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The Genetic And Environmental Aetiology Of Ocular Congenital Anophthalmos, Microphthalmos And Uveal Coloboma

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DECLARATION

I, Danny Morrison, have composed this thesis and the work was conducted as a member of a research group to which I made a substantial contribution. Any assistance received is acknowledged and clearly indicated. All the work contributing to this thesis was conducted while in post at the University of Edinburgh, Scotland.

This thesis has not been submitted in candidature for any other degree, diploma or professional qualification.

PREFACE

'While holidaying in the Highlands in August of 1919, I was asked by the parochial authorities to report on the condition of the eyes of the family of A.C., a labouring man dwelling in the Benderloch.

The parents were young, healthy-looking individuals, and lived in a cottage on the loch-side. The external appearance of their eyes was quite normal, and they had no complaint of visual defect. Apart from the condition of their eyes the four children appeared quite healthy. ...The local doctor, Dr. McNichol, who visited the house with me, informed me that there had been no inflammation of, or discharge from, the children's eyes at or after birth. The appearance of their eyes was entirely one of underdevelopment...there had been no similar condition of the eyes of the parentage on either side.

C.C., female, 3 years of age. The right eye was undeveloped, the cornea absent, and the eye blind. The left eye was more developed, the cornea was clear, and there was a downward coloboma iridis congenitalis. The child was able to distinguish a penny-piece held out to her.

J.C., male, 3 years of age, was absolutely blind. The right socket was empty, anophthalmos, and the cavity was contracted. The left eyeball was undeveloped, the cornea being absent.

C.C., male, 2 years of age. This boy was also practically blind. A small dark spot in the centre of the front of the right eyeball represented the cornea, and through this light and darkness could be distinguished. The left socket was empty, another case of anophthalmos.

M.C., 1 year old. The right eye resembled those seen in the other children, undeveloped, opaque, and blind. The left eye was the most normal-looking in any of the children. It was clear, bright, and healthy looking. ... The child could see well enough to take hold of objects held out to her.

I recommended that an attempt should be made to educate the eldest and the youngest children in the ordinary way, and that the second and third should be educated in a blind asylum. The parents are young and evidently prolific. Whatever the cause may be, the outlook for any future children is to me simply appalling.'

Lewis McMillan MD., British Journal of Ophthalmology, 1921.

ABSTRACT

The Genetic And Environmental Aetiology Of Ocular Congenital Anophthalmos, Microphthalmos And Uveal Coloboma

Introduction

Microphthalmos, anophthalmos, and uveal coloboma (MAC) are a related group of congenital eye abnormalities that may result in significant visual impairment. The aetiology of these eye conditions is poorly understood and a variety of genetic and environmental causes have been postulated.

This thesis argues that microphthalmos, anophthalmos and uveal coloboma have not been adequately defined. The difficulties in trying to establish a genetic or environmental cause of these complex conditions have been exacerbated by poor definition. The correct investigation of a cause relies on first establishing a phenotypic definition.

Methods

Scottish children born between 1981 and 1996 with a reported diagnosis of MAC were traced (n=338) and examined (n=152) for congenital eye and systemic anomalies. Eye (globe) size was determined using B-scan ultrasound (n=86). In selected cases, DNA was extracted from blood samples or mouthwashes. Mutation screening studies were done on the homeobox genes *PAX6* and *CHX10,* both of which are candidate genes for these eye conditions.

Results

The birth prevalence of MAC for Scotland for the period 1981–1996 was 1.78 per 10,000 live births. The prevalence in each of the fifteen health board regions was calculated.

An ultrasound study on 86 eyes showed that eye size cannot be used to define microphthalmos.

A new phenotypic classification of MAC, not dependent on eye size, is presented. The term microphthalmos does not feature in the new classification.

The prevalence of retinal detachment in eyes with uveal coloboma was calculated at 3.96% and the association of iris coloboma with iris heterochromia was firmly established, occurring in 17% of cases.

Systemic congenital anomalies were common and affected 37% of those examined. More than one case was confirmed within eight families, which suggested possible modes of inheritance. The empirical recurrence risk of inheritance was calculated for the purpose of genetic counselling.

PAX6 gene mutations were not found in 83 selected individuals, using denaturing high performance liquid chromatography. A *PAX6* gene missense mutation was found in one individual with an ocular phenotype similar to aniridia. In three subjects tested, no *CHX10* gene mutations were found.

Conclusions

Microphthalmos cannot be defined easily as it represents a number of different structural eye malformations. Microphthalmos cannot be defined by measuring the size of the eye. The term microphthalmos has very limited use.

A new phenotypic classification of MAC is proposed, based on the structural eye abnormality and without reference to eye size. The term microphthalmos does not feature in the new classification.

The large number of congenital systemic anomalies associated with the eye anomalies studied, and the occurrence of cases with a family history, suggests a complex genetic and environmental aetiology, with both factors possibly acting in combination.

Empirical recurrence risk estimates have been calculated for genetic counselling.

It is unlikely that *PAX6* gene mutations play a major role in the aetiology of MAC.

Further studies on these eye anomalies are indicated using this well phenotyped clinical and genetic database. This could include screening for other proposed candidate genes, studies on microdeletions and monitoring of birth prevalence. Large families of affected individuals are being sought for linkage analysis.

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CHAPTER ONE

ANOPHTHALMOS AND MICROPHTHALMOS: CAN THEY BE DEFINED?

'Sed infanti nuli oculi erant.'
But the child had no eyes. (Bartholini, 1657).

Summary

Anophthalmos and microphthalmos have not been clearly defined and are not easy to define.

Current definitions based on eye size alone are inadequate. Eye size alone is inappropriate to define microphthalmos. The term microphthalmos has very limited use and conveys no specific meaning.

The terms anophthalmos and microphthalmos represent clinically inseparable entities.

There is a continuous spectrum from true anophthalmos to mild microphthalmos.

Colobomas resulting from defective optic fissure closure occur in both normal-sized and microphthalmic eyes.

ANOPHTHALMOS

The fact that a case of anophthalmos was well described more than 300 years ago by Thomas Bartholini (Bartholini, 1657) is of more than just historical interest. The child had no eyes, with additional malformations (face, polydactyly of hands and one foot), and normal parents.

Many features of Bartholini's case report have a broad similarity to those written up more recently. The chapters to follow will demonstrate the significance of observations then and now with regard to the aetiology of this condition.

Anophthalmos (anophthalmia, Greek 'eyeless') is the congenital absence of an eye, which can be unilateral or bilateral. To a layperson, such a diagnosis would appear to be straightforward, but this is not so (Sorsby, 1934). True anophthalmos is a histological (post-mortem) diagnosis. It is the complete absence of any recognisable eye tissue or embryological remnant, and this can only be confirmed by serial sections of the orbital contents. Consequently, the more useful term *clinical* anophthalmos (Duke-Elder, 1964) was introduced to describe the apparent absence of an eye. This term is not always being used as it should, and is applied to many cases of extreme microphthalmos.

It should already be apparent that anophthalmos and extreme microphthalmos are being conflated. There is some justification for grouping them together but, as we shall see in the discussions of microphthalmos, there is great confusion over the diagnosis of microphthalmos, be it 'extreme' or 'severe' microphthalmos, 'remnant only' microphthalmos (Tucker et al. 1996), or 'mild' microphthalmos. The result is

that anophthalmos and all forms of microphthalmos are now commonly grouped together as the same condition, which they are not.

Before turning to the classification of anophthalmos, it is necessary to review the relevant stages of early eye development with respect to anophthalmos and the tissues that will eventually give rise to the eye. These tissues are derived from surface ectoderm, neural ectoderm, neural crest and mesodermal mesenchyme. Developmentally and functionally the eye is an extension of the central nervous system. The central nervous system differentiates from the ectoderm on the dorsal surface of the developing embryo, in an area which has thickened to form the neural plate, the cells of which are known as the neural ectoderm (Bron et al. 1997). A depression develops in the neural plate to form a groove, and the neural folds then grow and converge toward each other, forming the recognisable neural tube (eighteen days of gestation).

The first appearance of anlage of the future eyes, the *optic sulcus*, is before closure of the anterior end of the neural tube. These small lateral grooves are apparent at twenty days' gestation (2 mm stage). The sulcus enlarges to form the *optic evaginations* (*optic pits* internally), which later become the *optic vesicles*.

The optic vesicles enlarge and appear as hollow, symmetrical hemispherical outgrowths on the lateral side of what is now the forebrain vesicle. The cavity of the hollow optic vesicle therefore communicates with that of the forebrain, which will develop into the telencephalon (future cerebral hemispheres) and the diencephalon. It is from the side walls of the diencephalon that the paired optic stalks arise.

Returning now to anophthalmos, Ida Mann (Mann, 1959) described three types:

In *primary anophthalmos* there is failure of the optic pit to deepen and form an optic outgrowth from the forebrain.

In *secondary anophthalmos* there is an abnormality of the whole of the forebrain so that absence of the eyes is a consequence of a wider developmental abnormality of the forebrain.

The third type, *consecutive (degenerative) anophthalmos*, is when the optic vesicle formed but has subsequently degenerated and disappeared. Consecutive anophthalmos links up with extreme microphthalmos, and the two are only distinguishable microscopically.

Histopathology of anophthalmos

As mentioned earlier, histological examination of the orbital contents has confirmed the complete absence of an eye in several cases (Duke-Elder, 1964). However, in many cases of anophthalmos, a nodule representing some of the ocular tissues is embedded near the apex of the orbit. Within this nodule there may be several types of tissue mixed up and present in varying amounts: fibrous scleral tissue, pigmented and vascularised uveal tissue, cartilage. If neuroectodermal elements are found, then technically the case is one of extreme microphthalmos. This distinction has always been considered an academic one not of clinical importance. As we shall see in later discussions on aetiology and its investigation, when describing individual cases and syndromes, and when looking at the prevalence of anophthalmos, the lack of an adequate definition combined with varying diagnostic criteria has been detrimental to the process of understanding anophthalmos.

The histological findings within the orbit are extremely variable, and have been reported by several authors (Treacher Collins, 1887). Typically, the extraocular muscles are present and well formed and supplied by their appropriate nerves; insertion is into a nodule or mass of fibrous tissue, which represents the sclera. The muscles are not often sharply differentiated and may be inserted in an indiscriminate and tangled manner, altering their movement pattern. The lacrimal gland and apparatus are usually present, along with the orbital fat.

In consecutive (degenerative) anophthalmos, formation of the optic vesicle is followed by degeneration (Brownstein et al. 1977). In these cases, fragments of tissue derived from neuroectoderm (neurosensory retina, retinal pigment epithelium, choroid) will be found. The lens or lens elements (often calcified) are typically absent but may be present (Pearce et al. 1974).

The histopathology and postmortem findings posterior to the globe are just as variable. The absence of any optic nerve and the optic tracts/chiasm at post mortem is further confirmation of a case of true primary or secondary anophthalmos (Hesselberg, 1951). The optic foramen is absent or obliterated in a few cases. Replacement of the optic nerve by a rudimentary optic nerve or fibrous band is described in some cases. These abnormalities are reflected in the lateral geniculate nuclei and visual cortex being small or rudimentary (Brunquell et al. 1984).

The post-mortems of the nineteenth century (Hesselberg, 1951) and the later part of the last century (Duke-Elder, 1964) have been superseded by high resolution modern imaging techniques, and it is to these which we shall turn briefly.

Computerised tomography (CT) and magnetic resonance imaging (MRI) have confirmed many of the earlier pathological descriptions (O'Keefe et al. 1987) as well as revealing any associated brain pathology (Albernaz et al. 1997). Caution must be exercised in interpreting CT or MRI of the orbital contents for the presence of true ocular tissue because of the varying absorptions of the orbital tissues and its condensation.

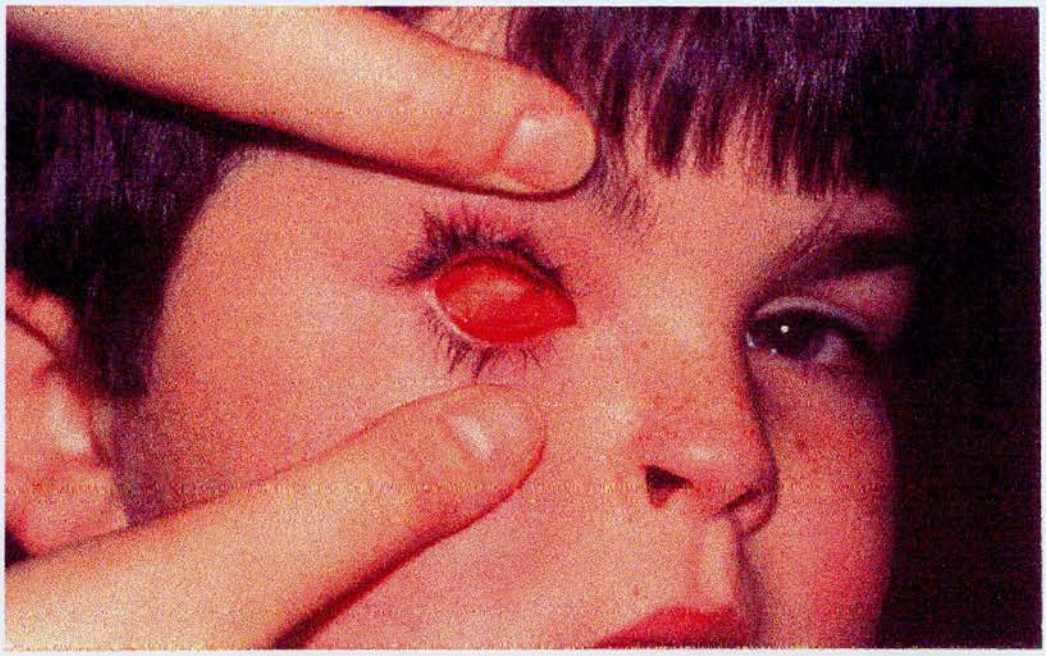
Clinical appearance of anophthalmos

As stated in the opening paragraph, extraocular congenital abnormalities are not uncommon with anophthalmos. This aspect will be discussed in chapter two (aetiology).

The external facial appearance of a neonate or small child with anophthalmos may be unremarkable, but there are sometimes signs or the typical features of a closed sunken eyelid with the 'eye' appearing to be deeply set. The palpebral fissure length is usually significantly reduced, with normal eyelid margins, lacrimal puncta and glands. Epicanthic folds may be present. The orbit is reduced in size, and is lined with conjunctiva. The lacrimal gland is usually present and functioning (Brunquell et al. 1984).

In all cases of anophthalmos there will be no vision at all (no perception of light) on the affected side.

Figure 1.1: Right clinical anophthalmos



Laterality of anophthalmos

Both unilateral and bilateral anophthalmos occur, and the possible significance of this will be discussed in the section on aetiology (chapter two). Treacher-Collins (Treacher Collins, 1887) summarised the cases of the earlier literature. He collected 30 bilateral and 12 unilateral cases. Von Hippel (von Hippel, 1899) added a further 34 bilateral and 11 unilateral cases. The impression is that bilateral anophthalmos is more common, but it may be that reporting of what is a far more disabling defect is more frequent. However, as we shall see later, a normal fellow eye does not always accompany unilateral anophthalmos.

Congenital cystic eye

Closely related to congenital anophthalmos is *'congenital cystic eye'* (Hesselberg, 1951). Returning to the stage of early eye development discussed earlier in this

section, it will be recalled that at the 2 mm embryo stage the optic vesicles appear as hollow outgrowths from the wall of the forebrain vesicle (Bron et al. 1997). At this stage (3–4.5 mm, 24–27 days) each optic vesicle will normally invaginate to form an *optic cup* by the anterior part of the vesicle lying in apposition to the posterior part. This anterior layer of the vesicle will go on to form the neuroretina, and is apposed to the posterior part (the future retinal pigment epithelium). A potential space between these two layers persists. The partial or complete failure of the optic vesicle to invaginate can result in the formation of a cyst in the potential space between the two layers of the optic cup, which may replace the globe of the eye. The cyst can vary between very small, presenting as clinical anophthalmos, and large, occupying the whole orbit and causing the eyelids to bulge (Dollfus et al. 1968). Congenital cystic eyeball has also been called *'orbital cyst with anophthalmos'*. However, this name is incorrect: histologically, the anterior wall of the cyst is made up of primitive retinal cells, with the posterior wall being a layer of primitive retinal pigment epithelium (RPE). The cyst is lined by an epithelium identical with ependyma. No primitive lens is present, confirming the fact that this type of malformation dates to before the 7 mm stage of embryonic development.

From the discussion above, it is already apparent that anophthalmos is variable in its clinical appearance and at the microscopic level, with eye tissue being present in quantities varying from nil to remnants. The point at which clinical anophthalmos transforms into extreme microphthalmos has yet to be established. It would therefore seem appropriate to start here with a description of microphthalmos, from which the lack of a current satisfactory definition should emerge.

MICROPHTHALMOS

Microphthalmos (microphthalmia, Greek 'small eye') is, quite simply, an eye that appears to be congenitally small. As with anophthalmos, the uninitiated might expect the definition and diagnosis to be straightforward, but this is far from the case. A number of significant factors have meant that no adequate definition exists:

1. As discussed under anophthalmos, there is no precise point at which clinical anophthalmos becomes extreme microphthalmos.

2. The transition point between microphthalmos and the normal eye, with respect to size, has not been established.

3. Microphthalmos is a very heterogeneous condition encompassing many different structural ocular abnormalities.

4. Both specialists and non-specialists are loosely applying the term 'microphthalmos' in the clinical sense.

Each of these points will be discussed in turn.

The fact that clinical anophthalmos and extreme (severe) microphthalmos (Figure 1.2) are very similar has long been recognised (Hesselberg, 1951). In extreme microphthalmos, only a small remnant of the globe is visible, and CT scans may be required to confirm the presence of a globe.

Figure 1.2: Right extreme clinical microphthalmos and left iris coloboma with clinical microphthalmos (ID 97)

Microphthalmos, according to Duke-Elder, was a term 'used to include a great variety of conditions and is justified in that in practically all cases the eye is smaller than normal'. This statement is true, but such a loose definition has contributed to the vast number of eye conditions in which microphthalmos has been diagnosed. All of the abnormalities resulting after the budding of the primary optic vesicle may be classed under the comprehensive category of microphthalmos (Duke-Elder, 1964). (Classification of microphthalmos will be discussed later in this chapter). The transition point between the normal eye and the microphthalmic one has yet to be determined. The size of the globe can be measured ultrasonically by A or B scans (Weiss et al. 1989b), CT or MRI (Wright et al. 1987). An effort to define microphthalmos has been made by Weiss (Weiss et al. 1989b), and this definition has been generally accepted by those who have recognised the need for a more rigorous diagnostic criterion (Warburg, 1993). Microphthalmos is said to be present when the axial length, adjusted for age, is at least two standard deviations (SDs) below the mean. In adults, this equates to an axial length of less than 18.5 mm. The 22 affected

patients whose data were used to establish the definition of microphthalmos (Weiss et al. 1989a) in fact had nanophthalmos ('simple', or 'pure', microphthalmos), which is a structurally and clinically distinct eye condition (see nanophthalmos below). When 'complex' microphthalmos was studied (Weiss et al. 1989b), microphthalmos was diagnosed on the basis of an adult axial length below 20.9 mm. However, subjects were selected for inclusion on the basis of this predetermined definition. A better study method would be to determine clinically or designate eyes as microphthalmic and then to measure the axial length. Many of the microphthalmic eyes in Weiss's study do not fulfil the author's own criteria, and a scatter graph has arbitrarily excluded some of the tabulated data. Microphthalmos has therefore been based erroneously on this definition. Furthermore, was this definition correct, refinement would be required by the addition of the phrase 'in an eye with a structural malformation.' The authors did state this but the phrase appears to have been ignored by those embracing the definition. This important point is a clear acknowledgement that it is the structural abnormality which is of significance, a point to which we shall return repeatedly. If microphthalmos were diagnosed on the basis of the definition above (more than two SDs below the mean), 2.5% of the normal population's eyes would be microphthalmic. This is considerably more than the estimated birth prevalence of microphthalmos of 1 in 10,000 live births, i.e. about 0.01% of the population (chapter two). Furthermore, there would not necessarily be any structural abnormality or visual impairment. We need to consider more accurate or appropriate means of 'diagnosing' microphthalmos.

Eye growth and size

Normal eye growth has been studied by a number of authors. The axial length of the eye has been measured in newborns, children and adults. The mean axial length (anterior corneal surface to retina) of the newborn eye is approximately 17 mm (Larsen, 1971). In 80 newborn boys the mean axial length was 16.78 mm and in 80 newborn girls slightly less, at 16.40 mm. The full adult size is almost attained by age thirteen years (Larsen, 1971). A small but significant increase in axial length of 0.4–0.5 mm occurs during puberty (Fledelius, 1982b). The adult eye has a mean axial length of 23.16 mm and 22.68 mm for males and females respectively (Larsen, 1971). Fledelius (Fledelius, 1982b) gives male and female axial lengths of 24.19 and 23.73 mm respectively.

Most (about two thirds) of the postnatal growth of the eye with respect to axial length occurs in the first 24–30 months of life (Fledelius and Christensen, 1996). The growth of the eye can be divided into three growth phases: a rapid postnatal phase with an increase of axial length of 3.7–3.8 mm in the first eighteen months of life, a slower childhood phase lasting to age five years with increase of 1.1–1.2 mm, followed by a juvenile phase up to the age of thirteen years with an increase of 1.3–1.4 mm (Larsen, 1971).

One factor that exerts a constant influence on the axial length of the eye from birth to adult life is low birth weight (Fledelius, 1982a). Low birth weight (defined as less than 2000 g) results in a reduction of adult axial length of 0.4-0.5 mm. This general and permanent influence on all eye dimensions is present throughout childhood and adolescence.

Corneal diameter

The concept of the eye appearing to be small rather than actually being small (as defined by axial length) is an important one. As stated above, many cases of true microphthalmos are correctly diagnosed on this basis clinically. The appearance of a small eye is strongly suggested by a real or apparent reduction in corneal diameter. Horizontal corneal diameter (HCD) increases with eye growth, but not in the same way as axial length (Hymes, 1929). One cannot directly correlate HCD with axial length. Microcornea is a term often used to denote a cornea of reduced size and this may occur in several eye conditions including oculodentodigital dysplasia (Judisch et al. 1979), aniridia (David et al. 1978), congenital cataract (Salmon et al. 1988), and retinopathy of prematurity (Kelly and Fielder, 1987) but the definition of what size of HCD constitutes microcornea has not been established. Duke-Elder (Duke-Elder, 1964) defined microcornea as an adult corneal diameter of less than 11 mm. Many, but not all, cases of microcornea do in fact have a reduced ocular axial length (Judisch et al. 1979; Bateman and Maumenee, 1984). Weiss (Weiss et al. 1989b) found a significant correlation between corneal diameter and axial length in subjects with complex microphthalmos. Several authors have determined values and normal ranges for HCD (Hymes, 1929). Nearly all of the postnatal growth, from 10 mm in the newborn, to about 11.8 mm in the adult, takes place in the first two years of life (males). Values for females are generally smaller by about 1 mm compared to males.

Clinical microphthalmos

The current accepted definitions of microphthalmos are inadequate. Consider how the diagnosis is made clinically. The clinical diagnosis is suggested by inspection and the label 'microphthalmos' is given. Microphthalmos, when extreme or severe, is easy to recognise. However, it is not necessarily the eyeball or globe size that is the first feature to be noticed. What is in fact noticed is the often-reduced palpebral fissure length, a flattening of the upper eyelid, and the suggestion or appearance of a reduced corneal size/diameter (Bateman and Maumenee, 1984). It is these features which when seen clinically make the appearance of microphthalmos. An eye may appear small when there is facial asymmetry, ptosis, or blepharophimosis (Warburg, 1995). A real reduction in size of the globe may occur in these situations. However, many cases of microphthalmos do not in fact have a small eye (Pallotta et al. 1998), but externally the eye does look small. Other reasons for an eye appearing small include its size relative to the other eye: it may be that the fellow eye or cornea is enlarged, such as in congenital glaucoma. The presence of other structural anomalies of the anterior segment, such as a cataract, may give the impression of a smaller eye.

So there are small eyes that are small and small eyes that are normal in size and small eyes that are big. They can only be distinguished by CT/MRI or ultrasound images. In practice, it is not possible to do this in every child with microphthalmos, nor would it be desirable. The diagnosis is therefore based on a clinical impression. This raises the very important question: how much does the size of the eye matter? Far more important than size is the presence of a structural abnormality in the eye. Later, the argument will be proposed that the term microphthalmos does not serve any useful

purpose as currently used. A definition is required for many purposes, such as documentation in congenital malformation registers. One could argue that precision is not vital here, and that as long as there is a consistent over- or underestimation, then the trends and overall changes in prevalence are still reliable. However, as will be seen in the next chapter (aetiology), precise numbers do matter. These are extremely rare congenital eye disorders, and the addition or subtraction of a single case can have a significant influence on the overall distribution or prevalence in even a large population. A major problem we are faced with is inconsistency in making the diagnosis of microphthalmos. Because of this inconsistency, it is impossible to determine from one clinician to another (or even from the same clinician) what is actually meant by microphthalmos. What has the clinician actually seen? Microphthalmos is a heterogeneous condition and, unless it is very extreme, structural eye abnormalities can be distinguished. The structural abnormalities differ from one case to another. The microphthalmos is a secondary feature and is in addition to the underlying structural defect of the eye. It may be that the reduced eye size is the result of a process common to the different structural eye conditions associated with microphthalmos. It could also be that the different structural eye abnormalities cause microphthalmos by a different mechanism.

Therefore, we are presented with no adequate clinical definition and no adequate scientific definition of microphthalmos.

Classification of microphthalmos

The most recent classification is that by Warburg (Warburg, 1993)(Table 1.1). Both phenotypic and aetiological classifications are proposed, and it is the recognition of

structural abnormality and phenotypic differences which makes the classification useful. Phenotype and structural abnormality is a key topic to which we will return in subsequent chapters. (The aetiology of microphthalmos is introduced in chapter two). The phenotypic classification includes apparent (or clinical) anophthalmos and congenital cystic eye. Simple microphthalmos (nanophthalmos), mentioned earlier, is also included (Brockhurst, 1974). Nanophthalmos is characterised by a small, typically hypermetropic eye. Axial length is usually around 16 mm, and hypermetropia of +10 dioptres is not unusual. Some of the underlying structural abnormalities include reduced corneal diameter, a disproportionately large lens and choroidal thickening. Complications can occur in adult life as a result of these structural abnormalities: angle closure glaucoma, a distinctive macular appearance of yellow pigmentation (Serrano et al. 1998), crowded optic discs, scleral thickening, uveal effusion, choroidal detachment and secondary (non-rhegmatogenous) retinal detachment. Nanophthalmos may be sporadic, as well as dominant and recessive inheritance occurring (Cross and Yoder, 1976). Nanophthalmos is a separate and distinct clinical entity from microphthalmos and, despite the wealth of literature written on the subject, clinicians have failed to recognise this. As a result, the confusion over the definition has been exacerbated.

Table 1.1: Phenotypic classification of microphthalmos (Warburg, 1993)

I Total microphthalmos
(A) Congenital cystic eye
(B) Apparent microphthalmos
(C) Simple microphthalmos (nanophthalmos)
(D) Microphthalmos with intraocular malformations
(1) Congenital cataract
(2) Anterior chamber malformations
(3) Coloboma
(a) Coloboma of the uvea
(b) Coloboma of the optic nerve
(c) Cystic coloboma
(E) Multiple ocular malformations
II Partial microphthalmos
(A) Anterior segment microphthalmos
(B) Posterior segment microphthalmos

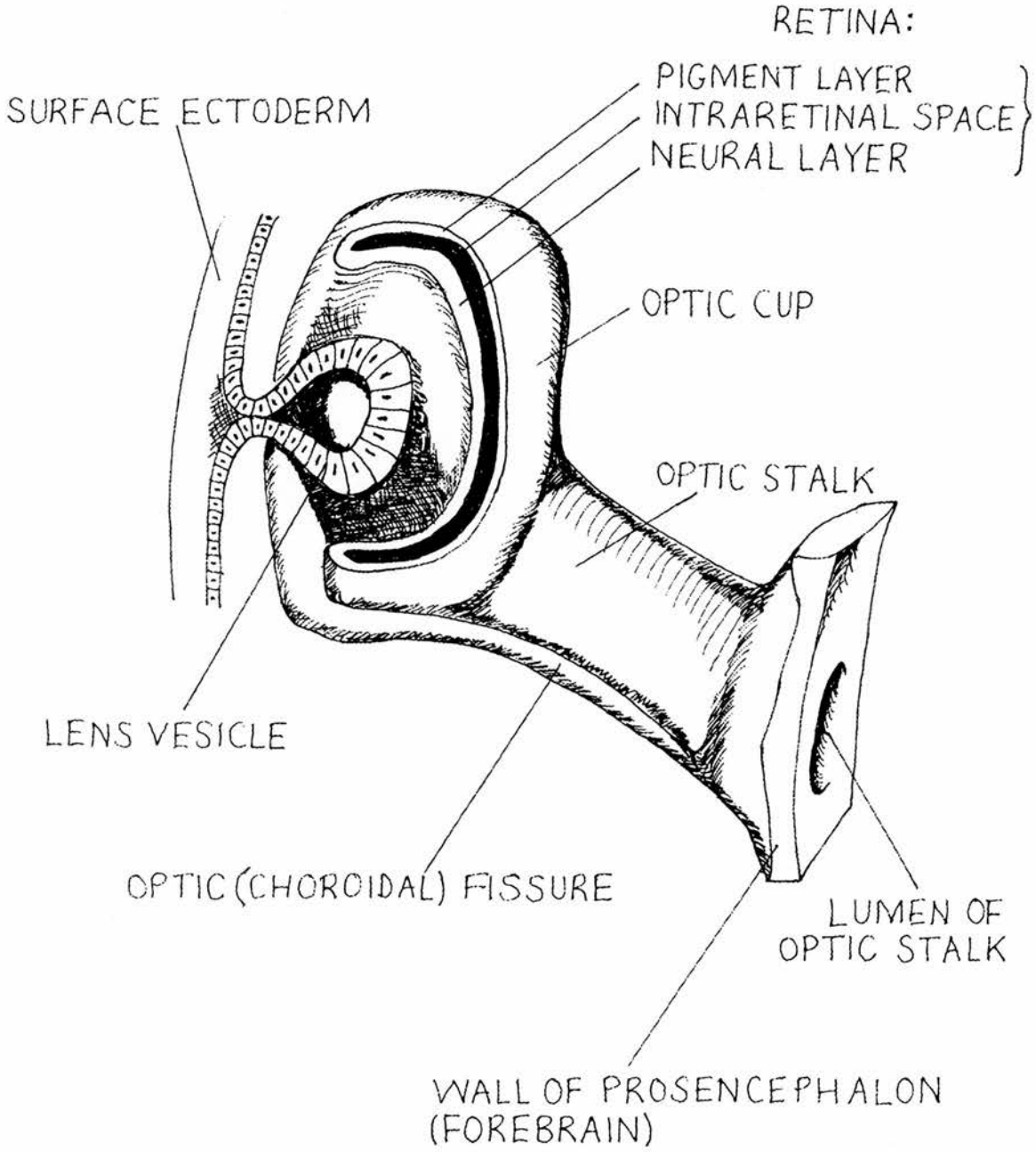
Microphthalmos is always associated with structural eye abnormalities and this has formed the basis of Warburg's phenotypic classification (Warburg, 1993). By far the commonest structural abnormality seen in microphthalmic eyes is a coloboma -more specifically, a uveal coloboma (Warburg, 1993). So significant is the occurrence of coloboma in microphthalmos that Bateman's classification considers microphthalmos as colobomatous or non-colobomatous. Warburg includes colobomatous microphthalmos as a subgroup of microphthalmos with intraocular malformations.

The literal meaning of coloboma is 'mutilation'. To understand the mechanism of how this structural ocular defect is thought to arise, we must again return to embryology and early eye development.

As described above, when the embryo is around the age of 24–27 days (3–4.5 mm stage) each optic vesicle will normally invaginate to form an *optic cup* by the anterior part of the vesicle lying in apposition to the posterior part (Bron et al. 1997). This anterior layer of the vesicle will go on to form the neuroretina, and is apposed to the posterior part (the future RPE). The optic vesicle remains in contact with the surface ectoderm and continues to be connected with the forebrain by a constriction, the *optic stalk*. The cavity of the diencephalon, which will later form the third ventricle, is continuous with that of the optic stalk. At about 27 days of gestation (4.0–4.5 mm) the surface ectoderm lying over the optic vesicle thickens to form the lens placode, which gradually invaginates to form the lens vesicle. Eventually, the lens vesicle separates from the surface ectoderm.

With the development of the optic vesicles into optic cups up to day 28 (7.6–7.8 mm stage) there is differential growth and movement of the cells of the optic vesicle. This differential growth causes the temporal and lower walls of the optic vesicle to move inward against the upper and posterior walls. The two laterally growing edges of the cup and stalk meet ventrally, with a resulting fissure, known as the *embryonic, fetal, choroidal,* or *optic fissure* (Figure 1.0). The precise events involved in closure of the optic fissure, which is such a critical event and crucial to normal eye development, have not been documented as well as one would expect. This is partly because of the early timing of the event. The result of errors in closure of the optic fissure provide explanations for much of the pathogenesis of some of the congenital eye conditions to be studied and discussed below and in later chapters: coloboma, microphthalmos with cyst, and orbital cysts. Studies of animal models have provided some insight into the

Figure 1.0: Diagrammatic representation of the development of the optic cup and optic (choroidal) fissure in a 7.5 mm human embryo. The optic vesicle and cup have been partly cut away and the lens vesicle is sectioned.



mechanism of closure (Geeraets, 1976). Cells at the margin of the fissure become inverted into the fissure and fuse after multiple appositional contacts develop. At the same time as fusion, there is programmed cell death at the sites of fusion. The closure of the optic fissure is generally accepted to occur at 5–6 weeks of gestation (10–13 mm stage) (Pagon, 1981). This timing can be considered in more detail, i.e. does closure begin at five weeks and end at six weeks, or does the timing refer to the range of onset of the time of closure? How long the process of closure actually takes may be minutes or days. It is also relevant to consider the actual mechanism of closure of the fissure with regard to direction and orientation. This is of great importance when considering the range of pathology that is thought to arise as a direct result of errors in the process of optic fissure closure. The lips of the fissure/cleft were once believed to meet in the central part (Duke-Elder, 1964), followed by anterior or posterior closure, a type of 'zipping up'. It is almost certain that the lips of the fissure meet at any point from anterior to posterior, that the point of initial closure is variable (Suzuki et al. 1988), and that the process of fusion is simultaneous. This, as we shall see later, explains the variety of pathologies that occur from the anterior end of the fissure (the iris and anterior segment of the eye) to the posterior end (the retina and optic disc).

In summary, the closure of the optic fissure probably takes place over a period of 1–2 days, commencing at some point between five and six weeks' gestation. This wide range of a number of critical events in eye development gives a huge scope for a variety of errors to occur during this process, with an equally wide range of pathological outcomes.

Microphthalmos with cyst

Having understood the process of normal optic fissure closure, we can now return to one of the variants of microphthalmos often mentioned, microphthalmos with cyst. The mechanism by which this occurs is quite different from the congenital cystic eye described earlier, although similarities in terminology, aetiology, and clinical appearance have lead to confusion.

Microphthalmos with cyst (microphthalmos with orbital cyst, orbito-palpebral cyst, cystic coloboma) occurs as a result of faulty closure of the optic fissure (Duke-Elder, 1964).

Clinically, the appearance of microphthalmos with cyst is very variable (Weiss et al. 1985). Typically, there is distension of the (usually lower) eyelid with cystic masses that usually transilluminate. The cyst may also be small and not detectable clinically, and is usually located inferonasally to the globe, although orientation is not always easy to determine. In contrast to congenital cystic eyeball, a small displaced globe will be present in addition to the cyst, and the two communicate, although the channel of communication may have closed off. The eye is usually only apparent after imaging or surgical exploration. With large cysts, ultrasound or CT scan usually detects a microphthalmic globe. The microphthalmic eye, if it is large enough to visualise detail within it, will often have a number of different structural abnormalities, most significantly a coloboma of the uvea (see coloboma page 22). Other abnormalities described frequently are corneal opacification and cataract. Within the microphthalmic globe, calcification may be seen (Porges et al. 1992). Rudimentary optic nerves are

present. The pathology of the cyst is a cellular lining of primitive retinal cells and RPE (Foxman and Cameron, 1984).

The pathophysiology of microphthalmos with cyst is a failure of normal closure of the optic fissure (Waring et al. 1976). In simple terms, the lips of the optic fissure are composed of an inner layer of neuroectoderm (which will later become the neuroretina) and an outer layer of neuroectoderm (later to become the RPE). Between these two layers of neuroectoderm, which make up the optic cup, is a potential space. The improper fusion of the lips of the optic fissure results in a number of complex abnormalities. If the optic fissure fails to close, a defect remains (a coloboma) that is lined only by the immature retina and retinal pigment epithelium. The outer layer of the coloboma will consist of a layer of sclera, which will be present to varying degrees and is ectatic. It is this scleral ectasia and herniation that produces the cystic structure, the cyst being in continuity with the contents of the globe. A second mechanism of cyst formation is if the neuroectoderm at the lips of the fissure proliferates: cysts can form at one or both edges of the fissure, which is continuous with the space between the two layers of the optic cup. Such a cyst can also originate from the optic nerve.

That the eye in microphthalmos with cyst is microphthalmic is probably secondary to the presence of the attached cyst. Possibilities are that the large cyst has inhibited growth of the eye, or that the cyst is a direct consequence of the eye being small due to another cause, or that the same factor producing the cyst has produced a microphthalmic eye.

COLOBOMA

The significance of uveal colobomas and defects of closure of the optic fissure to this discussion of microphthalmos is vital. That the pathophysiology of extreme microphthalmos and anophthalmos is a result of events taking place in the early development of the eye has already been demonstrated. It is tempting to separate the subject of extreme microphthalmos and anophthalmos (which blur into one another) from coloboma on the grounds of aetiology (chapter two) or pathophysiology. However, we have already seen that microphthalmos and coloboma frequently occur in the same eye (Gopal et al. 1996). To this we must add the huge number of descriptions in the literature of coloboma occurring in one eye and anophthalmos or microphthalmos (with or without coloboma) occurring in the fellow eye of the same individual or affecting different members of the same family (Porges et al. 1992). This suggests that the pathological mechanisms and aetiology (chapter two) are fundamentally similar, and that the vast array of differences that exist can be explained by subtle changes in the timing of events during ocular embryogenesis. Therefore, no discussion of anophthalmos or microphthalmos would be complete without a description of coloboma.

Microphthalmos with cyst illustrates clearly how one particular variety of microphthalmos occurs as a consequence of a failure of closure of the optic fissure. Here, we see that the primary defect or phenotype is probably the coloboma, and that the microphthalmos is most likely to be a consequence of this malformation. What then of colobomas occurring without microphthalmos? Is there something that distinguishes colobomatous microphthalmos from coloboma without microphthalmos?

There does not appear to be any difference in the basic pathophysiology of these two broad categories. Certainly, 'cystic coloboma' of the choroid exists in eyes of 'normal' size, these types of cyst often being referred to as staphylomas (Warburg, 1993). The condition occurs by the same mechanism as microphthalmos with cyst, but for a change in terminology. Here, the emphasis and significance is (appropriately) placed on the presence of the coloboma, the size of the eye not being a notable feature. That the eye is 'normal' size is debatable since a common outcome of this structural abnormality is an eye of increased axial length: *macrophthalmia* (Bateman and Maumenee, 1984).

As stated earlier, the term coloboma indicates a condition in which a portion of a structure of the eye is lacking (Duke-Elder, 1964). In ophthalmology the word refers to any notch, gap, defect, hole or fissure in any ocular structure. Consequently, the word has developed a broad meaning that often lacks specificity.

There are many types of coloboma, only some of which are relevant to this discussion. The colobomas with which we are concerned are those due to or at least considered to be caused by defects resulting from faulty closure of the optic fissure. Warburg (Warburg, 1993) classified coloboma according to phenotype, but this does not correlate with the underlying pathophysiology or the aetiology (Table 1.2).

Table 1.2: Phenotypic classification of coloboma, from Warburg 1993. Classification of microphthalmos and coloboma (Warburg, 1993)

I Typical
(A) Coloboma of the iris
(a) Complete (keyhole)
(b) Partial
(1) Peripheral
(2) Notch in the pupil
(3) Pigment epithelium defect
(4) Heterochromia of the iris
(B) Coloboma of the ciliary body
(C) Coloboma of the choroid
(1) (a) Partial (b) Complete
(2) (a) With (b) Without macular involvement
(3) (a) Cystic (b) Non-cystic
(D) Coloboma of the optic nerve
(1) Typical
(a) Cystic (b) Non-cystic
(2) Special
(a) Pits of the optic nerve
(b) Pedler coloboma
(c) Morning glory
(d) Optic disc hypoplasia
II Atypical
Coloboma outside the optic fissure
III Macular 'coloboma'

Considering those colobomas not relevant to this discussion first, these would be those regarded as 'special' colobomas of the optic nerve: pits of the optic nerve, Pedler coloboma, morning glory disc, and optic nerve hypoplasia. Pits are crater-like

depressions of the optic nerve head, situated mostly at the temporal side (Corbett et al. 1980). The difference between an optic pit and an optic disc (nerve head) coloboma can be quite subtle at times, and this has caused confusion in the literature with regard to the correct naming and description of these abnormalities when seen (Sugar, 1967). Pedler coloboma (Pedler, 1998) is an abnormality of the optic nerve head that gives the impression of a tumour. There is elevation and distortion of the adjacent peripapillary retina. The underlying defect may be in the formation of the posterior sclera. Equally rare is the morning glory malformation (Pollock, 1987). Here, the optic nerve head is funnel-shaped, contains a dot of white tissue in its centre, and is surrounded by an elevated annulus of chorioretinal pigment disturbance. The retinal vessels appear as multiple narrow branches at the edge of the disc.

None of these congenital defects of the optic nerve occur with anophthalmos, microphthalmos, or true uveal coloboma (Pollock, 1987), or in the fellow eye of a patient affected with one of these abnormalities.

Another form of coloboma that appears in the literature is the 'macular coloboma'. This refers to a wide range of abnormalities that are characterised by a pigmented lesion or malformation at the anatomical macula (Warburg, 1993). They are not colobomas caused by or resulting from defects in closure of the optic fissure.

The colobomas that remain to be discussed are those thought to arise as a result of defective closure of the optic fissure. However, we shall see that the mechanisms by which some of these abnormalities arise are not entirely clear.

Uveal coloboma arises from an abnormal closure of the optic fissure and the subject has been well reviewed (Pagon, 1981). Therefore, the defect is likely to occur around

the stage of closure of the optic fissure. As has already been discussed, this is likely to be at five to six weeks' gestation. However, what is not known is whether the factors affecting fissure closure, which are not understood, take effect before or during the period of abnormal closure. We will return to the subject of causes in chapter two. For example, it is possible that in some circumstances where abnormalities arise, there is a delayed onset of fissure closure, a prolonged process of closure, or an accelerated or premature closure. All of these mechanisms would produce different structural abnormalities.

It was Duke-Elder (Duke-Elder, 1964) who classified colobomas into *typical* and *atypical*. Typical coloboma referred to those colobomas due to defective closure of the optic fissure, whereas atypical included all those ocular malformations of a different (usually unknown) aetiology (Brodsky et al. 1988). However, we shall see that the pathogenesis of some of the atypical colobomas might best be described or explained in terms of optic fissure defects (Duke-Elder, 1964). The term 'typical coloboma' has not been applied consistently. Warburg's 1993 classification sub-classifies the special colobomas of the optic nerve (pits, Pedler coloboma, morning glory disc, optic nerve hypoplasia) under the *typical* (defective optic fissure closure) category although, as discussed above, the pathophysiology of these colobomas is not explained (Warburg, 1993).

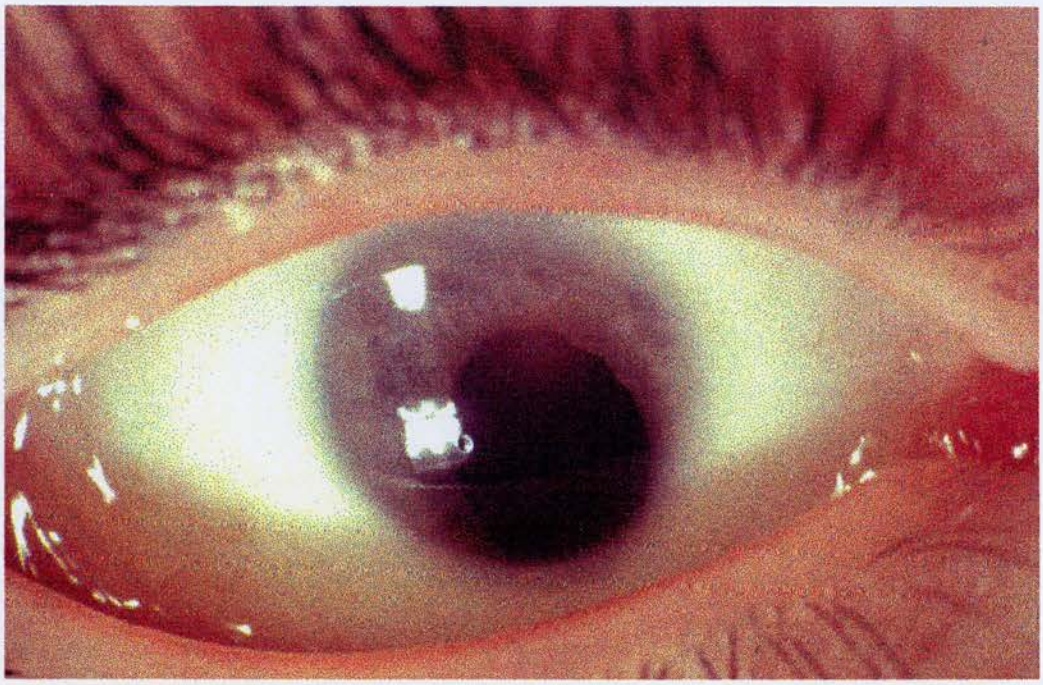
The optic fissure runs from the anterior segment of the eye and extends posteriorly along the infero-nasal aspect of the eye as far as the optic disc, continuing as a fissure/cleft of the optic stalk. A typical coloboma may therefore affect any of the

structures along this line. Colobomas affecting all of the aforementioned structures have been called *complete*, and those which are less extensive *partial*.

Iris colobomas are shown in Figure 1.3 and Figure 1.4. The usual position is inferior or nasal, which is consistent with the site of the optic fissure. The defect may extend to the limbus, where it varies in width, and when this occurs it almost invariably continues onto the retina.

Figure 1.3: Left iris coloboma

Figure 1.4: Right iris coloboma with clinical microphthalmos



Coloboma of the retina (or fundus coloboma) is a misnomer, since the coloboma is not just of the retina but refers to the absence or disruption of the retina and RPE that overlies the abnormal outer scleral coat.

The closure of the optic fissure was described above and errors in this process can lead to colobomas with cysts, by a mechanism similar to that in microphthalmos with cyst.

It is the variable nature of coloboma that has caused so many other conditions to be incorrectly labelled as coloboma and being confused with uveal coloboma. An example of this is the iris defect that occurs in Rieger's anomaly (Pearce and Kerr, 1965), in which full thickness iris defects interrupt the pupillary margin. Similarly, the absence of large sectors of the iris in aniridia has led to the use of the term coloboma (Hittner et al. 1980). The mechanism of such defects is not well understood, but they

are not considered to be related to closure of the optic fissure. Another congenital iris abnormality, which can be confused with an iris coloboma at first glance, is corectopia (Atkinson et al. 1994).

Before describing the pathology of coloboma, a brief mention of normal iris formation is necessary.

Three successive waves of neural crest cells contribute to the formation of the anterior segment of the developing eye (Bron et al. 1997). It is the second wave of neural crest cells, as mesenchymal cells, which will form the iris stroma and pupillary membrane. The lens vesicle separates from the surface ectoderm after 33 days and it is at this stage that an anterior chamber is first visible. The iris is the anterior part of the uveal tract, which is made up of the ciliary body and choroid more posteriorly. The uveal tract is derived from the neural crest cells, neuroectoderm and vascular channels. Following the mesenchymal tissue, the neuroectoderm of the optic cup margin's anterior layer will develop into pupillary sphincter and dilator muscles. The posterior layer of the iris, the posterior pigmented epithelium, is a continuation of the neuroectoderm that forms the neural retina.

In iris coloboma, the abnormality arises due to failure of optic fissure closure. Normal iris development is dependent on complete closure of the fissure and is associated with the formation of the anterior portion of the tunica vasculosa lentis, the vascular channels of which arise at about the sixth week of gestation from an annular vessel that encircles the rim of the optic cup. These vascular channels extend into the mesenchyme covering the anterior surface of the lens.

Duke-Elder (Duke-Elder, 1964) favoured a vascular event being the cause of coloboma. Vascular events are an essential component of iris development and could explain the presence of isolated iris coloboma arising in the absence of any retinal involvement. Conversely, defects in fissure closure could cause abnormal iris vascularisation leading to an iris coloboma.

Due to the fact that an iris coloboma represents the absence of tissue, little is understood about the histopathology. Clinically, a full thickness iris defect, which includes the pupil margin, is seen. The defect may extend partly to the limbus, and may be bridged by strands of iris stromal tissue. A persistent pupillary membrane is not uncommon (Pagon, 1981). It is possible that stromal defects of the iris in the infero-nasal position are minimal expressions of iris coloboma (Pagon, 1981).

A further type of coloboma, 'atypical coloboma', needs to be discussed, as its mechanism is so poorly understood. It refers to iris defects not located in the 'typical' infero-nasal position. These are not generally considered to be due to faulty closure of the embryonic fissure. Examples include the 'coloboma' of Rieger's anomaly, and some of the pupil abnormalities in aniridia. We shall return to this later (chapter six classification of coloboma), as many aspects of atypical coloboma are not easily explained. The site of the optic fissure is not absolutely constant, with the optic vesicle being invaginated from below and slightly medially. It is probable that the line of the fissure can become altered by unequal growth of the two sides of the retina. Therefore, it is possible that some so-called atypical colobomas may be typical, that is, due to defective optic fissure closure. Other complex factors could stimulate the

fissure to appear in a different position, or produce an accessory fissure (Duke-Elder, 1964).

Retinal colobomas (fundus colobomas, chorioretinal colobomas) have a very broad spectrum of clinical appearances (Gopal et al. 1996). The first indication of the existence of a retinal coloboma is the presence of a typical iris coloboma, although this is not always present. Other external features may be nystagmus or a squint, both of which are direct consequences of the visual impairment (see causes below), a 'white' fundus reflex, caused by the reflection of light from the sclera that is deficient in its pigmented layers. It is the retinal appearance that is so variable. An attempt has been made to classify this according to the degree of optic disc involvement, itself very variable (Gopal et al. 1996). Retinal colobomas usually appear as white defects in the inferior or nasal retina. Almost the entire posterior pole of the eye may be involved and the retinal coloboma may be accompanied by varying degrees of optic disc involvement (see optic disc coloboma below). The edge of the defect is well demarcated, bulging posteriorly to varying degrees, forming a staphyloma (bulge of the sclera) when severe (Weiss et al. 1985). Sometimes at the edge there is a variable amount of irregular pigment from the atrophic RPE. The floor of the coloboma is irregular. Retinal vessels may pass over the coloboma, but are usually disturbed and irregular in distribution. They frequently disappear from view as they curve over the edges of the coloboma onto the area of unaffected (or at least not colobomatous in appearance) retina. This unaffected retina, often described as normal, is not normal in the strict sense. The retinal vascular pattern is irregular, often with straightening of the vessels, and the macula or anatomical fovea, when not directly involved by the

coloboma, is often indistinct. Within the coloboma, choroidal vessels may be seen. The coloboma often extends anteriorly to involve the ciliary body and so the anterior edge is not seen. The width of the coloboma anteriorly does not correspond in size with the width or exact position of any iris coloboma defect, if this is present.

