GENETIC EPIDEMIOLOGY OF COMMON EYE DISEASES: A TWIN STUDY

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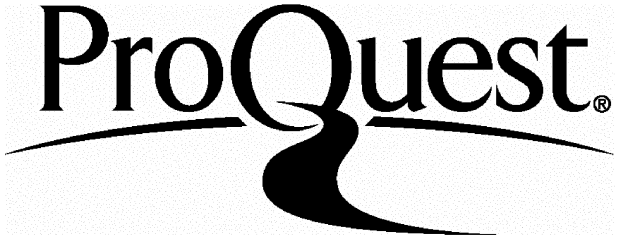

Abstract

Cataract and age-related macular degeneration are important causes of blindness and visual impairment, and refractive error is highly prevalent and considerable time and expense is directed at its correction. Epidemiological studies have identified environmental risk factors for all these condition, while other studies have demonstrated familial aggregation. Twin studies, which compare the concordance of phenotypes in monozygotic and dizygotic twin pairs, can be used to elucidate the genetic epidemiology of eye disease – i.e. determine the relative importance of genes and environment.

This thesis describes a classical twin study of 506 twin pairs (280 dizygotic and 226 monozygotic) with a mean age of 62 years. When they volunteered through national media campaigns, they were unaware of a potential eye study. Twins were comprehensively ascertained for refractive error using an autorefractor, and for cataract using subjective and objective grading techniques. Age-related macular degeneration was graded from stereoscopic macular photographs. Quantitative genetic model fitting, based on comparison of the covariance (or correlation) in the phenotype measurement between monozygotic and dizygotic twin pairs, determined the heritability, which is the ratio of genetic variance to total phenotypic variance.

Mean scores were similar, but monozygotic twins were more concordant than dizygotic twins, for all phenotypes. This suggested genes are important in common eye diseases, even those age-related traits such as cataract, and was confirmed by modelling. The heritability of spherical equivalent was 84-86% and that of astigmatism 42-61%. The heritability of nuclear cataract was 48% and it was 53-58% for cortical cataract, depending on the grading system used. The heritability of early age-related maculopathy was 54%. Both astigmatism and cortical cataract appear to involve dominant inheritance.

The heritability of age-related eye disease is substantial, and these results encourage identification of susceptibility genes through linkage and candidate gene studies, to further understand the mechanisms of disease.

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Publications arising from this work

Hammond CJ, Snieder H, Spector TD, Gilbert CE. Genetic and environmental factors in age-related nuclear cataracts in monozygotic and dizygotic twin pairs. *New England Journal of Medicine* 2000; **342**: 1786-1790

Hammond CJ, Duncan DD, Snieder H, de Lange M, West SK, Spector TD, Gilbert CE. The heritability of age-related cortical cataract: the Twin Eye Study. *Investigative Ophthalmology and Vision Science* 2000; *in press*

Hammond CJ, Snieder H, Spector TD, Gilbert CE. Factors affecting pupil size after dilation: the Twin Eye Study. *British Journal of Ophthalmology* 2000; *in press*

Submitted

Hammond CJ, Snieder H, Spector TD, Gilbert CE. Genes and environment in refractive error: the Twin Eye Study.

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Abbreviations

a^2	heritability attributable to additive genes
A	additive genetic latent variable used in modelling
AMD	late-stage age-related macular degeneration
ARM	age-related maculopathy
BDES	Beaver Dam Eye Study
BMES	Blue Mountains Eye Study
c	regression coefficient of observed variable on C in model
C	common environment latent variable used in modelling
χ	chi- as in chi-squared statistic relating to measure of fit
CI	confidence interval
COL2A1	type 2 collagen α1
d	regression coefficient of observed variable on D in model
D	dominant (non-additive) genetic latent variable used in modelling
df	degrees of freedom
DHRD	Doyne Honeycomb Retinal Dystrophy
DNA	deoxyribonucleic acid
DZ	Dizygotic
e	regression coefficient of observed variable on E in model
E	individual (unique) environment latent variable used in modelling
h^2	heritability attributable to additive and dominant genes
LOCS	Lens Opacity Classification System
MES	Melton Eye Study
μ	microns
MZ	Monozygotic

OCCCGS	Oxford Clinical Cataract Classification and Grading System
Oxford	cortical cataract (cortical spoke) score from OCCCGS
p	probability
r	correlation coefficient
SD	standard deviation
V	variance
WHO	World Health Organisation
Wilmer	cortical cataract score of Wilmer Automated Grading system

1.0 Introduction

Epidemiology can be defined as the quantitative measure of the distribution, determinants and control of disease in human populations.[1] There has been considerable epidemiological research on eye disease, examining the causes of low vision and blindness, particularly for age-related disease. Blindness is increasing in both the developed and developing world, particularly due to aging of the population. Several large population-based epidemiological studies have concentrated on environmental risk factors. While research into the genetic diseases affecting the eye has been the vanguard of genetic research, there has been little work on the genetic aspects of age-related eye diseases, with most genetic research being undertaken in single gene mutation syndromes and diseases.

Genetic epidemiology is the study of the genetic basis of disease in human populations, and uses quantitative statistical methods to study the role of genes in disease causation and severity. Eye problems such as refractive error, and age-related diseases such as cataract and macular degeneration, are believed to be complex diseases, with no clear method of inheritance. They are probably multifactorial, involving several genes as well as several environmental factors. This makes their study difficult. Most diseases, including those mentioned above, exhibit familial clustering, and it is difficult to disentangle the role of the shared environment from that of the shared genes within a family.

Twin studies offer a way of dissecting the relative roles of genes and environment in disease, to allow estimation of the importance of each. Knowledge of genetic epidemiology allows further research to be directed, in particular the identification of genes involved in disease. Once the genetic architecture of a disease is known, not only may the pathogenesis be further elucidated and potential treatments derived, but also significant environmental effects may become evident for particular gene mutations, which previously might have been lost in the multifactorial "noise" of a population study of the disease.

This thesis describes a study of a unique population of volunteer twins from the UK adult twin registry held by the Twin Research and Genetic Epidemiology Unit at St Thomas' Hospital. The aim of the study was to examine the genetic epidemiology of common eye conditions, which are important either because they are potentially sight-threatening or economically important, and to examine the whole spectrum of disease including early asymptomatic cases to estimate the heritability in a UK population. The prevalence of the conditions studied was ascertained from estimates derived from population studies and this data was used to determine the number of twins recruited. To achieve sufficient power for the quantitative genetic analysis in this population-based research, phenotypes that were relatively common, and which preferably could be measured on a continuous scale or at least categorised into several categories of severity, were required. Before the diseases studied are outlined, the following section details the epidemiology of low vision and blindness, to determine the common and sight-disabling conditions affecting the population.

1.1 The Epidemiology of Blindness and Low Vision

Prevalence is a measure of disease frequency and is the proportion of people with a certain disease in a population at a given time. Incidence is the number of new cases that occur in a specified time. Prevalence depends both on incidence and duration of a disease until recovery or death and so is not as useful a measure of disease frequency as incidence: for example a disease with high mortality and high incidence will have a low prevalence. Prevalence data are easier to obtain, requiring only one survey of the population rather than longitudinal studies. However, as blindness and low vision probably have a stable incidence and do not greatly affect life expectancy, prevalence figures give a useful indication of both incidence and burden of disease in a population.[2]

Worldwide it is estimated that at least 38 million people are blind and 110 million have severe impaired vision, and these numbers are rising as the world's population increases and ages.[3] The World Health Organisation (WHO) in conjunction with collaborating nongovernmental organisations has set up a Global Initiative for the Elimination of Avoidable Blindness by the Year 2020, to combat the estimated 7 million new cases of blindness each year. This is causing worldwide numbers of blind to increase each year by 1-2 million.[3]

1.1.1 Definitions of Blindness and Low Vision

Data about blindness comes from various sources: population-based sample surveys conducted using strict criteria are the best source, but other sources such as blindness registration statistics (especially in developed countries) and data from blind schools (useful for studies of childhood blindness) are used. Many countries have different definitions of blindness and low vision, which can make comparisons difficult. Most surveys now use the WHO criteria, although most data from England derive from blindness and low vision registration, which use different definitions. Table 1 summarises the different definitions of blindness and low vision.

Table 1 Definitions of blindness and low vision

	WHO Criteria	USA Criteria	England Criteria
Blind	acuity < 3/60 or field loss to < 10°	acuity ≤ 6/60	"unable to perform any work for which eyesight is essential" • acuity < 3/60 • better acuity <6/60 & field loss • very constricted (esp. inf.) field
Low Vision	acuity < 6/18	acuity < 6/12	"substantially and permanently handicapped" • 3/60-6/60 & full field • <6/24 with field contraction • ≥6/18 with gross field defects

The acuities quoted above are for best corrected acuity in the better eye, but in practice many studies have used the current refractive status of the subject in order to establish the level of functional impairment in a community. Note that the WHO criteria do not include field defects in their definition of low vision. Murdoch and others have shown in a study of onchocerciasis,[4] that serious visual impairment due to visual field constriction may be missed using this definition.

1.1.2 Prevalence of Blindness

Worldwide

The prevalence of blindness correlates closely with the degree of poverty, and so there is a wide variation world-wide. Four out of five cases of blindness occur in the developing world, and many of the causes are preventable.[5] Available prevalence surveys have been pooled, and Table 2 lists the estimated prevalence of blindness in various World Bank Economic Regions.[6] This suggests the prevalence varies

between 0.3% in established market economies, and 1.4% in sub-Saharan Africa. A useful summary of available data on blindness is published by the WHO Programme for the Prevention of Blindness, most recently in 1995.[6]

Table 2 Prevalence of Blindness by World Bank Economic Region

Economic Region	Prevalence (%)
Established market economies	0.3
Latin America and the Caribbean	0.5
Former socialist market economies	0.6
China	0.6
Middle Eastern crescent	0.7
Other Asian countries and islands	0.8
India	1.0
Sub-Saharan Africa	1.4

The prevalence of blindness increases with age: for example, the Beaver Dam Study in the USA of subjects aged 43-86 showed an overall blindness prevalence of 0.5%, but 2.0% in those over 75 years old.[7] In India, a recent well-conducted study suggested a prevalence of blindness of 3% in those over 30 years old, in keeping with the overall estimate of 1%, as only about 30% of the Hyderabad population where this study was performed are over 30.[8]

England

There has been no comprehensive population-based prevalence survey performed in this country, but there have been several studies looking at acuity in selected sample groups. A recent study in North London suggested a prevalence of blindness (<6/60 acuity) of 5.9% in a group of 1547 people aged 65 and older with current refractive aids.[9] The original cross-sectional prevalence survey in Melton Mowbray of those aged over 75 estimated 3.8% to be blind (≤6/60) after best correction.[10] The national registration system (the BD8 form) offers some useful epidemiological information about causes of blindness and trends, but it is not useful for prevalence studies, due to considerable under-registration.[11] The RNIB has estimated, from a population-based self-reporting survey, that blindness may be under-reported by up to 64% and

suggests a prevalence of 0.7%.[12] A hospital-based study suggested non-registration of blindness of 26% in a sample of patients attending clinics.[13]

It seems England probably has a similar rate of blindness to the other established market economies, of around 0.3%. Wormald, using registration data, has worked out a prevalence of 0.48% of blindness and partial sight for the figures to March 1991.[11]

1.1.3 Prevalence of Low Vision

Worldwide

Some prevalence surveys have established the proportion of low vision in their populations: based on the WHO global data on blindness, the prevalence of low vision is between 1.3 and 7.8 times the prevalence of blindness, with a mean of 2.9. Using the American criteria, the Framingham Eye Study found a prevalence of low vision of 2.4% (and blindness 0.9%), and 9.7% and 3.3% for those over 75.[14] More recently the Beaver Dam Eye Study showed a prevalence of visual impairment (<6/12 but >6/60 acuity) of 4.7% overall and 19.1% in those over 75 years, with respective blindness prevalences of 0.5% and 2.0%. Within communities in America the figure is variable: the Baltimore Eye Survey found visual impairment prevalence was 2.7% in whites and 3.3% in blacks, an age-adjusted relative prevalence of 1.75.[15]

England

There were 245 517 people registered as blind or partially sighted in England and Wales in March 1991, which is certainly an underestimate.[11] The North London study of people over 65 showed 30% had visual impairment by American criteria, 15% by WHO criteria (in addition to the 5.9% blind).[9] Similarly the smaller study by Wormald of people of the same age in inner London showed a prevalence of 10.6% and 7.7% respectively.[16] The Melton Mowbray study of those over 75 showed 25.6% had visual impairment (< 6/18) after refraction.[10] Registration of people with non-

reversible low vision is lower than for those who are blind.[12; 13] These figures show that there is still a high level of treatable visual impairment in the community, even in England today with reasonable, usually local, resources: 88% of the 30% of people with cataract in North London were not in contact with eye services.[9]

1.1.4 Incidence of Blindness and Low Vision

There are virtually no data on the incidence of blindness and low vision. Based on new registrations for the year ending March 1991 it has been estimated that the incidence rate is 58 per 100,000 population per year in England.[11] Rosenberg and Klie demonstrated an incidence rate for those aged 60-99 of blindness due to AMD as 140:100,000 per year for females and 66:100,000 per year for males using blind registration data in Denmark, although there is no way of knowing how complete these data are.[17] Incidence rates have been estimated from the Framingham data[18] although again these are not true incidence data.

1.1.5 Causes of Blindness and Low Vision

Global Blindness

Estimation of the global causes of blindness is difficult: not only are assumptions made when extending data from a few small surveys to an entire region, but also there is often incomplete information. Many surveys have been conducted by trained field workers without facilities for dilated fundal examination. Table 3 lists a current estimate of the numbers of people blind from WHO and other figures[1]. Note that age-related macular degeneration (AMD) and diabetic retinopathy, both important causes in the established market economies, do not appear. This is because of the inadequacy of data in their estimation.

Table 3 Global causes of blindness[1]

	Millions	%
Age-related cataract	15.83	42
Trachoma	5.87	15
Glaucoma	5.12	14
Childhood	1.45	4
Onchocerciasis	0.27	1
Leprosy	0.40	1
Trauma	1.50	4
Others	7.46	19
Total	37.90	100

It is certain that cataract is not only the commonest cause of world blindness but also the most treatable. The relative proportions of the three commonest causes vary throughout the world. Recent doubt has been cast on the results of some of the surveys. Dandona and others have recently published a survey of an urban Hyderabad population sample using more sophisticated dilated fundal examination and visual field analysis rather than acuity measured by field workers.[8] They dispute the accepted wisdom (based on a 1986-89 national survey) that 80% of India's blindness is due to cataract. Their causes of blindness in those aged over 30 is listed in Table 4.

Table 4 Causes of blindness in Hyderabad study[8]

Causes of blindness	Percentage blindness
Cataract	34.3
Retinal disease total	22.4
Retinitis pigmentosa	14.2
Chorioretinitis scar	4.4
Macular atrophy	1.4
Myopic degeneration	1.2
Retinal detachment	1.2
Corneal disease total	20.1
Opacity after childhood fever	11.8
Chemical injury	4.1
Traditional eye medicine	3.2
Oedema	1.0
Refractive error total	0*
Glaucoma	15.2
Primary open angle	9.8
Primary angle closure	5.4
Optic atrophy	6.4
Trauma	1.6

*refractive error is zero because the WHO's criteria of blindness with best correction is used. If blindness is defined as acuity at presentation <3/60 or field of less than 10^{o}, then refractive error comprised 9.6% of blindness.

Dandona et al conclude that although cataract is still the most important eye disease to target, national blindness prevention programmes should not ignore the other causes.

Causes of blindness and low vision in Britain

There has been little recent data about causes of blindness in England. The published analysis of blind registrations (1980-81) found AMD to be the most common cause of blindness, in 37% of those registered, but cataract still caused 9% of blindness[19]. These are similar to the causes reported in the West of Scotland in 1983[20]. The 1990-91 figures suggest macular degeneration to be the cause in 48% of new registrations[21]. A more recent follow-up analysis of the West of Scotland (1996/97) has reported

cataract to be no longer a significant cause of blindness, but AMD is an increasing burden of blindness[22]. This is to be expected as the population ages: 75% of registrations in the West of Scotland study were for people over 65 years, and 62% over 75 years old. The three studies are summarised in Table 5. Figures for Denmark from those registered in 1993 are included for comparison[23].

Table 5 UK Causes of Blindness (in percentages) from blindness registration

Cause	Scotland 1979[20]	England 1980-1[19]	England 1990-1[21]	Scotland 1996-7[22]	Denmark 1993[23]
Macular degeneration	30	37	49	52	71
Glaucoma	15	13	12	19	5
Diabetic retinopathy	6	8	3.5	7	8
Cataract	10	9	3.3	1	0.5
Optic neuropathies	4	5	12		2.4
Refractive error		5			
Myopic degeneration	6				2

Although these figures represent the total numbers of people newly registered, obviously the relative proportions will vary with age. Diabetic retinopathy is the most common blinding condition in people of working age. Causes of blindness in the Danish Study (with admittedly small numbers of 113 people aged between 20 and 59 registered in 1993 compared to 1452 over 60) are shown in Table 6.

Table 6 Percentage Causes of blindness in England, ages 16-64, 1990-91[21] and in Denmark, age group 20-59, 1993[23]

Cause	England 1990-91*	Denmark 1993
Diabetic retinopathy	12	37
Optic nerve atrophy	9	19
Higher optic pathway lesion		7
Myopia	3.4	5
Pigmentary retinopathy	11	5
Glaucoma	5	4
Age-related macular degeneration	11	4
Uveitis		4

**BD8 figures for England show 32% of "other conditions", and higher optic pathway lesions and uveitis are included in these.*

Although registration figures give useful information about the causes of blindness, they do not necessarily reflect the prevalence of visually impaired people in the community. The sobering North London survey demonstrated in a sample of 1547 people aged 65 and over that 30% had bilateral visual impairment (acuity less than 6/12), and 18% had better eye acuity less than 6/18 and 5.9% less than 6/60.[9] 72% of those with impairment were classified as potentially remediable, mostly due to cataract, although 9% had refractive error causing visual impairment (again<6/12 in one or both eyes). It seems even in established market economies there is much untreated pathology in the community.

1.1.6 Childhood Blindness

Childhood blindness is usually due to one of four factors: hereditary or genetic causes, intrauterine causes (such as congenital rubella), perinatal factors (such as retinopathy of prematurity) and childhood causes (such as corneal scarring as a result of measles and vitamin A deficiency).[24] The estimated prevalence and magnitude of the problem, from WHO figures for 1992,[25] is listed in Table 7.

Table 7 Childhood blindness

Region	Population 0-15 (million)	Blindness Prevalence (per 1000)	Etimated Number of blind children
Africa	240	1.1	264 000
Asia	1200	0.9	1080 000
Latin America	130	0.6	78 000
Europe/USA/Japan	240	0.3	72 000
Total			1494 000

The relative importance of the different factors varies across the world: childhood causes, in particular measles and vitamin A deficiency causing corneal scarring, are responsible for up to half the blindness in African children, while perinatal factors, in particular retinopathy of prematurity, may cause up to 20% of blindness in South America. Hereditary factors are most common in Europe, with higher standards of antenatal and perinatal care and better nutrition in childhood and have been cited as responsible for 30-50% of cases in the literature[24].

Table 8 lists the major causes of blindness as defined by anatomical site. It suggests that corneal scarring, which is largely preventable with measles immunisation, and diet and/or vitamin A supplementation in the case of vitamin A deficiency and measles, is the most important cause of blindness in childhood.[26] The trend of childhood blindness is that as socioeconomic factors improve there is less corneal scarring, and as perinatal care improves there is less retinopathy of prematurity, resulting in greater proportion of genetic diseases causing childhood blindness, as is currently the case in developed countries.

Table 8 Causes of childhood blindness by anatomical site

Site of abnormality	Estimated number blind	%
Corneal scarring/phthisis	500 000	33
Retina	300 000	20
Cataract	200 000	13
Optic nerve	100 000	7
Glaucoma	300 000	7
Other	300 000	20
Total	1 500 000	100

1.1.7 Trends in Low Vision and Blindness

The main trend is that the world's population is aging. The WHO estimates the world's population will increase from 5.8 billion in mid-1996 to 8 billion by 2025.[27] 380 million of these are over 65 years old, but by 2020 it is estimated that this will have risen by 82% to more than 690 million. Life expectancy was 48 years in 1955, 59 in 1975 and 65 in 1995. As a consequence there will be more age-related cataract and age-related macular degeneration as well as glaucoma. The incidence of diabetes is believed to be increasing in countries such as India, where many people are moving away from traditional diets to more western ones, and diabetic retinopathy may be a greater problem. Many of the preventable causes of blindness, such as corneal scarring are less important as socioeconomic factors and public health improve, and provision of cataract surgery remains the major priority in reducing world blindness.

Figure 1 graphically represents some of the trends of causes of blindness in England over the last 50 years from registration data, showing a decline in causes such as cataract and the increase in registration due to macular degeneration.

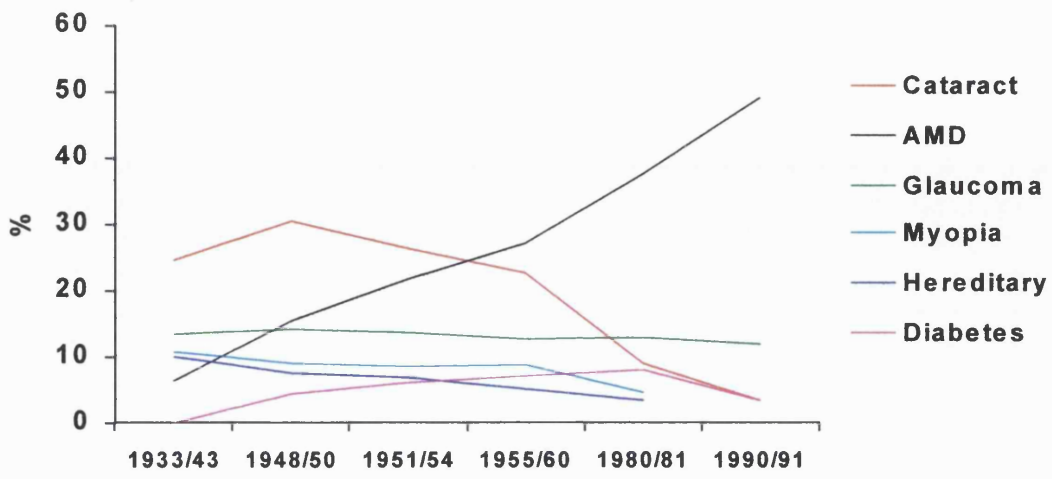
1.1.8 Summary

Blindness and low vision are increasingly prevalent in all populations in the world, with cataract the most important cause of world blindness. AMD is the most

important cause of blind and partial sight registration in the United Kingdom and seems to be increasing. The aetiology of both these conditions is largely unknown apart from age, and further knowledge about the genetic epidemiology would help in future research. Refractive error remains an important problem, not only because it is common and increasing in prevalence, but also because failure to correct it is still a significant cause of reduced vision in the community both in the western and developing worlds. There is evidence that the extreme end of the refractive spectrum (high myopia) is responsible for a significant proportion of blindness in some western countries, such as Ireland, where it is the second commonest cause of blindness in the working age population.[28]

Age-related cataract, AMD and refractive error were the main conditions investigated in this twin study, as they are common and the first two are important causes of visual impairment. In addition the phenotype or trait can be measured quantitatively, which improves the statistical power. Although glaucoma is an important disease, its prevalence (up to 2%) is not common enough for meaningful statistical analysis in a twin study of this size. There are many difficulties with diagnosis of glaucoma, as visual field analysis is time-consuming, expensive and difficult to interpret (a series of field analyses may be required to confirm repeatable field defects) and was not possible within the time constraints of this study. Intraocular pressure was not measured routinely, as the epitheliopathy caused by local anaesthetic drops may interfere with macular photographs (A.C. Bird, personal communication). Before current knowledge on the roles of genes and environment in the epidemiology is discussed for each of these three conditions, the use of twin studies, potential biases, statistical power and previous twin studies in ophthalmology are discussed in the next section.

Figure 1 Causes of blindness in England and Wales 1933-1991[11; 21]



1.2 Twin Studies

Quantitative genetics considers a phenotype as the sum of effects of both a genotype and an environment, and attempts to dissect the relative importance of each. Twin studies are based on the fact that MZ twins share identical genes, but DZ twins share, on average, 50% of the same genes. Assuming that MZ and DZ twins share the same common family environment (eg diet, school, age and position in the same family), any greater similarity between MZ twins is due to their additional shared genetic effect.

1.2.1 History of Twin Studies

Galton has been credited as the first scientist who recognised the potential of twins to study nature versus nurture: "There are twins of the same sex so alike in body and mind that not even their own mothers can distinguish them... This close resemblance necessarily gives way under the gradually accumulated influences of differences in nurture, but it often lasts until manhood".[29]

The first reports outlining the use of classical twin studies to compare concordance in identical and non-identical twin pairs have been quoted as published in 1924. Siemens, a German dermatologist, examined naevus counts and found a correlation of 0.4 in identical twins and 0.2 in non-identical,[30] and Merriman, an American educational pychologist, found the similarity in intelligence test results was markedly higher for identical than for non-identical twins.[31] However, Sorsby[32] reports a pioneer study by Jablonski in 1922, comparing similarities of refraction in 28 identical and 23 non-identical twins.[33] Subsequently, many hundreds of classical twin studies have been reported.

1.2.2 Uses of Twin Studies

In addition to the classical twin study of heritability, which compares disease concordance rates, or correlations of continuous traits, in MZ compared to DZ twins, there are other study designs which can be used to explore risk factors, disease frequency etc. Examples include:

1 Disease incidence, prevalence or outcome in twins compared to singletons
2 Co-twin cohort studies or controlled trials comparing disease rates in twins who are discordant for developmental, lifestyle, environment or medical care factors
3 Co-twin case-control studies comparing levels of exposure to potential risk factors in disease-discordant MZ pairs
4 Genetic linkage and association studies using DZ pairs as a special application of the sib-pair design
5 Drug trials and pharmacogenetic studies
6 Studies into gender effect

1.2.3 Identification of Twins

There are several ways of identifying twins, with different relative advantages and disadvantages. The "best" method in epidemiological terms is the population-based twin register as in nationwide registers in several Scandinavian countries.[34] These registers are difficult to set up and maintain, but the major advantage is that prevalence rates are comparable to that of the general population, which is important for generalising to the target population. Similarly twin sets systematically identified, such as military recruits, have been used. Other methods include media appeals for twin volunteers, which may result in unrepresentative prevalence figures but do not require a register. Appeals for twins with a particular disease or characteristic, or case series of twins presenting to clinicians with a disease of interest, may result in bias due to selection of twin pairs.

1.2.4 Potential biases in twin studies

Potential biases in twin studies may be divided into selection and ascertainment bias, and confounders also have to be considered.

Selection Bias

Selection bias occurs when there is a distortion in the effect measure that results from procedures used to select subjects, and will always be present when the ascertainment of twins is non-random.

Twin ascertainment bias

The method of selection of twins may influence concordance due to concordance-dependent bias. It has been shown, for example, that many early twin studies of diabetes mellitus and multiple sclerosis were flawed because case-series were used and concordant twins with disease were more likely to be ascertained than discordant pairs, resulting in an upward bias of concordance.[35]

Volunteer bias

Lykken et al have identified the "rule of two thirds" in twin volunteer samples[36]: two-thirds will be women, two thirds young and two thirds MZ. Since the proportion of twins in the population is approximately 1:2 MZ:DZ, and the sex distribution of twins should be approximately 1:1 for male:female twin pairs, it can be seen that volunteers are not completely representative of the whole twin population.

Geographical bias

In a volunteer study, there is a potential that twins widely separated geographically may be less likely to volunteer, and that twins living near each other may do so because of greater similarities to each other.

Information bias

Information bias arises from errors of measurement of all variables, including risk factors, outcomes and confounders, and therefore occurs in all epidemiological studies, especially if subjective measures are used. Random measurement errors would be expected to bias genetic risk estimates to be lower than they should be. However, some errors such as recall bias may affect concordance; recall may be more common for a concordant effect, and telescoping, in which recalled distant dates are moved forward towards the present, may bias recall-related results. Similarly non-independent ascertainment in which co-twins of affected probands are more likely to be ascertained than co-twins of unaffected individuals, may cause bias. This is likely to be reduced if there is comprehensive ascertainment.

Missing data may cause bias if there are systematic reasons for the absence of data. In addition, data may need to be considered as censored if there is any evidence that, for example, MZ twins develop a disease earlier than DZ twins with the risk of a spurious genetic risk being generated.

Twin studies can reduce this bias by using objective measures wherever possible, interviewing the twins separately when recall is required, and recruitment of twins unaware of any hypotheses being studied.

Confounding

A variable distinct from a disease being studied might result in a spurious association attributed to genetic causes: for example if smoking is more concordant in MZ than DZ twins then a smoking-related disease might be given a higher genetic correlation than is really the case. Similarly, if MZ and DZ twin groups were imbalanced, for example in age, then an age-related condition might give rise to a spurious genetic association.

Table 9 below lists some of the advantages and disadvantages of the relative twin study designs.[37]

Table 9 Different twin study designs.

Design	Advantages	Disadvantages
Clinical Case Series	No requirement for twin register	No estimate of disease prevalence
	Efficient, particularly for rare diseases	Selective ascertainment of concordant pairs
	Comprehensive for twin status and zygosity	Arbitrary and inflexible case definition
Volunteer twin series	No requirement for twin register	Bias towards concordant pairs
	Higher response to surveys and tests	Unrepresentative prevalence figures
	Flexibility in case definition	Zygosity may be incompletely confirmed
Population-based twin register	More representative prevalence figures	Often difficult to set up and maintain
	No inherent bias towards concordant pairs	Incomplete response may bias prevalence
	Flexibility in case definition	Zygosity may be incompletely confirmed
Record linkage to routine data	Highly efficient if available	Ascertainment may be incomplete
	Usually representative	Not immune to biases in concordance
	Comparison of twins to singletons	Inflexible case definition
Questionnaires or tests	Ascertainment systematic (may be incomplete)	Non-response may bias prevalence
	Flexible case definition (& objective if tests)	Inefficient (especially if tests needed)
	Less prone to concordance-related biases	

1.2.5 Assumptions of Twin Studies

Generalisability of Twin Studies

Twins are on average 1000g lighter than singletons and they are born approximately three weeks pre-term with greater neonatal complications. It has been shown that twin neonatal morbidity and mortality is higher than singleton, and so results from twin studies in paediatric problems cannot necessarily be generalised. For other

diseases and traits studied, it is important to test that the study disease or trait is the same in twins as in singletons, and to ensure that there is no association between disease and zygosity, to try to support generalisability of results to the general population.[38] In general, most results confirm the generalisability of twin studies, such as the Danish study of mortality in twins over the age of six which was found to be the same as singletons[39], or those looking at diabetes.

Equal environment assumption

The equal environment assumption is the most important assumption of the classical twin study.[38; 40] It assumes that both MZ and DZ twins share their common environment to an equal extent: they share the same womb, the same early environmental risk factors and are raised in the same family at the same age. This is also the most-criticised assumption of twin studies,[41] and may be of particular concern in behavioural and psychological studies, where MZ and DZ twins might not be treated the same. However, studies looking at mislabelling of zygosity (where twins have been reared assuming the wrong zygosity) and looking at MZ twin pairs who were reared apart or who did not look identical have all shown behavioural similarities more like the true zygosity, supporting the equal environment assumption.

Foetal programming: the Barker hypothesis

The twin model assumes the greater similarity between MZ and DZ twins is due to genetic factors, but recently some doubts have been cast by the "Barker hypothesis" of foetal programming.[42] This hypothesis suggests that the known association between low birth-weight and diseases such as hypertension, non-insulin dependent diabetes mellitus and coronary heart disease, reflects impaired foetal nutrition or oxygen supply which in itself is not thought to be genetic. The foetus is "programmed" by the undernutrition to alter metabolic balance, possibly through changes in the hypothalamic-pituitary axis, leading to later risk of cardiovascular disease. MZ twins are a little lighter and born a few days earlier than DZ (or more specifically monochorionic twins compared to dichorionic twins) and would therefore

be expected to have more of the diseases mentioned above (and more than singletons), which has not been shown in population studies. However, it is likely that with greater vascular anastamoses monochorionic twins may share more hormonal factors, resulting in more similar programming, causing the greater similarity in tendency to disease.[43] A twin study has confirmed the association between low birth weight and high blood pressure, with no difference between MZ and DZ twins, suggesting the difference is due to foetal environmental changes, although the size of this effect is relatively small.[44]

It has been shown that the angle of branching in retinal vasculature is related to birth weight (and this reflects a higher later risk of cardiovascular disease).[45] However, other studies (of the same population of subjects born in North Hertfordshire between 1920 and 1930) have failed to find an association between birth weight and nuclear cataract,[46] intraocular pressure and glaucoma,[47] and visual acuity and age-related eye disease.[48]

Twin-twin interaction

Another assumption of twin studies is that there is no twin-twin interaction, in which the actual zygosity may in some way influence the subsequent behaviour or characteristic being measured. This again may be of more relevance to behavioural studies of twins in childhood than to the present eye disease study. Twins have delayed language development and more behavioural and less emotional problems compared to singletons,[38] but these generally resolve by adolescence.

Assortative mating

Most genetic studies assume the absence of assortative mating, which means non-random mating. Assortative mating assumes that "birds of a feather flock together", and for a study of age-related eye diseases such as cataract and age-related macular degeneration is a reasonable assumption that mating is random. However, it has been shown that, for example, spouses do not mate randomly for height and tall people tend

to marry tall people. This could reduce estimates of heritability, as MZ correlations would be unaltered but DZ correlations raised because they are first-degree relatives.

1.2.6 The classical twin study

A greater similarity between MZ twins than DZ twins is explained as being due to genetic effects, assuming equal environmental influences. In the classic method, the difference between intraclass correlations for MZ twins and those for DZ twins is doubled to hestimate the heritability: this is known as the Falconer formula.[49] The remaining population variance can then be attributed to environmental effects. These estimates have low power and large standard errors and do not make use of information available in variances and covariances. In recent years, model fitting has become standard in twin research.[50] Model fitting approaches involve solving a series of simultaneous structural equations in order to estimate genetic and environmental parameters that best fit observed twin correlations. Model fitting has a number of advantages, including making assumptions explicit, and estimations of goodness-of-fit and quantitative genetic parameters and their standard errors. In addition, the fit of different models and multiple variables can be analysed in addition to a single variable (multivariate versus univariate analysis).

The observed phenotypic variance of a population (V_P) can therefore be separated into the variance due to genetic and environmental components. Additive genetic variance (V_A) is the variance that results from the additive effects of alleles at each contributing locus. Dominance genetic variance (V_D) is the variance that results from the nonadditive effects of two alleles at the same locus summed over all loci that contribute to the variance of the trait. Shared (common) environmental variance (V_C) is the variance that results from environmental events shared by both members of a twin pair (eg rearing, school, neighbourhood, diet). Specific (unique) environmental variance (V_E) is the variance that results from environmental effects that are not shared by members of a twin pair and also includes measurement error.

Expressing it as an equation, $V_P = V_A + V_D + V_C + V_E$

If each of these effects (A, D, C & E) is conceived of as a latent factor with zero mean and unit variance, h, d, c and e are factor loadings of the observed variable on the latent factors, indicating the degree of relationship between latent factors and the phenotype. Because the latent factors have unit variance, squaring the factor loading yields the variance explained by various components ($V_A = h^2$, $V_D = d^2$, $V_C = c^2$, $V_E = e^2$). Therefore, $V_P = h^2 + d^2 + c^2 + e^2$

These contributions are often reported as the standardised form, which is done by dividing the specific variance component by the total phenotypic variance (eg $h^2 = V_A / V_P$, where h^2 is the heritability). Further details of analysis of twin studies are given in the Methods of Analysis section of the Subjects and Methods chapter (Section 2.8).

1.2.7 Adoption Studies

Another tool to examine whether there is a significant genetic effect is the adoption study of twins separated at birth and reared in different environments. This means the equal environment assumption of the classical twin study is not required. If there is greater concordance for MZ and DZ twins reared apart than for unrelated individuals, and this concordance is greater in MZ than DZ twins, a genetic aetiology is suggested. These adoption studies, however, are rare as there are few twin pairs separated at birth or shortly after.

1.2.8 Statistical power in twin studies

Many twin studies reported in the literature have been case reports or small series of monozygotic twins concordant for the disease or trait described. While they are in themselves interesting and are used to suggest genetic influence, they are not proof of genetic effect as MZ twins share the same early family environment as well as genetic influences: a similar study of DZ twins is required to compare concordance rates to confirm a genetic effect.

Many studies comparing MZ and DZ concordance, however, have been too small to provide sufficient power to assess heritability. The analysis of twin studies was revolutionised in 1970 by publication of a classical paper by Jinks and Fuller,[51] further extended by subsequent researchers. For a common trait or disease showing high heritability, at least 200 pairs of twins need to be examined to obtain accurate estimation of the heritability. For less common traits or those with lower heritability, many more are required. For rare diseases, it may be more efficient to study small selected samples of diseased individuals, but these need to be drawn from a large unselected population (hence the use of volunteer and population twin registers[34]) and care is needed to avoid ascertainment bias. Early medical twin studies which ascertained diseased twins through clinics and therefore potentially doubly ascertained concordant twin pairs came up with much higher heritabilities than are believed to be true (eg diabetes and multiple sclerosis).[35]

1.2.9 Twin Studies in Ophthalmology

There have been several twin studies of eye disease, with the larger ones in particular related to refractive error. Many of the other studies have been case reports or small series of MZ twins concordant for the eye disease in question. As suggested above, these may provide some insight into potential genetic causes, but are no proof of this.

Refractive error

There have been many studies of the heritability of refractive error, with the earliest dating back to Jablonski in 1922.[33] The early studies confirmed more similar refractions in identical compared to non-identical twins. More recent studies using quantitative techniques to estimate the heritability have mainly concentrated on myopia, summarised in Table 10. However many of these studies were small and often selected myopic twins (with selection criteria not clearly stated).

The Finnish study of myopia by Teikari[52; 53] was based on a population register, but refraction data came from spectacle correction sent in from postal questionnaire, so

discordant twins with one not requiring correction might be underestimated. Nance's study of Norwegian twins was also a population-based postal questionnaire, and required completed questionnaires from twins and their spouses before inclusion. Unaffected individuals were therefore included, although it is not clear whether actual spectacle correction or simply the need for correction was assessed.[54]

The largest study of refractive error to date by Sorsby et al[32] studied twins with all refractive errors (but "twins available for study" is the only selection criterion mentioned). He documented the refractions meticulously in his monograph (Figure 2), enabling subsequent calculation of the heritability of 0.87.[55] Most studies estimated a high heritability for myopia, between 0.58 and 0.87. The low heritability in the study by Angi et al[56] of 0.11 was unusual in that children studied were aged 3-7 (myopia usually appears between 6 and 15 years of age) and children with form-deprivation myopia due to congenital cataract were included. Most twin studies, however, have estimated a high heritability for myopia.

