field trips which took place in each major centre in Tasmania over weekends.

There were five masked examiners, each of whom assessed one parameter of glaucoma by following the standard clinical examination protocol for each patient (Appendix B); one member of the research team took a history, obtained consent (Appendix A), refracted and measured visual acuities; another examined visual fields; another measured IOP and performed gonioscopy; and two independent ophthalmologists scored the optic discs. Finally, fundus photographs were taken and DNA samples collected (venous blood).

In addition, several hundred elderly nursing home residents were examined by the GIST team, to collect a series of normal control individuals for use in the study. I participated in almost every field trip in 1995 and 1996 and also performed home visits with Associate Professor Mackey on patients unable to attend the clinics, representing 50 days (400 hours) of patient examinations.

The nursing home controls were needed primarily as a disease depleted control group set for genetic analysis. Although we considered using these as a control group for incidence of a family history of glaucoma, my analysis showed that they were not age matched to the total GIST population. In addition, many had early dementia and were unable to provide reliable details about a family history of glaucoma.

It is difficult to obtain large series of individuals without glaucoma, where a family history of glaucoma has been questioned and cross-referenced. The closest comparison available to act as a control group for this study is the cohort from the Twins Eye Study in Tasmania, which consists of unaffected, age-matched twins. This data was collected to cross-reference twins with glaucoma pedigrees. The participants in this study have undergone a comprehensive ophthalmic assessment, including a family history of eye disease and a complete eye examination.

Associate Professor David Mackey gave me access to the data from this study. I have also assisted in the clinical examination of some of the participants in the Twins Eye Studies. I reviewed the family history information sheets and compiled the data used in the control comparison. Information regarding a family history of glaucoma was extracted from the existing database. As it was not possible to examine the family members of the controls to confirm the diagnosis of POAG, we were forced to rely on the

family history as reported by the study subjects at the time of their assessment.

CLINICAL ASSESSMENT

Participants attended various eye clinics throughout Tasmania or were visited at their homes if they were unable to attend a clinic. A detailed questionnaire and a standard interview were administered (Appendix B), covering knowledge of family history, demographic data, medications (including drug names and frequency of use), and medical history of systemic disorders such as hypertension, diabetes, migraine, corticosteroid use and systemic vascular disease. Problems with vision, past eye disease or eye treatment, and ocular symptoms were also included. Patients were asked to bring all their medications or their physicians' medical summaries to the interview to improve the accuracy of reporting.

A detailed eye examination was performed and included the following:

- Subjective refraction and best corrected visual acuity using a Snellen chart.
- Visual field testing. This was performed with a standard Humphrey automated perimeter (Humphrey, Inc, San Leandro, CA) using a 24-2 array, a size III target, and full threshold test system. Both eyes were tested consecutively with a short break between each eye and using the appropriate near correction for 1/3 metre. The testing was

monitored by trained staff present in the room. Results were reviewed for reliability using fixation losses, false-positive errors, false-negative errors and short-term fluctuations, and defects were detected using pattern deviation analysis as the field needs to be adjusted for any shift in mean sensitivity (eg. from cataract) (Coote, McCartney, Wilkinson et al 1996).

- Intraocular pressure measurement using the standard calibrated Goldmann applanation tonometer (Haag Streit AG, Bern, Switzerland) with a drop of fluorescein 2.0% tear film enhancement and topical local anaesthetic (Chauvin Pharmaceuticals Ltd, Essex, UK). The IOP was not standardised for time of day. In some patients such as those who were bed-bound or in geographically isolated locations, it was not possible to perform Goldmann tonometry. In such cases, IOP readings from portable devices such as Perkins (Clement Clarke, Harlow, Essex, UK) or Tonopen (Mentor, Norwell, MA, USA) were accepted as satisfactory alternatives.
- Anterior segment examination. Any anterior chamber, iris or lens abnormalities were noted.
- Gonioscopy
- Optic disc analysis. Pupils were dilated with tropicamide 1% and phenylephrine 10% (Chauvin Pharmaceuticals Ltd, Essex, UK) and assessed using slit-lamp biomicroscopy under magnifying binocular stereo vision using a 78 or 90 dioptre non-contact lens or a fundus contact lens. The following features were noted by two independent

clinicians and were ranked according to the GIST scoring system (Coote, McCartney et al 1996):

a) Size of scleral canal (horizontal and vertical)

b) Presence and amount of peripapillary changes to retinal pigment epithelium and choroidal vasculature

c) Consistency and depth of retinal nerve fibre layer up to one disc distance from the disc edge

d) Vascular branching pattern

e) Presence of 'Drance' type nerve fibre layer haemorrhages

f) Neuroretinal rim width, consistency and colour

g) Focal defects in the rim or pits not contiguous with the central cup

h) The vertical and horizontal cup-disc ratio as judged on contour (noting the phenomenon of 'overpass cupping'), 'bayonetting' of emerging nerve head vasculature, widening of the interstices of the lamina cribrosa, and posterior bowing of the lamina.

- Stereoscopic optic disc photography using a Nidek 3-Dx/F fundus camera (Nidek Co. Ltd, Japan) and Kodachrome ISO 64 film processed by Kodak (Eastman Kodak Co, Rochester, NY). Each participant had bilateral 30 degree colour retinal stereophotographs taken centred on the optic disc and macula. 35 mm slide transparencies were mounted in clear plastic sheets, allowing close apposition of stereo pairs.
- Optic discs were measured from stereoscopic photographs using a Pentax stereo viewer II (Asahi Optical Co. Ltd, Japan). All optic discs or high-quality stereophotographs of the discs were scored independently by at least two glaucoma specialists based upon the GIST score protocol

(Coote, McCartney et al 1996). If there was disagreement, a consensus between the ophthalmologists was reached.

- Venesection. Venous blood was obtained for DNA extraction.

Patients with any signs other forms of glaucoma, trauma, inflammation, pseudoexfoliation, pigment dispersion, angle dysgenesis or other significant anterior segment pathology, or of occluded or potentially occludable angles on gonioscopy were excluded from the study. Other exclusion criteria were the presence of a field defect caused by a condition other than POAG eg. macular degeneration or vascular/thrombotic events, and optic disc pathology eg. optic disc drusen.

DEFINITION AND CLASSIFICATION OF GLAUCOMA AND THE GIST SCORING SYSTEM (Coote, McCartney et al 1996).

As discussed in the introductory chapter, the diagnosis of glaucoma can be difficult and the classification for research purposes can be contentious. The diagnosis generally takes into account the level of IOP, the optic disc appearance and the visual field. Previous genetic linkage studies on juvenile open angle glaucoma pedigrees have relied upon an analysis of definitely affected individual using the 'single best diagnosis' convention. Studies of adult-onset POAG have been complicated by limited numbers of unequivocally affected members identified even in very large pedigrees due to the later onset of the disease. Many members of the pedigree may have equivocal clinical features or be too young to show signs of the disease.

A scoring system was thus developed for this study, both to define the criteria to be used to diagnose glaucoma and to adjust for age.

The GIST score was developed to facilitate the study of families with glaucoma. It is a numeric value between 0 and 1, where 0 is clinical certainty of absence of the disease and 1 is the definitive diagnosis of POAG.

The score is sequentially developed. The first part is the clinical examination and assigning a value to these findings which contribute to the raw score. This is followed by the translation of the "raw score" into the pedigree probability or the GIST score which includes a component of probability of unaffected status.

Clinical features that are consistent with glaucoma are scored based on a point system, with a maximal possible raw score of 5. One point is available from the IOP and one from the visual field; two points are available from the appearance of the optic disc. In any of these categories, one additional point is available for a feature highly consistent with and typical of the pedigree pattern. Only one additional point may be awarded per individual, giving a maximal raw score of 5. Those members of the pedigree who demonstrate no clinical evidence of glaucoma have a raw score of 0.

The scoring system assumes autosomal dominant transmission. Thus, first-degree relatives of affected individuals are assumed to have a 50% risk of inheriting the trait. To convert to the final GIST score each point of the raw

score increases the GIST score by 0.1. Members of the pedigree are given a starting probability of 0.5 in the GIST score, which reflects this risk. Therefore, each point of the raw score raises the GIST score from 0.5 to a maximum score of 1. The score is developed for the individual, not for each eye separately. The eye with the highest raw score is used in the development of the GIST score. When the raw score is 0, the GIST score is decreased by units of 0.1 depending on the age of the individual and the age of onset of disease in that pedigree. The minimum GIST score is 0.

Development of the raw score

- Intraocular pressure: Elevated IOP score $\geq$ 22 mmHg 1 point. Grossly elevated IOP (>4 standard deviations above the mean ie. 28 mmHg or higher) may score the additional point.

- Optic disc analysis: Optic disc changes suggestive of glaucoma score 1 point and changes highly suggestive score 2 points. Changes considered as highly consistent with the pedigree may score the additional point (total 3).

- Visual field: Reliability indices are taken into account. The field scores 0 if it is normal or if there is a defect not considered significant for glaucoma. There is a score of 1 for a significant field defect consistent with glaucoma or if markedly degraded and consistent with

glaucoma. An additional point can be allocated if the defect is especially consistent with the pedigree or with the disc appearance.

Conversion to the GIST score

To convert to a GIST score, each point of the raw score increases the GIST score by 0.1 to a maximum score of 1. If the raw score is zero, then the GIST score is decreased by units of 0.1 depending on the age of the individual and the age of onset of disease in that pedigree.

Definition of POAG

For the purposes of this study, individuals with a GIST score of 0.5 or lower were regarded as normal or unaffected, whilst those with a score of 0.7 or higher were regarded as POAG cases.

Those with a GIST score of 0.6 were regarded as glaucoma suspects.

To a limited extent, the GIST score correlates positively with the severity level of glaucoma in a given individual.

DATA MANAGEMENT & STATISTICAL ANALYSIS

All data were entered into a password-verified Microsoft Access database. Microsoft Excel was used for tabulations and graphics. Statistical analyses were performed using the SPSS statistical package version 10 (SPSS Inc., Chicago, USA). If there was at least one other affected family member confirmed on clinical examination, the individual was classified as having familial glaucoma. If there was no known family history of glaucoma, the individual was classified as having sporadic glaucoma. The data were stratified by GIST scores in the familial and sporadic glaucoma groups, and by closest degrees of relatives with POAG in the familial glaucoma group.

"Degree" of relationship to known glaucoma sufferers was identified on a four level categorisation.

First-degree relatives are father, mother, son, daughter, and siblings. Second-degree relatives are grandparents, grandson, granddaughter, aunt, uncle, nephew and niece. Third-degree relatives are first cousins, great-grandparents, great aunt/uncle, great grandson or great granddaughter. Fourth-degree relatives are more distant relatives, including second cousins' children and great great grandparents.

RESULTS

The Glaucoma Inheritance Study in Tasmania is an ongoing study whose database is continually updated. The results reported in this thesis are derived from the database as it existed in December 1999.

Invitations were sent to 3800 patients and family members who had been investigated or treated for glaucoma as identified via the clinical notes of all ophthalmic practices in Tasmania over the preceding 15 years. A team of researchers examined the patients in the Glaucoma Inheritance Study, and their relatives. Most participants were seen on average for 2 occasions. A total of 2444 patients were examined. If a patient did not meet the criteria for the diagnosis of glaucoma then they were not included in the study. This left 1702 glaucoma cases.

With the assistance of a professional research genealogist using computerised family tree databases available in Tasmania, 309 pedigrees were constructed. A total of 2444 subjects were examined, from which 1702 POAG patients were identified (GIST score of ≥ 0.7).

1014 patients belonged to families in which other members were affected (familial glaucoma).
688 patients did not have any family members known to be affected (sporadic glaucoma) (Figure 9).

486 individuals were assigned a GIST score of 0.6 and were classified as glaucoma suspects.

From these results it can be concluded that in Tasmania 59.6% of POAG is familial.

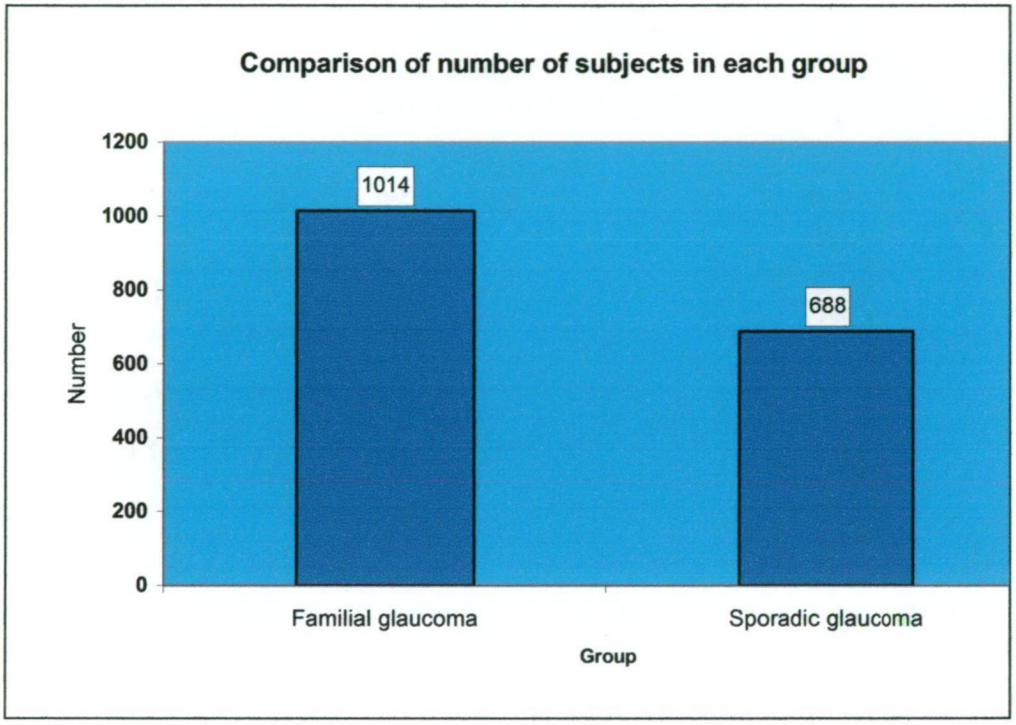


Figure 9. Bar graph showing distribution of cases between the two groups

In the control group taken from the Twins Eye Study in Tasmania, of 155 pairs of twins studied, 38 (24%) had a family history of glaucoma (1^{st} – 4^{th} degree relative affected).

9% had a first-degree relative, 13% a second-degree relative and 2% a third-degree relative affected.

PEDIGREES

The largest pedigrees identified by the study are included on the following pages.

KEY:

□ Male

○ Female

■ ● Affected with glaucoma

⊠ ∅ Deceased individuals

? Possibly affected

Partially filled boxes are glaucoma suspects

The number of glaucoma affected individuals within pedigrees varied from 2 to 29. There were 3 pedigrees containing 20-29 affected members, 3 pedigrees containing 10-19 affected members, and 301 pedigrees with fewer than 10 affected members. Only those family members who underwent clinical assessment were included, so the size of the pedigrees may have been larger, had every family member been examined.