Much milder and less extensive forms of coloboma may be seen, where the optic disc and macula are not involved and the anatomy of the posterior pole and its vascular pattern is essentially normal. The coloboma begins some distance from the optic disc and extends anteriorly. It may appear as a localised defect anywhere along the line of the (presumed) optic fissure.

As stated earlier, colobomas of the retina are due to primary pathology in both the neural retina and retinal pigment epithelium that overlies the abnormal outer scleral coat. The sclera is thinned by the loss of its inner layers, hence the ectasia. The choroid is absent, only the larger choroidal vessels sometimes being seen. The neural retina is absent, or atrophic, thinned and stretched to varying degrees to form a membranous sheet (Duke-Elder, 1964). The abnormal and ectatic sclera is important to note, as it is this which may give rise to an irregular profile or shape of the eye on ultrasound, CT or MRI (Fledelius, 1996), or produce an unexpectedly long axial length (Bateman and Maumenee, 1984) or axial myopia (see below).

Before returning to the subject of microphthalmos, it is worth considering the causes of visual impairment that occur as a result of coloboma. This will enhance our understanding of coloboma and emphasise its significance as a structural abnormality that occurs in spite of microphthalmos (Duke-Elder, 1964).

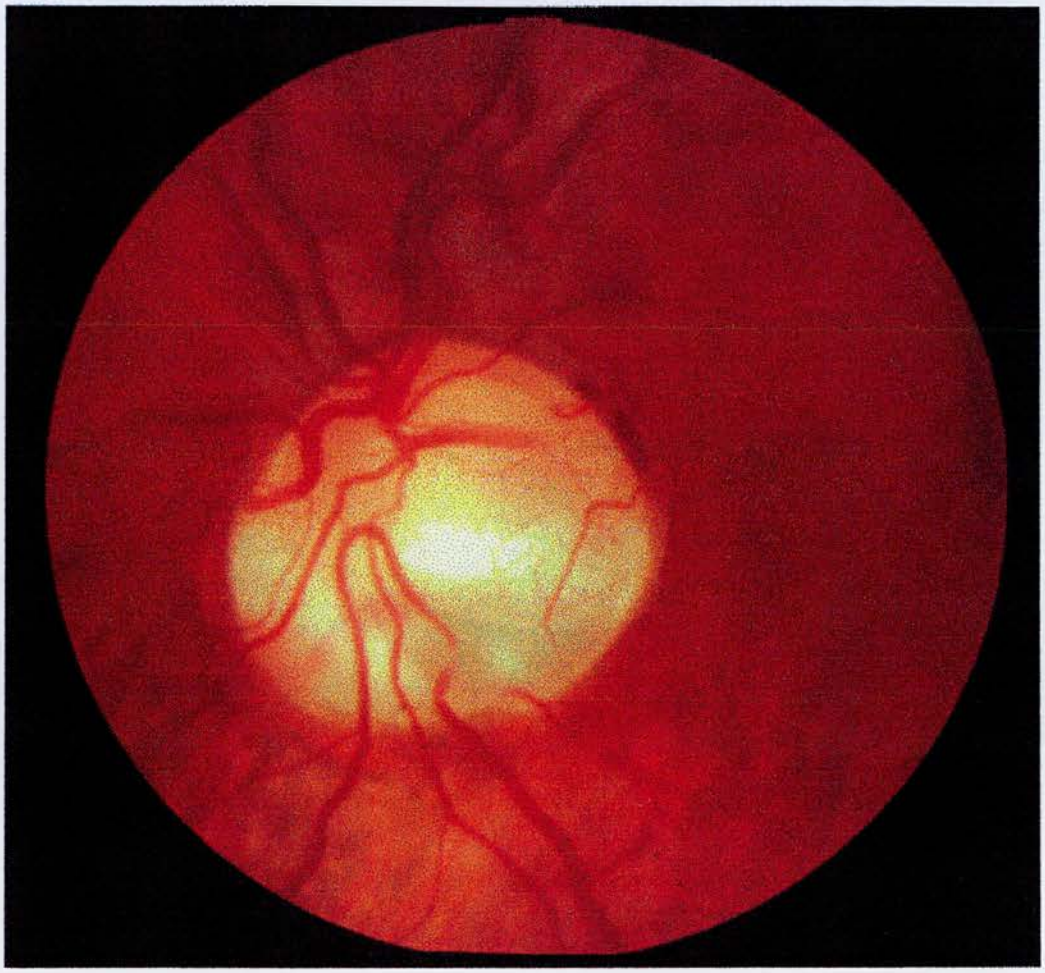
That the cornea is often reduced in size has been established. One effect of this can be to increase the curvature and cause myopia or astigmatism, which in turn may lead to amblyopia if uncorrected. Cataract or lens opacities do occur with colobomas, but they are not usually visually significant. Mild photophobia often occurs with iris coloboma due to an inability of the pupil to constrict in the absent segment. The retinal involvement ranges from a small to a large field defect, and retinal detachments do occur in a small but significant number of cases (Daufenbach et al. 1998). Involvement of the anatomical macula and/or fovea leads to impaired acuity, but the precise effect on vision is not easy to predict (Olsen et al. 1996). A staphyloma or ectasia may cause axial myopia. Optic nerve involvement probably causes significant visual impairment, but this is often difficult to assess since it is often present in conjunction with the other abnormalities described. Recently, Brodsky (Brodsky, 1999) has demonstrated hypoplasia of the intracranial optic nerve on MRI in patients with retinochoroidal coloboma.

Optic disc coloboma (optic nerve head coloboma, optic nerve coloboma, coloboma of the optic nerve entrance), Figure 1.5

This requires separate discussion. As stated above, some colobomas are probably not related to defective optic fissure closure and some of these were described briefly. However, the inclusion of these congenital structural eye anomalies under the broad and non-specific heading 'coloboma' is what has led to such difficulty in trying to establish the aetiology (genetic and/or environmental) of these conditions. We will see

(chapter two) that efforts to establish a cause have been hampered by this loose terminology and lack of definition.

Figure 1.5: Left optic nerve head coloboma

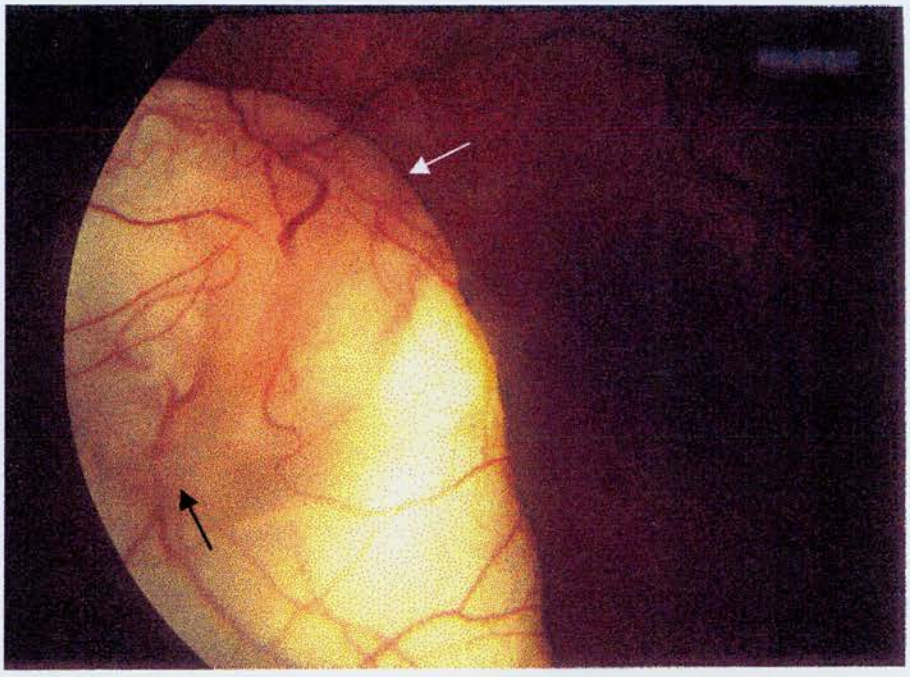


Optic disc coloboma is as variable in its manifestations as anophthalmos or microphthalmos. Furthermore, unlike the vast majority of iris and retinal colobomas, it is difficult to attribute their presence to defective optic fissure closure or to another mechanism. The pathophysiological cause of pure (isolated) optic disc coloboma is not well understood, although it would seem to be a defect in closure or formation of

the head of the optic nerve and/or along the optic groove, manifesting itself clinically as a structural abnormality at the optic nerve head (Savell and Cook, 1976).

For those optic disc colobomas which occur in eyes already having an iris and/or retinal coloboma it is easy to explain the mechanism as being due to defective optic fissure closure. Such optic disc colobomas are seen to be within or are adjacent to retinal colobomas (Figure 1.6) (Gopal et al. 1996). The colobomas vary considerably in clinical appearance and are not easily classified (Duke-Elder, 1964): the defect is usually larger than a normal optic disc, may occupy almost the entire posterior pole, and is oval, round or irregular in shape. Within the white colobomatous area the optic disc, often distorted or tilted and sometimes its typical pink colour, may be seen. In other cases, no recognisable disc is apparent. The disc may be excavated, usually has no central blood vessels, and the blood vessels that do emerge are displaced to the edge of the colobomatous area and tortuous in appearance. Hyaloid remnants may appear at the optic disc. Cases of crescented myopic disc or tilted disc are frequently (incorrectly) labelled as colobomatous. It is important to realise that the optic disc may be within the retinal/fundus coloboma, but normal in appearance when inspected closely.

Figure 1.6: Left retinal coloboma involving the optic disc. The edge of the coloboma is indicated with a white arrow and the origin of the optic nerve by a black arrow



In isolated optic disc coloboma, the clinically gross structural abnormalities are confined to the optic nerve itself, the nerve head appearing to be excavated, often quite deeply. The entire disc area may be involved, with only a thin rim of neural tissue (Savell and Cook, 1976). Comparisons with deep glaucomatous cupping have justifiably been made (Gopal et al. 1996).

Having introduced and discussed the subject of colobomas, it is time to return to the classification of microphthalmos according to Warburg (Warburg, 1993). (See page 17 and Table 1.1: Warburg's phenotypic classification of microphthalmos.) Here, 'microphthalmos with intraocular malformations' is given a separate category. It is this category of microphthalmos which encompasses such a broad phenotypic spectrum, so extensive are the number of congenital eye conditions which have been

described as belonging to this category (Table 1.3). The result of this is that the term microphthalmos carries no specificity, and can include several eye conditions that are clearly not of the same pathological aetiology.

Table 1.3: Congenital ocular malformations associated with microphthalmos.

Congenital eye anomaly	Reference
Anterior segment dysgenesis	(Ghose et al. 1991)
Cataract	(Walpole et al. 1990)
Corneal opacification/cloudy corneas	(Siber, 1984)
Peters' Anomaly	(Traboulsi and Maumenee, 1992)
Persistent Hyperplastic Primary Vitreous	(Haddad et al. 1978)
Retinal folds	(Young et al. 1987)
Retinopathy of Prematurity	(Kelly and Fielder, 1987)
Sclerocornea	(Bessant et al. 1998)

All of the conditions in this category of microphthalmos with other ocular malformations have abnormalities which would be better described with the primary diagnoses/phenotypes in their own right, as attaching the term microphthalmos conveys no further information of use other than that the eye appears to be small (which may or may not be true). A real or apparent reduction in corneal size has already been mentioned as being highly suggestive of a small globe. It would be preferable if the clinically descriptive term was dropped completely or (since it would be too much for this drastic change in perception to occur overnight without great

disruption) at least appended to these clinical conditions, e.g. 'Peters' anomaly *with* microphthalmos'.

Colobomatous microphthalmos is not like the above tabulated ocular malformations, since it occurs within individuals having anophthalmos in the fellow socket (Pagon, 1981), and also within different affected members of the same family or pedigree (McMillan, 1921). The weight of evidence is that colobomas resulting from defects of the optic fissure are related to anophthalmos by a similar aetiology or pathophysiology (Bateman, 1984), probably acting at different times.

So far, discussion of the classification of microphthalmos has centred on Warburg's classification system (Warburg, 1993). Duke-Elder (Duke-Elder, 1964) used the term 'complicated microphthalmos' to describe the heterogeneous group of congenital ocular malformations in which microphthalmos is a feature (Table 1.3). Bateman (Bateman, 1984) categorised microphthalmos as colobomatous or non-colobomatous. Like these other classifications, François (Francois et al. 1983) recognised the significance of colobomatous microphthalmos as a separate group.

Cryptophthalmos

This unusual and rare condition is often mentioned in discussions of anophthalmos or microphthalmos. Cryptophthalmos is a term used to describe an eye covered with continuous sheets of skin extending from forehead to cheeks. There are three ocular variants of cryptophthalmos, as well as the occasional coexistence of cryptophthalmos with specific systemic features: cryptophthalmos syndrome, François syndrome or

Fraser syndrome. In the first variant of cryptophthalmos, *complete cryptophthalmos*, the lids are replaced by a sheet of skin running from forehead to cheek with absence or poor development of the eyebrow. The covered globe gives rise to an elevation in the overlying skin, which is adherent to the underlying cornea. In *incomplete cryptophthalmos*, rudimentary lids are present with a palpebral aperture of about one-third of normal length. The globe is almost completely covered by skin. In the third form of cryptophthalmos, *abortive cryptophthalmos* or *congenital symblepharon*, the upper lid does not have a defined margin and covers and adheres to most of the cornea. There is no upper lid punctum or conjunctival fornix (Walton et al. 1990).

Microphthalmos or a small globe is said to occur sometimes in all three variants, although closer inspection of several case reports (Thomas et al. 1986; Brazier et al. 1986) does not bear this out. It seems reasonable to assume that detailed examination of a globe covered by skin has not been possible, and that the narrow palpebral aperture in many cases has given the impression of a small eye (see page 14). Cryptophthalmos arises because of a failure of lid fold development: the ectoderm overlying the cornea is converted into skin and no conjunctival sac forms. The association between cryptophthalmos and multiple congenital malformations such as syndactyly, renal agenesis and abnormal genitalia has been well reviewed by Thomas (Thomas et al. 1986). Many cases of cryptophthalmos seem to have an autosomal recessive mode of inheritance (Thomas et al. 1986).

Summary, chapter one

- Anophthalmos and microphthalmos have not been satisfactorily defined and are not easy to define.
- The current definition of microphthalmos, based on eye (globe) size, is inadequate and eye size cannot be used to define microphthalmos.
- Anophthalmos and microphthalmos are quite distinct structurally, but are also very difficult to separate.
- There is a continuous spectrum from true anophthalmos to mild microphthalmos.
- Microphthalmos is not a useful term and conveys no specific meaning.
- Colobomas resulting from aberrant optic fissure closure occur in normal-sized eyes and microphthalmic eyes. Emphasis here is placed on the structural abnormality (coloboma), not the eye size.
- Anophthalmos and uveal colobomas result from errors in the early stages of eye development. It is difficult to narrow this period down precisely, there being a relatively large 'window' in which things can go wrong (gestation weeks three to eight approximately).

We are still faced with the problem of meaningful names for the structural eye anomalies discussed above. Clearly, anophthalmos and mild microphthalmos (at opposing ends of the spectrum) are very different. Clinical anophthalmos remains a clinically useful and specific term. However, microphthalmos, severe microphthalmos, extreme microphthalmos and mild microphthalmos are not useful, as these terms can attach a different meaning to the same structural congenital eye abnormality (or the

same meaning to different structural congenital eye abnormalities). It is the structural ocular abnormality, or phenotype, which carries significance and meaning, and it is this term that should be used. The term anophthalmos, although varying in timing and possibly in cause, cannot be refined any further as a clinical ocular phenotype, as no structural abnormality is present to be described. This seems an obvious point, but is well worth stating.

As we will see in the next chapters, appropriate terminology would allow progress to be made in our understanding of the aetiology of these distressing eye malformations.

From the discussion within this section, in which a case has been put forward that anophthalmos and microphthalmos are almost impossible to define, we are now faced with the difficult problem of establishing aetiology for these very same and loosely defined congenital eye anomalies. This is the subject of chapter two, in which the current evidence for a genetic and/or environmental aetiology is presented.

CHAPTER TWO

ANOPHTHALMOS/MICROPHTHALMOS: GENETIC OR ENVIRONMENTAL?

'Misery loves company.'

Zeiter, in a discussion about the frequent occurrence of extraocular abnormalities in microphthalmos (Zeiter, 1963). This is a topic that concerns much of this chapter.

Summary

Anophthalmos/microphthalmos has features that suggest both a genetic and an environmental aetiology. The failure to define adequately these conditions has made it increasingly difficult to determine a cause.

In chapter one, it was proposed that anophthalmos and microphthalmos have no clear definition. The problems that this absence of a satisfactory definition has presented to clinicians from a wide range of specialities, as well as to scientists, will be illustrated. Lack of definition and specificity is a subject that will recur.

The discussion that follows summarises the evidence for anophthalmos and microphthalmos being genetic or environmental in aetiology.

Epidemiology of anophthalmos/microphthalmos

Before presenting some of the genetic and environmental evidence, it is useful to consider how the birth prevalence and incidence of these congenital eye malformations is estimated. The quality of data is very poor for a number of reasons:

1. Anophthalmos/microphthalmos has never been properly defined (Dolk et al. 1998). There is a wide use (and abuse) of these terms by both specialists and non-specialists.
2. The diagnosis may not be apparent within the time period within which notification must be made, usually the first ten days of life (Working Group of the Registrar General's Medical Advisory Committee, 1995).
3. Late diagnoses/notifications cannot be included (Working Group of the Registrar General's Medical Advisory Committee, 1995).
4. Notification is voluntary (Working Group of the Registrar General's Medical Advisory Committee, 1995).

5. The diagnosis may be seen but not documented if the 'cause' is presumed to be known or understood. This occurs when it is part of a well-recognised syndrome (Bianchi et al. 1994). An example of this is trisomy 13 (below).

At best, therefore, one can only consider current data to be crude estimates. In itself, this would not be such a problem, as one could look at trends and fluctuations. However, there is no consistent pattern of over- or under-diagnosis from one clinician to another, or within a geographical region. Case ascertainment is highly inconsistent. The rarity of these conditions (as few as 1 in 100,000 live births for anophthalmos) means that a single case can have a profound effect on the overall prevalence for a country.

The congenital malformations (anomalies) registers, in their current forms in Scotland (Scottish Morbidity Record, SMR) and England and Wales (Office of Population Censuses and Surveys, OPCS), are not suited to some of the uses for which the data is being applied (Working Group of the Registrar General's Medical Advisory Committee, 1995). These registers were originally established for the purpose of surveillance and rapid reporting of congenital malformations. This came about in 1964 following the thalidomide epidemic (Working Group of the Registrar General's Medical Advisory Committee, 1995; Office of Population Censuses and Surveys, 1995). For many reasons, including some of those listed above and specific to eye malformations, these registers are unsuitable for detailed analysis.

Some of the data are summarised in Table 2.1. Methods of data acquisition vary widely from one country to another, as does the inclusion and definition of, for example, stillborns, neonatal deaths and therapeutic abortions. Some data are

presented for coloboma, which, as we have seen, overlaps extensively with the category of microphthalmos.

Table 2.1: Birth prevalence of congenital anophthalmos and/or microphthalmos and iris coloboma. Birth prevalence is per 10,000 live births unless otherwise stated.

Reference	**Anophthalmos & microphthalmos combined**	**Anophthalmos**	**Microphthalmos**	**Iris coloboma**
OPCS, Wales 1976-1985*	0.3			
(Fujiki et al. 1982)			0.39	
(Clementi et al. 1992)		0.6 per 10,000 live and still births		
(Stoll et al. 1992)		0.3	1.8	0.7
(EUROCAT Working Group, 1995) (1990-94)	1.1	0.2	0.9	
(EUROCAT Working Group, 1994)	1.57	0.26	1.31	
(Bianchi et al. 1994) (Spagnolo et al. 1994)		0.35	0.83	
(Kallen et al. 1996)	1.50			
(Stoll et al. 1997)		0.23	1.7	1.4
ICBDMS 1998 (1996)**	0.26	0.08	0.18	
(Dolk et al. 1998)	1.0			

*(Welsh Office, 1985).
**(International Clearinghouse for Birth Defects Monitoring Systems, 1998).

Anophthalmos and microphthalmos are responsible for a small but significant amount of blind and partially sighted registrations (BP1 forms in Scotland, BD8 in England and Wales). However, they are not a good source of information on these conditions, since the time between diagnosis and certification (voluntary) is very variable, and only bilaterally affected children will be recorded. Between April 1990 and March 1991 in England and Wales, congenital anophthalmos was the documented cause of registration in 3 children (age 1–15). For the same group, microphthalmos was listed 11 times (Evans, 1995).

In a Japanese survey on the causes of visual handicap among schoolchildren, microphthalmos was ranked third (Fujiki et al. 1982).

Sex ratio of anophthalmos/microphthalmos

Kallen did not find any significant difference between the two sexes (Kallen et al. 1996).

Clusters of anophthalmos/microphthalmos

In recent years, much of the research involving anophthalmos/microphthalmos, whether genetic or environmental, has sprouted from the suggestion that the prevalence is increasing or that there are geographic clusters. The research on which some of this thesis is based is no exception. Certainly, it is beyond the scope of this thesis to discuss the subject of clustering at length and to provide an answer, but clustering is relevant to the subject of establishing a cause for these congenital eye anomalies.

In the early 1990s, reports began to appear in the national press (Paduano et al. 1993) that anophthalmos and/or microphthalmos were increasing in prevalence, that in parts of Scotland (Fife) and England (Lincolnshire) there were 'clusters' in time and place, and that the alleged cause of these clusters was pesticide use in the environment (Dyer, 1996). This was not the first occasion on which concern had been raised about possible clusters of anophthalmos/microphthalmos (Lenihan, 1985; Scottish Home and Health Department, 1988).

The publicity, concern and debate around this issue, which continue to the present day, highlighted the inadequacy of current congenital malformation registers in the UK (Watterson, 1993) and Europe, as no definite or rapid answers were available (Dolk and Elliott, 1993).

The presence or absence of clusters, whilst being of immense interest and a significant public health and legal issue, does not in fact alter significantly the correct approach to finding a cause for anophthalmos/microphthalmos. Rarely does cluster analysis, itself an extremely complicated science, lead to the finding of a cause (Olsen et al. 1996). Cluster analysis does remain important in addressing public concern with the possible adverse effects of environmental pollutants (Olsen et al. 1996). This is a subject that we shall return to later in this chapter.

There is no data at present that suggests that there are clusters of anophthalmos/microphthalmos in time or space. The registers that do exist are not accurate enough (see above) to detect any statistically significant variation. Increased awareness of these conditions will probably have an effect on the level of reporting, although the difference may not be measurable.

The cause of anophthalmos/microphthalmos is considered by many to be environmental. Environmental means any external agent that can cause harm to a developing foetus during the critical early stages of eye development and maternal factors (illness, drug intake). Paternal exposure to chemicals before conception is a theoretical possibility (Robaire and Hales, 1993), though a case has not been reported. Clearly, and this will become more apparent later, the complete separation of causes into environmental or genetic, if one is to believe there is more than one cause, is artificial but is convenient for the purpose of discussion. It is equally possible that these two major factors could be interacting (Kaprio, 2000). This chapter begins with the statement that there are some features of congenital anophthalmos/microphthalmos that suggest the causes are environmental and genetic. Where possible, anophthalmos and microphthalmos are discussed separately. As should be apparent from chapter one, the possible inaccuracy of these diagnoses and reporting does mean that close inspection of any clinical case report is necessary, but this may not always be possible. One can be fairly certain of what the author is conveying by the term anophthalmos, which at least is likely to be extreme microphthalmos, but microphthalmos, as we have seen, could represent any one of a large number of ocular phenotypes.

What are the 'environmental' features, that is, those characteristics that together suggest an environmental aetiology?

External factors and the environment have for a long time been considered as a possible cause of anophthalmos (Treacher Collins, 1887). The factors are as follows:

1. The sporadic nature and rarity. It is very unusual for these conditions to occur in an individual with a previous family history or with a sibling with a similar or related eye condition. Such sporadic cases have been reported many times (Treacher Collins, 1887; Brownstein et al. 1977; O'Keefe et al. 1987).
2. Frequent reports of the occurrence of these eye conditions in the presence of or with a good history of exposure to a potential toxin or illness at a critical stage of embryogenesis. This could of course be coincidental, but the rarity, particularly of anophthalmos, makes the evidence for such substances (Tables 2.2 and 2.3 page 51) as being harmful very convincing. Such reports should always be taken seriously.
3. Clusters allegedly related to environmental pollutants, pesticides being the most commonly cited (Dolk and Elliott, 1993). The scientific evidence for the toxic effect of pesticides is the effect of large doses fed experimentally to pregnant rats. A significant number of the rat embryos developed microphthalmos and some were anophthalmic, with additional brain malformations (Hoogenboom et al. 1991).
4. The fact that these eye conditions are so variable in appearance/phenotype possibly implicates an environmental cause/influence.
5. The occurrence of so many unilaterally affected individuals (Tucker et al. 1996) (but see later discussion on laterality under genetic causes).
6. The fact that anophthalmos/microphthalmos so frequently occurs in the presence of other (extraocular) congenital malformations, as unrecognisable and unexplainable new 'syndromes', with unusual constellations of physical signs or dysmorphology (Treacher Collins, 1887; Brownstein et al. 1977; O'Keefe et al. 1987). O'Keefe's series of 15 cases of clinical anophthalmos found multiple anomalies in 9 cases.

Warburg (Warburg, 1993) went as far as to classify anophthalmos/microphthalmos according to aetiology. This seems rather ambitious in view of the fact that it is not easy in the majority of cases to attribute a likely 'cause', a point which the author herself acknowledged. 'Prenatally acquired' was used for a group which included disruptions due to drugs/irradiation or maternal disease.

Table 2.2: Possible causes of anophthalmos

Maternal drug ingestion of teratogens
Thalidomide (Smithells, 1973)
Carbamazepine(Sutcliffe et al. 1998)
Retinoids (Autret et al. 1997)
Ethambutol (Perz, 1987)
LSD (Lysergic Acid Diethylamide) (Margolis and Martin, 1980)
Maternal illness
Nutritional factors
Vitamin A deficiency (Sarma, 1959)

It is not a useful exercise to discuss at length the environmental causes of microphthalmos, as it has already been made clear that microphthalmos is not a single diagnosis or recognisable phenotype but a group of great heterogeneity. However, some of the causes are listed, as the cases described may have been recognisable as clinical anophthalmos or colobomatous.

Table 2.3 Possible causes of microphthalmos

Maternal drug ingestion of teratogens
LSD (Bogdanoff et al. 1972)
Thalidomide (Smithells, 1973)
Carbamazepine (Sutcliffe et al. 1998)
Maternal exposure during pregnancy
Oxydemeton-methyl (organophosphate) (Romero et al. 1989)
Chlorpyrifos (Dursban) (Sherman, 1996)
Maternal illness/intrauterine infection
Rubella (Gregg, 1941)
Cytomegalovirus (Miklos and Orban, 1964; Tarkkanen et al. 1972)
Varicella (Kriss et al. 1997)
Toxoplasmosis (Kriss et al. 1997)
Parvovirus B19 (Weiland et al. 1987)

Some of the more familiar established 'causes' of microphthalmos warrant discussion, as many of these are based on questionable data or assumptions which have continued to be passed on. For example, it has long been accepted that microphthalmos is a feature of the fetal alcohol syndrome. However, it is the typical facies of short palpebral fissures that gives the impression of microphthalmos. The ptosis that can occur is also a feature that can give the misleading impression of microphthalmos (Stromland, 1987).

Congenital TORCH infection, an acronym for Toxoplasma, Rubella, Cytomegalovirus and Herpes virus, is one of the most frequently stated causes. Again, closer inspection of the literature does not demonstrate this. The eye abnormalities associated with most of these infections are quite specific, and the pathology is usually very recognisable.

It was Gregg (Gregg, 1941) who summarised the characteristic ocular features of congenital rubella syndrome (typical cataract, corneal haze, pigmentary retinopathy, *mild microphthalmia*) (Alfano, 1966; Wolff, 1972). In Wolff's series of 30 eyes

described as microphthalmic, none was without other evidence of ocular congenital rubella. It is notable that these features only occur within the context of the systemic congenital rubella syndrome. Of course, it is possible that the very characteristic ocular pathology could occur as an isolated ocular lesion. The microphthalmos that Gregg described was 'very mild' and in all cases there was typical 'rubella' cataract. Again, we are reminded of the inadequacy of the term microphthalmos. Microcornea would be a more suitable description in these cases (Wolff, 1972).

Congenital toxoplasmosis and congenital varicella are both listed in standard textbooks as causes of microphthalmos (Lambert, 1997). In toxoplasmosis, it is the retinochoroiditis that is the diagnostic feature (Noble and Carr, 1982).

Intrauterine cytomegalovirus infection manifests itself in the eye as a chorioretinitis, usually (but not always) within the context of the systemic features.

Congenital varicella is more notable for the chorioretinitis and/or cataract. It is more than likely that these are the primary phenotypic features.

Neonatal herpes simplex infection following intrauterine infection has not been described as causing microphthalmos.

Anophthalmos, iris or fundus coloboma due to defective optic fissure closure, have not been described in any of the TORCH infections.

Summarising the TORCH infections, they have never been documented as a cause of anophthalmos or extreme microphthalmos, and when they do cause microphthalmos, it has a typical 'mild' appearance, the other ocular features being the primary feature and of diagnostic importance. However, it is theoretically possible that sub-clinical infection could be responsible for these eye defects, although there is no evidence for this.

The mild forms of microphthalmos that result from some of these infections are probably the result of a generalised inhibition of ocular growth in the later stages of development secondary to the infective process and intraocular inflammation of a structurally normal eye. Infection at an earlier stage of embryogenesis (i.e. around the time of optic fissure closure) would almost certainly result in far more severe disruption to the eye.

If environmental factors were causative or influential, one would expect that they would exert their effects during the critical early stages of eye development. Such an effect would not have to be sustained to inflict serious damage. The human embryo shows the earliest signs of eye development at the 2 mm stage. Only a very small amount of toxin (e.g. a virus) would be required to exert a major influence on an organ that is a fraction of a millimetre in size.

Possible causes of coloboma

Due to the large degree of overlap between microphthalmos and coloboma, and the fact that most cases of microphthalmos are colobomatous, the two are similar and difficult to separate (see Table 2.3 for microphthalmos).

Vitamin A deficiency has been mentioned as a cause (Lamba and Sood, 1968) as well as thalidomide (Smithells, 1973). Like microphthalmos, TORCH infections have been overemphasised as a possible cause of coloboma (Mintz-Hittner et al. 1976), and probably account for an insignificant number of sporadic cases.

Genetic aetiology

There is little doubt that in a number of cases, anophthalmos and microphthalmos have an aetiology that one would consider to be 'genetic' (Box 2.1). This is a word that needs to be defined and explored further. 'Genetic' encompasses a number of very different mechanisms. Firstly, that the underlying mechanism is inherited in some way. Secondly, there may be a chromosome abnormality that can be identified on laboratory testing. Thirdly, there may be no identifiable chromosome abnormality but a previously recognised group of systemic malformations in conjunction with the eye abnormality that has a known pattern of inheritance (a clinical genetic syndrome), or a clinical genetic association. These clinical genetic aetiologies do overlap to a degree, and the separation is slightly artificial. Categorisation does however assist understanding.

If anophthalmos/microphthalmos were genetic in aetiology, one would expect to see some or all of the following features:

1. Families in which more than one individual was affected with the same (or related) structural eye abnormalities. These would follow recognised patterns of Mendelian inheritance (dominant, recessive, X-linked). If recessive, one would expect there to be a high proportion of consanguineous parental relationships with respect to the proband.
2. An excess of bilaterally affected individuals (see environmental causes above). It may be that there is underreporting of unilateral cases, especially if the fellow eye is normal (Tucker et al. 1996). Bilateral pathology is not a prerequisite to having a genetic cause. Many individuals with a known (probable) genetic cause are unilaterally affected (McMillan, 1921; Francois, 1968; Pagon et al. 1981b), Briggs

quoted in Sorsby (Sorsby, 1934; Brunquell et al., 1984). Conversely, many individuals with no detectable genetic cause (presumed non-genetic) are bilaterally affected.

3. The presence of chromosomal abnormalities in individuals and their parents, if the parents are also affected.
4. Well-recognised ocular clinical genetic syndromes and associations. These are supposed to exist but closer examination doesn't confirm this, as it may be that features are being made to 'fit' a previously described syndrome e.g. in Lenz's syndrome or Waardenburg's syndrome (see below).

Box 2.1: Possible genetic aetiologies of microphthalmos/anophthalmos

Mendelian inheritance
Chromosomal abnormality (e.g. rearrangement)
Chromosome microdeletion
Clinical genetic syndrome
Clinical genetic association
Complex multifactorial

In one of the earliest cases of anophthalmos described at the beginning of chapter one, (Bartholini, 1657) the child was affected with other (systemic) congenital abnormalities. This is of fundamental importance since it suggests that the eye abnormality is part of a wider developmental defect that has manifested itself in more than one major organ. As stated earlier, this could be considered as strengthening the case for an environmental insult. From a genetic point of view however, it can be argued that one or many genes may be involved or are interacting to produce complex

patterns of malformation. A single gene could be involved in multiple developmental pathways. In both cases (genetic and environmental) one could explain the inconsistency and variation by considering variable gene expression or doses of teratogen. The presence of eye malformations in so many different clinical genetic syndromes is not easily explained. The environment can mimic the effects of inheritance.

In many of the previous cases and reports and descriptions, an attempt has been made to distinguish cases in which the congenital abnormalities were confined to one or both eyes (isolated), with no systemic anomaly. This is not always possible as systemic examination may be incomplete or not sufficiently well documented to do this with certainty.

Anophthalmos has been described in several families and pedigrees. The better quality published cases are summarised in Table 2.4. Listed alongside each case is the likely/presumed pattern of inheritance. Other congenital malformations are listed in some cases. None are documented to have a chromosomal abnormality.

Table 2.4: Anophthalmos occurring in families and pedigrees

Relationship of probands and Laterality	Presumed or likely form of Mendelian inheritance	Other malformations	Notes	Reference
Sisters, bilateral	Recessive			(Walker, 1831)
Father and son, unilateral	Dominant			(Treacher Collins, 1887)
Bilateral	Recessive (parents 1st cousins)	Dysmorphic face, hand and foot deformities		(Treacher Collins, 1887)
4 sibs, mixture of anophthalmos, iris coloboma, microphthalmos, all asymmetrical	Recessive			(McMillan, 1921)
Mixture of anophthalmos, iris coloboma, microphthalmos, all asymmetrical. Large pedigree, consanguinity.	Recessive			(Porges et al. 1992)
Bilateral	Parents 1st cousins			(Hesselberg, 1951)

Relationship of probands and Laterality	Presumed or likely form of Mendelian inheritance	Other malformations	Notes	Reference
3 sibs, bilateral	Recessive	All died of congenital heart disease		(Pearce et al. 1974)
3 male sibs, bilateral	Recessive or X-linked recessive			(Joseph, 1957)
18 individuals, bilateral	Recessive, widespread consanguinity			(Kohn et al. 1988)
1 unilateral, 3 bilateral	Recessive, widespread consanguinity			(Da Silva and De Sousa, 1981)
1 unilateral, 1 bilateral. Mixture of anophthalmos, iris coloboma, microphthalmos	Dominant			(Pagon, 1981)
1 unilateral, 1 bilateral. Mixture of anophthalmos, iris coloboma, microphthalmos	Dominant	Mental retardation (bilateral case)		(Francois, 1968)
Brother and sister, bilateral	Recessive	Mental retardation		(Ashley, 1947)

Relationship of probands and Laterality	Presumed or likely form of Mendelian inheritance	Other malformations	Notes	Reference
3 sibs bilateral, 1 unilateral	Recessive			Briggs, 1813 in:(Sorsby, 1934))
3 sibs, bilateral	Recessive			Monteath, 1821 in: (Sorsby, 1934)
1 unilateral, 3 bilateral (2 brothers, maternal uncle, cousin)	X-linked recessive	Mental retardation		(Hoefnagel et al. 1963; Hoefnagel et al. 1963)
2 sibs, bilateral	Recessive, parents 1st cousins	Dysmorphic, syndactyly of toes	Waardenburg's recessive anophthalmia syndrome	(Traboulsi et al. 1984)
Bilateral	Recessive, parents 1st cousins	Dysmorphic, hand and toe syndactyly	Waardenburg's recessive anophthalmia syndrome	(Richieri-Costa et al. 1983)
4 sibs, 3 bilateral deceased, 1 unilateral proband (normal fellow eye)	Recessive, parents 1st cousins	Fusion of hand metacarpals, toe syndactyly (proband only)	Waardenburg's recessive anophthalmia syndrome	(Richieri-Costa et al. 1983)
2 female sibs, unilateral	Recessive, parents 1st cousins	Bilateral malformations of hands and feet	Waardenburg's recessive anophthalmia syndrome	(Waardenburg et al. 1961)

Relationship of probands and Laterality	Presumed or likely form of Mendelian inheritance	Other malformations	Notes	Reference
Bilaterally affected male, maternal aunt bilateral, mixed abnormalities in maternal male cousin and maternal uncle		Pyloric stenosis, mental retardation, ear abnormality (proband only)		(Tucker et al. 1996)
Bilaterally affected male, maternal aunt unilaterally affected				(Tucker et al. 1996)
Bilateral male sibs	X-linked recessive	All with mental retardation, one with pre-auricular skin tags and cleft soft palate	Localisation of gene to Xq27–Xq28	(Graham et al. 1991)
Bilateral (2 sibs, 1 cousin)	Dominant			(Fryns, 1995)
3 unilateral, 1 bilateral	Dominant			(Russell-Eggitt et al. 1985)
Unilaterally affected male, maternal uncle same		Spina bifida		(Tucker et al. 1996)

Waardenburg's recessive anophthalmos syndrome

Waardenburg's recessive anophthalmos syndrome (see Table 2.4) is useful to discuss as it reveals much about the inadequacies of our understanding of the aetiology of anophthalmos.

In 1935 Waardenburg described the occurrence of bilateral anophthalmos with limb defects (syndactyly) in two girls, the offspring of first cousins. This familial recessive syndrome has since been described in two other families. However, the additional systemic anomalies vary from case to case. Traboulsi reported a male propositus with simian creases, absent prepuce and widely spaced nipples (Traboulsi et al. 1984). Richieri-Costa's first case (Richieri-Costa et al. 1983) describes widespread dysmorphism (low set ears, hypoplastic upper helix, retrognathia, high arched palate). These varying observations may reflect different levels of reporting, but may also reflect a different phenotype. The real difficulty arises when new cases are described and the features 'fitted in' to comply with the (ill-recognised) syndrome (Pallotta, 1985). The spectrum of features of the clinical genetic syndrome is then expanded to accommodate each case, often with the mention of major and minor diagnostic criteria. It can be worthwhile with respect to prognosis and parental reassurance to fit a severely disabled child into a named/recognised syndrome or diagnosis, but sometimes this is not helpful in counselling or risk assessment.

How does one explain such variation of expression? It could be that these cases described are different conditions, or if they are the same condition there may be a disturbance in temporal synchronisation and sequential progression of the affected developmental fields (eye and distal limb). It is possible that a single gene could cause

not anophthalmos *with* limb abnormalities, but a disturbance of eye *and* distal limb formation with a resulting variable phenotype (Richieri-Costa et al. 1983).

It is interesting that Bartholini's case report of 1657 describes a child with anophthalmos and polydactyly of the hands and one foot: Is this the first description of the syndrome named after Waardenburg? (Bartholini, 1657).

Chromosome abnormalities

Numerous chromosome malformations and rearrangements have been reported:

1. Translocation 7/15 [46, XY, t(7, 15)] delayed neuropsychomotor development, translocation 4, 14.(Wajntal et al. 1978).

2. 14 (q22.1–22.3) deletion (Elliott et al. 1993), 14q22q23 (Bennett et al. 1991).

3. Trisomy 13.

Since the case first described by Patau, (Patau et al. 1960) trisomy 13 has been a recognised cause of anophthalmos and microphthalmos. (The case described by Patau had apparent anophthalmos.) Patau's syndrome is useful to discuss with respect to both anophthalmos and microphthalmos, because it typifies some of the difficulties in diagnosis and accurate documentation in cases of multiple severe congenital anomalies. The frequency of these eye deformities is far from 100% of cases. However, accurate or precise documentation of eye anomalies is (understandably) not necessarily of the highest priority in these very sick babies. Taylor (Taylor, 1968) looked in detail at 27 cases of Patau's syndrome (clinical and post mortem findings). 88% had eye defects, 76% microphthalmos, and 33% iris coloboma. Facial deformities such as epicanthic folds and eyelid oedema in these cases can certainly

make the assessment of apparent eye size difficult (chapter one). In Taylor's series, there was just one case of anophthalmos ('Eyes not visible, anophthalmia suspected'). In summary, trisomy 13 probably accounts for a small but significant number of cases of apparent anophthalmos. The frequency of occurrence of anophthalmos in trisomy 13 is probably approaching nil (Hoepner and Yanoff, 1972).

Genetic aspects of microphthalmos

It is not possible to summarise the vast body of literature on the hereditary aspects of microphthalmos. This is because of terminology and the imprecise meaning of the word microphthalmos. Even careful examination of each paper or clinical report does not often lead to an adequate phenotypic description, and photographs, where present, are unreliable and difficult to interpret. This problem is not confined to the older literature and descriptions, as some of these are documented in sufficient detail to recognise a structural eye abnormality. Again, it is the structural eye abnormality that is of fundamental importance when trying to understand the genetic aetiology.

With this in mind, a brief attempt will be made to discuss some of the work on the clinical genetics of microphthalmos. No apology is made for exclusions or criticism, and the reader is reminded that such an argument lies at the core of this chapter, and indicates why determining aetiology is so difficult.

That microphthalmos represents a number of separate eye conditions, each with a distinctive phenotype, was discussed in the last chapter. Coloboma and colobomatous microphthalmos will be discussed separately, and special mention of these cases will be made where possible.

As with anophthalmos, there is no easy way to classify or categorise the genetics of microphthalmos. Warburg (Warburg, 1991) states that there are more than 100 genetic traits with microphthalmos and coloboma. At the time, there were approximately 50 different autosomal dominant syndromes, 67 autosomal recessive syndromes, and 16 X-linked syndromes. It is a great pity that coloboma and microphthalmos are so often 'lumped' together, as this makes the task of sifting out the reports on coloboma very difficult. An ocular malformation described as a 'coloboma' will more than likely be accurate and actually represent the ocular phenotype of uveal coloboma although, as described in the previous chapter, this is a term also subjected to misuse.

Abnormal karyotypes are common in patients with microphthalmos and coloboma associated with delayed mental development and minor abnormalities (Warburg and Friedrich, 1987).

Warburg reviewed coloboma and/or microphthalmos in chromosomal aberrations (Warburg and Friedrich, 1987). In the 500 papers reviewed, abnormalities were reported in almost every chromosome. The authors concluded that deletions and duplications were non-specific for these disorders when trying to determine loci for the Mendelian types of colobomas or microphthalmos. This study could have been considerably improved and made more specific by phenotypically classifying and separating the various ocular malformations. This may have led to determination of some loci (Brewer et al. 1998).

It is practically impossible to attempt to separate those disorders in which eye malformations are the only pathology, there being no systemic anomalies. The justification for doing this (or not) was discussed above (page 56 item 4).

Many descriptions are erroneous. For example, closer inspection of some papers will suggest that the microphthalmos described is in fact nanophthalmos (Wolff, 1930). This distinctive ocular malformation was described in chapter one.

The On-line Mendelian Inheritance in Man (OMIM) (http://www3.ncbi.nlm.nih.gov/Omim) refers to 'microphthalmos-cataract' and lists a paper by Zeiter (Zeiter, 1963). This paper describes autosomal dominant cataract. Capella (Capella et al. 1963) is also listed under 'microphthalmos-cataract' and this paper describes both X-linked and dominant cataract. Microphthalmos and microcornea are key words in the paper's title, but certainly the microphthalmos is not remarkable in the phenotypic description. Comparison with previous work in these papers and summaries of the literature at the time of publication reflect the confusion that is bound to arise when obviously different conditions are compared and contrasted. This pattern has continued, with very recent papers apparently identifying the locus for autosomal recessive microphthalmos (Bessant et al. 1998). This is admirable and diligent work but the ocular phenotype in this particular paper is in fact sclerocornea. This simple fact seems to have been lost in the title, the paper disappearing into the mass of literature already existing on the subject of 'microphthalmos '.

Many papers referring to congenital microphthalmos and its inheritance patterns are concerned with colobomatous microphthalmos, which is possibly no different to 'ordinary' or non-colobomatous microphthalmos.

Chromosomal disorders may present with microphthalmos as a feature and trisomy 13 was discussed earlier. Closer examination of the eyes in cases of chromosomal abnormality with microphthalmos reveals that there is a range of different but distinct

ocular phenotypes (Ginsberg and Bove, 1974) rather than just microphthalmos. Yokoyama (Yokoyama et al. 1992) describes autosomal dominant congenital cataract and microphthalmos with a familial t (2;16) translocation. Again, the primary pathology in this family is the cataract described.

Taylor's series (Taylor, 1968) of 27 cases of trisomy 18 (Edwards' syndrome) records 29% as having an eye defect, the same percentage with microphthalmos. Notably, iris coloboma was not present in any case.

Warburg (Warburg and Friedrich, 1987) postulated that the reason for autosomal chromosome aberrations producing such a wide range of systemic malformations is due to disturbance of the neural crest developmental field. The differentiation of the neural crest cells is influenced by an environment in which (defective) non-neural crest cells carry important genetic information.

Immense difficulty arises when performing literature searches on databases such as The London Dysmorphology Database for inherited conditions or syndromes (Winter and Baraitser, 1997) and a term such as microphthalmos is introduced as a search feature or criterion. Microphthalmos encompasses so many diagnoses that the term is a hindrance. The situation produced then requires consideration of several clearly unsuitable diagnoses for that particular case, as very likely the previous literature will have described at some time in the past 'a case of (almost any well-known or rare syndrome) *with* microphthalmos'. Many of these case reports are worthy attempts to further delineate or define well-known or barely recognised syndromes. However, there is certainly the danger of artificially introducing new or incorrect diagnostic criteria. Even with good clinical data it is not an easy task to recognise or clarify a new syndrome (Baraitser and Winter, 1988).

To illustrate this point one or two of the better-known microphthalmic syndromes are discussed further.

The Lenz microphthalmos syndrome (Traboulsi et al. 1988): In 1955 Lenz (Lenz, 1955) described an X-linked malformation syndrome characterised by microphthalmos, mental retardation, and aural, digital, skeletal and urogenital abnormalities. Traboulsi (Traboulsi et al. 1988) reviewed the post mortem findings on Lenz's original patient and reviewed the twelve cases recorded in the literature up to that time, which included a description of two of their own cases. The first describes a boy with bilateral colobomatous microphthalmos, head circumference/weight/height all below fifth percentile, delayed mental development, abnormal ears, widely interspaced teeth, small right hand with hypoplastic thumb and clinodactyly of left toes. Mother had 'a number of similarly affected male relatives none of whom were available for examination.' The second case described was a boy with bilateral colobomatous microphthalmos (the published photo and dimensions would suggest unilateral), ear abnormalities, conductive/neural deafness, imperforate anus and hypospadias. The right hand had pre-axial duplication of the thumb, the left partially so. The right foot had an appendage to the second toe and syndactyly of toes three and four, the left foot syndactyly of toes two and three. Family history was unremarkable. The authors are mistaken here, as firstly they have described two patients, one of whom has a questionable family history and the other none at all. So to state that the condition is X-linked (or familial at all) is incorrect. Secondly, the two cases have few systemic features in common, either with each other, or with Lenz's original description. What they do have is uveal colobomas with systemic

abnormalities. Chromosome results were not stated for the first case; the other is recorded as normal.

The postmortem findings from Lenz's original patient, who died of uraemia aged 28 years, included bicuspid aortic valve with mild coarctation of the aorta, aplastic left kidney, ureter and renal vessels, hydronephrosis of right kidney, cryptorchidism, and spina bifida occulta. The ocular features listed (translated from German) included microphthalmos, microcornea with corneal diameter approximately 4 mm and cloudy remains of lens. There is no mention of any type of ocular coloboma.

The authors (Traboulsi et al. 1988) summarised the systemic features in a table showing some ten different categories, including 'other' anomalies. Closer examination reveals that not only do the ocular phenotypes differ (including Lenz's original case), but also only six of the cases in the table have any family history at all.

In summary, we have a poorly defined syndrome, of uncertain prognostic outcome. Most of these cases are not definitely Lenz's syndrome, or any other syndrome for that matter. For example, the proband of Hoefnagel et al (Hoefnagel et al. 1963) listed in the table had bilateral clinical anophthalmos. The geneticist is therefore not equipped with the quality of information required to make anything more than the crudest assessment of recurrence risk.

The many publications (Francois et al. 1983) that have inappropriately adopted the term microphthalmos would be impossible to list and discuss here: for example, the MIDAS syndrome (Microphthalmos, Dermal Aplasia, and Sclerocornea), where the phenotype is simply sclerocornea (Happle et al. 1993). It is possible that in some cases microphthalmos has been diagnosed on the basis of finding blepharophimosis (Happle et al. 1993). Similarly, the X-linked Nance-Horan syndrome (cataracts with

distinctive dental features and characteristic facies) is distinguished by the ocular findings of cataract, with microphthalmos or microcornea not being diagnostic criteria (Walpole et al. 1990).

Microdeletions

The reporting of normal chromosomes (usually by G-banding) does not exclude microscopic chromosome deletions that are now being recognised more frequently (Shapira, 1998). The deletion or duplication may have been previously unrecognised because the abnormality of the chromosome was not detected at the level of resolution of standard chromosome analysis. The features of deletion and microdeletion syndromes result from loss of one copy of a gene or genes contained within the deleted segment. In some conditions, the deleted gene is dose-sensitive, such that haploinsufficiency occurs because of only 50% activity coming from the remaining copy of the gene. In other conditions, e.g. retinoblastoma, deletion of the gene results in unmasking of recessive alleles or polymorphic variants in the remaining copy of the gene. In some deletion conditions, the phenotype arises as a consequence of loss of the only 'functioning' copy of an imprinted gene. Such a case exists in Angelman syndrome, where the deletion is maternally derived.

Iris coloboma has been described in the Rubinstein-Taybi syndrome (Guion-Almeida and Richieri-Costa, 1992), now considered to be caused by a microdeletion of chromosome 16p13.3 (Hennekam et al. 1993). However, in the paper by Hennekam, none of the described affected subjects had structural eye abnormalities.

Genetic aspects of coloboma

In chapter one the significance of coloboma as an ocular malformation was described, as well as its frequent occurrence within the context of clinical microphthalmos. Deliberately, no attempt has been made to distinguish coloboma from colobomatous microphthalmos/macrophthalmos (Bateman and Maumenee, 1984) or from colobomatous microphthalmos with cyst. Emphasis is placed on the structural abnormality and not the (often subjective or arbitrary) assignment of microphthalmos.

Mendelian inheritance of coloboma

In many of the cases described the ocular abnormalities vary extensively, from a simple iris coloboma to clinical anophthalmos (Pagon, 1981).

Autosomal recessive: ocular abnormalities only (Pagon et al. 1981b).

Autosomal dominant: often with variable penetration and expressivity (Duke-Elder, 1964; Francois, 1968; Bateman and Maumenee, 1984).

X-linked: with microcephaly, short stature and mental retardation (Goldberg and McKusick, 1971).

Incomplete documentation of ocular and systemic abnormalities, particularly in the older literature, and over-reporting of families with particular forms of over or under-expression, make it difficult to calculate the penetration (Pagon, 1981). Maumenee empirically estimated the recurrence figures for the subsequent child of healthy unrelated parents being affected with coloboma as 9%, and for the children of an affected person as 46% (Maumenee and Mitchell, 1990). For all new cases, new

sporadic mutations, reduced penetrance and non-genetic aetiology need to be considered.