Figure 2 Front cover and page from Sorsby's classic twin study of refraction in twins

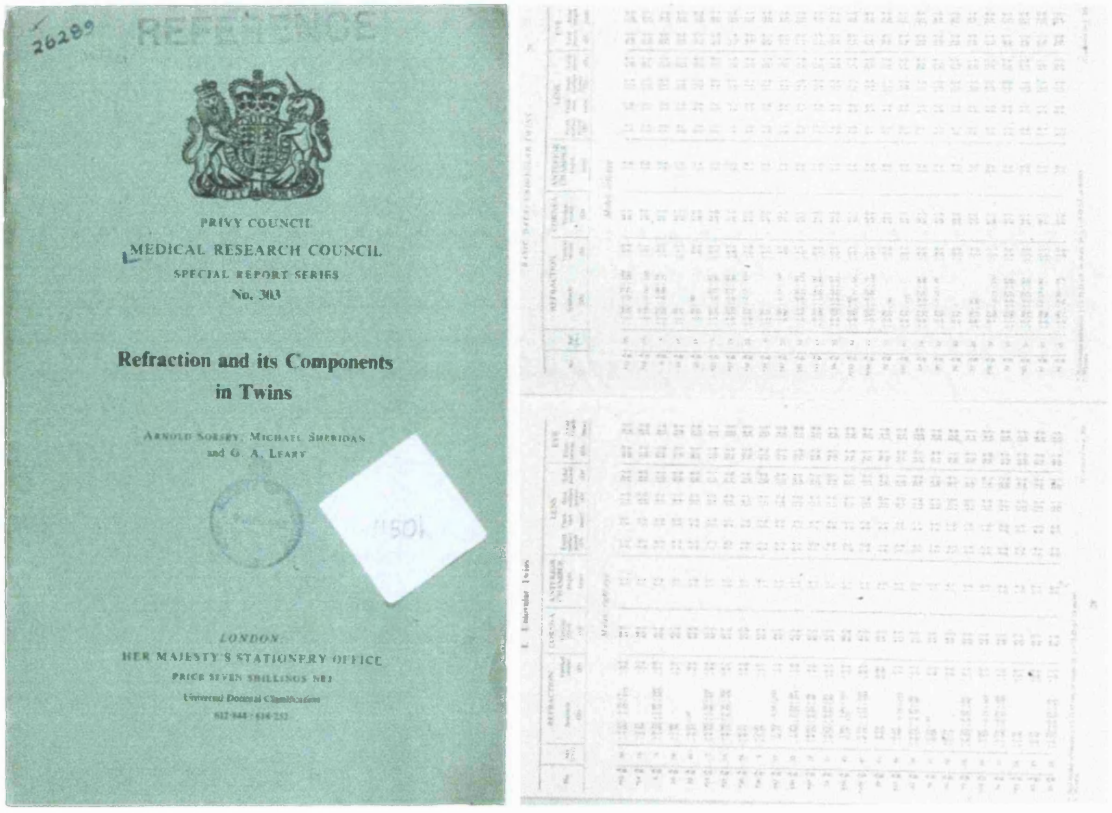


Table 10 Twin studies of myopia

Study	h^2	No. pairs	Age	h^2 formula	Comments
Sorsby, 1962 [32]	0.87	MZ=78 DZ=40	4-63	$(r_{mz}-r_{dz})/1-r_{dz}$	Calculation by Goss et al, 1998 [55]
Nakajima, 1963[57]	0.83	MZ=39 DZ=10	12-17	$(\Delta_{dz}-\Delta_{mz})/\Delta_{dz}$	
Kimura, 1965[58]	0.80	MZ=33 DZ=16*	15-20	$(\Delta_{dz}-\Delta_{mz})/\Delta_{dz}$	
Awetissow, 1980 [59]	0.70	MZ=61 DZ=51	?	$2(r_{mz}-r_{dz})$	
Hu, 1981 [60]	0.61	MZ=49 DZ=37*	7-19	$(r_{mz}-r_{dz})/1-r_{dz}$	
Nance, 1982[54]	0.92	MZ=86 DZ=61	Adult	"tetrachoric correlation"	
Knobloch, 1985 [61]		MZ=18 DZ=8	Adult		Adoption study
Lin, 1987[62]	0.25	MZ=90 DZ=36	7-23	$2(r_{mz}-r_{dz})$	
Teikari, 1991 [53]	0.58	MZ=54 DZ=55	30-31	$2(r_{mz}-r_{dz})$	
Angi, 1993[56]	0.11	MZ=19 DZ=20	3-7	$2(r_{mz}-r_{dz})$	

Abbreviations: h^2 = heritability, * = different as well as same-sex DZ twins included, r_{mz}= intrapair correlation coefficient for MZ twins, r_{dz} = intrapair correlation coefficient for DZ twins, Δdz & Δmz not defined by authors.

There have been fewer twin studies of hyermetropia and astigmatism. Sorsby's twin study[32] included hypermetropes in the calculations observed in the table above, and for astigmatism established a high concordance for MZ twins versus DZ twins. He did not quantify this further. Teikari et al sent a postal questionnaire to 1200 twins aged 30-31 from the Finnish population registry in a study of hypermetropia[63]; data from 191 twin pairs suggested a heritability of 0.75. Nance's Norwegian twin study of 65 twin pairs suggested a heritability of 0.82.[54]

The Finnish twin registry's study of astigmatism in 72 twin pairs[64] found no difference between MZ and DZ concordance, arguing against a heritable component. Although these studies were performed on a sample of the twin population, they only included data on twins who both sent in spectacle refractive error. This potentially underestimated discordant twin pairs, and also the amount of astigmatism as many low astigmatic individuals are not prescribed their full correction. The Norwegian study, based on 223 twin pairs with one or both reporting astigmatism, suggested a heritability of 0.62.[54]

There have been no twin studies of refractive error using objective measures of refractive error in combination with modern quantitative genetic modelling techniques to estimate heritability and model the relative roles of additive and dominant genes as well as common and unique environment. In addition, previous studies have not been population-based or included large enough numbers for sufficient statistical confidence.

Cataract

There have been no studies on age-related cataract in twins, as far as can be established. Congenital cataract in MZ twins, concordant in galactokinase deficiency[65] and aniridia[66] or discordant in a sporadic case[67] have been reported. While genetic congenital cataract is important in the industrialised countries, little is known about the relative importance of genes and environment in age-related cataract.

Age-related macular degeneration

There has been no published series involving a population based twin study, but several individual case reports of concordant exudative AMD in monozygotic twin pairs have been reported [68-70]. Klein et al reported 9 MZ twin pairs: 8 had similar advanced AMD fundal changes and visual impairment and in the 9th pair one had exudative AMD and the other confluent large soft drusen[71].

The largest twin study by Meyers et al[72; 73] reported 134 twin pairs (98 MZ and 38 DZ) and two triplet sets examined prospectively. 25 of 25 MZ twin pairs with AMD were concordant, compared to only 5/12 DZ twin pairs, suggesting an important genetic contribution to AMD. Although initial recruits knew the purpose of the study was study of AMD, ascertainment bias was reduced for the majority of subjects by not informing them of the reason for eye study, and twins with and without AMD were examined.

Recently an Icelandic population-based study of 50 MZ twin pairs suggested a high concordance ("90%" – including those with and without ARM) in these twins compared to twins and their spouses ("70%").[74] This is not an accepted statistical method, but 9 pairs of the 50 MZ twins were concordant for ARM and 5 discordant (pairwise concordance 0.78), compared to 2 concordant (and 14 discordant) spouse-pairs of the 47 spouses (concordance 0.22). Although this suggests a genetic influence as opposed to environmental influence later in life, it does not exclude the childhood shared environment of twins, which could explain the greater similarity. Figures from DZ twin pairs are needed for comparison to exclude shared family environment effects.

Seddon et al[75] have reported in abstract form a potentially large population-based twin study contacting the 14000 elderly (69-79 year old) twins in the Veterans' Twin Study and asking about a diagnosis of AMD; retinal photographs of both pairs of any twins responding positively will be graded. Although the study will be biased towards those with late AMD and visual loss, it may be the only way of collecting enough twins with disease to allow confident statistical analysis, and the results are awaited with interest.

Glaucoma

Cases have been reported of MZ twins concordant for congenital glaucoma,[76] primary open angle glaucoma,[77-79] low tension glaucoma[80] and simultaneous closed angle glaucoma.[81] Genetic influences have been suggested for intraocular pressure, which

was more similar in 61 pairs of MZ twins than 32 pairs of DZ twins,[66] and for the cup/disc ratio in a study of 10 MZ and 7 DZ normal twin pairs.[82]

Teikari linked the Finnish national twin registry and the registries of those receiving free medication for chronic open angle glaucoma and hospital discharges,[83] and found 114 cases of chronic open angle glaucoma, primary or "capsular" (pseudoexfoliation), from 108 twin pairs (29 MZ and 79 DZ). Only 3 MZ and 3DZ pairs were concordant, resulting in a heritability of 13%, which is surprisingly low. Other diseases included in the record linkages[84] were not common enough (or required no hospital admissions/free medication) to allow meaningful analysis.

An Icelandic study examined 20 twin pairs (7 MZ and 13 DZ) at least one of whom had a diagnosis of closed angle glaucoma from the Finnish registry.[85] Only two (MZ) pairs were concordant.

Strabismus

Twin studies in strabismus have been reviewed by Paul and Hardage, who summed concordance from the various studies included in that review and found that MZ twins had a concordance of 73% and DZ 35%,[86] suggesting a role for genetic factors. They report the largest series of 126 pairs studied by Waardenburg in the 1950s (which included heterophorias), who found 83% of MZ twins concordant compared to only 9% of DZ twins. A more recent population-based family study showed that in multiple births a child had a 17-fold increased risk of exotropia if their sib was exotropic, and MZ concordances were higher than DZ for for esotropia and exotropia.[87]

Other eye conditions

Many case reports of MZ twin pairs concordant for diseases have been published, such as giant retinal tears,[88] Brown syndrome,[89] and steroid response,[90] and some discordant for disease such as aniridia,[91] keratoconus[92] and retinoblastoma.[93] Classical twin studies of lens thickness in diabetes,[94] iris colour changes in

childhood[95] and iris characteristics[96] have been published. A small study of only 10 MZ twin pairs examined macular pigment, which may be related to macular degeneration.[97] The results were somewhat confusing and identified 5 pairs as being very similar and 5 pairs different, concluding that macular pigment levels are likely to be environmental in origin, which it does not prove at all.

Adoption studies

There has only been one eye study in adopted, separated twins, from the well-known University of Minnesota project.[98] It found (of 18 MZ and 8 DZ pairs) more similar refraction in MZ (concordance "75%") than DZ (concordance "50%"), similar time of onset in 3 pairs of MZ twins concordant for esotropia, and very concordant cup:disc ratios in the MZ twins.

1.2.10 Summary

Twin studies are a unique method of quantitatively assessing the different roles of genes and environment. Care is required in selection of twins to avoid falsely high levels of concordance due to dual ascertainment of affected twin pairs. Assumptions of the twin model, in particular that of equal environment, have been tested and found generally to be true. Twin models have been used in ophthalmology, particularly for refractive error, but there have been few population-based studies of sufficient power to use modern quantitative genetic modelling techniques to assess heritability and subdivide the constituents of the variance. There have been no twin studies of age-related cataract previously, and twin studies of AMD have concentrated on end-stage disease with serious visual loss already present. The choice of the three phenotypes to be used in this twin study therefore seems justified.

The next section details current knowledge of the epidemiology and genetics of the phenotypes to be asssessed in this study.

1.3 Epidemiology of Refractive Error

Much has been written about the epidemiology of myopia, which is seen as an increasing public health problem. There has been less research on astigmatism and less still on hypermetropia. There has been long-standing debate on the relative importance of "nature versus nurture" in refractive errors, particularly myopia.[99] Family studies on ocular refraction and its components showing a high degree of concordance (for example in the work of Arnold Sorsby[100] and twin studies in Finland[52]) have suggested genetic factors are important. However, recent dramatic changes in prevalence, particularly in the Far East,[101] have moved the focus of research towards environmental causes.

Myopia tends to be studied as a separate entity rather than being treated as part of the spectrum of refractive errors, and as no "refractive error genes" have been identified, it is not clear whether this approach is valid. There is a spectrum of refractive error, approximating a normal distribution with leptoskurtosis (the high myopes), such as that seen in the Baltimore Eye Survey from the late 1980's, a cross-sectional population survey of those aged 40 and older.[102] The Baltimore Eye Study showed the odds ratio for 12 years of education was 1.36 in myopia and 0.67 in hypermetropia.[102] This again suggests that artificial categorisation of spherical equivalent into myopia and hypermetropia may be inappropriate: they are probably subject to the same spectrum of genetic and environmental influences.

1.3.1 Epidemiology of Myopia

Introduction

Myopia is the most common eye condition, and its costs are enormous. Defined as a state of refraction in which parallel rays of light are brought to focus in front of the retina of a resting eye,[103] it has been classified as either physiologic or pathologic (higher levels of myopia associated with other pathology). Myopia may rarely be

congenital, is usually school-age onset (ages 6-17) and less often adult-onset. Coincident with the increasing levels of refractive surgery for myopia, there have been advances in the understanding of the control of eye growth. Animal studies have suggested that eye growth is regulated by the quality of retinal image ("emmertropisation"),[104] and if humans have the same mechanism (as is likely) then human myopia may occur if a child inherits a dysfunctional emmertropisation mechanism.[105]

Prevalence of myopia

Different studies are difficult to compare as many have used an arbitrary cut-off of 0.25D or 0.5D, or one based on acuity, and many have not involved cycloplegic refraction which may overestimate myopic error particularly in children and young adults due to the pseudomyopia of accommodation.[103]

Population studies in the United States in the early 1970's estimated an adult prevalence of between 17.5% (in the Framingham Eye Study[106]) and 25% (in NHANES: the National Health and Nutrition Examination Survey[107]). More recent population studies performed in the late 1980's suggest that myopia is more common: the Baltimore Eye Survey estimated a prevalence in 43-54 year-olds of 48.1% compared to 25.5% in NHANES.[102] Similar figures were obtained by the Beaver Dam Eye Study which showed a decreasing prevalence of myopia with age from 43% in the age group 43-54 years to 14.4% in those over 75.[108] It is unclear whether this reduction is a cohort effect or a true effect of less myopia with age. NHANES suggested that the prevalence of myopia rises until the early teens, then is fairly stable until the forties and then decreases.[102] A study of 208 selected myopes from an eye clinic followed for 20 years or more suggested the change per patient age decade were: 20s, -0.60D; 30s, -0.39D; 40s, -0.29D; 50s, +0.28D; 60s, +0.41D.[109]

Prevalence does seem to be rising, particularly in the Far East. A study in Singapore, for example, demonstrated a rise in the prevalence of myopia in young adults from 26% to 43% over a decade, reaching 65% in university graduates.[101] This has been replicated elsewhere. Since genetic factors cannot change in a generation and

generally change very slowly, there must be an important environmental influence causing this change in prevalence, which may be mediated through susceptibility genes.

Incidence of myopia

There are little population-based cohort data or longitudinal data on the incidence and progression of myopia. Studies have suggested that myopia stops progressing on average around the age of 15-16, but that there is a gradual elongation of axial length and increase in myopia in some people after this.[103] The Beaver Dam Eye study has examined changes in refractive error over a 5 year interval. Changes in women were +0.23 dioptres in those aged 55-64, -0.01 in those aged 65-74 and –0.37 in those over 75 years.[110] This is likely to be due to the induced myopia of nuclear sclerosis.

Family Studies and Twin Studies

Numerous family studies have shown a strong association of myopia in families, such as the Framingham Offspring Eye Study.[111] This large cross-sectional study determined a prevalence of myopia (of –1.0D or more) in 57% of those aged 23-34 and 20% in those over 65, and found the strength of the sibling association depended on the age difference between youngest and oldest. An odds ratio of 5 was found if there was only 2 years' age difference, 3.9 if the difference was 5 years, and 2.5 if it was 10 years. If the presence of myopia was purely genetic, one would expect the odds ratio to be the same whatever age difference, so this suggests siblings nearer in age to each other share some more common environmental effects. This study also confirms the strong association between age and myopia.

The Orinda Longitudinal Study of Myopia, a community-based study of a cohort of children aged 6-14, has suggested that children with two myopic parents have longer eyes and less hyperopic refractive error than children with only one myopic or no myopic parents.[112] This may mean that genetic factors are important, with growth commencing from a different starting point in those who are myopic. Their seven-

year follow-up will help answer some of the questions regarding rate of growth of the eye in myopes compared to normals.

Some authors, misunderstanding twin studies, have suggested that "unless twins are separated at birth and brought up in contrasting environments they provide no unambiguous genetic information on the etiology of their similar refractive outcomes".[113] This is wrong: twin studies comparing MZ and DZ concordance have suggested that additive genetic factors are important in development of myopia.[32; 53; 54; 56-60; 62] The one adoption study[61] confirmed this finding. The pioneering twin study was by Jablonski in 1922 in which he noted similar refraction in MZ twins and more different refraction in DZ twins.[33] Results of the twin studies are summarised in the section on Twin Studies in Ophthalmology (Section 1.2.9), with the majority finding high heritabilities of 60-90%.

Genetic studies in myopia

No genetic mutations have been described for simple myopia so far, but recent genomewide scans have identified loci for familial high myopia,[114; 115; 115] raising the possibility of future identification of abnormal genes in refractive error. Mutations causing syndromes with associated myopia such as Col1 and Col2 mutations in Stickler syndrome have also been described. These are all obvious candidate genes for population studies of myopia, and raise the possibility of genetic predisposition to myopia.

Environmental risk factor studies

The "use-abuse" theory states that close work produces myopia, and this is reflected in the higher prevalence of myopia in people who are more intelligent, highly educated and of higher socioeconomic status.[103] Several cross-sectional studies have demonstrated a strong association between myopia and intelligence or years of school attendance. For example an Israeli study of more than 157 000 male military recruits aged 17-19 showed a prevalence of myopia of 8% in the group with the lowest intelligence scores and 27.3% in the highest, and 7.5% in those who completed

schooling of eight years or less compared to 19.7% in those who completed more than 12 years.[116]

The incidence of myopia rises between the ages of 6 and 14, as children attend school and start reading, and the increasing amount of myopia in the Far East may reflect the rising educational attainment levels there. It has been observed that myopia rapidly develops when remote communities are opened up and formal education occurs: for example there was virtually no myopia in the parents and grandparents of Alaskan Eskimos but a prevalence of 58% was observed in the offspring.[117]

Another study suggesting that close work is important was one conducted in Israel in two groups of schoolboys of identical genetic background: myopia prevalence was 81% in orthodox boys compared to 27% in those from general schools.[118] This could be due to the considerably greater time spent by the orthodox boys studying tiny print commentaries, and was not observed in girls who do not have the same amount of close reading.

More recently ambient light at night has been suggested as an additional environmental factor,[119] although the results from this study have recently been cast into doubt and it may be that myopic parents are more likely to leave night lights on.

Treatment

While the treatment of myopia has been refractive correction using spectacles or contact lenses, and now surgery (Radial Keratotomy, intrastromal corneal rings, phakic lens implants) and laser (excimer Photo Refractive Keratectomy and LASIK), there is increasing research on ways of trying to prevent progression using bifocal lenses, atropine eye drops (to block accommodation), intraocular pressure-lowering agents and drugs,[103] none of which are being used in routine clinical practice yet.

Summary

The two strongest risk factors for myopia are family history and close work. It seems likely that a combination of close work in a genetically susceptible individual causes myopia to develop. This correlates well with animal models and experiments in myopia. The exact inheritance and gene-environmental interaction remain unanswered, and gene-linkage studies will further unravel the continuing nature versus nurture debate.

1.3.2 Epidemiology of Hypermetropia

While there has been some research into hypermetropia in infancy,[120] there has been little research in adults. Hypermetropia or hyperopia can be defined as that form of refractive error in which parallel rays of light are brought to a focus some distance behind the retina.[121] Most cases are classified as simple hypermetropia, with relatively few having pathological hypermetropia associated with other conditions. 2-3D of hypermetropia is present in most infants in a normal distribution, which decreases steadily during early life as emmertropisation occurs.

Prevalence of hypermetropia

The prevalence of hypermetropia seems to have remained fairly constant, unlike myopia. Fifty years ago Duke Elder estimated some 50% of the population to be hypermetropic.[121] Using the population-based studies of people over the age of 43, the age, gender and education-adjusted figure for the Baltimore Eye Study was 43.9%[102] and the Beaver Dam participants 49.0%,[108] both using a definition of >0.5D.

Incidence of hypermetropia

There are no longitudinal studies of hypermetropia, so the natural history and the incidence of hypermetropia are unknown.

Family Studies and Twin Studies

Early family studies suggested either a dominant inheritance or an "irregular" dominant effect,[121] but more modern studies such as the Framingham Offspring Eye Study have only reported their myopia findings[111] and have not reported their hypermetropia data. Sorsby's classic twin study in fact included mainly hypermetropic twins - no pairs in his series of 40 DZ twins and only a quarter of the 78 sets of MZ twins were myopic.[32] Reanalysis of his data on right eyes suggests an even higher concordance for hypermetropia than the overall refraction concordance of 70% for MZ and 30% for DZ twins: the hypermetropia concordance is 83% in MZ twins and 34% in DZ twins, suggesting important genetic effects.

The only twin study reporting on hypermetropia was a Finnish study of 191 pairs of twins who returned a hyperopic prescription in a questionnaire survey of all their (1200) twins aged over 60.[63] This study estimated a heritability of 0.75, similar to that of myopia, although they do not state their definition of hypermetropia and again it is biased as only those wearing distance spectacles could have returned a prescription, presumably excluding some of those with a low dioptric power.

Genetic studies in hypermetropia

There have been no genetic studies into human hypermetropia, that this author has identified.

Environmental risk factor studies

There have been no specific risk factor studies for hypermetropia alone, but as stated above the Baltimore Eye Study suggested the odds ratio for years of education was 1.36 in myopia and 0.67 in hypermetropia, suggesting hypermetropia is inversely associated with education (opposite to myopia).[102]

Treatment

Treatment of hypermetropia is often not required if there is no associated strabismus or amblyopia in children and if accommodation can overcome the refractive error. However, as presbyopia develops, many hypermetropes are blurred for distance vision and require spectacle or contact lens correction. There are now algorithms being developed for excimer laser treatment of hypermetropia.

Summary

Genetic factors are important in the development of hypermetropia, and any definite environmental factors are unclear. It seems that hypermetropia and myopia are part of the same spectrum and that abnormal emmertropisation and/or close work may result in hypermetropia or myopia.

1.3.3 Epidemiology of Astigmatism

Astigmatism, from the Greek "a", absence; and "stigma", point, occurs when parallel rays of light entering the eye are not focused on a single point. Both corneal factors and non-corneal factors (such as lenticular changes) may contribute, although it is felt that larger degrees of astigmatism are largely caused by an aspheric anterior surface of the cornea.[122]

Prevalence of astigmatism

The Baltimore Eye Study suggested a prevalence of around 32% for more than 0.5D of astigmatism,[102] and other studies have suggested a frequency of around 20% with equal or more than 1.0D.[122] Astigmatism is very common in infancy (mainly against the rule) and gradually decreases over the first four years of life.[120] It is not so clear when the mainly with the rule adult astigmatism develops, but adult studies suggest it

is fairly stable until the sixth and seventh decade when the prevalence increases again.[102] There are no adult incidence data.

Family and Twin Studies

There have been mixed messages from family and twin studies. Sorsby's twin study concluded that astigmatism, like the other components of refraction, had a strong genetic basis.[32] However, the Finnish twin study (again using questionnaire data from twins who wore glasses) concluded that the correlations for MZ and DZ twins were not different and so there was no genetic component.[64] Mash et al used family studies and concluded that heritability of astigmatism was low,[123] but recently an Italian group used complex segregation analysis to identify evidence for a single major autosomal dominant locus.[122] This requires confirmation as only one of their two statistical methods was able to identify this model over any others.

Genetic studies in astigmatism

There have been no genetic studies into astigmatism, apart from the family studies described above.

Environmental risk factor studies

There is little in the human literature, but chick studies have suggested the developmental decrease in astigmatism appears more dependent on mechanical factors rather than visual ones (unlike myopia), and that they seem unable to compensate for imposed astigmatism using accommodation.[124]

Treatment

The mainstay of treatment is spectacle correction, but gas-permeable and toric soft contact lenses are being increasingly used. There is also considerable research into

astigmatism correction using the excimer laser (PRK or LASIK) using erodable masks.

Summary

The aetiology of astigmatism is uncertain, with the relative role of genes and environment being undetermined as the literature is conflicting about their relative importance.

1.3.4 Conclusions: genes and environment in refractive error

Genes seem to explain much of the variance of myopia and hypermetropia in the population. However environmental factors are still important and it seems likely that the amount of close work has a major influence on development of myopia, which is becoming a significantly worse problem every decade. The aetiology of astigmatism is less certain, and may involve genetic influences, but more research in this area is required.

1.4 The Epidemiology of Cataract

Cataract is a major public health issue. In 1997 the World Health Organisation estimated there are 38 million people blind in the world, approximately half due to cataract. On current projections there could be an estimated 50 million people blind due to cataract by 2020.[125] Cataract affects every country: in the industrialised world, there are 1.5 million cataract extractions performed each year in the USA at vast cost, and currently there is no treatment other than surgery.[126] Recently, the British Government has recognised the need to increase the amount of cataract surgery in the UK from 175 000 to 250 000 a year in its Action on Cataract initiative.[127]

1.4.1 Definition

Cataract is defined as an opacification of the crystalline lens of the eye. Dolin, in his excellent chapter on the epidemiology of cataract, has suggested that a cataract is opacification with "severe" vision loss; the term for cloudiness of the lens before this loss of vision is lens opacity.[128] However, there is no clear cut-off point and the distinction, in terms of prevalence and aetiologic epidemiological research, is artificial: in this thesis a definition of cataract as any opacification within the lens will be used.

1.4.2 Classification

Cataract can be classified by anatomic location ("histological" classification) or by aetiology. An aetiological classification, which consists of seven categories (age-related, congenital, traumatic, and associated with intraocular disease or systemic disease or noxious agents), has obvious attractions. Table 11 lists some classification systems. However, modern epidemiologic methods are discovering an increasing number of risk factors in "age-related" cataracts and it is difficult to assign a person's cataract, specifically in the elderly, to one specific aetiology. Cataract is likely to

have a multifactorial aetiology. Therefore a more appropriate classification system is one based on anatomical classification. It is recognised that there are three main types of cataract: cortical, nuclear and posterior subcapsular, each of which may carry different risk factors, and this is the most commonly used classification system.

Table 11 Classification systems of Cataract

Classified by:	Types	Examples
Anatomic Location	Cortical	
	Nuclear	
	Posterior subcapsular	
	Mixed	
Aetiology	Age-related	
	Congenital	
	Genetic/non-genetic	
	Traumatic	
	Associated with intraocular disease	uveitis, glaucoma, retinal detachment, retinal degenerations, persistent hyperplastic primary vitreous, aniridia, high myopia
Systemic disease association	Metabolic disorders	diabetes, galactosaemia, hypoparathyroidism, Wilsons, Fabrys, Refsums
	Skin disease	atopic dermatitis, congenital ectodermal dysplasia
	Connective tissue disorders	Myotonic dystrophy, Marfans
	Renal disease	Alport's, Lowe's
	Central nervous system	Neurofibromatosis II, Sjogrens
Noxious agent association	Ionising radiation	X-ray, ultraviolet
	Drug-induced	steroids, chlorpromazine

1.4.3 Prevalence

There are few true population-based prevalence studies for cataract, and as different studies have used different definitions, detection and grading techniques, they are difficult to compare: for example, the Framingham Eye Study[129] specified a visual acuity of less than 20/30 in its definition. However, there have been several studies using photodocumentation which have not included vision criterion in the definition of cataract, and these are probably the most accurate record of the prevalence of lens opacities in the population. The most elderly subset of the population is often underrepresented in epidemiological studies, so it is not always easy to project these figures to the general population. Cataract progressively increases with age such that it is estimated that some degree of lens opacity is present in 50% of those over 60 years and 100% in those over 80 years of age worldwide. Three population-based photodocumentation studies are the Beaver Dam Eye Study (BDES),[130] the Blue Mountains Eye Study (BMES),[131] and the Melton Eye Study (MES).[132]
Table 12 lists the studies' details and some of the prevalence data; note that the BDES examined subjects aged 43-84 years old and the BMES 49-96 years of age, so these are selected figures.

Table 12 Prevalence of cataract in recent population studies

Study	Location	Year	Sample	Cataract Prevalence for age (%)			
				Age	Nuclear	Cortical	PSC
BDES[130]	USA	1988-90	4926	55-64	6.6	10.9	4.3
				65-74	27.4	25.4	8.4
				75-84	57.0	42.4	14.3
BMES[131]	Australia	1992-94	3654	55-64	3.9	13.1	3.8
				65-74	21.8	28.4	6.5
				75-84	48.5	46.7	11.7
MES[132]	England	199?-95	1201 (560*)	55-74	?	36	11

*based on analysis of 560 subjects' eyes.

The BDES and BMES used the Winconsin grading system,[133] and the MES the Oxford Clinical Cataract Classification and Grading System[134] and the Lens Opacity Classification System III,[135] and so are difficult to compare directly. Details and comparison of the grading systems appear later in the secion on Cataract Grading. The higher prevalence of cortical and posterior subcapsular cataracts in the MES is attributable to different grading criteria: if similar criteria to the Winconsin system are applied, then the prevalence becomes 11% for cortical opacities and 2% for posterior subcapsular cataract.

These studies suggest that cataract is common in the community in the industrialised countries, and is probably even more common in less developed countries: it has been estimated that cataract may occur 10-15 years earlier in India than in industrialised countries.[128]

1.4.4 Incidence

If exact prevalence is difficult to define, figures are even less certain on incidence and progression. Podgor et al inferred 5 year incidence rates of any lens opacity from the Framingham Eye Study prevalence data,[18] suggesting 23% for those aged 65, 31% for those aged 70 and 37% for those aged 75. More recently three studies have used more modern grading systems to examine the question prospectively[136; 137]. The Italian-American Cataract Study Group[136] assessed a group of 1399 persons aged between 45 and 79 using the Lens Opacities Classification SystemII (LOCSII),[138] and the Longitudinal Study of Cataract[137] followed nuclear opacities in 764 subjects with a median age of 65 using the LOCSIII grading system.[135] The Beaver Dam Eye Study recently published data on its five-year incidence data using the Wisconsin grading system.[139] Figures from the recent studies are tabulated in Table 13.

Table 13 Comparison of incidence and progression data

Cataract type	Years	Italian-American (65-74 yr)		Longitudinal Study (>65 yr)		Beaver Dam (43-84 yr)
		Incid	Progr	Incid	Progr	Incidence
Nuclear	2	5.1	53.5	5.9	10.3	
	5	11.5	80.4	7.7	12.0	12.0
Cortical	2	11.5	37.1			
	5	28.2	66.9			8.0
PSC	2	4.4	42.5			
	5	9.6	65.5			3.0

Some of the differences can be explained by different definitions of change, different age structure of samples, or methodological difficulties. Indeed the Italian-American Study had considerable regression in grading (about 20%), suggesting either an inaccurate or a crude grading system had been used as it is accepted that there is little regression of lens opacities. Wisconsin grading in the Beaver Dam study was more reliable.

The studies show a steady incidence of lens opacities in the elderly age group, with progression of lens opacities in those already with cataract in over two-thirds over 5 years. The placebo arm of the antioxidant trials underway (such as the Age-Related Eye Disease Study AREDS) will also contribute to incidence and progression data.

1.4.5 Twin and family studies of age-related cataract

There have been no reported twin studies of age-related cataract. A segregation analysis within the Beaver Dam Eye Study showed significant sibling correlation and suggested a single major gene could account for up to 35% of the variability of nuclear cataract,[140] supporting the role of genes in age-related cataract. Similarly, a

further segregation analysis of cortical cataract in the Beaver Dam Eye Study suggested the best fitting hypothesis was a single major gene accounting for 58% of the variability of age- and sex-adjusted measures of cortical cataract.[141] With the variance sex dependent, they suggested this major gene could account for 75% and 45% of the total variability among males and females, respectively.

1.4.6 Genetics of age-related cataract

The possibility that genetic influences may be involved in the development of age-related nuclear cataract has up to now been largely ignored although mutations in congenital cataract[142] and in mouse models causing nuclear cataract[143; 144] suggest a role for genes. Differentially expressed genes from lens epithelia dissected from age-related cataractous and noncataractous human lenses have recently been described.[145] Expression of the homeobox gene SIX5 has been identified in the mature lens but not the fetal lens, and a mutation has been implicated in adult onset cataract associated with myotonic dystrophy.[146] Identification of further genetic abnormalities in age-related cataract will undoubtably follow.

1.4.7 Risk Factors

A wide range of risk factors have been reported for cataract, though for many of these the evidence is not conclusive as to whether the observed associations are causative. Studying risk factors for cataract is difficult, as lifetime measures may be required, as it is not known if there is a "critical period" for exposure, and measurement of ocular exposure can be difficult (eg uv light). In addition, confounding needs to be considered, and there may be interactions between different exposures.

Different conclusions can be drawn from the same data; Harding concludes that diabetes, glaucoma and myopia are major causes in Western countries, with severe diarrhoeal disease being more important in the developing world, but Young concludes that heat, oxygen and light are the major causes.[147] This review will

attempt to examine some of the most important factors identified in large-scale epidemiological studies.

Age

The strongest risk factor for cataract is age: the Beaver Dam Eye Study demonstrated rates of 1.5%, 1.5% and 1.6% for nuclear yellowing, cortical opacities and posterior subcapsular cataracts respectively in those aged 43-54, rising to 74.1%, 42.4% and 14.3% in subjects over the age of 75.[130] Although this cannot exclude cohort effects, every study shows similar findings.

Female Sex

There seems to be an excess risk in development of cataract in women, particularly cortical lens opacities, which has been verified by several cross-sectional[130; 131] and case-control[148] studies. For example the BMES[131] demonstrated nuclear opacities in 53.3% of women compared to 49.7% of men, cortical cataract in 25.9% of women compared to 21.1% in men, while PSC opacities were no different.

Hormones may be involved; Klein from the BDES has reported that use of hormone replacement therapy seems to be protective for severe nuclear opacities, and previous pregnancy may also protect against cataracts, as well as late onset menopause.[149] This potential role of oestrogen needs to be validated by other cohort studies.

Sunlight (ultraviolet irradiation)

Sunlight has been associated with cataracts, although the evidence is conflicting, with many studies not examining individual exposure. The Chesapeake Bay watermen study specifically calculated individual lifetime dose of UV-B in 838 subjects and determined that doubling exposure to UV-light increased the risk of cortical cataract by 60%, but found no association with nuclear cataract.[150] Dolin has reviewed the epidemiological evidence relating to UV-B[151] and concludes that while animal experimental evidence shows a link between cataract and UV-B, there is limited

evidence that solar UV-B causes cortical and subcapsular cataract, and consistent evidence that nuclear cataracts are not associated with UV-B. West concurs with this view also in a more recent review.[152]

Smoking

There is now fairly consistent evidence that smoking is related to nuclear[148; 153] and posterior subcapsular cataracts. The London City Eye Study reported that smokers of more than 25 cigarettes a day were three times as likely to develop cataracts than non-smokers,[153] and ex-smokers had an intermediate risk, lending support to a causal relationship. It has also been shown that cigarette smoking increases the risk of progression of nuclear opacities.[154]

Diabetes

Although clinic-based case-control studies have reported diabetes as a risk factor,[148] they may be susceptible to selection and definition biases. But population-based studies have confirmed that diabetes is a risk factor for cataract.[155] An example is the Framingham Eye Study,[14] which found cataract in 19% of diabetics compared to 12% in non-diabetics, and found the risk only in those under the age of 65. There is also in vitro and in vivo evidence of the causation of cataract by elevated glucose levels and osmotic changes.

Steroids

The cataractogenic nature of steroids has been well described, both in epidemiological surveys[148; 156] as well as in clinic and case series. The hallmark of the steroid-induced cataract is the posterior subcapsular cataract, which has been linked to dose and duration of treatment.[157] Posterior subcapsular cataracts are uncommon (less than 10% of cataract) but because of their position in the lens and their sometimes rapid development, they have a large impact on vision and constitute a greater proportion of the surgical case-load.

Socio-economic factors

Low education in terms of years at school has been associated with cataract[14; 148; 156] in diverse populations, even attempting to correct for occupational, nutritional and environmental factors. An excess of cataract has been found in rural populations, for example in the NHANES survey,[155] after correcting for ultraviolet exposure. Non-professionals had a higher rate of cataract than professionals in the Lens Opacities Case-Control Study.[148] These factors are difficult to disentangle from other factors such as diet, alcohol consumption and smoking, and at present there is no obvious biochemical or physiological explanation, and so should be treated with some caution.

Height, weight and body mass

Although the Indian Case-Control Study[158] demonstrated low height as a risk factor for cataract (confirmed in the Framingham Eye Study[14]) as well as low body mass index, this has not been confirmed in other studies,[148; 155; 156] and may reflect short stature as a marker of chronic malnutrition at an early age. The interesting finding that weight at one year of age is inversely related to nuclear cataract 60-70 years later[46] may support the hypothesis that early nutrition is important in age-related nuclear cataract.

Alcohol

Alcohol consumption has been reported to be a risk factor in some studies,[159; 160] although it has not been confirmed in other studies.[148; 156] Harding's Oxfordshire case-control study[159] found that people drinking more than four units a day had twice the risk of cataract, and other researchers have suggested a J-shaped curve, similar to alcohol's cardiovascular effects.

Diarrhoea and severe dehydration

Minassian and others showed in two case-control studies in India that severe diarrhoea and dehydration, resulting in confinement to bed for at least three days, carried a three to four-fold risk for developing cataract in later life. This has not been

confirmed in other studies in India, for example the US-India case-control study,[158] although they did not use such a stringent definition. Further research in this area is required, as it may be an important modifiable risk factor in the third world.

Hypertension

Hypertension is another risk factor with conflicting evidence as to its significance. The Framingham Eye Study[14] and the India-US case-control study[158] associated cataract with hypertension, particularly systolic hypertension, but other epidemiological surveys failed to do so.[148; 155; 156] More recently, allowing subtypes to be graded, the Beaver Dam Eye Study concluded that people with hypertension were more likely to have posterior subcapsular lens opacities with an odds ratio of 1.39 (95% confidence interval 1.05,1.84).[161] The mechanism by which this may operate is unclear.

Antioxidants

Oxidation of lens proteins is associated with cataract formation, and it follows that high levels of antioxidants, such as vitamins, may be protective. The evidence is varied and no clear consensus emerges. The Lens Opacities case-control study in Boston found regular intake of multivitamins protective of all types of cataract,[148] while prospective data of 50,000 nurses in the United States determined the risk of cataract extraction to be 45% lower in women taking vitamin C supplements for at least 10 years.[162] However other studies have not supported this finding.[156; 158] Vitamin E, again found to have a protective effect in the Boston series[148] and in animal experiments, had no significant effect in the Nurses Health Study, which did show a protective effect for carotenoid levels, but not β-carotene in particular.

Two nutrition intervention trials, the Linxian Cataract Studies, demonstrated a 36% reduction in the incidence of nuclear cataract in those aged 65-74 taking multivitamins, and a 44% reduction in this age group in those receiving riboflavin/niacin supplementation.[163] There are several multicentre prospective randomised trials underway to answer the question whether vitamin supplementation may be protective against cataract.