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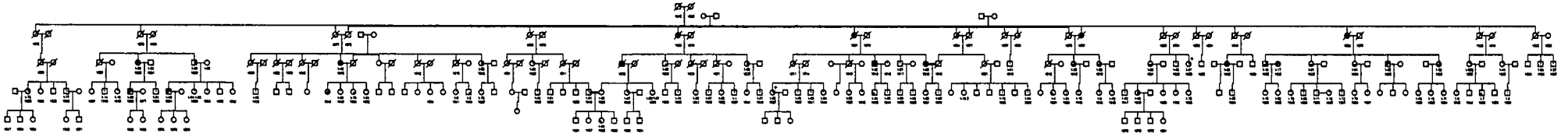

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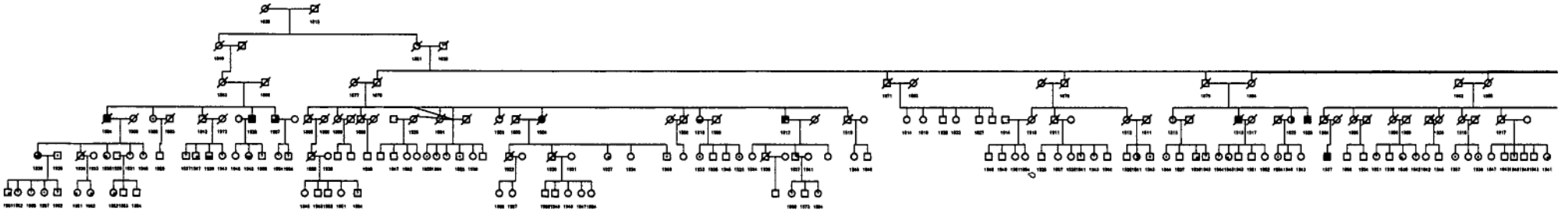

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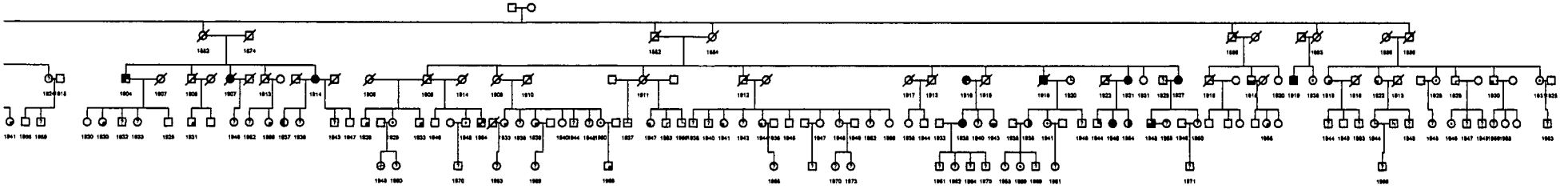

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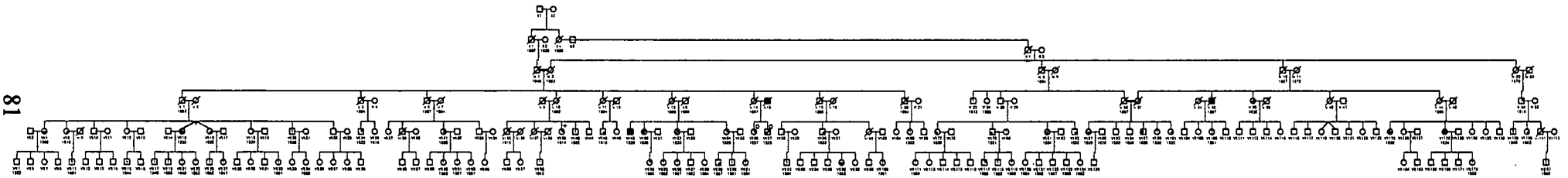

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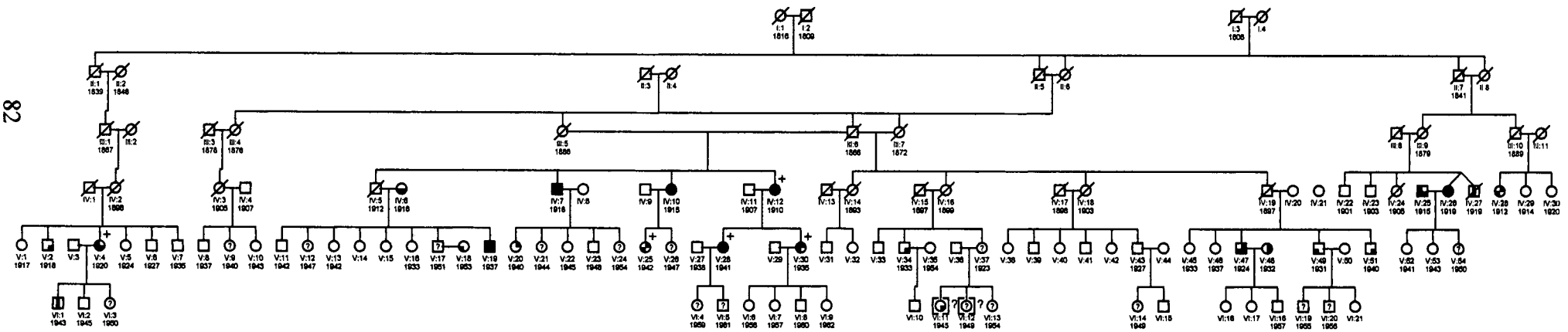

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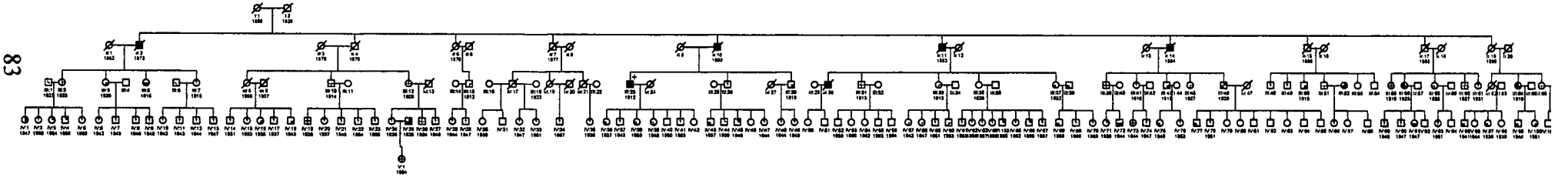

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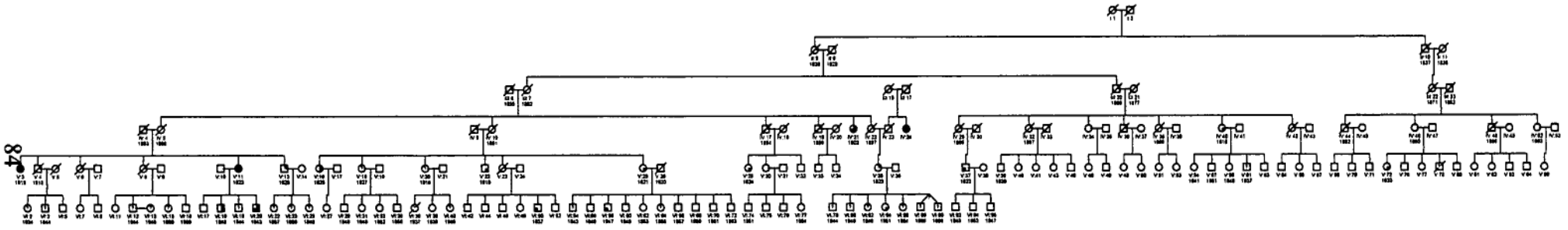

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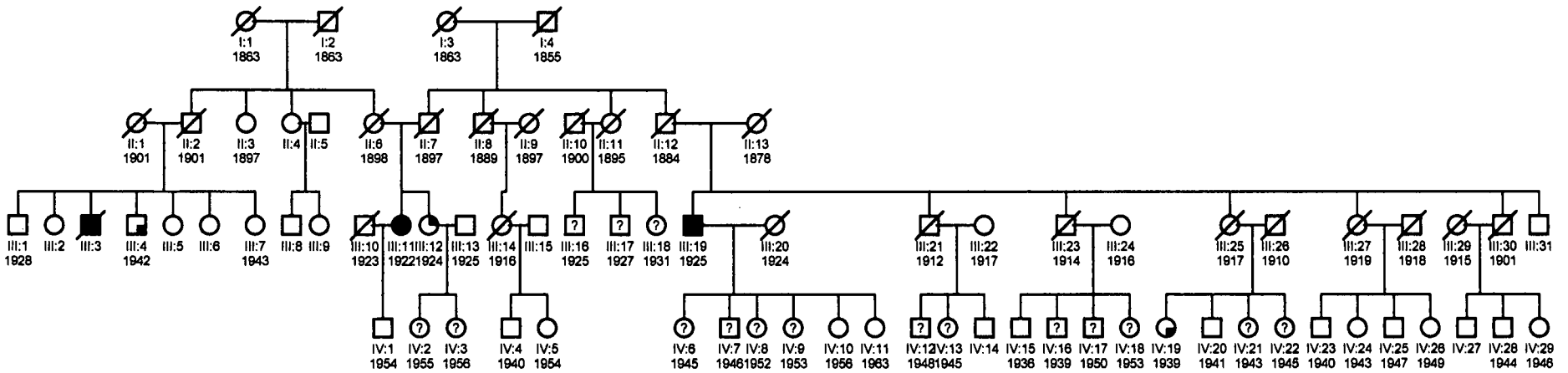
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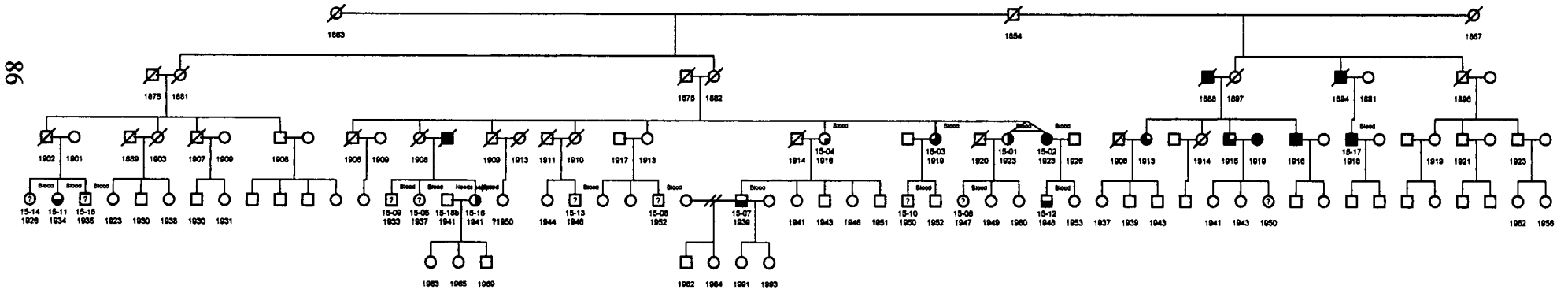
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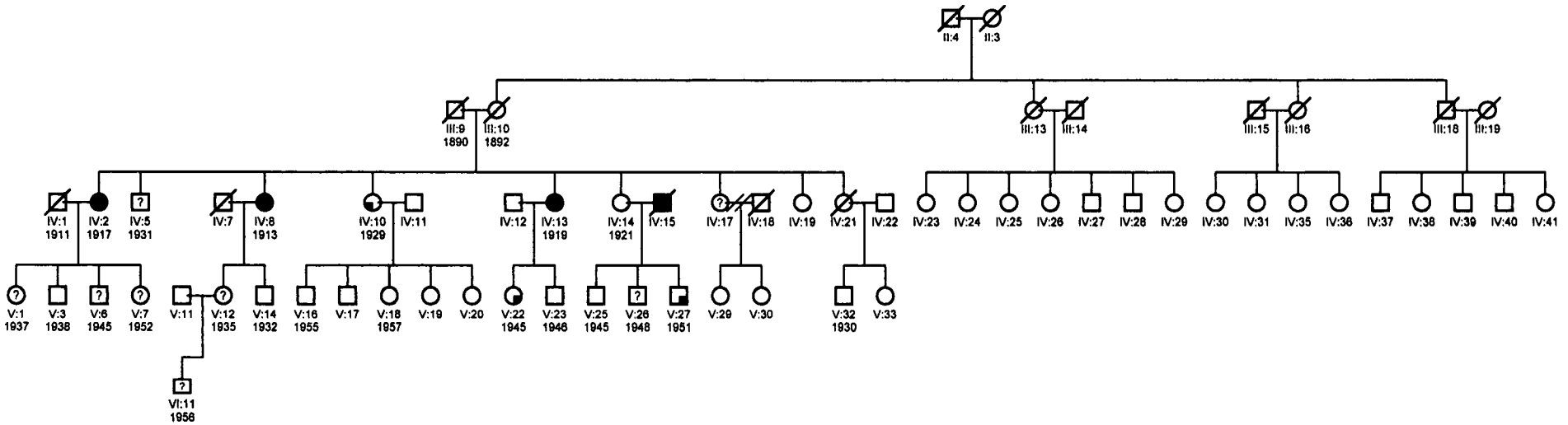
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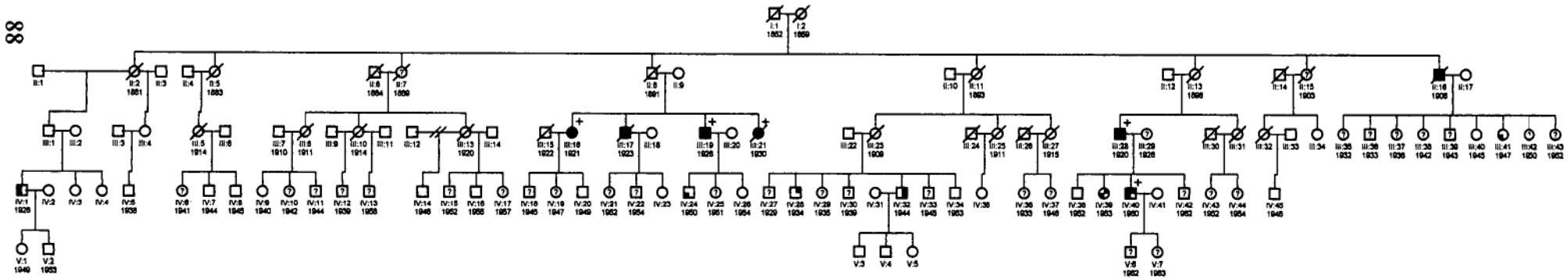
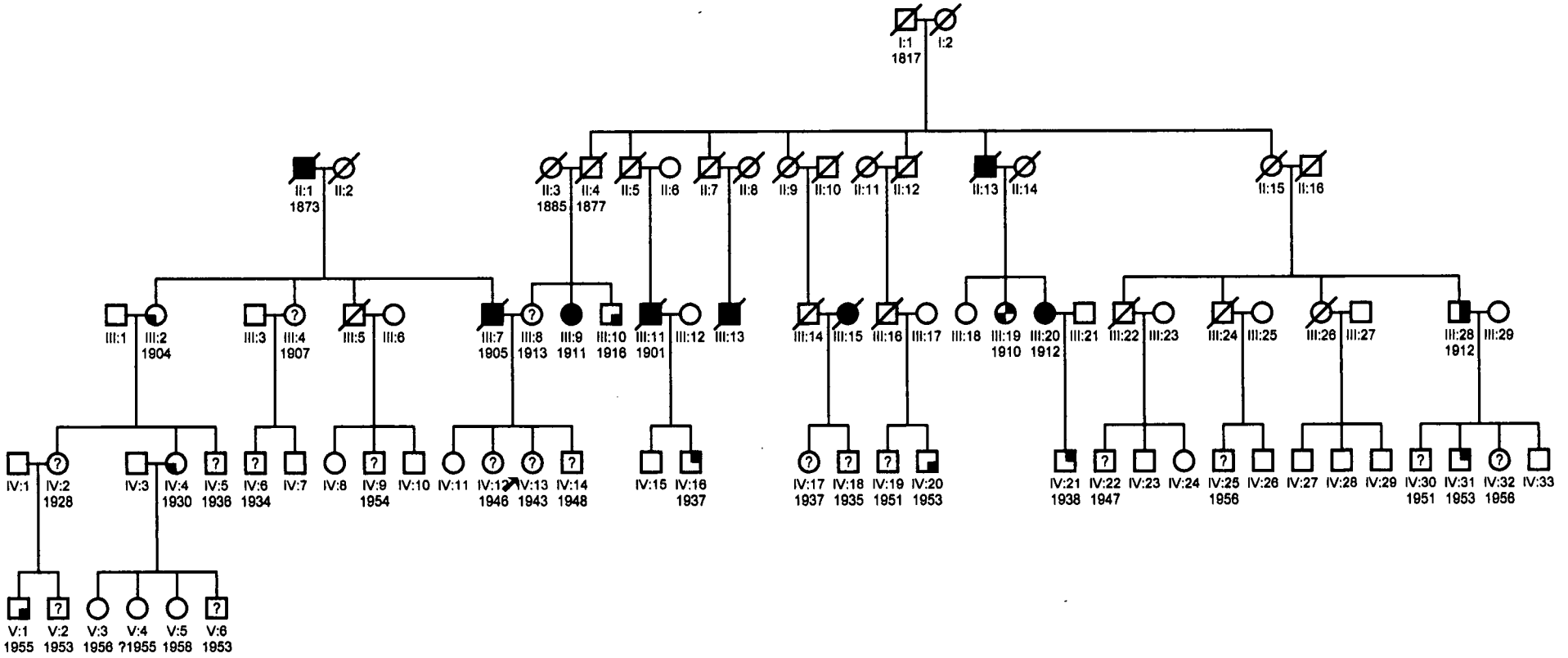
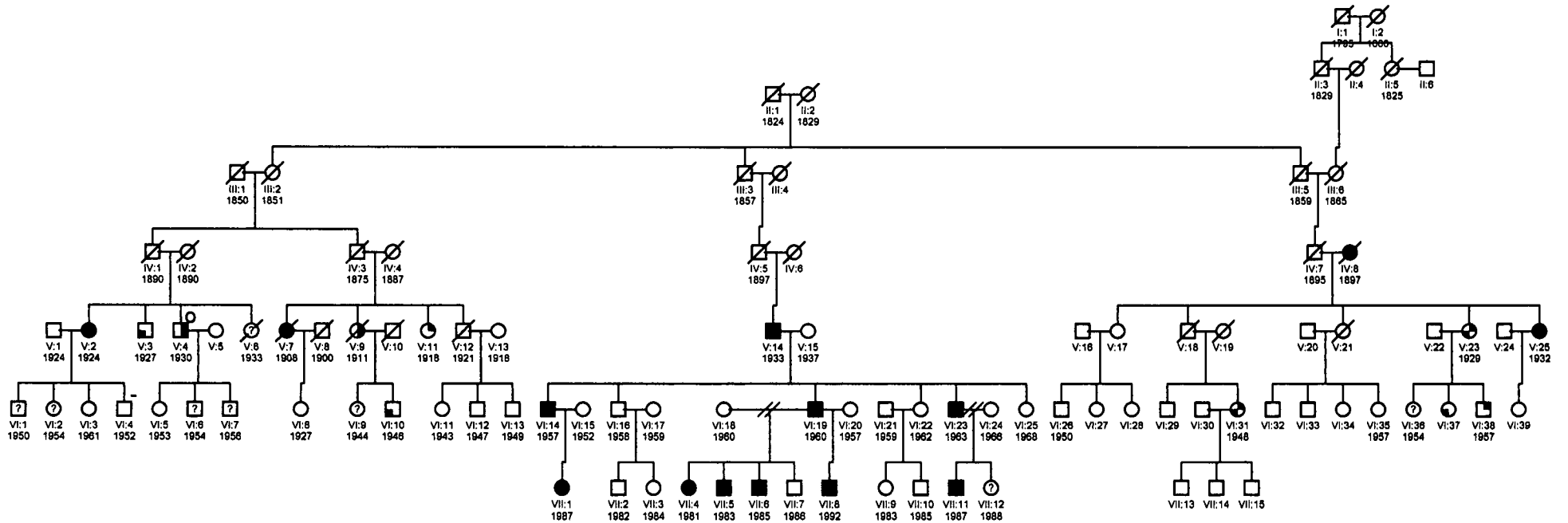