No single gene for colobomatous malformation has been identified to date. This is understandable given the absence of large pedigrees and the lack of significance of chromosomal aberrations as pointers in the search for a gene. Developmental fields were briefly mentioned when anophthalmos and its presence in so many chromosomal abnormalities was discussed. Developmental fields are body parts that can be affected in an identical manner by different insults (genetic or environmental). Maumenee (Maumenee and Mitchell, 1990) proposed that single gene defects exist which act in the early stages of the developing embryo before definition of 'limb', 'axial' and 'ocular' fields. This mechanism would exist in parallel with the likely existence of pathogens that may affect the different tissues during the sensitive developmental period.

Many systemic associations are associated with colobomatous defects, as either part of a chromosomal abnormality and/or clinical genetic syndrome. The extensive overlap of these categories and poor understanding of their aetiology makes classification difficult. The systemic malformations have been listed in several reviews (Kriss et al. 1997) or case series (Leppig and Pagon, 1993) and are listed in OMIM. As with microphthalmos, but much less so as the phenotype is relatively distinct, the description of coloboma in some of these papers is questionable. For example, closer inspection of the literature reveals that the 'retinal colobomas' described in Joubert's syndrome, the most characteristic feature of which is neonatal tachypnoea, are in fact macular hypoplasia and retinal pigment epithelium changes (King et al. 1984).

Some of the chromosomal aberrations associated with coloboma include:

Trisomy 13 occurred in 33% of 18 cases (Taylor, 1968) and in more than half of the cases reported by Hoepner (Hoepner and Yanoff, 1972).

Trisomy 18 is very rarely associated with coloboma (Mullaney, 1978).

Other types of chromosome rearrangement, including translocations (Friling et al. 1995) and inversions (Pallotta, 1991) are frequently reported.

The Cat Eye Syndrome is the association of iris coloboma with anal atresia (Cory and Jamison, 1974). Patients have an extra acrocentric chromosome that is small and abnormal in appearance. Additional major and minor congenital malformations occur frequently.

Clinical genetic associations are non-random concurrences of several particular, but variable, malformations which are not pathogenetically related, or which are not known to have the same principal aetiology. Associations may be the result of teratogens that act at a certain time on a certain area in a person who responds in a certain way. Therefore, associations could represent embryological relationships and timing, not specific causes. It follows that associations can have either a genetic or an environmental aetiology.

The CHARGE association

It was Pagon (Pagon et al. 1981a) who proposed the acronym CHARGE, having described 21 patients with choanal atresia, ocular coloboma or both, who had associated anomalies. CHARGE = Coloboma, Heart disease, Atresia Choanae, Retarded growth and development and/or CNS anomalies, Genital hypoplasia and Ear

anomalies. The colobomas described ranged from iris coloboma without visual impairment to clinical anophthalmos. A cause or pathological mechanism has yet to be defined.

In conclusion, none of the many systemic malformation and dysmorphology syndromes described with coloboma (whether accurate or not), as varied as focal dermal hypoplasia (Goltz syndrome) (Temple et al. 1990) or the branchio-oculo-facial syndrome (Fielding and Fryer, 1992) have contributed significantly to our knowledge of how these eye defects arise.

Genetic aspects of optic nerve head type coloboma

Optic nerve head coloboma, in the absence of iris or retinal coloboma, was discussed in chapter one. The developmental basis of this abnormality is unclear, and it is uncertain whether it arises as a result of a defect in optic fissure closure. Autosomal dominant inheritance has been well described (Savell and Cook, 1976). More recently, dominant optic nerve coloboma and renal disease have been shown to arise as a result of *PAX2* gene mutations. (Sanyanusin et al. 1995). It is possible that *PAX2* gene mutations are responsible for isolated optic nerve coloboma (Cunliffe et al. 1998).

Summary, chapter 2

- Epidemiological data is of poor quality and inconsistent diagnostic criteria and variation makes much of the data difficult to draw useful conclusion from. The congenital malformation registers, in their present state, do not meet all the needs for which they are now used, i.e. detailed scrutiny and statistical analysis of very rare congenital eye disorders.

- Anophthalmos/microphthalmos and coloboma have features that suggest both a genetic and environmental aetiology.
- One cannot easily distinguish environmental from genetic causes. In a sporadic case, significant similarities are present in the absence of an obvious 'genetic' cause.
- Genetic features of anophthalmos/microphthalmos include family histories consistent with dominant, recessive and X-linked inheritance. Other genetic features include the frequent association of these eye malformations with chromosomal disruptions, clinical genetic syndromes and associations.
- Environmental features of anophthalmos/microphthalmos include the sporadic nature of occurrence, associated congenital malformations not fitting into a recognised pattern or syndrome, the frequent description of occurrence with teratogens, maternal drugs or maternal illness, and the wide variation of ocular phenotype.
- Bilateral and unilateral defects are equally common in genetic cases as in sporadic/environmental ones. Unilateral pathology does not imply or increase the likelihood of a genetic cause.
- Microphthalmos has been overused in the literature when describing many phenotypically distinct ocular abnormalities occurring in systemic syndromes.
- The failure to define adequately anophthalmos/microphthalmos has made it difficult to determine a cause.

This chapter has concentrated on the environmental and genetic background of anophthalmos/microphthalmos and coloboma. In chapter three, the use of mouse genetics to explore human eye developmental disorders is introduced.

CHAPTER THREE

SEARCHING FOR GENETIC CAUSES OF DEVELOPMENTAL EYE DISORDERS IN HUMANS: MOUSE MODELS AND CANDIDATE GENES

'A human is like a mouse, only more so' (Erickson, 1989).

Much of the discussion in this chapter is based on mouse models of human genetic disease. Mouse genetics is one of many approaches to finding the genetic basis of an inherited condition. As with humans, the choice of inappropriate terms for phenotypic description has not always been helpful and confusion does arise.

The proper investigation into a cause rests on establishing a phenotypic definition. This is equally relevant whether searching for genetic or environmental causes. All terms must be clearly understood, with no ambiguity. Names should be based on the structural abnormality present and not be highly subjective or irrelevant to the defect. This is not easy. Ocular malformations must be phenotyped and described in detail. It may be useful or necessary to reclassify structural abnormalities to maintain consistency with current and emerging understanding of development and the genetics of development.

Searching for genetic causes of eye disorder in humans

By 'genetic' is meant locating a specific gene at a chromosomal location, in which a gene mutation causes a particular eye abnormality either by itself or in combination with other genetic or environmental factors

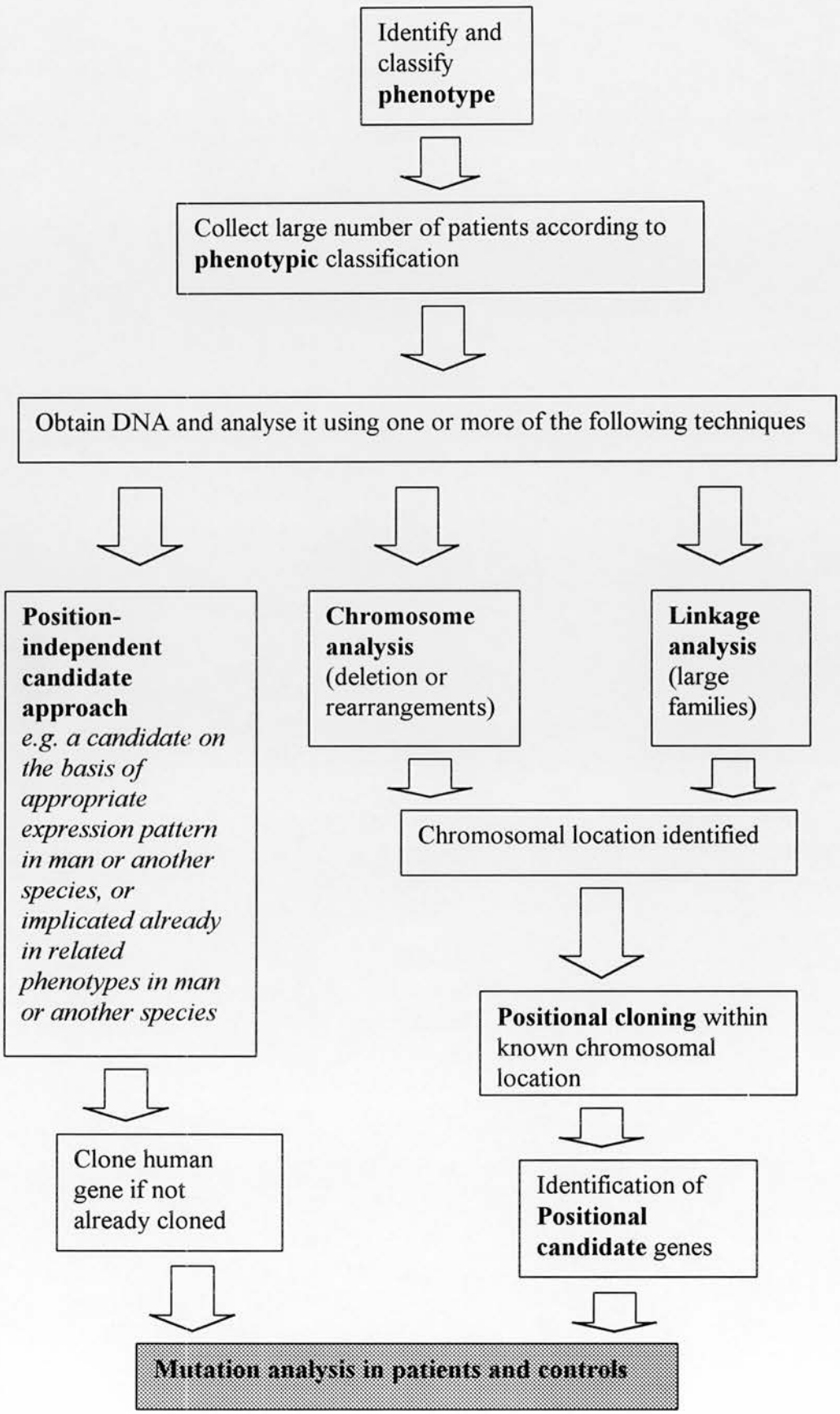
To look for genetic causes, certain information is required. This information is listed below and a possible sequence of events is illustrated in Figure 3.1.

1. A phenotype is identified, defined and classified.
2. The collection of tissue samples for DNA extraction from correctly phenotyped affected individuals.
3. DNA analysis (methods/techniques below).
4. Localise gene i.e. link to specific chromosome region.
5. Sequence the gene.
6. Identify disease-causing mutations. In mice, these can be natural mutations or experimental models with induced mutations. Human mutations are natural.

Failure on the first point, phenotypic definition and characterisation, can make progress difficult or impossible.

A number of well-established methods (Box 3.1) can be used to determine whether or not a disease has a genetic component and if so how to identify the disease-causing gene. The classical method is where large pedigrees are available for gene mapping by linkage analysis. These techniques work well for Mendelian-inherited (single gene) eye diseases. Other approaches used are positional cloning and the candidate gene approach. These techniques and their limitations are described briefly below.

Figure 3.1 Summary and flow chart of some of the methods used to determine the gene causing a disorder



Box 3.1 Methods to determine the gene causing a disorder

1. Linkage analysis
2. Positional cloning
3. Position independent candidate gene
4. Positional candidate gene
5. Chromosomal deletions or rearrangements
6. Mutation analysis

1. Linkage analysis

Genetic linkage analysis is often the first step in identifying a disease-causing gene. The aim is to compare, within a family, the inheritance of a disease gene with the inheritance of specific DNA segments (markers). Co-inheritance of the disease markers with the disease-causing gene links the disease (localises it) to a specific chromosomal region (Musarella, 1992).

2. Positional cloning

Through linkage studies, a gene is located solely on the basis of map position (without any knowledge of the gene's function). The genomic region implicated by the linkage analysis is covered with a 'contig' (contiguous DNA clones) of genomic clones, which may be cosmids, BACS (bacterial artificial chromosomes), PACS (P1-artificial chromosomes) or YACS (yeast artificial chromosomes). Genes are identified from the sequencing. All genes identified in the vicinity are considered as possible candidates.

3. Position-independent candidate gene

The gene is a candidate for the disease of interest. The disease gene is searched for on the basis of the *function or likely role* of the protein involved, its amino acid

sequence, or the biochemical or pathophysiological consequences. It may be that the gene is expressed in the tissues of the affected organ. Unfortunately, for most eye disorders, there is little information about the disease gene or its function. This approach is discussed later in this chapter.

4. Positional candidate

Often, because so little is known about eye disease genes and their function, a combined strategy may be used. A gene is mapped to a chromosomal region by linkage analysis, and then attractive candidates in the area are surveyed. Some genes mapping in that area may already be known, and sequencing can identify new ones. This approach is considered further in the second half of this chapter.

5. Chromosomal deletions or rearrangements

These can help localise a gene by the occurrence of the disorder with the missing or disrupted segment e.g. retinoblastoma and 13q14, aniridia and 11p13 (Churchill and Booth, 1996). Once a genomic region has been implicated by a chromosomal rearrangement, the same positional cloning or positional candidate approaches may be used as for linkage. Some chromosomal rearrangements already described in individuals with anophthalmos and colobomas are listed in chapter two page 68. Chromosomal microdeletions were also discussed in chapter two.

6. Mutation analysis

The above methodologies merge into one another and are not always used in isolation. They are summarised in Figure 3.1. The approach used for anophthalmos

and microphthalmos and *PAX6* (described in chapter nine) is 'pure candidate gene'. Often, a combination of techniques is employed, based on increasing knowledge and location of human genes.

Relative risk studies (chapter eight), which have not been done for anophthalmos/microphthalmos and coloboma, estimate the risk to sibs versus the general population, and can provide a measure of the genetic component.

There are several possible reasons why the genes causing human anophthalmos/microphthalmos have not been localised:

1. To detect linkage, there needs to be a sufficient number of affected individuals within a pedigree to provide the statistical power. This also depends on inheritance pattern and the structure of the pedigree. Rarely are there sufficient affected relatives in anophthalmos/microphthalmos.
2. Anophthalmos/microphthalmos does not always demonstrate the clear patterns of Mendelian inheritance that typify single gene disorders. They are complex traits, which may be due to multiple gene interactions or gene and environment interaction.
3. The gene(s) responsible may have variable expression and penetrance.
4. There are possibly several genes which when mutated can cause anophthalmos/microphthalmos but the phenotypes are lumped together, the phenotype being way 'downstream' of the failed developmental step. Therefore, it is difficult to assess from the phenotype the degree of involvement of different genes.

Some genetic aspects of eye development

Eye development involves two intertwined processes (Graw, 1996). One is an ongoing series of inductive signals, which determines the initial architecture of the major components of the eye. The other is the co-ordinated differentiation of these components. The action of transcription factors and inductive signals ensures the correct development of the different eye components. Some of the knowledge of how genes control human eye development has come from studies in mice, when spontaneous and induced mutations have been analysed. Mutations affecting the eye can be easily identified and many mouse mutants have been described.

Before discussing some of these mouse mutants and the role of particular genes in development of some eye structures (lens, retina, optic nerve), some of the shortcomings, assumptions, and difficulties of using mouse models are considered.

Mouse models do not always replicate the human disease associated with the same mutations. Erickson has suggested three general mechanisms why this is so (Erickson, 1989):

1. Different biochemical pathways between mouse and man, such as the human inability to synthesise vitamin C. Different genetic defects may result in a similar disease phenotype but the animal model is not a precise one, since the deletion or elimination of an additional factor necessary for the regulation of other unlinked genes could occur.
2. Different developmental pathways between mouse and man. The size difference influences some developmental pathways: e.g. carbonic anhydrase (CA II) deficient mice do not develop osteopetrosis like CA II deficient humans due to differences in

bone remodelling. The different nervous systems in mice and man reflect variation in neurodevelopment.

3. The fact that some pathological processes possibly occur at the same absolute rate in man and mice instead of being related to life span. A time dependent rate of onset may explain the mild phenotype of *mdx;* this is the probable mouse homolog of the dystrophin locus, the human X-linked locus at which some mutations cause the childhood onset of severe Duchenne muscular dystrophy, while others cause a milder adult muscular dystrophy (Becker's). The symptoms in *mdx* hemizygous mice suggest that the mutation has resulted in either a Becker-like disease with a lifespan-related onset or a Duchenne-like disease with an absolute time-related onset, since humans do not usually show symptoms until several years of age, a length of time greater than the usual survival of mice.

Although mice carrying mutations homologous to those causing human disease can differ from their human counterparts, even imperfect models can be useful. This subject will be discussed later in this chapter, and is well illustrated by previous work on human and mouse paired box genes.

Aspects of early eye development relevant to anophthalmos and coloboma were described in chapter one. It is to these particular aspects of genetics and eye development that attention must be paid: development of the optic vesicle, optic cup, and iris.

A brief description follows of some of the many mouse mutants in which the early stages of eye development are affected (Graw, 1996). It is worth noting at this point

that the lack of rigour in defining ocular phenotypes is not confined to humans, and that many of the names applied to the mice are also misnomers.

Eyeless (*ey1, ey2*).

Eyeless causes anophthalmos. Its chromosomal localisation is unknown. The apposition of optic vesicles and surface ectoderm, a process thought to be critical to lens induction, is affected. Homozygous *ey1* mutants have a smaller than normal lens which is decentred from the optic cup.

The interaction between optic cup and overlying surface ectoderm is a very important step in eye development, this interaction being affected in a number of mutants, as well as in the *Pax6/Sey* mutation (see below).

Aphakia (*ak*)

Autosomal recessive mice mutants have an abnormal extracellular material form between the lens and optic cup during invagination of the optic cup, with abnormal deposits of material between lens epithelial cells. The rudimentary lens becomes disorganised.

Head blebs (*heb*)

Heb mice produce abnormal or absent eyes due to prenatal blebs, usually on the head.

Myelencephalic blebs (*my*)

In mouse mutants with myelencephalic blebs, extracellular matrix forms between the optic vesicle and overlying presumptive lens ectoderm. An increased concentration of glycosaminoglycan in the extracellular matrix later causes rupture of the lens capsule. Other defects affect the neural retina. By E14, the cornea and other structures of the eye cannot be identified.

Extra-toes (*Gli3*)

Mice homozygous for the dominant mutation Extra-toes die perinatally with multiple malformations of the eye and other organs. In one class of homozygote the developing eye has distorted optic cups and small lens vesicles, and goes on to develop coloboma and small eyes secondary to failure of optic fissure closure. In another category the optic cup and lens placode are not formed and there is anophthalmos (Franz and Besecke, 1991). The mutations have been mapped to chromosome 13 and are due to a deletion within the gene *Gli3*, which is a transcription factor and oncogene.

Small eye (*Pax6*) and ocular retardation (*Chx10*) are described later in this chapter.

Having identified a mouse mutant, the next task is to determine which chromosome is involved, the locus of any proposed genes, and the nature of any mutations responsible for a particular phenotype. In addition, detailed knowledge of the expression pattern of the gene products (proteins) at different stages of embryogenesis is required.

Returning to *Gli3* as an example, this gene is a candidate for the autosomal dominant human disorder Greig cephalopolysyndactyly syndrome (GCPS). Mutations in one allele of *Gli3* cause GCPS. Gli3 is widely expressed in human tissues and GCPS is characterised mainly by post-axial polydactyly of the hands and pre-axial polydactyly of the feet, craniofacial defects including macrocephaly and broad nasal root. Defects in heterozygous mice are not dissimilar (Vortkamp et al. 1991; Hui and Joyner, 1993). Similarities between mouse and human mutants can lead to important discoveries regarding the genetic aetiology of congenital eye defects, although in the case of GCPS there is no ocular abnormality.

That the term microphthalmos carries no clearly defined meaning in mouse molecular genetics is illustrated by consideration of the mouse microphthalmia (*Mi*) mutation. The eyes of the mutants develop poorly (small or absent eyes) because of defects in the retinal pigment epithelium, making the primary pathology abnormal retinal development. Other cell types affected are the neural crest derived melanocytes, which cause deafness due to lack of inner ear melanocytes. (Jackson and Raymond, 1994; Steingrimsson et al. 1994).

A human homologue has been identified for the mouse *Mi* gene, referred to as *MITF*. Mutations within *MITF* have been found in patients suffering from Waardenburg Syndrome type 2, a dominantly inherited syndrome associated with hearing loss and pigmentary disturbances such as a white forelock and iris colour defects. No microphthalmia exists in this condition.

That microphthalmia is a descriptive term applied so frequently in mouse developmental genetics, with little regard for the precise structural phenotype, is well illustrated when some of the genes controlling development of the lens, retina and optic nerve are considered.

As with humans, mouse eye conditions in which the primary ocular pathology is abnormal lens, retina or optic nerve development may have (or appear to have) smaller eyes, but only as a consequence of the primary ocular abnormality. The question we should be asking ourselves is:

Is it 'microphthalmia' or 'something else *with* small eyes'?

Several other important mouse genes and their human counterparts illustrate this confusion. Homeobox genes are important because they are highly conserved in the animal kingdom. Two such homeobox genes are *Chx10* and *Pax6*:

Very small eyes are produced in mice homozygous for the gene *ocular retardation (or)* (Truslove, 1962). It was noted subsequently that the most significant morphological pathology was the effect on the neuroretinal layer, degeneration of which somehow impairs the overall development of the eye. Ocular retardation is caused by a mutation in the *Chx10* gene (Burmeister et al. 1996). Although these mice are microphthalmic, the primary pathology is probably within the bipolar cells of the inner nuclear layer, along with absence of the optic nerves. This information was ascertained by careful examination of the expression pattern of the Chx10 protein in the developing mouse eye being localised to these cells. *Chx10* will be discussed in more detail in chapter nine.

Small eye: what's in a name? The _Small eye_ mouse and _Pax6_

Pax6 belongs to the paired-like class of developmental genes first described in *Drosophila* (Bopp et al. 1986). Mutations in the *Pax6* gene cause mouse small eye (*Sey*) (Hill et al. 1991). *Sey* is a semidominant mutation that in the homozygous condition results in complete lack of eyes and nasal primordia. Heterozygotes have a reduced eye size. However, of more significance may be the delayed closure of the optic cup in embryos that alters the shape of the opening. There is little further elaboration of the phenotypic description, and this is surprising, given that a more precise description would almost certainly enhance our understanding of the function of this gene and its protein, a transcription regulator. The other structural abnormalities described in the small eye mouse are failure of or incomplete separation of the lens from the cornea, lens abnormalities such as reduced size and vacuolation, and reduced anterior chamber size. The word coloboma is used to describe the

phenotype but, as is the case with clinicians in humans, the structural abnormality seen has not been made clear. It is likely that the coloboma is some form of defect of the iris or of the whole eye (Theiler et al. 1978). Observations on the small eye phenotype show that the iris is thin and diaphanous, i.e. like tissue paper (Jordan et al. 1992). This is not too dissimilar to the human aniridia phenotype and illustrates the point that small eye is a slight misnomer: a better term would be 'mouse aniridia' or 'mouse iris hypoplasia'. 'Small eye' tells us little, if anything, about the mouse ocular phenotype.

In humans, mutations of the *PAX6* gene cause aniridia (Ton et al. 1991; Jordan et al. 1992), as well as mutations being found in several other human eye conditions, each having relatively indistinct phenotypes (Hanson et al. 1994). The *PAX6* gene is of fundamental importance to this thesis, and will be discussed in more detail in chapter nine.

PAX2 is also a member of the *PAX* gene family, characterised by containing a paired box of highly conserved genes first identified in *Drosophila melanogoster.* The human *PAX2* gene maps to chromosome 10q24–q25. *PAX2* is expressed in the developing eye and kidney. Heterozygous mutations of the *PAX2* gene are associated with renal-coloboma syndrome (Sanyanusin et al. 1995; Sanyanusin et al. 1995). However, the phenotype associated with *PAX2* mutations shows a great deal of variability and it is possible that mutation of *PAX2* causes a proportion of isolated optic nerve colobomas (Cunliffe et al. 1998).

The second part of this chapter concerns one of these techniques that may help to identify the causative gene(s) for anophthalmos, microphthalmos and coloboma. This is the candidate gene approach.

THE CANDIDATE GENE APPROACH TO GENETIC AETIOLOGY

The different approaches to finding a genetic cause and locating a gene have been outlined. This section is an introduction to the candidate gene approach. A more detailed exploration of why particular candidate genes were selected is discussed in methods and results (chapter nine). Included in this section is some discussion of the current status of progress using the candidate gene approach and methodology.

How candidate genes can be used to consider the genetic aetiology: luck or judgement?

Phenotypic similarities are one of the most important factors when using the candidate gene approach to find the cause of a genetic abnormality. Identifying a disease gene can start with the assumption of a candidate gene: a particular gene is hypothesised to be the locus for the disease, and the hypothesis is then tested by checking for evidence that the candidate gene is associated with the disease.

The candidate gene approach may be based on particular properties of the product of the candidate gene which are consistent with its involvement in the pathogenesis, e.g. by appropriate gene expression pattern in embryological tissue sections demonstrated by in situ hybridisation against messenger RNA (mRNA). The candidate genes may be suggested for some disorders without knowing the subchromosomal location of the disease gene and/or candidate gene (position-independent candidate gene approach). However, the confidence in a particular candidate disease gene is increased if it can be shown to map to the same subchromosomal region as the disease gene (positional candidate gene approach). An example of the positional candidate gene approach is

the identification of the rhodopsin gene in autosomal dominant retinitis pigmentosa (Dryja et al. 1990).

If an animal phenotype shows a striking similarity to a human disorder, then it might result from mutations in the animal ortholog of the human disease gene. Knowledge of the animal ortholog can be used to help identify and characterise the human gene. This can then be tested for its involvement in the disease. The animal model may have originated spontaneously or have been created artificially (X-ray or chemical mutagenesis or gene targeting).

Mutations at more than one locus may produce the same clinical result. In some cases, the phenotypes caused by mutations in different genes can be distinguished, in others not (e.g. *PAX6*). If a gene is identified as the locus for one such disease, then genes loosely related to it in sequence or function may be candidates for closely similar diseases e.g. *PITX2*, *PITX3*.

Mouse models as an illustration of how to find the cause

Candidate genes can be used to establish the genetic basis and exact cause of developmental eye disorders. Using knowledge gathered from other species- *Drosophila melanogoster* and mice in particular, has made this possible. On morphological grounds the relationship between the human eye and the *Drosophila* eye is not immediately apparent, but from the developmental gene point of view, there are several important genes that are similar in sequence, i.e. there is structural and (probably) functional similarity. Such genes are highly conserved across several species (human, *Drosophila*, mouse, zebrafish) and are thought to be 'protected' on the basis of their fundamental importance.

Many developmental genes contain a segment of DNA called a homeobox. This DNA sequence (motif) encodes a DNA binding motif called a homeodomain that controls the production of mRNA by other genes at a precise time during development and tissue differentiation. Mammalian *PAX* genes were given their name because of sequence homology to the *Drosophila* segmentation gene *paired* (Bopp et al. 1986). To date, nine *PAX* genes have been identified in humans (Traboulsi, 1998). The *PAX* genes are characterised by a conserved DNA sequence, the paired box. Some *PAX* genes, such as *PAX6*, also contain a homeobox. The *PAX6* gene is homologous to human aniridia, mouse small eye and *Drosophila eyeless* genes. The *Drosophila eyeless* gene initiates eye formation (Halder et al. 1995).

Because of homology between humans, mice and *Drosophila*, *PAX6* and other conserved genes can be used to increase our understanding of the role of these genes in eye development. Having a gene which when mutated naturally or experimentally causes a known defect allows parallels to be drawn between different species and organisms. *PAX6* is therefore thought to have a fundamental role in the control of eye morphogenesis and terms such as 'the master control gene' are frequently used (Quiring et al. 1994; Halder et al. 1995). *PAX6* gene function (normal and mutant genes) can be studied in great detail by looking at its expression in various tissues (which implies function of some sort) in relation to different stages of development (Walther and Gruss, 1991; Nishina et al. 1999). The gene can be manipulated in *Drosophila* (Halder et al. 1995) or mice (Schedl et al. 1996); the production of model mutants or 'knockouts' furthers understanding of gene and protein function. However, one must be aware of the limits of the conclusions that can be drawn from such experiments (see earlier discussion in this chapter).

Therefore, the genes conserved in *Drosophila* and vertebrates such as mice are likely to be of great importance in human eye development. One then hopes to find or demonstrate naturally occurring eye mutations with a phenotype that is recognisable. Not all eye development genes fit these criteria, as the human gene may not have been discovered or recognised, but those that do are most amenable to intense study. Such genes are the first to become candidates for human eye conditions. It is likely that humans and mice have many important eye development genes that have not been found, or which are involved in other aspects of development e.g. *Rx* in *Drosophila* (see below).

There is a huge number of developmental genes recognised in *Drosophila*, mice and other vertebrates, whose function in humans has yet to be fully elucidated and which are the subject of intense interest and speculation. Examples are *Six3*, a murine homolog of the *Drosophila sine oculis* gene (Oliver et al. 1995), the *Rx* homeobox gene which is conserved in *Xenopus* and mice (Mathers et al. 1997), and mouse *Hox-7.1* and *Hox 8.1* which are related to the *Drosophila Msh-like* family (Monaghan et al. 1991). The *Sox1* gene is important in lens development, partly through its action on γ-crystallin gene expression. When homozygous mice have been targeted with mutations they are microphthalmic with abnormal and opaque lenses (Nishiguchi et al. 1998), yet another example of what is primarily a lens problem manifesting as the 'microphthalmos' phenotype.

Therefore, the first group of candidates are those mouse eye development genes in which naturally occurring eye mutations occur with a recognisable phenotype, and in which the orthologous human gene is known and mutations may have been identified (Table 3.1).

In the second group (Table 3.2) one can include mouse and vertebrate eye development genes in which the orthologous human gene is not yet known or in which human mutations have not been described. This table excludes several mouse mutants (described earlier, page 84) with no gene identified e.g. *myelencephalic blebs*. Listed in Table 3.2 are the strong candidate genes.

Table 3.1: Eye development genes with (natural) mutations in mouse and human

Gene (human location)	Group/Family	Mouse Gene Name	Mouse Phenotype	Human Gene And Ocular Phenotype
PAX2 (10q24-q25)	*PAX*	*Pax2 (Krd)* kidney and retinal defects (Sanyanusin et al. 1995)	(*Krd* transgenic mice) retinal hypocellularity, altered retinal function, kidney defects (Keller et al. 1994)	Renal-coloboma syndrome (Sanyanusin et al. 1995; Sanyanusin et al. 1995).
PAX6 (11p13)	Homeobox	*Sey, small eye*	Thin or absent iris, reduced eye size	Aniridia (Ton et al. 1991). *Plus other ocular phenotypes - chapter nine
MITF 3p12-p14.4	Basic-helix-loop-helix-leucine zipper (bHLH-ZIP)	*microphthalmia (mi)* (Steingrimsson et al. 1994)	Retinal pigment epithelial abnormalities, reduced eye size	Waardenburg's Syndrome (WS2). Iris colour defects (heterochromia) (Hughes et al. 1994).
SIX3 2p21	Homeobox (SIX/sine oculis)	*six3* (Oliver et al. 1995)	Not known	Holoprosencephaly, iris coloboma, persistent hyperplastic primary vitreous (Wallis et al. 1999)
PITX2/RIEG1 (4q25) (13q14) (Phillips et al. 1996)	*Bicoid*-related Homeobox	*Pitx2*	Knockout mouse heterozygote has variable, severe Rieger syndrome (Gage et al. 1999)	Axenfeld/Rieger's syndrome (Semina et al. 1996), IGDS, familial iris hypoplasia
PITX3 (10q25)	Homeobox	*Pitx3*	Not known	Autosomal dominant cataract, anterior segment dysgenesis (Semina et al. 1998)

Gene (human location)	Group/Family	Mouse Gene Name	Mouse Phenotype	Human Gene And Ocular Phenotype
Gli3	Zinc finger	*Extra toes* (Franz and Besecke, 1991)	Coloboma with small eyes, anophthalmos	Greig cephalopolysyndactyly syndrome: no eye defects (Vortkamp et al. 1991)
FKHL7 (FOXC1) (6p25)	Forkhead	*Mf1* (Kidson et al. 1999).	Anterior segment anomalies, hypoplastic trabecular meshwork and small Schlemm's canal	Anterior segment dysgenesis, Axenfeld-Rieger anomaly, Iridogoniodysgenesis anomaly (Gould et al. 1997; Mears et al. 1998)

Table 3.2: Eye development genes with mutations in mouse but no human mutation or phenotype

Gene	Family	Mouse Name	*Mouse Phenotype*
CHX10 (14q24)	Homeobox	Ocular retardation (Burmeister et al. 1996)	Optic nerve aplasia, thin hypocellular retina, reduced eye size.
SOX1 (13q34)	*Sox* family (Nishiguchi et al. 1998)		(Knockout mice) cataract, reduced eye size
DRES93 (*VAX2*) (Barbieri et al. 1998)	Homeobox, EST (expressed sequence tag)	Not known	Not known

Anterior Segment Dysgeneses

The embryology of the anterior segment was described in chapter one. Anterior segment dysgenesis (mesenchymal dysgenesis) is a group of closely related developmental eye disorders, with some overlap of phenotypic features (Churchill and Booth, 1996). Axenfeld, Peters' anomaly, Rieger's anomaly, and sclerocornea are all thought to be due to faulty migration or function of neural crest cells. Peters' anomaly is characterised by congenital corneal opacity with underlying defects in the posterior corneal stroma, Descemet's membrane, and endothelium. Iris synechiae are frequently present, passing from the collarette to the periphery of the corneal opacity, as well as keratolenticular strands. Half of patients with Peters' anomaly develop early onset glaucoma. Rieger's syndrome consists of Rieger's anomaly plus facial and systemic abnormalities: maxillary hypoplasia, hypodontia and peg shaped teeth, and periumbilical hernia.

The genes for anterior segment dysgenesis are autosomal dominant, fully penetrant, and variable in expressivity. All four anterior segment iris-hypoplasia disorders result in glaucoma in over half the cases.

The nomenclature and terminology used in describing the various anterior segment dysgeneses is (understandably) confusing. This stems from the fact that there is extensive phenotypic overlap (Shields et al. 1985). To further complicate the picture, genetic heterogeneity is now becoming apparent as more genes are identified and implicated as the cause underlying these eye defects.

Whilst the genes *PITX2, PITX3* and *FKHL7* may not be the cause of anophthalmos or microphthalmos, the extensive phenotypic overlap and variable expression of this group of developmental eye anomalies and the complexity of their genetics is well documented. Some of the findings are summarised below, with abbreviations in Box 3.2.

Box 3.2 Anterior Segment Dygeneses

ARA: Axenfeld-Rieger Anomaly ARS: Axenfeld-Rieger Syndrome IGDA: Iridogoniodysgenesis Anomaly IGDS: Iridogoniodysgenesis Syndrome

Both IGDA and ARA map to the same chromosomal location, 6p25, and are therefore possibly allelic (Gould et al. 1997). There is also the possibility that two or more genes are located at 6p25 and that mutations of the different genes underlie the related ARA and IGDA phenotypes. A major locus for development of the anterior segment of the eye, IRID1, is therefore located at 6p25. *FKHL7 (FOXC1)*, a member of the evolutionarily conserved forkhead/winged helix transcription factor gene family, has been mapped to 6p25. Mutations of this gene have been identified in some,

but not all, patients with Axenfeld-Rieger Anomaly. An additional locus involved in eye development is probably located at 6p25 (Mears et al. 1998).

The ARS gene has been mapped by linkage to 4q25 and mutations of this gene, *PITX2 (RIEG)*, a homeobox transcription factor gene, underlie the phenotype (Semina et al. 1996). IGDS has been mapped to 4q25 so this again raises the possibility that ARS and IGDS are allelic variants of the same disorder (Walter et al. 1996). A second locus for ARS has been mapped to 13q14 (Phillips et al. 1996).

PITX3 is a human homeobox gene and is the human homologue of the mouse *Pitx3* gene (Semina et al. 1998). It is a member of the *RIEG/PITX* homeobox gene family, and mutations have been found in families with anterior segment dysgenesis.

It is worth remembering here that the genetic aetiology of the anterior segment dysgeneses is not so straightforward, as *PAX6* mutations have been identified in some cases of Peters' anomaly (Hanson et al. 1994) (see chapter nine, *PAX6*).

Summary, chapter three

- Several different approaches to finding a genetic cause of eye malformations have been introduced. The reasons why these have not been fruitful or applicable to anophthalmos, microphthalmos and coloboma have been discussed.
- There is some overlap and misuse of terms used to describe different ocular phenotypes in mouse developmental eye disorders. This can make comparisons with the homologous human eye condition difficult.
- Some mouse eye anomalies and the associated gene have been described.
- Homeobox genes and the *PAX6* gene have been introduced.

- The candidate gene approach has been introduced. Some of the eye development genes with (natural) mutations in mouse and humans that could be investigated in this way have been listed. An important point is the similarity between an animal (usually mouse) phenotype and a human eye disorder.

This chapter has been an introduction. In the second half of this thesis, the practical application of the candidate gene approach on a phenotypically well-defined population is described is (chapter nine). The next chapter describes the methods used to obtain such a population.

CHAPTER FOUR

MATERIALS AND METHODS: OVERVIEW

'Mystery of babies with no eyes'

The Observer, January 1993 (Paduano et al. 1993)

In the introductory section, chapters 1–3, four key points were raised which form the main arguments of this thesis. In this second part, divided into six chapters consisting of four parallel studies, the data collected will be examined with reference to the four arguments. Each chapter and study is distinctive enough to stand alone with its own background, methods, results and discussion.

The introductory arguments in chapters 1–3 were:

1. **Anophthalmos/microphthalmos have not been defined adequately** (chapter one). The case was argued that there is no satisfactory definition. Data presented in chapter seven, the classification of the eye defects, and chapter eight, the study on axial length and corneal diameter, will demonstrate this.
2. **Anophthalmos/microphthalmos and coloboma have features that suggest both genetic and environmental aetiology** (chapter two)**.** Data presented in chapter six (description and classification of eye defects) and chapter eight (systemic abnormalities, family history, recurrence risk) will reinforce this argument.

3. **The correct investigation for any cause rests on establishing a structural/phenotypic definition** (chapter two). A classification based on a phenotypic definition is described in chapter six.
4. **The candidate gene approach can be used to consider the genetic aetiology of anophthalmos/microphthalmos and coloboma** (chapter 3). These investigations are described in chapter nine for *PAX6* and *CHX10*.

Background and history

In 1996 the Scottish Office Department of Health (Chief Scientist's Office) provided the funding for a two-year study into anophthalmos and microphthalmos in Scotland, The Scottish Microphthalmia Study. The chief investigator and grant holder is Dr Harry Campbell (Grant number K/OPR/2/2/D303).

The origins of the study date back to 1985, when the Scottish Home and Health Department produced the Bonnybridge/Denny Morbidity Review (Lenihan, 1985). In 1983, a farmer living near Denny (north west of Glasgow) reported unusual morbidity in his herd of dairy cattle. The farmer attributed this problem to atmospheric emission from a nearby chemical waste incineration plant (Re-Chem International Ltd). The chemical incineration plant was disposing of polychlorinated biphenyls (PCBs). In 1984, there were complaints by the public about the emissions having an adverse effect on their health. In May 1984 the figures for cancer registrations in the area of Stirlingshire covering the towns of Bonnybridge, Denny and Larbert were published in the press, showing an apparently greater rate of increase of cancer in this area (1977–81) than in Scotland as a whole. It was later in 1984 that two babies were reported as

being born within four weeks of each other, to parents living within a mile of each other, with similar but rare congenital eye defects.

In view of the public concern about these reports, on 26 June 1984 the Secretary of State for Scotland (House of Commons, 1984) set up an independent review of morbidity in the Bonnybridge/Denny area under the chairmanship of Professor John Lenihan:

> To review any unusual features of morbidity recorded in the Bonnybridge/Denny area and in the surrounding district; to take full account of other available studies of statistical variations in morbidity in areas of comparable size; to report on the significance of any abnormal findings and on any other relevant information that is available; and to advise whether further studies are required.

From 1976–1983 in the area studied (population 38,000) there was one reported case of microphthalmos, which was not particularly unusual, plus two recent cases of microphthalmos/anophthalmos in 1984. Three further cases were outside the study area but within the Forth Valley Health Board area between 1981 and 1984. Comparing this data with a 79% sample of Scotland as a whole, it was considered that the number of cases was 'sufficiently striking to merit further investigation' and that 'in view of the absence of an established cause in most cases of microphthalmos which have been reported in the literature, we suggest the possibility of hitherto unrecognised environmental factors should also be examined by comparing the incidence of microphthalmos in the Forth Valley Health Board area with the incidence in other areas where similar environmental conditions are likely to exist.' Although hospital consultants and general practitioners did not report unusual morbidity other than the incidence in 1984 of microphthalmos, it was advised that the full data for 1983 onwards should be monitored (Lenihan, 1985).

In 1984 in South Wales there was similar public concern about the Re-Chem industrial waste plant at Pontypool. The Secretary of State for Wales immediately instructed Welsh Office officials to examine health data for the Torfaen area, which lies within Gwent, similar to that which had been examined and published by the Scottish Office for the Denny/Bonnybridge area. The recorded incidence of anophthalmos and microphthalmos (1974–83), derived from OPCS notifications and Welsh Hospital Activity Analysis system (HAA), was lower than that for Wales as a whole. No cases were revealed for Torfaen (Welsh Office, 1985).

In response to the recommendations of the Lenihan report, in 1988 the Scottish Home and Health Department reported on microphthalmos in the Forth Valley Health Board Area (Scottish Home and Health Department, 1988). This was a working party under the chairmanship of Professor J. Strong. Prevalence data in the Forth Valley for microphthalmos, congenital cataract and coloboma were compared with five other health board areas. The data was obtained from several sources to maximise case ascertainment (Busby et al. 1998). The prevalence rate for microphthalmos in the Forth Valley (1971-1985) was comparable to the other areas. No clustering of cases was observed in the area of the chemical incineration plant. The highest prevalence rate occurred in Greater Glasgow, an area with the most highly organised recording system. The study concluded that the available data did not allow a firm conclusion to be drawn about the occurrence of microphthalmos in Forth Valley compared with other areas of Scotland. Of equal significance in the conclusion was that the difficulties of collecting sound information on a relatively rare and untreatable congenital abnormality affecting a small but very visible organ were clearly demonstrated. The present surveillance system in the neonatal period identified less

than a third of cases of microphthalmos and there was no national system for the consistent reporting of congenital abnormalities recognised after the neonatal period.

In 1993, newspaper reports (Paduano et al. 1993) of an apparent cluster of children with anophthalmos/microphthalmos or coloboma in North East Fife prompted an investigation by the Fife Health Board (Rowarth, 1995). The birth prevalence, using a variety of information sources, was relatively high, there being 22 cases observed against the 15 expected for the period studied (1981–1993). This report recommended further local study and continued monitoring of the birth prevalence of coloboma and/or microphthalmos in Fife.

The study on which some of this thesis is based came about as a result of continued public anxiety and some of the recommendations of the Forth Valley Area and Fife Health Board Reports (Scottish Home and Health Department, 1988; Rowarth, 1995). It was recommended that there should be continued monitoring of the birth prevalence of anophthalmos/microphthalmos and coloboma, and that data be recorded in such a way as to allow identification of cases notified from more than one source.

The Scottish Microphthalmia Study was the name chosen for this project, which was approved by all the relevant local research ethics committees and health boards. The Scottish Microphthalmia Study set out to achieve a number of objectives:

1. To identify all the cases of microphthalmos, anophthalmos, and coloboma (MAC) in Scotland from 1/1/81 to 31/12/96, using multiple sources of information. This was to include live births and stillbirths. The result would allow calculation of the birth prevalence.

2. It was hoped that if sufficient cases were identified, that the data would provide the basis of a future case control study into environmental aetiology (principal investigator and grant holder Dr Harry Campbell, University of Edinburgh).
3. To develop a simple case definition for registration purposes, applicable and understandable by non-specialists.
4. To consider the validity of defining microphthalmos based on axial length of the eye.
5. To produce a case register and get it established and running.
6. To examine clinically as many cases as possible and confirm or make an accurate ocular diagnosis, as well as documenting any systemic anomalies.
7. Where clinical examination is not possible, to confirm the ocular diagnosis as accurately as possible using case records and notes.
8. To examine the distribution of MAC in Scotland for the period 1981–1996 inclusive.
9. Where possible, to extract DNA from blood samples or mouthwashes, and to examine this DNA for mutations in the *PAX6* gene. In selected cases, to establish lymphoblastoid cell lines in Edinburgh or at the European Collection of Animal and Cell Cultures (ECACC) in Wiltshire, England for storage and transformation.
10. To calculate the recurrence risks for inheritance of anophthalmos, microphthalmos, or coloboma, for the purpose of counselling.
11. Collaborative studies: to examine selected DNA samples for *CHX10* mutations (Professor R. McInnes, Toronto, Canada). Other candidate genes identified by laboratories willing to undertake the analysis would also be considered.

Multiple sources of information were used for a number of reasons, the primary aim being to capture the entire population. This method provided data for further studies:

(i) To compare the accuracy and completeness of ascertainment for the different sources, and the completeness and quality of data held at the Information and Statistics Department (ISD) Scotland. Comparisons would also be made against the data from local registers, when permissible, such as the Glasgow Eurocat register.

(ii) To estimate by capture-recapture analysis the total population of MAC.

The following sources of information were used:

1. **The Scottish Morbidity Record (SMR1, SMR10 and SMR11)**

The data was provided with the permission of ISD Scotland, consisting of a list of 263 names. The SMR11 is the neonatal discharge record, SMR1 the hospital inpatient statistic, and SMR10 is the school health service medical record. This data was provided by ISD. Eye disorders are coded according to the World Health Organisation (WHO) International Classification of Diseases (ICD) 9. A search was made under the three codes: 743.0, 743.1 and 743.4 (Table 4.1). The search could have been extended to include 743.5 (congenital anomalies of the posterior segment) but the likelihood of this diagnostic code picking up any additional cases of true fundus coloboma would be almost nil (see chapter six results, eye findings), the more likely outcome being a huge number of 'false positives' from other unrelated conditions. Some of the conditions confused with fundus coloboma of the uveal tract were discussed in the three introductory chapters of part 1. Some of the conditions causing false positives are listed in the results section, chapter six.

Data from ISD Scotland included the following:

Case ID number

Surname

Case reference number: a code unique to hospital's record system

Sex (1 for male, 2 for female, 9 for unspecified)

Date of birth

Outcome description: alive, died 1–23 hours, died 1–6 days, died 1–3 weeks, stillbirth

Postcode

Hospital code

Area of residence (health board)

Diagnostic code (ICD-9): up to 12 codes listed

Contact with all children and their parents was always with the consent of the General Practitioner. This was important for several reasons, since the GP would be in a position to confirm that the child's date of birth and address were correct, that the child was still alive, and also make us aware if there were any special circumstances which might prevent the child taking part in the study, e.g. social or multiple health problems. The GP would also, from their records, confirm the ocular diagnosis. The child's address and the GP's name and address were identified by contacting the relevant health board. Letters to GPs were sent with reply-paid envelopes.

Individual children were traced systematically by a number of different methods. Closer inspection of the ISD tables and subsequent letters showed that errors in the recording of data, e.g. incorrect date of birth, misspelling of surname and duplicate entries, had occurred. This did create major problems in some cases. Other difficulties

were changes of surname, such as the child being recorded under the mother's maiden name rather than her married name.

Following a reply from the patient's GP, a letter was sent to the child's parents inviting them to take part in the study, with written consent forms for both parents and the child. All letters included reply-paid envelopes.

An appointment letter was then sent to the parents and child for examination at their local or nearest ophthalmology department, or at home in some cases. Use of ophthalmic outpatient clinics all over Scotland was by permission of the local hospital consultants and NHS trusts management.

Non-responders

All non-responders were sent a second letter by recorded delivery.

Table 4.1: Classification of congenital eye anomalies (reproduced from ICD-9)

743.0 Anophthalmos
Agenesis of eye
Cryptophthalmos
743.1 Microphthalmos
Aplasia of eye
Dysplasia of eye
Hypoplasia of eye
Rudimentary eye
743.4 Coloboma and other anomalies of the anterior segments
Aniridia
Anisocoria, congenital
Atresia of pupil
Coloboma of iris
Corectopia
Corneal opacity, congenital
Microcornea
Peters' anomaly
Rieger's anomaly
743.5 Congenital anomalies of the posterior segment
Coloboma: fundus
Coloboma: optic disc
Congenital retinal aneurysm
Congenital vitreous opacity

It is apparent that no specific code exists for every eye condition, and that the same condition may be coded differently. However, the search is wide enough to cover all the conditions in which the malformations of anophthalmos, microphthalmos or uveal coloboma might be the diagnosis.

ICD-10 (Table 4.2) is now in use (since April 1996) (World Health Organisation, 1977) and whilst this represents an improvement in terms of diagnostic specificity and more detailed coding (e.g. coloboma of iris has its own unique code), there is still overlap between the different codes.

Table 4.2: Classification of congenital eye anomalies (reproduced from ICD-10)

Q11 Anophthalmos, microphthalmos, and macrophthalmos
Q11.0 Cystic eyeball
Q11.1 Other anophthalmos
Agenesis of eye
Aplasia of eye
Q11.2 Microphthalmos
Cryptophthalmos *not otherwise specified*
Dysplasia of eye
Hypoplasia of eye
Rudimentary eye
Excludes: cryptophthalmos syndrome
Q11.3 Macrophthalmos
Excludes: macrophthalmos in congenital glaucoma
Q13 Congenital malformations of anterior segment of eye
Q13.0 Coloboma of iris
Coloboma *not otherwise specified*
Q13.1 Absence of iris
Aniridia
Q13.2 Other congenital malformations of iris
Anisocoria, congenital
Atresia of pupil
Congenital malformation of iris *not otherwise specified*
Corectopia
Q13.3 Congenital corneal opacity
Q13.4 Other congenital corneal malformations
Congenital malformation of cornea *not otherwise specified*
Microcornea
Peters' anomaly
Q13.5 Blue sclera
Q13.8 Other congenital malformations of the anterior segment of eye
Rieger's anomaly
Q13.9 Congenital malformation of the anterior segment of eye, unspecified
Q14 Congenital malformations of posterior segment of eye
Q14.0 Congenital malformation of vitreous humour
Congenital vitreous opacity
Q14.1 Congenital malformation of retina
Congenital retinal aneurysm
Q14.2 Congenital malformation of optic disc
Coloboma of optic disc
Q14.3 Congenital malformation of choroid
Q14.8 Other congenital malformations of posterior segment of eye
Coloboma of fundus
Q14.9 Congenital malformation of posterior segment of eye, unspecified

2. Ophthalmologists

A letter was written to each of the 75 consultant ophthalmologists in Scotland. The list was obtained from ISD Scotland.

3. Paediatricians

A letter was written to each of the 138 consultant paediatricians in Scotland. The list was obtained from ISD Scotland.

4. Clinical geneticists

A letter was written to each of the eight consultant and senior registrar clinical geneticists in Scotland.

5. Pathologists

A letter was written to each of the consultant pathologists in Scotland. The list was obtained from ISD Scotland.

6. Publicity campaign

A press release was issued in February 1997 via the University of Edinburgh to publicise the research. Dr Harry Campbell was interviewed on the two main Scottish evening news terrestrial TV channels, as well as on Radio Scotland. Articles appeared in the *Herald* and the *Edinburgh Evening News* (Robertson, 1997).

7. Micro and Anophthalmic Children's Society (MACS)

This national support group was written to and informed of the study. Parents and children who contacted us were volunteers with fully informed consent.

8. Directors of education of special schools

For each health board, the directors of education of all special schools for all the Scottish local education authorities were contacted. The 34 head teachers and/or official correspondents of the grant-aided special needs schools were contacted.

9. Blind registration data (BP1)

Record cards were searched by hand for two health boards (Lothian and Borders).

10. Glasgow ophthalmology outpatients

Permission was granted from the Sick Children's Hospital in Glasgow to search the diagnostic codes (ICD-9) for the outpatient clinics.

11. Glasgow Department of Clinical Genetics

Access was granted to search the computerised database and examine records of patients seen with eye complaints within this department.