Myopia and Glaucoma

Although myopia and glaucoma have been reported as strong risk factors in Oxfordshire case-control studies[164] these have not been duplicated elsewhere and further research is necessary before any conclusions can be drawn.

1.4.8 Treatment

Currently the only proven treatment of cataract is surgical extraction: there are an estimated 8 million operations per year, over 1.5 million in the United States.[126] It has been estimated that by 2020 over 30 million operations per year will be required to reduce cataract blindness to less than a million. Good epidemiological research is required to look at risk factors and treatments which might delay onset of cataract: back in 1984 it was estimated that if cataract could be delayed by 10 years, the amount of surgery could be reduced by 45% with huge cost savings.[165] There is currently a large deficit in cataract surgery across the UK, particularly with the aging population,[166] and the Government is starting to address this.[127]

As stated earlier, there are currently trials of antioxidant vitamins underway to see whether cataract can be prevented or progression slowed. Although aspirin might theoretically prevent cataract, evidence from large randomized trials of aspirin has been disappointing and has shown no benefit, so this cannot be recommended at present.

1.4.9 Conclusion: genes and environment in age-related cataract

Cataract is a multifactorial disease, in which age, female sex, diabetes, smoking, steroids and (probably) sunlight have been shown to be definite risk factors. There are numerous other possible risk factors for which there is conflicting evidence, and further epidemiological studies, in particular large randomised prospective studies, are required to find out if there is any way of preventing or slowing progression of

cataract. If there is significant genetic risk, which this study hopes to determine, susceptibility genes require identification.

The differing risk factors between studies underline the fact that most ophthalmic epidemiological studies have examined populations within a specific narrowly-defined area, which may result in the population being overmatched, and true environmental effects may be underestimated because the population has been uniformly exposed to a particular risk factor. Further research is required to compare different populations in different environments; it may be, for example, that diet has not appeared important in many western well nourished population studies, but this may be very different in less well-fed populations.

1.5 Grading of Cataracts

One of the main factors limiting epidemiological research into cataract has been the lack of an objective, reproducible and standardised method for detecting and grading the lens opacity. As changes occur with normal aging, it is important to determine whether these are appropriate for a given subject's age or whether this represents cataract. Because of the slow pace of change, any method used to detect progression of cataract in epidemiological research (whether it is studying risk factors for progression or an intervention trial) must be sensitive enough to detect that change. Methods must be reproducible and reliable, particularly when used by several investigators in the same study. It is important to monitor inter- and intraobserver agreement.

When grading techniques are compared, it is useful to have a "gold standard". For example fluorescein angiography is used as the standard for assessing methods of screening for diabetic retinopathy. Unfortunately, there is no such standard for cataract grading. It is therefore important to compare grading systems with one another to see if the same thing is being measured. There has been little comparison performed, despite the presence of several grading techniques and photographic methods. This leads to some difficulty identifying the "best" grading system or measurement method.

As I will explain later, in order to generalise from twin studies it is important to ascertain whether data obtained from this "healthy volunteer" population is representative of the population as a whole. We have chosen to compare data with the Melton Mowbray Eye Study, a population study of cataract (and ARMD) in England[132] that covers a similar range of ages and, like our study population, largely comes from a white European background. They also are using the same techniques for grading cataracts, so we will be able to compare directly.

Table 14 lists the methods of detection and grading of cataract that can be used, along with some pertinent references to these techniques. While discussing the different

methods, I will attempt to explain some of their advantages and disadvantages, as well as the rationale for our choice of grading technique.

1.5.1 Subjective Methods

Subjective methods, using multiple ophthalmologists performing a standardised examination, or if the survey is small enough a single ophthalmologist to eliminate interobserver variation, are useful for "field" surveys and have been the most widely used in epidemiological research.

Visual acuity/function

Some early studies, for example the Framingham Eye Study in the USA[129] and the Nepalese survey,[167] included impairment of visual acuity as part of the diagnostic criteria for cataract, but it has been recognised that not only must other causes of impaired vision be ruled out, but also that early cataracts and some types of cataract (e.g. cortical cataracts) may not impair the vision sufficiently to be detected by a drop of Snellen acuity. More subtle methods of glare and contrast sensitivity may be more sensitive than simple acuity alone,[168] but they are psychophysical tests which may be difficult in field conditions or when screening an elderly population. Macular function tests enable an assessment of retinal function in the presence of cataract, in order to try to eliminate retinal causes of loss of vision.

Clinical Examination in the Field

Handlight examination or ophthalmoscopy has been used to detect cataracts, especially in difficult field conditions such as the Nepalese Eye Study.[129] There is good interobserver agreement, and the method is quick and cheap. However it is very difficult to detect early lens opacities, and the only grading system possible is a very crude one, giving little more information than prevalence of advanced disease.

Slit lamp clinical examination

Descriptive

Slit lamp examination allows a more descriptive technique detailing the position of lens opacities and an attempt to grade their severity. The Framingham Eye Study[167] used a fairly basic slit lamp examination by ophthalmology residents. However without clear references there was substantial interobserver variation in detection and grading.

Grading

The introduction of photographic standards for comparison in the form of standardised slit lamp, Scheimpflug, colour and retroillumination photographs enabled a more accurate way of grading cataracts. All methods have recognised that the three important types of cataract (nuclear opacity, cortical and posterior subcapsular cataract) must be included in a grading system,[134; 135; 138; 169-171] and in addition most include a further category of nuclear colour,[134; 135; 138; 169; 171] as it is recognised that brunescence and nuclear opalescence are not the same.

The most widely used grading system is probably the Lens Opacities Classification System (LOCS). Originally designed for use in a case-control study of risk factors by Leo T Chylack and others at Harvard,[169] the four categories of grading were introduced in LOCS II.[138] This grading system, which used five grading categories for nuclear opalescence, seven for cortical, four for posterior subcapsular and three for nuclear colour, is not only reproducible[138; 172] but also has proved useful in studies of progression of cataract.[173]

LOCSIII was developed to correct some of the difficulties in LOCSII:[135] namely problems with grading nuclear colour, uneven scaling and high 95% tolerance limits, making the system insensitive to change. LOCSIII has expanded sets of reference photographs and used decimalized grading, making it more sensitive than LOCSII.

An increased number of grading intervals reduces the kappa, but vastly increases sensitivity to change.[174] It is now the standard system used.[137; 175]

The main other slit lamp grading method, the Oxford Clinical Cataract Classification and Grading System (OCCCGS) also grades the four elements of nuclear opalescence ("white scatter"), nuclear colour ("brunescence"), cortical opacities ("cortical spokes") and posterior subcapsular cataract.[134] However, it grades an additional six features: anterior clear zone thickness, waterclefts, vacuoles, retrodots, focal dots and anterior subcapsular opacity. The importance of all these features is not clear, but evidence is emerging that some of them are related to each other[176-178] and may be early signs of cataract, important to grade for in longitudinal studies. The LOCS team felt that increasing complexity reduced reproducibility and reliability,[138] but the OCCCGS has been validated.[179]

Further refinements of OCCCGS now include the addition of grading coronal flakes, and decimalization of the grading, again to increase sensitivity to change.[180] Although it is the most complex, we have adopted the OCCCGS for this study as there is only one investigator, eliminating interobserver differences,[181] and our study population will have generally early lens changes. LOCSIII and OCCCGS have now been compared and seem to relate reasonably well for the four important features of nuclear colour, opalescence, cortical and posterior subcapsular cataracts.[181]

These subjective grading systems have been criticised as having high interobserver variation, as there is no standard for all the grades, and grading of early lens opacities is still very difficult[182]. The subjectivity may obviously also introduce problems, particularly in a twin study where zygosity is obvious and examination of pairs of twins was performed together, introducing the potential for bias with a subjective test. Therefore there has been an attempt to introduce more objective methods of grading.

1.5.2 Objective methods

The need for objectivity has resulted in development of photographic techniques, which have been used to obtain more objective grading as well as a permanent record of the phenotype for comparison studies, such as long-term intervention studies.

Slit lamp photography

Brown in Oxford among others developed the technique of anterior slit lamp photography to photograph the nucleus,[183] and LOCSII,[138] LOCSIII[135] and the Winconsin[133] grading systems have successfully used slit lamp photographs, which have been shown to be reproducible and reliable in the detection and grading of cataract.[184-186] There is good agreement between observers, but because all layers of the nucleus are not in focus, the analyses are not precise enough for clinical trials.

Modified slit lamp photography

Scheimpflug

Slit lamp cameras have been modified along the Scheimpflug principle to obtain photographs with the entire anterior segment in focus. When an object plane (the slit beam), objective plane (the camera lens) and image plane (film or charged coupling device [CCD] element) intersect at one point (usually 45 degrees), this results in a photograph with a deep field of focus. Scheimpflug photographs can be used in analysis of cataract as well as anterior segment biometry.[187] The nuclear changes can then be graded using trained readers to read the photographic images, or densitometric analysis of the optical density.

This form of photography allows more objective analysis of cataract, but is mainly limited to nuclear changes: cortical and posterior subcapsular cataracts are difficult to grade using Scheimpflug images. Densitometry of video-grabbed CCD images should provide high sensitivity for change in longitudinal studies, although at present

they are black and white and so only optical density rather than colour can be analysed.

There are four main Scheimpflug systems with software for densitometric analysis available: the Topcon,[188] Zeiss,[185] Nidek[189] and Oxford[190] systems. They have been shown to reproducible and reliable,[184; 191-193] and have the advantage of objective agreement, good repeatability and the potential to use different classification systems on the same images. There are some disadvantages, however: they are light-sensitive and not very portable, and so not very suitable for field studies. In addition they are expensive, particularly for densitometry and software, and there are no adequate grading criteria or comparison with the slit lamp grading systems at present. There is also a problem with standardisation between different machines and so results are not yet directly comparable.

We have chosen to use a system based on the Oxford system: the Marcher Case 2000 series (Marcher Enterprises, Hereford, UK) which is a combined Scheimpflug and Retroillumination camera system, with images grabbed by a video-CCD system and analysed by proprietary biometric and densitometric software.

Retroillumination systems

Images of cortical and posterior subcapsular cataracts are best obtained using retroillumination cameras, particularly the NeitzCTR[194] and Oxford[195] cameras. There is good agreement between observers reading images, and the photographs may be better than clinical examination at detecting early changes.[196] Densitometric analysis to calculate, for example, area covered by cataract, allows objective assessment with good repeatability,[197; 198] and is good at detecting subtle changes.

There are some difficulties with retroillumination analysis: it may not be precise enough for clinical trials, and in particular the automated densitometry analysis may not distinguish different lens opacities, for example posterior cortical and posterior subcapsular cataract. Again densitometric support software is required, and more studies are needed on reproducibility, and analysis and grading criteria. There is no standardisation, and many images require manipulation or enhancement, as the red

reflex on which the image is based is often asymmetrical due to the position of the optic disc.

1.5.3 Conclusion

Scheimpflug and Retroillumination image analysis may determine, with reasonable accuracy, nuclear opalescence, cortical and posterior subcapsular cataract, and slit lamp photography may document nuclear colour, but other features as those in the OCCCGS cannot be reliably or accurately photographed at present, making these measurements still subjective. Some of the pertinent references relating to grading of cataracts are summarised in Table 14.

As a general principle it is not advisable in epidemiological studies to use more than one method of measuring variables, particularly the main outcomes of interest. If the two different measures give a different result one is left with not knowing which is "true". However, the methods used in analysing continuous data from twin studies (Mx pathway modelling, see section 2.8) allow values from different methods of measurement to be fitted in the model, increasing power. Therefore I elected to use both subjective and objective gradings in the cataract assessment of the twins.

Table 14 Pertinent References for Grading of Cataracts

Method				Description	Validation	Examples	Comparison	
I Subjective Methods								
A	Visual acuity/function							
	1	Snellen/EDTRS						
	2	Glare/contrast sensitivity						
	3	Macular function						
B	Clinical examination in field			167	167			
C	Slit lamp clinical examination							
	1	Descriptive		129		129		
	2	Grading						
		1	LOCS	135; 138; 169	172	137; 173; 175	181; 199	
		2	Wilmer	170				
		3	Oxford	134	179	132; 176; 177; 200		181
		4	Japan	171				
II Objective Methods								
A	Slit lamp photography							
	1	Regular		183	184; 185	186	184	
	2	LOCS		135; 138				
	3	Winconsin		133				
B	Modified slit lamp photography							
	1	Scheimpflug						
		a	Topcon	188	184	201	184; 202	
		b	Zeiss	203		204		
		c	Nidek	189	191; 192	189; 200	191; 202; 205	
		d	Oxford	190	193	175		
	2	Retroillumination						
		a	Neitz CTR	194	197		185; 194	
		b	Oxford	195	198	178; 206		

1.6 The Epidemiology of Age-related Macular Degeneration

1.6.1 Introduction

Age-related macular degeneration is the most commonly-cited cause of low vision and blindness registration in the Western world. The incidence may also be increasing in the United Kingdom at a rate higher than would be expected on the basis of aging alone.[19; 21; 22; 207] There is little information on the natural history of age-related maculopathy (ARM),[208] partly due to difficulties of disease definition and grading, and also due to concentration on treatment of exudative ARM.

An international group proposed the overall term "age-related maculopathy" (ARM) to encompass both early age-related macular changes (including soft drusen >63μm, hyper and/or hypopigmentation) and late changes. Late changes (neovascularisation and geographic atrophy) are described by the term age-related macular degeneration (AMD) that will be used in this thesis.

1.6.2 Prevalence

Comparisons between many studies published before the 1990s are difficult because of the different definitions and classification systems used. All studies, however, have shown a marked increase in prevalence with increasing age. The Melton Mowbray study of 484 people over the age of 75 estimated a prevalence of ARM (using Framingham criteria) of 39% in those aged 75-84 years, and 53% in those aged 85 and older.[10]

Some recent prevalence data using macular photograph grading systems are detailed in Table 15. The Beaver Dam Eye Study (BDES), the Blue Mountain Eye Study (BMES) and Rotterdam study all used a grading system based on the Wisconsin

Grading System,[209] which will be discussed later, and the Chesapeake Bay watermen study used its own grading system.

Table 15 Prevalence of ARM in recent population studies

Study	Location	Year	Sample	ARM Prevalence for age (%)			
				Age	Early ARM	Late ARM	Soft drusen
BDES[210]	USA	1988-90	4771	55-64	13.8	0.6	
				65-74	18.0	1.4	
				75+	29.7	7.1	
BMES[211]	Australia	1992-94	3654	55-64	2.6	0.2	
				65-74	8.5	0.7	
				75-84	15.5	5.4	
				85+	28.0	18.5	
Rotterdam[212]	Netherlands	1990-95	6251	55-64		0.2	
				65-74		0.8	
				75-84		3.7	
				85+		11.0	
Chesapeake [213]	USA	?1987	755	50-59			6.0
				60-69			13.0
				70-79			26.0
				80+	13.6		

The use of a standardized grading system in the future, the International ARM Epidemiological Study Group classification,[214] should improve comparability of studies, as differences in the stuidies summarised in Table 15 may be real, or simply due to differences in grading.

1.6.3 Incidence

Until recently, there were little data on the incidence of ARM, with virtually no long-term follow up studies. Sparrow et al performed a seven year follow up of the original cohort of the Melton Mowbray Eye Study patients,[208] and documented for 88 survivors a 7 year incidence (regression) of 30.6% (20.0%) for drusen, 54.5% (8.8%) for RPE degeneration, increased pigment, and 1.3% for each of subretinal haemorrhage, subretinal scar/fibrin and geographic atrophy.

More recently the large epidemiological studies have reported incidence data,[215] and these are detailed in Table 16.

Table 16 5 year incidence of age-related macular degeneration

Study	ARM	AMD
BMES	7.7	1.1
BDES	8.2	0.9
Rotterdam		0.6

There is evidence that ARM is becoming more common,[207] and a clinical impression that, for example in Japan, the incidence is rapidly increasing and there may be phenotypic differences between populations in different geographic locations (Bird AC, personal communication).

1.6.4 Family and twin studies of ARM

There is evidence that family members of individuals with early ARM and AMD are more likely to have the disease than unrelated subjects, supporting the role of genetics.[216] Several sibling case-control studies (not all with fundus photographic grading) have shown a greater risk for siblings of those with disease than those without, for example Silvestri's study from Belfast identified a relative risk of 19.[217] Seddon[218] and Hyman[219] have published similar results from the United States, as has Klaver from the Rotterdam Eye Study (using only sibs of those with late AMD).[220] The results from these are summarised in Table 17. Klaver's study also examined offspring of those with AMD in her study, and found overall odds ratios for first degree relatives of 4.8 for ARM and 19.8 for AMD. Family studies therefore suggest genetic influence in ARM and AMD but cannot completely exclude the possibility of shared environmental effects.

Klaver has recently reanalysed the Rotterdam Eye Study data, using a family score method, in which the risk in siblings of affected individuals also takes into account the expected rate, based on age/sex/risk factor specific population based data.[221] She

found that there is heterogeneity of genetic risk; 3% of families (of 64 cases) had a highly increased risk, with 13% demonstrating a moderately increased risk and 67% no increased risk.

Table 17 Prevalence of siblings affected by ARM or AMD in case-control studies

Study	Sibs of affected		Sibs of unaffected	
	No. affected/total	%	No. affected/total	%
Silvestri	20/81	25	1/78	1
Seddon	35/98	36	15/112	13
Hyman	29/146	20	12/152	8
Klaver	25/49	51	15/92	16

Heiba, again working on data from the Beaver Dam Eye Study, examined 546 sibships and concluded, using segregation analysis, that genetic effect could not be excluded, and that a single major gene could account for 55% and 57% of the variability of right and left eyes respectively.[222] It seems surprising that such a single major gene has not yet been detected. This analysis may have overestimated the effect of a single gene or underestimated the potential number of genes involved.

Twin studies, initially small case series of largely monozygotic twins, have shown remarkable degrees of concordance, in the order of 90%, particularly in late ARM.[68-72; 98] However, these can be criticised as being not population-based and therefore subject to a high risk of ascertainment bias. The largest twin study of macular degeneration recruited 134 pairs of twins and demonstrates how ascertainment can cause bias. The prevalence of macular degeneration was 42% in twins recruited 1986-1991 when the study was advertised as being about AMD, while the prevalence was 21% subsequently when twins were asked to have "an eye test".[73] Even so, the study showed a complete pairwise concordance of 1.0 in 25 pairs of MZ twins with ARM (although the stage of ARM was not always the same) and a concordance of 0.59 in the 12 pairs of DZ twins with ARM, confirming a role for genes. The only population-based twin study from Iceland found a pairwise concordance of 0.78 for MZ twins (and 0.22 for their spouses) but unfortunately did not study DZ twins.[74]

This reduces the likelihood of environmental factors in later life being the sole cause of ARM, but cannot exclude the early shared family environment of MZ twins as the cause rather than the shared genetic effect.

Twin and family studies published to date therefore suggest a genetic component, but it is difficult to quantify the relative roles of genes and environment at present; no "heritability" study has been published. A population-based twin study is required to reduce ascertainment bias. The difficulty is in recruiting numbers of twins of sufficient age to have enough power to detect a significant heritability. Seddon's group, examining fundal photographs of twins from the 14000-strong Veterans register one or both who have a diagnosis of ARM, will hopefully have such power to analyse the late, vision-reducing stages of ARM.[75]

1.6.5 Genetics of ARM

Much hope in identification of candidate genes for ARM has rested in finding the genes causing hereditary retinal dystrophies which have a similar phenotype to that of ARM, such as Stargardt's disease, Best macular dystrophy and Doyne honeycomb retinal dystrophy (DHRD). Mutations have been found in the ABCR gene, which encodes a rod outer segment protein called rim protein, responsible for Stargardt's disease. There was excitement when mutations were found in the ABCR gene in ARM,[223] suggesting this as a candidate gene for ARM. However, much of this hope has faded, as the initial study's control matching techniques were questionable; subsequent series have shown no greater mutation rate in ARM patients than controls.[224] More recently, after identification of the gene causing DHRD (an identical single nucleotide mutation in all affected famies), none of 494 patients with ARM were found to have the mutation.[224]

Similarly, the mutations involved in other single gene-mutation retinal dystrophies have not been shown to be significantly associated with ARM.[216] Klaver has reported an association of AMD with polymorphisms in the Apolipoprotein gene ApoE (significantly associated with Alzheimer's disease),[225] from the Rotterdam Eye Study population, although this association has not yet been reported by any other groups.

A genome-wide screen was performed using a family with autosomal dominantly inherited age-related macular degeneration, with the disease locus mapping to chromosome 1q25-q31.[226]

It seems that identification of causative gene abnormalities for a disease as complex as AMD is going to be difficult, particularly as the pathogenesis of AMD is not yet clearly understood, and candidate genes are currently largely limited to conditions which have a similar phenotype.

1.6.6 Risk Factors

The body of evidence from epidemiological and laboratory studies implicates the following four pathogenic mechanisms: oxidative damage, photochemical damage from ambient light, increased thickness in Bruch's membrane, and reduced foveolar choroidal circulation. The environmental risk factors for ARM and AMD have been reviewed recently in a comprehensive review by Jennifer Evans.[227] Results from some of the recent large epidemiological studies are summarised here, as well as data published after the above review; these no doubt reflect publication bias (as well as this reviewer's bias) of positive associations.

Age

Age is well-recognised as one of the major risk factors for macular degeneration, and rises dramatically with age, such that over the age of 75 approximately 10% of people have AMD in at least one eye, which may rise to 30% over the age of 85.[227]

Female sex

ARM has been reported as more common in women than in men in some studies, but not in others.[228; 229]

Smoking

Smoking is now well-established as a risk factor for ARM, particularly the exudative form of wet AMD. Large population studies such as the POLA study from France (Relative Risk 2.2),[230] the Rotterdam Eye Study (RR 6.6)[231], the Beaver Dam Eye Study (RR 2.5 for women, 3.29 for men)[232] and the Blue Mountains Eye Study (RR 3.92)[233] have confirmed the relationship between smoking and neovascular AMD. The effect of smoking was also seen in the Eye Disease Case Control Study comparing 421 cases with 615 controls with a relative risk of 2.2 (95% CI 1.4-3.5) for current compared to non-smokers.[234] Prospective studies of women (the Nurses Health Study) and men (the Physicians Health Study) have shown a higher risk in current smokers.[235; 236] In addition the Blue Mountain Eye Study found a higher risk (RR 1.75) for early ARM in current smokers[233] which the other studies did not. Finally, five-year incidence data from the Beaver Dam Eye Study support an association between smoking and large drusen.[237]

Hypertension and vascular factors

The data on cardiovascular risk factors and the risk of ARM and AMD are conflicting, but large studies have shown no significant associations. The Beaver Dam Eye Study showed no strong association between hypertension and cardiovascular disease and ARM,[238] although it did support an association between high dietary fat and cholesterol intake and exudative AMD.[239] The Eye Disease Case-Control Study also found no relationship between cardiovascular disease and AMD, although it did have a positive association between serum cholesterol and AMD,[234] inverse to that of the Beaver Dam Eye Study. Recently, the Blue Mountains Eye Study also showed an increased risk of ARM in those with high dietary cholesterol intake, and a lower risk for those with high fish oil intake.[240]

Klein examined the relationship between cardiovascular risk factors and the incidence of ARM prospectively in the Beaver Dam five-year follow-up study. He found no strong associations, only a weak correlation between cardiovascular factors and

retinal pigmentary abnormalities.[241] The Blue Mountains Eye Study also showed no clear connection between cardiovascular disease and risk factors, apart from one associated with increased fibrinogen levels.[242]. However, the Rotterdam showed a significant risk for exudative AMD with atherosclerosis, as assessed by examination of carotid artery plaques (relative risk 2.5, with 95% CI 1.4-4.5), and two recent case-control studies identified hypertension or poorly-treated hypertension as a significant risk factor.[243; 244]

There are problems exploring risk factors (particularly in such an age-related condition as ARM) that are associated with a higher mortality rate – those exposed to the risk factor may die before they get the disease, or die before they can be included in the study.

Sunlight/ultraviolet radiation

Personal lifetime ultraviolet/sunshine exposure is extremely difficult to measure, particularly with so many other factors affecting the retinal dose such as hat and sunglasses behaviour, absorption by the lens and facial anatomy. Data from the Beaver Dam Eye Study showed a modest increased risk (RR 2.26 for exudative AMD) in those spending the most time outdoors compared to those spending the least time,[245] as did the smaller study of Chesapeake Bay watermen.[246] Many other studies, such as the Eye Disease Case-Control study, have shown no association between sunlight exposure and AMD.[234] Skin sensitivity may play a part in sunlight behaviour and therefore risk of AMD, and some of the studies are discussed in the next section.

Eye colour

The Blue Mountains Eye Study found blue eyes to be significantly associated with both early ARM and late AMD, as well as abnormal (high and low) sensitivity to the effects of the sun,[247] which has been shown before, as has been shown before.[227] Case-control studies have give conflicting evidence: the Eye Disease Case-Control Study showed no association,[234] whereas a large French study of 1844 patients and 1844 controls did.[244] A British case-control study of 101 patients and 102 controls

found an odds ratio of 5.5 (95% CI 2.0-15.9) for those who observed (obviously retrospectively) that their iris colour had become lighter compared to those who did not.[248] The significance of all these findings is unclear, but may represent the role of ocular melanin in preventing oxidative damage of the retina.

Antioxidants

Results have been inconsistent on the effect of dietary antioxidants and supplements in the prevention of AMD. The Eye Disease Case-Control Study found dietary carotenoids (particularly lutein and zeaxanthin) were associated with reduced risk of AMD but not vitamins A, C and E,[249] and also serum carotenoid levels (but not zinc) were associated with reduced AMD.[234] Lutein and zeaxanthin are localised at the macula in the retina (the macula pigment), but few studies have specifically investigated whether these pigments, which absorb blue light and are powerful antioxidants, are protective. The Blue Mountains Eye Study found high serum alpha-tocopherol and beta-carotene were not protective for AMD or early ARM,[250] while data from the Baltimore Longitudinal Study of Aging suggested a protective effect of high levels of plasma alpha-tocopherol.[251] Incidence data from the Beaver Dam Eye Study suggested only high dietary vitamin E and pro-vitamin A carotenoids (lycopene) were inversely associated with drusen (and zinc with pigmentary changes).[252]

Alcohol

There seems to be little risk involved in alcohol consumption regarding ARM: although subanalysis of the Beaver Dam Eye Study data concluded that beer intake may be a significant risk factor.[253] This has not been replicated in other studies which have shown no association between alcohol intake and ARM, including the Blue Mountains Eye Study[254] and the Eye Disease Case-Control Study.[234] Incidence data from the Beaver Dam Eye Study did not support alcohol or beer as an aetiological agent.[255]

Oestrogens

There are still not enough data to have a clear idea of the effect of oestrogens on ARM and AMD. The Beaver Dam Eye Study found no change in risk of AMD with oestrogen replacement (odds ratio 0.96, 95% CI 0.85-1.09) or with hysterectomy (odds ratio 0.95, 95% CI 0.85-1.05).[256] The Blue Mountains Eye Study has suggested an increased risk in those with longer time between menarche and menopause.[228] However, the Rotterdam Study found a twofold increased risk of AMD in those who had menopause before the age of 45,[257] and the Eye Disease Case-Control Study found the use of post-menopausal oestrogen replacement associated with a lower risk of neovascular AMD.[234]

Other risk factors

Other risk factors reported have been high body mass index for early ARM,[242; 258] low hypermetropia[259] and cataract surgery.[260; 261]

1.6.7 Treatment

The only proven treatment is laser photocoagulation of choroidal neovascular complexes, which is beneficial to a very small minority of patients with ARM who present early with exudative AMD. Photodynamic therapy and radiotherapy have offered some hope for future treatment, and there are other experimental treatments. However, until the genetic mechanisms and environmental interactions are better understood, there seems little hope for effective treatment for the vast majority of sufferers with AMD, and currently there are no interventions for prevention.[262]

1.6.8 Conclusion: genes and environment in ARM

It seems likely from family and twin studies that there is an important genetic component to AMD. However, the relative contributions of genes and environment have not yet been fully determined, particularly for early ARM. Age is a major risk

factor for the condition, and smoking seems to be a consistent risk factor for neovascular AMD. Other risk factors have not been consistent over studies, and once susceptibility genes have been identified, different subgroups susceptible to different environmental effects may be determined.

1.7 Grading of Age-related Macular Degeneration

1.7.1 Introduction

Like cataract grading, comparison between different population studies of ARM has been difficult because of the different classification systems and even different terminology for the same changes. Early studies, such as the Framingham Eye Study[14] used a visual acuity cut-off (<= 20/30) and diagnosed "senile macular degeneration" using ophthalmoscopy.

Later grading systems have used no visual acuity cutoff, and used a grading system based on photographs of the macula. Stereoscopic fundal photographs assessed with a rigid protocol offer considerable advantages over ophthalmoscopy. These include the fact that photography is rapid and non-invasive, often detects subtle abnormalities easily overlooked by ophthalmoscopy, and can be used for longitudinal studies. In addition, reliability and replication of results can be assessed, quality control is feasible and multicentre studies can be monitored centrally. Therefore the more recent studies have used stereoscopic fundus photograph grading. Some grading systems also included fluorescein angiography in their diagnostic criteria.[263]

Two large epidemiological studies of ARM in the 1980's developed grading systems; the Chesapeake Bay watermen study[213] and the Beaver Dam Eye Study.[209] The latter grading system, the Wisconsin grading system, defined three subfields 500, 1500 and 3000 micrometres diameter centred on the macula, with the outermost two divided into four by radial lines, resulting in 9 subfields. This system was taken up by other large studies such as the Blue Mountains Eye Study,[264] and the Rotterdam Eye Study,[212] and was shown to be reproducible in different self-taught units, with kappa scores showing moderate to good agreement.[265] However, even between these studies, the definition of ARM varied, leading to difficulty comparing the actual results.

It became apparent that a consensus method of grading ARM was required, in order to compare the prevalence and phenotype in different populations, and for use in analytical, genetic or intervention studies. Therefore the International Age-related Maculopathy Epidemiological Study Group Classification was developed,[214] which has become the benchmark for grading macular degeneration. It is this grading system that has been used in the Twin Eye Study.

1.7.2 The International Age-related Maculopathy Epidemiological Study Group Classification

The classification system grading is based on the reading of stereoscopic 30 degreee macular photographs centred on the fovea and also centred on the temporal margin of the disc (based on the Airlie protocol for diabetic retinopathy photography) and three concentric circles as defined by the Wisconsin grading system. It aims to establish the following signs in people over the age of 50 with no coexisting pathology which could cause the lesions (defining ocular trauma, retinal detachment, chorioretinal inflammation or infection, or choroidal dystrophy), and uses no visual acuity cutoff. The definitions are:

Early ARM

- Soft drusen >63μm
- Areas of increased pigment or hyperpigmentation (in the outer retina or choroid) associated with drusen
- Areas of depigmentation or hypopigmentation of the RPE, most often more sharply demarcated than drusen, without any visibility of choroidal vessels, associated with drusen

Late ARM (=AMD)

Geographic atrophy (dry AMD)

- Any sharply delineated roughly round or oval area of hypopigmentation or depigmentation or apparent absence of the RPE in which the choroidal vessels are more visible than in surrounding areas, which must be at least 175μm in diameter.

Neovascular AMD (disciform, exudative or wet AMD)

- RPE detachment(s) which may be associated with neurosensory retinal detachment, associated with other forms of ARM
- Subretinal or sub-RPE neovascular membrane(s)
- Epiretinal (with exclusion of idiopathic puckers), intraretinal , subretinal, or sub-pigment epithelial scar/glial tissue or fibrin-like deposits
- Subretinal haemorrhages that may be nearly black, bright red or whitish-yellow and that are not related to other retinal vascular disease
- Hard exudates (lipids) within the macular area related to any of the above and not related to other retinal vascular disease

1.8 Conclusion

This thesis therefore describes a twin study established to determine the genetic epidemiology of common, important eye diseases, and in particular refractive error, age-related cataract and age-related macular degeneration have been examined. Environmental risk factors have been identified in all these conditions, and family studies have shown aggregation, suggesting a role for genetic factors. However, with the exception of some previous studies of myopia, the genetic architecture of these diseases is not known, and in particular the relative role of genes and environment in their aetiology in unknown. While interest in the genetics of these conditions has increased in recent years, much of the research has been based on environmental risk factors.

The next section, on the Subjects and Methods of this study, details the setting up of the twin study, with an emphasis on modern, objective measures of grading of the phenotypes of interest. In light of potential biases of previous twin studies, the selection of twins for this study will also be discussed.

2.0 Subjects and Methods

2.1 Subjects

2.1.1 The St Thomas' UK Adult Twin Registry

The twin pairs recruited to the Twin Eye Study were taken from the St Thomas' UK Adult Twin Registry. This is a volunteer twin registry compiled from twins volunteering to help medical research, and recruited from national media campaigns in the United Kingdom, and, more recently, Ireland.[34] The Registry was initially set up to study osteoporosis and osteoarthritis,[266] and has now been extended to examine the genetics of common chronic diseases. So far, over 2800 pairs of twins have been seen and examined with regard to a wide range of diseases ranging from hypertension, skin naevi to MRI disc and spine degenerative changes. Up until the Twin Eye Study, no eye assessment had been performed. Twins were seen from all over the United Kingdom, and no payment was made for visits, although all travelling expenses for the twins were refunded. Since the Registry initially studied osteoporosis and osteoarthritis, all subjects initially recruited and examined were women. Subsequently, male twins have been accepted on the register, but the majority of volunteers are overwhelmingly female, which is not unexpected as the "Rule of Two Thirds" applies in twin volunteer studies:[36] two thirds of volunteers for twin studies are female, MZ and young. The current figures are that 4000 pairs of twins have volunteered, and around 2800 pairs have been examined. They have been recruited predominantly from printed press news and advertisements, in national and local newspapers and womens' magazines. There have also been television appeals, timed to coincide with other twin stories the unit has been involved in. The majority of women seen have been DZ twins (1900 cf 900) as the Twin Research and Genetic Epidemiology Unit has moved to using DZ pairs for sibpair genetic studies. Twins recruited have ranged between 18 and 75, and only 240 male pairs are on the registry. Twins on the register come from all over the UK, and now Ireland too, with a south-east/central bias.

2.1.2 Sample size calculation

Sample size was calculated prior to the start of the study; 600 pairs of twins (300 MZ and 300 DZ) were estimated to have 95% power to detect a difference between the two (20% heritability) at the 5% significance level. It was estimated that 18% and 45% of probands would have ARM and lens opacities respectively (108 and 270 individuals of each zygosity). For ARM, which has the lower prevalence, it was estimated that for concordance rates of 50% for MZ and 20% for DZ twins, 27 pairs

of the 108 MZ individuals with ARM would be concordant for ARM (27/(27+27+27)=33%) compared to 11 pairs of DZ twins (11/(11+43+43)=11%). These sample sizes are for the binary estimates of ARM, and for the continuous measures of refractive error and cataract, the power is greater, so the binary data was the "limiting factor" of the study's power.

2.1.3 Inclusion Criteria

Many of the outcomes being examined by the Twin Eye Study may be more common in women such as nuclear and cortical cataract[128] and AMD.[256] As stated above, there are not enough men on the register for meaningful analysis in a twin study this size and to examine whether there are different genetic and environmental influences in men and women, so it was decided to restrict the Twin Eye Study to same-sex women pairs only. Twins included in the study were examined at St Thomas' Hospital between January 1998 and July 1999.

The main aim of the Twin Eye Study was to quantify the effects of genes and environment on age-related diseases such as cataract and AMD, so the older twins were asked to participate. The intention was to approach all twins over the age of 60, and then twins aged 50-60 to make up the number seen to 600 pairs, with an even split between MZ and DZ twins. Only twins interviewed and examined by the St Thomas' registry were included in the eye study, as baseline data on risk factors and other potential confounding variables were collected by the main osteoporosis/arthritis study, to reduce duplication and length of the eye assessment visit. Some twins were seen for the eye study on the same day as their full assessment, others up to 18 months after their initial (or repeat) visit. As the main study has been concentrating on genetic analysis, they have examined more DZ than MZ twins (1400 pairs compared to 600), so all MZ twins examined in the correct age range were approached, and then a balancing number of DZ twin pairs, selected at random from lists of the twin register.

Twins were unselected when approached about the eye test; they were selected on age criteria alone. Although initially twins near to London were recruited to reduce cost, subsequently twins from all over the UK were approached if they fitted the age criteria. Recruiters were instructed to advise twins that past ocular history was irrelevant to the study, to attempt to reduce selection bias, and to encourage individuals with or without eye problems or spectacle/contact lens wear to attend for an eye test. The twins were not informed of outcomes being assessed at recruitment, only that the eye test involved pupil dilation so they should not drive for a few hours after the tests.

The vast majority of twins volunteered for the Twin Registry unaware of the possibility of an eye examination. However, as there was some publicity associated with the start of the Eye Study, there was a potential bias of twins with eye problems or family history volunteering for the eye examination. Therefore the twins were asked whether they had volunteered for the eye study in response to a telephone call from the Twin Research Unit (those already registered from other sources) or in response to the eye publicity, to establish if the latter were any different, resulting in potential bias.

2.1.4 Exclusion criteria

Twins were excluded if they did not fall into the correct age category. For each part of the study, a pair of twins was excluded if one of the pair was unable to be assessed. If one twin, for example, had previous cataract surgery or other potentially refractive procedure to both eyes, or if they had corneal changes making autorefraction impossible, then they and their twin were excluded from the refractive error study part of the Twin Eye Study. However, if one twin's right eye was unable to be assessed but their left was, then this could be included in "worse eye" comparative analysis, or in analysis comparing twins' left eyes, depending on the analysis performed. Details of numbers of twin pairs assessed for each disease or trait is given in the results section.

2.1.5 Zygosity

Zygosity was determined by standardised questionnaire concerning similarity in childhood.[267] This has been shown to be very discriminatory, and is based on how difficult friends and relatives found it to tell the twins apart, with the most discriminatory question being "were you as alike as two peas in a pod?" Where there was any doubt, or if the researcher was uncertain on appearances, zygosity was confirmed by DNA short tandem repeat fingerprinting. This was performed in approximately 40% of twin pairs and resulted in changing 9 of the 506 pairs' recorded zygosity compared to their answers to the questionnaire. Interestingly, around 15 of the twin pairs had spent their lives believing themselves to be different to the zygosity suggested by the questionnaire and confirmed by DNA fingerprinting, usually because of medical advice to their mothers around the time of birth, based on the number of placentas.

2.2 Consent

Ethics approval from the Guys and St Thomas' Ethics Committee was obtained prior to the start of the Twin Eye Study. Twins of the relevant ages were telephoned by

administrative staff of the Twin Research Unit, and asked if they wished to participate in the eye study, with a short description of the eye test. Those who accepted were invited to St Thomas' Hospital for an eye test. The twins attending were asked for consent to undergo a dilated eye examination, using the standard consent form used by the Twin Research Unit **(appendix)**. Some underwent the eye examination the same day after undergoing the other tests, but the majority attended specifically for the eye examination.