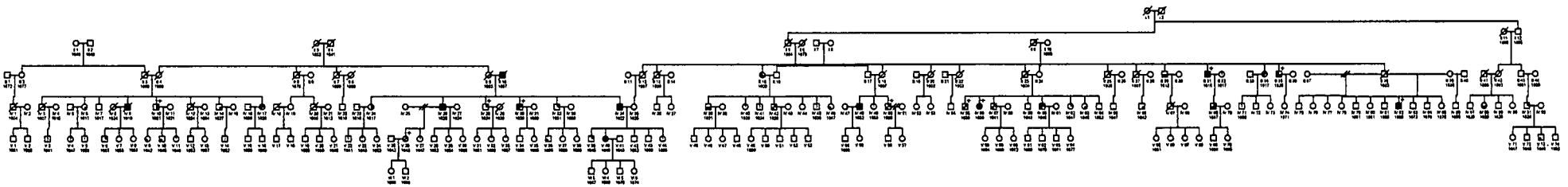
GTas26


I:1 1817
I:2
II:1 1873
II:2
II:3 1885
II:4 1877
II:5
II:6
II:7
II:8
II:9
II:10
II:11
II:12
II:13
II:14
II:15
II:16
III:1
III:2 1904
III:3
III:4 1907
III:5
III:6
III:7 1905
III:8 1913
III:9 1911
III:10 1916
III:11 1901
III:12
III:13
III:14
III:15
III:16
III:17
III:18
III:19 1910
III:20 1912
III:21
III:22
III:23
III:24
III:25
III:26
III:27
III:28 1912
III:29
IV:1
IV:2 1928
IV:3
IV:4 1930
IV:5 1936
IV:6 1934
IV:7
IV:8
IV:9 1954
IV:10
IV:11
IV:12 1946
IV:13 1943
IV:14 1948
IV:15
IV:16 1937
IV:17 1937
IV:18 1935
IV:19 1951
IV:20 1953
IV:21 1938
IV:22 1947
IV:23
IV:24
IV:25 1956
IV:26
IV:27
IV:28
IV:29
IV:30 1951
IV:31 1953
IV:32 1956
IV:33
V:1 1955
V:2 1953
V:3 1956
V:4 ?1955
V:5 1958
V:6 1953

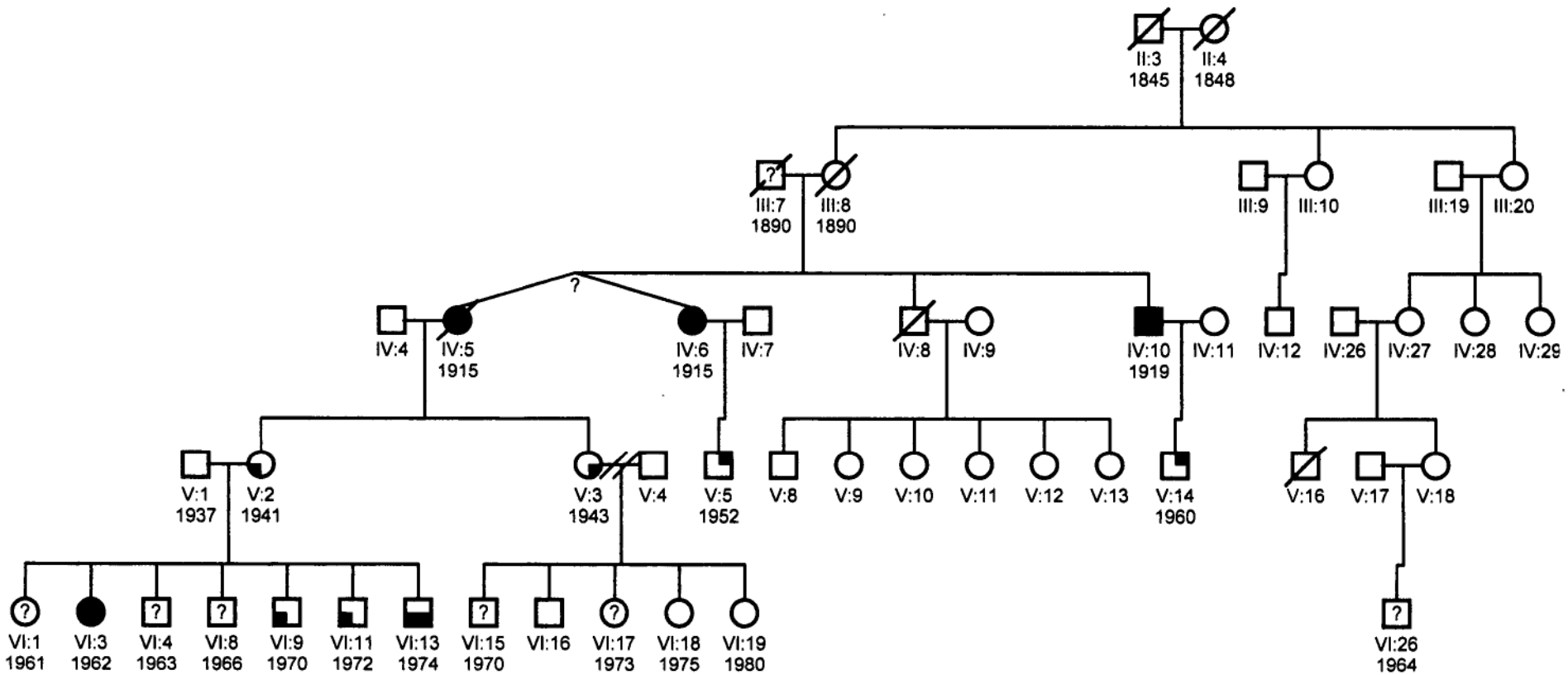
GTas35


I:1
I:2
II:1 1824
II:2 1829
II:3 1829
II:4
II:5 1825
II:6
III:1 1850
III:2 1851
III:3 1857
III:4
III:5 1859
III:6 1865
IV:1 1890
IV:2 1890
IV:3 1875
IV:4 1887
IV:5 1897
IV:6
IV:7 1895
IV:8 1897
V:1 1924
V:2 1924
V:3 1927
V:4 1930
V:5
V:6 1933
V:7 1908
V:8 1900
V:9 1911
V:10
V:11 1918
V:12 1921
V:13 1918
V:14 1933
V:15 1937
V:16
V:17
V:18
V:19
V:20
V:21
V:22
V:23 1929
V:24
V:25 1932
VI:1 1950
VI:2 1954
VI:3 1961
VI:4 1952
VI:5 1953
VI:6 1954
VI:7 1956
VI:8 1927
VI:9 1944
VI:10 1946
VI:11 1943
VI:12 1947
VI:13 1949
VI:14 1957
VI:15 1952
VI:16 1958
VI:17 1959
VI:18 1960
VI:19 1960
VI:20 1957
VI:21 1959
VI:22 1962
VI:23 1963
VI:24 1966
VI:25 1958
VI:26 1950
VI:27
VI:28
VI:29
VI:30
VI:31 1948
VI:32
VI:33
VI:34
VI:35 1957
VI:36 1954
VI:37
VI:38 1957
VI:39
VII:1 1987
VII:2 1982
VII:3 1984
VII:4 1981
VII:5 1983
VII:6 1985
VII:7 1986
VII:8 1992
VII:9 1983
VII:10 1985
VII:11 1987
VII:12 1988
VII:13
VII:14
VII:15

GTas37



GTas40


II:3
1845
II:4
1848
III:7
1890
III:8
1890
III:9
III:10
III:19
III:20
?
IV:4
IV:5
1915
IV:6
1915
IV:7
IV:8
IV:9
IV:10
1919
IV:11
IV:12
IV:26
IV:27
IV:28
IV:29
V:1
1937
V:2
1941
V:3
1943
V:4
V:5
1952
V:8
V:9
V:10
V:11
V:12
V:13
V:14
1960
V:16
V:17
V:18
VI:1
1961
VI:3
1962
VI:4
1963
VI:8
1966
VI:9
1970
VI:11
1972
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1974
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1970
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1973
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1975
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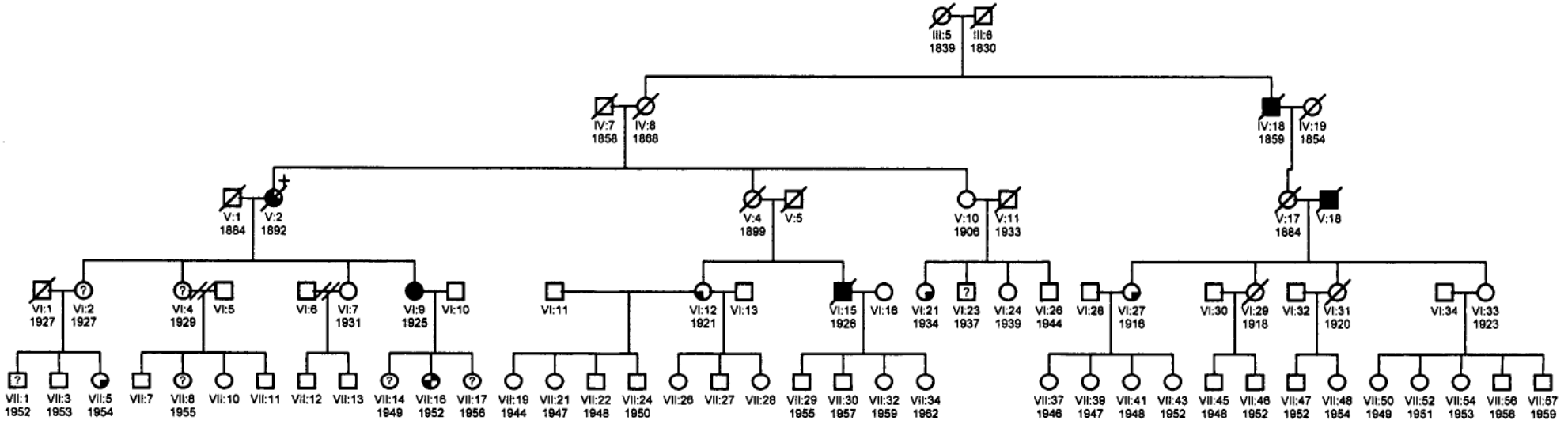
1980
VI:26
1964