12. Royal Blind School, Edinburgh

With permission, the records were searched of Scotland's only blind school, with further assistance from the medical (Dr Brian Fleck, Consultant Ophthalmologist, Royal Infirmary of Edinburgh) and nursing staff. Subjects were contacted via their GP.

13. School clinical medical officers

A total of 59 school clinical medical officers were written to.

Data Collection

Data collection form

Because of the wide brief of the study, the data collection forms had to be developed to serve many purposes. The forms were also to be used in cases where information may have been extracted from medical case notes, such as in neonatal deaths.

Therefore, some of the questions asked may at first glance seem irrelevant (some examples are given below). A copy of the 17-page data collection form is in the Appendix.

Features of note on the data collection form:

Page 1: The study was on children born in Scotland. Both the address at birth registration and the hospital of birth were included (page 3).

Page 3: Mothers were specifically not asked about illnesses before or during pregnancy or medications taken in order not to prejudice the validity of the questionnaire for the future case control study into environmental factors.

Study identification number

All children notified to the study were assigned a unique identification (ID) number.

Photographs

Where possible, and with written consent, each child had a basic set of photographs taken: eyes, face, profile, hands and feet. Additional photographs were of any congenital anomalies noted and to confirm/make any difficult diagnoses or dysmorphological comments (Dr David FitzPatrick, Clinical Geneticist).

Blood samples and mouthwashes

Where possible, and with written consent, a sample of venous blood was withdrawn for DNA extraction and/or to create a lymphoblastoid cell line (see chapter nine). In some cases, blood was also sent for chromosome analysis. Alternatively, a mouthwash was obtained for DNA extraction from buccal mucosal cells (see chapter 9).

Ultrasound scans

Where possible, an ultrasound B scan was done to determine the axial length of both eyes. The measurements were taken as part of an investigation into eye size and the definition of microphthalmos (chapter seven).

Not all hospitals or clinics had ultrasound B scanners available; scans were done in Edinburgh (Princess Alexandra Eye Pavilion), Dunfermline (Queen Margaret's Hospital), Glasgow (Yorkhill), Dundee (Ninewells), Aberdeen (Royal Infirmary). In Glasgow, the late Dr Anne Hollman, Consultant Radiologist, did B scans in the X-ray department.

Ocular diagnosis

Children were recruited into the study based on a prior medical diagnosis of anophthalmos, microphthalmos, or coloboma. On completion of the eye examination or review of medical notes, an accurate phenotypic clinical diagnosis was made if possible. In some cases, this meant excluding the child from the study on the basis of the eye abnormality not being clinical anophthalmos, microphthalmos, or coloboma. The colobomas did include both uveal and optic nerve 'colobomas' (see chapter one). Based on knowledge before the start of the study, the following congenital eye conditions were excluded (Box 4.3):

Box 4.3: Congenital ocular malformations associated with microphthalmos (see chapter 1 page 37)

Anterior segment dysgenesis
Cataract
Corneal opacification/cloudy corneas
Peters' anomaly
Persistent hyperplastic primary vitreous
Retinopathy of Prematurity
Retinal folds
Rieger's Anomaly
Sclerocornea

The ocular diagnosis and reasons for exclusion were documented. The collected data is summarised in the next chapter (results, chapter five).

DISCUSSION

The methods used to collect the data were as comprehensive as possible, from as many sources as could be identified. Blind registration data was searched through for only two health boards. These records had to be searched manually and the pick up from this source is extremely low since only bilaterally affected cases with low vision are listed (see chapter two) (Evans, 1995). Additional cooperation from some regions or departments (e.g. Glasgow) did not necessarily produce further cases.

There are difficulties with using different sources, some of which are national and some local. Not all sources have an equal chance of identifying an affected individual. The clinical examination and review of medical records ensured that duplicate dates of birth or errors resulting from changes of name were eliminated, thus avoiding multiple inclusion of the same individual. Some of these issues are considered in a separate publication (Campbell et al. 2002).

Summary, chapter four

- There have been major concerns about chemical hazards being a possible cause of congenital eye anomalies in Scotland since 1983 when a farmer in Bonnybridge noticed some increased morbidity in his cattle.
- The Scottish Microphthalmia Study arose from continued public anxiety and some of the recommendations of the Forth Valley Area Report and Fife Health Board Report, which recommended that there should be continued monitoring of the birth prevalence of anophthalmos/microphthalmos and coloboma.
- The multidisciplinary nature of the study is emphasised.
- An outline of the methods of case ascertainment is described, followed by the methods of data collection and documentation.

The next chapter (five) is an overview of some of the study results.

CHAPTER FIVE

RESULTS: OVERVIEW

'Future epidemiological studies on anophthalmia and microphthalmia should include the detailed ophthalmological and dysmorphological assessment of affected patients and their families to define cases accurately and identify those of known cause. Such assessment would also provide the opportunity to investigate chromosomal and genetic factors, with potential benefits for genetic counselling and antenatal diagnosis.'

(Gilbert, 1993)

This thesis was the first national clinical population study of anophthalmos/microphthalmos and coloboma.

Summary

A total of 152 Scottish children were clinically examined. Of these, 122 were included in the study and this group formed the subset for detailed analysis. A further 61 children are believed to be affected, based on examination of clinical records.

The total number of children affected is believed to be 183.

135 children were excluded from the study, based on clinical examination or detailed review of records.

The combined minimum birth prevalence of anophthalmos, coloboma and microphthalmos in Scotland for the period 1981–1996 was 1.19 per 10,000 live births. This birth prevalence increases to 1.98 per 10,000 if the cases not clinically confirmed are included.

There were seven children with clinical anophthalmos. The birth prevalence of anophthalmos for the period 1981–1996 was 0.68 per 100,000 live births.

Birth prevalence was highest in the health board region of Fife (2.59 per 10,000).

Birth prevalence was lowest in the Western Isles (0 per 10,000).

28% of children were registered as blind. More than half of the children aged three or over had special educational needs.

RESULTS

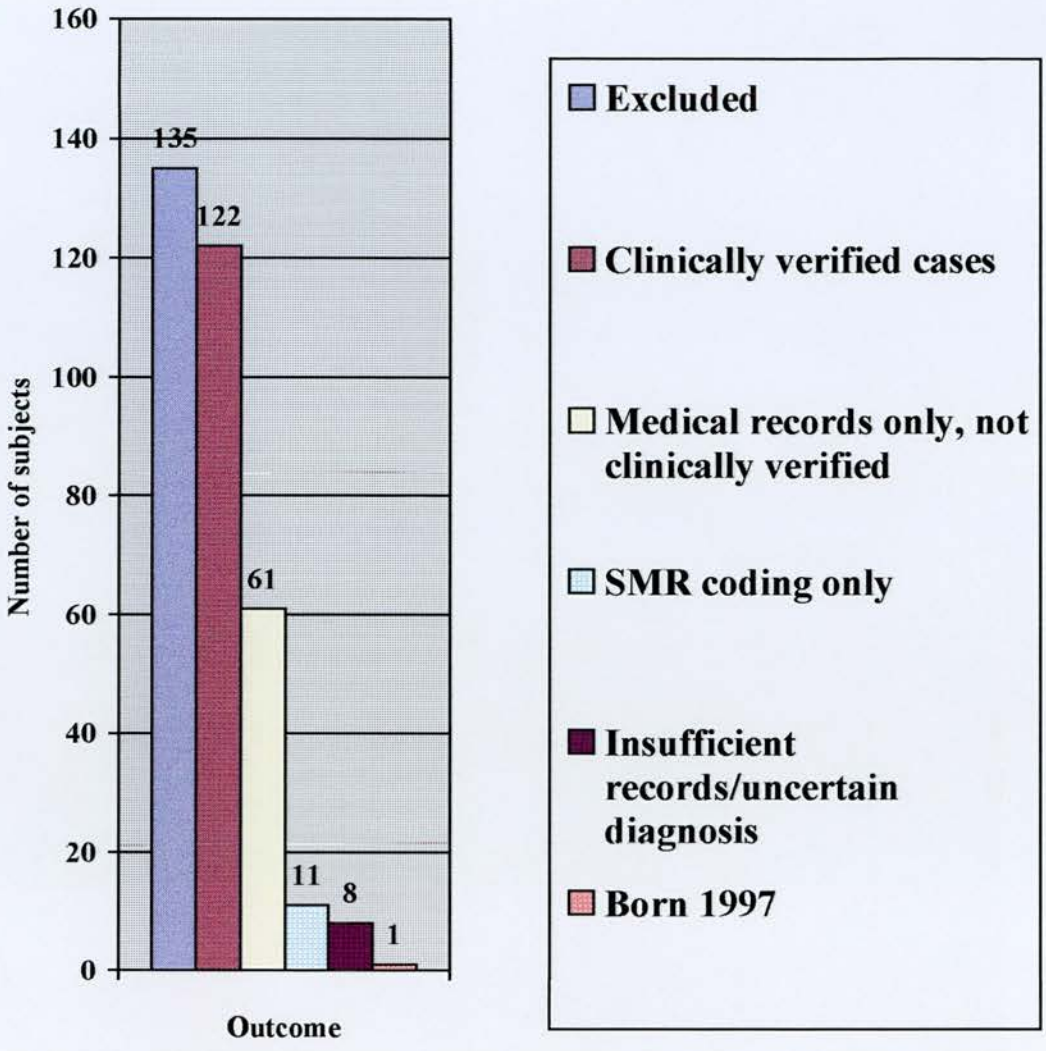
The study continues to add names to the database. The database is continuous and allows diagnoses to be verified clinically and not to be based solely on medical notes (general or ophthalmic records).

At the end of February 1999, 338 ID notifications of possible cases were in the database (Table 5.1). The total number of affected children assigned an ID number by the end of February 1999 was 203. The total number excluded from the study by the end of February 1999 was 135. All children were born between 1 January 1981 and 31 December 1996. The breakdown was as follows and is summarised in Figure 5.1.

Table 5.1: Status of 338 notifications to study in February 1999

Total	**338**
Excluded from study	135
Clinically examined	122
Medical records only, not clinically confirmed	61
SMR coding only	11
Insufficient records/uncertain diagnosis	8
Born 1997	1

Figure 5.1: Status of 338 notifications to study in February 1999



123 cases were examined in detail by the author. One child, ID 271, was born in 1997 and was examined because the referring consultant was particularly interested in the study. This child is included in the table but not in any analysis of prevalence. Some clinical information is used from this case.

The 122 children form the subset that was subjected to detailed analysis.

In another 61 cases in which only records were available, the notes were examined by the author (DM) (Table 5.2).

Table 5.2: Ocular diagnosis by records only

ID	Date of birth	Ocular diagnosis from records; comments
2	22 September 1983	right iris coloboma
10	17 December 1988	optic disc coloboma
12	26 October 1990	bilateral iris coloboma/cataracts; Patau's syndrome (trisomy 13)
13	22 January 1992	left ocular malformation and retinal atrophy
21	28 July 1991	bilateral iris/fundus colobomas; microcephaly, phenylketonuria
25	26 July 1991	right iris and simple retinal coloboma
27	04 July 1985	bilateral iris and simple retinal colobomas; multiple congenital anomalies
28	23 June 1990	coloboma left iris, sib affected (ID 32)
31	31 August 1987	bilateral microcornea/microphthalmos, corneal opacities, ?PHPV
32	08 November 1985	bilateral uveal coloboma, right microphthalmos; sib affected (ID 28)
46	28 September 1993	optic disc colobomas; CHARGE association
49	29 October 1991	right cataract/microphthalmos, retinal pigment/scar; foetal varicella
56	12 May 1989	left iris and retinal coloboma
68	09 April 1994	right iris coloboma, bilateral retino-choroidal coloboma; microcephaly
71	26 November 1985	bilateral iris and right retinal coloboma
72	29 December 1987	bilateral anophthalmia; delayed development
74	03 July 1989	right cataract with microphthalmos, squint
78	16 April 1994	bilateral uveal/fundus colobomas/microphthalmos; CHARGE association
82	25 May 1990	right microcornea/microphthalmos, detached retina

ID	Date of birth	Ocular diagnosis from records; comments
88	09 July 1995	right iris coloboma
95	18 February 1994	small right eye, no vision right eye, cataract
103	02 January 1987	microphthalmos (congenital toxoplasmosis)
108	14 November 1991	bilateral colobomas/microphthalmos, right enucleation
112	14 November 1992	bilateral optic nerve head colobomas
116	07 February 1984	right microphthalmos
118	17 May 1988	right chorioretinal scarring and inferior coloboma
128	10 January 1996	bilateral uveal coloboma, microphthalmos; CHARGE/VATER association
130	29 May 1983	bilateral iris colobomas only.
136	23 November 1984	right microphthalmos
139	04 August 1996	complicated microphthalmos
149	28 February 1990	left iris/fundus coloboma
154	01 May 1994	right iris and fundus coloboma; spina bifida
157	29 March 1981	a 'degree' of microphthalmos, fetal alcohol syndrome
160	03 August 1982	left microphthalmos
161	05 May 1983	bilateral iris and fundus colobomas, detached retina
163	29 July 1996	microphthalmos
173	16 May 1991	bilateral microphthalmos; bilateral acetabular dysplasia
176	28 March 1992	bilateral aphakia, right squint, microphthalmos
184	10 July 1995	left coloboma
191	15 October 1994	right microphthalmos
193	08 May 1981	small right eye, coloboma
195	14 March 1983	left iris/fundus coloboma; delayed development, dysmorphic
196	07 December 1983	bilateral fundus coloboma/microphthalmos; patent ductus arteriosus, delayed development
198	07 July 1983	coloboma right eye
207	25 June 1996	left iris coloboma

ID	Date of birth	Ocular diagnosis from records; comments
208	25 February 1982	cataract, superior left iris coloboma, microphthalmos
211	11 July 1981	microphthalmos; ventriculoseptal defect
227	17 January 1984	bilateral retinal dysplasia; microcephaly
229	09 February 1987	left microphthalmos ?PHPV
231	10 August 1985	right retinal coloboma, left microphthalmos
248	16 July 1994	bilateral optic disc coloboma
266	21 October 1992	left eye coloboma
278	23 August 1991	right microphthalmos
281	27 July 1996	left iris/fundus coloboma; congenital heart disease
289	06 April 1993	left squint, microphthalmos
291	03 December 1995	bilateral iris/chorioretinal coloboma
341	08 July 1993	right iris/optic disc coloboma. left hypoplastic disc
343	25 March 1995	small retinal coloboma left eye
344	13 July 1995	left inferior chorioretinal coloboma
348	03 July 1996	iris coloboma; Noonan's syndrome
352	07 July 1989	right retinal dystrophy, microphthalmos

In 11 more cases, the diagnosis was derived only from the diagnostic coding, and was not verified clinically or from records.

A further 8 cases were referred from other sources, or the GP replied but with insufficient or no clinical data.

Total 122 + 61 +11+ 8= 202

Six deceased individuals are amongst the two groups (four from the SMR and two from the other sources.

Therefore, of the 202 subjects, 122 are definitely affected as these have been clinically verified. This proportion increases by 61 to 183 of the 202, if the diagnostic records

examined are accurate. These 61 records were only included because there was little doubt that the diagnosis was correct. In nearly all cases the information was obtained from the comments and notes based on examination by a consultant ophthalmologist.

There were several reasons why it was not possible for some children to be examined, and these ranged from subjects not giving consent, which was rare, to failure to attend an appointment on more than one occasion (Table 5.3). In some cases, although consent for a clinical examination was not given, permission was granted for access to medical records.

Table 5.3: Reasons for children not having a clinical examination (total 81)

28	No reply to letter
11	Untraced (from SMR)
10	Refusal
6	Resident in England (no consent to contact)
6	Untraced
6	Did not attend appointment
6	Deceased
3	GP refusal to approach patient
3	Refusal but with permission to examine records
2	Search still in progress
81	**Total not clinically examined**

A number of children are in Table 5.1 on the basis of the SMR coding only, no other information having been obtained. The SMR diagnostic codings are unreliable in both quality and quantity (see comments below on ascertainment). The majority of the exclusions (Table 5.4) were obtained from the SMR data from ISD Scotland, and the actual ocular diagnosis can differ quite significantly from the SMR coding.

In addition to the above, 135 names were excluded from the study on the basis of the ocular diagnosis not being microphthalmos, anophthalmos or coloboma (definition

below). These are listed in Table 5.4. Of the 135 exclusions, the author clinically examined 29. In 24 cases both eyes were normal with no congenital abnormality. A further 9 children were born and registered outside Scotland, usually in England.

Table 5.4: Exclusions from study

ID	Date of birth	Ocular diagnosis; comment	Normal eye examination	Examined by DM
7	October 27, 1992	blepharophimosis, myopia		Examined by DM
9	February 4, 1992	corectopia and posterior embryotoxon		Examined by DM
11	September 18, 1983	born in England		
14	August 9, 1993	Peters' plus: multiple and severe congenital abnormalities		
16	January 26, 1992	Rieger's syndrome and congenital glaucoma; mother also affected		
22	November 10, 1984	born in England		
23	August 12, 1985	retinopathy of prematurity, born 26 weeks, both eyes small		
24	May 31, 1986	retinopathy of prematurity, born 25 weeks, is blind		
36	January 20, 1993	aniridia		
41	August 31, 1994	bilateral corneal opacities		Examined by DM
42	August 12, 1991	congenital glaucoma		
43	July 24, 1996	normal eyes	normal eyes	
44	October 20, 1990	optic nerve hypoplasia, septo-optic dysplasia		
52	July 20, 1993	narrow palpebral apertures	normal eyes	
54	August 21, 1986	anterior segment dysgenesis, familial		
57	June 6, 1988	anterior segment dysgenesis (Rieger's-like), congenital glaucoma		Examined by DM
59	May 15, 1982	left congenital cataract		Examined by DM
65	January 22, 1993	born in England		
69	March 19, 1988	no eye problems at all; brother has Rieger's	normal eyes	
73	August 21, 1986	anterior segment dysgenesis, familial		
80	January 4, 1993	Rieger's anomaly		
81	March 17, 1993	corneal ulcer/anterior segment dysgenesis		Examined DM
86	June 7, 1991	squint, ptosis; dysmorphic		Examined by DM

ID	Date of birth	Ocular diagnosis; comment	Normal eye examination	Examined by DM
87	January 13, 1984	Peters' anomaly		Examined by DM
92	November 15, 1992	septo-optic dysplasia; hypothalamic hypopituitarism		
98	December 6, 1994	squint		
99	August 19, 1994	facial asymmetry, body asymmetry, toe abnormalities	normal eyes	Examined by DM
104	February 5, 1989	left corneal/conjunctival/scleral dermoid, eyelid coloboma		Examined by DM
105	March 1, 1994	optic nerve hypoplasia, septo-optic dysplasia		
110	October 31, 1993	pale optic discs, unusual choroidal vessels		
114	March 15, 1986	retinopathy of prematurity, born 26 weeks		
119	July 7, 1984	bilateral corneal scarring and maculopathy		
120	September 16, 1982	born in England		
123	March 27, 1983	nystagmus, optic nerve hypoplasia, squint		
124	June 15, 1990	Rieger's anomaly		
125	June 9, 1990	not born in Scotland		
127	August 28, 1982	unilateral Peters' anomaly		Examined by DM
131	January 8, 1984	blue sclera; osteogenesis imperfecta	normal eyes	Examined by DM
133	March 12, 1984	Peters' anomaly, left eye enucleated		
135	December 7, 1984	epibulbar dermoid; Goldenhar's		
137	March 5, 1985	left convergent squint		Examined by DM
141	March 20, 1986	no eye abnormality	normal eyes	
143	May 1, 1986	whites of eyes were greyish at birth only	normal eyes	
144	July 8, 1986	Norries syndrome		
146	November 26, 1986	right cataract, probably mild form of persistent hyperplastic primary vitreous		
147	August 30, 1988	anterior segment dysgenesis		
148	February 24, 1988	had cyst on left eyeball at birth	normal eyes	Examined DM
151	January 9, 1993	optic nerve hypoplasia		Examined by DM
152	February 8, 1993	aniridia bilaterally		Examined by

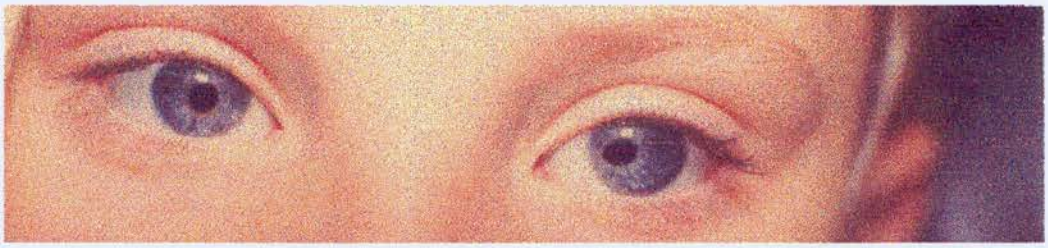
ID	Date of birth	Ocular diagnosis; comment	Normal eye examination	Examined by DM
				DM
156	September 6, 1990	right vitreous opacity and right squint		
158	November 12, 1981	no eye abnormality; Robinow's syndrome	normal eyes	
164	November 22, 1984	cortical blindness		
165	March 8, 1985	retinopathy of prematurity		
167	January 8, 1985	corneal opacity		
168	November 21, 1985	anisocoria, anisometropia, ptosis		
169	August 23, 1986	born in England		
171	October 1, 1987	optic nerve hypoplasia		
177	December 3, 1992	congenital glaucoma, Axenfeld/Rieger's		
178	March 17, 1994	retinopathy of prematurity, high myopia		
179	January 7, 1994	aniridia		
180	September 29, 1995	no eye problem	normal eyes	
185	May 4, 1992	prematurity, cataract, probably persistent hyperplastic primary vitreous		Examined by DM
194	May 5, 1982	familial aniridia (sib ID 197)		
197	December 31, 1983	familial aniridia (sib ID 194)		
201	December 30, 1985	anterior segment dysgenesis		
202	February 3, 1987	sporadic aniridia		Examined by DM
203	March 25, 1987	nystagmus, squint		
205	March 16, 1990	sporadic aniridia		Examined by DM
209	January 21, 1994	optic nerve hypoplasia, right convergent squint.		
212	October 13, 1986	anterior corneal staphyloma		
215	December 13, 1989	Axenfeld's anomaly, congenital glaucoma		
218	September 29, 1990	corneal dystrophy		
221	October 6, 1991	congenital glaucoma		
223	January 20, 1993	right third cranial nerve palsy		
225	February 26, 1994	eyelid coloboma and angular dermoid		
226	May 18, 1983	left eyelid ptosis		
235	April 7, 1987	aniridia		
237	September 20, 1986	Rieger's anomaly; Hirschprung's, chromosome translocation (4, 13).		Examined by DM
240	February 4, 1992	no eye abnormality	normal eyes	Examined by DM

ID	Date of birth	Ocular diagnosis; comment	Normal eye examination	Examined by DM
245	July 16, 1987	blepharophimosis syndrome		
246	June 9, 1988	aniridia (familial)		
247	October 15, 1994	optic nerve hypoplasia, septo-optic dysplasia		
250	November 21, 1988	born in England		Examined by DM
255	February 28, 1992	not born or conceived in Scotland		
256	December 6, 1982	optic nerve hypoplasia, septo-optic dysplasia		
257	September 8, 1990	corectopia		
259	August 27, 1985	bilateral optic atrophy, multiple congenital abnormalities		
260	September 16, 1982	septo-optic dysplasia		
265	February 20, 1981	septo-optic dysplasia		
270	April 28, 1988	cortical blindness		
273	July 14, 1987	anterior segment dysgenesis, no pupil, secondary glaucoma		Examined by DM
274	February 23, 1988	unilateral congenital cataract		
279	June 8, 1985	retinopathy of prematurity, born 28 weeks, left cataract, detached retina		Examined by DM
280	January 18, 1985	no eye abnormality	normal eyes	
283	November 29, 1989	retinopathy of prematurity		
285	April 22, 1991	retinopathy of prematurity left eye		
286	November 20, 1993	prematurity, born 26 weeks		
288	December 1, 1992	retinopathy of prematurity, born 28 weeks, myopia and astigmatism		Examined by DM
292	August 30, 1992	astigmatism and refractive ambylopia left eye		
298	July 6, 1982	not born in Scotland. Left iris/ fundus coloboma		Examined by DM
300	December 24, 1985	aniridia (familial)		
301	August 22, 1987	no eye abnormality	normal eyes	
302	February 16, 1993	optic nerve hypoplasia, holoprosencephaly, micropcephaly		
303	May 27, 1992	Rieger's anomaly		
304	October 23, 1982	no eye abnormality	normal eyes	
305	September 25, 1986	no eye abnormality	normal eyes	
306	October 15, 1981	no eye abnormality	normal eyes	
307	March 12, 1984	no eye abnormality, had a sticky eye at birth	normal eyes	
308	August 10, 1989	no eye abnormality	normal eyes	
309	February 25, 1993	optic nerve hypoplasia, cerebral palsy		

ID	Date of birth	Ocular diagnosis; comment	Normal eye examination	Examined by DM
310	February 13, 1995	no eye abnormality	normal eyes	
311	October 30, 1995	not affected		
312	November 28, 1987	narrowed palpebral aperture		
313	September 12, 1995	limbal dermoid cyst		
314	December 7, 1984	no eye abnormality	normal eyes	
315	March 30, 1990	no eye abnormality	normal eyes	
316	August 25, 1985	not affected		
317	November 21, 1984	aniridia (familial)		
319	September 17, 1985	premature, born 28 weeks, has cataracts		
320	July 7, 1983	premature, no ocular abnormality		
321	June 22, 1987	septo-optic dysplasia; holoprosencephaly		
322	October 28, 1985	no eye abnormality	normal eyes	
323	October 2, 1981	iris heterochromia		
336	June 26, 1989	aniridia		
339	December 6, 1982	Rieger's/glaucoma (familial)		
340	June 6, 1988	no eye abnormality	normal eyes	
342	September 21, 1986	born in England, bilateral iris coloboma, brother affected (ID 267)		
345	July 21, 1988	aniridia		
346	March 14, 1996	no eye abnormality	normal eyes	
347	May 24, 1996	aniridia		
349	September 9, 1996	Peter's anomaly		
350	November 11, 1996	no eye abnormality		
351	July 31, 1996	aniridia/glaucoma		
353	December 9, 1984	anterior segment dysgenesis, familial		
356	August 29, 1985	born in England, optic disc coloboma, familial, sister of ID 102		Examined by DM

Common diagnoses in those excluded from the study included aniridia, anterior segment dysgenesis (Peters' or Rieger's anomaly), retinopathy of prematurity and strabismus (squint). Corectopia is illustrated in Figure 5.2.

Figure 5.2: Corectopia left pupil (ID 9, excluded from study)



It will be recalled from the last chapter that SMR diagnostic codes were used to search for these eye conditions and that in the case of coloboma, a search was made under ICD9 743.4. This includes 'Coloboma and other anomalies of the anterior segments'. It is therefore not surprising that so many major and minor disorders affecting the front of the eye are included in the exclusions (Table 5.4). Another notable feature which is not surprising is the number of rare and unusual congenital abnormalities affecting the retina or optic nerve and therefore only visible with an ophthalmoscope. An example of this is that there were more than a dozen cases of optic nerve hypoplasia. It is reasonable to expect that at the time these abnormalities in very young babies were first suspected and the names placed on diagnostic registers, to make a more precise clinical diagnosis without detailed specialist opinion would have been difficult. In clinical practice, an examination under anaesthesia is sometimes required.

Ascertainment of cases and sources of information (chapter four)

The levels of case ascertainment and the effectiveness of the different sources are beyond the scope of this thesis and will form the basis of a separate publication. Cases were obtained from all sources. Most of the cases were obtained from the SMR data, ophthalmologists and paediatricians. There was considerable overlap between

sources. It should also be noted that some sources were more reliable than others in the quality or accuracy of the data obtained, and this is not surprising. Also, not all sources have an equal chance of capturing a case. Blind registration data can only yield those children with bilateral and significant visual impairment. These points have been considered in a publication on capture-recapture (Campbell et. al, 2002).

The birth prevalence is similar to that obtained in previous studies and suggests that case ascertainment was extremely high.

Birth prevalence of microphthalmos, anophthalmos and coloboma (MAC)

This was estimated for Scotland as a whole and for each of the 15 health boards. The total number of live births (all genders) in Scotland from 1981–1996 was 1 023 027. Based on the lower figure of clinically validated diagnoses (122), the minimum birth prevalence of MAC in Scotland over this period is 1.19 per 10,000. The birth prevalence of anophthalmos (n=7) was 0.68 per 100,000 live births. The figures are comparable with other studies (chapter two, Table 2.1 page 46). (Data for live births from ISD, Scotland). No corrections have been made for births within the same family.

If the higher figure of 202 cases is used the birth prevalence is 1.98 per 10,000 live births. Based on 183 validated diagnoses, including 61 records (but excluding those with only SMR codes), birth prevalence overall is 1.78 per 10,000 live births.

Further analysis of 122 cases examined clinically

The minimum birth prevalence based on 122 is 1.19 per 10,000. The birth prevalence for each of the 15 health boards ranged from 0.74 to 8.02 per 10,000 live births

(Table 5.5). The unusually high figure in the Shetland Isles, where the total number of live births was 4,982, is accounted for by 3 of the 4 affected children being siblings. In Fife, the health board region that has attracted so much attention, the overall birth prevalence was 2.58 per 10,000 live births. The figure is reduced to 2.44 per 10,000 when the two affected sibs are counted as one, reducing the number of confirmed affected children from 18 to 17. In view of such intense publicity and attention in this area, it is no surprise that a high number of cases were found.

No affected children were reported or found in the Western Isles (total number of births 4,909). Study questionnaires and letters were returned from this region. The small population of this region is the most likely explanation for the complete absence of cases.

Table 5.5: Birth prevalence of microphthalmos, anophthalmos, and coloboma in Scotland 1981–1996, by health board

Health board area	Number of clinically verified cases	Number of live births	Prevalence per 10,000 live births (95% confidence interval)
Argyll and Clyde	9	88 812	1.01 (0.35–1.67)
Ayrshire and Arran	11	73 724	1.49 (0.61–2.37)
Borders	2	17 844	1.12 (0.00–2.67)
Dumfries and Galloway	6	26 956	2.23 (0.45–4.01)
Fife	18	69 531	2.59 (1.40–3.78)
Forth Valley	9	52 782	1.71 (0.60–2.28)
Grampian	9	103 007	0.87 (0.30–1.44)
Greater Glasgow	16	196 027	0.81 (0.42–1.20)
Highland	3	40 653	0.74 (0.00–1.58)
Lanarkshire	9	116 920	0.77 (0.27–1.27)
Lothian	11	147 642	0.75 (0.31–1.19)
Orkney	1	3 745	2.67 (0.00–7.90)
Shetland	4	4 982	8.03 (0.16–15.90)
Tayside	14	74 461	1.88 (0.90–2.86)
Western Isles	0	4 909	0
Scotland	**122**	**1 023 307**	**1.19 (0.98–1.40)**

Distribution of MAC births by year (Table 5.6 and Figure 5.3)

There are about 60,000 live births each year in Scotland, the numbers having fallen slightly since 1981 (source: Registrar General's Office, Scotland). In this study, the birth prevalence of MAC was lowest in 1988 and highest in 1993 (0.60 and 2.23 per 10,000 live births respectively).

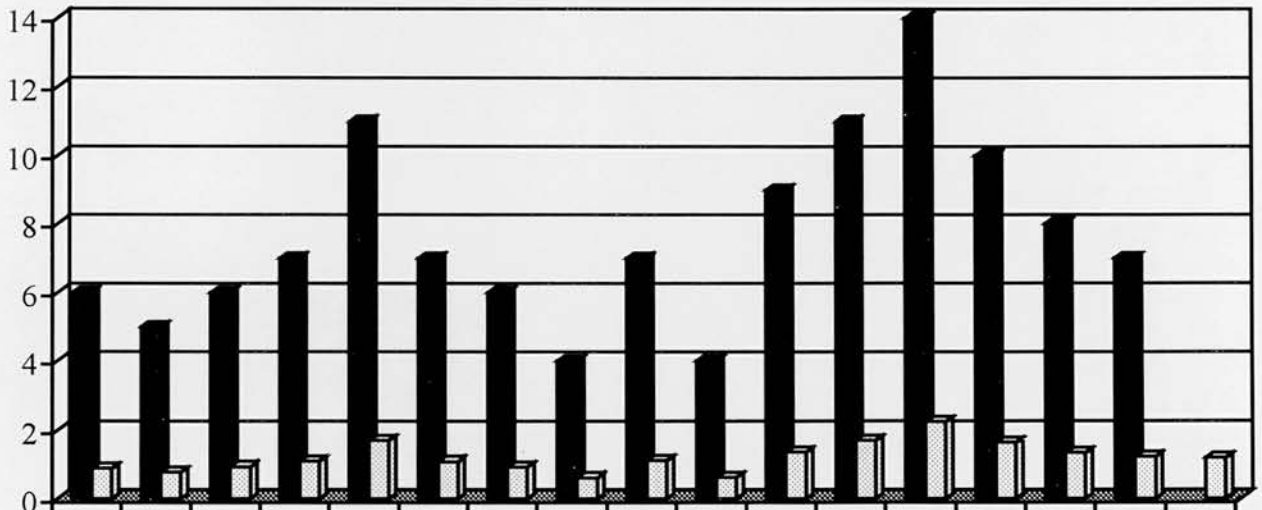
The distribution of births by year is shown in Table 5.6. A significant number of cases was ascertained from every year back to 1981 and the maximum number from 1993,

the year in which news of anophthalmos and microphthalmos hit the national papers (Paduano et al. 1993).

Table 5.6: Birth prevalence of microphthalmos, anophthalmos, and coloboma in Scotland 1981–1996, by year

Year of birth	Total number of clinically verified cases	Number of live births	Prevalence per 10,000 live births
1981	6	68 055	0.88 (0.17–1.59)
1982	5	65 197	0.77 (0.10–1.44)
1983	6	64 232	0.93 (0.19–1.67)
1984	7	64 512	1.09 (0.28–1.90)
1985	11	65 281	1.69 (0.70–2.68)
1986	7	65 038	1.08 (0.18–1.88)
1987	6	65 307	0.92 (0.18–1.66)
1988	4	65 799	0.61 (0.01–1.21)
1989	7	63 347	1.11 (0.29–1.93)
1990	4	64 963	0.62 (0.02–1.22)
1991	9	66 371	1.36 (0.47–2.25)
1992	11	64 671	1.70 (0.69–2.71)
1993	14	62 732	2.23 (1.06–3.40)
1994	10	60 911	1.64 (0.62–2.66)
1995	8	59 485	1.34 (0.41–2.27)
1996	7	57 406	1.22 (0.32–2.12)
Scotland 1981-1996	**122**	**1 023 307**	**1.19 (0.98–1.40)**

Figure 5.3: Clinically confirmed cases and birth prevalence of microphthalmos, anophthalmos and coloboma in Scotland, 1981-1996



	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	Scotland
■ No. of clinically confirmed cases	6	5	6	7	11	7	6	4	7	4	9	11	14	10	8	7	
□ Birth prevalence/10,000 live births	0.88	0.77	0.93	1.09	1.69	1.08	0.92	0.61	1.11	0.62	1.36	1.7	2.23	1.64	1.34	1.22	1.19

Sex distribution

A similar number of male and female cases were found. Of the 122 clinically verified cases, 67 (55%) were male and 55 (45%) were female. The difference is statistically significant (chi-square test 0.50 > probability > 0.10).

Clinical anophthalmos data

The data obtained from the national register for congenital anomalies held at ISD Scotland is currently the only source from which any estimate of the birth prevalence of anophthalmos can be made. To illustrate some of the difficulties (small numbers, rare condition) in using this data to draw conclusions, a closer inspection of the number of cases of anophthalmos follows. The findings of the 13 names coded as ICD9 7430 (anophthalmos) are listed in Table 5.7. Of these 13, 10 were traced and examined. In only six was the actual diagnosis (anophthalmos) correct. Two children died within the first six days of life and one was stillborn. Two children had colobomatous microphthalmos. In one case examined there was no structural eye abnormality at all (see table of exclusions). Included in the table (ID 47) is a child with anophthalmos coded as microphthalmos at birth. Even for a condition as apparently obvious as anophthalmos, the accuracy of 7/13 (54%) with no guarantee of complete ascertainment has implications. Conclusions about data drawn from the congenital anomalies register need to be interpreted with great caution.

In Fife health board region only one confirmed case of anophthalmos (ID 166) was found over a 16-year period. Two children were incorrectly coded on the SMR register as 743.0 (anophthalmos).

Table 5.7: New cases of anophthalmos in Scotland, 1981–1996, based on SMR data

ID	Sex	Date of birth or year of birth	Status	Diagnosis (SMR coding)	Diagnosis on clinical examination
166	F	20.4.85	Alive	7430	Left anophthalmos
	F	1985	Died 1-23 hrs	7430	?
280	M	18.1.85	Alive	7430	No eye abnormality
306	F	15.10.81	Alive	7430	No eye abnormality
72	F	29.12.87	Alive	7430	Bilateral anophthalmos
51	M	17.11.89	Alive	7430	Coloboma, microphthalmos
204	M	24.3.89	Alive	7430	Left anophthalmos
	M	1990	Died 1-6 days	7430	?
117	F	31.10.90	Alive	7430	Anophthalmos /microphthalmos
	M	1991	Still birth	7430	?
62	F	10.5.92	Alive	7430	Coloboma, microphthalmos
47	M	3.9.93	Alive	7431*	Left anophthalmos
67	F	4.7.94	Alive	7430	Left anophthalmos
239	F	19.6.94	Alive	7430	Left anophthalmos

*7431 is microphthalmos. This case included in table for completeness since all other known cases of anophthalmos are included.

Unilateral or bilateral eye defects

69 (57%) children had a unilateral eye defect and 53 (43%) were bilaterally affected.

Fife health board report 1995

This 1995 report is a further illustration of the limitations of data based on reporting without clinical examination or uniformity of definition. An investigation was carried out into the apparent clusters of microphthalmos/anophthalmos/coloboma in North East Fife (Rowarth, 1995). After exclusion of children with chromosomal

abnormalities or an obvious family history link (see discussion below as to the logic and validity of doing this), 22 cases of MAC were observed in the study period 1981–1993. Multiple sources of ascertainment were used. It was concluded that the birth prevalence of MAC in Fife was relatively high. Inspection of the tables of 27 cases listed in the report, most of whom were traced and clinically examined during the Scottish Microphthalmia Study, reveals that the data obtained is mostly accurate, but with some errors which are significant when dealing with such small numbers. One child had a rare congenital iris anomaly (corectopia), one pair of twins had partial aniridia, another child had Peters' anomaly, and another had normal eyes. Of the 27, only two children were not traced in this study, which again confirms the likely high degree of case ascertainment achieved for this region. That the prevalence was highest in the health board region of Fife (2.59 per 10,000) is partly explained by the particular attention drawn to this condition in the area.

Vision and registration

Out of 122 examined, 34 (28%) were registered as blind (less than 3/60 best corrected Snellen acuity for distance).

Seven children on the blind/partially-sighted register had a unilateral eye defect with a normal fellow eye. There is no simple explanation for this. One would expect a population such as this, which was not selected based on vision, to express the full range of visual potential. Studies on populations selected from hospital clinics or blind registration data will yield a different clinical spectrum.

Education and social needs

There were 106 children aged 3 or over, and over half of them received help with special educational needs: 23 (22%) attended a special school and a further 31 (29%) received special assistance within a mainstream school. However, the reasons for being in a special school may not be based solely on vision, but on the presence of other congenital anomalies. Some form of disability living allowance was received by 46 children (38%).

Ocular phenotype

A summary of the ocular phenotypes in each of the 122 clinical examinations is listed in Table 5.8. A total of 72 children were classified as having clinical microphthalmos in either eye (94 eyes). Clinical microphthalmos was defined as the clinical impression of a small eye. All colobomas (iris, fundus and optic nerve) were included, a total of 130 eyes. Colobomatous microphthalmos (eyes with coloboma which were classified as microphthalmic) was present in 53 eyes. Six children had clinical anophthalmos in at least one eye.

Table 5.8: Ocular phenotypes of the 122 clinical examinations

ID	Ocular phenotypic diagnosis
1	bilateral extreme microphthalmos, right corneal opacification, iris abnormalities
3	bilateral atypical iris 'colobomas', 'aniridia-like', bilateral anterior subcapsular cataracts
4	right iris coloboma only, left minor pupil margin abnormality
5	bilateral iris and fundus colobomas, bilateral microphthalmos
6	left iris and fundus coloboma, microphthalmos, iris heterochromia
8	left iris coloboma, corneal opacity, microphthalmos
15	right optic disc coloboma, right ptosis, blepharophimosis
17	bilateral iris colobomas, bilateral fundus colobomas, right lens opacities, high myopia

ID	Ocular phenotypic diagnosis
	myopia
18	bilateral iris colobomas, right simple retinal coloboma
19	left microcornea/cataract, retinal pigmentation, microphthalmos
20	right microphthalmos, right ptosis, possible retinal dysplasia
26	bilateral iris and fundus colobomas, microphthalmos, affected sib ID 106
29	left optic disc coloboma and simple retinal coloboma
30	right iris coloboma and simple retinal coloboma
33	right iris and fundus coloboma, bilateral microphthalmos
34	bilateral iris, lens, and fundus colobomas
35	right fundus coloboma/ microphthalmos, left optic disc coloboma and thin vessels, affected twin ID 96
39	bilateral iris and simple retinal colobomas, bilateral cataracts (lensectomies) bilateral microphthalmos
40	bilateral iris and fundus colobomas, left microphthalmos
45	bilateral iris colobomas, ptosis
47	left anophthalmos, right iris 'coloboma' nasally (atypical and atrophic), 'aniridia-like', dysplastic retina, optic disc hypoplasia
48	left extreme microphthalmos
50	left extreme microphthalmos, right nystagmus, right optic nerve head/disc 'coloboma'
51	bilateral iris and fundus colobomas/microphthalmos, iris heterochromia, left post subcapsular cataract
53	right sclerocornea, left cataract, bilateral microphthalmos
55	bilateral iris colobomas/microphthalmos, left cataract, right fundus coloboma
58	left iris coloboma, bilateral fundus colobomas/microphthalmos
60	bilateral nanophthalmos/microphthalmos, high hypermetropia, left divergent squint, Schwartz-Jampel syndrome, has twin
61	left iris and fundus coloboma, detached retina, left microphthalmos
62	left microphthalmos, right optic disc/macular coloboma, abnormal right retina
63	bilateral iris and fundus colobomas, left microphthalmos, right cataract, right mucocele nasolacrimal duct
64	right iris abnormal/dysplastic, left iris coloboma
66	bilateral fundus colobomas/microphthalmos, left iris coloboma, affected sib ID 296
67	left anophthalmos

ID	Ocular phenotypic diagnosis
70	right iris coloboma
75	bilateral iris colobomas, right iris fissure/cleft, right disc/macular coloboma, left microphthalmos, left fundus coloboma
76	bilateral iris and fundus colobomas
77	left microcornea, cornea opaque, extreme microphthalmos
79	bilateral iris and fundus colobomas, bilateral microphthalmos, iris heterochromia, right cataract, high myopia
83	right iris fissure-like 'coloboma', left iris coloboma, bilateral fundus colobomas, iris heterochromia, left microphthalmos
84	right cataract, extreme microphthalmos
85	right iris and simple retinal coloboma, microphthalmos, iris heterochromia
89	bilateral iris colobomas, left iris fissure-like 'coloboma', right simple retina coloboma
90	bilateral iris and colobomas/microphthalmos
93	bilateral complicated microphthalmos, vitreous and retina disorganised, bilateral lensectomies
94	bilateral extreme microphthalmos, corneal opacification, right iris coloboma (right lensectomy/vitrectomy, retinal detachment failed surgery)
96	bilateral fundus colobomas, affected twin ID 35
97	left iris coloboma, left retina abnormal, bilateral extreme microphthalmos
100	right cataract and severe microphthalmos, possibly complicated by prematurity, born at 30 weeks
101	right cataract and corneal scar, right microphthalmos, simple retinal coloboma, anterior scleral staphyloma
102	bilateral optic disc colobomas, mum and sister affected
106	right iris and fundus coloboma, iris heterochromia, affected sib ID 26
107	right lens opacity, complex optic disc/macular coloboma, microphthalmos
109	bilateral cataracts (lensectomies), bilateral optic disc colobomas, right microphthalmos
111	left iris coloboma, left retina detached, microphthalmos
113	bilateral iris colobomas, right simple retinal coloboma, left fundus coloboma/microphthalmos
115	left microphthalmos, possible persistent hyperplastic primary vitreous (PHPV), left lensectomy/vitrectomy, blocked left nasolacrimal duct
117	left anophthalmos, right extreme microphthalmos
121	bilateral iris 'colobomas', aniridia-like

ID	Ocular phenotypic diagnosis
122	bilateral iris colobomas, bilateral optic nerve hypoplasia (from records)
129	right iris and fundus coloboma, right microphthalmos
132	right iris and fundus coloboma, iris heterochromia, right microphthalmos, affected sibs ID 216, ID 299
138	bilateral iris and fundus colobomas, bilateral cataract, right microphthalmos, high myopia
140	left iris coloboma, lens coloboma, iris heterochromia
142	left iris stromal coloboma/'furrow'
145	bilateral iris colobomas, left cataract, lens 'notches'/colobomas
150	right iris coloboma and simple retinal coloboma
153	left iris coloboma
159	bilateral iris coloboma, cortical lens opacities, affected sib ID 297
162	left atypical superior iris coloboma, left cataract and lens coloboma, left simple retinal coloboma (atypical position)
166	right anophthalmos, left nystagmus
172	right cataract and extreme microphthalmos
174	left iris coloboma
181	bilateral iris fissure/coloboma, bilateral microphthalmos
187	bilateral and unusual iris 'colobomas', 'aniridia-like', small right lens opacity
188	left iris pupillary membrane remnant, left cataract, bilateral fundus colobomas, left microphthalmos
189	bilateral sclerocornea, bilateral microphthalmos
192	bilateral iris and fundus colobomas/microphthalmos, bilateral small corneal opacities
199	bilateral corneal opacification, bilateral cataracts, extreme microphthalmos
200	right iris coloboma
204	right anophthalmos
210	right corneal scar, absent pupil, right microphthalmos
213	left microcornea and corneal opacification, retinal dysplasia/coloboma (eye enucleated: records)
216	right iris coloboma and simple retinal coloboma, affected sibs ID 132, ID 299
217	bilateral iris colobomas, bilateral simple retinal colobomas
219	left cataract and microphthalmos, possibly PHPV
222	right iris coloboma, bilateral simple retinal colobomas
224	right optic disc coloboma/dysplasia, right microphthalmos

ID	Ocular phenotypic diagnosis
228	bilateral iris and fundus colobomas
232	left cataract, iris and fundus colobomas
234	bilateral iris colobomas/simple retinal colobomas
236	right iris coloboma, right minor lens opacities, right fundus coloboma/detached retina, right microphthalmos , left simple retinal coloboma
238	left iris and fundus coloboma, left cataract, microphthalmos, iris heterochromia
239	left anophthalmos, right nystagmus
241	right iris stromal coloboma/'furrow'
242	right iris coloboma and simple retinal coloboma
243	left iris coloboma/fissure
244	left iris atrophy, pupillary membrane, microphthalmos, pigmentary retinopathy, probably inflammatory/infective
251	right atypical superior iris coloboma, right simple retinal coloboma
252	right iris coloboma, cataract, extreme microphthalmos, probable detached retina
253	left extreme microphthalmos
254	right cataract (aphakic) and microphthalmos, possibly PHPV
258	right iris and fundus coloboma, right microphthalmos, iris heterochromia
261	left corneal opacity and cataract, left extreme microphthalmos, possibly PHPV
263	left iris coloboma and simple retinal coloboma
264	right iris and fundus coloboma, left optic disc abnormally shaped, iris heterochromia
267	right iris coloboma and microphthalmos, left sclerocornea and microphthalmos
268	right iris and fundus coloboma, microphthalmos, affected twin ID 269
269	left iris and fundus coloboma, microphthalmos, affected twin ID 268
272	left iris and fundus coloboma, left microphthalmos
275	right atypical superior iris and fundus coloboma (in typical position) right limbal dermoid excised
276	left cataract and microphthalmos
277	left persistent pupil membrane, iris heterochromia, microphthalmos
284	right corectopia, left anterior segment malformed, bilateral microphthalmos
287	bilateral iris coloboma, left iris 'fissure-like' defect
290	left iris coloboma, left simple retina coloboma
296	left optic disc coloboma affected sib ID 66
297	left iris fissure/coloboma, affected sib ID 159

ID	Ocular phenotypic diagnosis
299	bilateral cataracts and retinal colobomas (?) and microphthalmos, abnormal anterior segments, affected sibs ID 132, ID 216
324	right iris and fundus coloboma, right microphthalmos
335	right iris coloboma and simple retinal coloboma
337	left iris and fundus coloboma, iris heterochromia

Extraocular congenital malformations

These are discussed in more detail in chapter nine. There was significant developmental delay in 23 children (19%), 40 children were considered to be facially dysmorphic (Dr David FitzPatrick, Consultant Clinical Geneticist), 11 children had significant congenital heart disease, and a total of 45 had another (extraocular) congenital anomaly. In 13 children there was a recognised clinical genetic syndrome, including 3 with the CHARGE association. Seven children had a positive family history of a related eye defect and this data is considered further in chapter eight.

DISCUSSION

Data for this study was collected retrospectively, using multiple sources of ascertainment. The birth prevalence is therefore a minimum value. Ascertainment levels were probably very high and there appears to be consistency, since similar numbers were obtained for each of the 16 years back to 1981. The study continues to add to the database, although very few 'new' cases continue to be added.

Some sources of ascertainment were not fully explored, although this may have had little or no effect on the outcome, since not every source has the same chance of identifying a case. Blind registration data was used for only two health board areas.

Cases in which the eye abnormality was minor with no effect on vision may not have come to the attention of any medical service.

An individual ophthalmologist rarely sees these eye conditions in clinical practice, and it is likely that most cases will be remembered and the notes or details would be retrievable. Most departments did not have a formal method of retrieving cases.

In children with multiple congenital anomalies, it is understandable that there may be a failure to document an eye abnormality, either because of time constraints, ill health due to a life-threatening malformation, or there not being sufficient space to log all abnormalities on the recording form (there is space for eight diagnoses on the SMR11 neonatal discharge record). With multiple malformations, it may be assumed that the eye anomaly is present as part of a clinical syndrome e.g. trisomy 13, so the eye defect is not documented. Conversely, there may be an excess of ocular diagnoses made in children with multiple congenital anomalies.

The large number of exclusions and incorrect diagnoses illustrates some of the difficulties of relying on registers for data analysis. Some of the lack of specificity of the ICD-9 codes will be improved by ICD-10 (see chapter four).

In England, the combined prevalence of anophthalmos and microphthalmos has been estimated as 1.0 per 10,000 births (Dolk et al. 1998). The prevalence of clinical anophthalmos in this study is less than that found in an Italian study of 0.60 per 10,000 births (Clementi et al. 1992).

It is difficult to compare the findings of this study with other work, as each case was clinically validated. A recent study in England (Dolk et al. 1998) was based on a postal survey and questionnaire. A period of just six years was covered (1988–1994). Problems with this approach included the failure to use a definition that could be

applied consistently and the confusion of case ascertainment by excluding cases of 'mild' microphthalmos or where the 'severity' of microphthalmos was unknown, whatever these incompletely defined terms may be. In the English study, cases of assumed aetiology based on clinical appearance in combination with other congenital abnormalities, or maternal infection, were excluded. That 'genetic' and 'environmental' cases cannot easily be distinguished has been made clear in chapter two.

This study included all cases, which were selected based on the presence of an eye abnormality without reference to vision, other congenital anomalies, or cause. Cases with chromosome abnormalities and multiple malformations have been excluded in some studies (Kallen et al. 1996).

The question of clustering has not been considered since the small numbers involved in each health board region would not stand up to detailed analysis, and it is not within the scope of this thesis. Only a small change in the numbers would have a marked effect on prevalence, and the data is not sufficiently complete to allow useful conclusions to be drawn on these matters. Furthermore, the place of birth has been used for geographical location, but this may not be the most significant factor, as prenatal development and factors back to at least the point of conception need to be considered.