2.3 Questionnaire

After an initial explanation of the aims of the study, twins were asked about previous eye history using a standardised questionnaire **(appendix)**. The twins were both present when each individual was asked questions, which might lead to a recall bias, but experience has shown the twins are not keen to be separated for the tests. The second twin was asked their questions 10-15 minutes after the first to reduce this. The questionnaire was designed to establish previous eye history (such as strabismus, spectacle wear) and possible exclusion criteria (such as refractive or cataract surgery). Family history was also established, as well as any eye medication the twins may have used. General health questions and systemic medications were established in the original visit of the twins to the Twin Research Unit, and so were not included in the eye questionnaire. These data were recorded, and the Twin Research Unit has data on relevant exposures that include smoking, menopausal status and hormone replacement therapy, alcohol intake and measured variables including blood pressure, weight and height, and lipid levels. However, these have not been further analysed in this thesis: firstly, the data analysed are complex and there was not time to perform additional analysis, and secondly the aim of the study was to examine the heritability, which sums the overall effects of genes and environment. Many risk factors such as smoking are more concordant in MZ twins than DZ twins, and there are many twin studies showing that many aspects of personality have an important genetic component, even smoking (ref: Benowitz NL. The genetics of drug dependence: tobacco addiction. *New England Journal of Medicine* 1992; **327**: 881-883). This further complicates attempts to dissect out the effects of specific risk factors in such a study. Twins who are either exposure discordant or outcome discordant can be used for more specific risk factor analysis, not addressed in this study.

2.3.1 Data Entry

Data for the eye questionnaire as well as eye examination findings were entered onto a proforma **(appendix)**. This was subsequently inputted into a personal computer-based Access database by single entry, all by the study investigator. Quality control consisted of 3-monthly checking of data entered with an administrative assistant, auditing a random selection of 1/10 of data entered. During these checks mistakes noted were rectified and documented. On all occasions, the error rate was

approximately 0.1% of entries. In addition, after all data had been entered, all extreme values in the data being analysed were rechecked back to the original forms.

2.4 Eye examination

As part of the eye examination, all twins underwent monocular visual acuity testing using the logmar visual acuity chart, as used by the ETDRS studies. Acuity testing was performed with spectacle correction or with pinhole. Cover testing was performed at 6 metres, and stereopsis was assessed using the TNO stereotest. Pupil reactions were then assessed using a pentorch. Intraocular pressure was not measured in this study, because of concerns about the procedure jeopardising the quality of the macular photographs. The retina was examined on the slit lamp using an indirect biomicroscopy lens after pupil dilation, and if the optic disc appeared suspicious for glaucoma, then the intraocular pressure was measured after fundal photography. Any findings requiring further investigation or treatment resulted in an immediate letter given to the twin on their day of examination addressed to their general practitioner, asking for referral locally.

2.5 Reproducibility

30 unselected twins were measured on two occasions (between 1 and 6 months after the initial visit) to study the reproducibility of the measurements, where appropriate. The investigator did not see their original measurements or photographs until after the second visit. Fundus photographs were not repeated but all other tests were performed as at the first examination, detailed below

.

2.6 Refractive error measurement

A Humphrey-670 automatic refractor was used to assess refractive error. An automatic refractor measures refractive error by detection of infrared light aligned through the pupil and reflected back by the retina. A sphere (optometer) mirror is moved and stokes lens sets adjusted until the null point is found and the light is

reflected back on itself. At the null point the optics represent the prescription of the subject. Keratometry readings were obtained by capture of a CCD frame with reflections from 9 source LEDs and the corneal curvature is calculated from distortions of the reflection (Carl Zeiss Ltd, personal communication).

Two measures of refractive error were recorded for each eye: spherical equivalent (the spherical component of the refraction plus half of the cylindrical component) and total astigmatism (with its minus-cylinder axis). Corneal astigmatism was calculated from the keratometry readings. The keratometry readings were not recorded from all subjects, as the autorefractor recording these was unavailable for 6 months of the study during which another autorefractor was used which did not measure the keratometry. The two machines were compared on a sample of 20 twins and found to give very similar readings on measures of spherical equivalent and total astigmatism. Corneal astigmatism was calculated as the difference between the two axes of the keratometry readings obtained by the autorefractor in those twins with data. All readings were recorded in dioptres.

Most practising ophthalmologists are aware that autorefractors are very accurate at assessing the angle of astigmatism, but do differ a little with absolute amounts of spherical equivalent and astigmatism compared to subjective refraction. However since the twin pairs were examined together and the examiner could not be masked to the zygosity (MZ twins are very identical, even when they are 70 years old!), it was decided to use the objective autorefractor rather than subjective refraction with retinoscopy.

2.7 Cataract assessment

Pupils were dilated with one drop of 1% tropicamide followed 45 seconds later by one drop of 10% phenylephrine. After the questionnaire had been completed, twins were sent off for a coffee break to return for cataract assessment at least 50 minutes following instillation of eyedrops to allow for maximal dilation.

2.7.1 Oxford Clinical Cataract Classification and Grading System (OCCCGS)

Subjective grading of cataract was obtained using the OCCCGS.[134] 10 different features of the lens are graded by the OCCCGS by an observer at the slit lamp using standardised settings, based on comparison with reference standards drawn on a flip chart attached to the slit lamp. The OCCCGS has been changed to include decimalised steps,[180] to improve detection of differences.[174] Most scores are graded from 0 to 5, in steps of 0.1. The OCCCGS has been shown to be reproducible.[179]

Nuclear cataract

Two of the 10 components graded by the OCCCGS reflect nuclear cataract: white scatter (light scattered back when shone into the lens) and brunescence (brown discoloration seen in lenses with cataract). The subject's lens was viewed in a standardised fashion (slit illumination on full power at 45 degrees with slit width 0.3mm using the same slit lamp for all subjects) and compared with five reference standards, based on standard Munsell color samples for brunescence and neutral density grey scale samples for white scatter, which are shown in Figure 3 and Figure 4. Shades and colours are not exact due to reproduction of the templates. Each lens was given a score from 0 to 5 for brunescence and for white scatter. Brunescence and white scatter scores are comparable to nuclear colour and nuclear opalescence in other subjective grading systems, e.g. the Lens Opacity Classification System (LOCS).[135; 181]

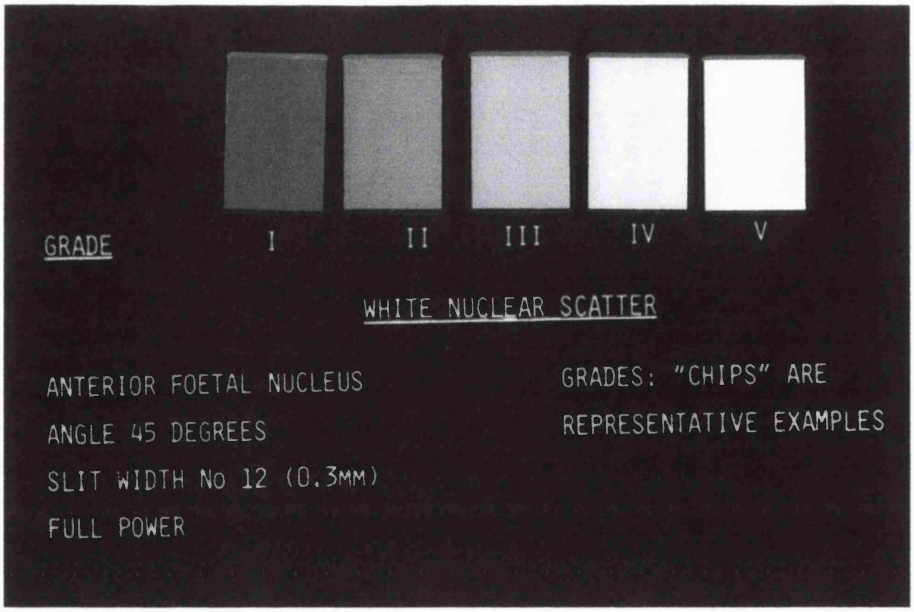


Figure 3 Standard grading template for white scatter with OCCCGS

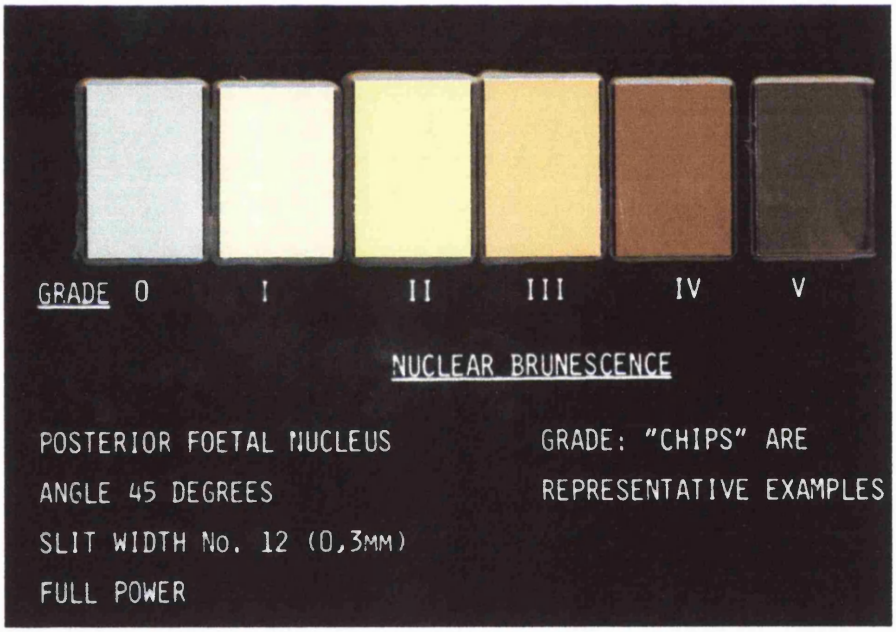
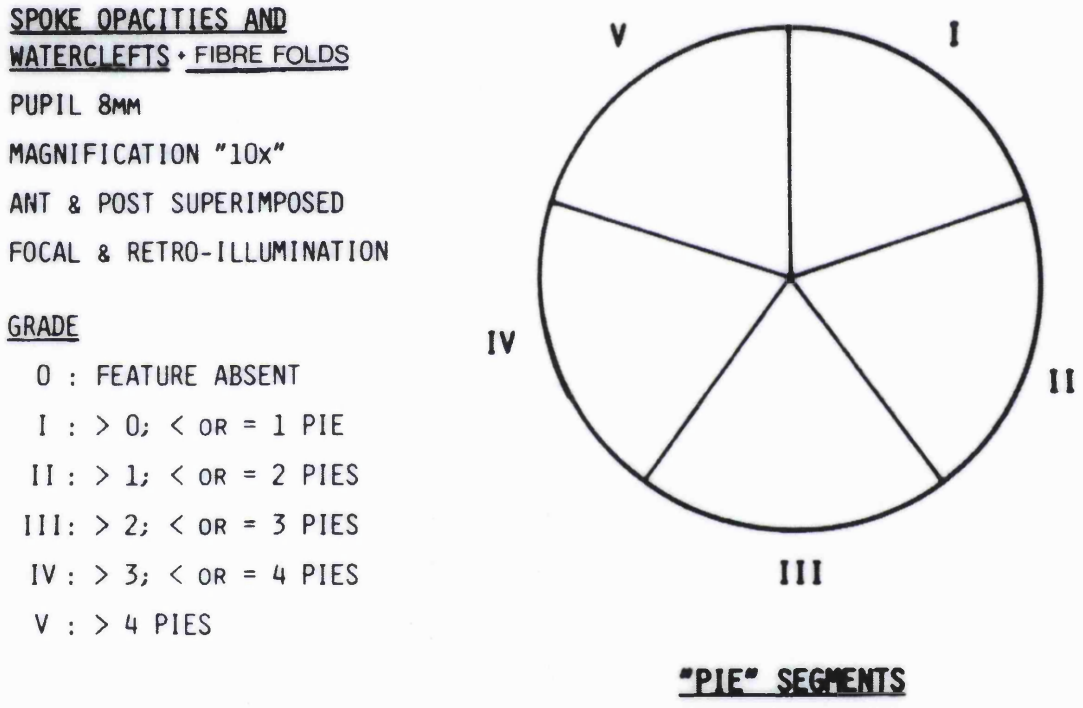


Figure 4 Standard grading template for brunescence with OCCCGS

Cortical cataract

The amount of cortical cataract was assessed using the OCCCGS which divides the total area of the lens visible within the pupil into five "segments" of a pie chart, as in Figure 5. The grader assesses the approximate area covered by the cortical spokes seen, again resulting in scores from 0 to 5 in steps of 0.1.

Figure 5 Grading standard chart for cortical spoke opacities in OCCCGS



Other features

Other features assessed by the OCCCGS include vacuoles, retrodots, waterclefts, focal dots, etc. These features rarely affect vision such that they result in cataract extraction, and so are not included in other grading systems such as the LOCSIII

system. Their significance is not entirely certain, but the features were graded as part of the study.

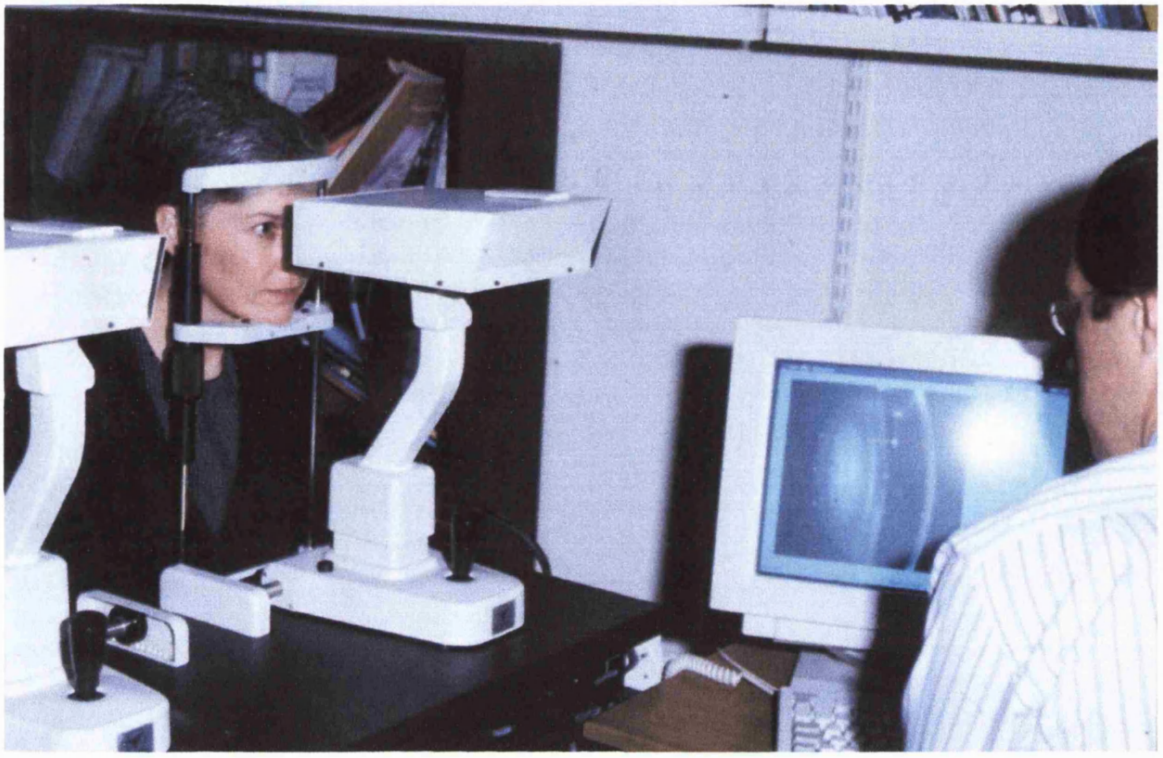
Training in OCCCGS grading was provided by Mr John Sparrow at the Bristol Eye Hospital, who pioneered the grading system. In addition, the study investigator visited the epidemiology unit at Leicester University (Mr James Deane) and had discussions with the team there who have considerable experience in cataract and AMD grading.

2.7.2 Scheimpflug lens imaging

An objective grading system was also used because of difficulty grading early lens opacities in subjective grading,[182] and potential bias due to knowledge of twins' zygosity when seen together. The Scheimpflug charge couple device (CCD) camera system developed in Oxford was used (Marcher Enterprises Ltd, www.marcher.co.uk),[190]and is seen in Figure 6. It is based on a slit lamp camera modified along the Scheimpflug principle to obtain photographs with the entire anterior segment in focus.[187] Digitised CCD images were taken in a dark room with standardised gain and exposure, and stored on computer. Densitometric analysis of these images results in reproducible nuclear cataract scores.[193]

Two images were taken of each lens of each subject. The two images were taken with a different gain but which were the same for all twins' examinations, to allow comparison of results. Software incorporated in the machine, the Marcher Case 2000 system, allows semi-automated densitometric analysis. Three scores were extracted from the images: central nuclear dip and anterior peak from the first image and nuclear average (which overlaps with the other two to an extent) from the second.

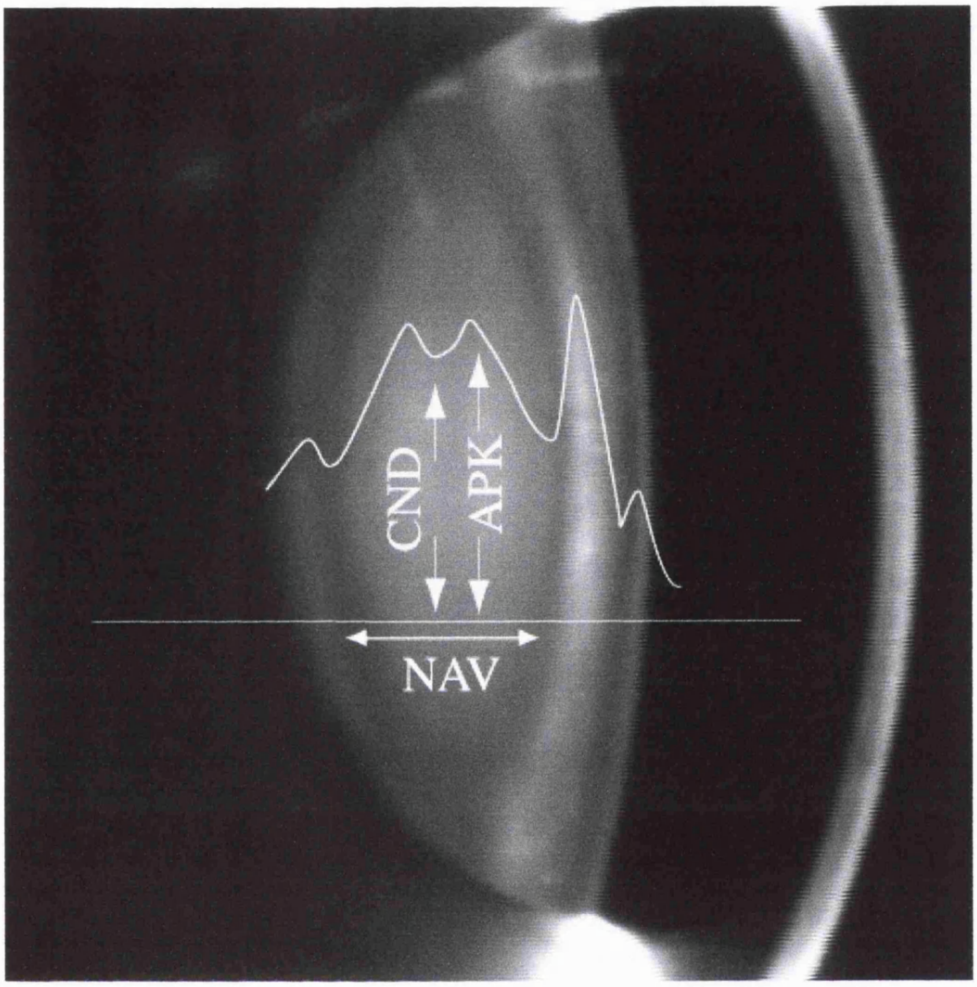
Figure 6 Case 2000 digital CCD camera for Scheimpflug and retroillumination photographs of subject's lens.



Densitometry scores were automatically saved in the Case 2000 database, and transferred to the Eye Study Access database attached to the eye questionnaire data.

An example of a Scheimpflug image of the lens of one subject is shown in Figure 7, with the densitometric measures superimposed. The superimposed white line represents the pixel density of the photograph in a strip 20 pixels high through the axial centre of the lens. The three scores measured from each photograph are illustrated: CND=central nuclear dip, APK=anterior peak, NAV=nuclear average. These three scores are likely to be strongly correlated, but as they have all been used in different cataract studies[175; 190; 204] and there is no consensus on the "best" score to assess the degree of cataract, all were included in this study.

Figure 7 Example of a Scheimpflug lens photograph.



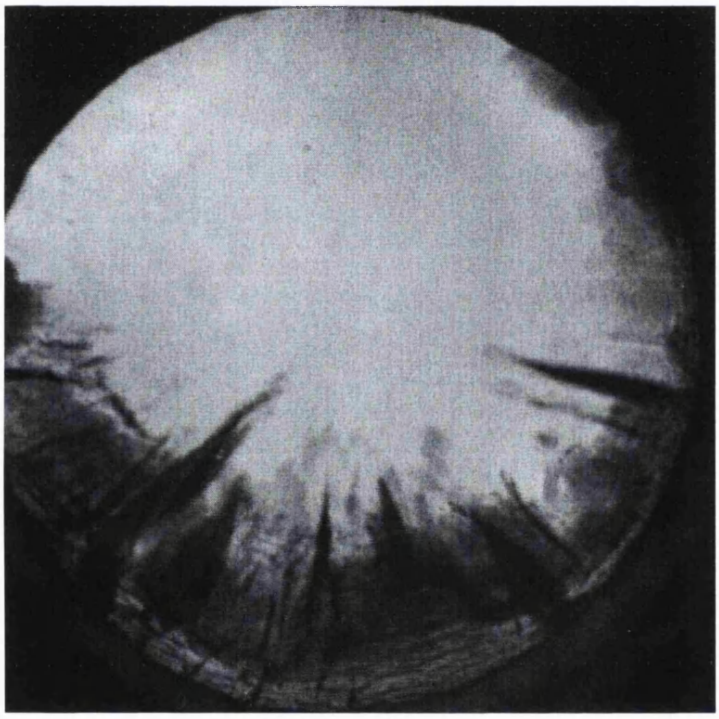
2.7.3 Retroillumination images

The Marcher Case-2000 system, which also has a retroillumination camera, was used for photography of cortical and posterior subcapsular cataracts as they cannot be assessed from cross-sectional Scheimpflug images. Two images were taken for each eye of each subject; one focussed on the anterior lens surface (most cortical spokes are in the anterior cortical part of the lens) and one focussed on the posterior lens to photograph posterior subcapsular cataract. Gain was not standardised: it was adjusted

for each image to allow for the best quality image with greatest contrast between clear and cataractous areas. Figure 8 shows an example of cortical cataract in the right eye of a twin.

Automated rather than subjective analysis of these images is difficult, because of the artefacts of uneven illumination across the image due to refractive error and uneven retroillumination because of the asymmetric optic disc.

Figure 8 Example of retroillumination photograph showing cortical lens opacities.



Automated analysis of retroillumination images

Retroillumination images were sent to the Wilmer Eye Institute at Johns Hopkins University for automated grading of cortical cataract. The system has been developed by Don Duncan in the Applied Physics Laboratory, in collaboration with Prof Sheila West of the Dana Center of Preventive Ophthalmology. Ours was the first dataset it has been used on. The programme involves sophisticated techniques to detect the pupil edge, detect pathology using secondary segmentation and extract the relevant

metrics. The Wilmer system has a great advantage in that the analysis of the images is totally automated, and involves no human decisions during the analysis, as many image analysis systems do.

Pupillary segmentation was performed using "snakes" and "balloons" deformable contours; snakes contract until external force is the same as the change in image intensity, and balloons expand until the internal pressure is the same as the change in image intensity. This is illustrated in Figure 9; when the "snake" on the left meets the "balloon" on the right, the pupillary margin is defined. If the two do not meet, then pupillary segmentation has failed.

Secondary segmentation takes account of the morphology and texture of the opacification to decide what is cataract and what is not (Figure 10 and

Figure 11). It is followed by a clean up process to extract measures such as spherical vacuoles or long, thin strands such as pupillary strands. The resulting opacification (in sixteenths of the pupil area) is extracted. The system can provide data on different pupillary diameters if standardisation is required (for example, in a longitudinal study).

Figure 9 Snakes (left) and balloons (right) are used to detect the pupil margin

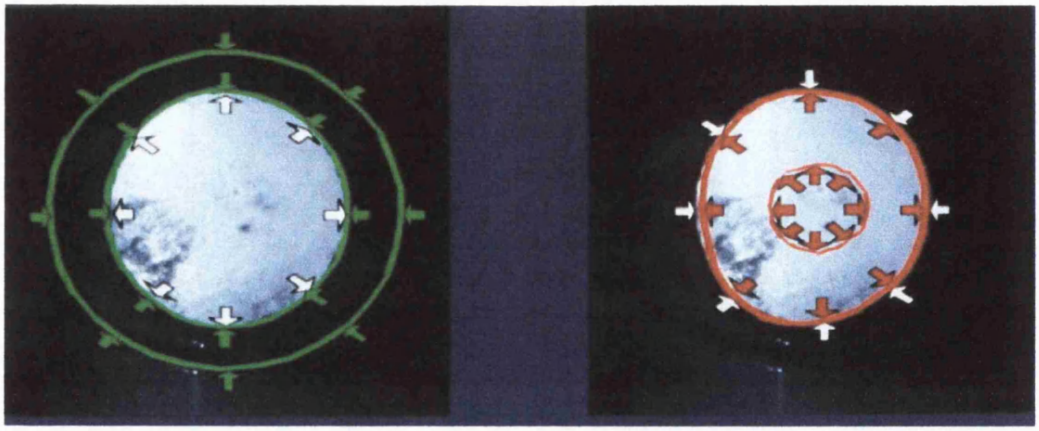


Figure 10 Secondary segmentation defines the areas of significant opacity

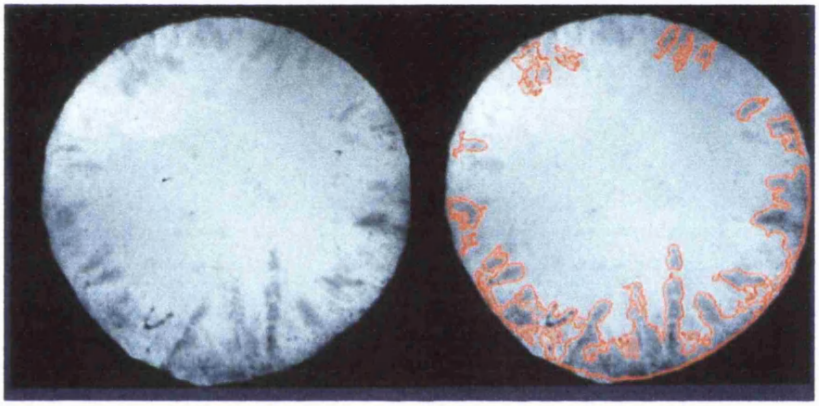
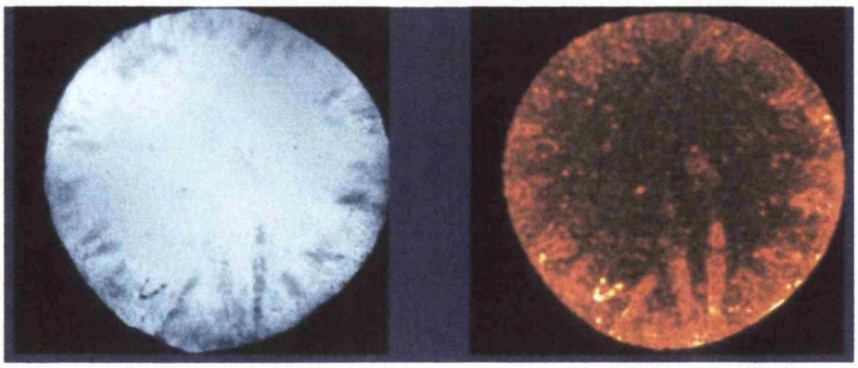


Figure 11 Texture statistics (fractionation) used to further define cataractous areas



2.8 *Age-related macular degeneration assessment*

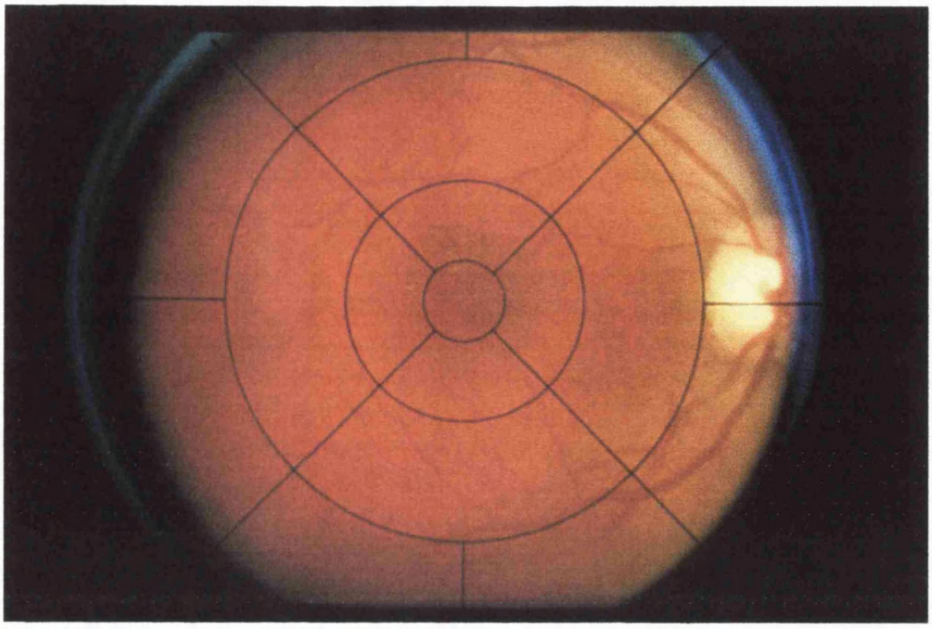
In order to assess the amount of ARM/AMD, stereoscopic macular photographs were taken of the twins. The fundus was photographed according to the International Classification guidelines,[214] by two photographs of each macula taken by a Kowa camera on a 30 degree width of field setting, and developed on Kodak Ektachrome 64 film. All film was processed by the same company, which processes all the fundus photographs at St Thomas' Hospital, to allow for as much consistency as possible.

Photographs were assessed using stereoscopic viewing spectacles on an x-ray viewing light-box, and graded according to the International Classification. Data was directly entered into a database attached to the eye questionnaire database. Training in grading was provided by Professor Alan Bird at Moorfields Eye Hospital, first author of the International Classification system, as well as the associate specialist working with him performing much of the grading, Miss Sarah Owens. Further training was obtained from the Leicester University group running the Melton Eye Study, based on the Winconsin grading training set of slides.

All macular photographs showing any abnormality, and a random sample of those judged to be normal, were assessed by another ophthalmologist unaware of the original grading. This was performed by Mr Andrew Webster, Wellcome Senior Research Fellow at the Institute of Ophthalmology and Honorary Consultant at Moorfields Eye Hospital, who was trained in grading macular photographs in association with Professor Bird. Where the two assessments differed, photographs were shown to Professor Bird for arbitration and these results were used in the analysis.

The classification system quantifies the size and type of drusen and their location and frequency within defined regions of the macula. In addition, areas of hypopigmentation and hyperpigmentation are noted, as well as late-stage disease including geographic atrophy or subretinal neovascularisation. Figure 12 illustrates the grid superimposed over the macula of a patient (from the Wisconsin example set,[209] not from this study) with ARM, centred on the fovea.

Figure 12 Grid from grading system superimposed over macula to define standardised regions



2.8 Methods of Analysis

Data was input into an Access database specifically written by the investigator for the Twin Eye Study. Data was exported to a statistical programme, STATA,[268] which was used to analyse means, standard deviations, correlations (for normally-distributed variables) and other general statistical applications.

Structural path equation modelling was performed with Mx.[269]

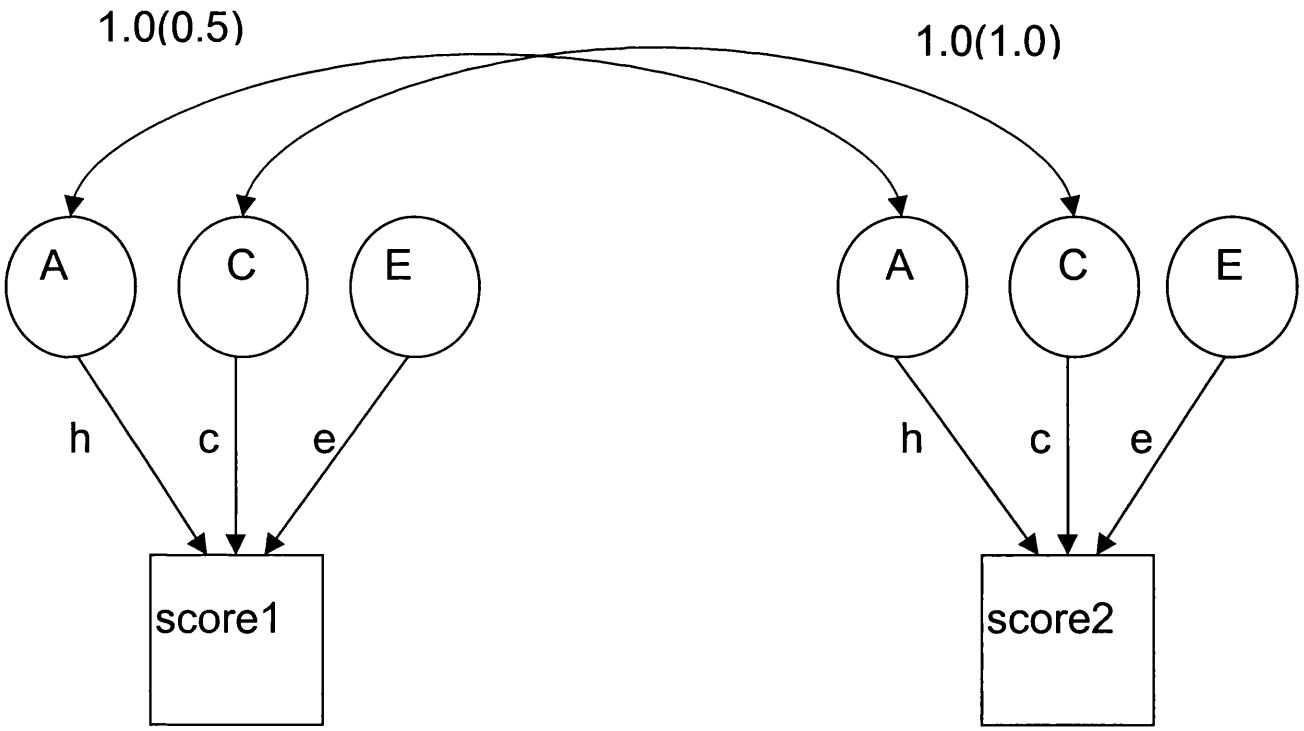
2.8.1 Analytical approach for continuous data

Details of model fitting to twin data have been described elsewhere.[50; 270] In short, the technique is based on the comparison of the variance-covariance matrices in MZ and DZ twin pairs and allows separation of the observed phenotypic variance into additive (A) or dominant (D) genetic components and common (C) or unique (E) environmental components using structural equation modelling. E also contains

measurement error. Dividing each of these components by the total variance yields the different standardised components of variance, for example the heritability (h^2) which can be defined as the ratio of additive genetic variance to total phenotypic variance.

Figure 13 below illustrates a path model for the observed scores for twin 1 and twin 2 (score1 and score2) which are represented in squares as they are measured variables. Latent factors are represented in circles: A, C and E are the additive genetic, common environmental and unique environmental influences. D, the dominant genetic influence, is omitted to simplify the diagram. The correlation between the latent genetic factors is 1 for MZ pairs and 0.5 for DZ pairs. For the dominant genetic factors it is 1 and 0.25 for MZ and DZ pairs respectively. Regression coefficients of the observed variables on the different latent factors are shown in lower case: h is the additive genetic effect, c the common environment effect, and e the unique environmental path coefficient.

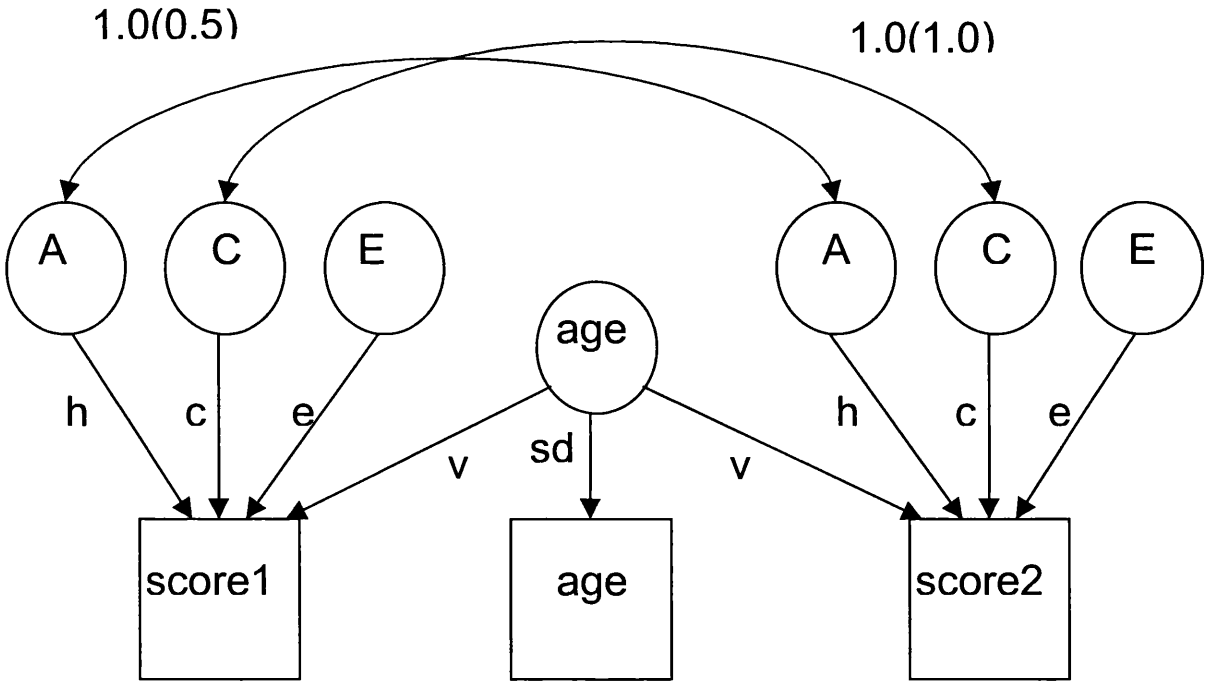
Figure 13 Example of a path diagram of an ACE twin model for measured variable of "score"



The effect of age on modelling

Age is an important risk factor in age-related diseases such as cataract. As twins share the same age, correlations for both MZ and DZ pairs will be inflated for age-related traits. If not accounted for, the effect of age is confounded with C, their common environment.[271] To eliminate this, and to allow estimation of its effect on the variance within the population, age was incorporated into the model. Figure 14 illustrates the twin model used for analysis, including age. In this case abbreviations are the same as above, plus v the age-effect latent factor and sd the standard deviation of age.