GTas46


III:5 1839
III:6 1830
IV:7 1858
IV:8 1868
IV:18 1859
IV:19 1854
V:1 1884
V:2 1892
V:4 1899
V:5
V:10 1906
V:11 1933
V:17 1884
V:18
VI:1 1927
VI:2 1927
VI:4 1929
VI:5
VI:6
VI:7 1931
VI:9 1925
VI:10
VI:11
VI:12 1921
VI:13
VI:15 1926
VI:16
VI:21 1934
VI:23 1937
VI:24 1939
VI:26 1944
VI:28
VI:27 1916
VI:30
VI:29 1918
VI:32
VI:31 1920
VI:34
VI:33 1923
VII:1 1952
VII:3 1953
VII:5 1954
VII:7
VII:8 1955
VII:10
VII:11
VII:12
VII:13
VII:14 1949
VII:16 1952
VII:17 1956
VII:19 1944
VII:21 1947
VII:22 1948
VII:24 1950
VII:26
VII:27
VII:28
VII:29 1955
VII:30 1957
VII:32 1959
VII:34 1962
VII:37 1946
VII:39 1947
VII:41 1948
VII:43 1952
VII:45 1948
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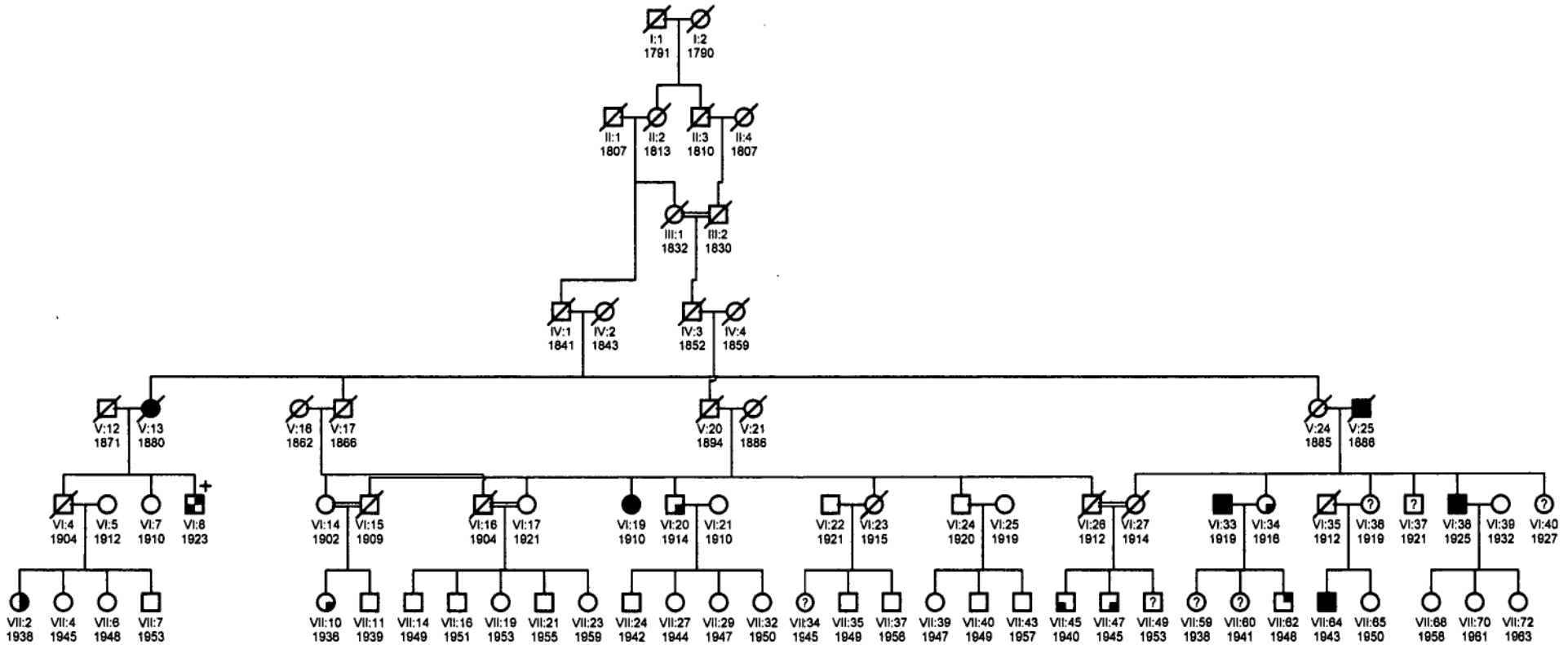
VII:47 1952
VII:48 1954
VII:50 1949
VII:52 1951
VII:54 1953
VII:56 1956
VII:57 1959

GTas51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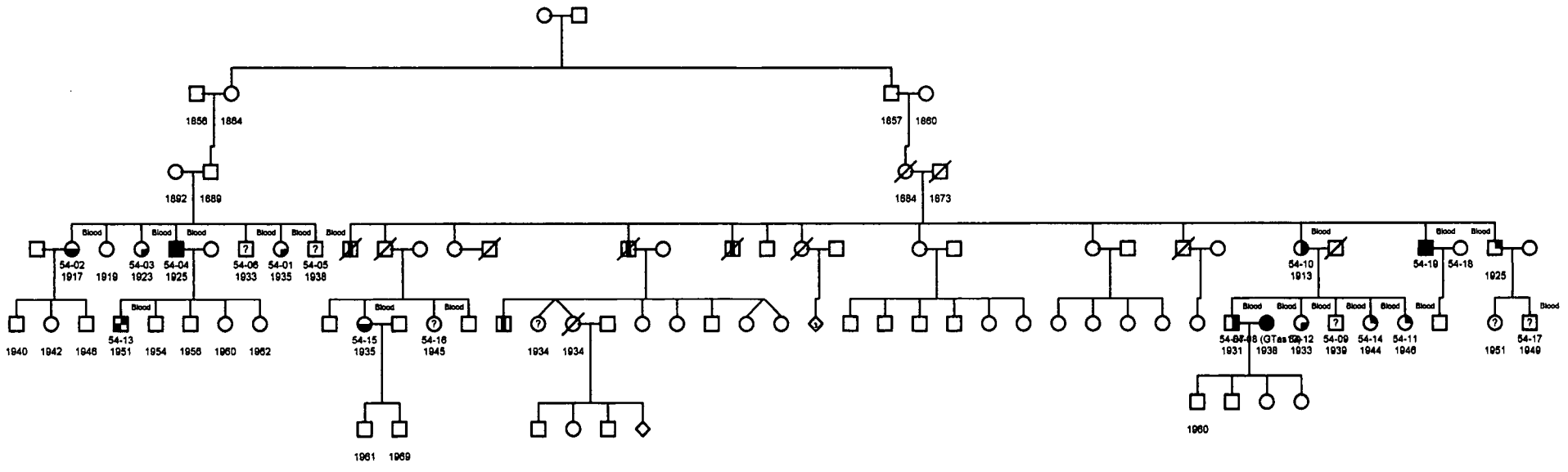
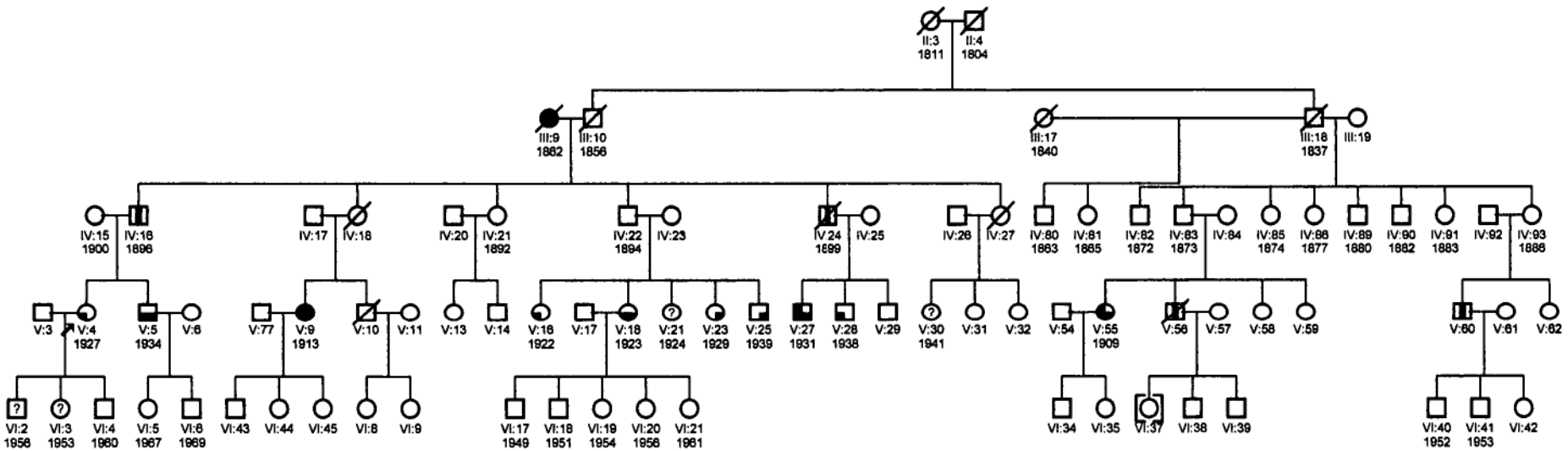
I:2 1790
II:1 1807
II:2 1813
II:3 1810
II:4 1807
III:1 1832
III:2 1830
IV:1 1841
IV:2 1843
IV:3 1852
IV:4 1859
V:12 1871
V:13 1880
V:16 1862
V:17 1866
V:20 1894
V:21 1886
V:24 1885
V:25 1886
VI:4 1904
VI:5 1912
VI:7 1910
VI:8 1923
VI:14 1902
VI:15 1909
VI:16 1904
VI:17 1921
VI:19 1910
VI:20 1914
VI:21 1910
VI:22 1921
VI:23 1915
VI:24 1920
VI:25 1919
VI:26 1912
VI:27 1914
VI:33 1919
VI:34 1918
VI:35 1912
VI:38 1919
VI:37 1921
VI:38 1925
VI:39 1932
VI:40 1927
VII:2 1938
VII:4 1945
VII:6 1948
VII:7 1953
VII:10 1936
VII:11 1939
VII:14 1949
VII:16 1951
VII:19 1953
VII:21 1955
VII:23 1959
VII:24 1942
VII:27 1944
VII:29 1947
VII:32 1950
VII:34 1945
VII:35 1949
VII:37 1956
VII:39 1947
VII:40 1949
VII:43 1957
VII:45 1940
VII:47 1945
VII:49 1953
VII:59 1938
VII:60 1941
VII:62 1948
VII:64 1943
VII:65 1950
VII:68 1958
VII:70 1961
VII:72 1963

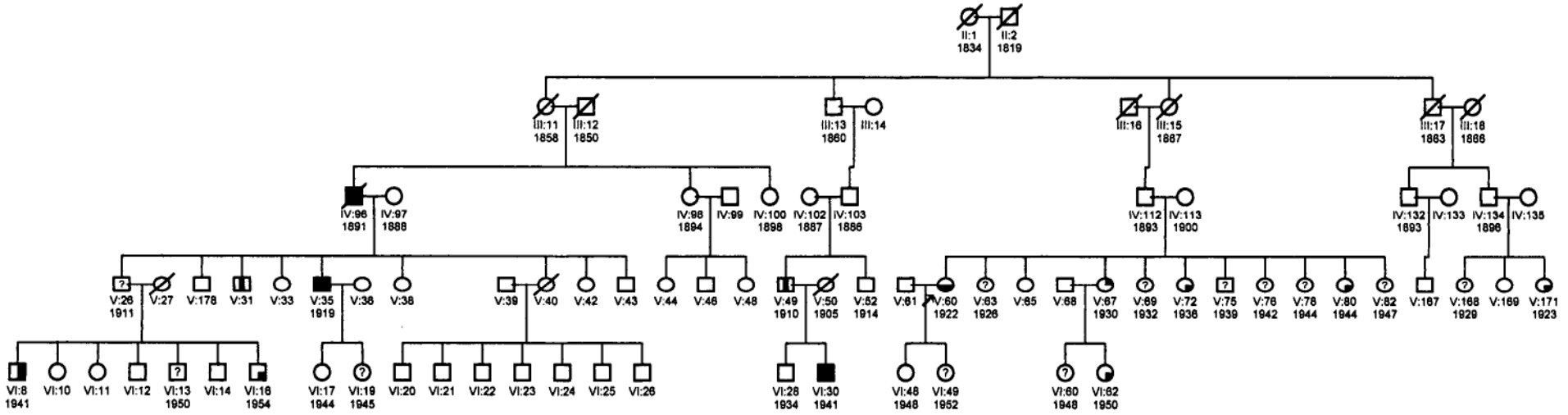
GTas54


1856
1864
1857
1860
1892
1889
1884
1873
54-02
1917
1919
54-03
1923
54-04
1925
54-06
1933
54-01
1935
54-05
1938
54-10
1913
54-19
54-18
1925
1940
1942
1948
54-13
1951
1954
1956
1960
1962
54-15
1935
54-16
1945
1934
1934
1931
1938
1933
54-09
1939
54-14
1944
54-11
1946
1951
54-17
1949
1960
1961
1969

