Developmental Biology ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Perspective

Utilizing the chicken as an animal model for human craniofacial ciliopathies

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ARTICLE INFO

Article history:
Received 1 July 2015
Received in revised form
13 October 2015
Accepted 21 October 2015

Keywords:
Chicken
Craniofacial
talpid^2
talpid^3
Primary cilia
Ciliopathies
Oral-facial-digital syndrome
Joubert syndrome

ABSTRACT

The chicken has been a particularly useful model for the study of craniofacial development and disease for over a century due to their relatively large size, accessibility, and amenability for classical bead implantation and transplant experiments. Several naturally occurring mutant lines with craniofacial anomalies also exist and have been heavily utilized by developmental biologist for several decades. Two of the most well known lines, *talpid*^{2} (*ta*^{2}) and *talpid*^{3} (*ta*^{3}), represent the first spontaneous mutants to have the causative genes identified. Despite having distinct genetic causes, both mutants have recently been identified as ciliopathic. Excitingly, both of these mutants have been classified as models for human craniofacial ciliopathies: Oral-facial-digital syndrome (*ta*^{2}) and Joubert syndrome (*ta*^{3}). Herein, we review and compare these two models of craniofacial disease and highlight what they have revealed about the molecular and cellular etiology of ciliopathies. Furthermore, we outline how applying classical avian experiments and new technological advances (transgenics and genome editing) with naturally occurring avian mutants can add a tremendous amount to what we currently know about craniofacial ciliopathies.

"A box without hinges, key, or lid, yet golden treasure inside is hid."– The Hobbit

1. Introduction

1.1. Avians as a model for craniofacial development and disease

There has been a long-standing relationship between the study of craniofacial development/disease and the avian model system. The chicken has provided insights into much of what is known about craniofacial development. Several seminal experiments performed in avian models furthered our understanding of craniofacial growth and patterning. Cranial neural crest, the progenitors of anterior facial skeleton and connective tissue, were first observed in the chick embryo (His, 1868). In addition to fate mapping the cellular contributions to the craniofacial complex (Couly et al., 1993), chickens have been used to identify the extent of neural crest cell plasticity (Couly et al., 2002, 1996, 1998; Köntges and Lumsden, 1996; Le Douarin et al., 2004), explore the molecular and cellular basis for species-specific facial patterning (Schneider and Helms, 2003; Tucker and Lumsden, 2004) and determine the requirements for tissue–tissue interactions during craniofacial development (Chong et al., 2012; Creuzet et al., 2004, 2006; Etchevers et al., 1999; Hu and Marcucio, 2009a). Furthermore, several naturally occurring avian mutants have been utilized to understand pathological conditions (Robb et al., 2011). Here, we review and compare two of the most utilized avian models of craniofacial disease (*talpid*^{2} and *talpid*^{3}) and highlight what they have revealed about the molecular and cellular etiology of a disease class that has a profound affect on craniofacial development: ciliopathies.

The avian embryo has been highly relevant for human craniofacial development due to the well-conserved organization and growth of the facial prominences between avians and mammalian species (mouse and human) during craniofacial development (Fig. 1). Facial development, regardless of species, begins with the formation and growth of five distinct facial prominences: a singular frontonasal prominence (FNP), paired maxillary prominences (MXP) and paired mandibular prominences (MNP) (Brugmann et al., 2006) (Fig. 1A–I). In humans, the FNP gives rise to

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http://dx.doi.org/10.1016/j.ydbio.2015.10.024
0012-1606/