Figure 14 Standard ACE twin model, incorporating age effects.



Model fitting procedure

A series of models were fitted to the variance-covariance matrices. The significance of variance components A, C, D and age was assessed by testing the deterioration in

model fit after each component was dropped from the full model, leading to a model with as few parameters as possible. Models constraining all genetic effects to be nonadditive (i.e. the DE model) are considered unlikely as they lack a sensible biological interpretation.[50; 272] Submodels were compared with the full model by hierarchic χ^2 tests. The difference in χ^2 values between submodel and full model is itself approximately distributed as χ^2, with degrees of freedom (df) equal to the difference in df of submodel and full model. Model selection was also guided by Akaike's Information Criterion (AIC = χ^2-2df). The model with the lowest AIC reflects the best balance between goodness of fit and parsimony.

Maximum likelihood modelling assumes a normal distribution of the variable in question, and where this was not the case for continuous data, such as astigmatism or nuclear cataract, then the data was transformed (usually with a log transformation) to render it more normal. By implication, data that is not normally distributed must be treated as categorical rather than continuous data, and its analysis will be discussed below.

Multivariate analysis

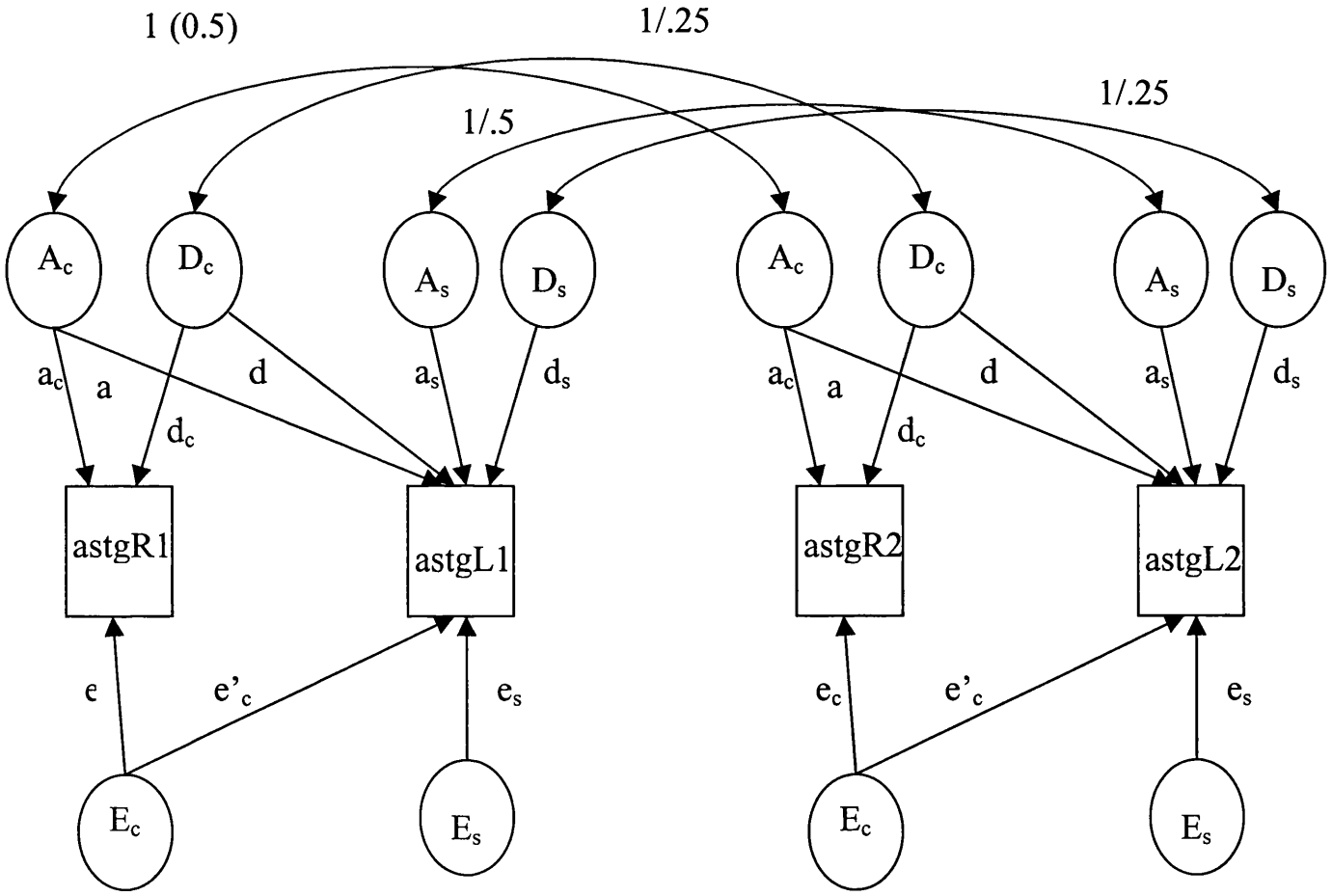
Extension of univariate to multivariate models allows for information from both eyes to be included into the model. Additionally some reasonable assumptions, such as the same genes influencing both eyes, can be incorporated into the model and tested for. The main advantage of multivariate modelling is an increase in power.[273]

Cholesky decomposition

A bivariate Cholesky decomposition[50; 274] was used to analyse measures for right and left eyes simultaneously. The Cholesky model allows exploration of the extent to which the different factors (A, C, D or E) can explain the variance and covariance of the outcome measures. Figure 15 illustrates a full Cholesky ADE model for astigmatism (astg) for right (R) and left (L) eyes for twin 1 and twin 2. The number of latent factors equals the number of variables: the first factor (A_c D_c or E_c) loads on both eyes, the second factor (A_s D_s or E_s) loads only on the second (i.e. left) eye in the

model. DZ twins share half the additive genes (A_c or A_s) and only a quarter of the dominant genetic influence (D_c or D_s) compared to MZ twins, so correlations between those latent factors are different for MZ and DZ twins (DZ figures in brackets).

Figure 15 Cholesky bivariate decomposition model for astigmatism



The genetic correlation between right and left eyes gives an indication of the amount of overlap between (sets of) genes influencing both eyes. Genetic correlation is calculated as the (additive) genetic covariance between the two eyes divided by the square root of the product of the total genetic variance components of each eye.[271]

Three submodels of the full Cholesky model were examined to test three different hypotheses. (1) Are both eyes influenced by the same set of genes? This is the case if specific genetic influences (a_s and d_s) can be set to zero without a significant reduction in fit of the model. (2) Is the size of the genetic effect the same in both eyes? This is the case if the genetic regression coefficients for left and right eye can be set equal (a_c=a'_c and d_c=d'_c) without a significant reduction in fit of the model. (3) Is the measurement error the same for both eyes? To test this hypothesis the influence of unique environment was reparameterized: this independent pathway structure is equivalent to the Cholesky structure in this case. The specific unique environmental influences can be set equal and the model tested for reduction in fit.

Factor analysis

In this study there were several potentially highly correlated variables, for example white scatter in nuclear cataract measured by the three Scheimpflug image scores and one OCCCGS score from right and left eyes. Factor analysis was used to test to what extent they could be summarised in one measure ("nuclear cataract" in this example). Factor analysis derives a number of unrelated linear factors from a number of variables which may be related to each other. Each factor is given an eigenvalue, which represents the amount of variance attributable to it. The number of factors may be the same number as the variables, but where the variables are related, insignificant factors can be excluded. Those factors with an eigenvalue greater than or equal to 1 are retained in the analysis.[275]

Factor loadings, equivalent to Pearson's correlation coefficients between each measure and each factor, were produced.[276] In addition, factor score coefficients were estimated for each of the retained factors and a single factor score for each individual was calculated as a weighted sum of the values of the standardised measures, using the scoring coefficients of the first factor as the weights. This factor score was then used as a continuous variable in univariate model fitting analysis of heritability, to produce a single heritability estimate for a score which had several measures taken of it, such as nuclear cataract.[277]

2.8.2 Analysis of non-continuous data

The maximum likelihood modelling methods used in twin analysis (modelling twin covariances) assume that the trait being analysed must be normally distributed. This is not true for cortical cataract or AMD where many subjects had no disease. The genetic and environmental contributions can, however, be quantified by assuming there is a continuous underlying liability to disease (involving multiple genetic and environmental factors). The correlation in liability among twins can be estimated from the frequencies of disease-concordant and disease-discordant pairs, using a multiple threshold model.[49; 50] Multiple thresholds were created by categorising the amount of cortical cataract into 8 categories for both Oxford and Wilmer grading systems, rather than using continuous data of cortical scores. Correlations between the twins can then be calculated using polychoric correlation matrices, using PRELIS.[278] Age, an important risk factor in cortical cataract and AMD as well as nuclear cataract, must be accounted for as before.[271] Therefore polyserial correlation matrices including correlations between age (a continuous trait) and cataract (categorical data) were calculated for MZ and DZ twin pairs using PRELIS.[278] These polyserial correlation matrices were used in the Mx genetic modelling program.[269]

2.8.3 Analysis of bivariate data

For bivariate data, ie those traits with "yes/no" answers (eg hyperpigmentation present or absent), analysis was performed using simple 2x2 contingency tables to calculate the pairwise concordance. The pairwise concordance, which is the risk of a twin developing a disease (or having a trait) if their cotwin already has that disease or trait, is calculated by the formula concordance=2C/(2C+D), where C is the number of pairs concordant for the phenotype in question, and D is the number of pairs discordant for that trait. A greater concordance for MZ twins (as seen using an MZ:DZ ratio) suggests that genetic factors are important. These 2x2 contingency tables can be incorporated within the Mx maximum likelihood modelling programme, with an assumption of an underlying normally-distributed liability, to provide estimates of the relative importance of genetic and environmental factors.[269] However, the loss of power with binary data is considerable compared to continuous data, and so it is

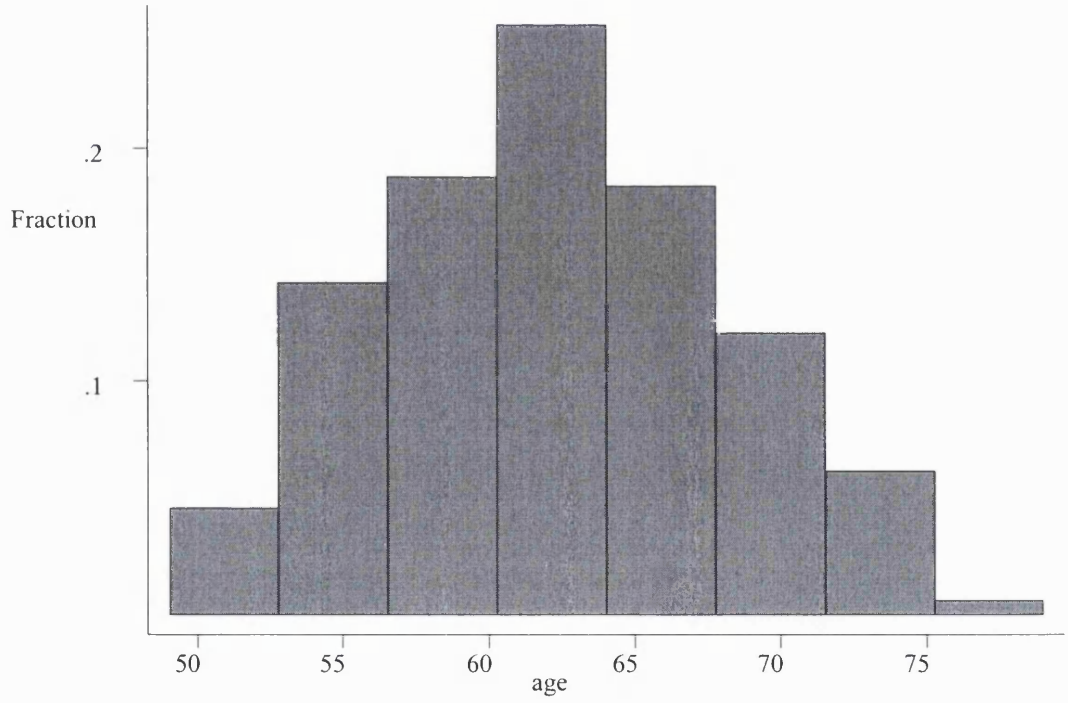
possible that even with over 500 twin pairs phenotyped in this study, there may not be suffficient power to distinguish between different models if the prevalence is not high enough.

3.0 Results

3.1 Study Population

The final numbers of twins examined for this study were 226 MZ twin pairs and 280 DZ twin pairs, making 506 pairs in total. The age distribution of the twins is detailed in Figure 16. The overall mean age was 62.2 years, with standard deviation 5.73, and the range of ages was from 49 to 79 years.

Figure 16 Frequency histogram of ages of twins seen in Twin Eye Study.



For age-related traits, it is important to ensure the ages of MZ and DZ twins are similar (in addition to other factors such as the variance of the outcome being measured) to allow valid comparisons and analysis. Table 18 shows that MZ and DZ

twin pairs were closely matched in mean age, as well as the proportions of each type of twin pair falling into different age categories.

Table 18 Numbers and age distribution of MZ and DZ twins seen in the Twin Eye Study

	MZ twin pairs	DZ twin pairs
Number seen	226	280
Mean (SD) age	62.4 (5.72)	62.1 (5.75)
Age range	51-75	49-79
Age groups	%	%
49-54	11	10
55-59	24	24
60-64	27	36
65-69	28	21
70-74	9	8
75-79	0.4	1

Table 19 below details the number of twins on the UK twin registry seen at the Twin Research and Genetic Epidemiology Unit, and the numbers in each group seen in this Eye Study. Two thirds of twins over 70 were seen, and approximately half of those aged 60-70 years were examined. Recruiting all twins over 60 was not possible as some twins had moved, withdrawn from the twin studies, or their details were not available to administrative staff when contacting the twins. Other twins have not been seen on the Twin Research Unit for a general baseline visit (only these twins were examined in the eye study in case other baseline data would be needed), and many more DZ twins have been seen than MZ twins (for sib-pair genetic studies). This study recruited most of the MZ twins available in the specified age group. Recruiters, unfortunately, have not kept data of refusals to attend, but report very few, at the rate of about 1 in 10-20 approached, usually due to logistic reasons of travel and time.

Table 19 Numbers of twin pairs seen in the Eye Study and number on the register of the Twin Research Unit

	Registered	Eye Study
Pairs seen	2815	506
Age groups		
50-54	485	53
55-59	402	121
60-64	361	162
65-69	216	122
70-74	66	44
75-79	4	4

20 pairs of twins initially agreeing to the eye study subsequently were not examined: 10 due to illness of one twin or within the family, 6 for no reason given to the Twin Research Unit, and 4 who changed their mind. The mean age of these 20 pairs was 59.3 years, but otherwise there seemed to be no difference from the other twins.

The age group initially decided on was 50-79 years, but this was extended from 50 years and over to 49 and over because two pairs of that age were given appointments for the eye study. One pair of twins were wrongly registered on the Registry database and were only 42 years old when they attended for the eye examination and so were excluded from analysis.

There was some publicity about the eye study at its commencement, so to examine if this caused any bias in the twins seen, they were all asked whether they had been recruited by the study administrators, or whether they had volunteered for the eye study in particular. 102 individuals reported knowledge of the eye study from this publicity and volunteered to attend the eye study (10%), 70 DZ and 32 MZ. Their mean age was 60 years compared to the overall average of 62 years, and their average scores (with overall average in brackets) were 2.0 (2.17) for white scatter, 0.39 (0.45)

for spherical equivalent, 0.66 (0.73) for astigmatism. These figures do not suggest a significant bias of twins with eye disease volunteering for the study having heard about the study rather than being recruited by the administrators.

3.2 Reproducibility

Thirty twins (15 pairs) were randomly selected and asked to return for a repeat visit 1-6 months after their initial visit for a repeat examination to determine how reproducible the measurements for refractive error and cataract were. It was decided on this interval to avoid the examiner remembering his original grading of the subjective cataract scores. It was decided not to rephotograph the maculae for ARM grading, as the twins reported it to be unpleasant due to the brightness of the flash, and we felt that the same photographs could be subject to regrading by the graders to assess their reproducibility instead.

3.2.1 Reproducibility of Refractive Error measures

Intraclass correlations (ICC) obtained for the 30 subjects are listed in Table 20. They show a high reproducibility for all measures recorded by the autorefractor.

Table 20 Reproducibility intraclass correlations (ICC) of autorefractor readings

Measure	ICC for Right eye	ICC for Left eye
Spherical equivalent	0.99	0.98
Total astigmatism	0.93	0.85
Keratometry 1	0.99	0.97
Keratometry 2	0.97	0.98

3.2.2 Reproducibility of Cataract Grading Scores

The intra-observer reproducibility study of 30 unselected twins from this series was performed, and showed high reproducibility for Scheimpflug image scores. Intraclass correlations for OCCCGS scores were good for cortical cataract, reasonable for white scatter, but less good for brunescence.

Table 21 Intraclass correlations (ICC) of cataract scores in reproducibility sample of 30 twins.

Measure	ICC Right eye	ICC Left eye
Central nuclear dip	0.97	0.96
Anterior peak	0.92	0.94
Nuclear average	0.98	0.97
White scatter	0.67	0.64
Brunescence	0.50	0.52
Cortical spokes	0.89	0.69

As the Wilmer grading for cortical cataract is completely automated, the reanalysis of the same images results in identical scores. For the repeat analysis of the twins included in the reproducibility study, the intraclass correlation for the worse eye score was 0.93.

Interobserver comparability

Although the grading was all perfomed by a single observer (CH) for this study, for the purposes of training my results were compared to the "gold standard" grader, Mr John Sparrow, Senior Lecturer and Consultant Ophthalmologist in Bristol, who developed the OCCCGS. In order to maximise pathology detected, a series of preoperative cataract patients were graded by both observers over three days: the first day was training, and the second and third involved blinded grading by both graders and comparison of results. 12 patients were assessed on these two days, and the

intraclass correlation for white scatter scores of the 19 eyes graded (5 were pseudophakic already) was 0.93 for the two graders.

Cataract grading drift

The OCCCGS involves subjective grading of cataract. In order to assess whether there was any drift during the study, the score for white scatter was plotted against the date of visit, illustrated in Figure 17. Although it does not look as if there is any drift, when age of the twins examined is plotted against date of visit, seen in Figure 18, it can be seen that the age of the twins examined tended to become lower during the study. Therefore the cataract scores, being age-related, should have become lower over the course of the visits. The Scheimpflug image scores (not shown) all showed a negative incline over the span of the study, suggesting no drift. The combined nuclear cataract score, used in the final analysis, plotted against the date of visit, has overall a slight negative slope (Figure 19).

Figure 17 White scatter score from OCCCGS (right eye) plotted against date of visit for each twin

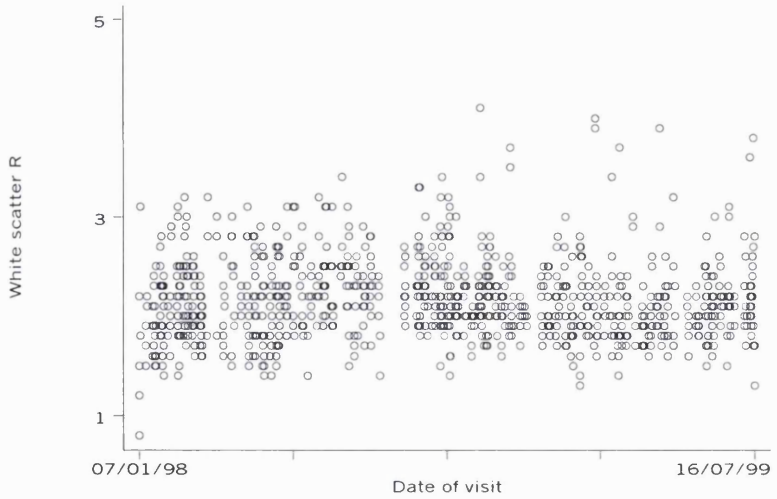


Figure 18 Age of twin plotted against date of visit

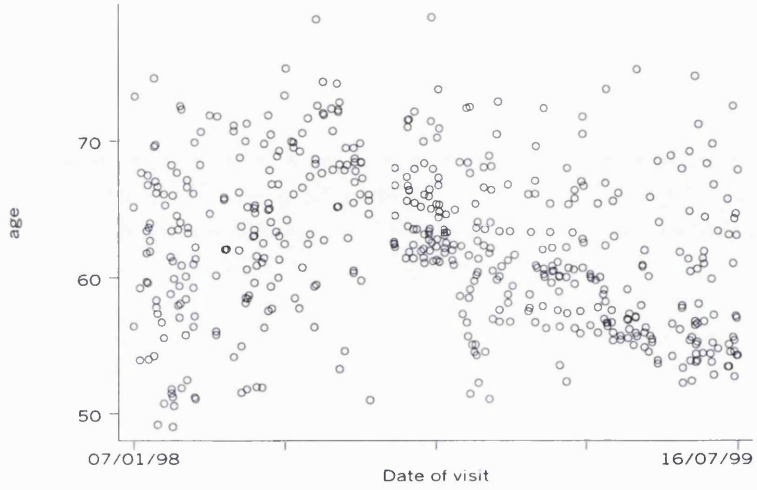
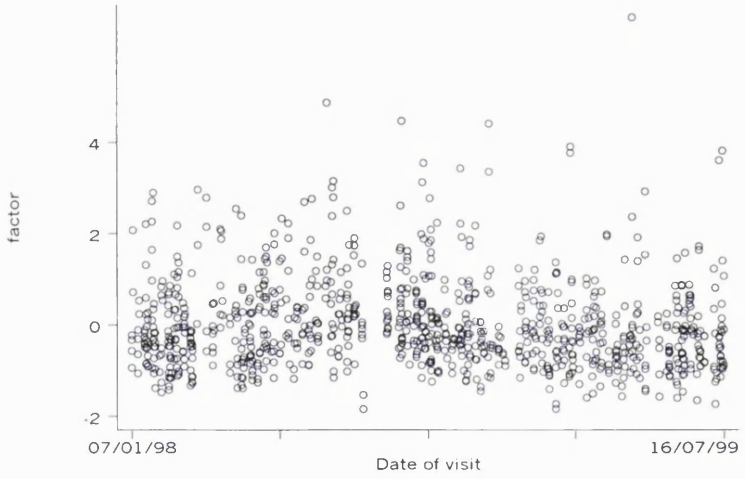


Figure 19 Factor score (combined nuclear cataract score) plotted against date of visit



To assess the impact of this grading drift, the generalised estimating equation was used. This statistical technique can include the non-independence of the twin pair results, and estimate the effect of the date of visit on the actual grade, taking into account the age of the subjects. The date of visit significantly affected the OCCCGS grades (all on the scale of 0-5) over the 18 months of twin assessment, amounting to a change in score of 0.14 (p=0.002) for white scatter (mean of twin scores was 2.1, standard deviation 0.4). The drift of brunescence score over 18 months was 0.20

($p<0.001$), which is highly significant given the standard deviation was 0.3 and mean 0.7 overall. For cortical spokes, the OCCCGS drift was 0.18 ($p=0.03$).

The drifts of the objective grading techniques used in the study were non-significant. For the effect of date of visit on Scheimpflug image scores of nuclear cataract, p values were 0.79-0.89, and on the Wilmer grading of cortical cataract the p value was 0.89. Assessing drift of the factor (combined nuclear cataract) score which was used in the final analysis and included both objective and subjective grading, the date of visit did not have a significant effect ($p=0.2$).

3.2.3 Reproducibility of macular degeneration grading

Stereoscopic macular photographs were not repeated, but each photograph with any question of an abnormality (apart from peripheral small hard drusen) was graded by two graders, myself (CH) and Andrew Webster (AW) as detailed in the Methods section. Photographs of 222 individuals (of the 1012 in the study) were graded separately by CH and AW, and any disagreements were reviewed by both graders together, and given a final grading. There were 132 separate disagreements (often small, such as 34 eyes graded as having small hard drusen <63 microns diameter by CH and not by AW, which did not influence the actual prevalence of ARM). Finally, CH and AW did not reach agreeement (or were uncertain of the grading) on 25 individuals' photographs, which were reviewed by Professor Bird for a "final" opinion and his grades entered as the final grades.

Table 22 is an example of the grading from CH and AW for largest drusen in the left eye of each of the 222 individuals separately graded. The diagonals (in bold) represent agreement between the two graders.

Table 22 Comparison of ARM grading for largest drusen size of left eye

		AW GRADES									
C	Grades	0	1	2	3	4	5	6	7	8	*Total*
H	0	**53**	0	2	3	0	1	2	0	0	*61*
	1	3	**0**	0	0	1	0	0	0	0	*4*
G	2	34	1	**37**	7	6	0	0	6	0	*91*
R	3	4	0	17	**9**	4	0	1	2	0	*37*
A	4	0	0	0	2	**13**	0	1	1	0	*17*
D	5	0	0	0	0	0	**0**	0	0	0	*0*
E	6	0	0	0	0	2	0	**2**	2	0	*6*
S	7	0	0	0	0	0	0	1	**0**	0	*1*
	8	2	0	0	0	0	0	0	0	**3**	*5*
	Total	*96*	*1*	*56*	*21*	*26*	*1*	*7*	*11*	*3*	***222***

Grades: 0= no drusen, 1=uncertain, 2=hard drusen only, 3=intermediate 63-125μ soft drusen, 4=large >125μ soft distinct drusen, 5= large >125μ soft indistinct drusen (crystalline/calcific/glistening), 6= large >125μ soft indistinct drusen (semisolid), 7= large >125μ soft indistinct drusen (granular), 8=cannot grade

As the data was graded categorically, the two graders' performance was compared using the weighted kappa statistic.[279] Kappa statistics of 0.21-0.40 indicate fair agreement, 0.41-0.60 moderate, 0.61-0.80 substantial agreement and 0.80-1.00 almost perfect agreement. Table 23 demonstrates the weighted kappa statistics for the various items graded: largest drusen size, hyperpigmentation and hypopigmentation for the two graders compared to each other (CH vs AW) as well as each grader compared to the final agreed grading (FINAL).

Table 23 Kappa values for comparison of graders for ARM grading

Item graded	Eye	AW vs CH	CH vs FINAL	AW vs FINAL
Largest drusen size*	L	0.50	0.79	0.68
	R	0.50	0.74	0.68
Hyperpigmentationϕ	L	0.63	0.86	0.57
	R	0.97	0.93	0.90
Hypopigmentationϕ	L	0.34	0.71	0.56
	R	0.42	0.76	0.61

Abbreviations: AW=Andrew Webster grading, CH=Christopher Hammond grading, FINAL=final agreed grading. * = grading from 0 to 8, see Table 22 for description. ϕ = pigmentary changes graded from 0 to 4: 0= nil, 1=uncertain, 2=present but <63μ size, 3=present and >63μ size.

3.3 Refractive Error

3.3.1 Autorefractor Results

Figure 20, Figure 21 and Figure 22 below show the distributions of refractive error, total astigmatism and corneal astigmatism from the autorefractor readings, with normal curves superimposed (all graphs shown are for figures for the right eye of each individual). They show a wide range of measures (in dioptres) for all categories, with spherical equivalent and corneal astigmatism being approximately normally distributed, but total astigmatism being left-skewed. The square root of this figure best approximated a normal distribution, and was used for subsequent analysis. Although modelling requires a near-normal distribution, further transformation (such as the log transformation of the spherical equivalent data) did not result in any significantly different results, so the raw data was used.

Figure 20 Frequency histogram of measures of spherical equivalent for right eyes of all twins examined.

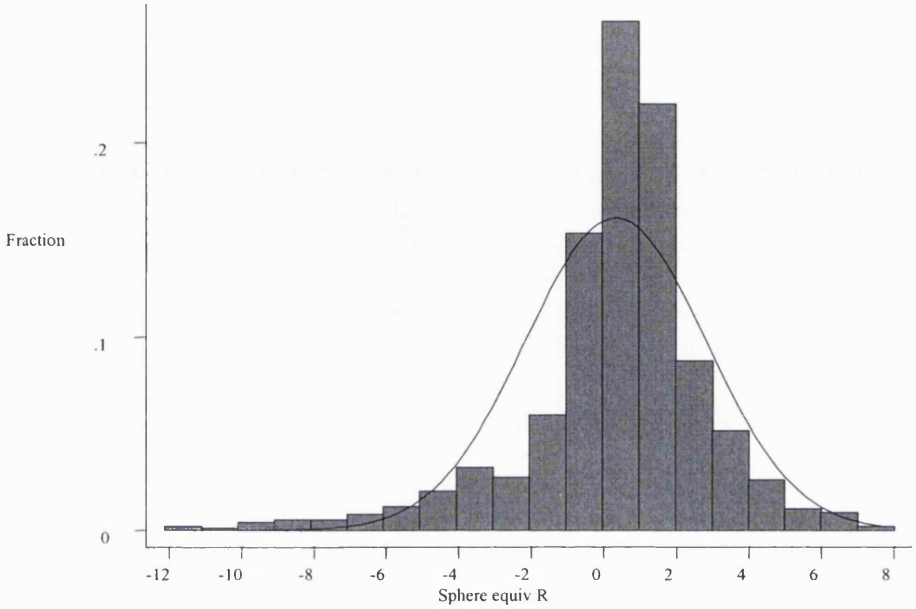


Figure 21 Raw values of corneal astigmatism values (calculated by subtraction of Keratometry2 reading from Keratometry 1)

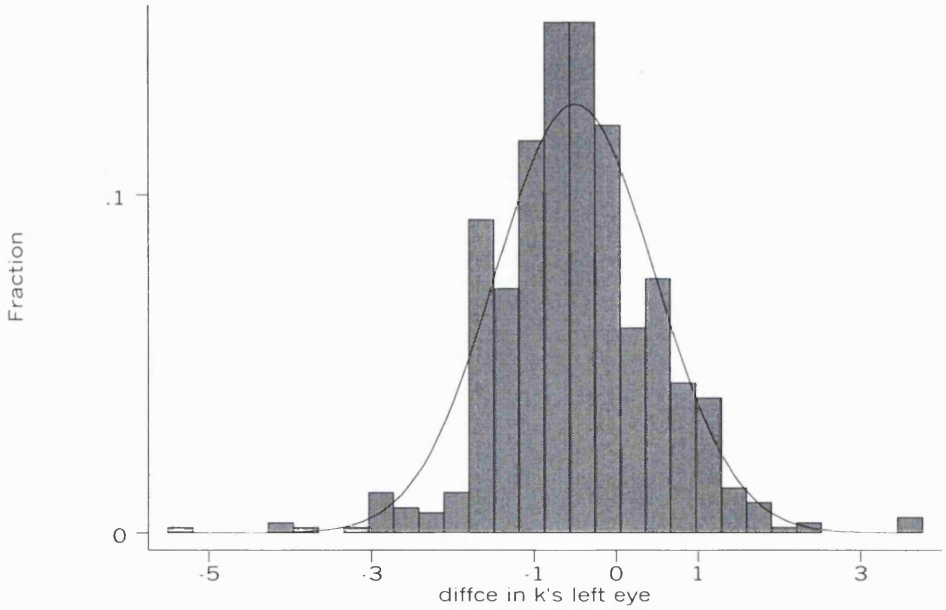
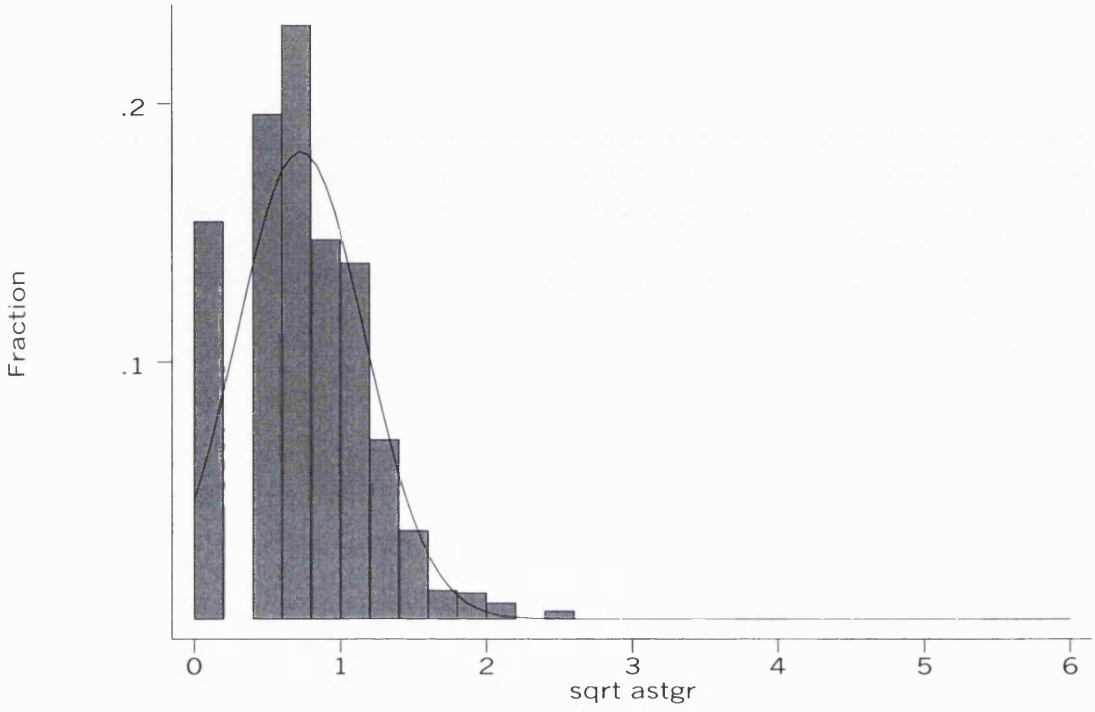


Figure 22 Total astigmatism for right eyes (in dioptres, above) and square root-transformed values (below) used in analysis



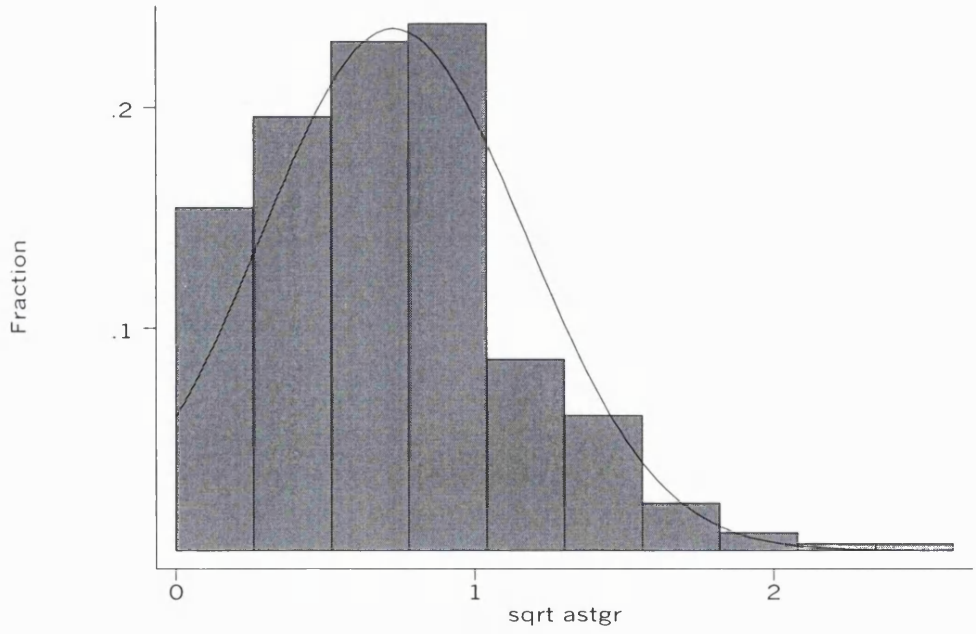


Table 24 shows mean values (SD) and ranges for spherical equivalent, total astigmatism and corneal astigmatism for right and left eyes in the two groups of twins. Values were similar for MZ and DZ twins and for right and left eyes. Only two-thirds of twins had keratometry recorded, resulting in fewer twin pairs with values for corneal astigmatism. This was because the data was not initially collected, as the initial autorefractor used did not have this capability. At the changeover of autorefractors, the two were compared on a sample of twins and compared well for all readings. Of the 1012 individuals, data was not available for 16 right and 17 left eyes. This was because 24 eyes were pseudophakic, and 9 were ungradeable due to corneal opacities, previous eye surgery or injury, which might have altered refraction. The resulting numbers of pairs of twins included in each analysis are detailed below.

Table 24 Results of autorefractor readings for MZ and DZ twin pairs, after exclusions

		MZ			DZ		
Measure	Eye	Mean (SD)	N	Range	Mean (SD)	N	Range
Spherical equivalent	right	0.31 (2.45)	215	-10.25 to +6.5	0.34 (2.51)	266	-12.12 to +7.25
	left	0.39 (2.44)	217	-10.37 to +7.25	0.49 (2.37)	263	-12.12 to +8.0
Total astigmatism	right	0.75 (0.77)	216	0 to 6.5	0.70 (0.77)	264	0 to 6.75
	left	0.75 (0.75)	217	0 to 5.25	0.70 (0.71)	262	0 to 5.5
Corneal astigmatism	right	-0.40 (1.07)	159	-4.0 to +5.5	-0.52 (1.0)	168	-5.75 to +3.75
	left	-0.43 (0.99)	162	-3.0 to +3.75	-0.56 (0.96)	166	-5.5 to +2.25

3.3.2 MZ/DZ correlations

The intraclass correlations for the measures are shown in Table 25. For spherical equivalent, the combination of a high correlation between MZ twins of more than 0.8 and DZ correlation approximately half that value suggests a strong additive genetic effect, as DZ twins share only half of the additive genetic effects compared to MZ twins. The correlations for astigmatism are lower for MZ twins, suggesting more

environmental (or measurement error) effects. For both measures of astigmatism, the DZ correlations approximate a quarter of the MZ correlations, which suggests a role for dominant genes, as DZ twins share only a quarter of the dominant genetic effect compared to MZ twins.

Table 25 Intraclass correlations within MZ and DZ twin pairs for measures of refractive error.

	Eye	MZ	DZ
Spherical equivalent	Right	0.86	0.47
	Left	0.83	0.48
Total astigmatism	Right	0.52	0.20
	Left	0.52	0.10
Corneal astigmatism	Right	0.70	0.13
	Left	0.61	0.20

3.3.3 Modelling Results

Univariate Analysis

The inferences above were confirmed by the results of model fitting. Results of univariate modeling are shown in Table 26. For spherical equivalent the best-fitting model is the AE model for both eyes: C and D can be dropped with no significant change in χ^2, but A cannot be dropped (ACE versus CE model: $\chi^2[1]=66.241$ for left eye and 102.386 for right eye, $p<0.001$ for both). The AE model (one ascribing variance due to additive genes and individual environment only) has the lowest AIC for spherical equivalent, representing the best-fitting and most parsimonious model.

Univariate analysis for total astigmatism shown in Table 26 suggests ADE is the best-fitting model for the left eye. AE is marginally more parsimonious for the right eye (a slightly lower AIC despite the ADE model having a lower χ^2), but the left eye fits the ADE model better (higher probability). Combining both eyes in multivariate analysis is likely to result in the ADE model showing the best fit. For corneal astigmatism, the ADE model again is the best-fitting model for both eyes, suggesting the inheritance of astigmatism involves additive and dominant genetic effects as well as individual environment.