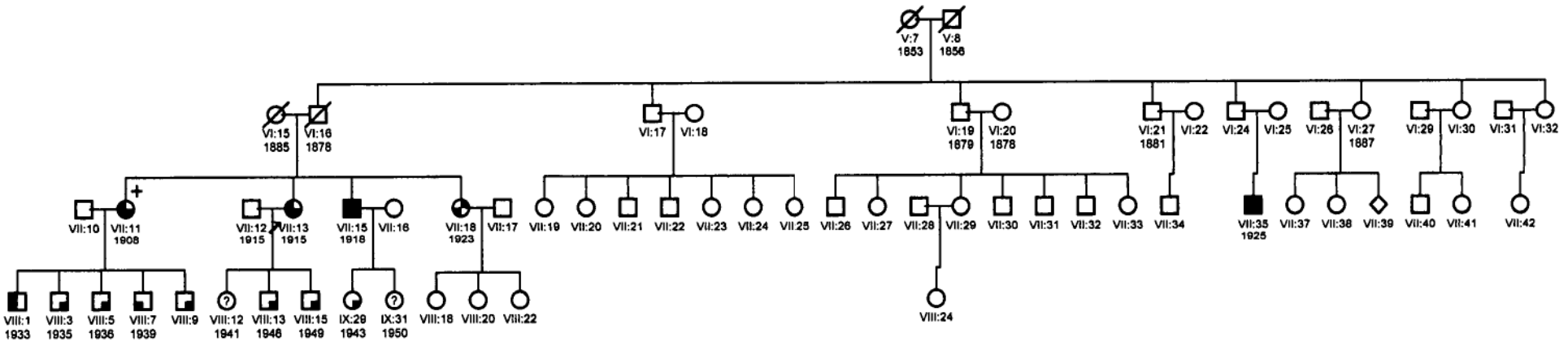
GTas57


II:3 1811
II:4 1804
III:9 1882
III:10 1856
III:17 1840
III:18 1837
III:19
IV:15 1900
IV:16 1896
IV:17
IV:18
IV:20
IV:21 1892
IV:22 1894
IV:23
IV:24 1899
IV:25
IV:26
IV:27
IV:80 1863
IV:81 1865
IV:82 1872
IV:83 1873
IV:84
IV:85 1874
IV:86 1877
IV:89 1880
IV:90 1882
IV:91 1883
IV:92
IV:93 1886
V:3
V:4 1927
V:5 1934
V:6
V:77
V:9 1913
V:10
V:11
V:13
V:14
V:16 1922
V:17
V:18 1923
V:21 1924
V:23 1929
V:25 1939
V:27 1931
V:28 1938
V:29
V:30 1941
V:31
V:32
V:54
V:55 1909
V:56
V:57
V:58
V:59
V:60
V:61
V:62
VI:2 1956
VI:3 1953
VI:4 1960
VI:5 1967
VI:6 1969
VI:43
VI:44
VI:45
VI:8
VI:9
VI:17 1949
VI:18 1951
VI:19 1954
VI:20 1956
VI:21 1961
VI:34
VI:35
VI:37
VI:38
VI:39
VI:40 1952
VI:41 1953
VI:42

GTas70


II:1 1834
II:2 1819
III:11 1858
III:12 1850
III:13 1860
III:14
III:16
III:15 1887
III:17 1883
III:18 1886
IV:96 1891
IV:97 1888
IV:98 1894
IV:99
IV:100 1898
IV:102 1887
IV:103 1886
IV:112 1893
IV:113 1900
IV:132 1893
IV:133
IV:134 1896
IV:135
V:26 1911
V:27
V:178
V:31
V:33
V:35 1919
V:36
V:38
V:39
V:40
V:42
V:43
V:44
V:46
V:48
V:49 1910
V:50 1905
V:52 1914
V:61
V:60 1922
V:63 1926
V:65
V:68
V:67 1930
V:69 1932
V:72 1936
V:75 1939
V:76 1942
V:78 1944
V:80 1944
V:82 1947
V:167
V:168 1929
V:169
V:171 1923
VI:8 1941
VI:10
VI:11
VI:12
VI:13 1950
VI:14
VI:16 1954
VI:17 1944
VI:19 1945
VI:20
VI:21
VI:22
VI:23
VI:24
VI:25
VI:26
VI:28 1934
VI:30 1941
VI:48 1948
VI:49 1952
VI:60 1948
VI:62 1950

GTas73


V:7
1853
V:8
1856
VI:15
1885
VI:16
1878
VI:17
VI:18
VI:19
1879
VI:20
1878
VI:21
1881
VI:22
VI:24
VI:25
VI:26
VI:27
1887
VI:29
VI:30
VI:31
VI:32
VII:10
VII:11
1908
VII:12
1915
VII:13
1915
VII:15
1918
VII:16
VII:18
1923
VII:17
VII:19
VII:20
VII:21
VII:22
VII:23
VII:24
VII:25
VII:26
VII:27
VII:28
VII:29
VII:30
VII:31
VII:32
VII:33
VII:34
VII:35
1925
VII:37
VII:38
VII:39
VII:40
VII:41
VII:42
VIII:1
1933
VIII:3
1935
VIII:5
1936
VIII:7
1939
VIII:9
VIII:12
1941
VIII:13
1946
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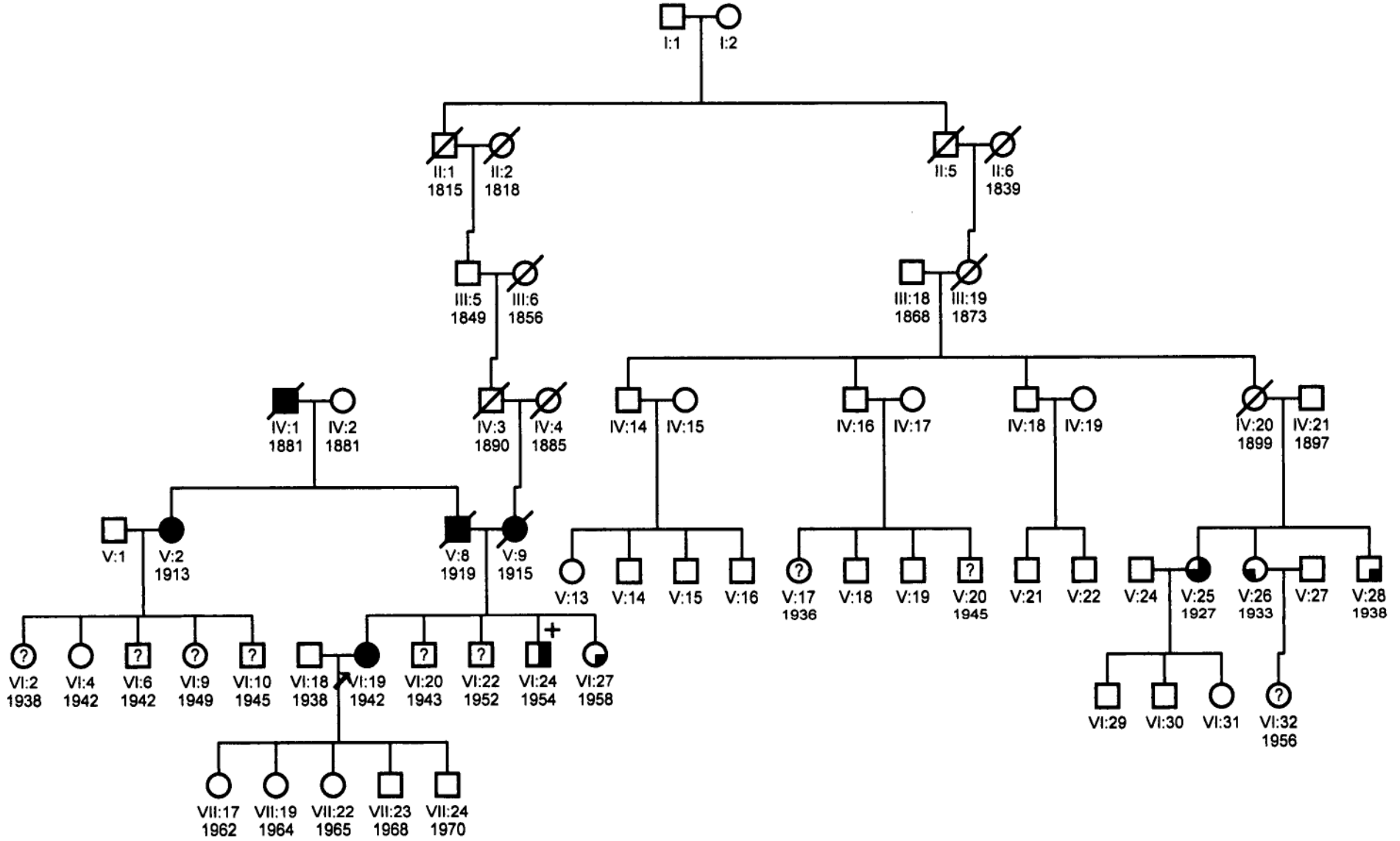
1949
IX:29
1943
IX:31
1950
VIII:18
VIII:20
VIII:22
VIII:24

GTas74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I:2
II:1
1815
II:2
1818
II:5
II:6
1839
III:5
1849
III:6
1856
III:18
1868
III:19
1873
IV:1
1881
IV:2
1881
IV:3
1890
IV:4
1885
IV:14
IV:15
IV:16
IV:17
IV:18
IV:19
IV:20
1899
IV:21
1897
V:1
V:2
1913
V:8
1919
V:9
1915
V:13
V:14
V:15
V:16
V:17
1936
V:18
V:19
V:20
1945
V:21
V:22
V:24
V:25
1927
V:26
1933
V:27
V:28
1938
VI:2
1938
VI:4
1942
VI:6
1942
VI:9
1949
VI:10
1945
VI:18
1938
VI:19
1942
VI:20
1943
VI:22
1952
VI:24
1954
VI:27
1958
VI:29
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1956
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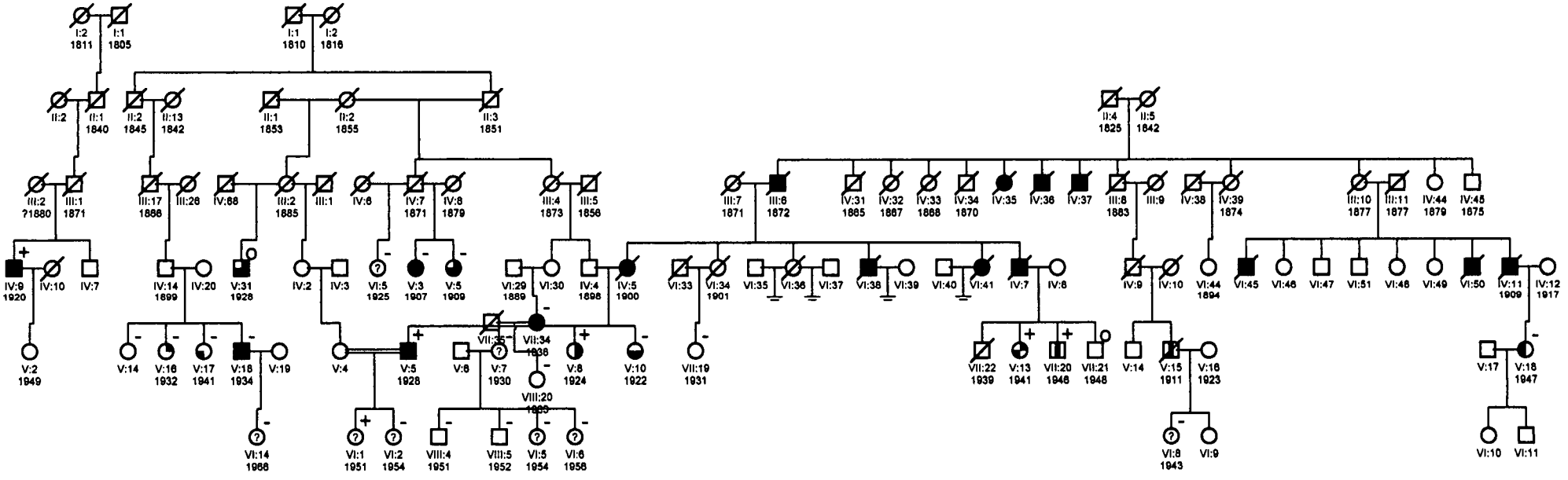
1962
VII:19
1964
VII:22
1965
VII:23
1968
VII:24
1970

GTas81


I:2 1811
I:1 1805
I:1 1810
I:2 1816
II:2
II:1 1840
II:2 1845
II:13 1842
II:1 1853
II:2 1855
II:3 1851
II:4 1825
II:5 1842
III:2 ?1880
III:1 1871
III:17 1888
III:26
IV:68
III:2 1885
III:1
IV:6
IV:7 1871
IV:8 1879
III:4 1873
III:5 1856
III:7 1871
III:6 1872
IV:31 1865
IV:32 1867
IV:33 1868
IV:34 1870
IV:35
IV:36
IV:37
III:8 1883
III:9
IV:38
IV:39 1874
III:10 1877
III:11 1877
IV:44 1879
IV:45 1875
IV:9 1920
IV:10
IV:7
IV:14 1899
IV:20
V:31 1928
IV:2
IV:3
VI:5 1925
V:3 1907
V:5 1909
VI:29 1889
VI:30
IV:4 1898
IV:5 1900
VI:33
VI:34 1901
VI:35
VI:36
VI:37
VI:38
VI:39
VI:40
VI:41
IV:7
IV:8
IV:9
IV:10
VI:44 1894
VI:45
VI:46
VI:47
VI:51
VI:48
VI:49
VI:50
IV:11 1909
IV:12 1917
V:2 1949
V:14
V:16 1932
V:17 1941
V:18 1934
V:19
VI:14 1988
V:4
V:5 1928
V:8
VII:35
V:7 1930
VII:34 1936
V:8 1924
V:10 1922
VIII:20 1993
VI:1 1951
VI:2 1954
VIII:4 1951
VIII:5 1952
VI:5 1954
VI:6 1958
VII:19 1931
VII:22 1939
V:13 1941
VII:20 1946
VII:21 1948
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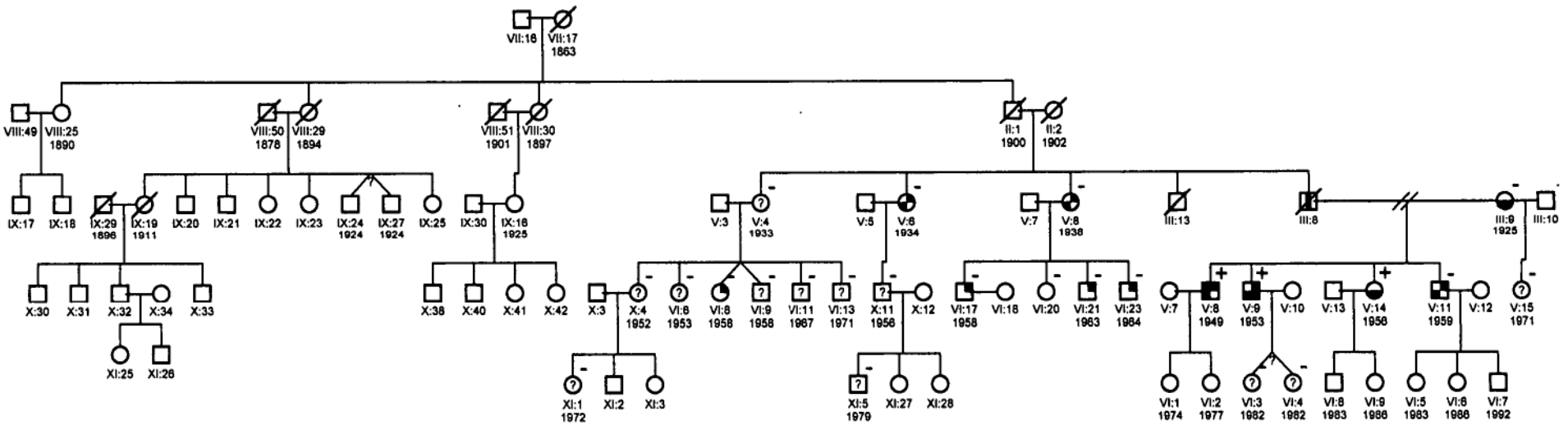
V:15 1911
V:16 1923
VI:8 1943
VI:9
V:17
V:18 1947
VI:10
VI:11