The evaluation of clustering itself does not add much towards our understanding of the aetiology of these eye defects (Rothman, 1990). An investigation into geographical variation and clustering of anophthalmos and microphthalmos in England did not find any evidence of clusters in babies born between 1988 and 1994 (Dolk et al. 1998). The prevalence was higher in rural and low-density population

areas compared to urban areas. Despite the shortcomings of the study, the observation of an almost twofold excess risk in rural areas does warrant further investigation and confirmation.

Summary, chapter five

- The co-operation from GPs, specialists and other health professionals was extremely good. Very few patients refused consent for the clinical aspects of the study, once contacted.
- Case ascertainment in this study used a wide variety of sources. The overall prevalence suggests that ascertainment levels were high.
- A significant number of children were excluded from the study after being found not to have any congenital eye defect.
- Birth prevalence was highest in the Fife health board area, an area which has received the most attention and which was the subject of a previous report.
- Birth prevalence in the Western Isles was nil. This health board region has one of the smallest populations and very few births. This is interesting in the light of suggested clusters in rural and low-density population areas.
- A register of validated cases has been produced which allows cases to be added and information updated.

CHAPTER SIX

CONGENITAL OCULAR MALFORMATIONS: CLASSIFICATION AND OTHER NEW FINDINGS

Summary

Eye defects were classified into three groups according to the presence or absence of a defect related to optic fissure closure, with a third category comprising eye defects without a recognisable phenotype. The term microphthalmos does not feature in the new classification, which is based on ocular phenotype.

A classification of coloboma is presented which highlights some of the difficulties in defining this condition and the limitations of our understanding. Some forms of coloboma are best described by the term 'optic nerve head disruption'.

Most of the eye defects were related to defects of optic fissure closure. All the bilateral defects were consistent with the new classification.

Eye pathology in bilaterally affected individuals was usually asymmetrical.

The pathophysiology of superior (atypical) iris coloboma is poorly understood.

A number of ocular phenotypes were seen which do not fall easily into any classification system.

The iris coloboma-heterochromia association has been characterised and is not uncommon (Morrison et al. 2000).

The prevalence of retinal detachment in chorioretinal coloboma has been estimated (Morrison and Fleck, 1999).

BACKGROUND AND METHODS

The reason for first grouping the eye defects into clinical anophthalmos, microphthalmos and coloboma at the outset of this study was to make the data comparable to previous studies. These diagnoses are the terms by which cases could be searched for on registers and when communicating with clinicians. The categories of coloboma and clinical microphthalmos (the latter often with coloboma) were predicted to overlap, and this also occurred to some extent with anophthalmos and microphthalmos (chapter one). As each of the terms was applied to the various structural ocular abnormalities, it became apparent that greater specificity and diagnostic (phenotypic) information was required, and this exposed the limitations and shortcomings of current terminology. Also, problems with definition arose as there is no accepted or applicable definition of microphthalmos (chapter one).

The different eye anomalies needed to be distinguished in order to make more detailed comparisons, e.g. of family history and recurrence risk (chapter eight), extraocular abnormalities (chapter eight), and mutation screening for candidate genes (chapter nine). The limitations and lack of precision of the term coloboma and its different varieties, the wide range of microphthalmic phenotypes, and the inability to characterise anophthalmos beyond clinical anophthalmos, all needed to be addressed.

An attempt was made to unify and classify the conditions of anophthalmos, coloboma, colobomatous microphthalmos and microphthalmos.

A new classification system would need to be simple, reliable, repeatable, and ideally would be based on development with respect to timings and structural abnormalities. It would also need to be clinically applicable. Classification based on a description of the anatomical site affected is over-complicated and not easily applied in practice.

This type of classification may not bear any relationship to the cause (Warburg, 1993). A classification related to genetic mutations is also a possibility, but the role and contribution of genetic factors is poorly understood. Ideally, future genetic studies (family history and pedigree analysis, clinical genetic syndromes, recurrence risk, mutation screening) would be consistent with the classification system.

Observations resulting from the ultrasound study of axial length made it clear that microphthalmos could not be defined on the basis of axial length (chapter seven). Making the diagnosis of microphthalmos based on appearance is easy for extreme microphthalmos but with 'mild' or 'moderate' degrees of microphthalmos it is problematic. For unilateral cases it is possible to comment on the relative difference on the assumption, possibly incorrectly, that the structurally normal fellow eye is indeed normal, but for bilateral cases the task is even more difficult.

The most common structural abnormality found in all cases was a uveal coloboma, and this is relatively easy to distinguish. The timing of the insult to the eye can also be narrowed down to a relatively short period of development (chapter one).

This created the first two categories, fissure (OFD) or non-fissure (non-OFD). The third category relates to those cases in which it could not be said with any certainty that a fissure defect was present or not. In such cases, there was no view of the iris or posterior segment, or no discernible eye structures due to an extremely small eye or remnant being present (clinical anophthalmos). This third group was unclassifiable (U). It should be noted that unclassifiable could mean an underlying OFD or non-OFD, but this can only be based on the status of the fellow eye.

An attempt was made to make a simple classification of coloboma based on the anatomical site, taking into account the present knowledge about formation of the

optic fissure (chapter one). Ideally, the new classification would be consistent with some of the understanding of the expression/involvement of developmental genes in different parts of the eye. However, abnormalities within different genes could cause a similar defect, or the same gene could be responsible for several different types of defect. A decision had to be made, based on the appearance of the optic disc, as to whether or not the defect was likely to be fissure-related.

The first step was to classify the eye as OFD or non-OFD. Only one eye needed to be a fissure defect to classify the individual as belonging to this category.

Any case of clinical anophthalmos or bilateral extreme clinical microphthalmos was unclassifiable. These unclassifiable cases cannot be phenotyped, unless the fellow eye expresses a defect.

The non-fissure defects were expected to be heterogeneous, and the small numbers involved would not allow further classification.

For a classification system to work, agreement would be expected between the two eyes of the same individual. Also, if the condition were thought to be inherited, the same basic structural defect would be present within any family or pedigree. The expected combinations would be OFD/OFD, OFD/U, non-OFD/non-OFD, U/U, U/non-OFD. The combination of OFD/non-OFD would not be predicted.

The above classification is not entirely 'new' in the sense that colobomatous and non-colobomatous microphthalmos has long been recognised (Bateman, 1984; Tucker et al. 1996). Non-colobomatous microphthalmos has been called 'complicated' microphthalmos due to its heterogeneous nature (Goldberg and McKusick, 1971). The key difference with the proposed classification is that defects are not classified

according to apparent or actual eye size (axial length), but according to phenotypic abnormality.

Microphthalmos is not a phenotypic diagnosis and does not feature in this classification. What was previously non-colobomatous microphthalmos is either not an optic fissure defect (non-OFD) or an unclassifiable eye malformation (U).

Optic nerve head coloboma and fissure defects

In chapter one, the term coloboma (literally, a defect) was shown to be an inadequate term for describing the poorly understood underlying processes. Optic nerve head colobomas have a highly variable clinical appearance (Olsen et al. 1996).

The origin of certain types of optic disc (optic nerve head) colobomas is not understood. It is possible that some types are related to closure of the optic fissure. Other unknown mechanisms related to optic nerve formation may be involved.

Optic nerve head 'colobomas' suffer from being assigned this limited term, since assumptions are made about the causative mechanism being related to fissure closure, being lumped together with iris or chorioretinal colobomas (Daufenbach et al. 1998). A separation or distinction would be helpful, with the word coloboma dropped. 'Optic nerve head closure disruption', 'optic stalk/groove head closure disruption', or 'optic nerve head disruption' would be more helpful to distinguish this group of abnormalities. From here on, the term 'optic nerve head disruption' will be used to describe these defects. It is important to emphasise that although the probable embryological mechanism is a defect of optic fissure closure, the genetic influences may be different, possibly due to the different anatomical location - the optic stalk/groove - as well as the timing.

RESULTS

Tables 6.1, 6.2 and 6.3 lists the ocular diagnoses along with the designated classification into one of the three categories OFD, non-OFD and unclassifiable. There were 85 individuals with an optic fissure defect, 12 non-optic fissure defect, and 25 unclassifiable (Table 6.4). This breakdown is represented in Figure 6.1.

Figure 6.1: Classification of congenital eye malformations in 122 Scottish children

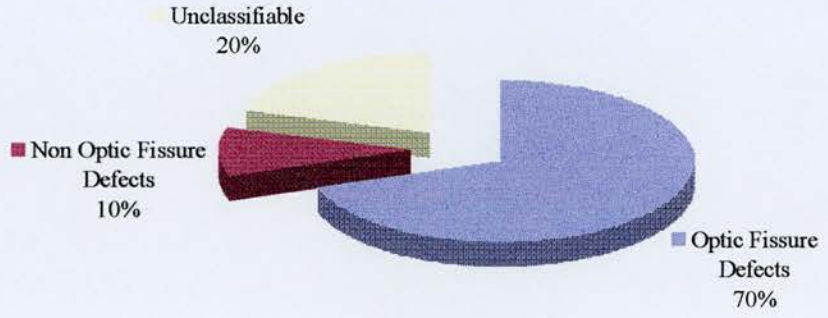


Table 6.1: Ocular phenotypes classified as left or right optic fissure defect (OFD)

ID	Ocular phenotypic diagnosis
4	right iris coloboma only, left minor pupil margin abnormality
5	bilateral iris and fundus colobomas, bilateral microphthalmos
6	left iris and fundus coloboma, microphthalmos, iris heterochromia
8	left iris coloboma, corneal opacity, microphthalmos
15	right optic disc coloboma, right ptosis, blepharophimosis
17	bilateral iris colobomas, bilateral fundus colobomas, right lens opacities, high myopia
18	bilateral iris colobomas, right simple retinal coloboma
26	bilateral iris and fundus colobomas, microphthalmos, affected sib ID 106
29	left optic disc coloboma and simple retinal coloboma
30	right iris coloboma and simple retinal coloboma
33	right iris and fundus coloboma, bilateral microphthalmos
34	bilateral iris, lens, and fundus colobomas
35	right fundus coloboma/ microphthalmos, left optic disc coloboma and thin vessels, affected twin 96
39	bilateral iris and simple retinal colobomas, bilateral cataracts (lensectomies) bilateral microphthalmos
40	bilateral iris and fundus colobomas, left microphthalmos
45	bilateral iris colobomas, ptosis
51	bilateral iris and fundus colobomas/microphthalmos, iris heterochromia, left post subcapsular cataract
55	bilateral iris colobomas/microphthalmos, left cataract, right fundus coloboma
58	left iris coloboma, bilateral fundus colobomas/microphthalmos
61	left iris and fundus coloboma, detached retina, left microphthalmos
62	left microphthalmos, right optic disc/macular coloboma, abnormal right retina
63	bilateral iris and fundus colobomas, left microphthalmos, right cataract, right mucocele nasolacrimal duct
64	right iris abnormal/dysplastic, left iris coloboma
66	bilateral fundus colobomas/microphthalmos, left iris coloboma, affected sib ID 296
70	right iris coloboma
75	bilateral iris colobomas, right iris fissure/cleft, right disc/macular coloboma, left microphthalmos, left fundus coloboma
76	bilateral iris and fundus colobomas
79	bilateral iris and fundus colobomas, bilateral microphthalmos, iris heterochromia, right cataract, high myopia
83	right iris fissure-like 'coloboma', left iris coloboma, bilateral fundus colobomas, iris heterochromia, left microphthalmos
85	right iris and simple retinal coloboma, microphthalmos, iris heterochromia

ID	Ocular phenotypic diagnosis
89	bilateral iris colobomas, left iris fissure-like 'coloboma', right simple retina coloboma
90	bilateral iris and colobomas/microphthalmos
94	bilateral extreme microphthalmos, corneal opacification, right iris coloboma (right lensectomy/vitrectomy, retinal detachment failed surgery)
96	bilateral fundus colobomas, affected twin ID 35
97	left iris coloboma, left retina abnormal, bilateral extreme microphthalmos
101	right cataract and corneal scar, right microphthalmos, simple retinal coloboma, anterior scleral staphyloma
102	bilateral optic disc colobomas, mum and sister affected
106	right iris and fundus coloboma, iris heterochromia, affected sib ID 26
107	right lens opacity, complex optic disc/macular coloboma, microphthalmos
109	bilateral cataracts (lensectomies), bilateral optic disc colobomas, right microphthalmos
111	left iris coloboma, left retina detached, microphthalmos
113	bilateral iris colobomas, right simple retinal coloboma, left fundus coloboma/microphthalmos
122	bilateral iris colobomas, bilateral optic nerve hypoplasia (from records)
129	right iris and fundus coloboma, right microphthalmos
132	right iris and fundus coloboma, iris heterochromia, right microphthalmos, affected sibs ID 216, ID 299
138	bilateral iris and fundus colobomas, bilateral cataract, right microphthalmos, high myopia
140	left iris coloboma, lens coloboma, iris heterochromia
142	left iris stromal coloboma/'furrow'
145	bilateral iris colobomas, left cataract, lens 'notches'/colobomas
150	right iris coloboma and simple retinal coloboma
153	left iris coloboma
159	bilateral iris coloboma, cortical lens opacities, affected sib ID 297
162	left atypical superior iris coloboma, left cataract and lens coloboma, left simple retinal coloboma (atypical position)
174	left iris coloboma
181	bilateral iris fissure/coloboma, bilateral microphthalmos
188	left iris pupillary membrane remnant, left cataract, bilateral fundus colobomas, left microphthalmos
192	bilateral iris and fundus colobomas/microphthalmos, bilateral small corneal opacities
200	right iris coloboma
216	right iris coloboma and simple retinal coloboma, affected sibs ID 132, ID 299
217	bilateral iris colobomas, bilateral simple retinal colobomas
222	right iris coloboma, bilateral simple retinal colobomas
224	right optic disc coloboma/dysplasia, right microphthalmos

ID	Ocular phenotypic diagnosis
228	bilateral iris and fundus colobomas
232	left cataract, iris and fundus colobomas
234	bilateral iris colobomas/simple retinal colobomas
236	right iris coloboma, right minor lens opacities, right fundus coloboma/detached retina, right microphthalmos , left simple retinal coloboma
238	left iris and fundus coloboma, left cataract, microphthalmos, iris heterochromia
241	right iris stromal coloboma/'furrow'
242	right iris coloboma and simple retinal coloboma
243	left iris coloboma/fissure
252	right iris coloboma, cataract, extreme microphthalmos, probable detached retina
258	right iris and fundus coloboma, right microphthalmos, iris heterochromia
263	left iris coloboma and simple retinal coloboma
264	right iris and fundus coloboma, left optic disc abnormally shaped, iris heterochromia
267	right iris coloboma and microphthalmos, left sclerocornea and microphthalmos
268	right iris and fundus coloboma, microphthalmos, affected twin ID 269
269	left iris and fundus coloboma, microphthalmos, affected twin ID 268
272	left iris and fundus coloboma, left microphthalmos
275	right atypical superior iris and fundus coloboma (in typical position) right limbal dermoid excised
287	bilateral iris coloboma, left iris 'fissure-like' defect
290	left iris coloboma, left simple retina coloboma
296	left optic disc coloboma affected sib ID 66
297	left iris fissure/coloboma, affected sib ID 159
324	right iris and fundus coloboma, right microphthalmos
335	right iris coloboma and simple retinal coloboma
337	left iris and fundus coloboma, iris heterochromia

Table 6.2: Ocular phenotypes classified as left or right non-optic fissure defect (non-OFD)

ID	Ocular phenotypic diagnosis
3	bilateral atypical iris 'colobomas', 'aniridia-like', bilateral anterior subcapsular cataracts
20	right microphthalmos, right ptosis, possible retinal dysplasia
60	bilateral nanophthalmos/microphthalmos, high hypermetropia, left divergent squint, Schwartz-Jampel syndrome, has twin
93	bilateral complicated microphthalmos, vitreous and retina disorganised, bilateral lensectomies
115	left microphthalmos, possible PHPV, left lensectomy/vitrectomy, blocked left nasolacrimal duct
121	bilateral iris 'colobomas', aniridia-like
187	bilateral and unusual iris 'colobomas', 'aniridia-like', small right lens opacity
244	left iris atrophy, pupillary membrane, microphthalmos, pigmentary retinopathy, probably inflammatory/infective
254	right cataract (aphakic) and microphthalmos, possibly PHPV
277	left persistent pupil membrane, iris heterochromia, microphthalmos
284	right corectopia, left anterior segment malformed, bilateral microphthalmos

Table 6.3: Ocular phenotypes classified as left or right unclassifiable (U)

ID	Ocular phenotypic diagnosis
1	bilateral extreme microphthalmos, right corneal opacification, iris abnormalities
19	left microcornea/cataract, retinal pigmentation, microphthalmos
47	left anophthalmos, right iris 'coloboma' nasally (atypical and atrophic), 'aniridia-like', dysplastic retina, optic disc hypoplasia
48	left extreme microphthalmos
50	left extreme microphthalmos, right nystagmus, right optic nerve head/disc 'coloboma'
53	right sclerocornea, left cataract, bilateral microphthalmos
67	left anophthalmos
77	left microcornea, cornea opaque, extreme microphthalmos
84	right cataract, extreme microphthalmos
100	right cataract and severe microphthalmos, possibly complicated by prematurity, born at 30 wks
117	left anophthalmos, right extreme microphthalmos
166	right anophthalmos, left nystagmus
172	right cataract and extreme microphthalmos
189	bilateral sclerocornea, bilateral microphthalmos
199	bilateral corneal opacification, bilateral cataracts, extreme microphthalmos
204	right anophthalmos
210	right corneal scar, absent pupil, right microphthalmos
213	left microcornea and corneal opacification, retinal dysplasia/coloboma (eye enucleated: records)
219	left cataract and microphthalmos, possibly PHPV
239	left anophthalmos, right nystagmus
251	right atypical superior iris coloboma, right simple retinal coloboma
253	left extreme microphthalmos
261	left corneal opacity and cataract, left extreme microphthalmos, possibly PHPV
276	left cataract and microphthalmos
299	bilateral cataracts and retinal colobomas (?) and microphthalmos, abnormal anterior segments, affected sibs ID 132, ID 216

As predicted, the categories of non-OFD and unclassifiable defects were both very heterogeneous. Some of the suggested phenotypic diagnoses are listed in Tables 6.4 and 6.5. Many of these diagnoses are extremely rare and a much larger population would need to be studied to yield sufficient numbers to form subgroups. It is hoped

that some of these conditions will eventually be distinguished genetically (chapter three).

Table 6.4: Phenotypic diagnoses of non-OFD group

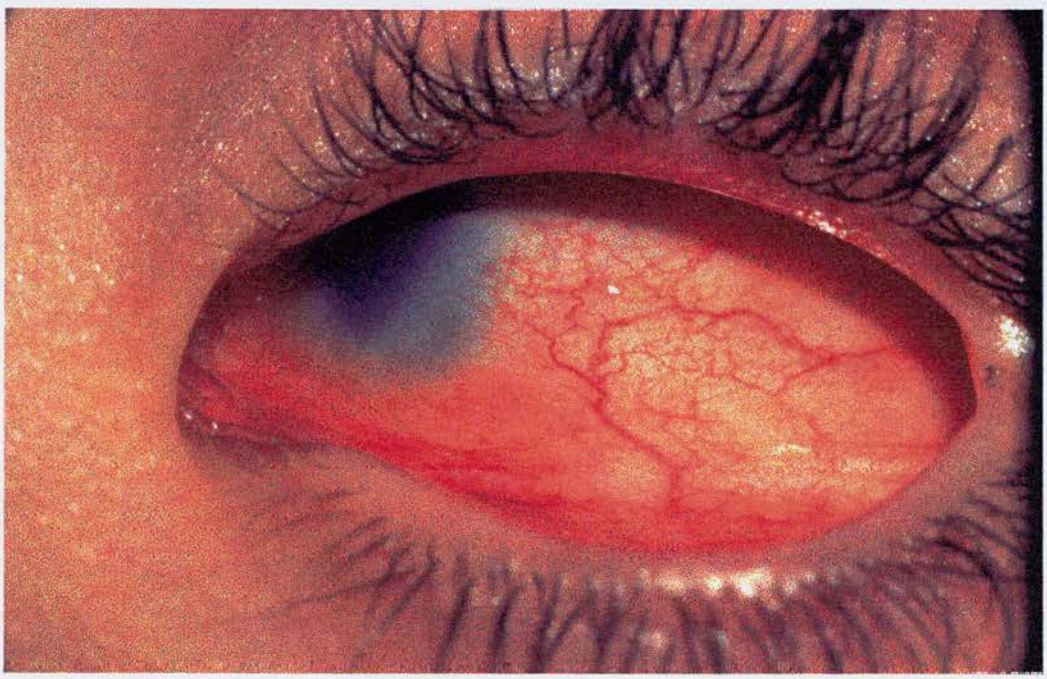
Phenotypic Diagnosis	ID
Possible aniridia	3, 121, 187
Retinal and/or vitreous dysplasia	20, 93
Nanophthalmos	60
Persistent hyperplastic primary vitreous	115, 254
Prenatal inflammation or infection	244
Persistent pupillary membrane	277
Corectopia	284

Figure 6.1: Possible aniridia, non-OFD. This subject also has a left esotropia (ID 121)

Table 6.5: Unclassifiable (no clear phenotype)

Diagnosis (no clear phenotype)	ID
Absent pupil	210
Anophthalmos	47, 67, 117, 166, 204, 239
Atypical (superior) iris coloboma	162, 251
Corneal opacification	1, 77, 199, 210, 213, 261
Cataract (no view of posterior pole)	19, 53, 84, 100, 162, 172, 199, 219, 261, 299
Extreme microphthalmos	48, 50, 77, 84, 100, 117, 172, 189, 199, 210, 219, 261
Iris abnormalities	1
Microcornea	19, 77, 213
Retinopathy of prematurity (suspected)	100
Sclerocornea	53, 189

Figure 6.2: Left extreme clinical microphthalmos (ID 48)



There were several other external eye abnormalities and defects noted (Table 6.6). Some of these could be described as secondary i.e. a consequence of the visual problem (e.g. strabismus). In many cases, they must be regarded as another

pathology. That other abnormalities coexist is not surprising and is a reminder of the complex aetiology (chapters one and two).

Table 6.6: External eye abnormalities noted

External Eye abnormality	ID
Blepharophimosis	20
Blocked nasolacrimal duct	115
Limbal dermoid	275
Mucocele	299
Nystagmus	50, 166, 239
Ptosis	15, 20, 45
Strabismus	60, 45

Lens coloboma

This was recognised in four cases (IDs 34, 140, 145, 162), all of whom had iris coloboma. The abnormality is subtle and appears as a small defect/impression in the lens, often with disruption of the adjacent zonular fibres (Pagon, 1981; Hornby et al. 2000).

Cataract

Lens opacities were seen in a significant number of cases. In many the opacities were not usually visually significant. (ID 159) (Pagon, 1981; Hornby et al. 2000). Six children had had cataract surgery (lensectomies).

Iris heterochromia-iris coloboma

This is described in a published paper. Nearly 20% of cases are affected (Morrison et al. 2000). Figure 7.8 page 193 and Figure 7.12 page 197.

Retinal detachment

There were several cases in which this occurred (IDs 94, 111, 236, 252). A letter has been published on this topic in which the prevalence was estimated at 3.76% (4 out of 101 affected eyes) (Morrison and Fleck, 1999).

Clinical anophthalmos (Figure 6.3)

This occurred in 7 cases (IDs 47, 67, 72, 117, 166, 204, 239). The cases had little in common and in none of them was there a family history of anophthalmos or coloboma. The systemic abnormalities present are discussed in chapter eight. Only one case was bilateral (ID 77). In ID 117 a very small ocular remnant could be seen in the fellow orbit by careful examination of a child documented as being bilateral anophthalmos. The distinction is academic (chapter one).

Figure 6.3: Left clinical anophthalmos

ID 47 is very unusual since the fellow eye has an atrophic iris with a defect nasally. The retina is whitish and dysplastic with a hypoplastic abnormal disc. This could well

have been a case of atypical aniridia or coloboma with anophthalmos affecting the other side. Blood was tested for *PAX6* and *CHX10* gene mutations and none were found (chapter nine). This boy was born with hydrocephalus (detected at 27 weeks of gestation) and cerebral atrophy and has developmental delay.

IDs 67 and 204 were the only cases of anophthalmos with a completely normal fellow eye.

The author did not examine ID 72 but the documentation and clinical records were accurate enough to be certain of the diagnosis. This included discussion with the child's artificial eye fitter.

ID 117 was the child born within a consanguineous marriage. Parents are first cousins (chapter two) and the mother had already had three miscarriages. This girl had microcephaly and developmental delay.

In ID 166 the fellow eye had reduced vision with nystagmus. Developmental delay and brain abnormalities meant that a more detailed assessment of vision was not possible.

ID 239: This three-year-old child also had nystagmus in the apparently normal fellow eye but was too young for a more detailed visual assessment.

Cases with a family history (IDs 26, 35, 66, 96, 102, 106, 132, 159, 216, 268, 269, 276, 296, 297, 299)

These are discussed from the combined systemic and ocular aspects in chapter eight.

IDs 26 and 106 are brothers with iris and fundus colobomas. The degree of involvement is different and one of the brothers is only affected unilaterally.

IDs 35 and 96 are identical twins. They are affected bilaterally but to different degrees with iris or fundus coloboma.

IDs 66 and 296: A girl with iris and fundus colobomas has a brother with normal vision and a retinal abnormality best described as a coloboma. Retinal architecture was normal. This would be classified as type 1 (see below). This coloboma would have remained undetected without fundus examination (see earlier comment).

ID 102: One of two sisters (the other was born in England) who come from a large family and are the third generation affected by an optic nerve abnormality best described as a coloboma. These have been classified in this study as being due to defects in optic fissure closure. This point is debatable. The optic nerve abnormality would be described as an 'optic nerve head disruption' (type 5, see below).

IDs 132, 216, 299: These three brothers are affected differently with defects of optic fissure closure. IDs 132 and 216 are unilaterally affected, but the vision of ID 216 is not impaired as the retina is involved to a lesser degree. ID 299 has a phenotype that could not readily be distinguished. This child is severely affected and is blind in both eyes.

ID 159 and 297: One brother is bilaterally affected with iris colobomas. The other (ID 297) has only a deep vertical fissure in the iris at the six o'clock position. This mild manifestation of an optic fissure defect has already been discussed above.

ID 268 and 269: These girls, the second pair of identical twins in the study, are affected symmetrically but on opposite sides. They are affected unilaterally. The ocular defect is therefore mirrored. The paternal grandmother has a well-documented iris coloboma.

ID 276: This girl has a unilateral cataract as the primary diagnosis and clinical microphthalmos. No view of the fundus was possible but detailed records from an examination at Great Ormond Street Hospital and from relatives confirmed that a first cousin born in England is affected unilaterally with the same condition.

With the exception of ID 276, all of the other seven cases above with a family history have an underlying defect of optic fissure closure.

Consanguinity

This was present in the parents of one child with bilateral clinical anophthalmos/extreme microphthalmos (ID 117, first cousins) and in the grandparents (suspected/complex?) of another (ID 109). ID 60 Schwartz-Jampel syndrome was a twin resulting from a consanguineous union. This condition is autosomal recessive (Pinto-Escalante et al. 1997).

DISCUSSION

Difficulties identifying fissure defects

IDs 15 and 102: Right optic disc coloboma refers to the 'optic nerve head disruption' (see above comments) and these were considered to be due to defects of optic fissure closure.

IDs 142, 181, 241: Iris fissure/grooves. These fissures or grooves also appeared in positions on the iris consistent with the location of the optic fissure in the fellow eye of classical coloboma (ID 64, 287) or in a sibling (ID 297, 159). For this reason they were judged to be optic fissure closure defects. However, in ID 243, discussed in more detail in chapter ten, a *PAX6* gene mutation was found.

ID 296 has a left optic nerve head disruption. This was classified as a fissure defect since an affected sibling (ID 66) had classical iris and fundus coloboma.

Difficulties identifying and classifying non-fissure defects

ID 3: The appearance was highly suggestive of aniridia. An ophthalmologist first saw this toddler when she was a small baby, and detailed examination was not possible due to the lack of cooperation in such a small child. The external appearance without the aid of a slit lamp makes it difficult to distinguish a coloboma from some forms of aniridia. It was hoped that the *PAX6* mutation study (chapter nine) would clarify this. A similar situation also applied to IDs 121 and 187.

Difficulties identifying and classifying unclassifiable defects

Most of these cases were clinically anophthalmic or had extreme microphthalmos with sclerocornea. No view of the iris or fundus was possible, e.g. ID 67, 84.

Fundus coloboma has a variable clinical appearance (Pagon, 1981; Gopal et al. 1996), and clinical classifications have been attempted before (Gopal et al. 1996), using the appearance of the optic disc and whether or not it is involved within the retinal coloboma. Their results did bear some relationship to visual function. However, more relevant to this study (aetiology, recurrence risk, molecular genetic studies) was that the defects all had the same underlying mechanism.

Classification of coloboma

Five types of uveal coloboma were recognised in this study. The five types were classified as being due to an underlying defect of optic fissure formation or closure.

The numbers of cases assigned to each group are listed in Table 6.7. The first three categories have iris involvement (iris coloboma).

1. **Iris coloboma only.**
2. **Simple fundus coloboma**. E.g. ID 18, ID 30. The iris was (nearly) always involved. The anatomy of the posterior segment of the eye was normal in terms of the vessel pattern and position of the optic disc. The disc was usually not affected and the eye not clinically microphthalmic. Many of these subjects had near normal visual acuity.
3. **Complex fundus coloboma.** Figures 6.4 and 6.5. The iris was nearly always involved, the anatomy of the posterior pole of the eye is indistinct, the vessel pattern is abnormal, the macula is indistinct or involved, and the optic disc is often involved within the coloboma. Staphyloma was common. Many of these eyes were clinically microphthalmic. Vision was usually poor, but often much better than the appearance suggested (see discussion below on visual acuity, extrafoveal function, and near visual acuity (Olsen et al. 1996)). These complex fundus colobomas may be a more extensive form of optic nerve head disruption, e.g. IDs 33, 51, 66.

Figure 6.4: Complex left fundus coloboma, type 3. The edge of the coloboma traverses the nasal region of the macula adjacent to the poorly defined fovea (arrow) (ID 51)

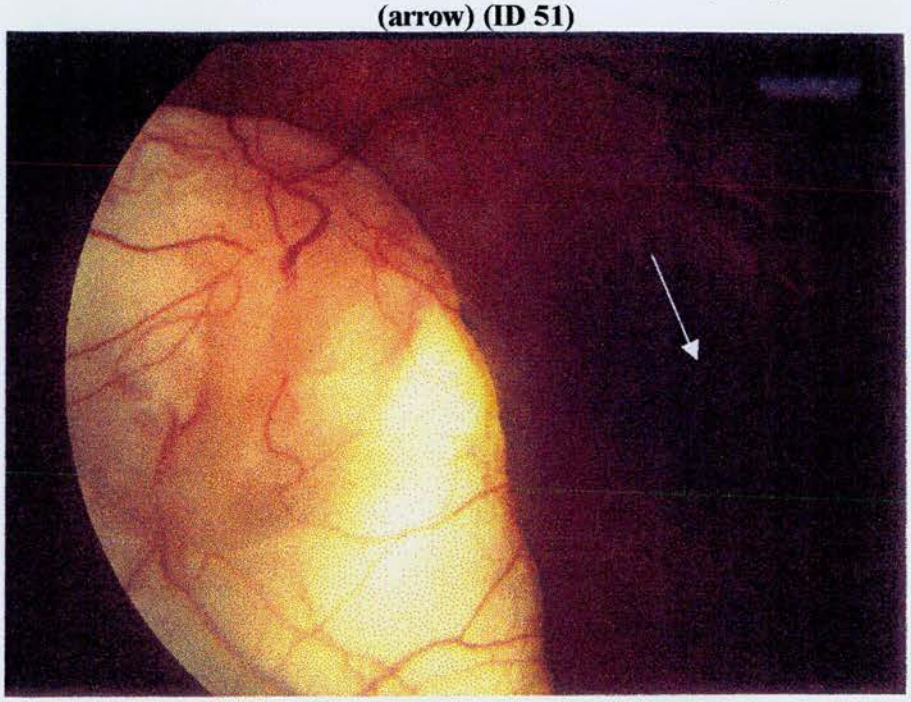
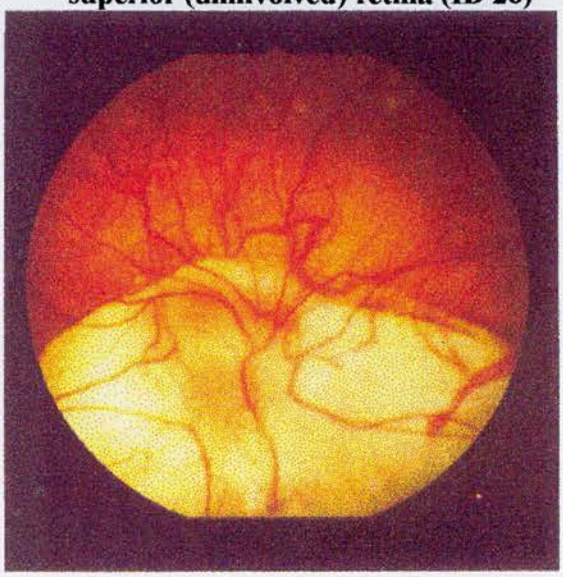


Figure 6.5: Right complex fundus coloboma involving the optic nerve and lower half of the retina, type 3. Note the irregular pattern of the blood vessels in the superior (uninvolved) retina (ID 26)

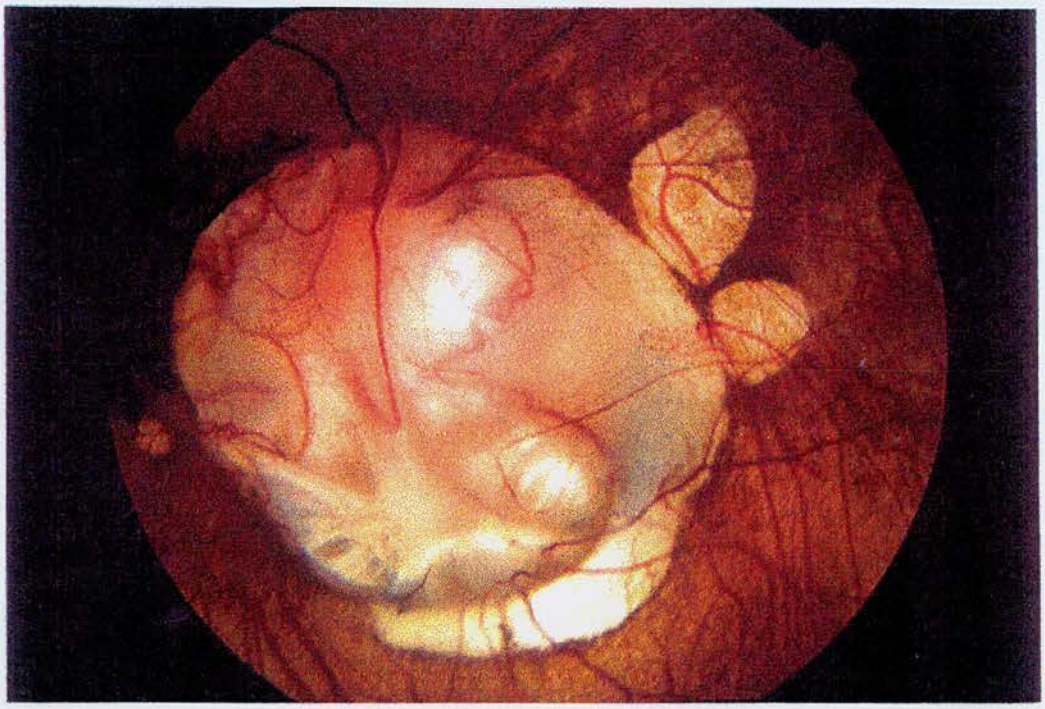


4. Irregular fundus/posterior pole coloboma. Figure 6.6. The iris was not involved.

The coloboma affects the anatomy of the posterior pole, there being no

recognisable optic disc or macula. Clinical microphthalmos was sometimes present. IDs 66, 96, 35, 188, 107.

Figure 6.6: Right irregular fundus coloboma (ID 107)



5. Optic nerve head disruption. Figure 6.7. The iris was not involved. Here, the disc is the only part of the eye involved (see above). The anatomy of the blood vessels and macula appears normal. Clinical microphthalmos was almost never present e.g. ID 15, 102.

Figure 6.7: Right optic nerve head disruption (ID 102)

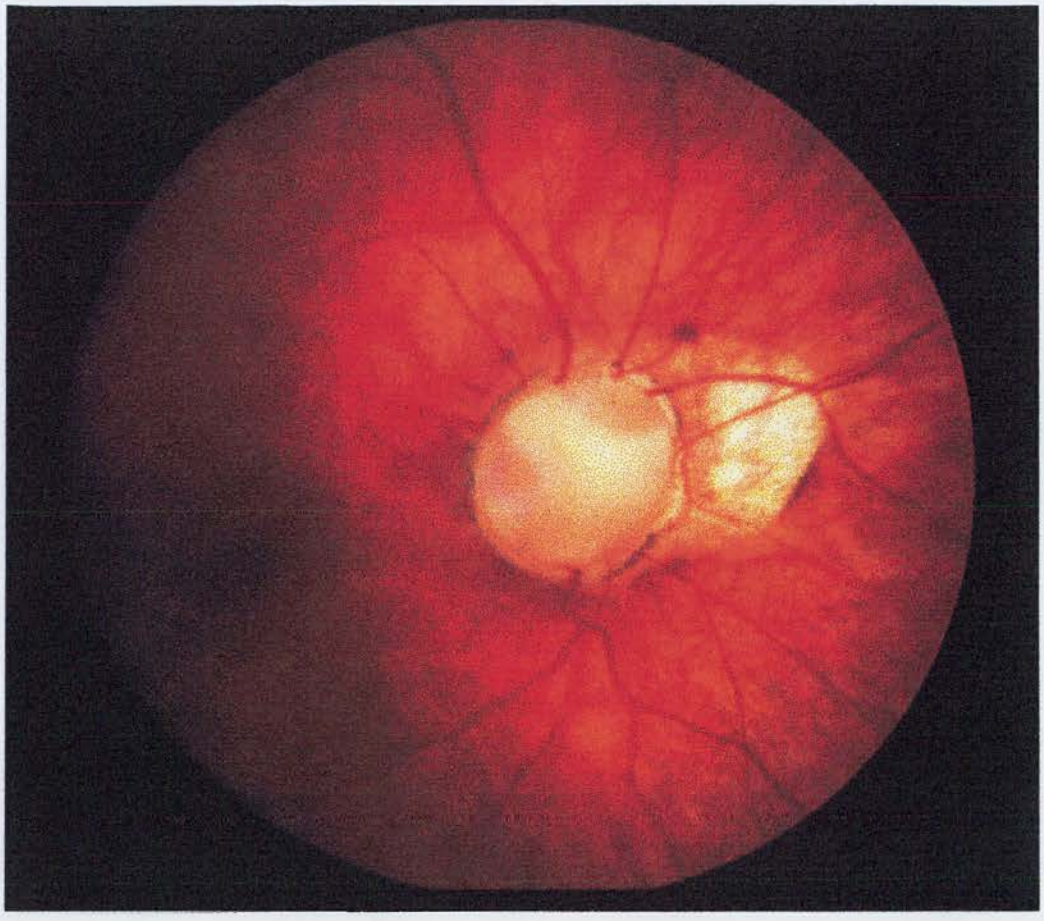


Table 6.7: Classification of colobomas: distribution amongst study group

Coloboma type	Number of eyes
I. Iris coloboma only	29
II. Simple fundus coloboma	22
III. Complex fundus coloboma	58
IV. Irregular fundus/posterior pole coloboma	7
V. Optic nerve head disruption	11

The coloboma types were identical in most bilaterally affected cases, although the symmetry of clinical appearance differed in most cases of bilateral eye pathology. In only four cases was there a difference in coloboma type. In ID 264, the types were type 3 (complex fundus coloboma) and type 5 (optic nerve head disruption) in each

eye. This does suggest that the underlying cause of some optic nerve head abnormalities is a defect of optic fissure formation or closure.

It is apparent that a simple classification of coloboma is not easy, and that the protean nature of this structural eye anomaly has made it difficult to apply any rules which do not have an exception.

The main difference between types 3 and 4 was whether the iris was involved or not. One important consideration is whether or not 'asymptomatic' retinal colobomas exist, and how common they are. The situation can exist where no iris coloboma is manifest and the vision is not significantly affected, and a retinal coloboma is present. This applies only to types 2 and 5 above. In only one case was this found, in the brother of a girl affected with classical coloboma (ID 66, 296). Therefore, it is highly unlikely that a significant number of cases were missed.

This reclassification of eye defects has deliberately moved away from classifying microphthalmos to classifying a group of structural defects. This recognises that microphthalmos is not a phenotypic diagnosis. Classification of microphthalmos in the past has not been easy or productive, and has done little to further our understanding of the causes (Bateman, 1984; Warburg, 1993).

The malformations that are not classifiable still present a problem. Because of the small numbers and rarity, it is difficult to recognise individual phenotypes. This should not deter us from sub-classifying each defect into a further category, though this would be on the basis that each category could later merge or disappear as more similar cases are found or patterns emerge. The key element here is adequate phenotypic detail. New groups or categories can emerge as our understanding of the genetics increases.

Other coloboma classifications

Coloboma has a wide and complex variety of clinical presentation (Steahly, 1990; Daufenbach et al. 1998). There have been several recent attempts to classify coloboma, based on the effect on vision and fundus appearance (Gopal et al. 1996; Olsen et al. 1996), or anatomy (Warburg, 1993). These classifications have all been excessively complicated, and are not easily applied in clinical practice. Gopal's classification concentrated on the variable appearance of the optic disc in fundus coloboma (based on photographs) and the author makes some correlation between disc appearance and visual acuity. Examination of fundus photographs by Olsen correlated normal foveal anatomy with improved visual acuity.

In this study, fundus photographs were not taken due to practical constraints. Drawings were made in the records of most cases, but these would not yield the detail required for the studies based on photographic evaluation.

Visual function

Observations in this study and recently published work (Olsen et al. 1996; Hornby et al. 2000) have made it clear that visual acuity is difficult to correlate with the structural eye abnormality seen. This is important since being able to predict the vision in a small baby or child is relevant to later development and education. The vision is also affected by factors other than the coloboma, such as corneal astigmatism and high refractive error and lens opacity. The reading and navigational vision can be surprisingly good, especially after optical correction with low vision aids (Hornby et al. 2000). In ID 51, for example, this boy has extensive left iris and fundus coloboma

(type 2) with optic disc involvement. Distance acuity is 6/18 but he is able to read small print (N5) with a spectacle correction of -2.00/+1.50 x 90.

A recent study proposed a phenotypic classification of coloboma, which correlated the groups with visual acuity, reading and navigational vision (Hornby et al. 2000). This study included diagnostic groups based on microcornea, which was defined (incorrectly) as being less than 10 mm horizontal corneal diameter, regardless of the patient's age. Microphthalmos was a further diagnostic category, and this was inappropriately defined based on axial length. These aspects are dealt with again in the next chapter (seven).

The above classification may require modification as not all the ocular phenotypes fit easily into it, and it is difficult to apply without having a photographic record. This makes application into clinical practice difficult. However, it is simple with just four categories. It is more easily understood and applicable than other classifications, and is not based upon the use of the terms microphthalmos or microcornea.

The classification will only have real importance if visual outcome or prognosis, or association with a specific genetic abnormality can distinguish the four groups.

As has already been stated, visual outcome in coloboma depends on factors in addition to anatomy, such as refractive error, strabismus, cataract, and extraocular problems such as developmental delay. This makes assessment extremely difficult. Although an attempt was made to document these factors, there are not sufficient numbers in this study with a complete data set for a valid comparison to be made. Data was collected on both near and distance vision of nearly 70 eyes affected with coloboma, but details of spectacle prescription or refraction were available in only 12 cases. None of the subjects were refracted due to time and equipment constraints.

Accurate vision assessment takes longer and detailed assessment needs orthoptic skill in younger children, when the most appropriate tests, e.g. Sheridan-Gardiner or Kay's pictures, can be used.

Asymmetry

Asymmetry was very common for fissure defects. Of 45 individuals bilaterally affected, 12 had symmetrical eye pathology. Also, all the individuals with a family history demonstrated asymmetry and variable ocular phenotypic expression (see above). There is no simple explanation for asymmetry. Each individual has two genetically identical eyes but because of stochastic factors (i.e. chance) the genetic defect, if it is indeed genetic, is expressed differently in the two eyes. This may be a result of critical gene dosage and effects of their protein products. If the eye defects were entirely environmental in aetiology then variable effects of toxins on the developing eye could explain the asymmetry. The alternative explanation is that genes and the environment are acting together (see chapter two). The gene(s) responsible for these eye malformations are known to have a very variable phenotypic expression. The varied expression does give clues as to the timing of gene expression during eye development (chapter three).

Fundamental to the classification of fissure and non-fissure defects is that no individual should have an eye defect in one eye not consistent with the same aetiology, i.e. there were no cases of OFD/non-OFD occurring together.

Atypical colobomas

In a few individuals, a 'coloboma' of the iris was seen in a position not usually recognised, that is, a typical coloboma of the iris is usually seen inferiorly (6 o'clock position) or inferonasally, consistent with the position of the optic fissure. In IDs 251 and 275, iris colobomas were atypical in position. There is no complete explanation for this but there are several possibilities:

The iris defect may not be a coloboma, but an anterior segment dysgenesis with a 'coloboma', or variant of aniridia. These would not be expected to have retinal or optic disc colobomas in the eye or fellow eye.

It may be the case that in some individuals the position of the optic fissure is different, or that an accessory fissure is present. In ID 251, the retina was affected in a position corresponding to the iris coloboma, and in ID 275 the fundus coloboma was in a typical (inferior) position away from the (superior) iris coloboma. Brodsky has described a unilateral superotemporal coloboma of the iris, ciliary body and choroid (Brodsky et al. 1988). The retina, macula and optic disc were normal.

ID 162, atypical superior iris coloboma with lens coloboma/notch and simple retinal coloboma, was unclassifiable (debatable).

This study has confirmed the highly variable appearance of ocular coloboma. Also, asymmetry of ocular pathology in bilaterally affected individuals was present in 33 of the 45 cases. Within individuals from affected pedigrees, there was asymmetry of eye pathology and cases with unilateral eye defects. This highlights the phenotypic variability and variable penetrance and expression of possible genes involved. Patterns

of inheritance suggested both autosomal dominant and autosomal recessive inheritance.

Non-OFD covers a number of different ocular phenotypes.

It is not possible to say whether any of those phenotypes grouped as unclassifiable had an underlying optic fissure defect and this group would need to be larger to sub-classify if further studies were undertaken.

Summary, chapter six

- A database and research resource of well phenotyped and clinically verified congenital eye malformations has been set up.
- Eye malformations previously described as coloboma and anophthalmos have been reclassified according to phenotype, based on the presence or absence of an optic fissure closure defect.
- A classification of coloboma is suggested, based on the assumption that some optic nerve head abnormalities are due to underlying defects of optic fissure closure. Difficulties remain with categorisation of optic nerve head abnormalities, which have been named 'optic nerve head disruption'.
- In 12 cases, external eye and lid abnormalities were present.
- There were eight cases with a family history confirmed by examination, and in seven of these the underlying eye malformation was a defect of optic fissure closure.

The structural eye anomalies in this study occurred not only as isolated eye defects, but also in individuals often with other systemic congenital anomalies. These are the subject of chapter eight.

The need for a new classification came about partly as a result of the findings in the next chapter (seven), where a definition for microphthalmos based on axial length was sought.

CHAPTER SEVEN

AN ULTRASOUND STUDY ON THE AXIAL LENGTH OF THE EYE IN CLINICAL MICROPHTHALMOS

'The diagnosis [of microphthalmos] frequently can be made by inspection alone. However, as microcornea may occur without microphthalmos and, conversely, microphthalmos may occur with a normal-sized cornea, a clinical diagnosis may be inaccurate. Ultrasonography, with precise measurement of the anteroposterior axis, now permits, and is in fact essential for, a biometric diagnosis in vivo.'

(Bateman, 1984).

This chapter will show that the latter part of the above statement is incorrect, since microphthalmos cannot be defined by the proposed methods.

Summary

Axial length of the eye is not a useful definition of microphthalmos.

More than half of all congenitally microphthalmic eyes have an axial length within or significantly beyond the normal range when corrected for age.

Most (not all) clinically small eyes have normal sized globes.

Some congenitally colobomatous eyes with microphthalmos are bigger than normal: congenital *colobomatous macrophthalmos with microcornea.*

Less than half of clinically microphthalmic eyes fulfil the commonly accepted definition of microphthalmos, which is based on an axial length of less than two standard deviations below the mean.

Reduced horizontal corneal diameter is found in most cases of clinical microphthalmos.

Reduced palpebral fissure length is found in most cases of clinical microphthalmos.

Ocular ultrasound B-scan seems to be well tolerated by children. Unlike CT or MRI, a general anaesthetic is not required for small children.

Ultrasound B-scan can yield additional clinical information by imaging the posterior segment in the presence of a lens or corneal opacity: microphthalmos with cyst, posterior staphyloma and retinal detachment. However, useful images are not always obtained with B-scans.

BACKGROUND

Making the diagnosis of clinical microphthalmos based on appearance is relatively easy for extreme microphthalmos but with mild/moderate microphthalmos it is problematic. For unilateral cases it is possible to comment on the relative difference on the assumption, possibly incorrectly, that the structurally normal fellow eye is indeed normal, but for bilateral cases the task is not so easy.

The current accepted definition of microphthalmos is based on the axial length of the globe being below the fifth centile (more than 2 standard deviations below the mean) when corrected for age, (Weiss et al. 1989b) (chapter one).

The aim of this study was to show that microphthalmos cannot be defined based on the axial length of the globe and that the current definition is not satisfactory (chapter one). These definitions continue to be used even in recent publications (Hornby et al. 2000).

METHODS

Eyes were judged as clinically microphthalmic or non-microphthalmic according to the records and diagnosis from the referring consultant ophthalmologist. Microphthalmos therefore included all apparently small eyes. An ultrasound B-scan was done on both eyes. All the eyes had an ocular phenotypic diagnosis, which included coloboma and clinical anophthalmos. Clinically anophthalmic eyes were not included in this part of the study for measurement.

Ultrasounds were done with a hand-held probe against the eyelid using contact gel. This does not require corneal anaesthesia or contact, making the procedure pain free and tolerable to young children. It was felt that other diagnostic information might be obtained by using ultrasound B-scan in preference to A-scans (Fledelius, 1996; Isenberg and Fishman, 1996). It is easier to obtain an axial length or a useful image in colobomatous eyes (often with staphylomas), eyes with cysts, dense lens opacities or vitreous abnormalities. Both eyes were measured where possible, including the normal fellow eye in unilaterally affected children.

Not all centres or clinics had ultrasound B-scanners available, so scans were only performed at the centres listed below.

A contact gel was applied to the outside of the upper eyelid, the scan performed, and an image printed. At least five different machines were used in different departments: Edinburgh (Princess Alexandra Eye Pavilion), Dunfermline (Queen Margaret's Hospital), Glasgow (Yorkhill), Dundee (Ninewells), Aberdeen (Royal Infirmary), and in each case it was the same operator (DM). The 14 ultrasound scans in Glasgow were done in the X-ray department of the Royal Hospital for Sick Children, Yorkhill.

These scans were performed under the supervision of the late Dr Anne Hollman, Consultant Radiologist.

Scans were assessed by Dr Brian Fleck, consultant ophthalmologist, who was unaware of the clinical diagnosis. The axial length of the eye was estimated from the printed image to the nearest 0.5 mm, using a calliper and the printed scale below each image.

All children had measurement of horizontal corneal diameter (HCD) and eyelid palpebral fissure length (PFL), where possible. These measurements were taken using a straight rule held as close to the eye and lids as possible.