Table 26 Model-fitting results for univariate analysis of spherical equivalent, (square root of) total astigmatism and corneal astigmatism

Measure	Eye	Model	χ^2	p	df	AIC
Spherical equivalent	right	ACE	18.392	0	3	12.392
		ADE	18.547	0	3	12.547
		AE	**18.547**	**0.001**	**4**	**10.547**
		CE	120.778	0	4	112.778
	left	ACE	3.924	0.213	3	-2.076
		ADE	5.788	0.12	3	-0.212
		AE	**5.788**	**0.22**	**4**	**-2.212**
		CE	70.385	0	4	62.385
Total astigmatism	right	ACE	5.801	0.12	3	-0.199
		ADE	4.556	0.21	3	-1.444
		AE	**5.801**	**0.21**	**4**	**-2.199**
		CE	24.21	0	4	16.21
	left	ACE	6.281	0.099	3	0.281
		ADE	**0.510**	**0.917**	**3**	**-5.49**
		AE	6.281	0.179	4	-1.719
		CE	27.784	0	4	19.784
Corneal astigmatism	right	ACE	21.506	0	3	15.506
		ADE	**14.388**	**0.002**	**3**	**8.388**
		AE	21.506	0	4	13.506
		CE	59.139	0	4	13.506
	left	ACE	15.996	0.001	3	9.996
		ADE	**13.656**	**0.003**	**3**	**7.656**
		AE	15.996	0.003	4	7.996
		CE	38.201	0	4	30.201

Abbreviations: χ^2 = Chi-square goodness of fit statistic; df = degrees of freedom, p = probability; AIC = Akaike's Information Criterion. See text for further abbreviations. Most parsimonious solution is printed in **boldface** type

Table 27 lists the standardised parameter estimates and 95% confidence intervals (95% CI) for the three measurements from the univariate analysis. Heritability is high for spherical equivalent (0.83 and 0.86 for the two eyes), and for astigmatism the

model estimates a significant effect of dominant genes (0.27-0.68) and lesser effect of additive genes (0-0.26), with unique environment responsible for the remaining variance (0.32-0.49). 95% CI are wide in the univariate ADE models, so the next step is to use multivariate analysis to try to increase power.

Table 27 Standardised parameter estimates and 95% confidence intervals of the best fitting models of univariate analysis of spherical equivalent, (square root of) total astigmatism and corneal astigmatism.

	Eye	a^2	95% CI	d^2	95% CI	e^2	95% CI
Spherical equivalent	right	.86	.83-.89			.14	.11-.17
	left	.83	.79-.86			.17	.14-.21
Total astigmatism	right	.26	0.0-.58	.27	0.0-.68	.47	.39-.57
	left	0	0.0-.64	.51	.11-.59	.49	.41-.59
Corneal astigmatism	right	0	0.0-.48	.68	.20-.75	.32	.25-.40
	left	.15	0.0-.64	.45	0.0-.68	.39	.31-.49

Abbreviations: a^2 = proportion of variance due to additive genes, d^2 = proportion of variance due to dominant genes, e^2 = proportion due to individual environmental effects, 95% CI = 95% confidence interval

Multivariate Analysis

The Cholesky bivariate decomposition is illustrated in Figure 15 and results are recorded in Table 28. The best-fitting full models from the univariate analysis, AE for spherical equivalent and ADE for astigmatism, were used. Figure 15 applies to astigmatism but can also apply to spherical equivalent if the dominant genetic effect is removed. For spherical equivalent, the genetic correlation between the two eyes is 0.98, suggesting the two eyes are controlled by the same genetic factors, although the submodeling (models 2 and 3) does not confirm this entirely.

Table 28 Model-fitting results of Cholesky decomposition for (square root of) total astigmatism, corneal astigmatism and spherical equivalent

Measure	Best-fit Model	Submodel	χ^2	df	AIC	$p(\Delta\chi^2)$	c. f.
Spherical equivalent	AE	1 Full	49.537	14	21.537		
		2 $a_s = 0$	54.526	15	24.526	0.03	1
		3 $a_c = a'_c$	58.467	15	28.467	0.003	1
		4 $e_s = e'_s$	49.762	15	19.762	n.s.	1
Total astigmatism	ADE	1 Full	12.550	11	-9.450		
		2 $a_s=0$, $d_s=0$	12.550	13	-13.450	n.s.	1
		3 $a_c=a'_c$, $d_c=d'_c$	19.555	15	-10.445	0.03	2
		4 $e_s = e'_s$	12.562	14	-15.438	n.s.	2
		5 $d_c=d'_c=0$	22.381	15	-7.619	0.007	2
Corneal astigmatism	ADE	1 Full	33.246	11	11.246		
		2 $a_s=0$, $d_s=0$	33.246	13	7.246	n.s.	1
		3 $a_c=a'_c$, $d_c=d'_c$	41.476	15	11.476	0.004	2
		4 $e_s = e'_s$	33.939	14	5.939	n.s.	2
		5 $d_c=d'_c=0$	40.206	15	10.206	0.03	2

For abbreviations see Table 26. . In addition A_s D_s E_s = specific variance component of A, D and E for left eye, with paths a_c & a'_c common additive genetic factor pathways to right and left eyes respectively, similarly d_c & d'_c from D_c to right and left eyes. $p(\Delta\chi^2)$ = probability of the change in χ^2 is zero. c.f. = model number compared to in this submodel.

In the full ADE Cholesky model for total astigmatism, genetic correlation is 1.0 and the specific additive and dominant genetic influences were estimated as zero. These observations were confirmed in the first submodel (model 2 in Table 28): both a_s and d_s can be set to zero with no loss of fit. However there is a significant loss of fit if the a_c and d_c paths are set the same (model 3), meaning that the size of the genetic effect for A and D may be different for the two eyes.

For corneal astigmatism, the full ADE model again is the best-fitting model, with model 2 suggesting that the same set of genes influences both eyes (model 2) but that size of genetic effect between A and D might be different for the two eyes (model 3).

There is no loss of fit if the specific environmental influence is set the same for right and left eyes for all three measurements of refractive error (model 4) – i.e. the measurement error is the same for both eyes.

The final model for astigmatism (model 5 for total and corneal astigmatism) sets the influence of dominant genes to zero resulting in a significant loss of fit. This confirms that the dominant genetic influence is significant, and illustrates the greater power of the multivariate modelling over the univariate modeling, in which the model for right eye total astigmatism allowed the dominant genetic influence to be dropped with no significant loss of fit (Table 28).

Table 29 displays the parameter estimates and 95% CI for the best-fitting models. For spherical equivalent, the heritability is 84-86%, with the remaining 14-16% of the variance due to unique environmental variance. Dominant genes explain a significant proportion of the population variance for astigmatism of 0.47 and 0.49 for total astigmatism in right and left eye, and 0.61 and 0.42 for corneal astigmatism (the wider 95% CI may reflect the smaller sample size of this measure). Additive genes explain a small proportion of the variance of astigmatism (0.01-0.18) and individual environment explains the rest of the variance (0.34-0.50).

Table 29 Standardised parameter estimates and 95% confidence intervals of the best fitting models of multivariate analysis of spherical equivalent, (square root of) total astigmatism and corneal astigmatism.

	eye	a^2	95% CI	d^2	95% CI	e^2	95% CI
Spherical equivalent	Right	.86	.83-.89			.14	.11-.17
	Left	.84	.81-.87			.16	.13-.19
Total astigmatism	Right	.05	.006-.13	.47	.37-.53	.48	.42-.56
	Left	.01	0.0-.07	.49	.42-.55	.50	.43-.58
Corneal astigmatism	Right	.04	0.0-.54	.61	.12-.71	.34	.29-.42
	Left	.18	0.0-.60	.42	.08-.66	.40	.33-.48

Abbreviations: see Table 27

Age Effect

The effects of age were considered as the myopic effect of early nuclear cataract might come into play with the older twins of this cohort. In fact the correlation between age and spherical equivalent is weak, with a correlation coefficient of 0.1 (Figure 23). When age is incorporated into the model for spherical equivalent, it only accounts for a modest 1.4% (95% CI 0.2-3.9) of the population variance. Similarly astigmatism is weakly correlated with age as seen in Figure 24, with a coefficient of 0.15 for both total and corneal astigmatism. Modelling again predicts that age accounts for a small proportion of the population variance of astigmatism of under 3%.

Figure 23 Changes in spherical equivalent of the right eye with increasing age.

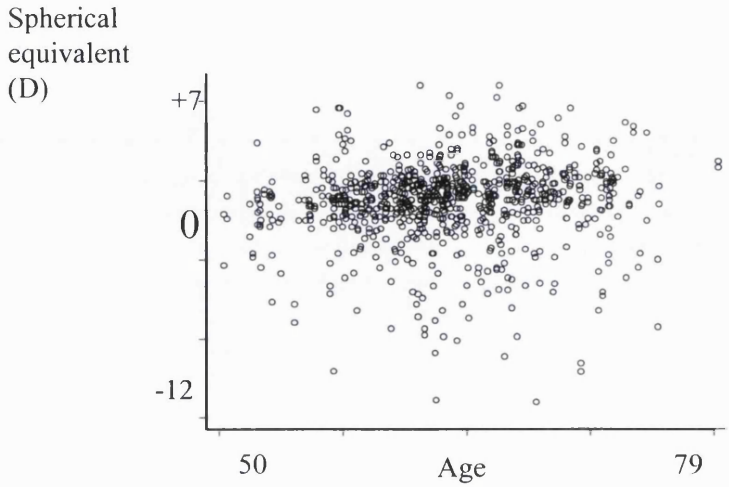
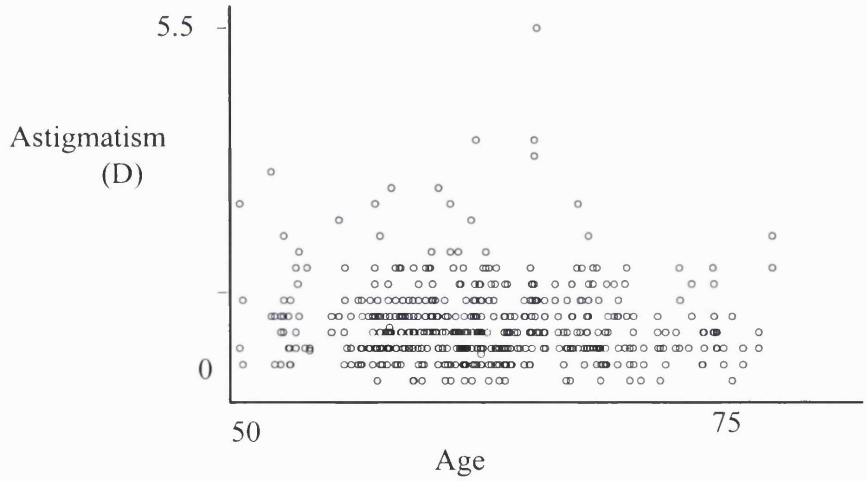


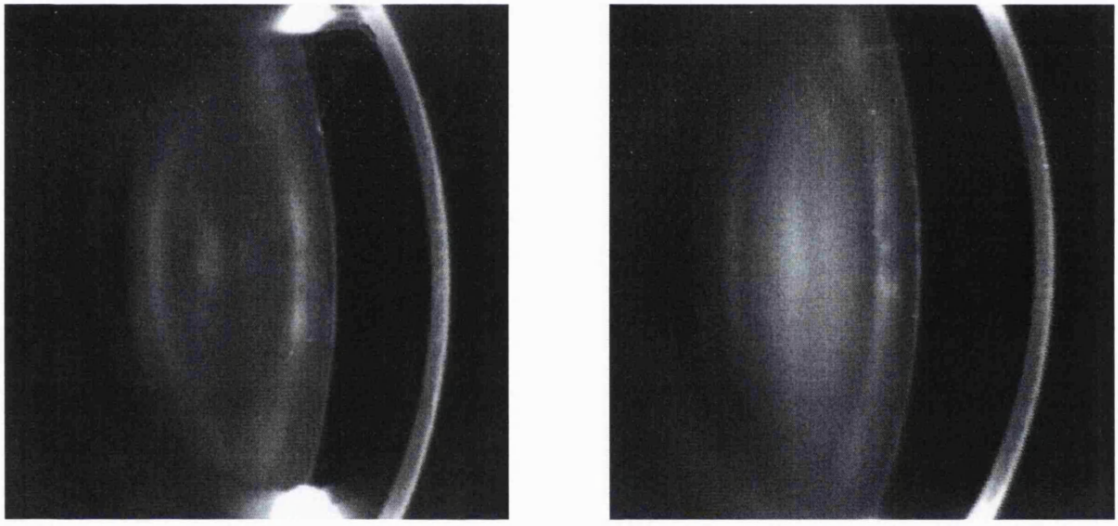
Figure 24 Astigmatism values plotted against age for right eye total astigmatism of all twins.



3.4 Nuclear cataract

Figure 25 illustrates a pair of 64 year-old twins, who were discordant for nuclear cataract. The twin in the left image has less nuclear white scatter than the twin in the right image. This scatter was converted into pixel densities (as illustrated in the Methods section 2.7.2) by measuring the pixel densities of various standardised points in the image.

Figure 25 Example of Scheimplug images of the right eye of a pair of twins discordant for nuclear cataract



3.4.1 Nuclear scores from Scheimplug and OCCCGS grading systems

Table 30 displays the mean (SD) scores for each of the measures of nuclear cataract included in each analysis. Scores were similar for right and left eyes and in MZ and DZ twin pairs. Of the 2024 eyes of 1012 twins, data from 49 eyes were excluded from full analysis. 24 eyes were pseudophakic, 11 could not be evaluated due to previous eye surgery or injury, and the Scheimpflug images for objective grading of 14 eyes were missing, although they were subjectively scored with the OCCCGS. The number of twin pairs resulting in each analysis is given in Table 30.

Table 30 Mean (SD) of nuclear cataract scores for MZ and DZ twin pairs in right and left eyes, with number of twin pairs analysed (N) after exclusions.

Measure	Eye	MZ		DZ	
		Mean(SD)	N	Mean(SD)	N
Central Nuclear Dip	Right	60.3 (15.8)	212	58.5 (14.1)	272
	Left	59.2 (15.6)	215	57.0 (13.7)	265
Anterior Peak	Right	68.6 (16.5)	213	67.1 (15.2)	272
	Left	66.2 (17.1)	215	64.2 (14.9)	265
Nuclear Average	Right	68.4 (13.5)	213	66.7 (11.8)	272
	Left	66.6 (12.9)	215	64.6 (11.0)	265
White Scatter	Right	2.19 (0.44)	217	2.13 (0.38)	274
	Left	2.17 (0.43)	221	2.12 (0.40)	269
Brunescence	Right	0.76 (0.38)	217	0.73 (0.31)	274
	Left	0.73 (0.35)	221	0.70 (0.29)	269

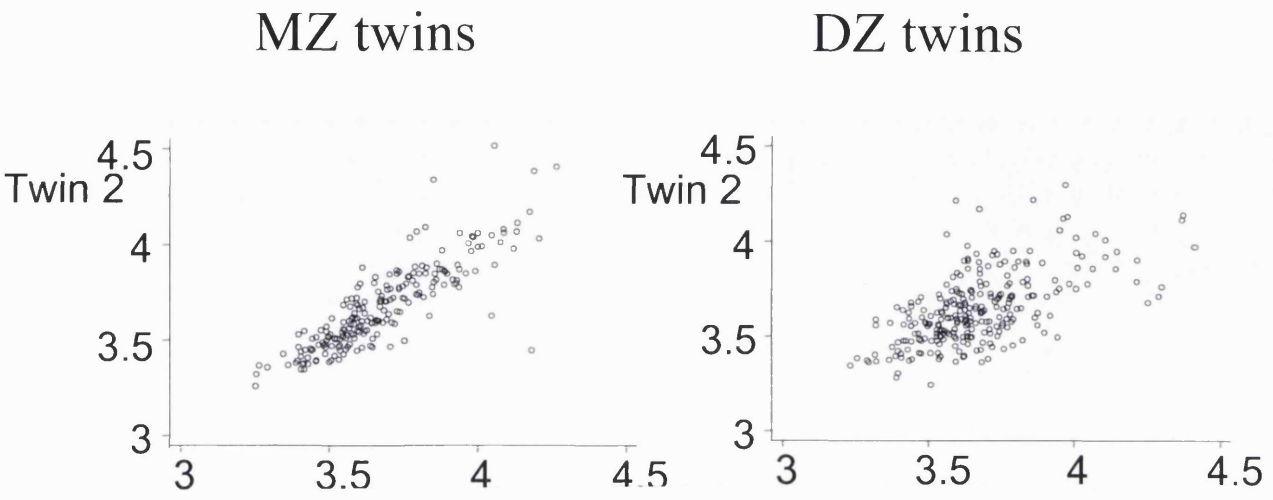
3.4.2 MZ/DZ correlations

Figure 26 represents the scatter plots of scores for central nuclear dip of the right eye for twin 1 plotted against twin 2. This shows a higher intrapair correlation for MZ than for DZ twin pairs, with less scatter compared to the DZ pairs.

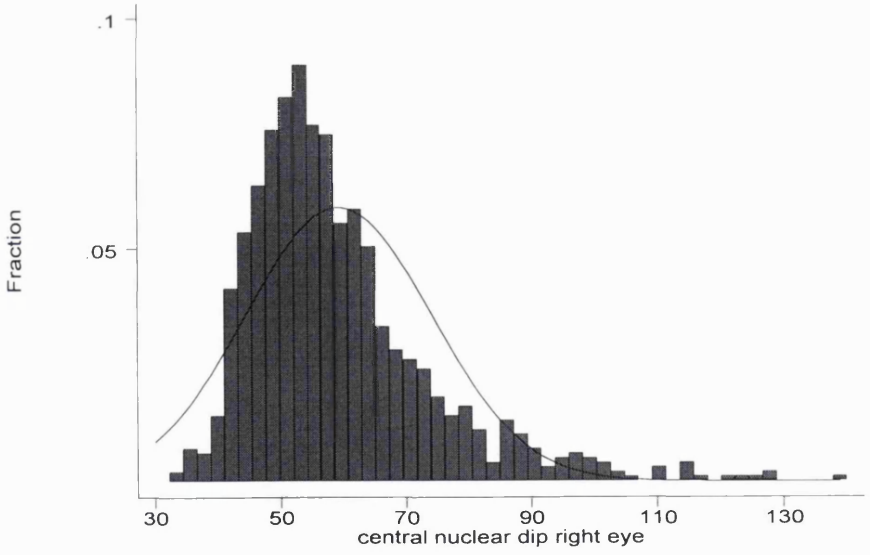
If a score was available for the right (or left) eye of both members of a pair, then this pair was included in analysis; if one or both had no score for an eye, then they were excluded.

Of the 24 pseudophakic eyes, 13 were DZ twins and 11 MZ. 3 pairs of twins were both bilaterally pseudophakic (2 DZ aged 68 and 79, 1 MZ aged 55), and 3 individuals were bilateral pseudophakes (2 MZ aged 64 and 65, 1 DZ aged 70), so no results from these twins and their pairs were included in analysis. However, the other 6 individuals (mean age 68 years) who were pseudophakic were phakic in their other eye, so the results of this eye in comparison with the same eye of their twin could be included in the analysis. The numbers of these twins are small enough and relatively equally distributed between MZ and DZ twin pairs to make little difference to the final analysis.

Figure 26 Scores of (log) central nuclear dip plotted for twin 1 against twin 2 in MZ and DZ twins



Distribution of nuclear cataract scores:



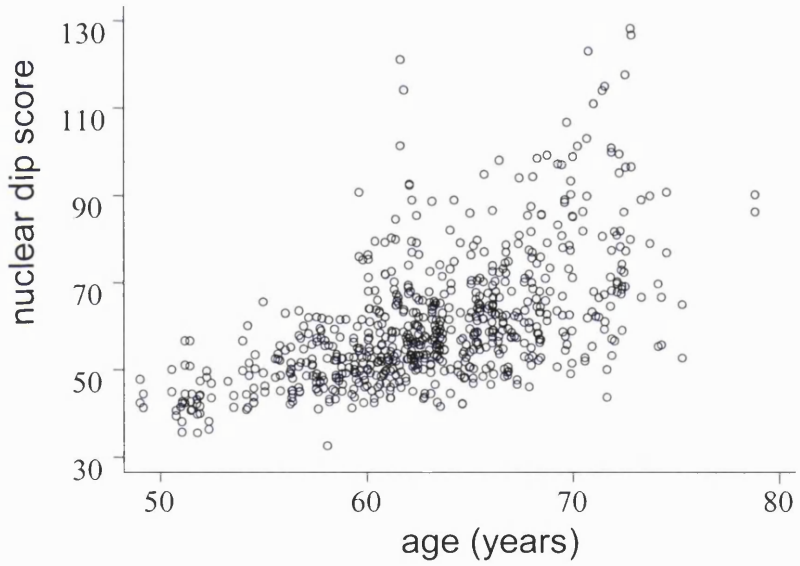
Nuclear Average	Right	0.85	0.58
	Left	0.87	0.51
White Scatter	Right	0.77	0.44
	Left	0.79	0.44
Brunescence	Right	0.91	0.73
	Left	0.88	0.74

3.4.3 Modelling Results

Age Effect

Nuclear cataract is strongly age-related, with age the strongest risk factor in epidemiological studies. Figure 27 demonstrates the close correlation between age and central nuclear dip right eye scores for the twins studied. The correlation coefficient (r) is 0.58. Therefore it was important to include age in the modelling for nuclear cataract. Models including and excluding age can be compared to confirm that age contributes significantly to the variance of this population (Figure 14).

Figure 27 Scatter plot of central nuclear dip scores for right eyes of twins plotted against age.



Univariate Analysis

Univariate modeling for each of the measures of nuclear cataract for each eye was performed and showed the best-fitting model for all the measures except brunescence is the AE model (additive genes and unique environment) including age. The best model for brunescence was ACE and age, suggesting additive genes, common and

unique environment and age all significantly contribute to its variance. Table 32 lists the standardised parameter estimates and 95% confidence intervals (95% CI) for these measurements. The three Scheimpflug image density values estimate very similar results, with a heritability of 44-47%, age accounting for 34-40% of the variance and unique environment for the remaining 13-22% only. White scatter scores estimate a heritability of 49-52%, with age and unique environment responsible for 25% of the variance each. For brunescence, the heritability is only estimated to be up to 22%.

Table 32 Standardised parameter estimates and 95% confidence intervals of the best fitting models of central nuclear dip, anterior peak, nuclear average, white scatter and brunescence.

Measure	Eye	h^2	95% CI	e^2	95% CI	age	95% CI	c^2	95% CI
Central nuclear dip	Right	.44	.38-.50	.17	.14-.21	.39	.33-.45		
	Left	.47	.41-53	.13	.11-.17	.40	.34-.46		
Anterior peak	Right	.44	.37-.50	.22	.18-.27	.34	.28-.40		
	Left	.46	.40-.52	.19	.15-.23	.35	.29-.41		
Nuclear average	Right	.46	.40-.52	.16	.13-.20	.38	.32-.44		
	Left	.45	.40-.52	.16	.13-.20	.39	.33-.45		
White scatter	Right	.49	.42-.55	.26	.21-.32	.25	.20-.31		
	Left	.52	.46-.59	.23	.19-.28	.25	.19-.30		
Brunescence	Right	.22	.14-.33	.11	.09-.14	.26	.21-.33	.39	.29-.49
	Left	.14	.05-.24	.14	.11-.17	.26	.20-.33	.46	.36-.55

Abbreviations: h^2 = proportion of variance due to additive genes (the heritability), c^2 = proportion of variance due to common environment, e^2 = proportion of variance due to individual environmental effects, age = proportion of variance due to age, 95% CI = 95% confidence interval

Multivariate analysis

Multivariate analysis of the four measures of nuclear cataract for each of two eyes was attempted, to try to obtain a single overall estimate of the heritability. However, using both Cholesky decomposition and its sub-decompositions, the Independent Pathway and Common Pathway models, it was not possible to obtain best-fit estimates using the Mx structural modelling programme. This is probably due to the close correlation between the measures: the matrix algebra underlying Mx relies on a maximum of up to 12 decimal places, and the Hessians were impossible to calculate.

Table 33 shows the correlations between the different measures of cataract. The three measures taken from the Scheimpflug images (central nuclear dip, nuclear average and anterior peak) correlated well, with coefficients 0.87-0.94. White scatter, which is essentially the subjective estimation of the same phenomenon measured by the image analysis, showed correlations with these measures with coefficients between 0.71-0.78. However, brunescence correlated less well with the others (0.41-0.46) and probably measures a different aspect of aging within the lens. Correlations of the scores between right and left eyes were high and ranged between 0.86-0.93 for all scores. Further analysis has subsequently not included brunescence, as the other measures are estimating the light scattering properties of the nucleus, rather than the colour.

Table 33 Correlations between different measures of nuclear cataract (data from right eyes below diagonal, data from left eyes above diagonal)

	CND	APK	NAV	WS	BR
CND	1.00	0.95	0.92	0.77	0.44
APK	0.94	1.00	0.90	0.76	0.41
NAV	0.90	0.87	1.00	0.73	0.44
WS	0.78	0.76	0.71	1.00	0.43
BR	0.45	0.42	0.46	0.41	1.00

Abbreviations: CND=central nuclear dip, APK=anterior peak, NAV=nuclear average, WS=white scatter and BR=brunescence.

Factor analysis

Factor analysis identified only one factor with a significant eigenvalue (6.67) for the eight measures of nuclear scatter (the three Scheimpflug scores and white scatter for right and left eyes), shown in Table 34. This factor explains over 92% of the variance, suggesting these measures are assessing the same phenomenon. Subsequent factors were insignificant; the second eigenvalue was 0.37.

Table 34 Results of factor analysis of eight nuclear scores for each individual

Factor	Eigenvalue	Proportion	Cumulative
1	6.67	0.925	0.925
2	0.37	0.052	0.977
3	0.18	0.026	1.003
4	0.08	0.011	1.014
5	0.01	0.001	1.015
6	0.003	0.0004	1.016
7	-0.04	-0.005	1.013
8	-0.07	-0.01	1.000

Table 35 lists the factor loadings for the first three factors from the factor analysis. All measures showed a strong positive loading (0.84-0.95) on the first factor, suggesting they all contributed significantly to the variance of this factor (more than 15% of the variance, equal to a loading of 0.40). Although factors 2 and 3 have little significance (eigenvalues less than 1), distribution of the loadings shows that factor 2 relates to the OCCCGS white scatter scores as compared to the Scheimpflug image scores, and factor 3 relates to right and left eyes.

Table 35 Factor Loadings for first three factors

Measure	Eye	Factor 1	Factor 2	Factor 3
central nuclear dip	right	0.95	-0.09	0.20
	left	0.96	-0.09	-0.18
nuclear average	right	0.91	-0.17	0.15
	left	0.93	-0.08	-0.16
anterior peak	right	0.93	-0.08	0.17
	left	0.94	-0.09	-0.17
white scatter	right	0.84	0.38	0.06
	left	0.84	0.38	-0.06

An overall score of nuclear cataract for each individual was calculated using weighted scoring coefficients for each measure from the first factor, multiplied by the measure for each individual. This single score was then used in univariate model fitting

analysis. This confirmed the AE model as best fitting (Table 36). Parameter estimates from this analysis resulted in a heritability of 0.48 (95% CI .42-.54); the remaining proportion of variance is explained by age (0.38, 95% CI .31-.44) and e^2, the unique environment (0.14, 95% CI .12-.18).

Table 36 Model-fitting results for univariate analysis of standardised nuclear score produced from all measures of nuclear scatter using factor analysis

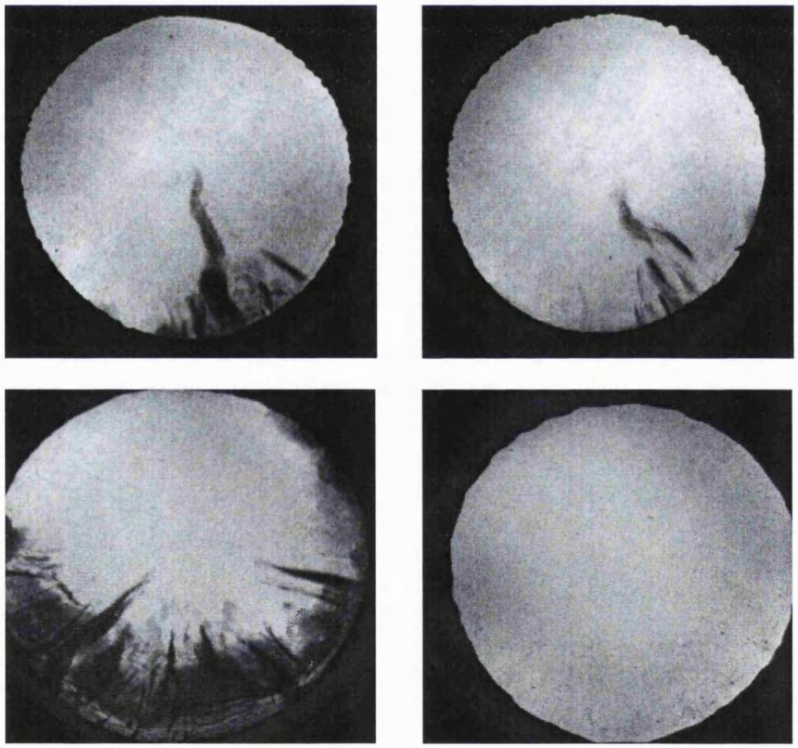
Model	χ^2	df	AIC	$p(\Delta\chi^2)$
ADE & age	26.374	7	12.374	
ACE & age	28.083	7	14.083	-
ACE lose age	308.426	8	292.496	<0.001
AE & age	**28.083**	**8**	**12.083**	**0.19**
CE & age	97.874	8	81.874	<0.001

Abbreviations: χ^2 = Chi-square goodness of fit statistic; df = degrees of freedom; AIC = Akaike's Information Criterion; $p(\Delta\chi^2)$ = probability that the change in χ^2 is zero compared to the full ADE model. See text for further abbreviations. Most parsimonious solution is printed in **boldface** type.

3.5 Cortical Cataract

Examples of some of the cortical cataract images obtained in the study are illustrated in Figure 28. The upper pair of images relate to the right eyes of a 63 year-old pair of MZ twins who have extremely concordant cortical cataract, for position, morphology and amount. By contrast, the pair of images below reflect extremely discordant cortical cataract in a pair of 58 year-old DZ twins.

Figure 28 Examples of retroilllumination images of cortical cataract from the study

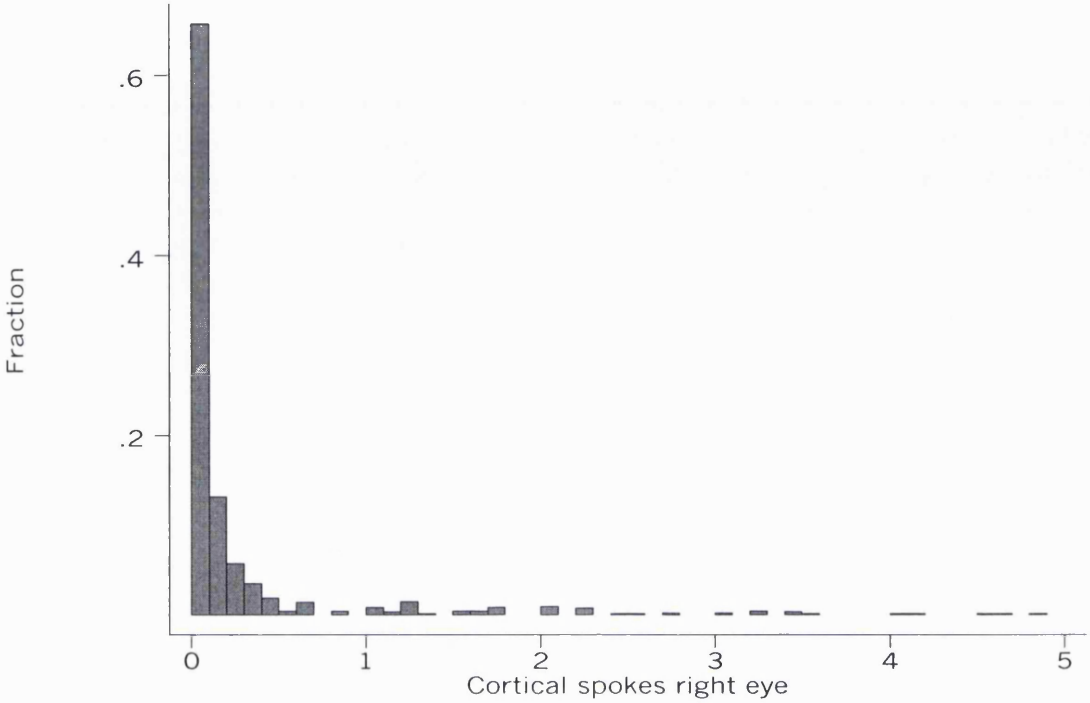


3.5.1 Cortical scores from Oxford and Wilmer grading systems

Of the 1012 subjects (2024 eyes), 35 eyes were excluded from the Oxford grading analysis: 24 eyes were pseudophakic and 11 were ungradeable due to previous eye surgery or injury. 1989 eyes remained, of which the images of 55 were unavailable for automated Wilmer grading, leaving 1934 eyes undergoing analysis by both techniques. To improve statistical power, the worse eye of each individual was used in the analysis of the cortical cataract data and will be used subsequently in the analysis for this thesis. This meant there were 991 individuals with a worse-eye Oxford score, and 957 twins with a worse-eye Wilmer score. Results for right and left eyes analysed separately were very similar, with less power than using the worse eye scores.

Cortical cataract scores were graded by the subjective slit-lamp based Oxford grading system, and the measurement of retroillumination images with the Wilmer automated system. Distribution of scores is clearly not normally distributed, as 65% of eyes had no cortical cataract at all on the Oxford grading, and only 160 eyes (of almost 2000 graded) had cortical cataract affecting more than 20% of the pupillary area (a score over 1.0 on the 0-5 scale). The distribution of scores is illustrated in Figure 29.

Figure 29 Distribution of cortical cataract scores (right eye) using the Oxford grading system



Cortical scores for the worse eye were used for each individual for the two grading systems and numbers of eyes graded by each are given in Table 37. Prevalence of significant cortical cataract (>5% area) was similar for MZ and DZ twins for both grading. 56% of individuals had no cortical cataract in either eye on the Oxford grading, although only 28% had no cortical cataract on the Wilmer grading system. The reasons for this difference are detailed in the discussion section. Including only those eyes with a significant cortical score (more than 5% of the lens area visible within the pupil), the median (SD) Oxford score was 1.1 (±1.0) for MZ and 1.0 (±1.0) for DZ twins (lens area 22% and 20%). For the Wilmer grading, it was 2.5(±2.2) for MZ twins and 1.8(±2.0) for DZ twins (lens area 15% and 11% respectively). It is not clear why the median MZ scores were higher than DZ on the Wilmer grading. Comparing the Oxford and Wilmer categorised grades (on a scale of 1 to 8) using

weighted kappa statistics results in a kappa score of 0.45, showing moderate agreement.[279]

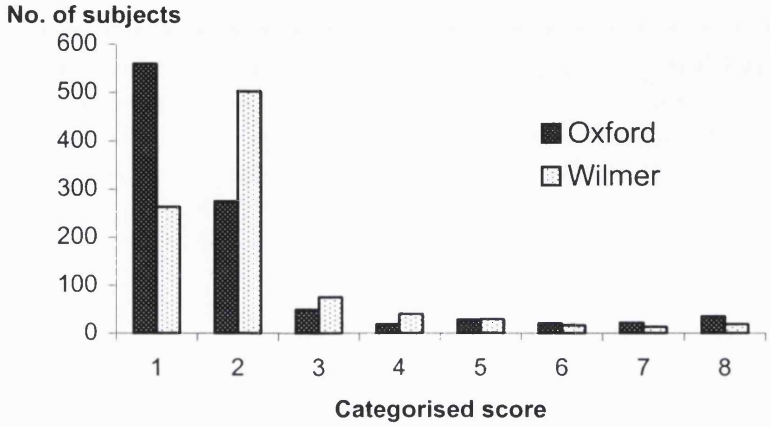
Table 37 Prevalence of Cortical Cataract for Monozgotic and Dizygotic Twin Pairs in the Worst Eye

Grading	Monozygotic twins				Dizygotic twins			
	N	Prevalence (%)			N	Prevalence (%)		
Area of Cataract		>0	>=5%	>=10%		>0	>=5%	>=10%
Oxford	443	42	19.4	13	548	45	20.6	13
Wilmer	424	74	24	16	533	71	23	14

N=number of subjects analysed.

Both scores were categorised into 8 categories to allow non-parametric comparison and modelling. The distributions of the Oxford and Wilmer graded scores are illustrated in Figure 30. Comparing the Oxford and Wilmer categorised grades, using a weighted kappa statistic, results in a kappa score of 0.47, showing moderate agreement between the two. Polychoric correlation coefficients were calculated, using PRELIS.[278] The correlations were significantly higher for MZ than for DZ twins, and were 0.74 and 0.36 for the Oxford scores and 0.64 and 0.20 for the Wilmer scores respectively. Correlation coefficients include all the twins concordant for no cortical cataract, which might make a correlation appear higher than the concordance of actual disease in twins. However, the prevalence was very similar for MZ and DZ twins, so it can be seen that the difference between MZ and DZ twins' correlation is highly significant – the MZ twins' correlation was more than 3 times higher than that of DZ twins for both scoring systems.

Figure 30 Categories of worst eye cortical cataract scores for Oxford and Wilmer grading systems



Grades are 1: no cortical cataract, 2: <5% area of lens covered by cataract, 3: >=5% & <10%, 4: >=10% & <20%, 5: >=20% & <30%, 6: >=30% & <40%, 7: >=40% & <50%, 8: >=50%

3.5.2 Modelling results

Results of the modelling analysis are illustrated in Table 38. They show that for both the Oxford and Wilmer grading systems, the best-fitting model was the ADE model including age. This means the variance of cortical cataract within this population is explained by the effects of additive and dominant genes, individual environment, and age. There was a significant loss of fit if any of these were excluded from the model, but if the effect of common environment (C) was removed the fit of the models did not change.

Table 38 Model-fitting results for analysis of cortical cataract scores using Oxford and Wilmer grading systems

Measure	Model	χ^2	$\Delta\chi^2$	df	vs	p
Oxford grading	1) ADE	4.752		-		
	2) ACE	8.829	4.077	-	-	-
	3) ACE no age	122.325	113.496	1	2	<0.001
	4) AE	8.829	4.077	1	1	0.04
	5) CE	57.904	49.075	1	2	<0.001
Wilmer grading	1) ADE	1.843		-		
	2) ACE	13.355	11.512	-	-	-
	3) ACE no age	90.012	76.657	1	2	<0.001
	4) AE	13.355	11.512	1	1	<0.001
	5) CE	48.587	35.232	1	2	<0.001

Abbreviations: A,D,C,E = additive genetic, dominant genetic, common environment and unique environmental effects respectively, χ^2 = Chi-square goodness of fit statistic; $\Delta\chi^2$ = change in χ^2 comparing submodel with full ADE or ACE & age model, df = change in degrees of freedom between submodel and full model, vs= model current model is comparing against, p = probability that $\Delta\chi^2$ is zero

Parameter estimates of the components and their 95% confidence intervals for the best-fitting models are given in Table 39. The broad-sense heritability (additive and dominant genetic effect combined) was estimated to be 58% (95% CI 51-64) for the Oxford grading and 53% (95% CI 45-60) for the Wilmer grading, which are very similar. Dominant genetic effects accountied for all the genetic effect in the Wilmer grading and 38% of the Oxford grading. Age explained 16% and 11% of the variance and individual environment 26% and 37% of the variance of cortical cataract in Oxford and Wilmer gradings respectively.