GTas87


VII:18
VII:17
1863
VIII:49
VIII:25
1890
VIII:50
1878
VIII:29
1894
VIII:51
1901
VIII:30
1897
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1900
II:2
1902
IX:17
IX:18
IX:29
1896
IX:19
1911
IX:20
IX:21
IX:22
IX:23
IX:24
1924
IX:27
1924
IX:25
IX:30
IX:16
1925
V:3
V:4
1933
V:5
V:6
1934
V:7
V:8
1938
III:13
III:8
III:9
1925
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X:31
X:32
X:34
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XI:25
XI:26
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X:40
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X:42
X:3
X:4
1952
VI:6
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VI:8
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1972
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1979
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VI:17
1958
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VI:20
VI:21
1963
VI:23
1964
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1949
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1953
V:10
V:13
V:14
1956
V:11
1959
V:12
V:15
1971
VI:1
1974
VI:2
1977
VI:3
1982
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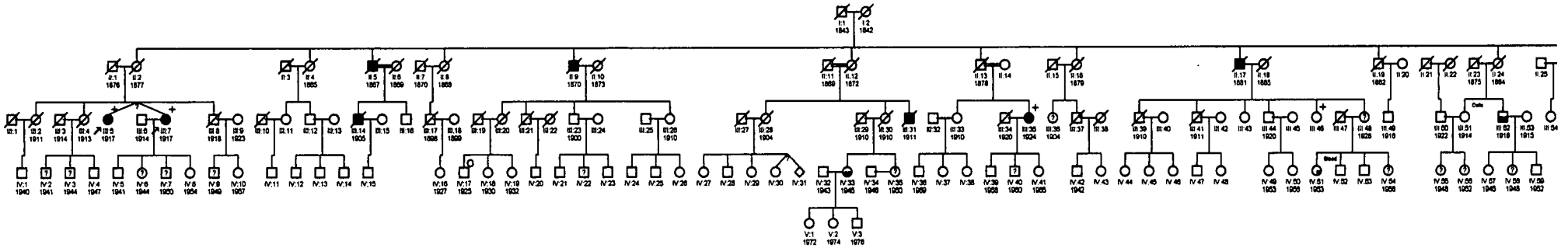
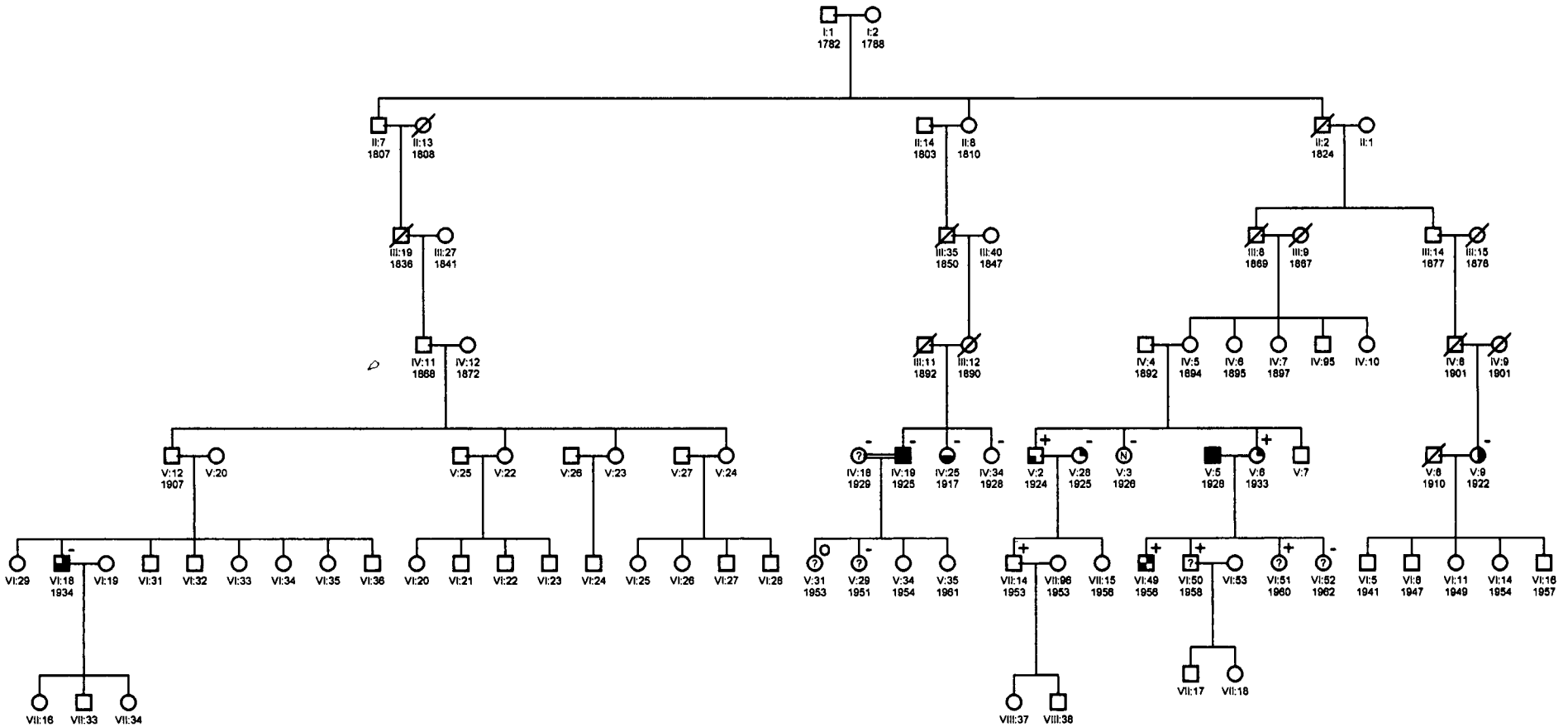
1982
VI:8
1983
VI:9
1986
VI:5
1983
VI:6
1988
VI:7
1992

GTas121



GTas255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II:14 1803
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II:1
III:19 1836
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III:35 1850
III:40 1847
III:8 1869
III:9 1867
III:14 1877
III:15 1876
IV:11 1868
IV:12 1872
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III:12 1890
IV:4 1892
IV:5 1894
IV:6 1895
IV:7 1897
IV:95
IV:10
IV:8 1901
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V:29 1951
V:34 1954
V:35 1961
VII:14 1953
VII:96 1953
VII:15 1958
VI:49 1958
VI:50 1958
VI:53
VI:51 1960
VI:52 1962
VI:5 1941
VI:8 1947
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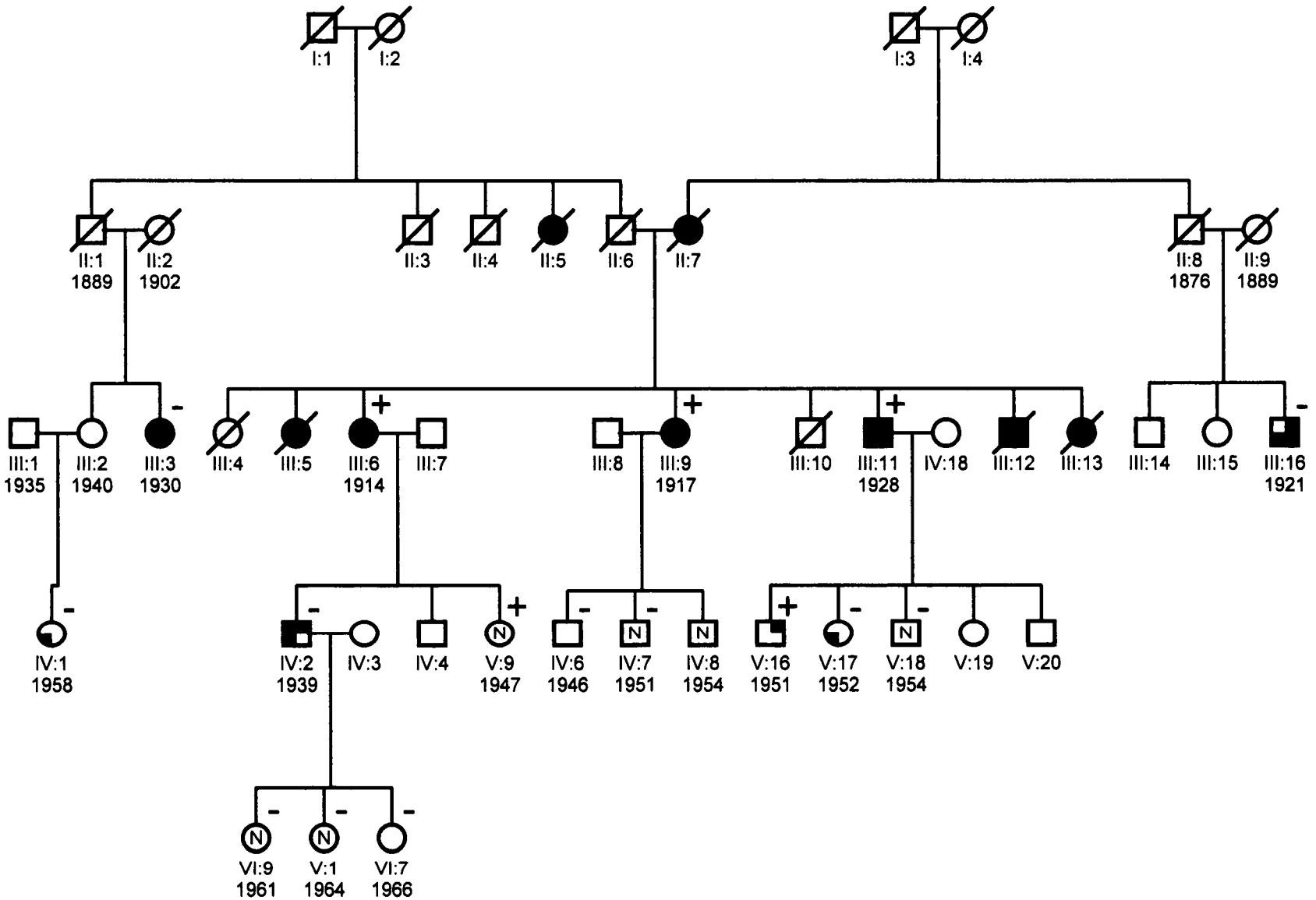
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GENDER

There was no statistically significant difference in gender distribution in the familial group compared with the sporadic group (chi-square value 0.14) (p= 0.7078) (Figure 10).

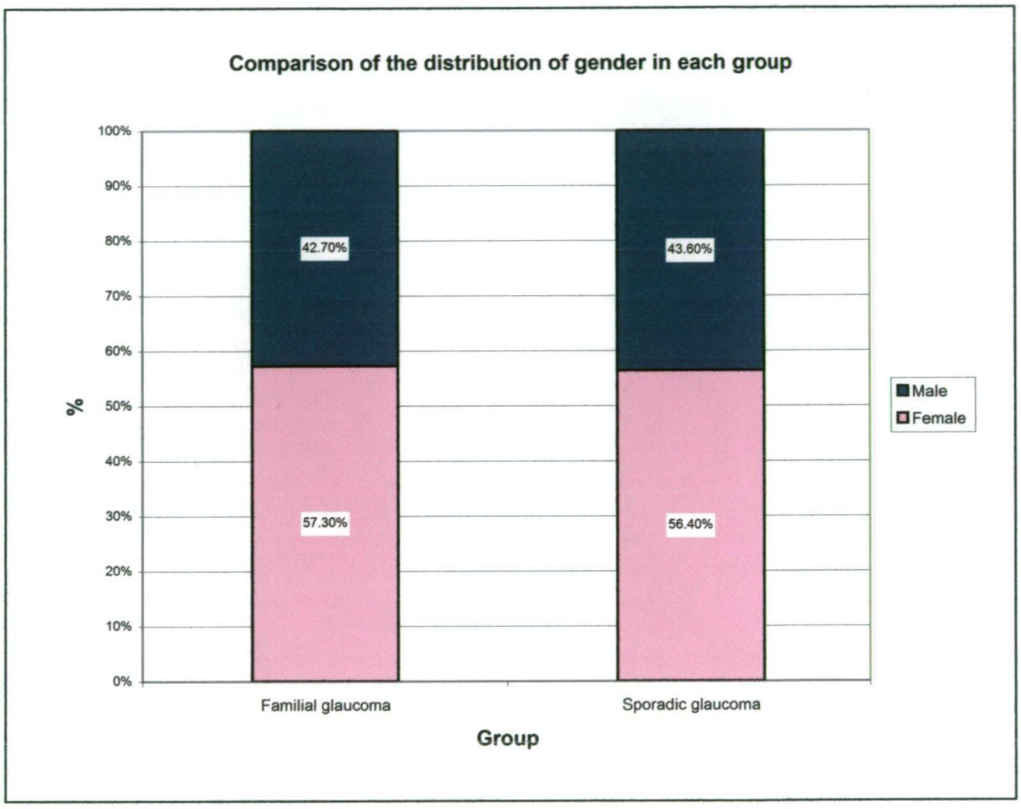

Figure 10. Bar graph showing gender distributions between the two groups

Within the familial group there was no statistically significant difference in the gender distribution when assessed according to the degree of the relationship (Figure 11).

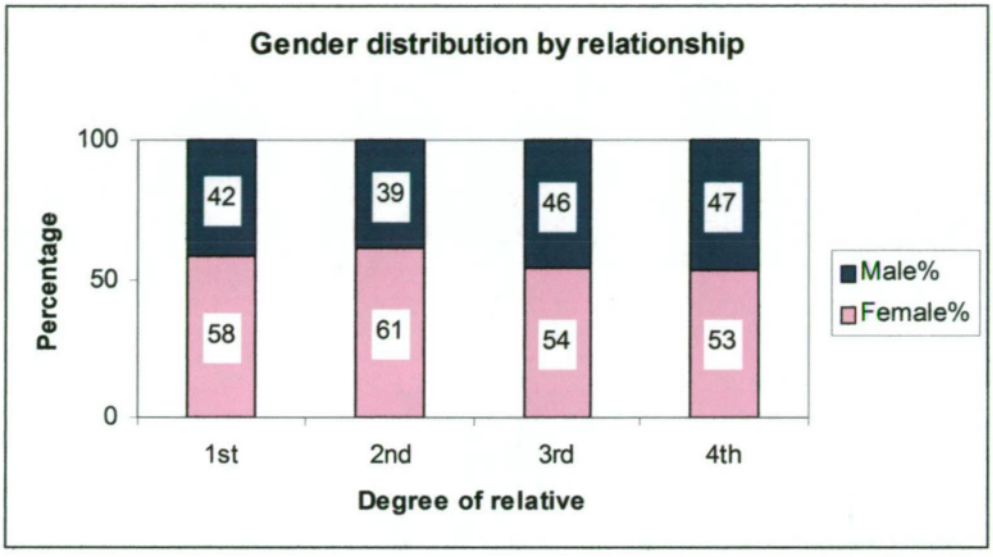

Figure 11. Gender distribution by relationship in familial group

AGE

Compared to the familial group, the sporadic POAG group was statistically significantly older at the age of examination by the study: 70.6 $\pm$ 12.6 years vs 72.6 $\pm$ 10.3 years (p= 0.001) and at the age of diagnosis: 61.4 $\pm$13.0 years vs 64.0 $\pm$ 12.6 years (p<0.001) (Figure 12).

