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THE CHICK AS AN ANIMAL MODEL OF EYE DISEASE

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Short Title: Chick models of eye disease

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ABSTRACT

A diverse range of chicken lines harbouring highly-penetrant, spontaneously-occurring mutations with an ocular phenotype have been identified over the past 40 years. These lines serve as models for human monogenic disorders including ocular albinism, retinal dystrophies such as Leber's congenital amaurosis, and coloboma, as well as the common complex traits glaucoma and myopia. Recent technical advances in gene targeting, mapping quantitative trait loci, and phenotypic characterisation of eye phenotypes offer exciting prospects for exploiting chicken genomic resources in fundamental and translational eye research.

INTRODUCTION

The domestic fowl has been widely used as an experimental animal during the last century for the study of fundamental processes of biology. As an experimental model it has many advantages: the embryo is readily accessible during a short incubation period, supplies of chicks are readily available and are easily accommodated in small pens or cages, sexual maturity occurs at 18-25 weeks, hens may produce as many as 300 offspring in a year and many varied genetic lines have been characterised.

This review will outline the genetic resources available for experimental research, describe the structure of the avian eye in comparison to that of the human, and discuss the use of chicks as models for defective ocular development. Mutant lines will be described and their use as animal models will be summarised. Finally, some well-established non-genetic chick models of eye disease will be described.

Advantages of the chicken as a model organism for translational research

Genetic resources

The chicken was the second **vertebrate** to be sequenced in 2004 [1] and the chicken genome has been regularly updated and comprehensively annotated since then. Synteny with human and mouse [2] and conservation of gene function between birds and mammals, make the publically available chicken genomic databases valuable resources for identifying gene candidates. A large number of single nucleotide polymorphisms have been detected and a 600K SNP chip is available commercially for rapid genotyping and fine mapping [3] as are gene expression arrays [4] and a retinal proteome database [5]. In addition, as discussed below, several mutant lines that inherit a recessive gene causing eye disease have been characterised that serve as animal models for investigating both gene pathways and potential therapies.

Experimental techniques

The chick has been the model organism of choice for the study of embryo development for many years, the embryo being readily cultured in open egg shells that permit the manipulation of cells and tissues in culture or *in ovo* [6]. Embryos can be manipulated to study eye development and cells and tissues can be transfected with transgenes by electroporation [7]; genes can be added to the germ line by lentiviral vectors to study gene function *in vivo* [8]; and transgenic chickens in which fluorescent protein reporters are expressed either ubiquitously or in targeted cell types are available to track the development of specific cells or tissues [9]. Improved methods for creating new mutants by gene editing, knock-in and knock-out in combination with the culture of primordial germ cells [10,11] make the chick a powerful model for the study of eye development in health and disease.

Structure and function of the avian eye

Vision is the primary sense in birds, and their visual system bears witness to this by virtue of a large eye size, excellent visual acuity and colour perception, and a degree of visual field overlap that permits binocularity [12,13]. The basic anatomy of the eye is similar to that of other vertebrates consisting of the cornea, anterior chamber, crystalline lens, vitreous and retina. In contrast to mammals, however, the retina is avascular and nutrition to the eye is supplied via the pecten, a highly invaginated vascular structure that projects into the ventral retina close to the optic nerve. The avian eye is cone dominated, in contrast to the rod-dominated human eye, although both share high acuity domains containing tightly-packed cones. The chicken retina contains 4 types of cones that contain coloured oil droplets that, together with the visual pigments, create a wide spectral sensitivity of 370-580 nm [14]. The basic wiring of the retina is similar to that of other vertebrates, as reviewed by Gütürkün [12]. Other anatomical differences between human and chick retina include the presence of scleral ossicles, a series of overlapping bony plates surrounding the cornea of birds, and a major contribution of changes in corneal curvature to the accommodation response.

In spite of differences in the gross anatomy of the chicken eye, the similarities and conservation of gene function make the chick a powerful animal model. The larger eye size, superior optical quality, better visual acuity, and ready accessibility of the embryo during development, make the chick an attractive alternative to mice and rats for investigating potential ophthalmic therapies and studying the developmental mechanisms of sight.

GENETIC MUTANTS AS ANIMAL MODELS

A large number of hereditary mutations in the chicken have been reported. Conditions that affected the eye and published before 1990 have been reviewed by Somes et al. [15] and a current list is available at the On-line Mendelian Inheritance in Animals website

(<http://omia.angis.org.au/home/>, accessed 21st October 2013). Recessive mutations

associated with blindness are listed in Table 1 for those listed as still available in 2005 [16] or that have been described in papers published since 1990. It should be noted that in avian species the female is the heterogametic (ZW) sex and is therefore affected by sex-linked recessive genes rather than the male (ZZ). Furthermore, because the two sex-determining systems have evolved separately in mammals and birds [17], synteny does not exist between the sex chromosomes of humans and chickens.