Table 39 Standardised parameter estimates and 95% confidence intervals of the best fitting models of cortical cataract for Oxford and Wilmer grading systems.

Measure	a^2	95% CI	d^2	95% CI	e^2	95% CI	Age	95% CI
Oxford grading	.20	0-.57	.38	.01-.64	.26	.22-.31	.16	.12-.21
Wilmer grading	0	0-.24	.53	.28-.60	.37	.30-.43	.11	.07-.15

Abbreviations: a^2 = proportion of variance due to additive genes, d^2 = proportion of variance due to dominant genes, e^2 = proportion due to individual environmental effects, e^2 = proportion due to age effects, 95% CI = 95% confidence interval

3.6 Posterior Subcapsular Cataract and other cataract phenotypes

3.6.1 Posterior subcapsular cataract

Prevalence of posterior subcapsular cataract (PSC) in the worse eye was, as expected for a population study, low, with prevalence of any PSC 4% and prevalence of significant PSC (>5% of lens area) of 2.5%. The numbers are small, with 4 pairs of MZ concordant for significant PSC and 9 pairs discordant (yielding a pairwise concordance of 0.46) and 0 pairs concordant for DZ twins and 8 pairs discordant (pairwise concordance 0). Although this might suggest a genetic influence, the numbers are too low for further analysis. Interestingly the prevalence in MZ twins was 3.8% and the DZ twins 1.4%, which could suggest a role for foetal environment, the Barker hypothesis. However, this analysis has not taken into account other environmental factors known to be important in PSC, such as oral steroid treatment into account.

3.6.2 Other cataract phenotypes

The other cataract phenotypes included in the OCCCGS were analysed as bivariate data as to whether they were present or absent, using 2x2 contingency tables. Table 40 details the prevalence of the cataract grades for each feature using the OCCCGS. For each phenotype (except anterior subcapsular cataract), the prevalence was also

calculated using a higher cut-off point which approximately halved the prevalence – individuals with a higher score are more likely to have "genetic" disease so the genetic component should be stronger (ie the MZ:DZ concordance ratio will be higher). For retrodots, which are almost universally present, two cut-off points of >1 and >3 were used. All cataract subtypes were graded on a decimalised scale of 0-5.

Table 40 Prevalence and twin concordances of cataract features in the OCCCGS

Phenotype	Grade	Prevalence	Concordance		
			MZ	DZ	MZ:DZ
Vacuoles	>0	0.49	0.62	0.58	1.05
	>0.5	0.20	0.48	0.38	1.27
Retrodots	>0	0.38	0.67	0.54	1.25
	>0.7	0.17	0.58	0.35	1.66
Focal dots	>1	0.67	0.87	0.77	1.13
	>3	0.07	0.71	0.19	3.76
Fibre folds	>0	0.13	0.41	0.30	1.36
	>0.3	0.10	0.44	0.27	1.64
Waterclefts	>0	0.26	0.65	0.60	1.08
	>0.3	0.12	0.60	0.46	1.29
Ant. subcapsular cataract	>0	0.05	0.5	0	-

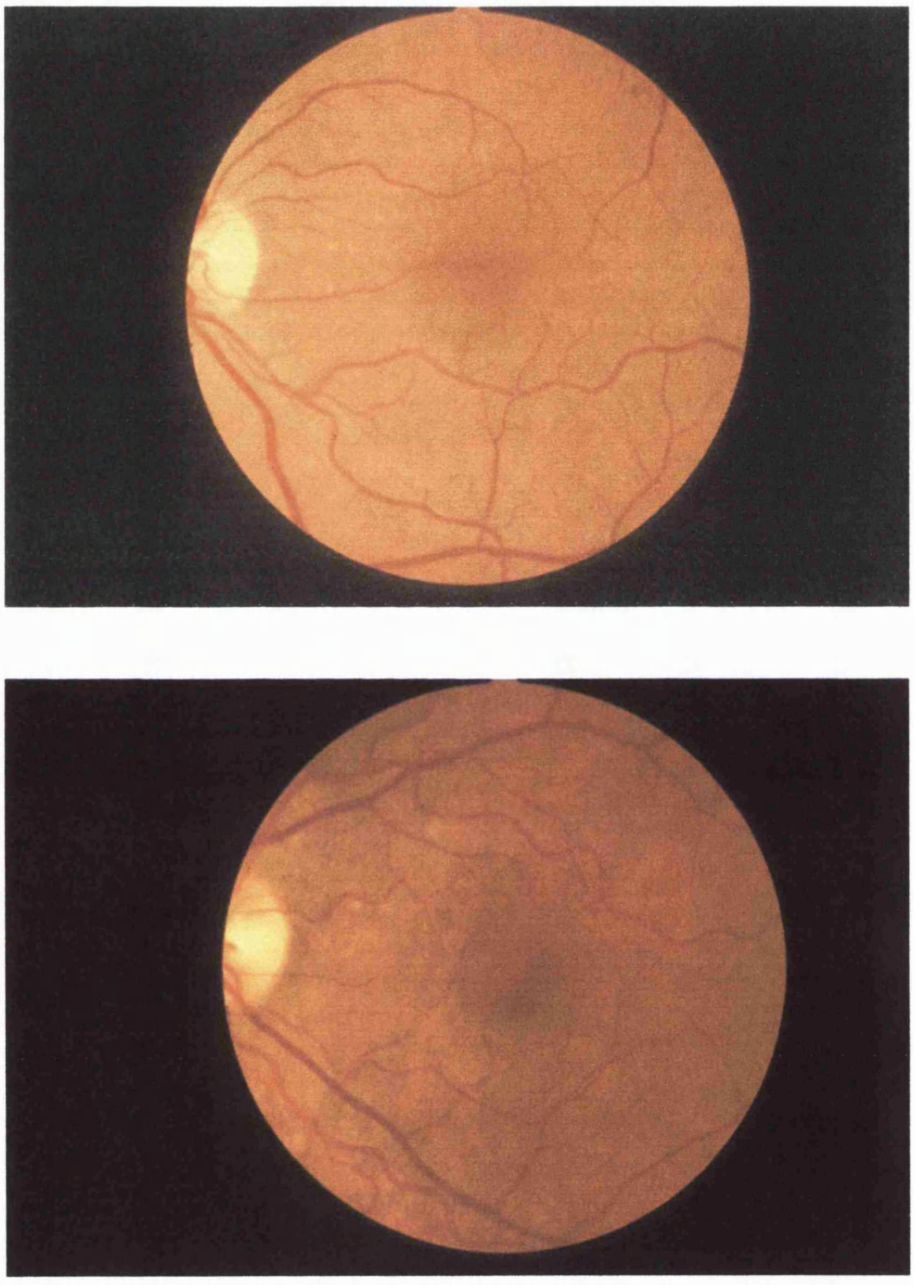
For the presence or absence of most of these traits, it can be seen that the MZ and DZ concordance was similar, indicating that a strong genetic influence was unlikely. Using a higher cutoff point resulted in the MZ concordances being higher than the DZ concordances, implying that there is some genetic influence on each trait the more severe that trait is. However, these higher cut-off points are arbitrary. The significance of all these phenotypes (except PSC) is unknown, and they cannot be objectively scored using a photographic method. As the genetic modelling of binary traits such as these is not very powerful, and because of these concerns, further modelling analysis was not performed. The prevalences were similar for MZ and DZ twins for all traits.

3.7 Age-related Macular Degeneration

The photographs were graded by the principal investigator (CH) and also by a medical retina specialist (AW) as detailed in the methods section. The levels of agreement and numbers of cases referred to the arbitrator (ACB) are detailed in the Reproducibility section 3.2.3. The results in this section are the most disappointing of the project, as fewer twins than expected had the disease phenotype, and the data was mainly binary with features present or absent, reducing the power of the study to model the heritability estimates which are potentially of such interest.

The fundus photographs of the left eye of a pair of 63 year old MZ twins are concordant for soft indistinct drusen, with the drusen seen between the optic disc and fovea in both twins, and are seen in Figure 31. The other eye was similar in both, but one twin had significant drusen at the fovea while the other did not.

Figure 31 Fundus photographs of a pair of twins concordant for ARM with soft indistinct drusen



3.7.1 Numbers of macular photographs graded

There were 1012 individuals from the 506 pairs of twins, and photographs were available for grading for 1006 individuals (99%): 6 missing sets of photographs were from both eyes; four came from two pairs of twins (1 camera failure, 1 lost), and 2 individuals from different pairs (photographs lost?). A further 24 photographs were judged ungradeable, 12 from right and 12 from left eyes. The reasons were 14 photographs of poor quality, 5 with media opacity resulting in insufficient detail being visible, 4 with coexistent retinal pathology precluding grading (2 central retinal vein occlusions, 2 previous retinal detachments) and 1 eye had been enucleated. However, from these 24 eyes the photographs from both eyes of only 1 individual were deemed ungradeable. Therefore, since the grading for the worse eye of each individual was used in the analysis, comparison data was available for 501 pairs of twins of the 506 pairs entered into the study (99%). These figures are summarised in Table 41.

Table 41 Numbers of photographs included in grading for AMD

	No. eyes (%)	No. individuals (%)	No. pairs (%)
Total number in study	2024 (100)	1012 (100)	506 (100)
Missing photos	12 (0.6)	6 (0.6)	4 (0.8)
Ungradeable	24 (1.2)	22 (2.2) – 1 eye	22 (4.3)
		1 (0.1) – 2 eyes	1 (0.2)
Remaining in analysis	1988 (98)	1005 (99)	501 (99)

3.7.2 Prevalence of ARM

The prevalence of ARM using the International Grading System was 13.7% overall. Details of the age-related prevalences of ARM as well as the different phenotypes are detailed in Table 42, with the BMES and BDES prevalence figures for women for comparison. The overall prevalence of pigmentary changes was 7.7%, and the prevalence of soft drusen >=C1 size was 5.3%. In addition, 48% of individuals had hard drusen, and 12% more than 20 hard drusen within the macula area. Some authors (such as Bressler) are now quoting numerous hard drusen to be an

independent risk factor for visual loss from AMD (AC Bird, personal communication). No individuals in this study had features of late AMD. Note that the prevalence figures cannot be directly compared as each study used slightly different criteria.

Table 42 Prevalence of ARM, pigmentary changes and drusen in the Twin Eye Study, BMES and BDES (data from women)

	No. of twins	Twin study#	BMES[211]	BDES[210]
ARM				
49-54	108	12.0	0.4	6.6*
55-64	562	11.5	2.2	12.2
65-74	326	17.2	9.3	18.3
75+	8	(38)ϕ	16.1	29.7
Any pigmentary change				
49-54		9.2	5.3	5.0*
55-64		6.0	7.1	9.2
65-74		9.5	13.0	13.6
75+		(25)ϕ	16.3	29.1
Soft drusen (distinct and indistinct >63μ size)				
49-54		8.3	2.7	6.6*
55-64		8.3	6.1	15.4
65-74		15.3	17.0	26.5
75+		(38)ϕ	27.2	42.7
Any hard drusen				
49-54		49		
55-64		50		
65-74		45		
75+		(12)ϕ		
>=20 hard drusen within macular area				
49-54		5.6		
55-64		11.5		
65-74		14.7		
75+		(12)ϕ		

ϕ = note only 8 individuals in this age category, so results have little significance

* = BDES age group 43-54; others as stated 49-54

= International classification[214]

3.7.3 Concordance between twins

Most phenotypes included in the International Grading System are binary: i.e. the feature (be it hyperpigmentation, or soft indistinct drusen) is either present or absent. While drusen are counted (and categorised in groups of 1-9. 10-19 and more than 20), the vast majority of the twins with soft drusen of >=C1 size had only 1-9 drusen, and so analysis using categorical data with multiple thresholds is not possible. Therefore the analysis was performed by calculating concordance tables, and using these as contingency tables within the maximum likelihood modelling.

The MZ and DZ concordances are detailed in Table 43, and show that there were few concordant MZ twins and even fewer DZ concordant twins. This will mean that the modelling is likely to have little power to discriminate between the different combinations of genetic and environmental effects. However, the higher MZ than DZ concordances for most phenotypes suggests a genetic component to the phenotypes assessed.

Table 43 Concordance of phenotypes within MZ and DZ twin pairs

	MZ twins		DZ twins		MZ:DZ concordance*
Phenotype: ARM by International classification					
	0	1	0	1	
0	178	17	204	35	
1	18	11	32	7	0.39:0.17
Phenotype: any pigmentary change					
	0	1	0	1	
0	191	17	238	20	
1	11	5	19	0	0.26:0
Phenotype: soft drusen (distinct or indistinct) >=125μ size					
	0	1	0	1	
0	206	6	247	15	
1	8	4	14	1	0.36:0.06
Phenotype: soft drusen (distinct or indistinct) >63μ size					
	0	1	0	1	
0	187	15	219	27	
1	15	7	26	6	0.31:0.18
Phenotype: any hard drusen					
	0	1	0	1	
0	80	54	72	60	
1	38	52	64	81	0.53:0.56
Phenotype: >=20 hard drusen in macular area					
	0	1	0	1	
0	193	9	206	34	
1	9	13	32	5	0.59:0.13

* Pairwise concordance calculated 2C/(2C+D) where C= number of concordant pairs with disease and D=number of discordant pairs.
For each phenotype 0=without specified phenotype, 1=with specified phenotype, 2x2 contingency table for twin 1 against twin 2

3.7.4 Modelling results

Univariate modelling results for the different phenotypes involved in ARM are detailed in Table 44. They show that with the small numbers of twins with disease, particularly the low numbers of concordant twins, the univariate technique using binary data has a low power to discriminate between the models. For ARM, using the International classification definition, the loss of fit when genetic effects are dropped reaches significance of p=0.03, and for soft drusen >=125μ the significance just reaches p=0.05 confirming a definite genetic effect on these phenotypes. However, for pigmentary changes and any hard drusen and soft drusen >63μ the model is able to drop genetic effects without significant deterioration in fit. These results mean that this twin study cannot completely exclude the fact that pigmentary disturbances of the macula and a few (<20) hard drusen in ARM might be purely environmental in origin and not related to any genetic effects. However, the trend is always to have a higher chi-square for the CE model (eliminating additive genetic effects) and in fact this result probably reflects the low power of this study, and there is a genetic effect for these phenotypes. Similarly, while dominant genetic effects can be eliminated with no significant loss of fit (except for >=20 hard drusen), this may reflect the low power rather than the actual absence of non-additive genetic effects on the phenotype.

Table 44 Univariate modelling results for phenotypes associated with ARM using International classification

Phenotype	Model	χ^2	$\Delta\chi^2$	df	vs	p
ARM	1) ADE	0.986		-		
	2) ACE	2.642	1.056	-	-	-
	3) AE	2.642	1.056	1	1	0.20
	4) CE	7.221	4.579	1	2	0.03
Pigmentary changes	1) ADE	6.240		-		
	2) ACE	7.584	1.344	-	-	-
	3) AE	7.584	1.344	1	1	0.25
	4) CE	9.616	2.032	1	2	0.15
Soft drusen >=125μ	1) ADE	0.96		-		
	2) ACE	2.338	1.378	-	-	-
	3) AE	2.338	1.378	1	1	0.24
	4) CE	6.033	3.695	1	2	0.05
Soft drusen > 63μ	1) ADE	0.785		-		
	2) ACE	0.996	0.211	-	-	-
	3) AE	0.996	0.211	1	1	0.65
	4) CE	3.258	2.262	1	2	0.13
Any hard drusen	2) ACE	8.076		-		
	3) ADE	8.213	0.137	-	-	-
	3) AE	8.213	0.137	1	1	0.71
	4) CE	8.559	0.483	1	1	0.49
>=20 hard drusen	1) ADE	5.123		-		
	2) ACE	11.190	6.067	-	-	-
	3) AE	11.190	6.067	1	1	0.01
	4) CE	27.573	16.383	1	2	<0.001

Abbreviations: see Table 38

Interestingly, the modelling results for hard drusen suggest there is no strong genetic component to scattered hard drusen, but strongly supports the idea that more than 20 hard drusen seem to be definitely genetic and that dominant genes are involved – "dominant drusen"?

Parameter estimates for the heritability (a^2) and the environmental influence (e^2) are given in Table 45. The estimates of the importance of dominant genetic effects are

included in the >=20 hard drusen category where they were significant. The confidence intervals are wide, reflecting the weakness of the study. For this calculation, the broad-sense heritability was used, combining the effects of both additive and dominant genes in the underlying liability, which enables the confidence intervals to be narrowed. The heritability of ARM was 54%, using the International classification criteria, and the heritability of soft drusen >125μ was estimated at 67% and that of pigmentary changes only 38%. The heritability of >=20 hard drusen is 83% and all of this is estimated to be dominant genetic effects.

Table 45 Parameter estimates (and 95% CI) of broad-sense heritability and environment effect in ARM

	h^2	95% CI	d^2	95% CI	e^2	95% CI
ARM (Internat grade)	54	28-74			46	26-72
Pigmentary changes	38	4-66			62	34-96
Soft drusen > 63μ	51	21-74			49	26-79
Soft drusen >=125μ	67	30-90			33	12-70
Any hard drusen	29	11-46			71	54-89
>=20 hard drusen	83	64-93	83	29-94	17	7-36

Abbreviations: h^2 = broad-sense heritability $(a^2 + d^2)$/total variance. For other abbreviations see Table 39

4.0 Discussion

4.1 Introduction

The discussion of this twin study of common eye diseases is divided into several sections: the heritability of the eye diseases studied will be discussed with regard to the data presented in the results section, and its significance as well as potential problems. Environmental factors and the effects of age will also be discussed. An important question about this twin study is its generalisability to the general population; to address this I will be assessing the validity of measures obtained in the study, and the representativeness of this data with regard to other population studies. Possible confounders will be introduced, and issues regarding the power of twin studies will be discussed. Finally, the possible applications of the knowledge obtained from this study will be discussed and the direction of future research will be summarised.

4.2 The heritability of eye diseases

This study has, surprisingly, suggested that genes are important in many aspects of common eye diseases, even those that are strongly age-related. Although there has been an increasing recognition of a likely genetic component to diseases such as AMD, research on refractive error and age-related cataract has concentrated almost exclusively on environmental effects. This emphasis has tended to maintain the impression among clinical ophthalmologists that the "cause" of refractive error is mainly environmental, while cataract is an inevitable aspect of aging, mediated by environmental factors. The results of this study are a reminder that an individual's genetic background is the most important predictor of whether they will develop a particular disease (or when).

Heritability, the "headline" figure from this twin study, is used as proof of genetic basis of disease to allow further studies of candidate genes and linkage analysis to

discover further loci. The establishment of a heritability for the different types of cataract (as well as astigmatism, not previously thought to be "heritable") will influence direction of future research into genetic defects and their possible interactions with environmental factors.

Discussion of the heritability results of the various components of this study are detailed below, with comments on the heritability calculations.

4.2.1 Heritability of refractive error

This study set out to define the heritability of refractive error, and has shown that an additive genetic effect is responsible for up to 86% of the variance of spherical equivalent in this population. Recent genomewide scans have identified loci for familial high myopia,[114; 115] raising the possibility of future identification of gene defects in refractive error. Dominant genes appear important in the inheritance of astigmatism, with a slightly lower overall genetic component of approximately 50% for total astigmatism and up to 60% for corneal astigmatism.

Spherical Equivalent: Myopia and Hypermetropia

The high heritability of spherical equivalent compares to previous twin studies, particularly the larger studies with reasonable power to detect heritability, which are listed in Table 10 of the Introduction. Sorsby's classic twin study[32] included 118 pairs of twins with a wide range of refractive error, yielding "concordance" rates (really the proportion of those twins within 0.5 diopters of each other) of 0.7 for MZ twins and 0.3 for DZ twins, suggesting an important genetic effect. Reanalysis of his data has resulted in a heritability of 87%,[55] a remarkably similar figure to the current study, performed on a similar cohort of (probably) caucasian English subjects. Nance's study of Norwegian twins yielded a heritability of 92%,[54] and Japanese twin studies heritabilities of over 80%.[57; 58]

Similarly Teikari's Finnish study of myopia, using spectacle correction sent by a sample of twins with a questionnaire, reported a heritability for women of 0.61

treating the trait as a dichotomous variable[53] and 0.75 for hypermetropia.[63] A Chinese study of myopia estimated the heritability to be 0.61.[60] These studies were not population-based, as they selected twins one at least of whom was myopic to study, and so cannot be extended to a general observation of the importance of genes in a population. A small study of twins reared apart with varying refractions demonstrated similar results.[61]

This is the first twin study to objectively examine an unselected population who wear and do not wear spectacles, and to use the complete population distribution of refractive error to estimate heritability and the relative importance of environment using modern model fitting techniques.

Recent myopia studies have concentrated on environmental effects, such as ambient light[119] and close work.[103] Our study shows that genetic effects contribute more to the overall population variance, but that is not to say that the 15% due to environmental effects is not important.

Astigmatism

Our study confirms the suggestion that astigmatism may be dominantly inherited, which was raised recently in an Italian family study.[122] This is of interest as twin studies are inherently weak at detecting dominance due to the DZ twins only sharing a quarter of the dominant effect as MZ twins.[50] The low power to detect dominance is especially true in univariate models.[280] We used information from both eyes in a multivariate model, optimising power to detect dominant genetic effect.[50; 273]

Despite using multivariate analysis, the 95% CI are wide for the estimates of the dominant effect: for total astigmatism the heritability estimates were 47% (95% CI 37-53) and 49% (95% CI 42-55) for right and left eyes, and 61% (95% CI 12-71) and 42% (95% CI 8-66) for corneal astigmatism. However, for all the measures, the D (dominant genetic effect) could not be dropped without deterioration in fit of the model, meaning that the dominant effect is a real one. The worse fit of the corneal

astigmatism is partly due to the fewer numbers of eyes assessed: 327 pairs of twins were assessed for corneal astigmatism compared to 480 pairs with data on total astigmatism.

The failure of the only other twin study from Finland to find a difference between MZ and DZ astigmatism correlations may be due to the fact that they studied only 72 pairs of twins both of whom wore glasses and had sent in their prescriptions from a questionnaire mailshot.[64] This could underrepresent discordant twins one of whom, for example, did not need glasses and those with low levels of astigmatism not requiring spectacle correction.

4.2.2 Nuclear cataract

This first twin study of nuclear cataract demonstrates that genes are important, too, in such an age-related condition, with a heritability of 48% for nuclear scatter. Age accounts for 38% of the variance in this population, and individual environment, which includes factors such as smoking, for only 14%. This small proportion may account for some of the difficulties in identifying environmental risk factors and obtaining significant outcomes in intervention trials in similar populations.

There are no comparable twin studies of cataract, but a family study associated with the Beaver Dam Eye Study involving segregation analysis has suggested a single major gene could account for up to 35% of the variability of nuclear cataract,[141] supporting the role of genes.

The best fitting AE model (Table 36) predicted additive genes, unique environment and age contribute to the variance of nuclear cataract in this population. As was discussed above, the classical twin study has low power to detect dominance. The difference in χ^2 of the ADE model compared to the AE model was not significant ($p=0.19$), allowing it to be dropped from the final model. However, the estimate of D (dominant genetic effect) in the ADE model was 19% of the total variance, and was not insignificant. Most inherited forms of congenital cataract are dominantly

inherited,[142] so it is possible that the heritability of nuclear cataract might include a dominant genetic effect which this study did not have sufficient power to detect.

The heritability of 22% for brunescence (the degree of brown discoloration of the lens) requires cautious interpretation. While common (shared family) environment may contribute 40% of the variance, this may also represent systematic bias or scoring error, overestimating the similarity for both MZ and DZ pairs and inducing a shared environmental effect. Brunescence is the most difficult of the subjective gradings to score accurately, particularly with low scores,[135; 182] as in our relatively young population with mean age of 62. There are only 5 reference standard photographs in the OCCCGS for brunescence (scores 0, 1.0, 2.0 etc), so with a mean score of 0.7 (SD 0.3) there were few standards to compare against. This was also reflected in the relatively low reproducibility of the measure, with an intraclass correlation coefficient of 0.50, and the drift of the grading. Brunescence in itself is strongly age-related and becomes much more common in the over-75 age group, few of whom were seen in this twin study.

4.2.3 Cortical cataract

Genes appear important also in cortical cataract, with a heritability of 53 to 58% in this population, and this inheritance appears to involve dominant genes (Table 39). Unique environment explained 26 to 37% of the variance. These figures compare with the heritability of 48% for nuclear cataract which had a lesser environmental effect of only 14% compared to cortical cataract. Age effects were more important in nuclear cataract, explaining 38% of the variance compared to 11-14% of the variance of cortical cataract.

These figures are interesting, suggesting that cortical cataract may in part be dominantly inherited. The previous family study of cortical cataract using commingling analysis showed two transformed distributions fitted better than one,[141] which would fit with a dominant transmission hypothesis (or a recessive hypothesis). However, complex segregation analysis predicted a major recessive gene accounting for 45% of the variance of women. There is an extensive set of assumptions in

complex segregation analysis, and violations of assumptions (eg skewness and kurtosis) can mimic the effects of a major locus. Twin studies do not estimate the number of genes involved, and assume a multifactorial causality (genes and environment) with underlying liability distribution.

Model fitting analysis of both methods of grading suggested dominant genes are important in cortical cataract inheritance (Table 38), reflected in the significant loss of fit when D, the effect of dominance, was dropped from the modelling. As discussed earlier, twin studies have low power to detect dominance due to the low DZ correlation,[280] which explains the wide confidence intervals. Genetic models assume the effect of dominance is additional to an additive genetic effect,[272] so the effect of removing additive genes from the model cannot be tested, even though the confidence intervals for estimation of A cross zero in the cortical cataract data.(Table 39)

4.2.4 Age-related macular degeneration

The low concordance between twins for the early features of ARM in this population sample is surprising, given the smaller series of twins which have shown a greater similarity. Meyers included in his definition of macular degeneration individuals with more than 20 hard drusen, and his 25/25 MZ concordance and 5/12 DZ concordance (of 134 twin pairs examined)[73] does not mention how concordant the twins were. This means that if one twin had only hard drusen and the other exudative AMD, they were classified as concordant. However, he did note concordance of exudative AMD in 4 MZ twin pairs and all 15 MZ pairs were concordant for non-exudative disease. The study may also be criticised as there were 98 MZ pairs and 38 DZ pairs examined, certainly not representative of the population, and recruitment at twin fairs may result in recruitment of twins more concordant as they hold a greater "twin identity". It may be that the mean age of our twins (62) means that only early disease was seen and that the twins may become more concordant with time – a ten year follow up would be very interesting. In fact Meyers' twins had a mean age of 64.9 for the MZ twins and 62 for the DZ twins, not dissimilar to this study.

Population-based twin studies always have a lower concordance than more selected twin samples, and often require huge numbers of twins to gain sufficient statistical power for calculation of heritability, particularly for binary traits. As an example, a recent pooling of all the Scandinavian twin and cancer registries analysed over 44 000 twins to estimate the heritability of cancers. For breast cancer, there were only 42 concordant MZ twin pairs and 505 discordant, and for DZ twins 52 concordant pairs and 1026 discordant. This resulted in an estimate of the heritability of breast cancer of 27% with 95% confidence intervals of 4 to 41%, which are wide despite the large numbers involved.[281]

Heiba attempted to improve power in his family segregation study from the Beaver Dam Eye Study by grading all individuals on a 15-point categorical scale, including all phenotypes from 1 hard druse through to soft drusen through to disciform scarring.[222] We decided not to do this with the twin study, as this form of categorisation is not supported biologically: patients do not progress through the stages and so they do not reflect a true categorical scoring – soft drusen (level 6) may occur without hard drusen (level 4) and who is to say that small hard drusen with pigmentary changes (level 9) are "more severe" than soft indistict drusen (level 7)?

The confidence intervals of the heritability estimates in this study are wide, reflecting the lack of power of a bivariate statistic, even in a study of this size. Despite the concerns about the power of this study of ARM, the parameter estimates that were significant suggest that genes are important in the heritability of soft drusen (67% heritability -Table 45) but not so for pigmentary changes (estimate of heritability 38% but 95% confidence intervals almost include 0% for genetic effect and 100% for environmental effect). This suggests that for candidate gene studies of AMD, more attention should be paid to the phenotype of soft drusen >=125μ size rather than pigmentary changes or indeed few hard drusen. These phenotypes seem much more likely to be environmentally mediated. In addition, more than 20 hard drusen appear genetic and dominantly inherited, with a heritability of 83%. The gene for dominant drusen associated with Malattia-Levinese/Doynes Honeycomb Retinal Dystrophy has been identified.[282] This gene was examined as a candidate gene in 494 individuals with AMD and none had a mutation. However, the definition of AMD was not

discussed in the published study, so we do not know whether the phenotype of AMD included multiple hard drusen, but clearly this particular gene is not involved in the aetiology of AMD.

4.2.5 Problems with heritability

Heritability estimates are a convenient headline for genetic epidemiology studies, but there is a danger if they are applied blindly. The heritability estimates of this and other studies reflect those heritabilities of the population studied, and so might be different for different populations. Therefore they are not per se "transferrable" to other populations. Similarly, as will be discussed with regards to age, selection of the population is critical: for an age-related trait such as cataract, the wider the age group of twins studied, the greater the effect of age, therefore reducing heritability. Conversely, if age effects are eliminated (by selection of twins the same age) then heritability estimates will be higher.

Other effects may influence heritability estimates, such as cohort effects: we have seen that the prevalence of myopia can change within a generation or two, and so heritability might be different for different people of different generations.

4.3 Environmental factors

Twin studies such as this estimate the overall effect of genes versus environment on a trait or disease. However, that is not to say that the environmental effect is not important: heritability estimates a population measure of the amount of variance explained by genetic effects, but this is not the amount of, say, cataract caused by genes in an individual. Environmental factors may be important in genetically susceptible individuals. The classic example of this is PKU (Phenylketonuria), a 100% genetic disease transmitted via a single gene, whose clinical phenotype can be entirely influenced by manipulation of environmental effects (in this instance, diet). In addition, many behavioural traits influencing the individual's environment such as smoking have been shown to be partly genetic in origin.

4.3.1 Refractive error

The heritability of 85% for spherical equivalent is remarkable given the focus of many studies on environment risk factors such as the "use-abuse" theory that close work produces myopia.[103] The Baltimore Eye Study showed the odds ratio for years of education was 1.36 in myopia and 0.67 in hypermetropia.[102] This suggests that artificial categorisation of spherical equivalent into myopia and hypermetropia may be inappropriate: they are probably subject to the same spectrum of genetic and environmental influences, justifying our use of the complete population distribution of refractive error.

There is little research into environmental factors in astigmatism, which this study has predicted may account for up to 50% of variance (Table 29). However, it must also be noted that the individual environment effect does also include the measurement error, and many clinicians feel autorefractors are least reliable at predicting the magnitude of astigmatism. Clearly, more research into genetic and environmental factors involved in the aetiology of astigmatism would be helpful.

4.3.2 Cataract

This study of heritability of cataract has not addressed individual confounders or environmental effects, the most important of which for nuclear cataract is smoking. Simulations have shown that for a disease with familial aggregation, familial clustering of environmental risk factors which impose a relative risk up to 10 are unlikely to influence the heritability significantly.[283] Reanalysis of this twin data, after eliminating the effects of smoking using regression, altered heritability by only 1 percent. Gene-environment interaction, assumed not to be present in the twin model, would not significantly alter the population heritability if it were present, although on an individual basis might allow prevention of a disease even if that disease is strongly genetic.

If ultraviolet light is a definite risk factor for cortical cataract, it might be that this population of British middle-aged women is likely not to have been exposed to extremes of sun exposure. Therefore a different population from, say, Australia, might be expected to have more cortical cataract and so the environment would have a greater influence on the variance. However, twin studies of naevi (another sun-related phenotype) have shown similar heritabilities in the United Kingdom and Australia.

4.3.3 Age-related macular degeneration

Environmental factors are known to be involved in the aetiology of exudative AMD such as smoking, as detailed in the Introduction. The relatively low power of the study has meant that no further analysis of environmental effects has been performed, although it would be interesting to study the discordant MZ twin pairs to see if there was any obvious difference in their environment to account for the different phentoype.

4.4 The effects of age

4.4.1 Refractive Error

The selection of older twins for the study of refractive error might be criticised because age might affect refraction, leading to bias (mean age of twins in this study was 62 years). In particular, the myopic effect of early nuclear cataract ("lens-induced myopia") might come into play with the older twins of this cohort. As was demonstrated in Figure 23 in the results section, the correlation between age and spherical equivalent is weak, with a correlation coefficient of 0.1. Therefore the younger twins are slightly more myopic than the older ones, which is shown in all cross-sectional cohort studies of refractive error in populations, as the prevalence of myopia seems to be rising. Nucleus-induced myopia seemed not to be significant in this population.

These results are supported by the study examining change in refractive error over 5 years in the Beaver Dam Eye Study.[110] The mean change in spherical equivalent for

women was small in those of similar ages to our twin population: for those aged 55-64 it was 0.23 dioptres, and –0.01 dioptres for those aged 65-74. The myopic shift of nuclear sclerosis only became important in those over 75, with the five year change of –0.37 dioptres, but as only 4 of the 506 twin pairs were over 75 in this study, this was unlikely to be significant overall.

The model fitting analysis (including age) for refractive error confirmed its small effect: age only accounted for 1.4% (95% CI 0.2-3.9) of the population variance for spherical equivalent, and for astigmatism 5% of the variance for total astigmatism (95% CI 2.2-8.5) and 2.9% for corneal astigmatism (95% CI 0.6-6.7). Therefore the age effects were not included in reporting the main results of the refractive error data.

4.4.2 Cataract

The strongest risk factor in most studies of cataract is age, and it would have been surprising if age was not an important contributor to the variance of the measures of nuclear opacity in this population spanning thirty years (twins studied were 49-79 years). The correlation between the measures of cataract and age was strong: for example the Pearson correlation coefficient of central nuclear dip and age was 0.58 (Figure 27). Spearman correlation coefficient rho was 0.66 for nuclear score (the combined factor score) with age compared to a rho of 0.35 for the cortical spoke score. The parameter estimates of the variance of the population due to age of 38% for nuclear cataract and of 11-14% for cortical cataract mirror the impression from epidemiological studies that age is more associated with nuclear cataract. Younger subjects may have cortical cataract alone which is "more" environmental (SK West, personal communication). Age therefore seems less important in cortical cataract than nuclear cataract.

Nuclear cataract does seem strongly related to age, and indeed mortality studies of cataract patients (individuals with cataract have a higher mortality than those who do not) suggest that nuclear cataract may be a marker of ageing. The selection of the age group studied (determined in this study largely by trying to examine all the older twins on the St Thomas' Adult UK Twin Register) clearly has an effect on the result,

which is why heritability figures are population-specific. If a pure sample of, say, 70 year olds was studied, the proportion of the variance due to age would be zero as they are all the same age. Similarly if a group of twins aged 20-90 were studied, the effect of age on the population variance would be greater and the heritability correspondingly reduced.

4.4.3 Age-related macular degeneration

Again, age is strongly related to this phenotype, and this is illustrated in the prevalence figures detailed in Table 42. The youngest age group of twins in the subanalysis, those aged 49-54 years, had as high a rate of ARM as those in the 55-64 years age group. This is unexpected given the other large ARM studies, and the increasing effect of age thereafter, and requires further investigation.

The prevalence rose with increasing age for both definitions of ARM, pigmentary changes and soft drusen, although hard drusen did not appear particularly age-related. Similarly, the Blue Mountains Eye Study, which found more hard drusen than this study (98% of individuals),[211] did not document an increase in prevalence of hard drusen with age. Unlike cataract, age has not been factored into the models because of the few concordant pairs for ARM; introducing a further variable would reduce statistical power further. However, using the methods of De Fries and Fulker involving multiple regression to analyse the same data (details not shown),[284] a similar heritability of 66% was obtained for soft drusen which reduced to 58% when age was regressed out, and for ARM the heritability dropped from 54% to 44% using the International classification definition.

4.5 Generalisability of the study: biases

In order for this twin study to be generalisable to the rest of the (singleton) population, several questions require answering: firstly, whether there was **selection** bias in that the twins who underwent an eye examination were in some way selected and are different to the normal twin population. Secondly, the measurements performed in this study should not only be reproducible (as discussed in the section on

Reproducibility) but also the measurements themselves should be **validated** and found to be the correct measures for the traits being examined. Finally, the question of **representativeness** should be addressed: is there any evidence that the twins involved in this study (and twins in general) are in some way different to the rest of the population?

4.5.1 Selection of the twins studied

As was discussed in the introduction, selection bias might influence the results if a non-representative sample of twins was examined, and as the twins in this study were volunteers they are already in some way selected. We attempted to minimise this bias by recruiting twins for the eye study from those who had volunteered in response to publicity about bone and other studies. They were therefore unaware of the potential of an eye study when volunteering and therefore we hoped to avoid twins specifically with eye disease from volunteering. In addition, the twins were not informed about the outcome measures in advance of the visit, just that they were receiving a full eye examination that involved pupil dilation and fundus photography. Some twin studies of myopia (such as the Chinese study of myopia that estimated a heritability of 0.61[60]) selected twins at least one of which was myopic, and so might not be generalisable to the whole population. Twins were paid their travel expenses, and so any geographical bias was hopefully reduced, but they were not paid any other fee, to reduce any selection bias towards those who wanted or needed payment.

As was stated in the section 3.1 about the study population selection, a record of twins who refused the eye test was not kept, so we cannot compare responders and non-responders. The study administrative staff who undertook the recruiting of twins (twins were telephoned from the database, selected on age and zygosity) have informed me that the usual reason for the few refusals was related to difficulties over the travel arrangements or infirmity of subjects or more particularly their spouses. The 20 pairs who initially consented to the eye test and subsequently did not have it performed seemed no different to the other twins in terms of demographics or reported disease and other problems, and had an average age of 59 years compared to the 62 years overall in the study.

Further selection bias might be evident if exposures such as smoking and alcohol intake in the twins are different to the general population, and as they are volunteers a selection bias towards health-orientated individuals might have taken place. Some of these exposures are discussed in the confounders section (Section 4.5.4) and show the twins not to be significantly different from a general practice-based sample of women from Chingford in Essex.

It must be remembered that the twin study attempts to examine the causes of variation within the population, and does not in fact explain the mean of the trait or disease in question. Therefore, if all individuals in a population have the same environmental exposures as each other, then the variation detected is likely to be due to genetic effects. Although the twins in this study were scattered from across the country, and all social classes, it may be that caucasian British women born 50-75 years ago experienced similar diet, education and other factors, and so in some senses are over-matched, so that little variation is explained by environmental factors. These environmental factors, however, may be important in explaining the level of disease in the population. However, clustering of environmental factors have been shown not to affect heritability significantly, and so the genetic effect determined in the heritability study in this thesis is likely to be valid for other populations. The best design would be to study twins from different populations and cultures, although the different genetic effects in populations might then cloud the issue further (for example, the four-fold increase in cortical cataract in blacks is likely to be genetic, the effect of which might be diluted by including black populations with white populations in the same analysis). Again the issue of population-specific heritability raises its head: we attempt to generalise the results from this study, but it must be done with caution.