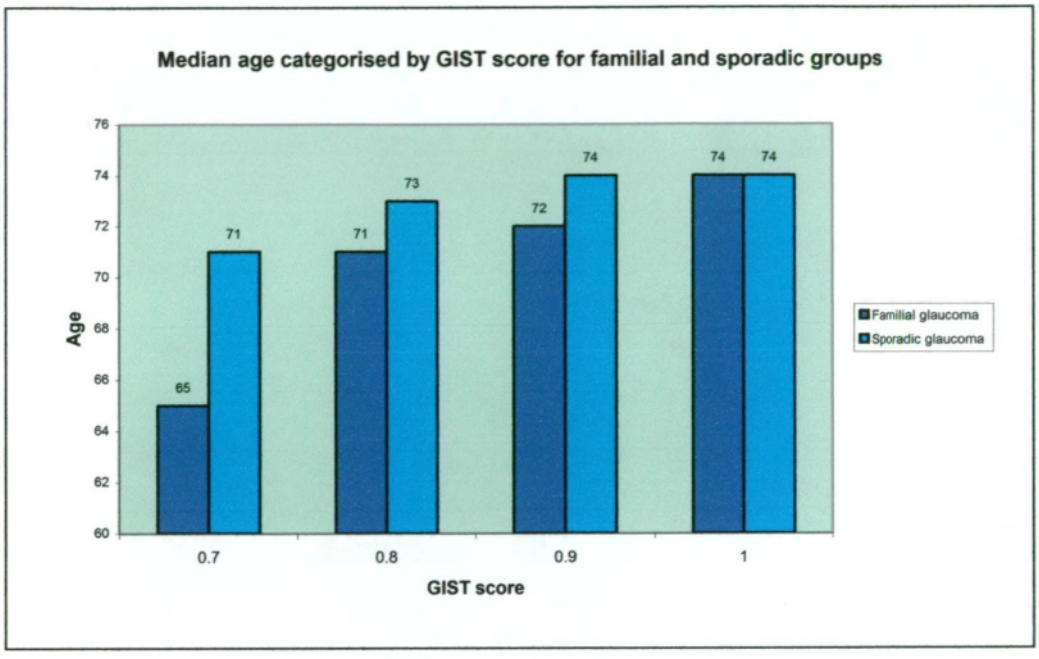

Figure 12. Bar graph showing age distributions between the two groups

RELATIONSHIP

Those with familial glaucoma were stratified according to the degree of the relationship of the affected relatives (Figure 13). The closest known affected relative was:

- a first-degree relative in 658 cases (64.9%)
- a second-degree relative in 110 (10.8%)
- a third-degree relative in 103 (10.2%)
- a fourth-degree relative in 143 (14.1%)

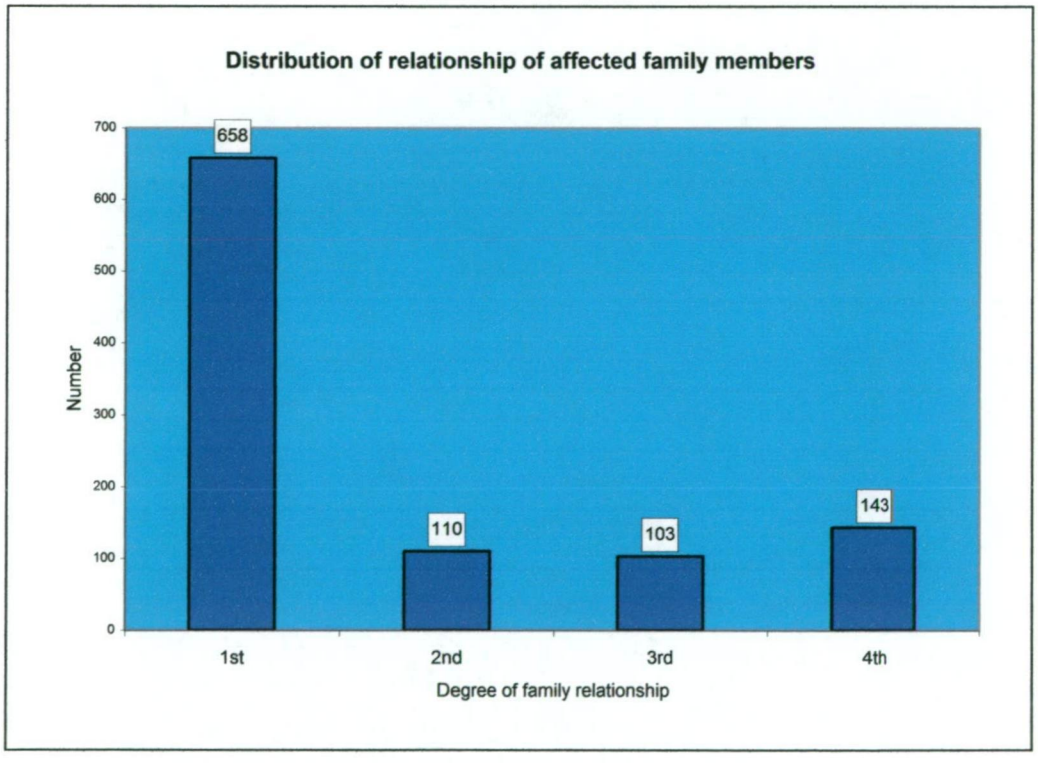

Figure 13. Bar graph showing distribution of relationships amongst familial group

GIST SCORES

A higher GIST score reflects more advanced disease. The familial glaucoma group appeared to have a greater proportion of subjects with a GIST score of 0.9 or 1.0 (38.16%) compared with the sporadic group (24.8%) (Figure 14). The Chi square test revealed a significant difference in the distribution of GIST scores between familial and sporadic cases of glaucoma ($p<0.0002$). This may reflect an earlier onset of and/or increased severity of the glaucoma found in the familial group.

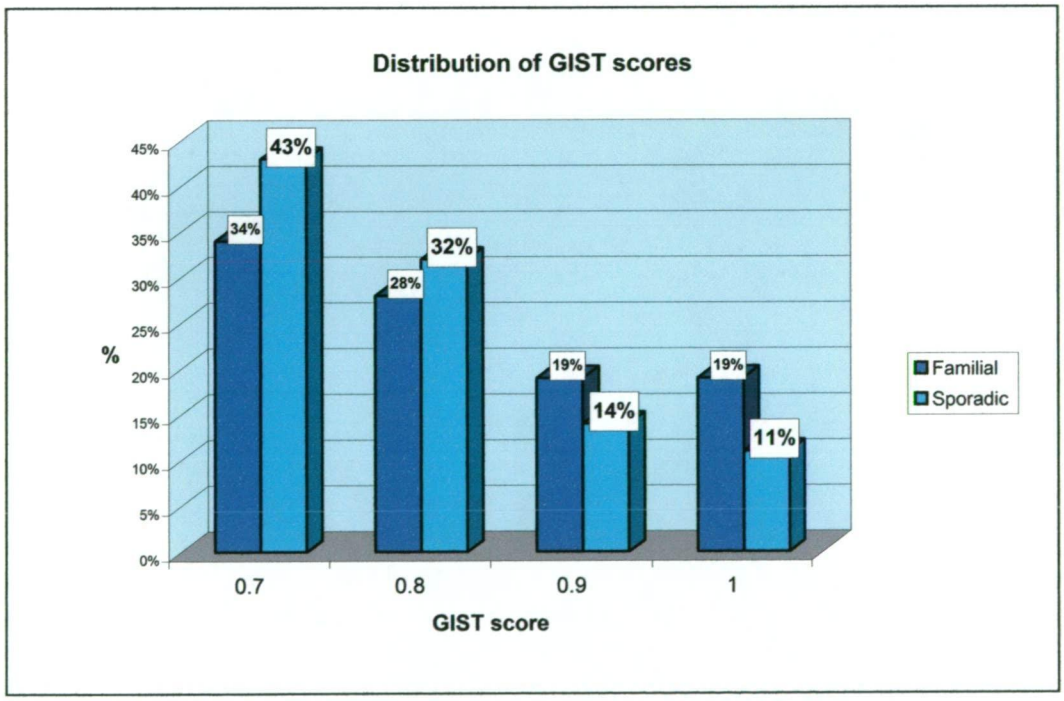

Figure 14. Distribution of GIST scores for familial and sporadic groups

DISCUSSION

The finding that almost 60% of POAG patients in Tasmania have at least one other family member affected is a higher percentage than that reported in most other studies (Charliat, Jolly and Blanchard 1994; Tielsch, Katz et al 1994; Wolfs, Klaver et al 1998; Mitchell, Rochtchina et al 2002).

Familial aggregation studies of POAG are subject to several possible biases. Clinic-based studies tend to report a higher prevalence of glaucoma in relatives than in population-based studies, owing to a possible differential use of eye care services by family members. Population-based studies create smaller sample sizes and thus decreased statistical power (Tielsch, Katz et al 1994; Wolfs, Klaver et al 1998). Most previously published studies, (Tielsch, Katz et al 1994; Nemesure, Leske et al 1996; Mitchell, Rochtchina et al 2002) with the exception of the Rotterdam Eye Study (Wolfs, Klaver et al 1998) have relied on family history ascertained by interview rather than examination. This raises the possibility of recall bias and also the variability of diagnostic criteria when diagnosing glaucoma. In addition, the participation rates for population-based prevalence studies have been variable, with some as low as 67% (Leibowitz, Krueger et al 1980) or 71% (Dielemans, Vingerling et al 1994). Many studies have only considered first-degree relatives and not more distantly related family members. This results in a likely under-estimation of the familial/genetic nature of glaucoma.

This study has several strengths:

- To the author's knowledge, it is the largest study of its kind reported.
- Whilst there was a clinic-based component to the study, the initial point of contact with patients was through several sources, including pharmacies, optometrists and their treating ophthalmologist as well as via community advertising through the media. Tasmania has a captive population and it is believed that almost all diagnosed glaucoma patients were identified and invited to participate. This study is not strictly a population-based study, but owing to the nature of the island and to the large numbers of participants, the sample is likely to have included a large proportion of the population. Even population-based studies have inherent difficulties and participation rates in most studies have varied from 67% to 83% (Leibowitz, Krueger et al 1980; Klein, Klein et al 1992; Dielemans, Vingerling et al 1994; Tielsch, Katz et al 1994; Mitchell, Smith et al 1996; Wensor, McCarty et al 1998).
- All relatives of probands were invited to participate and relatives more distant than just first-degree relatives were examined. This has added strength to the evidence that POAG is a familial disease and added information about the pattern of disease in the extended family.
- The study was performed over 6 years, which could have resulted in a higher number of patients and family members having been identified and possibly a higher number of previously undiagnosed cases being detected. In the Australian population, 50% of POAG cases remain

undiagnosed in the community (Mitchell, Smith et al 1996; Wensor, McCarty et al 1998). As result of the GIST being conducted over the last decade, a higher number of previously undiagnosed cases have been detected (McNaught, Allen et al 2000). This adds strength to the results.

- All the patients with familial glaucoma and family members were examined in a masked fashion and classified according to strictly defined criteria. Only patients who had been examined according to the study protocol were included in the database. This overcomes the difficulties with the inaccuracies of recall of a family history or the possibility of incorrect diagnosis, as well as ensuring uniformity of diagnosis. The difficulty of ascertainment had been overcome as the glaucoma cases included have definite glaucoma according to strictly defined criteria. The majority of relatives of apparently sporadic cases were not examined, nor were relatives of controls. The fact that this may introduce ascertainment bias described in other studies (Nemesure, Leske et al 1996; Mitchell, Rochtchina et al 2002) is acknowledged but could not be overcome in this setting with limited time, funding and resources.
- The number of glaucoma cases detected in Tasmania in this study correlates well with population-based projected numbers. The population of Tasmania does not differ greatly in its demographics from that of the population included in the Blue Mountains Eye Study (Australian Bureau of Statistics 2001) on the prevalence of glaucoma found in the Blue Mountains Eye Study and the Tasmanian Census

Population Data, the number of glaucoma cases likely in Tasmania was projected, as shown in the Table below (Prof Paul Mitchell personal correspondence). This study found a total of 1702 POAG affected individuals, which approximates the calculated projections.

Table 3. Projections of number of glaucoma cases in Tasmania

Projections of Glaucoma in Tasmania based on the Blue Mountains Eye Study

	<60yr	60-69yr	70-79yr	80+yr
M	55	219	528	346
F	126	265	811	1221

Total M + F 3571

(Half of these should be diagnosed =1785)

It is possible that factors could increase the prevalence of glaucoma in Tasmania, such as a large founder effect, and the fact that islands tend to reduce travel and result in a captive population. The data in above table suggest that the prevalence of glaucoma in Tasmania is comparable to that in the rest of Australia.

Owing to the inherent difficulties in performing such a study, we may never obtain an exact estimate of the familial prevalence of the disease. However, to the author's knowledge, this is the largest study of this nature published to date and many potential biases have been overcome.

The results show that we may have previously underestimated the familial nature of the disease.

A potential criticism of this study is the inclusion of IOP as one of the criteria used in the GIST score to determine the probability of the presence of glaucoma. It is acknowledged that this is not favoured by epidemiologists, but considerable controversy regarding the definition of glaucoma in epidemiological studies remains (Wolfs, 2000 Foster, 2002). Optic disc abnormalities are regarded as the gold standard for glaucoma diagnosis in epidemiological studies but the cut-off points for the definition of pathological disc cupping are yet to be fully elucidated, differ in different racial groups, are influenced by optic disc size and may also depend on the modality of disc imaging used. In a recent paper by Foster and co-authors discussing the definition and classification of glaucoma in prevalence surveys, IOP was not excluded as a diagnostic criterion in all circumstances, with allowance made for an IOP exceeding the 99.5^{th} percentile to be used as a diagnosis of glaucoma in the presence of a media opacity precluding the examination of the optic disc and the performance of a visual field test (Foster, 2002). The 'expert panel' that graded glaucoma cases in the Melbourne VIP was given information about elevated IOP when making a diagnosis (Wensor, McCarty et al, 1998).

The GIST is a genetic study rather than a purely epidemiological survey. In myocilin pedigrees, raised IOP is a feature of the

glaucomatous disease process and is one of the earliest signs of the onset of the disease (Fingert 2002). In using the GIST score, those patients with only slightly elevated IOP in the absence of glaucomatous disc cupping or a glaucomatous field defect would not be classified as having definite glaucoma. Only those with markedly elevated IOP (> 28 mmHg) would be classified as such and it could be argued that these individuals are at extremely high risk of developing glaucoma, especially in the presence of a family history of the disease, thus justifying their inclusion. This is borne out by results of the BMES, which found that 39% of subjects with an IOP $\geq$ 28 mmHg had glaucoma (Mitchell, Smith et al 1996).

Blindness and visual impairment cause significant morbidity and premature mortality in the population. There are well-established correlations between visual impairment and higher risk of falls, hip fractures, motor vehicle accidents and depression. The risk of death is increased almost three-fold for those over the age of 40 with visual impairment. The health costs of treating eye disease are high: AU$1.8 billion in 2004 in Australia. Glaucoma accounts for 3% of visual impairment and 14% of blindness in this country (Access Economics 2004).