Blindness enlarged globe (*beg*)

The *beg* chicken was identified in a commercial flock in 1982 [18]. Genotyping with the 600K SNP chip covering 31 chromosomes failed to detect the region containing the gene (unpublished results) and it is therefore assumed that the mutation occurs in one of 8 micro chromosomes that for technical reasons have not been sequenced. The *beg* homozygotes have significantly greater axial lengths and eye volumes than wild type controls, but no difference in intraocular pressure. Ophthalmic investigation showed an absent or sluggish pupillary response, atrophy of the pecten and peri-papillary retina and iris neovascularisation. Older birds had cataracts with prominent iris vessels and an irregular unreactive pupil, reminiscent of rubeosis iridis [19].

Chick albino (*ca*)

The albino chick phenotype is caused by a 6-bp deletion in the tyrosinase (*TYR*) gene that results in lack of pigmentation in the retinal pigment epithelium and consequent photophobia, reduced visual acuity, eye enlargement, myopia and astigmatism [20,21]. Whereas the albino chick does not exhibit nystagmus, a consistent feature of human albinism, *ca* chicks are non-the-less an animal model for eye disorders associated with albinism in humans [22].

Coloboma (*co*)

A recessive sex linked mutation in White Leghorns causes coloboma (*co*) of the eye and a number of other abnormalities including cleft palate, shortened limbs, defective digits and exteriorised viscera. The mutation is lethal in female embryos but might be useful for the study of comparable syndromes in human disease [23]. The mutation has been mapped to a relatively short section of 176 kb on chromosome X. Three candidate genes (SLC30A5, CENPH and CDK7) have been identified on the basis of this location and differential expression compared with the normal embryos [24].

Micromelia-4 (*mi-4*)

The *mi-4* mutation is an autosomal recessive which causes a reduction in eye size and is associated with high mortality [25]. Expression was stated to be limited primarily to females which appears to conflict with the high rates of reported mortality.

Pink-eye (*pk*)

The *pk* mutation reduces melanin deposition and death of ocular melanocytes in the presence of high concentrations of tyrosine [26]. Visual acuity is compromised as in the *ca* mutation but the two genes are distinct [27].

Pop-eye (*pop*)

The pop-eye mutation (*pop*) produces a mild to severe bilateral protrusion of the cornea in young chicks that increases in the adult. It is accompanied by an increased anterior chamber depth and scleral scarring and is associated, as in human keratoconus, with astigmatism, corneal thinning caused by age-related cell loss, and altered corneal curvature [28,29].

Retinal degeneration (*rd*)

The chicken retinal degeneration (*rd*) mutation causes a recessively-inherited retinal degeneration that results in blindness at hatch [30]. The disease is characterised initially by the loss of outer segments followed by degeneration of the inner segments and retinal pigment epithelium [31,32]. The phenotype is due to a null mutation in the photoreceptor guanylate cyclase (GC1) gene [33]. Affected birds have very low levels of retinal cGMP preventing phototransduction and are a model for Leber's congenital amaurosis. Interestingly, lentiviral expression of retinal GC1 restored vision in *rd* chickens, representing the first use of the chick as a gene therapy model for an inherited eye disease [34].

Retinal dysplasia and degeneration (*rd*)

The retinal dysplasia and degeneration (*rd*) mutation causes a sex linked recessive phenotype resulting from a C-T substitution that creates a premature stop codon in the multiple PDZ domain protein (*MPDZ*) gene [35,36]. Chicks are sighted at hatch but vision is clearly compromised by 9 weeks of age and the birds are completely blind at sexual maturity. Retinal degeneration is progressive and histologically involves undulations and thinning of the retina. There are no gross morphological differences between sighted and blind birds. Retinal protein and biochemical differences between sighted and blind *rd* chicks have been identified [37,38] but the role of *rd* as an animal model is unclear, as the homologous gene in humans is not currently implicated in causing a retinal dystrophy [36]. **However, a homozygous recessive C-T mutation in the human MPDZ gene resulting in the premature truncation of the protein has recently been identified that was associated with congenital hydrocephalus [39].**

Retinopathy globe enlarged (*rge*)

The *rge* mutation has been identified as a 3-bp deletion (D153del) in the gene for guanine nucleotide binding protein $\beta 3$ (*GNB3*) that is predicted to result in truncation of the full length protein [39]. Birds homozygous for the mutation are sighted at hatch but become blind after 9 weeks of age [40]. Montiani-Ferreira and colleagues [40-43] concluded that blindness was a secondary effect of the enlarged globe because no retinal pathology was observed that could not be explained by the increase in eye size. *GNB3* codes for the heterotrimeric G protein $\beta 3$ subunit (G $\beta 3$). The protein is usually expressed in cone photoreceptors and ON bipolar cells, but is entirely absent from the retina of affected birds [44]. Young *rge* birds show enhanced responses to hyperopic defocus, whereas myopic defocus is unable to counteract the eye enlargement of *rge* birds [45]. The *rge* deletion causes decreased phosphorylation in a tissue specific manner, whereas a common human polymorphism (825T) that increases phosphorylation is a predisposing factor for hypertension, obesity and coronary disease [46]. The *rge* is a potential model for understanding the role of G $\beta 3$ in ON bipolar cells in human myopia.