Axial length (AL) was compared to the age-corrected mean for sex (Larsen, 1971).

HCD was compared to age-corrected mean for sex (Hymes, 1929).

Palpebral fissure length was compared to the age-corrected mean (Hall et al. 1989).

RESULTS

Clinical microphthalmos was judged by the referring consultant ophthalmologists to be present in 56 eyes (42 subjects), and 30 eyes (22 subjects) were judged not to have microphthalmos (Tables 7.1 and 7.2). Both groups of eyes had structural abnormalities and the ocular phenotypes are listed in Table 7.3. The commonest phenotypic eye abnormality was uveal coloboma. A third group consisted of 21 eyes that were completely normal on examination and with normal vision. These 21 measurements were on the fellow eye of unilaterally affected individuals. The ID numbers are listed in Table 7.4.

Table 7.1: Clinically microphthalmic eyes: axial length (AL), horizontal corneal diameter and palpebral fissure length. Grey boxes represent eyes with an AL more than 2 SDs below the mean (matched for age and sex), blue boxes represent eyes with an AL more than 2 SDs above the mean, and the plain boxes represent eyes within the normal range

Case ID	Date of Birth	Sex M=1 F=2	Age at 1/1/98	Eye	Horizontal Corneal Diameter, mm	Axial Length, mm	Palpebral Fissure Length, mm
1	05-Sep-1984	1	13	r	not recorded	23.0	26.0
1	05-Sep-1984	1	13	l	6.0	17.0	26.0
6	28-Jun-1984	1	13	l	9.0	22.0	21.0
8	29-Nov-1992	1	5	l	9.5	22.0	21.0
20	19-Dec-1993	2	4	r	6.0	18.5	21.0
33	22-May-1985	1	12	r	5.6	22.0	24.0
33	22-May-1985	1	12	l	3.0	12.0	21.5
35	28-Feb-1993	2	4	r	9.5	21.0	18.0
39	06-Jun-1992	1	5	r	9.0	21.0	19.0
39	06-Jun-1992	1	5	l	8.0	20.5	19.0
48	29-Mar-1984	2	13	l	5.0	16.0	22.0
50	23-Jun-1996	1	1	l	3.0	13.0	18.0
51	17-Nov-1989	1	8	r	5.8	16.0	22.0
51	17-Nov-1989	1	8	l	10.5	18.0	26.0
55	17-Aug-1984	2	13	r	7.5	24.0	24.0
55	17-Aug-1984	2	13	l	8.0	23.0	24.0
61	05-Sep-1991	2	6	l	7.0	22.0	21.0
62	10-May-1992	2	5	l	8.0	15.0	22.0
66	19-Feb-1989	2	8	r	10.0	25.0	22.0
66	19-Feb-1989	2	8	l	6.5	21.0	22.0
75	01-Feb-1988	1	9	l	9.5	26.0	23.0
79	17-Jul-1991	1	6	r	9.5	32.0	21.5
79	17-Jul-1991	1	6	l	7.0	22.0	17.0
83	28-Feb-1982	2	15	l	10.5	24.0	26.0
84	15-Oct-1981	2	16	r	8.0	11.0	24.0
90	15-Oct-1993	1	4	r	9.5	22.0	22.0
90	15-Oct-1993	1	4	l	7.0	17.5	20.5
93	20-Aug-1981	2	16	r	10.0	13.0	24.0
93	20-Aug-1981	2	16	l	13.0	15.0	24.0
94	27-Aug-1987	1	10	r	7.0	13.0	19.0
94	27-Aug-1987	1	10	l	4.0	13.0	18.0
97	22-Jul-1982	2	15	r	1.0	14.0	23.0
97	22-Jul-1982	2	15	l	2.8	14.0	25.0

Case ID	Date of Birth	Sex M=1 F=2	Age at 1/1/98	Eye	Horizontal Corneal Diameter, mm	Axial Length, mm	Palpebral Fissure Length, mm
100	23-Aug-1992	2	5	r	5.0	not recordable	21.0
101	14-Dec-1985	1	12	r	10.0	22.5	26.5
107	23-Feb-1984	1	13	r	9.5	25.0	25.0
109	08-Mar-1987	1	10	r	9.5	26.0	23.0
111	22-Jun-1992	2	5	l	10.0	22.0	20.0
113	21-Jun-1995	1	2	l	8.0	22.5	20.0
129	27-Dec-1982	2	15	r	10.5	23.0	25.0
138	30-Jan-1982	2	15	r	11.0	24.5	28.0
172	07-Apr-1987	1	10	r	4.0	9.0	24.0
181	27-Dec-1995	1	2	r	8.5	22.0	not recorded
181	27-Dec-1995	1	2	l	8.0	24.0	not recorded
189	01-Jul-1992	2	5	r	not recorded	20.0	17.0
189	01-Jul-1992	2	5	l	not recorded	16.0	17.0
192	17-Nov-1989	2	8	r	7.0	22.0	18.0
192	17-Nov-1989	2	8	l	8.5	24.0	22.0
224	04-Aug-1993	1	4	r	9.0	22.0	16.0
252	24-Jan-1982	2	15	r	7.0	16.0	25.5
258	14-Nov-1990	1	7	r	9.0	24.0	21.0
261	26-Jul-1987	2	10	l	5.0	12.0	21.0
268	16-Feb-1996	2	1	r	7.0	20.0	not recorded
269	16-Feb-1996	2	1	l	7.0	20.0	not recorded
272	19-Aug-1991	2	6	l	8.0	23.0	not recorded
276	07-Nov-1991	2	6	l	9.5	21.5	20.5

Table 7.2: Clinically non-microphthalmic eyes: axial length, horizontal corneal diameter and palpebral fissure length. Grey boxes represent eyes with an AL more than 2 SDs below the mean (matched for age and sex), blue boxes represent eyes with an AL more than 2 SDs above the mean, and the plain boxes represent eyes within the normal range

Case ID	Date of Birth	Sex M=1 F=2	Age at 1/1/98	Eye	Horizontal Corneal Diameter, mm	Axial Length, mm	Palpebral Fissure Length, mm
18	04-Aug-1995	2	2	r	11.0	22.5	21.0
18	04-Aug-1995	2	2	l	11.0	21.0	21.0
34	27-May-1993	1	4	r	11.0	23.5	24.0
34	27-May-1993	1	4	l	11.0	24.5	24.0
35	28-Feb-1993	2	4	l	11.0	21.5	19.0
50	23-Jun-1996	1	1	r	11.0	20.0	21.0
62	10-May-1992	2	5	r	11.0	24.5	22.0
75	01-Feb-1988	1	9	r	11.5	22.0	23.0
76	31-Oct-1989	2	8	r	9.0	24.5	23.0
76	31-Oct-1989	2	8	l	10.0	27.5	23.0
83	28-Feb-1982	2	15	r	11.0	24.0	26.0
96	28-Feb-1993	2	4	r	not recorded	22.0	not recorded
96	28-Feb-1993	2	4	l	not recorded	19.5	not recorded
109	08-Mar-1987	1	10	l	10.0	26.0	23.0
113	21-Jun-1995	1	2	r	10.5	25.0	20.0
138	30-Jan-1982	2	15	l	11.5	29.0	28.0
140	25-Nov-1985	1	12	l	11.0	23.5	26.0
150	13-Jul-1992	1	5	r	12.0	20.0	22.0
153	09-Mar-1994	1	3	l	11.5	22.0	23.0
159	12-Dec-1981	1	16	r	11.0	21.0	26.0
159	12-Dec-1981	1	16	l	11.0	22.0	26.0
166	20-Apr-1985	2	12	l	not recorded	24.0	0.0
187	16-Dec-1996	1	1	r	12.5	20.0	18.0
187	16-Dec-1996	1	1	l	12.5	20.0	18.0
228	05-Apr-1994	2	3	r	9.0	26.0	22.0
228	05-Apr-1994	2	3	l	9.0	22.0	22.0
234	12-Nov-1991	2	6	r	11.0	24.0	22.0
234	12-Nov-1991	2	6	l	11.0	22.0	22.0
241	22-Nov-1981	2	16	r	12.0	24.5	28.0
275	29-May-1993	2	4	r	10.5	24.0	21.5

Table 7.4: Clinically normal eyes: axial length, horizontal corneal diameter and palpebral fissure length Grey boxes represent eyes with an AL more than 2 SDs below the mean (matched for age and sex), grey boxes represent eyes with an AL more than 2 SDs above the mean. The plain boxes represent eyes within the normal range

Case ID	Date of Birth	Sex M=1 F=2	Age at 1/1/98	Eye	Horizontal Corneal Diameter, mm	Axial Length, mm	Palpebral Fissure Length, mm
6	28-Jun-1984	1	13	r	11.00	23.0	23.0
8	29-Nov-1992	1	5	r	11.50	21.0	23.0
20	19-Dec-1993	2	4	l	11.50	18.0	21.0
48	29-Mar-1984	2	13	r	12.00	25.0	26.0
61	05-Sep-1991	2	6	r	not recorded	22.0	21.0
84	15-Oct-1981	2	16	l	12.00	24.0	26.0
101	14-Dec-1985	1	12	l	12.50	23.0	26.5
107	23-Feb-1984	1	13	l	12.50	24.0	26.5
111	22-Jun-1992	2	5	r	11.50	22.0	20.0
129	27-Dec-1982	2	15	l	12.00	22.0	25.0
140	25-Nov-1985	1	12	r	11.50	24.0	26.0
153	09-Mar-1994	1	3	r	11.50	22.0	23.0
224	04-Aug-1993	1	4	l	11.50	20.0	18.0
241	22-Nov-1981	2	16	l	12.00	24.0	27.0
252	24-Jan-1982	2	15	l	12.00	26.0	27.0
258	14-Nov-1990	1	7	l	12.00	21.0	24.0
261	26-Jul-1987	2	10	r	11.00	22.5	24.0
268	16-Feb-1996	2	1	l	10.00	20.5	not recorded
269	16-Feb-1996	2	1	r	10.00	21.5	not recorded
272	19-Aug-1991	2	6	r	11.00	23.0	not recorded
275	29-May-1993	2	4	l	10.75	23.0	21.5

Axial length (AL)

Table 7.1 lists the AL of 55 of the 56 clinically microphthalmic eyes. ID 100 had severe clinical microphthalmos with a clinically apparent globe and an ocular phenotype that included cataract and corneal scarring. The corneal opacification and density of the ocular media meant that no measurable ultrasound image was obtained. Only 20/55 (36%) clinically microphthalmic eyes fulfilled the proposed definition

criteria for microphthalmos. Almost half (42%) of the clinically microphthalmic eyes (23/55) had AL within the normal range when corrected for age and sex. Twelve eyes (22%) had AL more than 2 SDs above the normal range. Results are illustrated in Figure 7.1.

Figure 7.1: Axial length of 55 clinically microphthalmic eyes, showing number of eyes within two standard deviations of the age-corrected mean

Microphthalmos as defined by axial length	Axial length within normal range	Bigger than normal axial length (macrophthalmos)
20	23	12

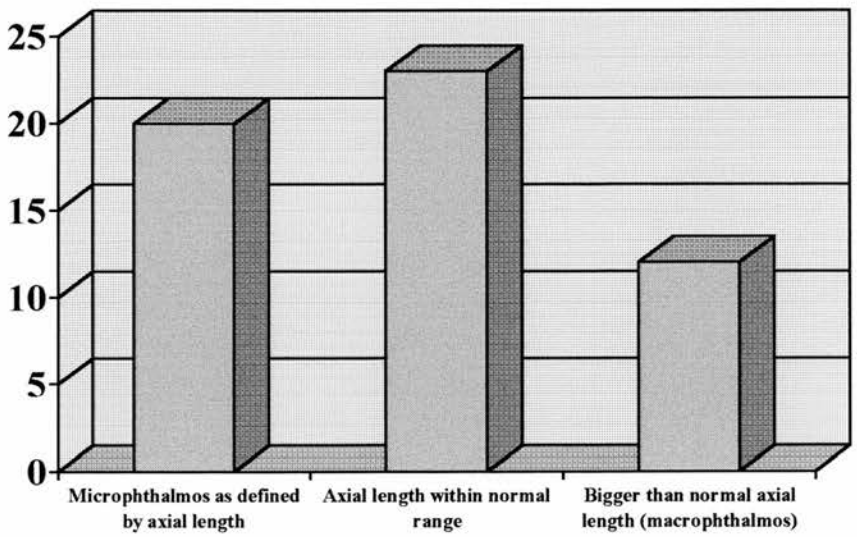


Table 7.2 lists the AL of 30 clinically non-microphthalmic eyes. Only 4 (13%) were below 2 SDs of AL when corrected for age and sex. Thirteen eyes (43%) were within normal range and 13 were above the normal range for AL.

Figure 7.1: Axial length of 30 clinically non-microphthalmic eyes with structural abnormality, showing number of eyes within two standard deviations of the age-corrected mean

Microphthalmos as defined by axial length	Axial length within normal range	Bigger than normal axial length (macrophthalmos)
4	13	13

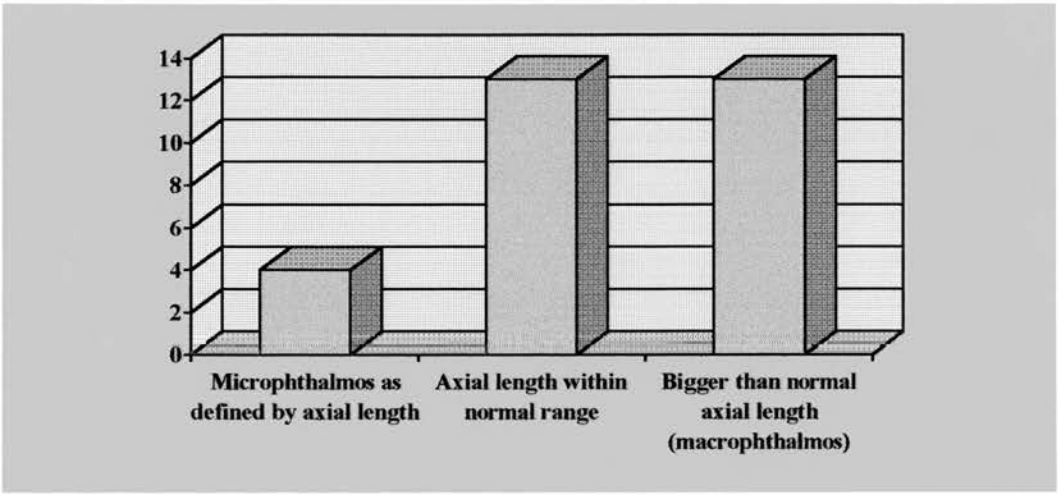


Table 7.3 lists the AL of 21 normal eyes. Two eyes (9.5%) fulfilled the definition of microphthalmos and 3 eyes were bigger than normal. Most of the normal eyes (16/21=76%) fell within the normal range of size.

Microphthalmos as defined by axial length	Axial length within normal range	Bigger than normal axial length (macrophthalmos)
2	16	3

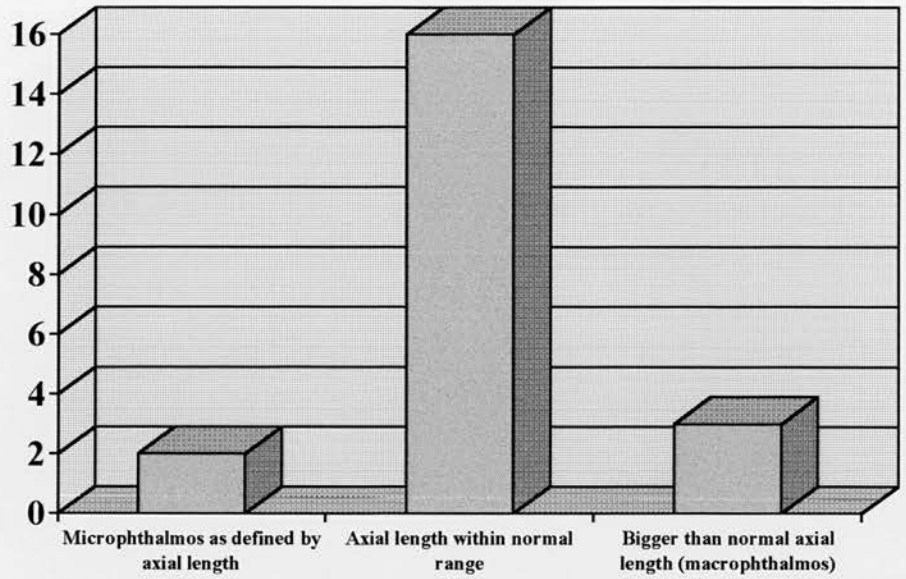


Figure 7.3: Axial length of 21 clinically normal eyes showing number of eyes within two standard deviations of the age-corrected mean

The B-scan ultrasounds confirmed many of the clinical findings. Other features noted were: staphyloma ID 76, 138, 258 (Figure 7.13, page 199), detached retina ID 94, 33 (Figure 7.15) (Morrison and Fleck, 1999), microphthalmos with cyst ID 50.

The cases in which the eye was clinically microphthalmic but which on measurement had AL more than 2 SDs above the normal range are *colobomatous macrophthalmos with microcornea* ID 55, 76, 79, 83, 192, 258. The increased AL in some cases may explain the presence of high myopia (ID 138).

Horizontal corneal diameter (HCD)

Horizontal Corneal Diameter (HCD) was measured in 53 clinically microphthalmic eyes (Table 7.1) and in 50/53 cases (94.3%) was more than 2 SDs below the mean, when corrected for age and sex. Only 3 eyes had a HCD within the normal range. In

the non-microphthalmic eyes (Table 7.2), HCD was measured in 27 cases and 9/27 (33.3%) were below the normal range with 16/27 within normal range. Two eyes had a HCD bigger than normal range. In the group of 20 normal eyes measured, HCD was within 2 SDs of the age-corrected mean in 90% of cases (18/20). In two cases with normal eyes, HCD was just below the normal age-corrected range (Table 7.3).

Eyelid palpebral fissure length (PFL)

The palpebral fissure length (PFL) of the eyelid was measured in 50 clinically microphthalmic eyes and was more than 2 SDs below the mean in 48 (96%) when corrected for age (Table 7.1). In 27 non-microphthalmic eyes measured, PFL was 2 SDs below the mean in 22/27 (81.5%) cases, with 5 cases within the normal range Table 7.2). Of 18 normal eyes measured, PFL was below the normal range in 13 (72.2%), Table 7.3.

To highlight some of the AL findings, several cases are discussed and illustrated in more detail:

Clinical microphthalmos: small eye measured on ultrasound

(i) Bilateral iris and fundus coloboma, ID 51 (Figure 7.4) Both eyes are clinically microphthalmic and have significantly shorter than normal axial lengths: right AL 16 mm, left AL 18 mm, right HCD 5.8 mm, left HCD 10.5mm.

Figure 7.4: Bilateral iris and fundus coloboma with clinical microphthalmos (ID 51)

(ii) Bilateral extreme clinical microphthalmos and left iris coloboma, ID 97 (Figure 7.5). ALs measured as small (14 mm). Right HCD 1 mm, left HCD 2.8 mm. An ultrasound B-scan of the left globe is shown in Figure 7.6.

Figure 7.5: Bilateral extreme clinical microphthalmos and left iris coloboma

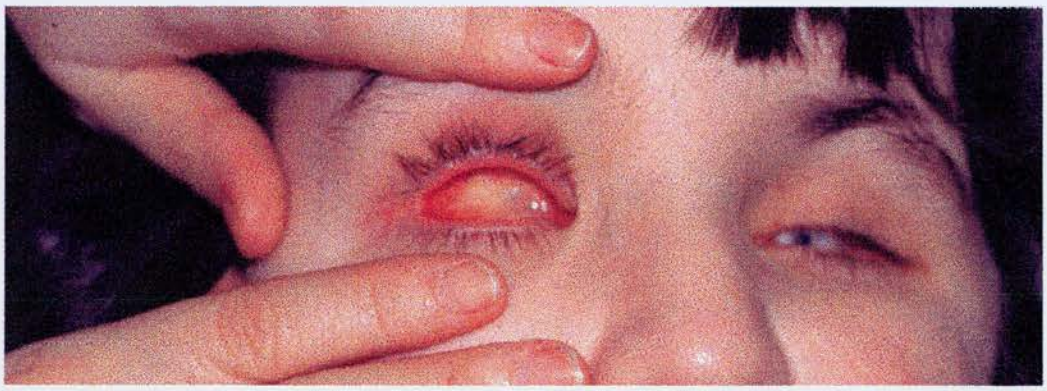
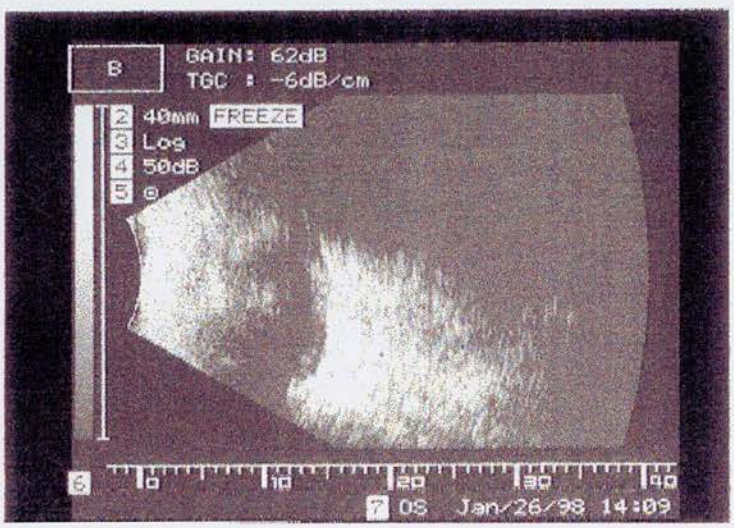


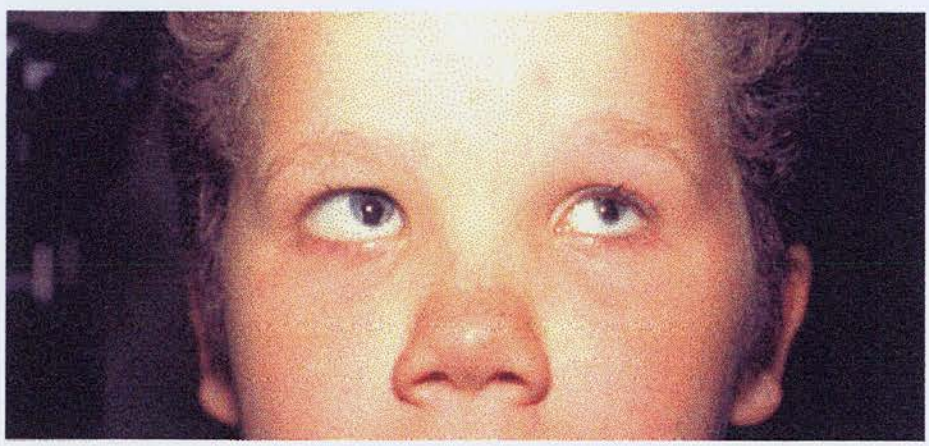
Figure 7.6: Ultrasound B-scan of left globe, ID 97. AL is 14 mm. The subject is pictured in Figure 7.5



Clinical microphthalmos: normal eye measured on ultrasound

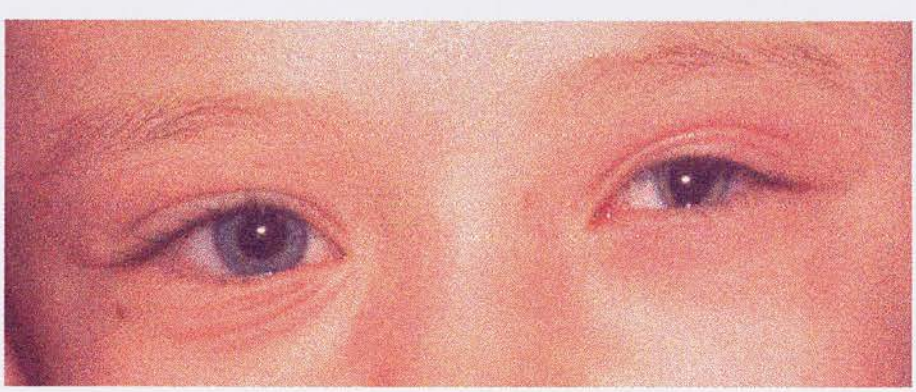
(i) Bilateral clinical colobomatous microphthalmos ID 79 Figure 7.7. Right AL 32 mm, left AL 22 mm.

Figure 7.7: Bilateral clinical colobomatous microphthalmos. The left AL of 22 mm is within the normal range. Right AL of 32 mm is big (ID 79)



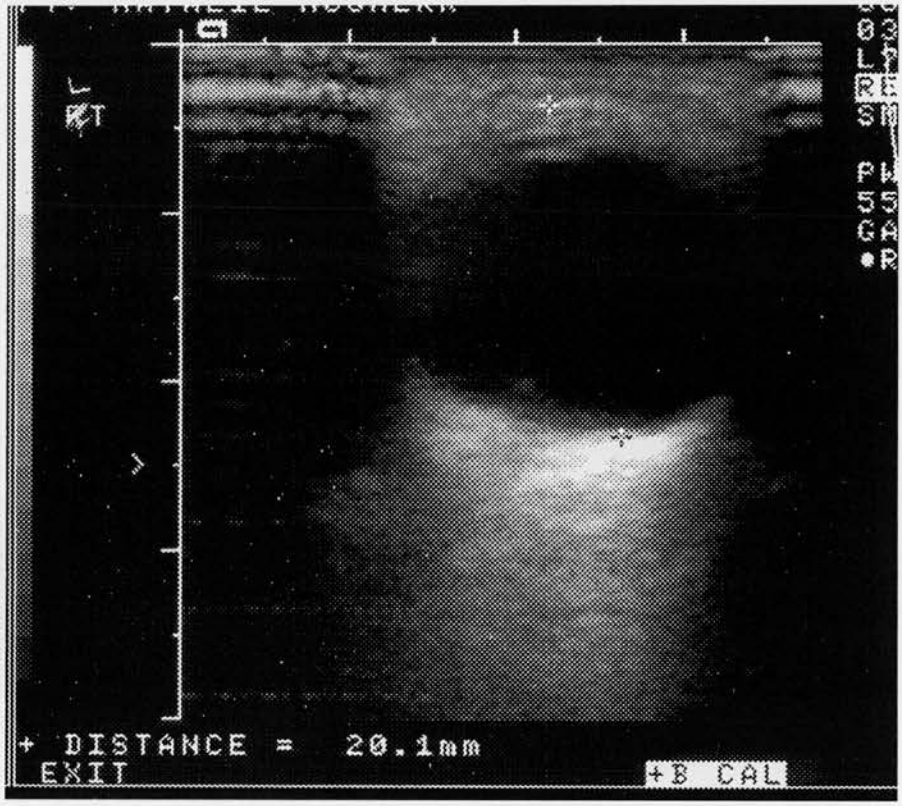
(ii) Left clinical colobomatous microphthalmos ID 272, Figure 7.8. AL 23 mm.

Figure 7.8: Left clinical colobomatous microphthalmos and iris heterochromia. The AL of 23 mm is within the normal range (ID 272)



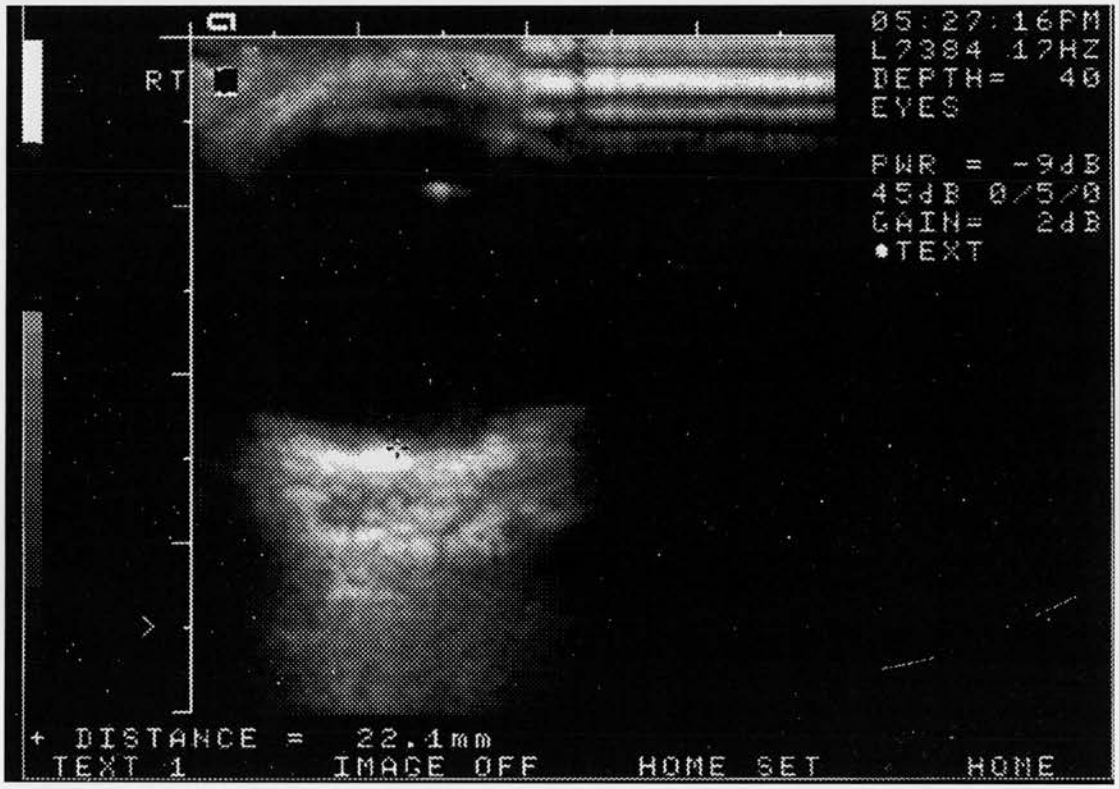
(iii) Left iris and fundus coloboma with clinical microphthalmos, normal right eye. ID 269. Right AL 21.5 mm, left AL 20 mm, left HCD 7.0 mm, Figure 7.9.

Figure 7.9: Ultrasound B-scan of left globe with left iris and fundus coloboma with clinical microphthalmos. The anterior corneal surface and the retina are indicated with crosses (ID 269)



(iv) Bilateral iris/fundus colobomas and bilateral clinical microphthalmos ID 90 Figure 7.10. Right AL 22 mm (normal), left AL 17 mm (small). Right HCD 9.5 mm, left HCD 7.0 mm.

Figure 7.10: Bilateral iris and fundus colobomas and bilateral clinical microphthalmos, Ultrasound B-scan of right eye. The anterior corneal surface and retina are marked with crosses (ID 90)



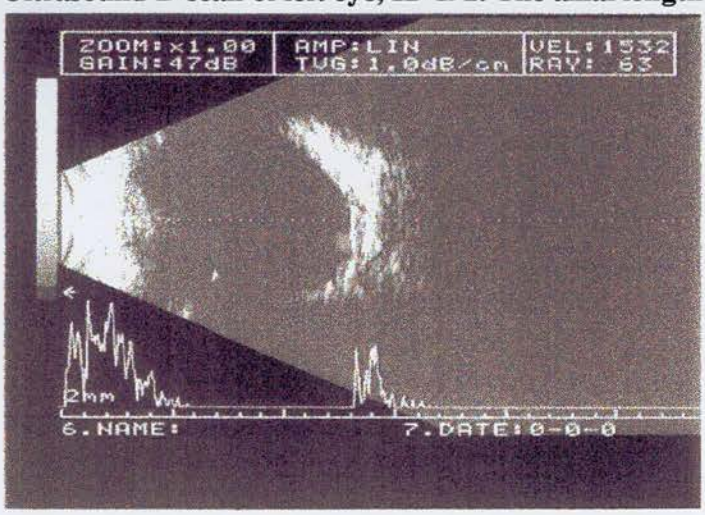
Clinical microphthalmos: big eye measured on ultrasound scan = *colobomatous macrophthalmos with microcornea*

(i) Right iris and fundus coloboma, clinical microphthalmos: ID 79 (Figure 7.7 page 193) Right AL 32 mm, right HCD 9.5 mm.

(ii) Bilateral iris and fundus colobomas, bilateral clinical microphthalmos: ID 192 (Figure 7.11). Left AL 24.0 mm, left HCD 8.5 mm.

Figure 7.11: Bilateral iris and fundus colobomas, bilateral clinical microphthalmos. The left AL of 24 mm is bigger than normal. The ultrasound B-scan is also shown (ID 192)

Ultrasound B-scan of left eye, ID 192. The axial length is 24.0 mm

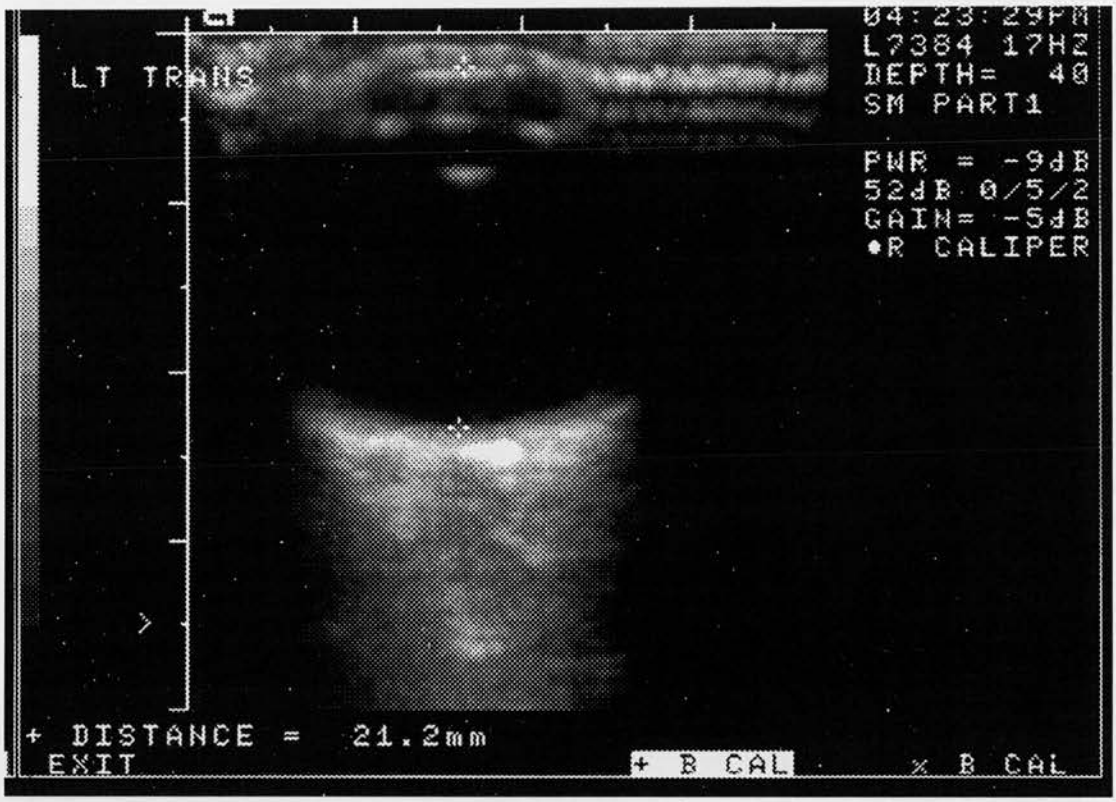


(iii) Right iris and fundus coloboma, right clinical microphthalmos: ID 258 Figure 7.12. Right AL 24.0 mm, HCD 9.0 mm.

Figure 7.12: Right iris and fundus coloboma, right clinical microphthalmos, iris heterochromia. Ultrasound B-scan of right eye shows irregular post pole. The B-scan of the normal left eye is shown at the bottom. AL right 24 mm (big), left 21 mm (normal eye in length and phenotype), HCD right 9.0 mm. (ID 258)

Right Eye, ultrasound B-scan (ID 258)

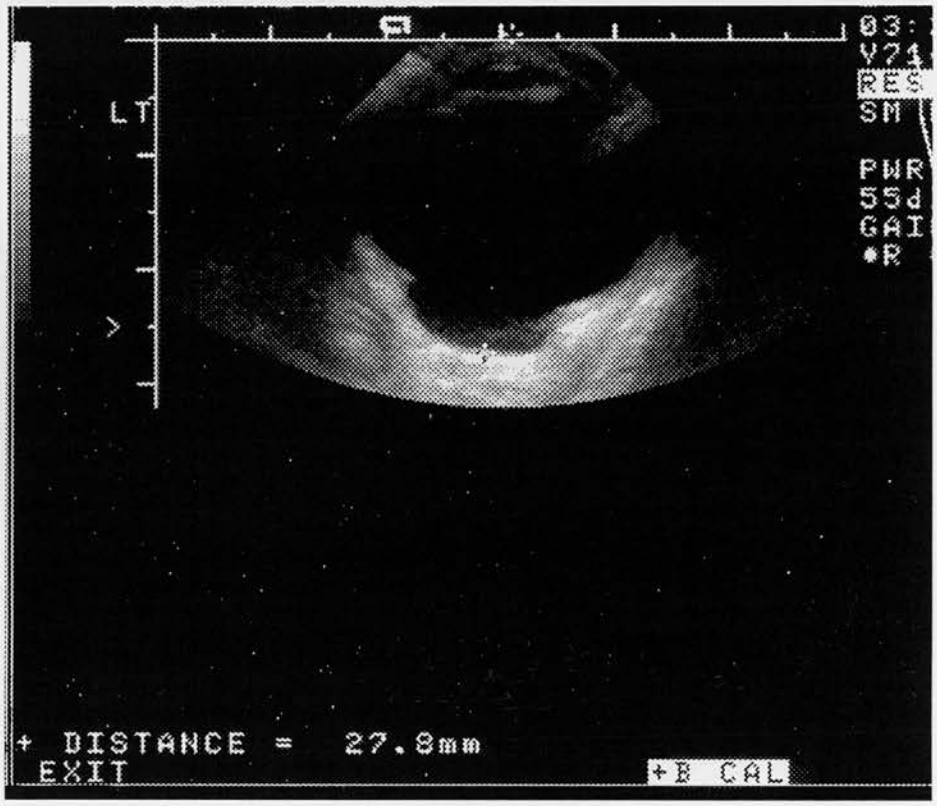
Left eye, ultrasound B-scan (ID 258)



Posterior staphyloma with or without macrophthalmos

(i) Bilateral iris and fundus colobomas, clinically non-microphthalmic Figure 7.13. The subject is an 8-year old girl. Right AL 24.0 mm (big), left AL 28.0 mm (big). Right HCD 9.0 mm left 10.0 mm (small). The symmetry of corneal size and the eyelid position makes the eyes appear to be normal size.

Figure 7.13: Ultrasound B-scan of left globe with iris and fundus coloboma, clinically non-microphthalmic. Note the ectatic and distorted posterior pole, typical of a staphyloma (ID 76)



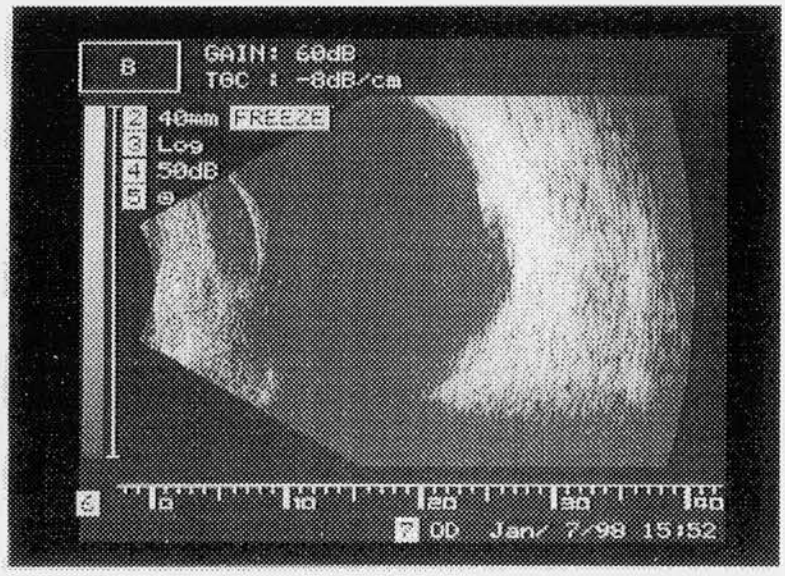
(ii) Bilateral clinical colobomatous microphthalmos ID 79 (see Figure 7.7). Right AL 32 mm, left AL 22 mm. The diagnosis is right *colobomatous macrophthalmos with microcornea.*

(iii) Bilateral iris and fundus colobomas, right clinical microphthalmos, ID 138 ultrasound B-scan shows staphyloma Figure 7.14, AL 24.5 mm.

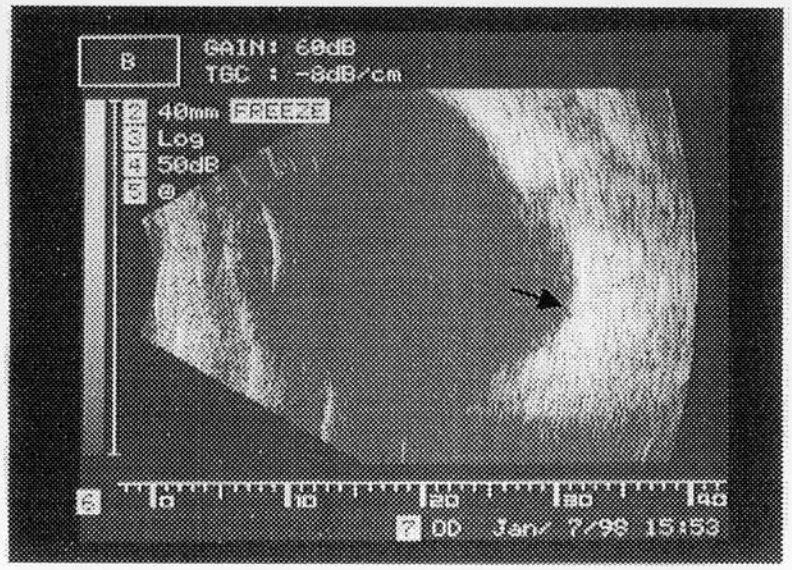
Diagnosis right *colobomatous macrophthalmos with microcornea.* Note that the left clinically non-microphthalmic eye has a staphyloma and AL 29.0 mm. Right HCD 11.0 mm (lower end of normal range), left HCD 11.5 mm. The bigger left cornea has made the right eye look small.

Figure 7.14A and 7.14B: Ultrasound B-scan of right and left globes. Bilateral iris and fundus colobomas, right clinical microphthalmos, left clinically non-microphthalmic. Left B-scan shows large staphyloma (arrow) (ID 138)

A (Right)



B (Left)



(iv) Bilateral iris coloboma, bilateral clinical microphthalmos, right fundus coloboma, left cataract ID 55 kimberley black. Right HCD 7.5 mm, left HCD 8.0 mm. Right AL 24 mm (big), left AL 23 mm (normal). Diagnosis: *right colobomatous macrophthalmos with microcornea.*

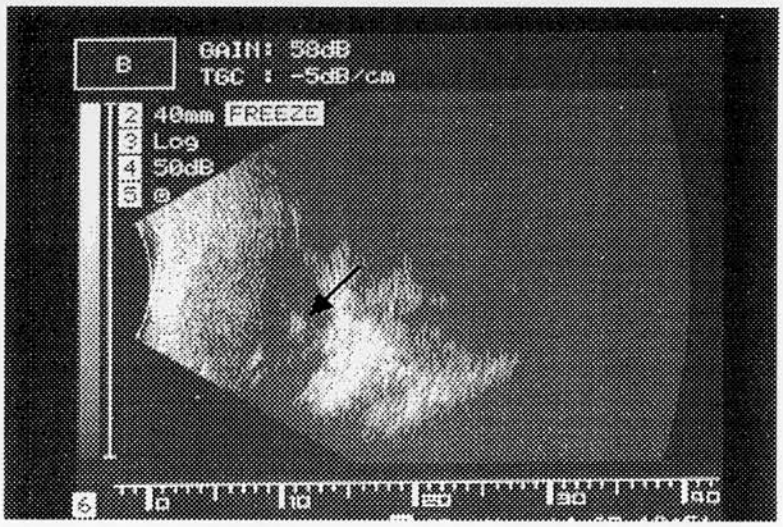
(v) Bilateral iris/fundus colobomas, left clinical microphthalmos: ID 83. Left AL 24 mm (big), right AL 24 mm (big).

Diagnosis: *colobomatous macrophthalmos with microcornea.* A staphyloma is present in left eye. Right HCD 11.00 mm, left HCD 10.5 mm. The difference in HCD has accentuated the appearance of left small eye. Both HCDs are just within normal range.

Retinal detachment

Right extreme clinical microphthalmos, bilateral corneal opacification and iris coloboma, Figure 7.15 (ID 94).

Figure 7.15: Ultrasound B-scan of globe with left extreme clinical microphthalmos, bilateral corneal opacification and iris coloboma. A retinal detachment is shown with an arrow (ID 94)



DISCUSSION

Not all eyes that look small are small when measured. The reasons for this were discussed in chapter one and several examples are illustrated in this study: reduced corneal diameter, reduced palpebral fissure length, ptosis and cataract, can all lead to the false conclusion of the eye being small.

This study has looked at a group of eyes that appear to be small and measured the AL with ultrasound B-scan. Only 36% of these clinically microphthalmic eyes fulfilled the definition criterion of having an AL more than 2 SDs below the age- and sex-corrected mean. Almost half of the eyes had normal ALs and a significant number had longer than normal axial lengths (*colobomatous macrophthalmos with microcornea*). In the group of 30 eyes with structural abnormalities but without the appearance of microphthalmos, only 4 were below the normal range of globe size, the rest being within or above the normal range. Therefore, some eyes that appear to be of normal size are small when measured. In the group of 21 normal eyes, the majority (72%) had eye sizes within 2 SDs of the mean.

For the definition of microphthalmos based on axial length to be useful, *all* eyes considered to be clinically microphthalmic would have to fulfil the criterion. Alternatively, the definition could be based on measurement and then to include only those eyes that fall below the normal range. But this would leave a group of eyes which look small but when measured are not. This is the case in 35/55 (64%) of the microphthalmic eyes in this study. It is neither practical nor useful in clinical practice to require measurement of AL, which is not necessarily indicated or relevant (see below).

In some of the colobomatous eyes with clinical microphthalmos, the AL was above the mean by more than 2 SDs. This *colobomatous macrophthalmos with microcornea* has already been described in the literature (Bateman and Maumenee, 1984; Pallotta et al. 1998), but in both papers was considered only to be part of an autosomal dominant familial trait. Only one of the cases in this study had any family history (ID 66) and the large number of cases (Table 7.1, ID 55, 75, 79, 107, 109, 113, 181, 192, 258) suggests that colobomatous macrophthalmos with microcornea is more common than previously recognised. The big eye explains the presence of high myopia in some cases, although hypermetropia is often found too (Olsen et al. 1996). Interestingly, microcornea (defined by the authors as HCD less than 11 mm) was not present in all of the cases described and was not always considered essential to make the diagnosis (Bateman and Maumenee, 1984). Bateman and Pallotta (Pallotta et al. 1998) found the eye anomaly of colobomatous macrophthalmos with microcornea in individuals with no systemic anomalies. In this study, colobomatous macrophthalmos with microcornea was found in individuals with (ID 55, 75, 109, 181, 192) and without (ID 66, 79, 107, 113, 258) systemic congenital anomalies, many of which are discussed in more detail in the next chapter (eight). The discussions by Bateman and Pallotta recognise the complex interrelationship between coloboma, corneal diameter, size of the globe and refractive error but, in an admirable attempt to categorise, simplify and define a 'new' clinical entity, they fail to realise that this cannot be achieved because of limitations in our understanding of why some of these structural abnormalities arise. Reasons for an overlong axial length include staphyloma and the ectatic distorted sclera that occurs in uveal colobomas affecting the posterior pole (See Figures 6.4, 6.5, 6.6, 7.13, and 7.14.)

The ultrasound B-scans confirmed some of the clinical abnormalities in cases such as retinal detachment and microphthalmos with cyst (Fisher, 1978; Weiss et al. 1985). The scans were well tolerated by all children but require a good degree of co-operation and patience on the part of the child. The ability to print an image is advantageous. The B-scan does not require clear media in order to obtain an image of the globe. In only one case where there was a clinically visible globe did the ultrasound machine fail to produce a useful image of the globe (ID 100 had a small cornea, cataract, vitreous opacification and extreme clinical microphthalmos, all of which probably contributed to the failure to obtain an image).

There are several disadvantages of this method of imaging when used to measure AL. A co-operative child is essential. To avoid corneal contact which would have required topical anaesthesia and since this might have caused corneal abrasion or trauma, ultrasounds were taken through the closed lid (Fledelius, 1996; Isenberg and Fishman, 1996). This makes it more difficult to align the probe with the visual axis, as there is no fixation target. All the scans were performed by the author and considerable care was taken not to indent the globe whilst performing the B-scans. The scans were measured independently by an observer (Dr Brian Fleck), who was not aware of the clinical diagnosis. Axial lengths were estimated by subtracting the thickness of the closed eyelid on the ultrasound, so there may have been errors in over- or under-estimation of eyelid thickness in the image. It is difficult to know how accurate the technique is, but it is unlikely to be as accurate as an ultrasound A-scan, most commonly used for biometry prior to cataract surgery. The A-scan requires the subject to fixate on a target or corneal contact, as well as relatively clear media so was not considered suitable for this study. The irregular posterior pole in many cases of

fundus coloboma would almost certainly have presented problems for an A-scanner, and pathology such as microphthalmos with cyst or retinal detachment would not have been detected. The A-scan also requires still more concentration and co-operation from the subject than the B-scan, and is therefore not suitable for smaller children.

The technical difficulties of measuring axial length of a globe with a large fundus coloboma or possible staphyloma include ensuring that the measurement of AL is along the visual axis, i.e. central corneal surface to fovea, which is not necessarily the longest axis of these eyes. Measuring the distance to the optic nerve head is not identical to the visual axis and, in the absence of a large coloboma or staphyloma affecting the optic nerve head, is only an approximation.

Many of the questions about the accuracy of measurements are dealt with by looking at the results from the group of 'normal' eyes, which is almost a type of 'control' group. There was no true control group as it was felt to be impractical to recruit children with no eye abnormalities from outside the hospital service to avoid any bias. The 'normal' group therefore consisted of the unaffected fellow eyes of children in the study with a unilateral abnormality. The results on AL suggest that the methods of measurement used in this study were accurate and repeatable, since the majority (72%) of these eyes had normal AL measurements.

The definition of microphthalmos based on AL arose as an attempt to reduce the confusion that exists about what microphthalmos is and how to discuss it within the context of various epidemiological or genetic studies. It has been made clear in the first chapter of this thesis and the previous chapter (six) that the term microphthalmos has very limited use. Instead, emphasis should be placed on the structural

(phenotypic) eye abnormality present. Intentionally therefore, no 'new' definition of microphthalmos based on AL will be proposed here. In theory, one could try and define microphthalmos clinically by looking independently at the HCD or eyelid PFL. The HCD, when reduced, certainly gives the impression of a small eye but, as this study shows, not all of the clinically microphthalmic eyes have a corneal diameter which is 2 or more SDs below the normal range. Unlike some recent publications (Hornby et al. 2000), this study has recognised that corneal growth and increase in HCD occurs mainly in the first few years, and that 'microcornea' does not represent a phenotypic abnormality. Like microphthalmos, microcornea is a descriptive term that applies to many structural eye abnormalities. HCD was therefore compared to age- and sex-matched tables. Not all eyes with reduced HCD have a reduced AL (Warburg, 1993). The measurements in this study, taken with a rule (not callipers, which more than likely would have been too difficult with small children), would appear to be accurate since nearly all (95%) of the HCDs were normal in the 'normal' group.