The best prognosis for treatment of glaucoma relies on early detection. Whilst great advances in genetic testing for glaucoma have taken place in the last decade, with genetic testing available for some individuals,

there is still a lack of biochemical and genetic markers for the disease in most cases. Clinical examination is the only method of early detection. This study confirms that those with a family history of the disease are at a greatly increased risk of developing glaucoma and suggests that familial forms of disease may be more severe.

Whilst the GIST score was developed as a research tool to assign probability of POAG in individuals in pedigrees of disease, it does also reflect disease severity, with a higher score correlating with more advanced disease. The distribution of patients with higher GIST scores appears to differ between the familial and sporadic groups, with a greater proportion of patients in the familial group having higher scores. This trend requires further research before it can be confirmed, but it does have important implications. The glaucoma patients with more advanced disease may be at higher risk of developing significant visual impairment or progressing to blindness. Since research has confirmed that even in advanced glaucoma, IOP lowering treatment is effective at slowing the progression of visual loss (Investigators- The Advanced Glaucoma Intervention Study (AGIS) 2000), it is important that those most at risk of blindness are identified in our community. As discussed in the introductory section, 50% of glaucoma remains undetected in the Australian population (Mitchell, Smith et al 1996; Wensor, McCarty et al 1998) and screening for glaucoma remains problematic. It is already known that a family history of glaucoma is a significant risk factor for developing the disease, but if we can identify those individuals at

greater risk of blindness through targeted case detection, it may be possible to reduce the impact on the community of blindness from glaucoma.

In the absence of proven, cost-effective population screening for glaucoma, it is appropriate to recommend regular examination of those at risk. Public awareness and education about glaucoma should be increased emphasising that the disease is frequently familial and that whilst it is potentially blinding it is treatable. Health care professionals should be educated regarding the significance of a family history of glaucoma and refer those at risk for regular assessment. POAG patients should be told to encourage other family members over the age of 40 to undergo regular eye examinations with an appropriately trained professional.

Mitchell and co-workers found a strong association between inhaled corticosteroid use and the presence of either glaucoma or elevated IOP (odds ratio 2.6%, confidence interval, 1.2-5.8) in individuals with a family history of glaucoma (Mitchell, Cumming and Mackey 1999). This suggests that taking a family history of glaucoma might form a valuable component of the workup of patients being considered for corticosteroid therapy and that corticosteroids should be used with great caution in persons reporting a family history of glaucoma. These patients need review by an ophthalmologist for the duration of the corticosteroid treatment and thereafter, as steroid responders have been

found to have an increased risk of subsequent glaucoma (Kitazawa and Horie 1981; Lewis, Priddy, Judd et al 1988). In the future, it might be feasible to perform tests for mutations in myocilin, or other glaucoma genes, as they become implicated, on patients before initiating corticosteroid treatment.

Glaucoma is an asymptomatic disease and difficult to diagnose in its early stages. As it emerges as a familial/ genetic disease, the possibility of DNA testing to identify individuals at risk is increasingly becoming a reality. Knowledge of glaucoma genetics is improving rapidly and views about genetic testing are likely to change correspondingly. At present, the currently identified glaucoma gene mutations are not common enough to justify DNA testing in the wider community (Mackey and Craig 2003). However, effective predictive testing for myocilin glaucoma has already been performed within a large Australian family, with a high level of acceptability (Healey, Craig et al 2004).

In a study by Craig and co-workers (Craig, Baird et al 2001) investigating the phenotype and age-related penetrance of POAG in Australian families with the most common Myocilin mutation (Gln368STOP), 7 of the 8 pedigrees studied contained one or more individuals with POAG who did not carry the mutation. This implies that other genes remain to be found in these large families as well as in the smaller pedigrees and sporadic cases.

Predictive DNA testing opens the possibility of community management of glaucoma by cascade genetic screening as discussed in the introductory section on glaucoma genetics. Further research is required to determine the best regimen for clinical screening of high-risk individuals and the most beneficial timing and methods of intervention. This is particularly relevant because glaucoma is an imminently treatable condition. At present, the majority of glaucoma patients do not yet have an identified genetic cause, with only about 5% of POAG being associated with an identified mutation (Stone, Fingert et al 1997). As further knowledge of the genetics of POAG is gained, more extensive genetic testing may become available.

Thorough data collection was possible in the GIST because it involved a relatively small population; however, conclusions from this study can be applied to much larger communities. The rate of diagnosis of glaucoma can be increased by asking individuals affected with POAG to find out if other family members are also affected. Taking a family history does not end with the first consultation but should be discussed at follow-up consultations. Those with POAG should also inform all their relatives that those family members over the age of 40 years (or younger if the age of onset of disease was earlier) should be examined.

The results of this study show that the familial nature of glaucoma is even greater than previously thought. At present there is substantial

impetus from government and heath care organisations to target the issue of blindness in the community and to improve detection and treatment of blinding disease. Public authorities should inform the general population that people with a positive family history of POAG should be screened for the disease. The results of this study could be helpful in the calculation of effectiveness and cost implications of disease detection in the Australian community.

CONCLUSIONS

The finding that almost 60% of POAG patients in Tasmania have at least one other family member affected is higher than previously reported.

This emphasizes the genetic nature of the disease and offers further evidence that as knowledge about the genetics of glaucoma increases, there will be an opportunity to apply this in the management of the disease.

The findings of this study offer opportunities to improve glaucoma case detection in the community. Of the 1702 glaucoma patients identified and examined in this study, 38% had a first-degree relative affected, with a smaller percentage having a more distantly related family member with the disease. This highlights the importance of ascertaining a family history of glaucoma. Identifying individuals at risk will allow early detection of the condition. This directly influences outcome, as the treatment of the disease is aimed at slowing the rate of progression of visual loss. Damage that has already occurred as a result of the optic neuropathy cannot be reversed. The evidence of an association with family history of glaucoma, use of inhaled corticosteroids and risk of the disease found by Mitchell, Cumming and Mackey (Mitchell, Cumming et al. 1999) is another example of how important it is for all clinicians and patients to be aware of the significance of a family history of POAG.

Initiatives to improve eye health in the community and prevent blindness should aim to improve awareness of POAG in the general population and health care providers. Education campaigns should highlight the familial nature of the disease and provision made for resources to be made available for the assessment of those at risk.

The identification of numerous pedigrees affected with glaucoma offers the possibility of identification of hitherto undiscovered genes and eventually possible genetic screening, either of those at risk or even a general population screening programme. Genetic screening in a family with a severe form of POAG associated with the MYOC gene has already been effectively performed with high acceptance from the family members demonstrating that there is already a role for genetic screening in appropriate circumstances.

Before genetic screening can be more widely applied, more research will be required to investigate the frequency, phenotype and origins of genes, the natural history of glaucoma in affected pedigrees and the nature genotype- phenotype interactions.

The cost-effectiveness of genetics screening will need to be weighed against the cost of conventional screening and the benefits of early treatment considered. In addition to clinical evidence of the value of predictive gene testing it is incumbent on those working in the field to

evaluate the acceptability of testing to patients and family members (Mackey and Craig 2003). Issues relating to insurance, ethics and confidentiality need to be taken into consideration (Mackey, Heon and Webster 2003).

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APPENDICES

Appendix A Patient information letter and consent for DNA testing

Appendix B Study proforma

APPENDIX A

GIST Glaucoma Inheritance Study in Tasmania a collaborative project of the
University of Tasmania, University of Melbourne,
Eye Department, Royal Hobart Hospital
Liverpool St, HOBART Tas 7000

Ph & Fax 03 62 22 8553

Department of Ophthalmology
Royal Victorian Eye & Ear Hospital
32 Gisborne St
EAST MELBOURNE Vic 3002
Ph 03) 9929 8713

12 January 2000

Dear Sir/Madam,

As you may be aware, researchers with the University of Tasmania and the University of Melbourne have been part of an international collaborative effort that discovered the first gene that causes inherited glaucoma. Dr David Mackey and his research team in the Glaucoma Inheritance Study in Tasmania (GIST) are investigating many families, in Tasmania and elsewhere in Australia, with glaucoma in order to discover other glaucoma genes. This means in the future it may be possible to predict those family members at risk for glaucoma. Thus this work should benefit future generations.

We believe that you have not yet been approached by the study, but that you may be interested in participating. You are under no obligation to participate in this project. If you do not wish to participate please indicate on the form over the page, or if you are interested please fill in the personal information sheet, and the family history information sheet. A copy of the consent form for DNA testing is on the reverse of this page. If you chose to participate then we will ask you to sign a copy of this and provide a sample of blood or if you prefer a mouth swab, which can be posted.

If you would like to be involved in the study please indicate on the page over and we will arrange another time to see you (usually at your eye clinic). There is no charge for the DNA test, which will involve around twenty minutes of your time. Alternatively we can post you a swab kit to brush the inside of your cheek and post this back to us.

If you would like further information or would like to contact us, please telephone Sue Stanwix on 6222 8553.

Thank you very much for your assistance.

Yours Sincerely,

Dr David Mackey
on behalf of the Glaucoma Inheritance Study (GIST).

Please tear off and keep this yellow page

Continued over page

GIST Glaucoma Inheritance Study in Tasmania
a collaborative project of the
University of Tasmania,
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CONSENT FOR DNA TESTING

Information for patients in the Glaucoma Inheritance Study in Tasmania (GIST).

The glaucoma inheritance study is looking for families with glaucoma to find the genes that cause glaucoma. We are inviting individuals and families who are affected with glaucoma to be involved in the study. This is at no cost to you. We wish to take a blood sample, or a mouth swab to test your DNA to see if we can find the mutations in the first gene that we have discovered that causes glaucoma. If this first gene is not affected we may use the DNA to help discover the other genes that lead to glaucoma. You are under no obligation to provide this and it may not carry any direct benefit to your glaucoma management, but it may assist us in understanding who else in your family is at risk of glaucoma.

The DNA will be tested and we may find: A change in the DNA, no change in the DNA, or be unable to find anything. You will be informed of the result of your test and be able to discuss this with us at any time.

The DNA will be stored at the Universities of Tasmania and Melbourne. The results of any scientific development will be owned by the Universities of Tasmania and Melbourne and their collaborators. You may ask to withdraw from the study at any time, without prejudice, and have your sample destroyed. We may do further studies on glaucoma at a later date and will of course inform you of your results. In our work we may find other abnormalities of the DNA and will discuss the results with you. This study will only be looking at genes related to glaucoma. We may also find that you are distantly related to other families that we have studied, based on the DNA findings. These results may all be published but will never identify you specifically.

We will give you a copy of this form to keep for future reference. For more details or any questions please contact Dr David Mackey on the above numbers or leave a message. The study is conducted in accordance with the NHMRC guidelines for human genetic research. If you have any questions about the ethical nature of this study you may contact Dr Rosalie Parton of the RHH ethics committee on 03)6222 8226.

Please sign this form to certify that you read and understood the Information sheet, and had explained the nature and possible outcomes from the DNA testing and your questions have been answered to your satisfaction.

I am happy to participate in the Glaucoma Inheritance Study GIST.

Name

Signed Date ...../...../2005

Witness Name

Signed Date ...../...../2005

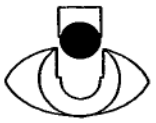
Address

Researcher Statement: I have explained the GIST project and its implications to this volunteer and I believe that the consent is informed and that he/she understands the implications of participation.

Name:

Signed Date ...../...../2005

APPENDIX B



GIST Glaucoma Inheritance Study:

Your Name Place of Birth

Spouses name

Please answer the following to the best of your ability. Leave blank if unknown.

Your father's name.....date of birth...../.../.....Place.....

Your father's father's name.....date of birth..../..../.....Place.....

Your father's mother's full name.....date of birth...../..../.....Place.....
(and maiden name)

Your mother's name.....date of birth...../..../.....Place.....
(and maiden name)

Your mother's father's name.....date of birth...../..../.....Place.....

Your mother's mother's full name.....date of birth...../..../.....Place.....
(and maiden name).

Names of your brothers and sisters.....date of birth
(First and Surnames
with married names of sisters;
please note if deceased.

If insufficient space please
use the reverse of this
sheet or attach list)

Names of your children.....Their dates of birth.....

Other Relatives affected with glaucoma (please note if deceased)

Name.....Relationship.....Address.....

Name.....Relationship.....Address.....

Name.....Relationship.....Address.....

Name.....Relationship.....Address.....

Name.....Relationship.....Address.....

Name.....Relationship.....Address.....

Is anyone tracing the family tree? Name and Address.....

Could you please attach or forward a copy of your family tree?
Thank you for your help with the Glaucoma Inheritance Study.

Glaucoma Inheritance Study GIST G /Registry Bleed

Please complete the following information to the best of your ability. If you do not know the answer please leave the question blank.

Today's Date...../...../..... Time.....

Surname.....

Maiden Name..... GP.....

First Name..... Ophthalmologist.....

Address.....Date of Birth...../...../.....

.....Age.....

.....Post Code.....

Phone.....

Do you have glaucoma?.....

When was the date of Diagnosis?..... Your age at Diagnosis?.....

The highest eye pressure if known?.....

Do you have a family history of glaucoma? Yes/No

Fathers side? Yes/No
Mothers side? Yes/No
Number affected

Please name your Glaucoma Medications.....

Have you ever had eye surgery or laser treatments for glaucoma? .....

What and When?.....

Do you have any other eye problems?.....

Have you had any other eye surgery? (What and When).....

or injury to your eyes?.....

Are you on any general medications?

Please name them if possible:.....

Do you have high blood pressure? Yes/No

Do you smoke? Yes/No

Do you have diabetes? Yes/No

Have you ever had a blood transfusion? Yes/No Why?

Have you ever had a heart attack, stroke or any other disease with hardening of the arteries? Yes/No

Do you get cold hands or feet? Yes/No

Have you had any thyroid problems? Yes/No

Do you suffer from migraine headaches? Yes/No

Have you ever been on Cortisone or steroid medication? Yes/No

Predilation Exam:	**Right**	**Left**	**Tick when done**
Acuity:			**Hx**
			Cons
Refraction: Distance			**Field**
			Press
and/or Readers			**Dilate**
IOP:			**Blood**
			Photo
Gonioscopy:			**Letter**
Anterior Segment			

Dilated Exam: No 1	**Right**	**Left**
Cup/Disc ratio		
Disc Size (S,M,L)	◯	◯
Other Disease		

Score R L signature

Stereophoto or Dilated Exam: No 2	**Right**	**Left**
Cup/Disc ratio		
Disc Size (S,M,L)	◯	◯
Other Disease		

Score R L signature

Stereophoto or Dilated Exam: No 3	**Right**	**Left**
Cup/Disc ratio		
Disc Size (S,M,L)	◯	◯
Other Disease		

Score R L signature

Field Score	**Right**	**Left**
Reliability	.....	.....
Score:A,B,C,D:	.....	.....
GIST Field Score	.....	.....

Concordance between field and discs? Yes/No

Glaucoma Type,Consistent with family's Type and other comments.

Field Score.....
Pressure Score
Disc Score.....

GIST SCORE.