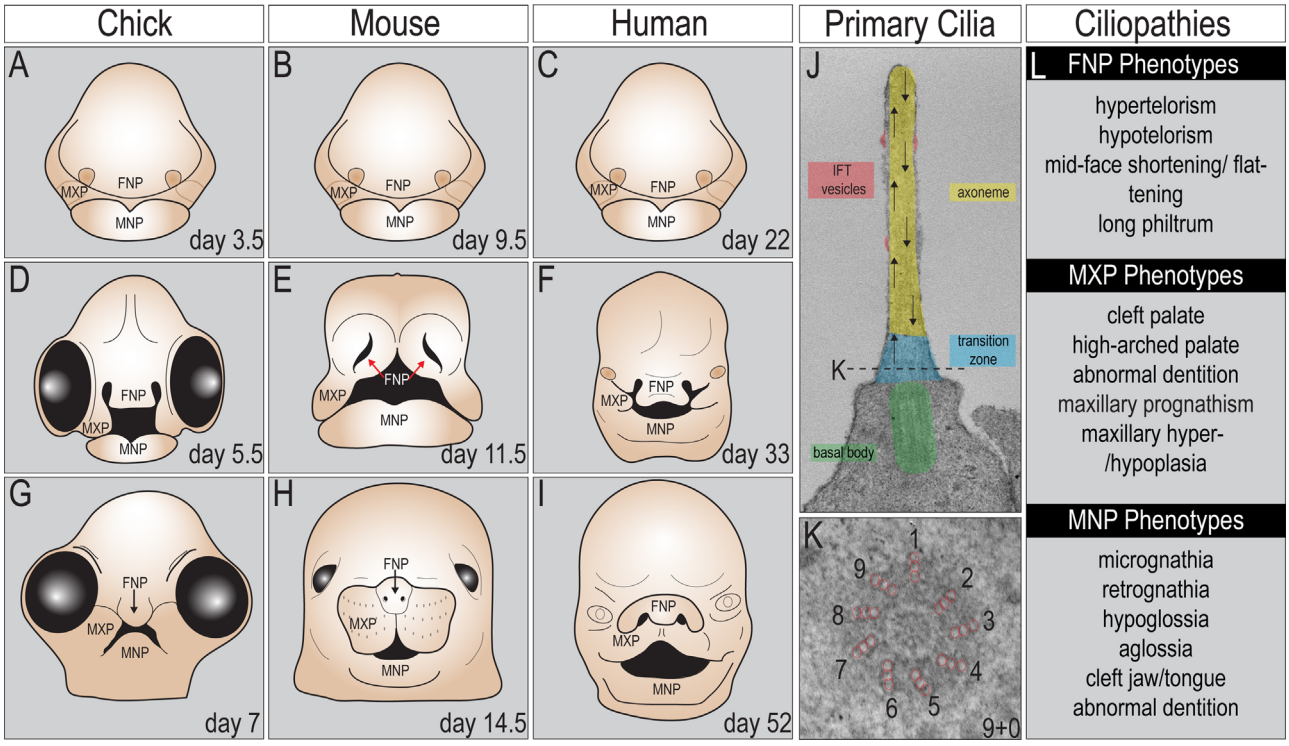

Fig. 1. Craniofacial development and primary cilia. (A–I) Development of the embryonic face in chick, mouse, and human. Note that following prominence fusion, species-specific differences become clear (e.g., beak in chicken, snout in mouse). (J) TEM of a primary cilium. Axoneme (yellow), basal body (green), IFT cargo (red), transition zone (blue). (K) Cross section of a primary cilium showing 9+0 microtubule arrangement. (L) Table of common phenotypes seen in ciliopathic patients arranged by facial prominence of origin.

midline features including the forehead, the middle of the nose, the philtrum of the upper lip, and the primary palate. In avians the FNP is also present and gives rise to the upper beak and primary palate (pre-maxilla). The other more lateral component of the frontonasal prominence, the lateral nasal prominences, contributes to the sides (alae) of the nose in humans and sides of the beak in avians. The MXP contributes to the upper lip, upper jaw and secondary palate in humans, and the lateral aspects of the upper beak and secondary palate in avians. In both humans and avians the MNP gives rise to the lower lip/beak, jaw, and the anterior two-thirds of the tongue (the 3rd and 4th branchial arches contribute to the posterior third and the intrinsic glossal musculature comes from the occipital somites (Parada et al., 2012)). The tissues that compose the facial prominences (surface ectoderm, neural crest, mesoderm and endoderm) are also highly conserved between avians and other mammalian species (mice and humans). This degree of morphological and cellular conservation has propelled the chicken into the forefront of animal models for human craniofacial disease, specifically craniofacial ciliopathies: a rapidly growing class of craniofacial anomalies caused by a defect in the structure or function of primary cilia.

1.2. Craniofacial ciliopathies and the avian model system

Primary cilia are microtubule-based organelles that dynamically extend from the cell surface (Fig. 1J and K). In the last decade, the cilium has gained increasing popularity due to their nearly ubiquitous presence on various cell types, their role as regulators of developmental signaling pathways (e.g., Sonic Hedgehog) and association with a number of syndromes collectively known as ciliopathies (Badano et al., 2006; Eggenschwiler and Anderson, 2007). Currently there are over 100 conditions that are either known, or likely ciliopathies (Baker and Beales, 2009). When evaluating these disorders, approximately 30% of the conditions, including Oral-facial-digital syndrome, Joubert syndrome, Bardet–Biedl syndrome, Meckel–Gruber syndrome, Sensenbrenner Syndrome (Cranioectodermal dysplasia) and Ellis–van Creveld syndrome (Zaghloul and Brugmann, 2011) (Table 1), are primarily defined by their craniofacial phenotype. These craniofacial ciliopathies frequently present with some combination of craniosynostosis, micrognathia, midfacial dysplasia and/or cleft lip/palate, thus significantly impacting the development of all facial prominences (Fig. 1L). In recent years several animal models, from various species, have been identified for the study of craniofacial ciliopathies (Table 1). Perhaps one of the most exciting discoveries focused on the identification of two avian models for human craniofacial ciliopathies. In the next section we discuss these mutants and how the unique features of the avian model system have contributed to understanding the etiology of craniofacial ciliopathies.

1.3. The talpids

The *talpid* mutants (*talpid, talpid*^{2}*, talpid*^{3}) are three distinct, autosomal recessive avian mutants with the shared phenotypes of polydactyly and severe craniofacial malformations. These mutants received the name "*talpid*" due to their polydactylous phenotype, reminiscent of the forelimbs of moles, shrews, and desmans belonging to the Talpidae family. The original *talpid* mutant was identified by Randall Cole in 1942. *talpid* had severe craniofacial defects, including: shortened inferior maxilla and general retarded growth of the facial structures (Cole, 1942). Sadly, before gene identification was possible, the original *talpid* went extinct. Years later, however, two separate avian lines, existing on different continents, with similar phenotypes to *talpid* would emerge. Aptly named *talpid*^{2} (*ta*^{2}) and *talpid*^{3} (*ta*^{3}), these two lines would serve as staples in the developmental biology communities for the study of limb and craniofacial development for the next half century. In