The palpebral fissure length results are interesting, since in many cases of clinical microphthalmos PFL is reduced. Also, the relationship between size of the globe and orbital growth is not entirely clear, although it is well recognised that the absence of a globe or a small globe probably inhibits eyelid growth (Lamb, 1970; Dunaway and David, 1996). A high proportion of all three groups studied (microphthalmos, non-microphthalmos and normal eyes) had eyelid PFLs that were more than 2 SDs below the normal range. It may be that in this particular group of children this was the case, and that the presence of a normal fellow eye did not prevent the eyelids, which are derived from different tissues in the embryo, from developing abnormally.

Alternatively, the measurements may have been underestimated as they were taken with a straight plastic rule, which may not have been held near enough to the face.

The reasons for not having the HCD and PFL measurements in a few cases (Tables 7.1–7.3) include lack of co-operation or not having consent, or difficulty in clearly defining the limbus for HCD measurements.

An ultrasound scan or image of the eye is not indicated in all cases of structural eye abnormality but, like all investigations, the decision is made based on the clinical findings and by considering many factors. In clinical anophthalmos, it is important to confirm the suspected diagnosis (Hodes and Snyder, 1978). Indeed, it may not be possible to open the eyelids easily in a very small baby when the condition is first suspected. Imaging may also reveal other pathology such as congenital cystic eye. A CT or MRI may provide even more information, including images of the brain and optic nerves (Albernaz et al. 1997). In an eye that appears to be smaller than normal, provided there is a good view of the vitreous and retina through a clear cornea and lens, imaging is unlikely to add further useful information. However, a detailed eye examination and refraction in a baby or small child will require a general anaesthetic. It is neither practical nor necessary for all eyes that appear to be small to have the axial length measured. If a diagnosis such as nanophthalmos is suspected (reduced HCD, high hypermetropia), an ultrasound A-scan may be more appropriate for measurement for the AL.

Clinically anophthalmic eyes were not included in this part of the study, since the primary concern was AL measurement. Ultrasound B-scan would only confirm the absence of a globe and not add significantly to whatever detail was already known.

An eye can appear to be small for several reasons, all of which contribute to the appearance: eyelid PFL and palpebral aperture, facial asymmetry, reduced HCD, any ocular asymmetry, abnormalities of the cornea or pupil or lens opacity.

Although AL of the eye cannot and should not be used to make a clinical diagnosis of microphthalmos, the size of the eye as determined by ultrasound may have some use in predicting visual function. Hornby et al looked at visual acuity in a large series of eyes with coloboma (Hornby et al. 2000). Sub-classification included the categories of microcornea with and without microphthalmos. Microphthalmos was defined using the definition of an eye smaller than 2 SDs below the mean, which is less than 18.5 mm AL in an adult. Such eyes tend to have extensive chorioretinal malformations, retinal detachment and vitreous opacification (IDs 93, 94, 97). In addition, microcornea (defined in Hornby's study as less than 10 mm HCD), was found to be a predictor of poor visual acuity when HCD was less than 6 mm. Neither of these two findings based on HCD or AL are at all surprising since the eyes with smaller anterior segments are more likely to be the most severely malformed and have posterior segment involvement. By arbitrarily defining microcornea as less than 10 mm HCD, many eyes with the appearance of clinical microphthalmos would be inappropriately excluded from further investigation, especially as they will have had structural abnormalities such as coloboma. The study by Hornby, in a worthy attempt to simplify a complex eye malformation, has created many artificial categories that overlap and cannot readily be distinguished (e.g. coloboma with microphthalmos, coloboma with microcornea). This approach also requires measurement of AL of every case of coloboma.

It is important to emphasise that a very small cornea often means a small eye (Bateman, 1984) but that there is no consistent relationship between corneal diameter and size of the globe. The results of this study make it quite clear that in many cases of small cornea, the size of the eye is normal, or even bigger than normal.

Summary, chapter seven

- Axial length of the eye is not a useful definition of microphthalmos.
- Axial length of the eye is very variable in the group of congenital eye defects studied, most of which had a uveal coloboma.
- Ultrasound B-scan is useful for visualising and confirming intra- and extraocular abnormalities, although there are limitations as to what can be seen.
- More than 42% of clinically microphthalmic eyes have an axial length within the normal range when corrected for age and sex.
- Some clinically microphthalmic eyes are bigger than normal: colobomatous microcornea with macrophthalmos.
- Colobomatous microcornea with macrophthalmos is not just an autosomal dominant familial eye condition, and occurs in individuals with systemic anomalies.
- Reduced eyelid palpebral fissure length and reduced horizontal corneal diameter are both common in clinical microphthalmos, but are not consistent findings.
- Not all structurally abnormal eyes need imaging: a specific indication should be sought.

Up to this point, the structural eye anomalies have been discussed as eye conditions only, with minimal reference to the individual in whom they occur. As described in the

introduction to this thesis (chapter two), no description of these eye anomalies would be complete without examining the possible relationship between the eye abnormalities and the many congenital systemic anomalies, clinical genetic syndromes and associations. This is the subject of the next chapter.

CHAPTER EIGHT

THE ASSOCIATED SYSTEMIC ANOMALIES, PATTERNS OF INHERITANCE AND RECURRENCE RISK

A prospective study that ascertains individuals with the entire spectrum of colobomatous defects from cystic eye to iris coloboma is needed to derive these types of empirical recurrence risks that exist for many other common congenital malformations. (Pagon, 1981).

Summary

121 children were examined for non-ocular congenital anomalies.

The colobomatous and non-colobomatous eye defects and the clinical anophthalmos studied were frequently associated with extraocular congenital anomalies.

Some previously recognised clinical genetic syndromes and associations were identified.

Many children were identified with multiple congenital anomalies that did not fit any previously described pattern or syndrome.

Mendelian patterns of inheritance were recognised in some individuals belonging to families with other affected members, with apparent autosomal dominant and recessive inheritance.

Children affected with these eye defects need careful evaluation by a paediatrician and clinical geneticist.

The classification system proposed in chapter six when applied to the 121 cases appears to be robust and consistent.

Recurrence risk estimates have been calculated for anophthalmos/microphthalmos and coloboma (Morrison et al. 2002).

It has not been possible to distinguish cases having a genetic aetiology from environmental/sporadic causes, based on examination.

ASSOCIATED SYSTEMIC ANOMALIES

BACKGROUND

Chapter two described how in previous studies involving clinical anophthalmos/microphthalmos and coloboma, a significant number of the affected individuals are reported to have systemic congenital malformations in addition to the eye anomaly (Pagon, 1981; Bateman, 1984; Maumenee and Mitchell, 1990; Clementi et al. 1992; Warburg, 1993; Leppig and Pagon, 1993; Kallen et al. 1996; Tucker et al. 1996; Daufenbach et al. 1998). These associated anomalies have been considered in various categories, some of which overlap extensively: clinical genetic (malformation) syndromes, multiple malformations but without a recognised syndrome, associations, chromosomal defects and single gene autosomal dominant, recessive or X-linked syndromes. (Pagon, 1981; Daufenbach et al. 1998). Some authors have listed the extraocular abnormalities as 'midline', 'limb' or 'digital' defects (Maumenee and Mitchell, 1990). In other reports, the anomalies are listed according to the organ system affected. (Kallen et al. 1996). Maumenee looked at 82 patients with colobomatous defects of the eye and identified a malformation syndrome

in 28 cases (34%) (Maumenee and Mitchell, 1990). Daufenbach studied associated anomalies in 48 children with chorioretinal coloboma and 18 (38%) had systemic abnormalities (Daufenbach et al. 1998). Because of problems with definition of these eye conditions and the differing primary aim of each of these studies (Clementi et al. 1992; Kallen et al. 1996), there is a huge variation in the percentage reported with extraocular systemic anomalies. The difficulties with definition are not confined only to the eye defects, as some systemic anomalies may be considered too minor to report or document (Leppig and Pagon, 1993).

In this study, a number of different approaches were used to document and to try and make sense of the systemic anomalies detected. It was hoped that the presentation of the raw data, without prior assumption of a 'genetic' or 'environmental' cause, might reveal new patterns or trends.

Classification system (chapter six)

As was made clear in the previous chapters, the term microphthalmos was not used as a diagnosis; instead, the phenotypic eye classification (described in chapter six) was employed. A further aim was to validate the classification system by seeing if the OFD was different in any way from non-OFD with respect to extraocular malformations.

Isolated eye anomalies

This is one of the most interesting concepts and various terms have been employed by other authors in an attempt to understand the aetiology of this entity more completely. Leppig refers to 'ocular coloboma without known cause', excluding subjects with an autosomal dominant condition, a known syndrome or chromosomal abnormality

(Leppig and Pagon, 1993). They did include patients with mental retardation and other malformations.

'Isolated' usually meant that the eye anomaly occurred without systemic anomaly and with no positive family history. An individual case like this can be considered to be 'sporadic' in aetiology. To some authors, this implies that the cause is 'non-genetic', but there is no evidence for this. As shown in chapter two, abnormalities may be confined to the eye in an individual with a positive family history.

METHODS

A total of 121 children were examined in detail and the ocular diagnoses are listed in Table 5.8, page 142). Systemic congenital anomalies were defined as any major deformity requiring treatment or surgical correction or causing morbidity or mortality, including significant developmental delay (Leppig and Pagon, 1993).

All previously documented congenital abnormalities and any named syndromes or provisional diagnoses used in the past in reference to the child were recorded. A full general examination of every child was done, including height, weight and head circumference. Photographs were taken of face, hands and feet. Additional photographs were taken of any anomalies seen. Supplementary information and previous investigations were confirmed by contacting general practitioners or by review of hospital medical records, with the parents' written informed consent.

The file, medical records and photographs of each child were reviewed with Dr David FitzPatrick, Consultant Clinical Geneticist. Any dysmorphological features were noted. In all cases with an extraocular congenital abnormality, a search for an identifiable syndrome or association was made on the London Dysmorphology

Database (LDDB) (Baraitser and Winter, 2000). A systemic clinical diagnosis was made wherever possible, and all cases were classified as isolated (i.e. eye anomaly only) or non-isolated (extraocular anomaly present).

All cases where there was a positive family history were recorded, including full details of the pedigree.

RESULTS

121 children were examined and included in this part of the study. The eye defects were divided into three groups: optic fissure defect, non-optic fissure defect, and unclassifiable (chapter 6, Tables 6.1, 6.2 and 6.3).

Extraocular (systemic) congenital anomalies

These covered the entire range of organs and systems. The individual results are summarised in Table 8.1, and the range of anomalies are listed in Table 8.2. Those abnormalities that occurred in significant numbers are discussed separately below. Extraocular malformations can be grouped into those affecting the brain, head and neck, chest, abdomen, and limbs. Most of the congenital anomalies were previously documented and investigated and treated as clinically indicated. For the brain defects described, reference was made to CT scan reports.

Table 8.1: Associated systemic congenital anomalies

ID	Sex 1=male 2=female	Systemic (extraocular) congenital anomalies
15	2	nasogastric feeding, congenital heart disease (VSD, PDA), trisomy 18
26	1	learning difficulties
33	1	postaxial polydactyly, proximal set thumbs
35	2	sensorineural deafness, tracheo-oesophageal fistula, congenital heart disease (PDA) short stature
39	1	hypospadias
40	2	left VIIth nerve palsy, choanal atresia, laryngomalacia, congenital heart disease (PDA), overriding toes 2/3
45	1	feet 4/5 toe syndactyly, cleft palate, micrognathia, inguinal hernias, horseshoe kidney
47	1	severe developmental delay, cerebral cortical atrophy, low set ears, doll-like facies
50	1	cerebellar cyst, left ear tag, left facial/scalp capillary haemangioma, scoliosis
53	1	microcephaly, right inguinal hernia
55	2	congenital heart disease (VSD), unilateral deafness, toes 2/3 syndactyly, 22q11 microdeletion
58	2	microcephaly
60	1	postaxial polydactyly R hand and both feet, psoriasis
62	2	agenesis of corpus callosum, ptosis, 4/5 hand syndactyly, broad toes
63	2	heminasal aplasia, pyloric stenosis, congenital heart disease (VSD)
67	2	hydrocephalus, bilateral radial aplasia, congenital dislocation of the hip
75	1	congenital heart disease (PDA), digitalised thumbs, maxillary hypoplasia
76	2	broad nasal tip, unusual facies (Kabuki-like)
90	1	congenital heart disease (PDA), low set lop ear
93	2	2/3 syndactyly left toes, high nasal root with broad tip, low set ears, short tented philtrum
94	1	congenital myopathy
96	2	tracheo-oesophageal fistula
97	2	hypotonia, ataxia, pharyngoplasty, 4/5 brachydactyly, deafness
109	1	bilateral cryptorchidism, low set posteriorly rotated ears
117	2	microcephaly
145	2	significant developmental delay
162	1	congenital heart disease (hole in heart), extra bones in feet
166	2	cleft palate, right deafness, notched alae nasi
181	1	hypotonia, right low set posteriorly rotated ear
192	2	abnormal vertebrae (C6/7), heart murmour
199	1	right hip subluxation, pyloric stenosis, cong heart disease (PDA)
204	1	heminasal hypoplasia
210	1	umbilical hernia, right inguinal hernia

ID	Sex 1=male 2=female	Systemic (extraocular) congenital anomalies
213	2	seizures, heminasal aplasia with proboscis (surgery) ophthalmic encephalocele
216	1	bilateral cleft lip and palate, glomerulonephritis
224	1	prominent ear lobes, small mouth, chest wall unusual shape
232	1	cleft plate, left inguinal hernia, cryptorchidism
238	1	congenital heart disease (Fallot's), nasal speech
239	2	hydrocephalus, abnormal premaxilla, lowset rotated left ear
253	1	osteogenesis imperfecta, umbilical hernia, camptodactyly
284	1	severe learning difficulties
299	1	severe learning difficulties, bilateral cleft lip and palate
324	1	congenital heart disease, developmental delay

Table 8.2: Extraocular (systemic) congenital anomalies

Head and neck	seventh cranial nerve palsy, laryngomalacia, unilateral deafness, cleft palate, cleft lip and palate, capillary haemangioma, nasal hypoplasia (Figure 8.1), choanal atresia.
Brain	cortical atrophy, microcephaly, cerebellar cyst, agenesis of corpus callosum, hydrocephalus, seizures, sleep apnoea, absences, petit mal.
Chest	ventriculoseptal defect (VSD), patent ductus arteriosus (PDA), Fallot's tetralogy.
Abdomen	umbilical hernia, inguinal hernia, inguinal hernia with cryptorchidism, cryptorchidism, glomerulonephritis, horseshoe kidney, pyloric stenosis, hypospadias.
Limbs	4/5 syndactyly of hand, bilateral radial aplasia, congenital dislocation of the hip, extra bones in feet, hypotonia, polydactyly hand and feet, post axial hand polydactyly, 4/5 syndactyly of feet, congenital hip subluxation, congenital myopathy, 2/3 toe syndactyly (Figure 8.2), scoliosis.
Skin	eczema, psoriasis, capillary malformation over lumbar spine.

Figure 8.1: (Right) nasal hypoplasia and clinical anophthalmos (prosthetic eye in place) (ID 204)

Figure 8.2: Bilateral toe syndactyly, toes 2 and 3 (ID 55)

Dysmorphic features

These are soft variations in morphogenesis and are not easy to categorise, and there is some justification to not including them in any analysis. In some cases they can be confirmed by measurement and rigid adherence to diagnostic criteria. It is not within the scope of this thesis to describe the art and science of dysmorphology as it applies to clinical genetics.

Several dysmorphisms were noted (Table 8.3), mostly affecting the head and neck. At least 40 children were judged to be facially dysmorphic. As already stated, there was

no control group and 'soft' dysmorphic features were noted in nearly every child. Ear abnormalities were documented in 20 cases.

Table 8.3: Dysmorphic features

Ears	posteriorly rotated ears, low-set, simple, cleft ear lobe **(Figure 8.3)**, prominent, large, preauricular pit, lop ear, ear tag.
Eyelids	up-slanting palpebral fissures, down-slanting palpebral fissures, epicanthus.
Nose	broad nasal root, high nasal root, notched alae nasi, nasal hypoplasia, small nose.
Lips	Cupid's bow upper lip, long philtrum, thick lips, thin upper lip, short-tented philtrum, smooth philtrum.
Teeth	Crowded.
Mouth	small, carp-shaped, Cupid's bow mouth.
Face	tall forehead, maxillary hypoplasia, facial asymmetry, hypertelorism.
Digits	broad thumbs, camptodactyly, 4/5 brachydactyly, tapering fingers, 4/5 finger clinodactyly, arachnodactyly, short 4/5 metatarsals, proximal set thumbs, digitalised thumbs **(Figure 8.4)**, short hallux bilaterally, saddle-gap toes, overriding toes 2/3.
Height	short stature.

Figure 8.3: Cleft earlobe (ID 187)

Figure 8.4: Digitalised left thumb (indicated with arrow) (ID 75)

A previously recognised clinical genetic syndrome or association or chromosomal syndrome was identified in 13 children (Table 8.3). Three of these had the CHARGE association (Pagon et al. 1981a).

Table 8.4: Recognised clinical genetic syndromes and associations

ID, Sex	Syndrome or Association	Systemic anomalies	Notes	Reference
60, M	Schwartz-Jampel syndrome	psoriasis, post-axial polydactyly right hand, polydactyly both feet, developmental delay	one of male twins, both affected. Consanguineous parents	(Pinto-Escalante et al. 1997)
15, F	Edwards' syndrome	congenital heart disease (VSD and PDA), severe developmental delay, nasogastric feeds	trisomy 18	(Taylor, 1968)
35, F	CHARGE association	congenital heart disease (PDA), developmental delay, tracheo-oesophageal fistula, short stature	identical twin	(Pagon et al. 1981a; Warburg, 1983)
96, F	CHARGE association	tracheo-oesophageal fistula, developmental delay	identical twin	
40, F	CHARGE association	congenital heart disease (PDA), choanal atresia		
53, M	Microcephaly	severe developmental delay, dysmorphic face		
55, F	Velo-cardio-facial syndrome	congenital heart disease (hole), 2/3 syndactyly of toes, dysmorphic with typical facial features (small mouth, dental crowding)	22q11 microdeletion detected	(Kelly et al. 1993; Wilson et al. 1993)

ID, Sex	Syndrome or Association	Systemic anomalies	Notes	Reference
117, F	Microcephaly/Micro syndrome	severe developmental delay	parents first cousins	(Warburg et al. 1993)
199, M	Prematurity	severe developmental delay, congenital heart disease (PDA), pyloric stenosis, subluxation of right hip		
67, F	Craniofrontonasal dysplasia	hydrocephalus, bilateral radial aplasia, congenital dislocation of the hip		
62, F	Aicardi syndrome	agenesis of corpus callosum, severe developmental delay, 4/5 hand syndactyly, broad toes		(Carney et al. 1993)
204, M	Hemi-nasal aplasia		unilateral anophthalmos	(van Kempen et al. 1997)
213, F	Hemi-nasal aplasia with proboscis	seizures		
63, F	Hemi-nasal aplasia	blocked nasolacrimal duct, severe nasal hypoplasia, congenital heart disease (VSD), pyloric stenosis		

Congenital heart disease

Congenital heart disease was present in 11 individuals (9%). Six of these had a patent ductus arteriosus (PDA), two a ventriculoseptal defect (VSD), and one tetralogy of Fallot. (see Table 8.6).

Significant developmental delay

This was the commonest congenital anomaly present, affecting 23 children (19%), most of these severely (Table 8.6).

Karyotyping: chromosomal abnormality on G-banding

Not all children in the study had karyotyping. In those cases where chromosomes had not been previously tested, G-banding was done on a blood sample. In 35 cases, chromosomes were tested. The indications for testing included any child with significant developmental delay and at least one congenital anomaly or growth failure or multiple craniofacial dysmorphisms. One case of trisomy 18 (47 XX, +18) was confirmed.

One child (ID 20) was noted to have increased chromosome fragility. This girl did not have developmental delay or any systemic congenital anomaly.

Several other individuals were strongly suspected of having chromosomal abnormalities, but none were detected on G-banding or when specific microdeletions were sought. For example, ID 166: severe developmental delay, cleft palate, unilateral deafness, dysmorphic (notched alae nasi, smooth philtrum).

Chromosome deletions and microdeletions

The second child with a significant chromosome abnormality was a girl with a 22q11 microdeletion (Table 8.4, ID 55). Chromosomes were normal on G-banding. Two other individuals (ID 97, 238) strongly suspected of having a 22q11 microdeletion were tested and no deletion was found:

ID 97: mild developmental delay, deaf, hypotonia, pharyngoplasty, hand (4/5) brachydactyly.

ID 238: Fallot's tetralogy, nasal speech, mild developmental delay.

A chromosome 4p deletion was suspected in ID 228 (Shapira, 1998). Ocular features were bilateral iris and fundus colobomas. Dysmorphic features included a broad nasal root and low-set ears. No 4p deletion was found on testing.

'Syndromic' but not recognised

Many of these were unusual combinations of congenital birth defects not previously described or readily explained. In this study, this group comprised those children in whom the clinical features strongly suggested a clinical genetic syndrome or association or that a chromosomal abnormality might be found. In none of these cases was a search on the London Dysmorphology Database productive (Baraitser and Winter, 2000).

ID 253: Male, age 8. Unilateral clinical anophthalmos (extreme microphthalmos) with osteogenesis imperfecta and umbilical hernia. Camptodactyly of fingers. No developmental delay.

ID 50: Male, age 4. Severe developmental delay, cerebellar cyst, left ear tag, preauricular pit, capillary haemangioma on scalp, scoliosis.

Several children were suspected of having a particular clinical genetic syndrome but when investigated none of these were proven, e.g. ID 45 suspected Rubinstein-Taybi syndrome but no 16p13 deletion detected (Hennekam et al. 1993).

Systemic congenital anomalies and clinical anophthalmos

As defined by the classification system, clinical anophthalmos was included in the category of unclassifiable defects i.e. it is not possible to know with certainty whether the defect has arisen due to an event occurring at the time of optic fissure formation. One would expect the range of systemic anomalies to be very different if the aetiologies were different. The systemic anomalies in the seven cases of anophthalmos are listed in Table 8.4. Although the range of extraocular congenital anomalies listed is diverse, in all cases the anomalies are of craniofacial origin and all but one child has severe developmental delay.

Table 8.4: Systemic anomalies associated with anophthalmos

ID, Sex	Ocular phenotype	Unilateral or bilateral pathology	Systemic congenital anomalies	Family history	Notes
117, F	right anophthalmos, left extreme microphthalmos	bilateral	microcephaly, severe developmental delay	mother had three previous miscarriages	parents first cousins
204, M	left anophthalmos	unilateral	left nostril hypoplastic	only child	
47, M	left anophthalmos, right atrophic iris ('aniridia' like) with retinal dysplasia	bilateral	hydrocephalus, cerebral atrophy	normal sibling	
166, F	right anophthalmos	unilateral	cleft palate, severe developmental delay	normal female sibling	left eye nystagmus
72, F	anophthalmos	bilateral	significant learning difficulties		
67, F	left anophthalmos	unilateral	left nasal atresia, craniofrontonasal dysplasia, limb defects	normal sibling	
239, F	left anophthalmos	unilateral	hydrocephalus left side of brain with malformed cortex, developmental delay, seizures, right hemiparesis	normal sibling	right eye nystagmus

Isolated eye abnormality

The majority (62.8%) of individuals examined were considered to have a congenital eye abnormality only (Table 8.6). This was defined as having no systemic congenital anomaly or developmental delay, no chromosomal abnormality, and not being significantly dysmorphic. This isolated group also included those with a confirmed family history (see below). There is an argument to exclude those with a family history as not being truly 'isolated' since a 'genetic' cause is strongly suggested. It is these apparently isolated eye abnormalities that cause some of the greatest difficulty in genetic counselling. (See recurrence risk below and discussion.)

Positive family history

Those individuals with a confirmed family history of an eye defect were identified in chapter six. Eight different family pedigrees were affected, one of these having identical twins (ID 268 and 269). The presumed or most likely pattern of inheritance is listed in Table 8.5 along with the clinical details. Seven of the eight affected pedigrees had defects related to closure of the optic fissure (Table 8.6). Both autosomal dominant and recessive patterns of inheritance are suggested. Within these affected pedigrees both unilateral and bilateral ocular pathology was found, e.g. IDs 26 and 106. Some affected members had congenital anomalies isolated to the eye and others did not, e.g. IDs 216 and 132.

Table 8.5: Associated systemic congenital anomalies in cases with a positive family history

ID	Ocular phenotype	Systemic congenital anomalies	Relationship	Likely pattern of inheritance	Notes
26, 106	iris and fundus coloboma ID 26 bilateral ID 106 unilateral	ID 26 developmental delay	brothers	autosomal recessive	
66, 296	ID 66 female: iris and fundus coloboma ID 296 male: retinal coloboma	isolated	brother and sister	autosomal recessive	brother 'asymptomatic', picked up on screening
102	optic nerve head disruption	isolated	sister (born in England), mother, maternal uncle and grandfather affected	autosomal dominant	see chapter six page 153, optic nerve head disruption
268, 269	unilateral iris and fundus coloboma. (opposite eyes: 'mirrored' defects)	isolated	identical female twins	autosomal dominant	paternal grandmother has iris coloboma, father has possible optic disc anomaly
159, 297	ID 159 bilateral iris coloboma ID 297 unilateral iris fissure	isolated	brothers	autosomal recessive	

ID	Ocular phenotype	Systemic congenital anomalies	Relationship	Likely pattern of inheritance	Notes
I32, 216 and 299	ID 132 unilateral iris and fundus coloboma ID 216 unilateral iris and retinal coloboma ID 299 unclassifiable.	ID 132 isolated ID 216 glomeruloneph ritis, cleft lip and palate ID 299 severe developmental delay	brothers	autosomal dominant	mother has cleft lip and palate, sister is normal
276	unilateral cataract	isolated	first cousins (female)	autosomal dominant	non optic fissure closure defect
35, 96	bilateral iris and fundus coloboma	CHARGE association	identical female twins		

Table 8.6: Systemic examinations of 121 individuals with congenital eye anomalies in relation to type of eye defect

Ocular developmental pathogenesis	Optic fissure defect (OFD)	Non-optic fissure defect (non-OFD)	Unclassifiable	TOTAL
Number	86	11	24	121
Bilateral eye pathology	45	6	10	61 (50.4%)
Discordance of pathogenesis in bilaterals	0	0	0	0
Isolated (ocular anomaly only)	58	8	10	76 (62.8%)
Positive family history	7	1	0	8
Other congenital anomaly (% of subgroup)	27 (31%)	2 (18%)	16 (66%)	45
Recognised congenital malformation syndrome or association (% of subgroup)	8 (9.3%)	1 (9%)	4 (16%)	13
Congenital heart disease (% of subgroup)	9 (10.5%)	0 (0%)	2 (8.3%)	11
Developmental delay (% of subgroup)	13 (15%)	2 (18%)	8 (33.3%)	23
Chromosome abnormality	2	0	0	2

DISCUSSION

Systemic anomalies

In this study, 37% (45/121) of children had an extraocular congenital anomaly and the findings are consistent with other studies. Previous studies have been at major referral centres or estimates from congenital anomaly registers (Maumenee and Mitchell, 1990; Leppig and Pagon, 1993; Kallen et al. 1996; Tucker et al. 1996; Daufenbach et al. 1998). Such studies tend to include the more severely affected individuals as far as the eye abnormalities are concerned. This study was based on an entire population and selection bias is therefore minimal.

The data from a large epidemiological study in Europe and California using congenital anomaly registers estimated that non-eye malformations were present in 73% of children with anophthalmos or microphthalmos after excluding all cases with a known chromosomal abnormality (Kallen et al. 1996). A review of the records of 48 patients found that 18 (38%) had other systemic anomalies (Daufenbach et al. 1998). In one retrospective study of the records of 82 patients in whom there was a colobomatous malformation, 27% had a 'midline' defect (clefting, cardiovascular, urogenital and intracranial malformations), 13% had digital anomalies, and between 15% and 24% had mental retardation. An identifiable syndrome was present in 28 patients, the CHARGE association being the commonest (Maumenee and Mitchell, 1990). In a retrospective review of ocular coloboma without known cause, defined as coloboma not occurring as part of a known syndrome or as autosomal dominant condition, 58 patients were examined for coexisting malformations. Mental retardation was present in 14/42 (33%) of those more than one year old. Other congenital malformations

were present in 43% of the patients. Of these 25 patients,10 had anomalies of the heart or great vessels and 18 had skeletal anomalies (Leppig and Pagon, 1993). A review of the medical records of 77 patients with anophthalmos or microphthalmos referred to Moorfields Eye Hospital found a 50% association with systemic anomalies in patients with congenital unilateral or bilateral anophthalmos and congenital unilateral or bilateral 'remnant only' microphthalmos. The percentages were lower in unilateral and bilateral non-colobomatous microphthalmic groups (30% and 0% respectively) and unilateral and bilateral coloboma microphthalmic groups (11% and 0% respectively) (Tucker et al. 1996).

Clinical genetic syndromes, associations and one case of a chromosomal malformation syndrome were identified in this population. In several children, the pattern of malformations was not previously described or recognised. Is this evidence of a genetic or environmental cause? It can be taken as evidence of either or both. Many clinical genetic syndromes have variable expression of the systemic features, and some sporadic (environmental) conditions are suspected and characterised by their random nature with respect to almost any organs or system being involved (see chapter two).

In this study, care has been taken to avoid ascribing a 'cause' or aetiology to any of the eye or systemic malformations described. Whilst it is important to recognise congenital malformation syndromes, such an exercise may detract from the search for a cause. This study avoided the temptation to describe new syndromes or fit those children into a previously recognised category. New combinations of congenital eye and systemic anomalies can lead to the recognition of a syndrome, but is only likely to

when such a combination occurs within a well-phenotyped pedigree, as opposed to the sporadic (isolated) cases seen in this study.

None of the parents in this study were questioned about the details of the pregnancy such as medications taken, diet, place of conception, and unusual events happening in early pregnancy. This information, whilst being of great interest, would not establish a causal role for the eye defects in any individual affected child. Mrs Sheena Macdonald at the University of Edinburgh is currently investigating these aspects in an environmental case-control study.

Family history

The affected individuals with family histories confirm that pedigrees with autosomal dominant and recessive inheritance exist, and X-linked inheritance has been described (chapter two).

Chromosomes

This study has once again highlighted the limitations of a 'normal' chromosome result by G-banding. Macroscopic chromosome studies on 35 patients revealed only one abnormality (trisomy 18). Leppig and Pagon studied the chromosomes of 21 of 58 patients with colobomatous malformations and only two were abnormal. One case was an inherited balanced translocation of chromosome 2 and 21 (inherited from a father whose eyes had not been examined) and the other was an individual with a low level of mosaicism for a small marker chromosome that could not be characterised.

This marker chromosome had been detected from peripheral blood and skin fibroblasts (Leppig and Pagon, 1993).

Further work on the study cohort could look at skin chromosomes for mosaicism, if it was felt that the likelihood of the chromosomes being abnormal was strong.

The paucity of macroscopic chromosome abnormalities (G-banding) in this study is interesting. It is likely that the contribution of chromosomal deletions and rearrangements has been overestimated in older studies since the eye diagnosis may be imprecise or not specified (see chapter two). Cases for this study may have been missed, particularly in children with multiple or life-threatening congenital malformations. Documentation of eye abnormalities may be incomplete and not appear on the congenital malformation registers if it is assumed that their presence is part of a recognised systemic association or syndrome (Bianchi et al. 1994; Kallen et al. 1996).

Why are systemic anomalies so common?

According to the developmental field concept (Opitz, 1985), in which body parts can be affected in an identical manner by different insults, one can consider the eye as a developmental field. If single gene defects exist, which act in the early stages of the developing embryo prior to the definition of 'limb', 'midline' and 'ocular' fields, the non-random occurrence of such malformations within syndromes is explained. Such a theory is also consistent with environmental pathogens being the cause of such defects during the sensitive developmental period (Maumenee and Mitchell, 1990).

Congenital heart disease occurred in 11 cases, 9 of these in the OFD group and the other two unclassified. This does suggest the possibility of a disruptive event occurring at similar stages of embryogenesis for both the eye and the heart. The major septa of the heart are formed between the 27^{th} and 37^{th} days of development, in parallel with optic fissure formation and closure (Langman, 1984).

How can unilateral eye defects be genetic or inherited?

There were 61 (50.4%) of individuals bilaterally affected, with the pathology being symmetrical in 17 of these cases. The asymmetry of ocular pathology is clearly demonstrated in this study. Significant differences are often seen between the two eyes of one individual, from completely normal, through minor eye abnormalities such as iris coloboma, to complete anophthalmos. Such insults occur at an early embryological stage, but repair could occur with the existence of pluripotent cells.

It seems reasonable to suppose that any individual has two genetically identical eyes but for stochastic reasons, these two eyes may express the genetic defect differently. For example, this could be the result of small differences in protein production by dosage sensitive genes such as *PAX6* (chapter nine).

Normal development depends on an ordered sequence of induction, so that growth, migration and differentiation begin in the right place, time and direction. This ordered sequence is regulated by genetic control, the genes being expressed during early development. It is possible that teratogens and environmental agents act on the genetic expression of early human differentiating cells. The genetic background of the embryo could presumably determine the sensitivity to external agents.

Classification of structural eye defects (see Table 8.6)

In chapter six a novel and simple classification system was proposed, grouping the eye malformations according to whether the primary embryopathology occurred during formation or closure of the optic fissure. The robustness of the classification system can be tested since one would predict the following:

1. Concordance of the type of eye defect in bilaterally affected individuals within the same sub-classification group
2. Concordance of the type of eye defect in family recurrences within the same sub classification group.
3. A similar spectrum of associated congenital anomalies and syndrome diagnoses within classification groups.
4. A different spectrum of associated congenital anomalies and syndrome diagnoses between classification groups.

This type of classification system would imply the existence of aetiologically distinct groups. This would improve genetic counselling and refine the analysis of epidemiological data and assessment of the role of the environment. A more rational approach to candidate gene selection and analysis is also possible.

Taking each of the above points (1–4) in turn, there was 100% concordance of the type of defect in bilaterally affected individuals. There were no cases of an OFD in combination with a non-OFD in the same individual (chapter six). In those cases with a family history (chapter six page 164 and Table 8.5 page 228), the type of eye defect

was consistent within the pedigree. Associations and syndromes were recognised within all three groups but the rarity of these cases makes any comparison between each group difficult (Table 8.6).

The associated congenital anomalies and syndromes were found amongst all three of the classification groups. By definition, all three cases of CHARGE association were from the OFD group. The percentage of each group affected by extraocular anomalies was between 18% (non-OFD) and 66% (unclassifiable). Malformation syndromes and associations were present in all groups but the rarity of these syndromes and the small group sizes do not make it possible to make any firm conclusion (Table 8.6). The small size of the non-OFD and unclassifiable groups does not justify statistical comparison. Also, these two groups are almost certainly heterogeneous. The unclassifiable group, consisting of those cases in which no ocular phenotype could be established (e.g. clinical anophthalmos, sclerocornea) might well include cases with an underlying optic fissure defect. The argument that the unclassifiable group is more severely affected is not helpful, since the severity of eye malformation could be determined by appearance (which is subjective), or visual function. Vision does not necessarily relate to the 'severity' of the eye defect and can be surprisingly good (Olsen et al. 1996). A study by Leppig did not find any relationship between the severity of ocular coloboma (graded into three categories of vision: good vision in both eyes, good vision in one eye and poor vision in both eyes) and the probability of associated systemic malformations or mental retardation (Leppig and Pagon, 1993).

Clinical implications

All children presenting with clinical anophthalmos or a uveal coloboma need careful assessment by a clinical geneticist or paediatrician. Investigation should be guided by the history and clinical examination with particular emphasis on dysmorphology and family history. Laterality is not an indicator of the cause being genetic or hereditary. Normal chromosomes do not exclude a genetic cause, and neither do congenital abnormalities isolated to the eye. There are implications here for genetic counselling (see below).

Further studies

It would be valuable to study in more detail a larger group of eyes without a fissure-defect (non-OFD), since the relatively small numbers in the study do not allow sub-classification or statistical comparison.

This study has contributed to understanding by the development and testing of a rigorous and simple classification that is robust and can be built on. However, the ill-defined 'optic nerve head disruption' defects ('optic disc coloboma') remain a debatable mystery, as well as the limitations of the non-OFD and unclassifiable groups since they represent different ocular phenotypes.

RECURRENCE RISK OF UVEAL COLOBOMA, ANOPHTHALMOS AND OTHER OPTIC FISSURE DEFECTS

INTRODUCTION

There are very few studies on the recurrence risk of inheriting uveal coloboma and anophthalmos (Pagon, 1981; Maumenee and Mitchell, 1990) and reliable information on which to base advice does not exist (Harper, 1998). One of the aims of genetic counselling is to be able to give the parents of an affected subject some likelihood or estimate of recurrence in another child or the offspring of the affected individual, and this was one of the most important aims of this study. This is the empiric risk. Some important points need to be remembered here. Firstly, the empiric risk is never nil, as there is always a sporadic risk to any individual in a population, regardless of family history. This equates to the birth prevalence of around 1 in 10,000 (0.0001%). Secondly, the recurrence risk depends on the presumed or most likely mode of inheritance based on as much information as possible from the pedigree of the proband.

It is not so difficult to give an accurate recurrence risk in these first two examples. The difficulty arises when the proband is the first child of unaffected parents, when the pattern of inheritance can be a new sporadic mutation, autosomal recessive, or 'not genetic' but environmental in aetiology. It is not possible, as the data above has shown, to distinguish these categories on examination. The presumption has always been made that cases bearing resemblance to some clinical genetic 'syndromes' are

more likely genetic. The problem lies in the fact that no genetic abnormality has been demonstrated to date (see chapter two).

The purpose was to determine the empiric risk regardless of the presence of extraocular anomalies. The numbers in the study and the very few affected individuals with presumed familial inheritance does not make it possible to separate 'isolated' from 'non-isolated' cases, and as has already been made clear in chapter two, such a separation may be unjustified. For an individual to have truly 'isolated' ocular abnormalities is a difficult definition to maintain. What is the significance, for example, of subtle dysmorphism in the absence of a recognisable syndrome or demonstrated chromosome abnormality?

The penetration of the genes responsible for optic fissure defects is considered to be less than 100% but it has never been precisely calculated or estimated, and gene expression is highly variable within the same pedigree (Pagon, 1981). One difficulty is that a coloboma may be manifest only on fundus examination, with no easily visible anterior segment abnormality such as iris coloboma, although this situation appears to be very rare (see chapter six).

The recurrence risk for the child of an affected person has been estimated as 46% (Maumenee and Mitchell, 1990). According to Pagon (Pagon, 1981), the apparently unaffected offspring of a parent with autosomal dominant coloboma has an 8.6% chance of having an affected child. Parents with normal eye examination and no family history having a child with isolated ocular coloboma have a recurrence risk of less than 25% but greater than the sporadic risk. Maumenee estimated this as 9%.

METHODS

In this study, an attempt was made to examine all first-degree relatives (parents and siblings if present) and when they were not present, confirmation of a normal eye examination in the past by an ophthalmologist or optometrist was sought. The remaining normal phenotypic classifications relied on family reports of normal eye structure and vision.

The 122 index cases were identified from 115 families. The individuals with a family history and the most likely mode of inheritance are listed in Table 8.5 (page 228). Cases with known chromosomal or single gene defects were excluded, as were cases with well-recognised patterns of malformation such as the CHARGE association.

Empirical recurrence risk

The empirical recurrence risk was calculated with the assistance of Dr David FitzPatrick, consultant clinical geneticist. Sibling recurrence risks were calculated by designating a single affected individual in each pedigree as the index case. Where two study cases were identified in the same family the eldest individual was taken as the index case. Two different segregation analyses were performed, taking into account incomplete ascertainment.

RESULTS

A total of 122 index cases were examined from 115 different families. For the recurrence risk analysis eight cases (seven families) were excluded: one adopted child (ID 324), four cases with recognisable patterns of malformation (three CHARGE

association [ID 35, 40 & 96; ID 35 and 96 were concordant monozygotic twins], Schwartz-Jampel syndrome with nanophthalmos only [ID 60]), and three cases with known chromosome anomalies (47,XX,+18 [ID 15]; 46,XX,del(22)(q11.22) [ID 55]; 46,XY,del(5)(q15;q22) [ID 45]). The remaining data set consisted of 114 index cases in 108 pedigrees. 1/114 cases (ID 109) was the offspring of consanguineous (first cousin) parents. Table 8.7 is a summary of the estimated empirical recurrence risks and related confidence intervals for all 108 cases of MAC and for the sub-group of cases with OFD. Details of the families with recurrences are given in Table 8.5 (page 228).

The empirical sibling recurrence risk was 6–8% in the whole group (OFD, non-OFD and unclassified) and 8–13% in the OFD sub-group (Morrison et al. 2002). Recurrence risks are higher than previous estimates.

All recurrences in siblings and first-degree relatives were in the OFD group. Although similar, the recurrence risk was 33% higher in those with bilateral disease (6–11%) compared with unilateral pathology (5–8%). The risk was significantly reduced (by 25–30%) if the parents did not have any structural eye defect. There were no recurrences in the unclassifiable group, this group being the one that included all the cases of clinical anophthalmos.

Table 8.7: Calculated sibling recurrence risks derived from segregation analysis

	Number of pedigrees	Total sibs	Number of affected sibs	Segregation ratio, assuming single incomplete ascertainment (95% CI)	Segregation ratio, assuming multiple incomplete ascertainment (95%CI)
OFD group	74	99	8	0.060 (0.000)	0.100 (0.001)
Non-OFD group	12	9	0	0.000	0.000
Unclassifiable	22	26	0	0.000	0.000
Whole group Total	**108**	**134**	**8**	**0.081 (0.001)**	**0.133 (0.002)**
Unilaterally affected	55	61	3	0.049 (0.001)	0.079 (0.002)
Bilaterally affected	53	73	5	0.068 (0.001)	0.117 (0.002)
Unilaterally affected, parents normal	55	61	3	0.049 (0.001)	0.079 (0.002)
Bilaterally affected, parents normal	50	69	2	0.029 (0.000)	0.043 (0.001)
Parent and child affected	3	4	3	0.750 (0.047)	0.857 (0.020)

DISCUSSION

The recurrence risk for unilateral and bilateral OFD is similar. This is not entirely surprising. The familial cases had unilateral as well as bilaterally affected individuals. The possible reasons for this were discussed earlier in this chapter (page 235). In none of the unilateral OFD recurrences was a parent affected, which may suggest the action of a mutation of low penetrance in these families.

The calculated figures are only a guide and may have a degree of bias, since all of the affected individuals, some of whom are relatively mildly affected, have survived and therefore will not be the most severely affected from a systemic point of view.

The recurrence risk for 'isolated' cases has not been calculated separately. This is justified on the basis that truly isolated cases cannot be defined or that any definition could be re-interpreted. Not all of the cases studied had cytogenetic tests, although the likelihood of any abnormality being detected in those not tested for a clinical reason is extremely low.

The recurrence risk for sporadic clinical anophthalmos is a challenging question. If clinical anophthalmos, an 'unclassifiable' defect, is the most severe form of optic fissure defect, then the risk is the same as that for OFD i.e. 3–8% when both parents are normal. There is, as discussed in chapter two, much evidence to suggest that clinical anophthalmos and simple iris coloboma are related.

For the first time, calculated empirical recurrence risk figures are presented for these rare but important eye malformations. The figures can be applied in counselling affected families and individuals.

The study data has been derived from a group of children. Longer-term follow up of their offspring would yield further information.

Summary, chapter eight

- The systemic congenital anomalies in 121 children with congenital eye anomalies are described.
- Common systemic associations were developmental delay and congenital heart defect.
- Systemic malformations accompanied all three major classes of eye defect: OFD, non-OFD and unclassifiable.
- Chromosomal abnormalities were rare, occurring in just two cases.
- Of the cases with multiple anomalies, some clinical genetic syndromes were recognised, most commonly the CHARGE association.
- Unrecognised or new patterns of clinical malformation were present in some individuals.
- Dysmorphism was common amongst this group of children.
- There were a significant number of familial cases; the pattern of inheritance was autosomal dominant or recessive.
- The sibling recurrence risk for optic fissure defects (iris and fundus colobomas) is 8-13%.
- The recurrence risk for anophthalmos is no more than that of OFD.

In the next and final chapter, this well phenotyped database of congenital eye anomalies is used to try and determine whether or not certain gene mutations play a role in the cause of the eye defects anophthalmos and coloboma. Modern molecular genetic techniques are employed, using the candidate gene approach.

CHAPTER NINE

AN INVESTIGATION INTO WHETHER *PAX6* GENE MUTATIONS ARE THE CAUSE OF HUMAN ANOPHTHALMOS, MICROPHTHALMOS OR UVEAL COLOBOMA

'Master control genes that act as developmental switches can be detected on the basis of their mutant phenotypes' (Halder et al. 1995).

Summary

84 subjects with a wide variety of ocular phenotypes from whom genomic DNA was available were screened for *PAX6* gene mutations using denaturing high performance liquid chromatography (DHPLC) and sequencing where indicated.

A single *PAX6* missense mutation was found in one subject, whose phenotype in retrospect is likely to be an aniridia phenotypic variant.

PAX6 gene mutations are unlikely to play a significant role in the aetiology of anophthalmos, microphthalmos or uveal coloboma.

Two subjects were screened by collaboration with a Canadian laboratory for *CHX10* gene mutations and none was found. This demonstrates the use of an accurate phenotypic database to screen for candidate gene mutations.

Cell lines have been established on 47 subjects with different ocular phenotypes.

INTRODUCTION

Why look for *PAX6* mutations in anophthalmos, microphthalmos and coloboma?

Sufficient evidence exists to suspect that there are *PAX6* mutations in human anophthalmos, microphthalmos and coloboma. The possibilities include new heterozygous mutations (especially missense) or homozygous mutations, although the latter is unlikely due to the severity of the predicted phenotype. Some of this evidence was introduced in chapter three and this is expanded below.

There is much evidence to suggest that the *PAX6* gene is of major importance to eye development, although enthusiasm to hail *PAX6* as the 'master control gene' of eye development (Quiring et al. 1994; Gehring, 1996) must be tempered with the recognition that the precise role of *PAX6* and its mechanism of action and interaction with other developmental genes is not fully understood. There are four main points of evidence, the strength of which gives sufficient reason to look for *PAX6* mutations in different congenital eye anomalies. These are:

1. Conserved role of PAX6 in eye development in many species.
2. Expression patterns of the *PAX6* gene.
3. The range of ocular phenotypes with *PAX6* gene mutations.
4. Haploinsufficiency and gene dosage effects.

Each of these points will be looked at in turn:

1. Conserved role of PAX6 in eye development in many species

The *PAX6* gene belongs to the *paired*-like class of developmental genes described in the fruit fly *Drosophila melanogoster* (Bopp et al. 1986). These segmentation genes play a key role in development by controlling pattern formation and morphogenesis, and their vertebrate counterparts appear to have a similar function (Walther and Gruss, 1991). The *PAX6* gene, which includes a paired box, has been highly conserved in evolution, being present in nematodes, zebrafish, *Xenopus*, chicken, mice and man. It contains a protein domain of 128 amino acids shown to be a novel DNA-binding motif. In addition to the paired domain, PAX6 contains a paired-type homeodomain. These DNA binding motifs suggest that PAX6 acts as a transcriptional regulator by binding to DNA.

The high conservation of PAX6 tells us that the protein has altered little over time, although it does not tell us what the precise function may be. The function of PAX6, at least in eye development, is conserved in many species.

Further evidence of a major influence of Pax6 on eye development has been provided by work on targeted expression of the *eyeless (ey)* gene in *Drosophila melanogoster*, which is homologous to the mouse *Small eye Pax6* gene and the human aniridia gene (Halder et al. 1995). Ectopic eye structures were induced on the wings, legs and antennae by targeted expression of *ey* DNA in various imaginal discs (other than the eye discs in which eye is normally expressed).

Small eye (Sey) mice homozygous for *Pax6* gene (*Sey/Sey*) mutations have no eyes and no nasal cavities and heterozygotes (*Sey/+*) have eyes with an abnormal optic cup, lens abnormalities and reduced eye size (Hill et al. 1991). The similarities

between mouse *Small eye* and aniridia extend to iris hypoplasia and corneal vascularisation (Jordan et al. 1992).

Mutations in the mouse (*Sey*) and *Drosophila* (*ey*) genes lead to a reduction or complete absence of all eye structures, and because these genes are similar in DNA sequence and in expression pattern even at the earliest stage of eye development, it has been suggested that *ey* and *Sey* may be the master control genes involved in eye development (Quiring et al. 1994; Halder et al. 1995).

2. Expression patterns of *PAX6*

The *PAX6* gene is expressed in the developing eye and central nervous system (CNS) and this has been demonstrated in both mice and humans (Walther and Gruss, 1991; Grindley et al. 1995; Nishina et al. 1999). The expression pattern suggests a regulatory role for *PAX6* in CNS and eye development. The temporal and spatial expression patterns in the early stages of mouse embryogenesis have been examined using mRNA *in situ* hybridisation (Walther and Gruss, 1991). Pax6 transcripts are detected in the neural tube, the forebrain, midbrain and hindbrain. Pax6 is also expressed in the pituitary and olfactory epithelium from its early pit stage to the adult-like nasal structure. In the eye, *Pax6* expression is first detected in the optic sulcus and at later stages in the eye vesicle, lens, differentiating retina and cornea. *Pax6* is expressed in the ectoderm overlying the optic vesicle before the lens has formed and continues to be expressed in the surface epithelium from which the cornea develops.

Examination of the expression pattern of *Pax6* in the *Small eye* mouse has shown Pax6 to be essential in the formation of lens placodes from surface ectoderm

(Grindley et al. 1995). The optic vesicle and surface ectoderm both express *Pax6* during normal development and both show abnormalities in *Sey/Sey* mice.

PAX6 expression in the developing human eye (6-22 weeks gestation) has been studied using immunohistochemistry and monoclonal antibodies reacting to PAX6 protein (Nishina et al. 1999) and also *in situ* hybridisation (Ton et al. 1991). In the early stages, PAX6 was expressed in the surface and neuroectoderm, including the corneal and conjunctival epithelium, lens, and optic cup, similar to murine expression. Pax6 expression continues in the differentiating cells in the cornea, lens, ciliary body and retina. In the retina, Pax6 is first expressed in all cells of the optic cup, and is then restricted to the ganglion cell layer and the inner and outer portions of the inner nuclear layer. The persistent Pax6 expression in the ganglion cells suggests an important role in retinal morphogenesis.

The early expression pattern of the *PAX6* gene is consistent with the idea that *PAX6* mutations might underlie severe ocular malformations, such as coloboma and anophthalmos.

3. The range of ocular phenotypes with *PAX6* gene mutations

Aniridia

The *PAX6* gene is the human aniridia gene. Aniridia is typified by almost complete absence of the iris, with the associated ocular malformations including corneal vascularisation (pannus), cataract, ectopia lentis, lens dislocation, glaucoma and foveal hypoplasia (Nelson et al. 1984). The human aniridia and mouse *Small eye* phenotypes arise from homologous defects in PAX6 (Ton et al. 1991; Glaser et al.

1992) and mouse *Small eye* is an excellent animal model for human aniridia. Aniridia is a semidominant disorder and the aniridia gene, *PAX6*, was isolated from the chromosomal region 11p13 by deletion analysis and positional cloning (Ton et al. 1991). *PAX6* gene mutations account for most cases of aniridia and occur in both the familial and sporadic forms (Glaser et al. 1992; Jordan et al. 1992; Hanson et al. 1993).

The mode of inheritance of aniridia is autosomal dominant, with a third of all cases being sporadic. The sporadic cases may occur as part of the WAGR (Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation) contiguous gene deletion syndrome involving band p13 of chromosome 11. Deletions are hemizygous.