The effect of publicity about the eye study seemed to have little impact, as the 10% of twins who knew about the eye study and volunteered in response to this had no significant differences to the rest of the population.

4.5.2 Measurement validity

Bias due to missing results was reduced by complete ascertainment of the twins: all twins attending received a full examination and therefore were phenotyped in the same manner. To avoid recall bias, measurement data was analysed rather than any recall data. However, further discussion of the measurements used in this study is warranted, to examine whether there was a potential subjective bias related to the examiner knowing the subjects' likely zygosity, and to examine whether the measurements used are valid.

Refractive error

Autorefraction

Most of the previous selective twin studies of myopia used subjective refraction (as did Sorsby's less selective study of 118 twin pairs[32]), resulting in potential bias due to the zygosity of twins being obvious at the time of refraction if they attended together. Autorefraction was used in our study rather than retinoscopy and subjective refraction to avoid this bias. Most ophthalmologists would not prescribe spectacles from autorefraction results, because of the subjective component to correction of refractive error, particularly the amount of astigmatism. However, the autorefractor provided an objective measurement, important in this study, and was highly reproducible. Spherical equivalent and keratometry readings correlated remarkably (ICC 0.97-0.99), and total astigmatism had lower but still extremely high correlations for a biological measurement of 0.85-0.93. Therefore I feel the measurement of refraction was valid in this study.

Astigmatism: a vector

Astigmatism is in fact a vector, which consists of a magnitude as well as its angle. In this study, only the magnitude of the vector has been analysed, similar to other studies of astigmatism.[122] The reason is that it is difficult to analyse variance, the basis of modelling, using both magnitude and angle at the same time, particularly as the axis is not normally distributed (and, for example, 1° is very close to 180°, but "opposite" 90°).

Using the principles of optical decomposition, it has been attempted to reduce the magnitude and vector of astigmatism to one relative value,[285] which Naeser has termed the polar value of net astigmatism.[286] His formula, $KP=M*(\sin^2\theta-\cos^2\theta)$ where KP is the polar value referable to the 90 degree meridian, M the magnitude and θ the angle of the astigmatism, allows a single number to be generated for astigmatism. Using this formula to calculate KP from our data, the same ADE model was shown to be best-fitting (data not shown). However the Naeser formula significantly reduces the relative values of oblique astigmatism which have less relevance in surgically induced astigmatism but may be important in a population study as ours. Application of the formula reduced our correlations for MZ twins from 0.5 to 0.3 for total astigmatism and DZ from 0.2 to 0.02, impairing the fit of the model and significantly reducing the power of the study to determine heritability. However, as the best-fitting model was the same, the use of the results of analysis of the magnitude seem justified.

Cataract

Different phenotypes of cataract were graded separately and analysed even in the presence of other types of cataract. Some classifiers have attempted, in the past, to categorise cataract into "pure" nuclear, cortical and posterior subcapsular cataract and mixed cataract for those with more than one type of opacity. This is because, although all cataract types get more common with age, the existence of one type of cataract is a risk factor for another type, so they are not "separate" diseases. However, this approach results in many mixed cataracts and fewer pure subtypes, with resulting difficulty in statistical analysis. It is well accepted now that risk factors may be different for different cataracts (eg smoking for nuclear, u-v light for cortical

and steroids for posterior subcapsular). Therefore for this study I have followed the example of most other studies and graded and analysed each subtype even in the presence of other subtypes of cataract.

Nuclear cataract

The scores for the Scheimpflug images correlated well with each other and had very similar estimates of heritability (44-47%). White scatter scores from the subjective grading system (OCCCGS) also showed a similar heritability of 49 to 52% (Table 32). The two methods of grading correlated well (Table 33). All these scores measure the same phenomenon, in which light transmitted into the eye is scattered back, which is a measure of the amount of opacification within the lens nucleus. Therefore combination of these measures using factor analysis to obtain a single heritability for nuclear cataract seems justified.

It seems that scores were slightly higher for right than left eyes, and slightly higher for MZ twins than for DZ twins (Table 30). The reason for this is not clear: while it might represent a real difference, it may be that there is some photographic artefact in the Scheimpflug nuclear scores (which are strongly correlated with each other). The Scheimpflug camera has its slit beam coming from directly ahead with the camera at 45 degrees on the left hand side (fixed, for both right and left eyes) and it may be that the nose in some way interferes with some of the luminant or reflected light. Another possible reason for this difference between the two eyes was the fact that right eyes were always tested before left eyes and luminance of non-laser light sources is known to vary with temperature. However, these differences are not clinically significant.

The differences in subjective grading between the beginning and the end of the study show that there was some drift in the grading of cataract, amounting to 0.14 for white scatter scores (on a scale of 0-5) over the 18 months of phenotyping the twins. This was not apparent in the objective Scheimpflug image scores and the Wilmer cortical cataract grading. Although this study was performed by a single observer, the drift, as well as potential interobserver variability for bigger and longer studies, shows how attractive a prospect automated, highly reproducible and repeatable grading systems are. The Marcher Case 2000 camera system, with its allied software for nuclear

scoring and the Wilmer software for cortical scoring performed extremely well, and compared well to the subjective OCCCGS grades. In addition, they require less training, can potentially be used by multiple operators following a standard protocol, and the concerns about grading shift and drift do not apply. For this reason I would recommend any future follow up study uses the automated systems only. However, at present the actual scores produced by different machines have not been compared and there is a suggestion that they are not transferrable between machines. Further research into calibration and comparison between different machines is required.

As stated in the discussion on the the heritability of nuclear cataract, the validity of the brunescence scores must be questioned. The very high MZ and DZ correlations suggest potential systematic bias, brunescence scores were the least reproducible of my grading, and there was considerable grading drift. This is because the mean was only 0.7 and standard deviation 0.3 in this group of twins, with the reference standards available only for scores of 1.0 and 2.0. Although other groups have managed to show high interobserver reproducibility for brunescence,[180] I found this the most difficult grading to perform personally, and with such a narrow spread of measurements feel any conclusions drawn about heritability of brunescence should be viewed with caution.

Subjects who had previously undergone cataract surgery were excluded from analysis (24 eyes), as no continuous data could be derived from them. Since nuclear scatter is a continuous measure it would be artificial to divide the subjects into those with and without cataract for analysis, despite the obvious loss from analysis of individuals who had significant cataract. Overall, however, the cataract scores seemed valid and reliable.

Cortical cataract

The similarity of results overall for both the Oxford and the Wilmer grading systems suggests there was no great subjective bias from the Oxford grading system, despite the fact that the zygosity of most of the twins was obvious to me at the time of the examination. The 95% confidence intervals for the broad-sense heritability estimates

for the two grading systems overlap almost completely, being 51-64% for the Oxford grading and 45-60% for the Wilmer grading.

This is encouraging, as development of an automated grading system for cortical cataract images is problematic, because of the difficulties detecting the pupil edge (particularly when there are opacities at the pupil margin), uneven illumination from the optic disc and retina, different effects on the reflected light due to refractive error, and the inability to distinguish cortical from other opacities such as posterior subcapsular cataracts. The development of the Wilmer system[287; 288] has been an important advance in automated grading of large population-based studies involving retroillumination images.

In the comparison between grading systems in our population, a total of 1989 eyes were examined. Standardising both scores for a scale of 0-10 makes direct comparison easier. For the slit-lamp based Oxford system, the mean opacification grade was 0.43 for both right (N=995) and left (N=994) eyes. For the automated reading of retroillumination images Wilmer system, the mean grades were respectively 0.35 (N=965) and 0.38 (N=969). Spearman correlation between the two schemes for all eyes was 0.62(N=1934). Differences between the two results were as follows: right eyes, mean = 0.07, SD = 0.83 (N=965); left eyes mean = 0.09, SD = 0.83 (N=969). Grade differences greater than 1 (10% total area) were found for 174 (9%) of the eyes. The differences are outlined below:

Reason:

1 Wilmer graded peripheral changes (focal dots, coronary flakes, arcus) as cortical cataract
2 Wilmer graded posterior subcapsular cataract as cortical cataract
3 Wilmer missed some opacities (generally with lots of cortical change of varying density, Wilmer did not grade subtly abnormal areas as cataractous)
4 Wilmer seems to have graded all opacities correctly on review: therefore difference due to Oxford grading scoring incorrectly or seeing changes in vivo completely invisible on retroillumination image

Differences:	Reason	No. eyes
Oxford>Wilmer	2	1
	3	45
	4	66
Wilmer>Oxford	1	56
	2	3
	4	3

While the Oxford grading estimated MZ and DZ twins to have the same amount of cortical cataract (mean and SD), the Wilmer system graded MZ twins' worse eyes to have slightly more cataract than DZ (Table 37), albeit not a clinically significant difference (difference between means 0.15 on a 0 to 16 grading scheme). The reason for this is not clear, as the two groups were well matched age-wise and in every other respect.

In summary, the two grading schemes were reasonably comparable, and in fact the weighted kappa statistic of the two categorised grading systems (from 1 to 8) was 0.47, showing moderate agreement between them. Although the Wilmer automated grading system still cannot fully discriminate between cortical and non-cortical opacities for "pure" cortical cataract assessment, both grading systems seem to reflect the amount of cortical cataract within the population, as far as one can tell. Further development of tertiary segmentation to remove edge artefacts will improve the Wilmer system's accuracy further. Certainly it has no drift, as demonstrated in the OCCCGS cortical spoke scores in this study, and can be performed on images potentially captured by different investigators to compare results directly.

Age-related macular degeneration

The lower prevalence of ARM than expected compared to other studies will be discussed in the next section on the representativeness of the results. The grading of ARM was performed according to the International grading criteria, and as all photographs with any abnormality were graded by both graders and then all disagreements reviewed and if necessary arbitrated by Professor Bird, it is felt the grading was a valid assessment of the degree of ARM. The two graders showed moderate to substantial agreement towards each other and towards the final grading

(Table 23). These kappa values are probably lower than they could be: drusen size grades 5-7, for example, are all large soft indistinct drusen and signify early ARM. The significance of the differences (calcified/glistening, serogranular and granular) is unknown and can be a "hard call" to make between these subtypes.

The assessment of ARM was valid in this study, judging by the low rate of ungradeable photographs (Table 41), and the fact that 501 of the 506 pairs in this study were included in the analysis. For the purposes of this analysis, the second grader AW did not grade the photographs that were judged to be normal or to have small hard drusen only. This was partly to avoid making the kappa scores of agreement artificially high, as 4/5 of the photographs fell into the category of having no features of ARM and so the likely agreement would have been very high. It is planned that he will examine all the photographs to confirm that CH did not miss any ARM cases in the cases graded by him as non-ARM before publication of these results. Any cases that he deemed doubtful in any way were graded by AW initially.

4.5.3 Representativeness

In general twins show similar morbidity and mortality to the rest of the population, and the assumption that they share equal environments has stood up to testing.[38] The twin volunteers seen at the Twin Research and Genetic Epidemiology Unit at St Thomas' Hospital, of which the twins seen in the eye study are a proportion, are very similar to a population sample of similarly-aged women from the Chingford Study, a longitudinal study of ageing and osteoporosis, osteoarthritis and fractures. These similarities extend to disease phenotypes as well as confounders and lifestyle factors such as smoking. (T.Andrew, personal communication) However, it is useful to compare the population results in this study with other published studies in similar populations, to see if the results are indeed representative.

Refractive error

There have been no recent population-based studies on the prevalence of refractive error in the United Kingdom, so it is difficult to find comparable data to establish how representative the twins' refractive errors are. It is known that refractive error is more

common in premature and low birth weight individuals, and twins fall into this group more than singletons. Figures taken from the Beaver Dam Eye Study suggested an age-adjusted prevalence of myopia in women aged 43-75 of 28% and of hyperopia 49% (±0.5D cutoff point). The corresponding figure for our twins was 24% myopes and 50% hyperopes, which are very similar.

Cataract

For cataract, the Melton Eye Study has studied a population of caucasian British men and women aged 54-75, a very similar group to the twins assessed in this study, using similar methods (the OCCCGS).[132] For nuclear cataract, the mean score in their study was 1.33 compared to 2.13 in this study (see Table 30). It appears that the twins have a higher degree of nuclear white scatter than singletons. While this may be true, it is probably unlikely and may simply be due to differences in the grading between me and the different graders in the Melton Eye Study (a grading shift). This reflects the difficulties comparing different studies when there is no clear "gold standard".

Other evidence that the twins' degree of cataract is representative include a similar age distribution of scores between the two studies, which increased at a similar rate between the studies, despite starting at a different point, as explained above. Also if twins had more cataract than singletons, a higher prevalence of pseudophakia would be expected than the general population. In this study only 17 individuals (24 eyes) of 1012 were pseudophakic, a prevalence of 0.016%, compared to the Melton Eye Study's 11 pseudophakes/aphakes out of 560 subjects (prevalence 0.019%). The prevalence of previous cataract surgery is therefore very similar.

For cortical cataract scores, 35% of the twins' eyes had some cortical cataract, similar to the prevalence of 36% in the Melton Eye Study subjects. The mean non-zero score was 0.34 for the Melton Eye Study and 0.65 for this twin study, which again could represent a genuine difference in twins, or could reflect different scorers rather than an actual difference in amount of cataract. On balance, the whole these data suggest that the degree of cataract in the twins is probably representative of that of the general population.

In conclusion, there is no evidence to suggest that the twins who volunteered for the eye study were significantly different to the general population. However, these data do emphasise how grading systems, despite being "standardised", can give different results in different populations with different graders, and the need for more objective methods to grade cataract to be able to compare between studies and populations.

Age-related macular degeneration

A concern of this twin study lies in the representativeness of the macular degeneration figures. The first comment is that there was no late AMD in this series of twins, where other studies have shown AMD in a similar age group. The overall prevalence of AMD in women was 1.9% in the Beaver Dam Eye Study and 1.7% in the Blue Mountain Eye Study. While it was substantially higher in the over 75 years age group, it was still 1.5% (95% CI 0.6-2.4%) and 0.9% (95% CI 0.2-1.6%) for those aged 65-74 years in the respective studies.[211]

It is difficult to compare the prevalence of ARM between studies accurately – although the prevalence figures are not dissimilar to major surveys of the BMES and BDES (Table 42), accurate comparison is difficult. This is because the definitions of ARM are different in each study: the BDES defined early ARM as the presence of soft indistinct or reticular drusen, or *any* drusen type (except "hard indistinct" drusen) with pigmentary changes.[210] The BMES defined early ARM as soft indistinct or reticular drusen, or *soft distinct* drusen with RPE abnormalities.[211] The International Grading criteria do not differentiate between soft distinct and indistinct drusen in the definition – any soft druse >63μ is included, and *any* drusen with pigmentary changes. Unfortunately, because the International classification does not distinguish between soft indistinct and distinct for drusen 63μ to 125μ diameter, I cannot calculate prevalence figures by the other studies' criteria. However, my impression is that the prevalence is lower than the American and Australian studies, if graded the same way. Unfortunately the Melton Eye Study have not published their prevalence data of a similar British population.

Several reasons may underly this lower prevalence figure for AMD and probably early ARM. Firstly, it may be an artefact relating to poor quality of retinal

photographs. This seems unlikely with such a low rate of ungradeable photographs (Table 41), and acknowledgement from experienced macular photograph viewers that photographs were of sufficient quality. A second reason may be a bias introduced in the prevalence figures because each pair of individuals was related and the population is therefore not a "true" sample. This can be overcome by selection of one twin at random from each pair. When this is done, the prevalence figures are not significantly different from the overall figures (which might be expected given such a low number of concordant pairs). A third possibility is that recruitment bias has occurred and that only twins with early disease have been recruited. It is possible that twins with late-stage AMD and visual loss might not volunteer to travel to London to be seen. However, the prevalence rates were probably also lower for the early asymptomatic ARM and the twins were not informed of the outcomes being studied, making it unlikely that serious recruitment bias occurred.

A fourth potential bias is that the twins are volunteers, and volunteers tend to be more aware of health than non-volunteers, so it is possible that they are healthier than a true sample of the population, which may have influenced the prevalence of ARM. However, smoking and drinking rates, as well as disease rates, in the twins are very similar to women in the Chingford population-based longitudinal study of osteoporosis and fractures (T.Andrew, personal communication), not supporting the argument that the twin volunteers are particularly healthy compared to a similar population-based cohort. The only consistent difference between twins is that DZ twins tend to be heavier than MZ twins, but similar weight to the general population, and this factor is not believed to be particularly important in the aetiology of ARM.

The twin prevalence might be lower than the other studies because the prevalence of ARM really is lower in the UK than in the USA and Australia. No prevalence data from a comparably-aged population in the UK are available, and we await publication of the Melton Eye Study results with interest. Finally, twins might have a lower rate of macular degeneration compared to singletons. This is difficult to explore, but since MZ twins are more likely to be monochorionic than DZ twins, if ARM was related to factors within the womb, a different prevalence between MZ and DZ twins might be expected if antenatal factors were involved. In this study the prevalence rate of ARM

was 12.8% (95% CI 9.8-15.8%) for MZ twins and 14.4% (95% CI 11.5-17.3%) for DZ twins respectively using the International criteria, not significantly different.

A question mark therefore remains as to how representative the twins are of the general population for ARM, but there is no evidence from other age-related eye diseases that they are significantly different.

4.5.4 Confounders

The groups of MZ and DZ twins were frequency age-matched to avoid any age-related difference between the two groups, important in the analysis of such traits as cataract (see Figure 16). Otherwise, it was assumed that confounders were similar for the two groups. Table 46 below shows that for the potential major confounders of smoking, smoking pack years in current smokers, alcohol intake, hormone replacement therapy and menopausal status, the groups of MZ and DZ twins were very similar. The DZ twins were slightly heavier than the MZ twins, but weight has not previously been shown to be important in the phenotypes measured in this study.

The conclusion is that the MZ and DZ twins were well-matched for confounders overall, although it is likely that the MZ pairs were more concordant for the smoking and alcohol data than the DZ twins. Some of this effect may have been included in the environmental effect, and some genetic (since these have been shown to be partially genetic), but as the calculation for nuclear cataract showed, taking smoking into account, familial clustering of environmental risk factors does not significantly alter heritability.

Table 46 has some comparison data from the Chingford longitudinal study of 1000 women examined between 1989-1999, a sample of women taken from a group practice register. Again, they are not a true population sample, but provide a sample of singleton women who are not volunteers, to compare the exposure prevalences to the twins. The exposures are broadly similar, although the twins were more likely to have used HRT, which may reflect the interest of the Twin Research Unit in osteoporosis. Thus the twins appear broadly representative.

Table 46: Potential confounders compared between MZ and DZ twins, women in Chingford study

Confounder		MZ (%)	DZ (%)	Chingford
Birth weight (kg)		2.28	2.34	?
Smoking	Never	55	56	54
	Current	14	14	21
	Ex-smoker	31	30	25
Mean Pack years	(in current smokers)	33.4	33.6	?
Alcohol	Never	14	14	18
	<= 10 units/week	74	73	72
	> 10 units/week	12	13	10
HRT	Never	60	55	76
	Current	21	26	7
	Ex-user	19	19	17
Postmenopausal?	% yes	97	96	90
Weight (mean)	Kg	64.6	67.3	67.4
Height (mean)	Cms	160	161	161

4.5.6 Foetal programming

Other potential biases discussed in the Introduction are unlikely to be of importance in this twin study such as twin-twin interaction and assortative mating. However, another potential bias is that of foetal programming, in which the foetal environment (more similar and more stressful in monochorionic as opposed to dichorionic pregnancies) may impact on later phenotypes: the Barker hypothesis.[42] The lighter of a pair of twins has been shown to have a higher blood pressure (with all the caveats of recall data).[44] Therefore MZ twins, who are more likely to be monochorionic and therefore may share more foetal environment than dichorionic (usually DZ) twins might be "programmed" to develop cataract or ARM later in life by their foetal environment. MZ twins are generally lighter than DZ twins (see Table 46), but in this study MZ twins had very similar measurements to DZ twins for all the phenotypes measured. There were some small differences for refractive error and cataract (see Table 24, Table 30, and Table 37), and the ARM prevalences were slightly lower for MZ than DZ twins (12.8% vs 14.4%), but on the whole the effect of foetal environment is likely to have been small for this cohort.

In other eye studies of foetal programming, examining a sample of a cohort of subjects born in Hertfordshire from 1920 to 1930, there was no association between birth weight and visual acuity and age-related eye diseases,[48] no association between birth weight or growth in the first year and intraocular pressure/glaucoma,[47] and no association between birth weight and cataract.[46] However, this last study did find an inverse association between nuclear cataract and weight at one year of age, which is an interesting finding, meriting further investigation. However, overall, there is little evidence for in utero environment significantly affecting age-related adult eye disease, and so foetal programming is unlikely to significantly confound a twin study.

4.6 Power of the twin study

The calculations for the power of this study during the design stage were based on an 95% power to detect a heritability of 20% at the 0.05 significance level, and predicted a requirement of 600 twin pairs to achieve this. Only 506 pairs were eventually seen in the study, due to a combination of difficulty in recruitment of the 600 pairs due to insufficient administration time, and a realisation that there was enough power in 500 pairs for the continuous outcomes studied here, apart from macular degeneration. The prevalence of ARM was lower in the twins than expected, based on the previously published literature, and 600 pairs would still have been insufficient. This twin study demonstrates the reduced power when a phenotype is reduced to binary data (as in the ARM results) rather than continuous data. Phenotyping of more twins would be required to increase the power of the study, but many more twins would be required in the elderly age groups to achieve this. I did not think this was practical in the timeframe and financial resources available to me.

In addition to this particular aspect of the study, there are two specific instances in which power questions should be asked of this twin study and twin studies in general: the power to detect dominant genetic effects and the power to detect common environment.

4.6.1 Dominant genetic effects

As stated earlier, twin studies have relatively low power to detect dominance. This is because MZ twins share all dominant effects but DZ twins are on average likely to share only one quarter of the dominant genetic effects.[50] This is illustrated in Figure 15. The low power to detect dominance is especially true in univariate models,[280] hence the value of multivariate models to optimise power,[273] as used in the astigmatism calculations (Table 28).

For the traits which demonstrated a dominant genetic effect, astigmatism (Table 28) and cortical cataract (Table 38), parameter estimates varied between different measures and confidence levels were wider than for additive only models. This is partly because 4 latent variables were being estimated (A,D,E and age for cortical cataract) compared to 3 for other AE models. Genetic models assume the effect of dominance is additional to an additive genetic effect,[272] so the effect of removing additive genes from the model cannot be tested, even though in some cases the parameter estimates for A were actually zero. For example, no additive genetic effect was estimated for the Wilmer grading while it was 20% for the Oxford grading. No additive genetic effect is implausible in the genetic modelling setting, so this parameter is reported even though the model estimates it at zero.

The nuclear cataract data, as discussed above in the section on the heritability of nuclear cataract, is a case in point in which some dominant effect might be involved, but this study did not have the power to detect it and under the most parsimonious "best-fitting" model the effect of D was removed. Although multivariate analysis was attempted, using the different measures, it came up with no greater significance of the effect of D.

4.6.2 The common environment effect

Although shared family environment might not have an effect on later age-related conditions such as cataract, it is surprising that all the models rejected any

contribution of family environment to any of the phenotypes measured in this study, apart from brunescence whose data is questionable. The absence of a common family environment effect for myopia is particularly surprising, as educational levels and socioeconomic factors have been shown to be important when one would expect the family environment (say, encouragement to read) would affect siblings similarly. The Framingham Offspring Eye Study found the strength of the sibling association depended on the age difference between youngest and oldest, half the odds ratio for a 10 year difference than for a 2 year difference in age.[111] This suggests siblings nearer in age to each other share some more common environmental effects. This does not square up to the twin data, although of course one of the advantages of twin family studies is that the siblings are by definition age-matched.

There is an inherent bias in the classical twin model that explains any greater similarity between MZ and DZ twins as due to genetic effect, because of the assumption of equal environment. It may be that trait-specific environmental effects could be more correlated in MZ than DZ pairs. This is often difficult to test in real life and many studies have not tried to examine the equal environment assumption. One way to test this might be to compare DZ with other sibling correlations, which should be the same if there is no shared environment effect. Regarding myopia and close work, I have not been able to find any data on the amount of time spent reading in twins. However, twin studies have suggested reading skills are largely genetic (one study of oral reading ability showed a heritability of 69% and common environment effect of only 13%), and a candidate locus for reading disability has been identified on chromosome 6.[289]

There is little statistical power under the classic twin model to detect shared environment effects, and the sub-modelling techniques employed to establish the "best-fit" or most parsimonious model may eliminate these effects when they are in fact real. It has been estimated that a twin study requires 500 pairs of MZ and DZ twins to detect a common environment effect explaining 25% of the variance (80% power, 0.05 level of significance).[290] Revisiting the data of this study for refractive error (see Table 26) the model does lose some fit when C is dropped for right and more so left eyes for spherical equivalent, so there might be some common environment effect which this study was not powerful to detect.

However, the data suggest that genetic influences are far more powerful than these environmental ones. For astigmatism, by contrast, there is absolutely no change in fit with the loss of C from the model, suggesting there is truly no common environment effect involved in the aetiology of astigmatism.

4.7 Application of the results from this study and future research

4.7.1 Genetic studies

These results have provided evidence that genes are important in age-related eye conditions, and indeed contribute more to the variance of these traits than the environment. This means that further research into the genes that cause these common complex or multifactorial diseases is required, so that a greater knowledge of the mechanisms of the disease can be obtained. Only when the actual mechanisms of disease are understood will possible preventive treatments or those designed to slow down disease progression be developed. In addition, possible gene-environment interactions may be determined, and particular individuals with a particular genetic make-up may be sensitive to a specific environmental agent, which could be avoided to reduce risk of a disease developing.

Genes involved in these diseases can be ascertained using the twin data by treating the DZ twin pairs as sib-pairs, who are used in association and linkage studies (usually with other family members, especially parents). In these age-related traits, establishing disease status and even obtaining DNA from parents is often impossible. DZ twins are ideal sib-pairs, as they are age-matched and matched for many other features. This means that difficulties in ordinary sib-pair analysis, where the younger sib does not have a disease but may develop it in time, are reduced. The fact that the DZ pairs, like ordinary siblings, share on average only half their DNA is used. MZ pairs share all their DNA and so cannot be compared and contrasted in these genetic studies. Two sorts of genetic study can be performed:

Candidate gene analysis involves testing the allelic status of the sibpairs for a known candidate gene, and to see whether disease associates with one particular allele overall in the DZ twin pairs. Obviously it involves prior identification of a gene that may be relevant to the trait being examined.

Linkage analysis using DZ twin pairs involves a whole genome screen looking at a series of genetic markers all along the DNA, and to see whether the trait in question is particularly linked to one particular marker or range of markers: quantitative trait linkage analysis.

With this in mind, two collaborations have already been started, looking at candidate genes in myopia and cataract, using the DNA extracted from the DZ twins examined in this study. We are collaborating with researchers at the Institute of Ophthalmology in London.

Myopia candidate gene study

In association with Mr Andrew Webster and Professor Shomi Battacharya at the Institute of Ophthalmology, we are examining candidate genes such as the Col2A1 gene, identified in Stickler's syndrome, and the fibrillin gene, identified in Marfans syndrome, both of which are associated with myopia. DNA has been extracted and the allele frequencies of the candidate genes and their frequency in the DZ twins is being identified, and will be linked with the refraction data to see if any of the alleles are associated with myopia in our population

Cataract candidate gene study

In association with Mr Peter Francis and Professor Shomi Battacharya at the Institute of Ophthalmology, we hope to examine candidate genes in cataract. There are several gene abnormalities identified in congenital cataract and these are ideal candidate genes for adult cataract. A grant application will be submitted shortly.

4.7.2 Epidemiological studies

The data from these twins can be used with data on the Twin Research Unit and Genetic Epidemiology database about risk factors and lifestyle details in a co-twin control study. An observational co-twin control study is possible to examine twins discordant for exposure to see if they have a different outcome. Similarly, a co-twin case control study can be undertaken, looking at twins discordant for disease and examining which environmental factors are different. In this study, as the MZ twins were so concordant for most of the features, the power of these studies may be limited.

Co-twin case control study

In association with Dr Bianca Stavola at the London School of Tropical Medicine and Hygiene and an MSc in Epidemiology student Ms Marta Romanengo-Panzeri, data from our twins relating to cataract and lifestyle factors such as birthweight, hormone replacement, smoking and alcohol are being analysed.

Longitudinal studies of ARM and Cataract

There is little published information on incidence and longitudinal outcomes in eye disease. This cohort is potentially useful as we hope to reexamine them when older (particularly relevant to the ARM side of the study) and to establish the heritability of progression of disease as well as the long-term follow up and incidence of ARM.

4.7.3 Further heritability studies

There are further avenues that might be explored with this unique twin cohort. The heritability of glaucoma, intraocular pressure and normal optic disc parameters is not known and there is currently considerable interest in the genetics of glaucoma.

Heritability of optic disc parameters

The fundus photographs taken in this study included stereoscopic views of the discs . It is hoped that the optic disc parameters will be analysed in association with Mr Richard Wormald at the Institute of Ophthalmology.

Heritability of macular pigment

Miss Clare Gilbert has submitted a grant proposal to study the heritability of macular pigment optical density in a fresh cohort of twins and to examine changes in density in response to nutritional supplements. The role of genes and environment in the level of macular pigment is debated. Macular pigment, which may have an important role in the prevention of oxidative and blue light damage to the retinal photoreceptors, can be measured with a continuous score, which should be ideal for twin modelling analysis.

It can be seen that the research outlined in this thesis has led to continuing research on this cohort of twins, and further possible cohorts from the Twin Research and Genetic Epidemiology Unit

4.8 Conclusions

This study has demonstrated that genetic effects are important in the development of common eye diseases such as refractive error, and even age-related eye diseases such as cataract and ARM. The highest heritability was 84-86% for myopia and hypermetropia, and the heritability of astigmatism, nuclear cataract and cortical cataract was 50-60%. Inheritance of astigmatism and cortical cataract predominantly involves dominant genetic effects. Age, as expected, is important in nuclear cataract (explaining 38% of the variance) and less so in cortical cataract (11-16%). These results offer exciting prospects in the search for susceptibility genes, which might allow prediction of those at risk for disease, as well as furthering the understanding of

the mechanisms and gene-environment interactions in the development of these important eye diseases.

Refractive error is becoming increasingly prevalent in all societies, and further understanding of the pathogenesis of myopia is required before possible treatments can be developed, to reduce progression or even prevent myopia in those at risk. Close work is increasing in modern society, and genes have been identified in familial high myopia[114; 115] but clearly susceptibility genes in simple myopia need to be identified. The high heritability identified in this study supports further efforts to identify them.

The understanding of how genetic mechanisms can result in age-related cataract may be advanced by the specific gene defects that are now being isolated in congenital cataracts,[142] and in specific adult-onset cataract syndromes such as that associated with myotonic dystrophy.[146] The results of our study encourage the search for genes in age-related nuclear cataract through linkage and candidate gene studies. This would further elucidate the pathogenesis of this common problem with the hope of future measures to delay its onset or progression. It has been estimated that if cataract onset could be delayed ten years, 45% less surgery would be required,[165] with a major financial and social impact.

For ARM, considerable resources are currently committed to further genetic research, and the results detailed in this thesis support this research and the role of genes in ARM. However, the results also confirm some of the difficulties of this research, and in particular the loss of power of genetic modelling (and other) techniques because the data are binary, and not continuous. Despite much effort in looking at single gene disorder phenotypes with a similar appearance to ARM, the results of genetic research in ARM have been disappointing.[224; 282] This study suggests that soft drusen are more heritable than pigmentary changes (and more than 20 hard drusen more so), and these phenotypes should be of special interest in looking for susceptibility genes.

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6.0 Appendix

Questionnaire and Data Entry Sheets

Lambeth, Southwark & Lewisham Health Commission
Guy's & St Thomas' Hospital Trust, St Thomas' Hospital

CONSENT FORM FOR PARTICIPATION IN RESEARCH PROJECTS & CLINICAL TRIALS

Title of Project: A STUDY INTO THE GENETICS OF VARIOUS CONDITIONS INCLUDING, BUT NOT LIMITED TO, OSTEOPOROSIS AND OSTEOARTHRITIS USING IDENTICAL AND NON-IDENTICAL TWIN PAIRS

Principal Investigator: DR TD SPECTOR **Other Investigator/s enrolling patients:**	**Ethics Committee Code No: EC95/041**

Outline explanation:

We are researching the genetic and environmental causes of osteoporosis and osteoarthritis although we would also like to look at the genetics of other conditions. This study is aimed at collecting information which will enable us to undertake such research.

Your visit today may involve a bone mineral density scan (of your spine, hip, forearm, whole body) and x-rays (of your knees, hands and pelvis) if you are over 40 years old. We may also perform an ultra sound scan of your heel, a spygmocardiography (this measures blood flow in your arteries via a small probe on your skin) and an ECG or Electro Cardiogram, which is a heart tracing. Lung function tests may be performed and we may ask you various questions about a wide range of subjects including family history and diet. Some twins will be asked to have a magnetic resonance imaging (MRI) scan to investigate the genetics of vertebral disc disease. We may also be performing some basic non-invasive analysis of skin, hair and teeth and some twins may be asked to have an eye examination. This involves inserting some eye drops to dilate the pupils and then taking some pictures of the back of the eye.

In addition, a blood sample will be taken. Your DNA and other genetic material and information may be extracted from the blood samples taken. DNA may be stored and subject to preservation procedures which will permit it to be more extensively analysed and used at a later date. A urine sample may also be taken.

We would like permission to use and retain for our own purposes, your blood, DNA, urine and the other characteristics that we measure on this visit to research conditions we are interested in now and in the future. Although you will cease to have ownership in them, all data and results will remain strictly confidential as we do not supply your personal details such as name, address or telephone number without contacting you beforehand for permission.

Other centres of excellence, including some companies, assist us with our research in exchange for various rights to our data and findings. Again, we do not supply your personal details such as name, address or telephone number without contacting you beforehand for permission.

Wherever possible every effort will be made to contact you if any results were found by us that indicated that medical intervention was required. However it must be highlighted that in most instances results are examined and tests are performed by nurses or research assistants not medically qualified doctors. Although the number of investigations and blood tests are numerous, these analyses may not be undertaken immediately and we do not investigate every system of the body fully. Accordingly, if you have any problem or query about your health you should contact your own General Practitioner.

Last amended 22/10/98

I (name) _____

of (address) _____ 1 6AZ

hereby consent to take part in the above investigation, the nature and purpose of which have been explained to me. Any questions I wished to ask have been answered to my satisfaction. I understand that I may withdraw from the investigation at any stage without necessarily giving a reason for doing so and that this will in no way affect the care I receive as a patient.

SIGNED (Volunteer) _____ Date 26/5/99

(Doctor/Research Nurse) C Hammond Date 26/5/99

(Witness, where appropriate) _____ Date _____

3 copies required:- one for researcher, one for patient/volunteer, one for patient's notes

Twin Eye Study: Questionnaire

Study number [4][4][3][2][1]-[][]

date [3][0]-[1][4]-[98]

Name (surname) [][][][][][][][][][][][][][]

(first name [][][][][][][] date of birth [12]-[06]-[57]

Previous Ocular History

- did you have a squint as a child or more recently? []no [con] []div []other
- do you have a lazy or weak eye? []no [Right] []Left
- do you have to wear spectacles to see clearly in the distance?
  []no []myope [hyperope] []astigmatism []not sure why
  what age? [][1]
- do you need to wear glasses for reading (or bifocals?) []no [yes:] what age? [4][0]
- do you have glaucoma (raised pressure in the eye)? [no] []yes: age diagnosed [][]
- do you have macular degeneration (poor central vision but normal surrounding field of vision?
  [no] []yes: age diagnosed [][]
- have you had a cataract operation? [no] []yes: age first eye [][] age second eye [][]
- have you had any other eye operations? [no] []yes: details (where/who)
  []glaucoma []retinal detachment []squint []other
- have you ever had an eye injury [no] []yes
- how were you recruited? []eye publicity [other]

Family History

	mother	father	any sister	any brother
• is there any family history of macular degeneration (poor reading and central vision but reasonable vision for getting about)?	[no] [] yes: age [][]	[no] [] yes: age [][]	[no] []yes: age [][]	[no] /[] yes: age [][]
• is there a family history of cataracts requiring an operation?	[no] [] yes: age [][]	[no] [] yes: age [][]	[no] []yes: age [][]	[no] /[] yes: age [][]
• is there a family history of glaucoma (raised pressure in the eyes needing eye drops or operation)?	[no] [] yes: age [][]	[no] [] yes: age [][]	[no] []yes: age [][]	[no] /[] yes: age [][]

Eye Colour

- what colour would you describe your eyes? [blue] []brown []green []hazel/other

have they changed since you were younger? [no] []yes: []lighter []darker []unsure

Ocular medications & duration

current drug	age	duration(m)	previous drug	age	duration (months)
[][].[]	[][]	[][][]	[][].[]	[][]	[][][]
[][].[]	[][]	[][][]	[][].[]	[][]	[][][]
[][].[]	[][]	[][][]	[][].[]	[][]	[][][]

Study number 9[1][4][3][2][1]-[][] date [3][0]-[0][4]-[9][8]

Ocular examination

			Right		Left
Autorefraction	SE	+■ -[]	[][7].[2][5]	+■ -[]	[][7].[2][5]
	K1		[4][4].[7][5]		[4][4].[7][5]
	K2		[4][5].[5][0]		[4][5].[5][0]
	astig		[0].[0][0]		[0].[2][5]
	axis (negative)		[][][9]		[][][4]
Vision (logmar: best corrected)		+[] -■	[0].[0][6]	+[] -■	[0].[1][4]

Cover Test []straight ■eso []exo []esophoria []exophoria []vertical []can't measure

TNO stereotest (mins) [9][9][7]

RAPD ■no []R []L

Lens		Right	Left
		[]pseudo []aphake []ungradeable	[]pseudo []aphake [] ungradeable
	thickness (C1)	[0].[7]	[0].[9]
	cortical spokes (C2)	[2].[3]	[2].[8]
	waterclefts (C2)	[0].[2]	[0].[2]
	fibre folds	[1].[1]	[2].[0]
	ASC (C1)	[0].[0]	[0].[0]
	PSC (C1)	[0].[0]	[0].[0]
	vacuoles (C2)	[0].[0]	[0].[0]
	retro-dots (C3,C4)	[0].[0]	[0].[0]
	focal dots (C2)	[0].[4]	[1].[0]
	brunescence (N)	[0].[2]	[0].[2]
	white scatter (N)	[2].[6]	[2].[8]
	other features?		
Disc appearance (vertical C/D ratio)		[0].[1]	[0].[1]
reticular pattern?		■no []yes	■no []yes
peripheral changes ?		■no []yes: details	■no []yes: details
other pathology ?		■no []yes: details	■no []yes: details
Intraocular pressure		[][]	[][]
Referral letter sent?		[]yes ■no	