The enlarged eyes of *rge* and *beg* chicks are associated with corneal flattening. The orientation of the collagen fibres in these lines has been investigated and was shown to be associated with progressive loss of alignment of circumferential collagen in the peripheral cornea and limbus whereas proteoglycan distribution was unaffected [47-49]. Boote et al. [50] used eyes from *rge* and control chicks to study the biomechanics of these ultrastructural changes and confirmed that material behaviour and response to intraocular pressure were affected by the changes in corneal collagen fibril orientation. These mutant lines have potential as animal models for quantifying the role of collagen architecture on the structure and function of the cornea and sclera.

Smoky Joe (SJ)

The name Smoky Joe refers to the colour of the plumage of a line of Leghorns that inherits a sex-influenced autosomal recessive gene causing retinal degeneration and blindness [51]. The birds have varying degrees of impaired sight at hatch and are blind at 8 weeks. Tran et al. [52] showed that the blind chicks had fewer retinal (particularly amacrine) cells compared with sighted SJ and that photoreceptors were almost absent in the blind birds.

Other avian genetic disorders

Shibuya et al. [53] reported a line of Fayoumi chickens with visual impairment, which they named GSN/1. Electrophysiological and anatomical findings suggested the disease was caused by developmental defects in the visual pathways, especially the optic tectum. The delayed amelanotic (DAM) line of chickens is characterised by early onset melanocyte dysfunction, which leads to severe retinal degeneration and visual impairment [54,55]. The disease is associated with several autosomal genes and is an animal model for autoimmune vitiligo where it is referred to as the Smyth line of chickens [56-58]. The Slate turkey is a traditional coloured breed that inherits a recessive gene, also called *pop-eye*, which is a model for secondary angle closure glaucoma [59]. The albino (*al*) mutation in Japanese quail (*Coturnix coturnix japonica*) exhibits raised intraocular pressure and thus may prove useful as an animal model for glaucoma. The disease is correlated with degeneration and dysfunction of dopaminergic amacrine cells and the metabolism of retinal indoleamine [60,61].

NON-GENETIC MODELS OF EYE DISEASE

The absence of circadian cues by exposure to constant light or darkness induces buphthalmos in chickens [62-64], while prevention of sharp vision during the light phase of the light/dark cycle induces globe enlargement and myopia [65]. Interestingly, globe enlargement does not

occur in blind chicks with degenerate retinas (*rd* and *rdd*) but does so in mutant lines that have a functional retina (*beg* and *rge*) (45 and unpublished data). The use of these lines collectively to investigate gene signals causing myopia would seem to be promising.

As well as the deprivation of sharp vision by means of lid suture or frosted lenses (so called “form-deprivation”) myopia can also be induced by fitting chicks with minus power (diverging) spectacle lenses [66]. This is a model of “emmetropisation”, the process which normally prevents myopia and hyperopia by means of a visually-guided feedback loop controlling the rate of eye growth [67]. Selective breeding has been used by Chen et al. [68] to produce chick lines with either high or low susceptibility to visually-induced myopia. A chick model of astigmatism has also been developed, in which a change in corneal curvature in a specific meridian can be induced by means of a low power cylinder spectacle lens [69]. A difference in eye size between the two progenitor lines of the Roslin Institute’s Advanced Intercross Line – a chicken resource population designed to facilitate the fine mapping of QTL (quantitative trait loci) – was proposed as a useful animal model for investigating genetic pathways mediating myopic eye growth, particularly the co-regulation of the eye’s component parts [70,71].

Cebulla et al. [72] used subretinal injections in young chicks as a model of retinal detachment. The accessibility of the chick embryo has been exploited in models of ethanol-induced microphthalmia [73] and steroid-induced cataract [74]. The chick cornea has also been proposed as a model for studying wound healing, for instance in response to surgical de-innervation [75] and corneal laser surgery [76,77].

A NOTE ON THE WELFARE OF BLIND BIRDS

Blind chicks and adults are maintained under relevant welfare legislation and local guidelines for animal experimentation. In contrast to the claims of Ali and Cheng [78], the welfare of

blind birds, and by inference those in which sight has been compromised by experimental manipulation, is adversely affected [79]. The numbers of birds used in these experiments should therefore be minimised and fully justified by the importance of the results in relation to the welfare costs to the birds.

CONCLUSIONS

The rapid development, accessibility, and techniques for experimental manipulation, combined with a comprehensive array of genetic resources, make the chick an invaluable animal model of eye disease. The large size of the eye, availability of genetic mutants, the ability to create transgenic chickens and the potential of methods for precise gene editing, make it a promising animal model for testing therapies.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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