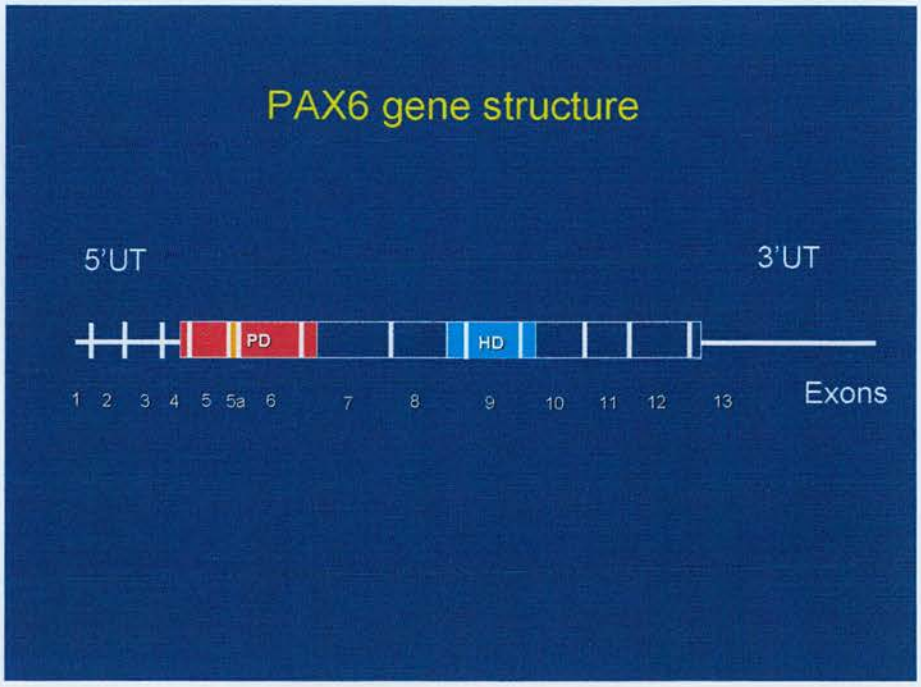
Heterozygosity for *PAX6* mutations produces the *Small eye* phenotype in mice and aniridia in humans. Homozygous mouse *Pax6* mutations produce the severe and lethal phenotype of almost complete absence of the eyes and nose (Hogan et al. 1986; Hill et al. 1991). A presumed case of a homozygous aniridia gene mutation (no analysis was performed) was described by Hodgson (Hodgson and Saunders, 1980) in a stillbirth resulting from the union of both parents having aniridia. The foetus had complete absence of the eyes and palpebral fissures, nose and adrenal glands. In 1994, Glaser (Glaser et al. 1994) demonstrated two different *PAX6* mutations in a compound heterozygote. The phenotype of the proband, who survived for eight days, was anophthalmia (absent eyes and eyelids fused) with multiple craniofacial defects (small head, external nose malformation and choanal atresia, multiple brain abnormalities including absent corpus callosum). The anatomical CNS abnormalities correlated well with mouse *Pax6* homozygotes.

Although typically in human aniridia there is no iris present, there is great phenotypic variability, with some cases having an almost complete iris with coloboma-like defect or thinning (Hittner et al. 1980; Negishi et al. 1999). This great variation in ocular phenotype means that cases of aniridia may be missed in what is a predominantly inherited condition.

***PAX6* gene and protein structure organisation** (Ton et al. 1991; Glaser et al. 1992; Epstein et al. 1994; Glaser et al. 1994). The *PAX6* gene spans 22kb and encodes a 422 amino acid protein that is probably a transcriptional regulator (Figure 9.1). The +5a alternatively spliced form encodes 436 amino acids. The gene has 14 exons with one alternative splicing site (exon 5a). The initiation codon is in exon 4 and the termination codon is in exon 13. The alternative splicing produces a second protein with a 14 amino acid insertion in the paired domain and arises by alternative mRNA splicing and exhibits unique DNA binding properties, an important determinant of transcriptional activity (Epstein et al. 1994). There are three protein domains. The paired domain (128 amino acids) is composed of two sub-domains, each consisting of three alpha-helices, with the second and third helices being arranged in a helix-turn-helix configuration. The N terminal half of the paired domain is thought to specify DNA binding. The homeodomain (61 amino acids) is separated from the paired domain by a 78 amino acid linker region. The homeodomain again is made up of three alpha helices, the last of these being responsible for sequence recognition and DNA binding. The 152 amino acid C-terminal segment of PAX6 is rich in the amino acids proline, serine and threonine and is called the 'PST-domain'.

The amino acid composition of this domain suggests that it is involved in transcriptional activation, and this has been supported by functional studies (Glaser et al. 1994).

Figure 9.1: Human *PAX6* gene structure



***PAX6* gene mutations: the spectrum**

By March 1999, over 100 *PAX6* gene mutations have been described and these are summarised in the database at http://www.hgu.mrc.ac.uk/Softdata/PAX6.

4. *PAX6* dosage and pathogenetic mechanisms

The molecular basis of aniridia is most likely to be haploid insufficiency for *PAX6*, caused by a simple loss of function of one copy of the *PAX6* gene by deletion of the gene or intragenic mutation. The eye appears particularly sensitive to *PAX6* dosage

(Hanson et al. 1993; Glaser et al. 1994) (Hanson and van Heyningen, 1995). Abnormal gene dosage is a common cause of spontaneous abortion and human birth defects, the best-known example being Down's syndrome (trisomy 21), in which the precise effect of increased dosage on the embryo is difficult to determine. In abnormalities caused by deletions or any other inactivating mutation, e.g. nonsense mutation, which leave only a single copy of certain critical genes such as *PAX6*, 'haploinsufficiency' occurs. Two copies are needed for a normal phenotype. Haploinsufficiency genes show a wide range of penetrance and expressivity (Fisher and Scambler, 1994) and there are a number of possible reasons. Some developmental pathways are particularly susceptible to dosage effects because of the sensitivity to the levels of certain proteins. The simplest effect of hemizygosity is insufficient protein being produced. Haploinsufficiency gene products are often proteins that take part in the assembly of intermolecular complexes that may have an exact stoichiometry, such as the proposed role of PAX6 as a transcription factor. Another possible explanation for the haploinsufficiency of PAX6 is that it has multiple DNA binding sites in any one cell; these binding sites are likely to be of different affinity with some binding PAX6 very tightly and some not so tightly. If the level of PAX6 protein is reduced by 50%, some targets may not become bound at all, and those genes will not be activated. Hemizygosity would therefore be particularly likely to result in dosage effects. The levels of expression of the non-deleted gene will also have an influence on phenotype. Other possibilities to account for the phenotypic variability are that hemizygosity could increase the sensitivity of the embryo to the effects of environmental insults

(infection, teratogen). Also to be considered is that cells hemizygous for a particular gene might be targets for a 'second hit' of the non-deleted gene.

The extraordinary degree of conservation of *PAX6*, which must have been maintained by selection, indicates that any amino acid change would be expected to be deleterious. In the next section, some of the published *PAX6* mutations are discussed.

Beyond aniridia: The spectrum of *PAX6* mutation phenotypes

PAX6 mutations have been found in Peters' anomaly (Hanson et al. 1994), autosomal dominant keratitis (Mirzayans et al. 1995), isolated foveal hypoplasia (Azuma and Nishina, 1996) and uveal ectropion (Azuma and Yamada, 1998). These studies, along with the range of aniridia phenotypes, have broadly implicated *PAX6* in human anterior segment malformations.

In the Peters' anomaly phenotypes (Hanson et al. 1994), one case described was the result of a deletion of the *PAX6* gene. A further case was described due to amino acid substitution from a point mutation within the paired box and this case is one of the few missense mutations described. It was also noted that a proportion of *Small* eye mice heterozygous for a murine *Pax6* nonsense mutation, have an ocular phenotype resembling Peters' anomaly (Hanson et al. 1994).

The autosomal dominant keratitis and corneal vascularisation phenotype described by Mirzayans (Mirzayans et al. 1995) does have features which overlap with the various forms of aniridia, including foveal hypoplasia. The condition was associated with a mutation in the exon 11 splice-acceptor site. Once again, a truncated protein is predicted.

A *PAX6* missense mutation (see missense mutations below) has been described in a case of isolated foveal hypoplasia in the C-terminal part of the paired domain (Azuma and Nishina, 1996). Foveal hypoplasia, which caused reduced visual acuity and nystagmus in these cases, is a well-recognised feature of aniridia, making this an interesting phenotype-genotype comparison.

Azuma et al (Azuma and Yamada, 1998) reported an isolated case of a non-specific anterior segment anomaly (corneal opacification, invasion of cornea with conjunctival epithelium, uveal ectropion of the pupil, and posterior embryotoxon). Here, a missense mutation occurred in exon 13, affecting the PST domain.

The vast majority of aniridia patients have intragenic *PAX6* mutations that are predicted to cause premature termination of protein translation. These consist of splice mutations, frameshift mutations and nonsense mutations. Much less common are in-frame deletions or splice mutations that would be predicted to delete part of the protein. Missense mutations (where one amino acid is substituted for another as a result of a single nucleotide substitution) are very rare in aniridia patients, with only two cases reported to 1999.

Missense mutations and *PAX6*

In 1998, the spectrum of *PAX6* mutations in aniridia was heavily biased towards premature protein truncation (nonsense, splicing, insertions and deletions), these making up 92% of all reported mutations. Only 2% of mutations lead to substitution of one amino acid for another (missense mutations) (Hanson et al. 1999). The

remaining 6% of aniridia mutations are caused by in-frame deletions or splice mutations that would be predicted to delete part of the protein.

The high conservation of the PAX6 amino acid sequence would predict that pathological *PAX6* missense mutations should be much more common. When *PAX6* missense mutations arise they are highly likely to be pathological and so because they are so rare in 'textbook' aniridia patients, they must be associated with other phenotypes. The few cases of missense mutations described for *PAX6* have tended to be non-aniridia phenotypes (see earlier) (Hanson et al. 1994; Mirzayans et al. 1995; Azuma and Nishina, 1996; Azuma and Yamada, 1998; Gronskov et al. 1999; Hanson et al. 1999). A major factor probably contributing to the lack of missense mutations is ascertainment bias: missense mutations seem to have a tendency to cause eye phenotypes that are not always considered for *PAX6* mutation analysis. This is further justification for screening patients with complex congenital eye disorders such as anophthalmos and coloboma. Since 1998, many more missense mutations have been found and there is increased awareness that variant eye phenotypes can be caused by *PAX6* mutations.

A missense mutation in the alternative splice region of the *PAX6* gene (exon-5a) within the paired-type domain has recently been described in combination with some unusual eye phenotypes: Peters' anomaly, congenital cataract, Axenfeld anomaly and foveal hypoplasia (Azuma et al. 1999).

Summary, *PAX6* mutations

- There is a bias towards nonsense, deletions, insertions, splice and frameshift mutations
- Few *PAX6* missense mutations have been described
- The *PAX6* missense mutations are often unusual phenotypes
- Several *PAX6* missense mutations have been described within the paired box

METHODS

DNA extraction

It was initially thought that a combined technique of DNA and RNA extraction could be used (Qiagen reagent kit), but this technique was abandoned after preliminary testing because the procedure was lengthy, complicated, and did not produce very large quantities of DNA suitable for PCR.

DNA was extracted from venous blood samples or mouthwashes within 48 hours of being taken. Venous blood was collected in sodium EDTA (ethylene diamine tetra-acetic acid) tubes. The genomic DNA was extracted using a kit (Nucleon, Biosciences) based on chloroform extraction.

DNA was extracted from mouthwashes, in 10–20ml of tap water, as follows: Spin for 10 minutes at 3000 rpm, discard supernatant and place the cell pellet in 50–200 micro litres of 0.05M sodium hydroxide. Mix then boil for 20 minutes. Add 25 micro litres of 1M TRIS (2-amino-2-(hydroxymethyl)-1,3-propanediol) buffer pH 7.7, mix and then spin briefly to remove cell debris from suspension. 5 micro litres of the supernatant is used for PCR. The DNA concentrations were checked using a spectrophotometer.

Lymphoblastoid cell lines

Venous blood was collected in EDTA. Lymphoblastoid cell lines were established or white blood cells stored from a number of subjects. Marie Robertson and Cath Davidson at the MRC Human Genetics Unit, Edinburgh, carried out this preparation and storage work. The purpose of these cell lines is to create a potentially limitless supply of RNA suitable for PCR and mutation screening.

Some samples were sent to ECACC (European Collection of Animal and Cell Cultures) in Wiltshire, England for storage and transformation.

Karyotyping

Where it was clinically indicated (significant delayed development plus another congenital malformation or dysmorphic appearance), venous blood was collected in heparinised tubes for G-banding of chromosomes (Cytogenetics Laboratory, Royal Hospital for Sick Children, Edinburgh). These results are discussed in the previous chapter (eight).

Polymerase Chain Reaction (PCR)

The genomic DNA samples were amplified by PCR. Samples were diluted to the recommended concentration (see below) and the *PAX6* exon-specific primers (oligonucleotides) used were those published (Love et al. 1998).

The polymerase used was *Pfu* (Stratagene). *Pfu* DNA polymerase is a proofreading DNA polymerase isolated from *Pyrococcus furiosus*. It has a very low rate of nucleotide incorporation errors.

The PCR conditions for optimal amplification of each exon had to be established in accordance with the manufacturer's instructions. This required alteration of different parameters (DNA concentration, annealing temperature, number of cycles, enzyme concentration). In some cases, 10% Dimethylsulfoxide (DMSO) was required. It is likely that DMSO helps keep the double stranded DNA denatured, particularly in the regions that are G-C rich. Additional help and assistance to refine the PCR conditions towards the end of this stage was from Dr Kathy Williamson PhD, MRC Human Genetics Unit.

Reaction volumes of 50 micro litres were used with a DNA template concentration of 25–50 nanograms, 2.5 μM of forward and reverse primers, 10mM of dNTPs, and the enzyme and buffer supplied by the manufacturer. Typically, PCR amplifications were for 30 cycles: 94^0C for 45s, Tm (melting temperature) for 30s and 72^0C for 1 min, final extension time 72^0C for 10 min.

In addition to the patient samples, genomic DNA was amplified from some 'controls' known to have *PAX6* mutations within the relevant exon.

Following amplification a small aliquot of each PCR product was analysed on 2.5% agarose gel electrophoresis to confirm and determine the efficiency of amplification.

Denaturing high performance liquid chromatography (DHPLC)

This mutation screening method was chosen on the basis that it would allow rapid screening of large numbers of PCR-amplified DNA samples (WAVE™ DNA Fragment Analysis System, Transgenomic). Technical problems with the machinery column and software delayed mutation screening by several months. The description that follows is a summary of the methods and laboratory work initiated. Dr. Kathy Williamson, PhD, completed this.

The DHPLC has a high mutation detection rate (Underhill et al. 1997; Liu et al. 1998). If analysis of the peaks shows a shift then a mutation is suggested and the sample is reamplified, then sequenced to find the underlying change in DNA sequence. DHPLC exploits the differential retention of double-stranded heteroduplex and homoduplex molecules, allowing the automatic comparison of PCR amplicons for variation. The technique was chosen as it was considered to be efficient and relatively inexpensive with high levels of automation. DNA segments of several hundred base pairs in length can be analysed (up to 1.5kb).

Mutation/polymorphism scanning by DHPLC involves subjecting PCR products to ion-pair reverse phase liquid chromatography in a column containing alkylated non-porous particles (a divinylbenzene derivative). Reverse phase refers to the fact that the column is hydrophobic and is the opposite phase to the hydrophilic DNA. The DNA is bound to the column using an ion-pairing reagent TEAA (triethylammonium acetate). Under conditions of partial heat denaturation within a linear acetonitrile gradient, followed by cooling and hybridisation, heteroduplexes that form in PCR samples having internal sequence variation have a reduced column retention time relative to

their homoduplex counterparts. The resolution is temperature-dependent. In the majority of cases the elution profiles for heteroduplexes are distinct from those having homozygous sequence, making the identification of samples containing polymorphisms or mutations a straightforward procedure.

The method requires heteroduplex DNA for detection of intrasample sequence variation. It is possible that mutations could escape detection where loss of a wild type allele occurs in combination with mutation of the remaining allele since the predominant double stranded DNA formed would be mutant homoduplex. Therefore, the DNA from individuals who have two mutant alleles (homozygous) must be mixed with wild-type DNA and hybridised. After this, a sample will contain a mixture of hetero- and homoduplexes.

Optimal separation of the DNA hetero- and homoduplexes depends on the gradient of buffer concentration. Buffer A is 0.1M TEAA, and buffer B is 0.1M TEAA and 25% acetonitrile. The acetonitrile elutes the DNA off the column in relation to DNA fragment size. The recommended gradient is a 2% increase in buffer B per minute.

The resolution of hetero- from homoduplexes depends on performing the separation at a partially denaturing temperature. The WAVE^{TM} utility software is used to help determine the correct temperature (melting or mobile phase temperature) for mutation scanning based on the sequence of wild-type DNA for each exon, accounting for length and G-C content.

DNA samples were used from all patients on whom samples were available, regardless of extraocular abnormalities. All the patients were a subset of the clinically verified and phenotyped database (chapter seven).

DNA samples

The DHPLC methods for optimal mutation detection for each of the 14 exons were tested on DNA samples from (mainly) aniridia patients already known to have mutations within that exon. Both empirical methods and software-based algorithms were used. DHPLC detected 100% of the known mutations, which included a variety of mutations (deletions, insertions and substitutions).

5 microlitres of each PCR product was used in the DHPLC.

Sequencing (Dr Kathy Williamson PhD, MRC Human Genetics Unit, Edinburgh)

Following DHPLC analysis the trace for each sample was inspected and where a shifted peak was seen, the DNA sample was sequenced using the Thermo Sequenase Radiolabelled Terminator Cycle Sequencing Kit (Amersham/USB Corporation).

RESULTS

DNA was screened for *PAX6* mutations in 84 subjects. A number of shifted peaks were detected, but upon sequencing these were nearly always found to be intronic polymorphisms which were considered unlikely to have any deleterious effect on the PAX6 protein. Only one patient, ID 243 (male), was found to have a heterozygous mutation. The mutation was a single nucleotide substitution (c.1087G>C) in which a single nucleotide is changed from G to C in exon 9, within the homeodomain of PAX6. This missense mutation is predicted to substitute a highly conserved arginine residue encoded by AGA at position 242 with threonine (encoded by ACA). Arginine

242 is located in the middle of the second alpha helix of the paired type domain. This second alpha helix stabilises the third helix, which makes sequence specific contacts with the DNA. The G1087C mutation was not detected in over 160 other individuals analysed. As already discussed, very few *PAX6* missense mutations have been described.

In several other cases there were abnormal profiles on DHPLC, but these were found to be neutral base changes i.e. not predicted to alter the PAX6 amino acid sequence.

DISCUSSION

PAX6 gene mutations are not likely to be a significant cause of anophthalmos, microphthalmos or coloboma in humans.

The finding of a new heterozygous *PAX6* missense mutation in one individual demonstrates the need to cast the net wide and that further *PAX6* ocular 'non-aniridia' phenotypes may exist, or that there is extensive variation in the aniridia phenotype.

The ocular phenotype of patient ID 243 with the G1087C mutation is unusual and is difficult to name or categorise retrospectively. The abnormality consists of a unilateral (left) partial thickness defect of the iris at 6 o'clock. Only the anterior layer of the iris was affected (Figure 9.2), leaving the darker posterior layer almost intact. The external appearance when observed with the naked eye is that of an iris coloboma. No other ocular abnormality was present. There were no systemic abnormalities. Family history was negative and maternal eye examination was normal. Examination of the father was not possible, but eye examination is reported as normal.

Figure 9.2: Probable aniridia variant with *PAX6* missense mutation. The left eye has a partial thickness iris defect inferiorly (ID 243)

It is very likely that the abnormality represents an aniridia variant or partial aniridia, although missense mutations of aniridia variants have tended to be within the paired domain, in contrast to the mutation in this case being within the homeodomain (Gronskov et al. 1999; Hanson et al. 1999). The mother's DNA showed the same heterozygous *PAX6* exon 9 mutation, making this mutation likely to be inherited rather than sporadic, with a low expressivity. It is unlikely that this mutation is a polymorphism since it has not been previously recognised, is predicted to alter the PAX6 protein, and is associated with an abnormal ocular phenotype. The substituted arginine residue is conserved in this position in other PAX6 proteins (all vertebrates, *Drosophila melanogaster*, nematode, squid and sea urchin) so it is likely to be very important. The replacement of a positively charged arginine residue with a neutral threonine predicts a change in function of this position, although the exact effect is unknown. A functional analysis of the mutant protein might provide further evidence of the significance of the c.1087G>C mutation.

No other mutations were found. Some of the eyes initially classified as coloboma did bear a strong resemblance to partial aniridia (ID 3, ID 121, ID 187). The finding of a *PAX6* mutation would have clarified the diagnosis.

This study has highlighted the difficulty in separating the ocular phenotypes and the need for detailed description. Only heterozygous mutations were screened for. Homozygous mutations are highly unlikely as the resulting phenotype would probably be lethal or non-viable (see above). Only true homozygous mutations, i.e. an identical base change at the same position in both copies of the gene, are not screened for, and such a mutation is so unlikely that it can almost be excluded. Two mutated copies of *PAX6* with different mutations (a compound heterozygote) would be detected by DHPLC analysis. To screen for and exclude true homozygous mutations the DNA samples would have required dilution with wild type DNA in order to form detectable heteroduplexes. However, this study has screened for the genotypes most likely to exist in this cohort, since homozygotes would probably be non-viable.

DHPLC is a relatively new technique and the mutation detection rate for the *PAX6* gene under these conditions is unknown. Other, more established techniques of screening for mutations exist such as single stranded conformational polymorphism (SSCP) but may not be any more sensitive (Axton et al. 1997; Axton et al. 1997). However, there is little doubt that for mass screening of large numbers of DNA samples, DHPLC is a suitable tool.

It is difficult to establish the significance of finding a *PAX6* gene mutation in a non-aniridia ocular phenotype. Do all *PAX6* mutations matter? Probably not. If not, why not? Subtle and clinically undetectable changes may be present in the phenotype. Gene interactions and the effect of other genes downstream may be just as important. One cannot always consider mutations in isolation. In addition, environmental effects on genes are a possibility. We do not know the significance of the c.1087G>C mutation.

The mutation is potentially of great interest as it is the first missense mutation to be found in the homeodomain. Considering the mild phenotype, it is possible that the homeodomain does not play as significant a role in eye development as the paired domain, which harbours many pathological missense mutations.

CHX10, OCULAR RETARDATION

Mice homozygous for the gene *ocular retardation* (*or*) have very small eyes (Truslove, 1962). *Ocular retardation* is caused by a mutation in the *Chx10* gene, a homeobox gene (Burmeister et al. 1996). The significant morphological pathology is the effect on the neuroretinal layer, degeneration of which somehow impairs the overall development of the eye, causing microphthalmic mice. Homozygous *or/or* mice have optic nerve aplasia, thin hypocellular retina, and reduced eye size. The primary pathology is probably within the bipolar cells of the inner nuclear layer. The expression pattern of the Chx10 protein in the developing mouse eye is almost entirely localised to the cells of the inner nuclear layer (Liu et al. 1994).

Human *CHX10* maps to chromosome 14q24.3 and has five known exons. *CHX10* is thought to be a transcription factor and contains a homeodomain of the paired class, along with a second domain, the CVC domain. Like the homeodomain, the CVC domain probably has a role in DNA binding (Burmeister et al. 1996).

The inability of *or/or* ganglion cell axons to exit from the eye may be due to abnormalities in the formation of the optic stalk, which would include delayed formation or closure (Burmeister et al. 1996).

Based on the ocular retardation phenotype in mice, recessive mutations in the human *CHX10* gene might be predicted to cause developmental abnormalities of the eye. It is for this reason that patients with optic nerve aplasia or optic nerve defects, defects in retinal development, and microphthalmos were considered for screening of *CHX10* mutations.

METHOD

The genomic DNA of two subjects in the study (ID 47, 234) were sent to Professor R. McInnes for mutation screening (Department of Molecular and Medical Genetics, University of Toronto, Canada). The phenotypes were as follows:

ID 47: Male. Right iris coloboma, dysplastic retina with coloboma, optic disc hypoplastic and pale. Left anophthalmos. Delayed development, cerebral atrophy (confirmed by CT scan), low set ears, doll-like features.

ID 234: Female, Bilateral iris and retinal colobomas, normal optic nerves. Broad nasal root, smooth philtrum, low set posteriorly rotated ears. Suffers from absences.

RESULTS

All exons were assessed for mutations by PCR amplification and SSCP (single stranded conformational polymorphisms). No mutations were found.

DISCUSSION

The *CHX10* collaboration demonstrates the use of an accurate ocular phenotypic database to screen for candidate gene mutations. That no *CHX10* mutations were

found may show that the wrong ocular phenotype has been looked at, or that the mutation detection technique was not sensitive enough. Phenotypes worthy of further consideration include optic nerve aplasia and cataract.

Professor McInnes' group have screened more than 300 samples for *CHX10* mutations, including many with clinical microphthalmos and optic nerve aplasia. In two families, recessive *CHX10* mutations were found in the homeodomain at the chromosome locus 14q24.3. The phenotype was clinical microphthalmia with cataracts and severe iris abnormalities. Whilst these findings do not establish *CHX10* as an anophthalmia gene, it reinforces the significance of *CHX10* as a possible regulator of eye development (Percin et al. 2000).

Summary, chapter nine

- In only one of the 84 subjects with the congenital eye anomalies of uveal coloboma, clinical anophthalmos or microphthalmos, was a *PAX6* gene mutation found, after screening DNA.
- Mutation screening of the DNA samples was by the DHPLC method, which has the potential to screen a large number of DNA samples very efficiently.
- A new *PAX6* gene missense mutation has been described in an individual with an ocular phenotype that is probably an aniridia variant.
- No *CHX10* mutations were found in two samples sent to Canada for analysis.

CHAPTER TEN

DISCUSSION AND CONCLUSIONS

Anophthalmos, microphthalmos, and uveal coloboma are congenital eye anomalies that can cause significant visual impairment. The introductory chapters of this thesis (1–3) set out a number of arguments:

Anophthalmos, microphthalmos and coloboma have not been adequately defined

In chapter one, the early development of the eye was outlined, with a description of current terminology. Anophthalmos is best defined as *clinical* anophthalmos, where no globe is apparent. *Clinical microphthalmos* refers to a heterogeneous group of eye anomalies, all sharing the appearance of the eye being small. There is no current accepted definition of microphthalmos and attempts to define microphthalmos based on the axial length of the eye have not been successful. The term microphthalmos is overused and has been applied as a phenotypic diagnosis, but the phrase clinical microphthalmos is only helpful in the identification of a gross or generalised congenital eye anomaly, pending a more detailed phenotypic eye examination.

There are many types of coloboma and, like microphthalmos, the term coloboma has been applied inappropriately, although the range of definitions is narrower. In the case of isolated optic nerve colobomas, there is a question mark about their relationship to uveal colobomas and whether or not they are defects of optic fissure formation or closure. There is a continuous spectrum from true anophthalmos to mild

microphthalmos that includes the terms clinical (apparent) anophthalmos, extreme or severe microphthalmos, microphthalmos and mild microphthalmos. Therefore, the words anophthalmos and microphthalmos can be and have been applied to the same condition.

Anophthalmos/microphthalmos have features that suggest both a genetic and environmental aetiology

In chapter two, the birth prevalence of anophthalmos, microphthalmos and coloboma in several epidemiological surveys was outlined. The quality of many of these surveys and reports is hampered by the eye anomalies being poorly defined. The poorly understood aetiology of anophthalmos, microphthalmos, and coloboma was described, listing a number of alleged environmental causes. That there is also a genetic component in the aetiology was demonstrated by a number of familial cases described from the literature. The ocular and systemic features of anophthalmos/microphthalmos and coloboma suggest in many cases both a genetic and an environmental component in their aetiology. Examples include the significance of these eye anomalies being unilateral or bilateral, and the presence of multiple extraocular (systemic) congenital anomalies.

Proper investigation and the search for a cause rest on establishing a phenotypic definition

In the second and third chapters, the point was reinforced that to investigate the aetiology of anophthalmos, microphthalmos or coloboma, be it genetic or

environmental, required the rigorous application of a phenotypic definition. The difficulties with definition were introduced in chapter one, and these were added to with a description of some of the similar problems encountered by laboratory scientists when studying eye development anomalies in mice. A simple, reproducible, consistent definition would greatly enhance the epidemiological surveys, recruitment of subjects for research, clinical grouping and analysis of cases, and clinical and molecular genetic analysis.

Candidate genes can be used to consider the genetic aetiology

The final part of chapter three, the last introductory chapter, described some of the many approaches to finding a genetic aetiology or component to congenital eye anomalies and identifying genes that may be involved. The candidate gene approach, and the current knowledge of genes thought to be significant in human eye development, was summarised. The human *PAX6* homeobox gene and mice studies were introduced to illustrate how the application of this information may lead to the identification of a genetic cause of anophthalmos, microphthalmos or coloboma.

The studies and investigations described in the next six chapters of this thesis (4–9) set out to substantiate the introductory arguments. In chapter four the overall framework of the Scottish Microphthalmia Study is described, along with an overview of the methods used to recruit patients into the study database. This national study involved public health, ophthalmology, clinical genetics, and molecular genetic approaches.

More than 300 names were put forward as possibly being affected with anophthalmos/microphthalmos or coloboma and born within the dates specified (1981–1996). The total number of affected children was calculated as 183, and 122 of these were examined clinically. The calculated birth prevalence in Scotland is 1.78 per 10,000. The birth prevalence of clinical anophthalmos in Scotland is 0.68 per 100,000. The rarity of this condition has been confirmed, with just seven live births in 16 years. Prevalence of anophthalmos/microphthalmos across the Scottish Health Boards ranged from zero in the Western Isles to 2.59 per 10,000 in Fife. Birth prevalence did not increase over the 16-year period studied and remained constant. Comparison of these results with previous studies suggests that case ascertainment levels were high. The difficulties of relying on a single source for case ascertainment based on records and without clinical verification were demonstrated by the large number of exclusions from the study of unaffected normal children and individuals with other ocular phenotypes.

The Scottish Microphthalmia Study had a number of aims (chapter four). As many cases as possible were identified using multiple sources of information gathering. The database has provided the basis for a case control study into the environmental aetiology of microphthalmos, anophthalmos, and coloboma in Scotland.

A simple case definition needed to be developed for congenital anomalies registration and clinical record keeping which is sufficiently specific to allow cases to be searched for and easily identified. Clinical anophthalmos is a term that has a useful meaning. Microphthalmos, microphthalmia, and clinical microphthalmos have very limited use and would be better dropped from current congenital anomalies registers or used as a

suffix only, for example, *corneal scar with microphthalmos*. Microphthalmos could retain use as a secondary descriptive clinical term. Iris coloboma and retinal coloboma are sufficiently accurate terms to identify and capture the abnormalities sought. Optic nerve head abnormalities will always be difficult to identify early because diagnosis requires highly specialist ophthalmic examination and knowledge. In most cases of optic nerve head abnormality the correct diagnosis is not made until months or even years after the birth of the affected child.

Before embarking on any discussion of cause or investigations into the genetic cause of the many eye defects observed in the study group, an improved definition was needed, and this was based on phenotype. The eye examination findings were reclassified on the basis of the presence or absence of a defect thought to be due to optic fissure formation or closure. Both the non-optic fissure defects and unclassifiable groups are phenotypically heterogeneous. This phenotypically based classification system has no need for the term microphthalmos. Defects of the optic nerve, previously called optic nerve coloboma or optic nerve head coloboma, are now collectively grouped as optic nerve head disruption. This removes the term coloboma that has continued to obfuscate the understanding of the underlying mechanism that may have caused such an unusual defect of the optic nerve head. Colobomas were classified into five types, although problems remain with certain types such as the superior ('atypical') coloboma, which does not easily fall into any classification system.

There was notable asymmetry of ocular malformations in bilaterally affected individuals both with and without a family history. This is significant when considering

the aetiology and the risk of recurrence, since it is likely that in the past too much emphasis has been placed on unilateral and bilateral eye defects. A unilateral abnormality does not necessarily seem to indicate a non-genetic defect, and a bilateral abnormality does not necessarily imply a genetic cause.

The prevalence of iris coloboma-iris heterochromia has been found to be much higher than previously thought, affecting almost one-fifth of cases. In less than 4% of cases of iris or fundus coloboma there was a retinal detachment, which appeared to have developed before birth.

Many eyes can look small. Some small eyes are normal in size, some are big, and some really are small. The failure to define microphthalmos reinforces the point that the term has limited clinical use. That there is no suitable definition has been demonstrated in the ultrasound study of 106 eyes in chapter seven, in which nearly half of the 55 clinically microphthalmic eyes had a normal axial length. The study also made it clear that clinical *macrophthalmos* is probably a lot more common than previously recognised. There is no value in further pursuing a definition of microphthalmos or clinical microphthalmos and, as with the classification system for coloboma, further time and effort should be directed towards identifying and recognising new or different ocular phenotypes.

The eye anomalies studied were put in a systemic context in chapter eight, when 37.2% of 121 clinically examined cases were found to have a non-ocular congenital anomaly. There was a significant number of individuals affected with congenital heart disease and developmental delay. Many children's systemic anomalies did not fit into any previously recognised syndrome, and cases with a genetic aetiology or family

history were not distinguishable from sporadic cases of unknown aetiology. The familial cases showed both autosomal dominant and recessive patterns of inheritance. Chromosome abnormalities were tested for in 35 cases, with only one child having an abnormal karyotype (G-banding).

Genetic counselling of families for these rare but sometimes devastating eye anomalies is difficult, and the family pedigrees described in chapter eight provided data for calculations of the empirical recurrence risk of anophthalmos and uveal coloboma. For unaffected parents giving birth to an affected child, the sibling recurrence risk is around 3–8%. The risk does not differ significantly for unilateral or bilaterally affected individuals.

In the final part of the study in chapter nine, the candidate gene approach was used in an attempt to further understand the genetic aetiology of anophthalmos, clinical microphthalmos and coloboma. The genomic DNA of 84 individuals was extracted from blood or saliva and screened for *PAX6* gene mutations. Lymphoblastoid cell lines were established in 50 cases. Denaturing high performance liquid chromatography (D-HPLC) was used for mutation screening. A single heterozygous *PAX6* missense mutation was found in one individual. A review of the ocular phenotype, a defect of the anterior iris in the 6 o'clock position, made it very likely that this particular eye defect was an aniridia variant. The missense mutation, a single nucleotide substitution, was in the homeodomain and has not been described previously. The conclusion is that *PAX6* mutations are unlikely to play a significant role in the genetic aetiology of clinical microphthalmos, coloboma or anophthalmos.

In a smaller collaborative study, two individuals had genomic DNA sent to Canada for *CHX10* gene mutation screening. No mutations were found in this homeobox gene.

The study has achieved the initial aims, which span from the identification and clinical examination of as many cases as possible of anophthalmos, microphthalmos and coloboma, to the calculation of the recurrence risk and identification of a *PAX6* gene mutation. Collaborative studies were established with laboratories in Canada and Belgium, and cell lines were set up in Scotland and England. A database and register of clinically verified diagnoses now exists, from which the birth prevalence of these congenital eye anomalies was calculated for Scotland.

The major strength and uniqueness of the study was the detailed clinical examination of so many cases drawn from an unselected population across a whole country. The selection bias common to so many specialist and tertiary referral centres was therefore not present. The large period of time studied (16 years) and the two-year study period helped to maximise the amount of data which could be retrieved and allowed a more thorough search for cases with, for example, second letters to non-responders and GP letters for further information.

A retrospective study will always be disadvantaged by the need to retrieve data and the problems associated with this. Attempts to enhance retrieval and improve case finding included multiple sources of information gathering including a nationwide publicity campaign and letters to special schools. Nevertheless, there is still a degree of reliance on the memories and quality of records of clinicians.

A prospective study would not necessarily have avoided many of the problems encountered, since many of the same sources would need to be contacted in a similar

manner. Continued surveillance and monitoring requires a lot of publicity, education of professionals, and updates. Ultimately, there is still a reliance on individuals to fill in reporting cards. For a condition that is so rare and produces so few cases annually, a study would have to take place over several years to yield sufficient numbers and reveal any trends.

One of the implications for governmental organisations arising from this study is the use of data in the congenital anomalies registers. In this study, much of this data has been shown to be inaccurate in terms of the actual diagnosis and severity of eye defects. These congenital anomaly registers should not be used for detailed data analysis or monitoring of prevalence of rare conditions. The clinical register of congenital eye anomalies produced has provided a research resource and a model for future studies in ophthalmology, epidemiology, clinical genetics and molecular genetics. Many of the results are significant to those involved in the planning of resources and provision of education, special needs facilities and social services for visually impaired children. It is important to recognise that in many cases there is more than just an eye problem since multiple disability is not uncommon.

The classification system into optic fissure and non-optic fissure defects establishes a pattern and facilitates future phenotype-genotype correlation studies. Such studies may ultimately lead to a genotypic classification of eye defects.

It is still difficult to assess the undoubted genetic contribution to the aetiology of these complex congenital eye anomalies. The easy answer is to ascribe the cause to many genes interacting, since there is as yet no evidence that a single gene is culpable. None of the seven cases of anophthalmos found in Scotland were familial, although one case

did arise within a consanguineous marriage, which increases the chance of autosomal recessive inheritance. This may mean that these cases are non-genetic in aetiology, but it could also mean that the genetic cause cannot be found.

The majority of cases of anophthalmos, clinical microphthalmos and coloboma present sporadically, with fewer but significant cases where familial recurrence is seen. There is a genetic component in the aetiology of these eye anomalies, although in many families there is clearly reduced penetrance and extremely variable expression. The latter observation is underlined by the frequently different phenotype in the left and right eye. One or more of the following factors at play may explain these observations: interaction with modifier genes; interaction with environmental factors; involvement of stochastic factors in the developmental process. This variability makes it more difficult to access the genetic component. There is clearly considerable genetic as well as phenotypic heterogeneity. Defining the genes and developmental pathways involved seems the only approach to understanding the aetiology of these abnormalities. Critical environmental components are most likely to be identified and understood in the context of knowing the genes implicated. The ultimate aim is to know the genes involved in order to offer genetic analysis and eventually prenatal diagnosis.

Colobomatous eye defects are seen in a wide range of chromosomal abnormalities and yet cytogenetic chromosomal abnormalities are rarely found on routine screening of individuals with only eye abnormalities and no developmental delay or systemic congenital malformation. Not all of the children in the study had their blood tested by

G-banding of chromosomes, as it was felt highly unlikely that any new abnormality would be detected.

Colobomatous eye defects so often seem to be the harbinger of another congenital abnormality so a careful examination is essential. It would seem that this particular aspect of eye development is extremely sensitive to any disturbance of normal development, and may have something to do with other events occurring at the time of closure of the optic fissure in the embryo, which may occur over a number of days or weeks. Chromosomal microdeletions remain as a possible cause and the 22q11 microdeletion detected in one individual in this study suggests that other microdeletions might be found if a more aggressive search is made.

The candidate gene approach was used as the basis of screening for *PAX6* and *CHX10* mutations, using a relatively novel screening method, denaturing high performance liquid chromatography (DHPLC). The technique allowed large numbers of DNA samples to be tested. It is important to remember that mutations may have been missed in the screening process. Further studies on selected individuals in the group have been undertaken to screen for other candidate genes, *PAX2* and *DRES93* (*VAX2*). These were in collaboration with laboratories in Belgium (*PAX2*) and Italy (*DRES93*). So far, no gene mutations have been found.

There is scope for further studies using the candidate gene approach. The DNA samples and the phenotypic database allow matching of proposed candidate genes to the ocular and systemic phenotype. Linkage analysis is still a possible route, but the rarity of large families of affected individuals has always hindered further progress using this method.

A relatively unexplored area is that of chromosomal microdeletions, and there is potential here for further work. Testing with probes using fluorescence in situ hybridisation (FISH) is a relatively straightforward procedure.

The clinical significance and new implications of this study are that in all cases of anophthalmos or clinical microphthalmos or coloboma, a careful eye examination should be done to confirm and refine the ocular phenotype. First-degree relatives should be examined in conjunction with a detailed family history. In specific cases, an ultrasound B-scan may be indicated to confirm suspected clinical findings such as a detached retina. Visual prognosis and later function in colobomatous eyes is very difficult to assess, and refraction and low vision aids should be considered as soon as such measurement is possible, in conjunction with any treatment for amblyopia.

In view of the large number of children with non-ocular abnormalities and dysmorphism, a paediatrician or clinical geneticist should review all new cases. Genetic counselling and advice should be appropriate for each case, with realistic and more accurate recurrence risks quoted for future siblings. The only consistent feature of a familial (and therefore probably genetic) case is the family history. All other cases are sporadic.

It is tempting to ascribe a significant environmental role to the cause of anophthalmos, clinical microphthalmos and coloboma, especially in the absence of hard genetic evidence. But when the alleged causes are examined closely, there is often a breakdown in the relationship between timing of exposure and the type of defect seen. This may be due to an over-simplified view of the timings of events in embryogenesis and of what effects the possible accumulation of toxins acquired before the onset of

critical events may have later on in development. It is here that the lack of our understanding of cause is further exposed, as little or nothing is known about the dose, timing and duration of exposure that might bring about such eye defects. The nature or type of environmental agents to which a parent might be exposed is unknown, and the size of the human embryo at this early stage of development could make it exquisitely sensitive to the tiniest concentration of a toxin. Some of these questions about exposure to potential toxins may be answered by the current case-control study in Scotland (Sheena Macdonald, Department of Public Health Sciences, University of Edinburgh).

In this study, the ocular features, systemic abnormalities, and family histories are all evidence of both genetic and non-genetic (environmental) aetiologies for clinical microphthalmos, anophthalmos, and coloboma. The eye findings of asymmetry in bilaterally affected individuals and individuals with unilateral defects could be interpreted as being due to an environmental agent. Conversely, bilaterally affected cases, especially when symmetrical, could be seen as an indication of a genetic cause. Extra-ocular congenital anomalies were not confined to the cases with a family history: multiple congenital abnormalities are considered by many to be evidence of a genetic aetiology but may also be interpreted as a sign of an external agent or toxin acting in the early stages of embryogenesis. That the environment could be affecting susceptible developmental genes to differing degrees is a real possibility. An understanding of the underlying genetic causes of eye defects is necessary for the optimal management of these conditions, for the counselling of the affected families

and in order to provide insights into the biological processes involved in eye development that may eventually lead to new prevention strategies.

There is a complex genetic and possibly an environmental aetiology of ocular congenital clinical anophthalmos, microphthalmos and uveal coloboma. By exploring the definition, closely examining the phenotype, studying the family history, and testing for specific gene mutations, this thesis has made a significant contribution to the search for causes of these eye defects.

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APPENDIX

THE SCOTTISH MICROPHTHALMIA STUDY

Clinical Genetics Department
Molecular Medicine Centre
Western General Hospital
Crewe Road
Edinburgh EH4 2XU

Tel: 0131 651 1012

Date: _ _ / _ _ / _ _ **ID No:**

Personal Details

Surname: **First name(s)**:

Date of Birth: _ _ / _ _ / _ _

Sex: male ☐ female ☐ unknown ☐

CHI/CI No: ☐☐☐☐☐☐☐☐☐☐

NHS No: ☐☐☐☐☐☐☐☐☐☐

Health Records ID No: ☐☐☐☐

Address at birth registration:

.....

.....

.....

Postcode: .....

Current Address:

.....

.....

.....

Postcode: .....

Health Board: .....

Guardian: Name ..... Title .....

Address .....

.....

Postcode .....

Tel. no .....

Relationship to index case .....

Hospital of birth: .....

Gestational age: .....weeks

Birthweight: .....kg

Race: Caucasian ☐ Black ☐ Asiatic Indian ☐ Oriental ☐
Other ☐ .....

Living: Yes ☐ No ☐ Date of Death: _ _ / _ _ / _ _

GP: Name .....
Address .....

Mother's Details

Surname: **First name(s)**:

Surname at birth of child:

Date of Birth: _ _ / _ _ / _ _

Obstetric History

No. of children:

No. of miscarriages: **Stillbirths**: **Terminations**:

Rubella vaccination: Yes ☐ No ☐ Unknown ☐

Scan: Yes ☐ No ☐
.....weeks Normal ☐ Abnormal ☐ Unknown ☐

.....

.....weeks Normal ☐ Abnormal ☐ Unknown ☐

Amniocentesis: Yes ☐ No ☐

..... weeks

Normal ☐ Abnormal ☐ Unknown ☐

.....

Chorionic Villus Sampling: Yes ☐ No ☐ .....weeks

Normal ☐ Abnormal ☐ Unknown ☐

.....

Father's Details

Surname: **First name(s)**:

Date of Birth: _ _ / _ _ / _ _

Family History

Parental consanguinity: Yes ☐ No ☐

Twin: ☐ Yes ☐ No **Twin affected**: Yes ☐ No ☐
☐ MZ
☐ DZ
☐ Unknown

Multiple Birth: Yes ☐ No ☐ Number:.....

Comment:.....

1 No. of siblings: **No. affected**: **ID No**:

2 No. of 1/2 siblings maternal: **No. affected**: **ID No**:

2 No. of 1/2 siblings paternal: **No. affected**: **ID No**:

Birth order (including stillborns):

Family Tree

To include 1/2 sibs, twins, uncles/aunts, 1/2 uncles/aunts, grandparents, 1st cousins, great aunts/uncles, nephews/nieces, 1/2 nephews/nieces.

.....

.....

.....

.....

.....

1 Mother affected: Yes ☐ No ☐

1 Father affected: Yes ☐ No ☐

2 No. of maternal aunts/uncles: **No. affected**:

2 No. of paternal aunts/uncles: **No. affected**:

2 Maternal grandmother affected: Yes ☐ No ☐

2 Maternal grandfather affected: Yes ☐ No ☐

2 Paternal grandmother affected: Yes ☐ No ☐

2 Paternal grandfather affected: Yes ☐ No ☐

2 No. of 1/2 maternal aunts/uncles: **No. affected**:

2 No. of 1/2 paternal aunts/uncles: **No. affected**:

2 No. of maternal nephews/nieces: **No. affected**:

2 No. of paternal nephews/nieces: **No. affected**:

3 No. of 1/2 maternal nephews/nieces: **No. affected**:

3 No. of 1/2 paternal nephews/nieces: **No. affected**:

3 No. of maternal 1st cousins: **No. affected**:

3 No. of paternal 1st cousins: **No. affected**:

3 No. of maternal great aunts/uncles: **No. affected**:

3 No. of paternal great aunts/uncles: **No. affected**:

Total 1st degree relatives..... Affected.....

Total 2nd degree relatives..... Affected.....

Total 3rd degree relatives..... Affected.....

Investigations

Karyotype: Yes ☐ No ☐

☐ Normal
☐ Abnormal .....
☐ Unknown

Virology (TORCH): Yes ☐ No ☐ Unknown ☐

Positive ☐ Negative ☐ Unknown ☐

Details .....

CT Scan of head: ☐ Yes Details:.....
☐ No .....
☐ Unknown

MRI: ☐ Yes Details: .....
☐ No .....
☐ Unknown

Ultrasound of eye: ☐ Yes Details: .....
☐ No .....
☐ Unknown

General Examination

Known or suspected syndromes: .....

.....

Diagnoses

1 .....

2 .....

3 .....

4 .....

5 .....

6 .....

7 .....

8 .....

9 .....

10 .....

Weight: .....kg

Height: .....cm

Head circumference: .....cm

General impresssion:.....

Nose ☐ .....

External ears ☐ .....

Hearing ☐ .....

Lips ☐ .....

Teeth ☐ .....

Palate ☐ .....

Chin ☐ .....

CNS ☐ .....

Neck ☐ .....

Chest ☐ .....

Cardiovascular ☐ .....

Abdomen ☐ .....

Genitalia ☐ .....

Hands ☐ .....

Feet ☐ .....

Skin ☐ .....

Eye Examination

R Visual acuity:
Near
Distance
Snellen ☐ SG ☐ Kay's ☐

L Visual acuity:
Near
Distance
Snellen ☐ SG ☐ Kay's ☐

R Iris: Normal ☐ Abnormal ☐

Coloboma Yes ☐ No ☐

L Iris: Normal ☐ Abnormal ☐

Coloboma Yes ☐ No ☐

R Lens: Normal ☐ Abnormal ☐
☐

Coloboma Yes ☐ No ☐

L Lens: Normal ☐ Abnormal

Coloboma Yes ☐ No ☐

R Pupil reaction:
direct: Yes ☐ No ☐
RAPD Yes ☐ No ☐

L Pupil reaction:
direct: Yes ☐ No ☐
RAPD Yes ☐ No ☐

Horizontal Corneal Diameter				
R Current	mm		L Current	mm
__/__/__	mm		__/__/__	mm
__/__/__	mm		__/__/__	mm
__/__/__	mm		__/__/__	mm

Axial Length				
R Current	mm		L Current	mm
__/__/__	mm		__/__/__	mm
__/__/__	mm		__/__/__	mm
__/__/__	mm		__/__/__	mm

Palpebral fissure length: Right .....mm

Left .....mm

Inner canthal distance: .....mm

Outer canthal distance: .....mm

Inter-pupillary distance: .....mm

Glasses: Yes ☐ No ☐

Refractive Error			
Right		Left	
sphere		sphere	
cylinder		cylinder	
axis		axis	
spherical equivalent		spherical equivalent	

Funduscopy

Retina: Right .....

Left .....

Macular: Right .....

Left .....

Disc: Right .....

Left .....

Fundus Drawing

Eye Surgery:

1 .....

2 .....

3 .....

4 .....

Whole eye prosthesis: Right ☐ Left ☐
Implant: Right ☐ Left ☐
Corneoscleral shell: Right ☐ Left ☐

Blind registration: Yes ☐ No ☐ Date: _ _ / _ _ / _ _

Special school: Yes ☐ No ☐

Special needs register: Yes ☐ No ☐

Special assistance within mainstream school: Yes ☐ No ☐

.....

Disability Living Allowance: Care ☐ Mobility ☐

Other ocular diagnoses

☐ Eyelid .....

☐ Cornea .....

☐ Cataract .....

☐ Glaucoma .....

☐ Retina .....

☐ Refractive Error .....

☐ Amblyopia .....

☐ Trauma .....

☐ Tumour .....

☐ Other .....

Summary

Anophthalmia: Right Yes ☐ No ☐ Unknown ☐
Left Yes ☐ No ☐ Unknown ☐

Microphthalmia: Right Yes ☐ No ☐ Unknown ☐
Left Yes ☐ No ☐ Unknown ☐

Coloboma: Right Iris Yes ☐ No ☐ Unknown ☐
Lens Yes ☐ No ☐ Unknown ☐
Retina Yes ☐ No ☐ Unknown ☐
Optic Disc Yes ☐ No ☐ Unknown ☐

Left Iris Yes ☐ No ☐ Unknown ☐
Lens Yes ☐ No ☐ Unknown ☐
Retina Yes ☐ No ☐ Unknown ☐
Optic Disc Yes ☐ No ☐ Unknown ☐

Unclassifiable malformation: Right Yes ☐ No ☐
Left Yes ☐ No ☐

Mother's eyes

Eye problem: Yes ☐ No ☐

Diagnoses: 1.....

2.....

3.....

Visual acuity: .....

Anterior segment: .....

Posterior segment: .....

Father's eyes

Eye problem: Yes ☐ No ☐

Diagnoses: 1.....

2.....

3.....

Visual acuity: .....

Anterior segment: .....

Posterior segment: .....

Consultants under the care of: name/hospital

Ophthalmologists 1. .....

2. .....

3. .....

Paediatricians 1. .....

2. .....

3. .....

Geneticists: 1. .....

2. .....

Other consultants: .....

.....

Checklist

Photo ☐

Consent forms ☐

Blood: Obtained ☐ Refused ☐ To be arranged ☐

For: Karyotyping ☐ DNA/RNA (Edinburgh) ☐ Canada ☐

Cell line (Edinburgh) ☐ Cell line (Porton) ☐ Mouth wash ☐

Maternal Blood ☐

Paternal Blood ☐

Permission to be approached for future interview ☐

Contact address and telephone number:

.....

.....

.....

Further examination needed: Yes ☐ No ☐

Name .....

.....

Do you wish to know results of any blood tests: Yes ☐ No ☐

Member of MACS support group: Yes ☐ No ☐

Special requests by family:

.....

.....

Additional notes:

Follow-up and Counselling

Result:	Sent to:	Sent by:	Date: