Variation, Genetics, and Evolution of the Primate Craniofacial Complex

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14.1 Introduction

A remarkable discovery was recently announced regarding the genetic influence on the vertebrate craniofacial complex (Abzhanov et al. 2006; Campas et al. 2010). The subject of the study was the genus *Geospiza*, better known as Darwin's finches, the poster genus for evolutionary adaptation. It is well known that the beaks of the various species of these finches vary in depth, width, and length, and that the resulting shapes correspond with the ecological niche of the particular bird. In 2006, Abzhanov and colleagues described how different levels of expression of calmodulin (CaM), a calcium mediator, account for the variation in beak length (Abzhanov et al. 2006). Following previous work demonstrating that variance in beak depth and width was similarly described by levels of bone morphogenetic proteins-4 (BMP4) (Abzhanov et al. 2004), this work provides an elegant description of the genetic mechanism of morphological differentiation of craniofacial structures. While, in one sense, a beak is a discrete anatomical unit, it is also true that it is a complex of multiple hard and soft tissues with geometric properties extending beyond length, depth, and width. The significance of this work lies in the identification of the relationship between, and relative independent action of, CaM and BMP4 with respect to specific metric traits.

In contrast to the advances in avian cranial genetics, the genetic mechanisms responsible for variation of the primate craniofacial complex are still poorly understood. The current understanding of the genetic underpinnings of the primate craniofacial complex comes primarily from three sources, extrapolation from developmental studies of fish or avian animal models, analysis of dysmorphic syndromes in humans, or from the application of modern quantitative genetic approaches including genome-wide linkage analyses. In this chapter, we explore the genetic influences on primate craniofacial morphology and examine the relevance to diverse fields from evolutionary biology to biomedicine.

14.2 Primate Craniofacial Diversity

The order *Primates* is represented by roughly 400 species exhibiting great diversity in body size, locomotor habit, and environmental

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adaptation. Craniofacial trends in primate evolution have included changes in orbital morphology and orientation related to an increased emphasis on visual cues, and a relative increase in cranial capacity (Ross and Ravosa 1993; Ross 1996). Figure 14.1 provides examples of craniofacial form in two cercopithecoid primates (vervet monkey and baboon), a ceboid (spider monkey), and a prosimian (indri). Most primates exhibit a generalized mammalian cranial form, although there are interesting exceptions such as the beaver-like aye–aye (*Daubentonia*).

Some of the most dramatic evolutionary changes in primate craniofacial form are seen among the *Hominini,* the tribe including humans and their ancestors. These include significant changes in each of the craniofacial components, most notably the dramatic expansion of the brain and neurocranium, the concomitant increase in flexion of the basicranium at the pituitary fossa (the craniometric point known as sella), and a reduction in dimensions of the splanchnocranium with a resulting orthognathic disposition of the face. Figure 14.2 presents a comparison of bisected human and chimpanzee crania where these differences are readily apparent.

14.3 Background

14.3.1 Structure and Development

The skull (cranium and mandible) is a complex anatomical structure, with a developmental history that includes osteogenic precursors derived from both neural crest cells and mesoderm, and a

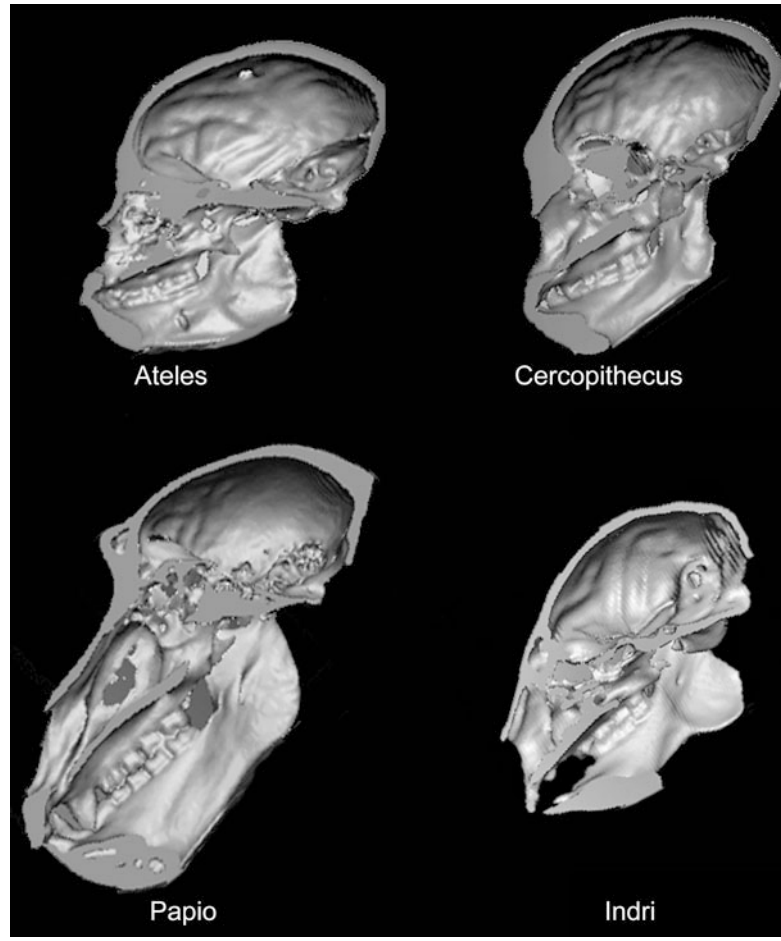

Fig. 14.1 CT reconstructions of the internal aspect of four primate taxa. All images are scaled to the distance from sella (the pituitary fossa) to nasion (the intersection of the nasal and frontal bones)

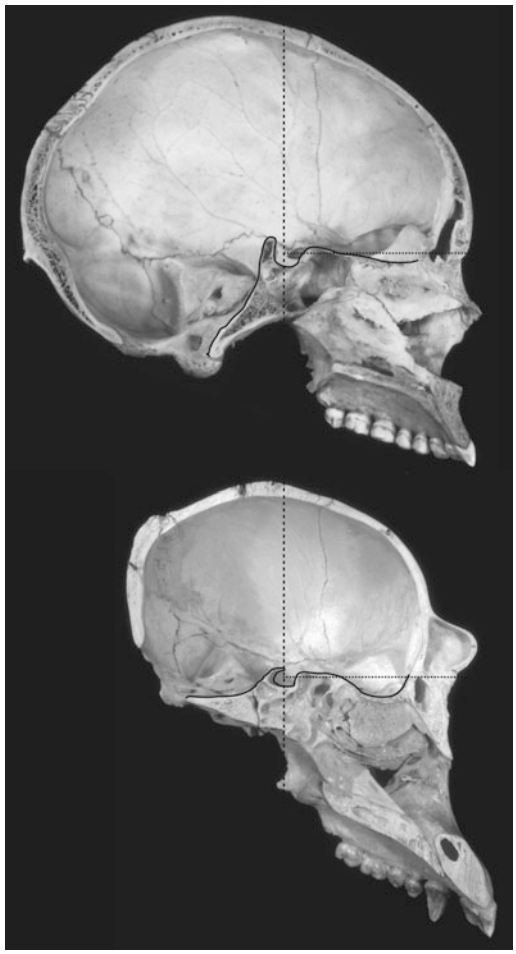

Fig. 14.2 Internal aspect of bisected human (*top*) and chimpanzee crania. Crania are aligned at sella (the pituitary fossa indicated by *vertical line*) and scaled to the distance from sella to nasion (*horizontal line*). Superior margin of basicranium is outlined in *black*

functional constituency including the housing of a diverse array of sensory and mechanical components. The craniofacial complex is frequently discussed in terms of developmental and functional components. The basicranium, which includes the sphenoid, ethmoid, and portions of the occipital and temporal bones, is phylogenetically the oldest component and is dominated by endochondral ossification during development. The neurocranium is identified as those bones surrounding the brain, such as the parietals and the squamous portions of the frontal, temporal, and occipital bones. The splanchnocranium is dominated by the zygomatics, maxillae, and mandible but also includes numerous small bones such as the nasals, the lacrimals, and the unpaired midline vomer. Of these components, the basicranium largely undergoes endochondral ossification; the neurocranium and splanchnocranium are predominately formed through intramembranous ossification, although several bones of the splanchnocranium demonstrate both forms of ossification (e.g., the mandible). It has been suggested that growth of bones derived from these two processes differs, with intramembranous bone largely governed by the surrounding mechanical environment, and endochondral bone regulated by the intrinsic genetic program within cartilaginous precursors (Enlow 1990; Lieberman et al. 2000). This suggested dichotomy, however, was problematic from the start because several cranial bones, such as the sphenoid, occipital, and mandible, utilize both forms of ossification for specific regions (Langille and Hall 1993).

The functional environment of the skull and surrounding soft tissues are also complex. Externally, the bones of the skull are subjected to biomechanical stresses imposed by nuchal, masticatory, and facial musculature and their associated tendons and fasciae. Internally, neurocranial growth has been hypothesized to be directed by brain size as well as fiber orientation of the meninges (Moss and Young 1960). In addition, the epithelium of the paranasal sinuses and the air spaces of the temporal bone may ultimately play a role in configuration of the associated bones and the distribution of mechanical strains within them (Sherwood 1999; Witmer 1997).

14.3.2 Paradigms for Genetic Research of Craniofacial Morphology

The past two decades have seen a considerable transition in the biological sciences largely as a result of the advances in genomic research. Craniofacial research, and most notably research into craniofacial anomalies, has moved from categorization of syndromes based on phenotypic patterns to the identification of specific gene mutations responsible for these syndromes.

While crude surgical approaches to the cranium and intracranial structures appear as early as 6500 BC, systematic interest in the anatomy of the craniofacial complex more likely began with the work of Herophilos (third century BC) or with the comparative anatomical approach of Galen (second century BC). The descriptive nature of anatomical observation was the dominant paradigm well into the nineteenth century, when quantitative analysis of cranial form began with the work of anatomists such as Blumenbach, Retzius, Broca, Morton, and Lombroso. This quantitative work was largely designed to describe differences between racial groups or, as with the case of the biological determinism of Lombroso, to predict potential criminal tendencies in individuals. The first studies in hereditary transmission of craniofacial features began with the pioneering work of Sir Francis Galton in 1875 (e.g., Galton 1885, 1876a, b), who was able to demonstrate heritable aspects of craniofacial form by examining sets of twins. Investigation of the growth of basicranial and intracranial structures began in 1931 with the application of the Bolton method standardizing radiographic technique allowing for consistent quantification of internal cranial structures (Broadbent 1931).

The descriptive paradigm that had dominated craniofacial research began to shift with the landmark paper of Moss and Young (1960), describing a functional approach to craniofacial biology (craniology in their terminology). This approach considered that cranial form closely reflects the functional demands of the associated hard and soft tissues and focused on the physical constraints placed upon the growing cranium. Importantly, functional craniology formalized the concept of the skull as a complex of both integrated and independent components.

As genetic methodology improved, the genetic basis for craniofacial form began to emerge as the dominant research topic. By the early 1980s Slavkin (1983) described the "genetic paradigm," as forming the basis for research into congenital defects. He defines this paradigm as recognizing the interaction between the gene and the environment in producing a phenotype. Importantly he stressed that

> not all traits that appear multiple times in the same family or pedigree are "genetic" in origin, and possible contributions from "non-genetic" factors (like mutagens, carcinogens, teratogens, nutritional status, environmental insults) must always be considered Slavkin 2001, p. 466).

Not surprisingly, with the rapid growth in genetic data, the perceived role of the environment began to diminish shortly thereafter. By the late 1990s, Moss (1997a), the father of functional craniology, was clearly concerned by the lack of consideration of nongenetic influences on craniofacial growth, identifying the "genomic thesis" as the dominant paradigm of morphogenesis. He suggested that the role of the environment was being overlooked in favor of genetic deterministic models, despite significant evidence for epigenetic/genomic interactions throughout development. Such decided shifts in thought are not uncommon following significant technological advances and, over time, there is typically a return to more synthetic approaches incorporating all available lines of evidence. This is currently evident in the increased attempts at a systems biology approach (Ideker et al. 2001a, b; Ideker and Krogan 2012), which is again advocating a more holistic approach integrating environmental, gene, and gene network data to provide a comprehensive view of the system under investigation.

The application of, and the need for, a systems biology approach to craniofacial biology was described in a recent review of gene discovery advances in craniofacial biology. Handrigan et al. (2007, p. 110) noted that current research is characterized by a piecemeal approach "focusing on one stage of development, one part of the face, or on just a few signaling pathways." The multifactorial basis of many syndromes, ranging from craniosynostosis to tooth agenesis, is becoming clear with new genetic components identified on a regular basis. Handrigan et al. note, "These manifold etiologies reflect the overriding integration and complexity of molecular regulation in craniofacial development and emphasize the need for exhaustive surveying of the involved genes and gene pathways." (Handrigan et al. 2007, p. 109–110).

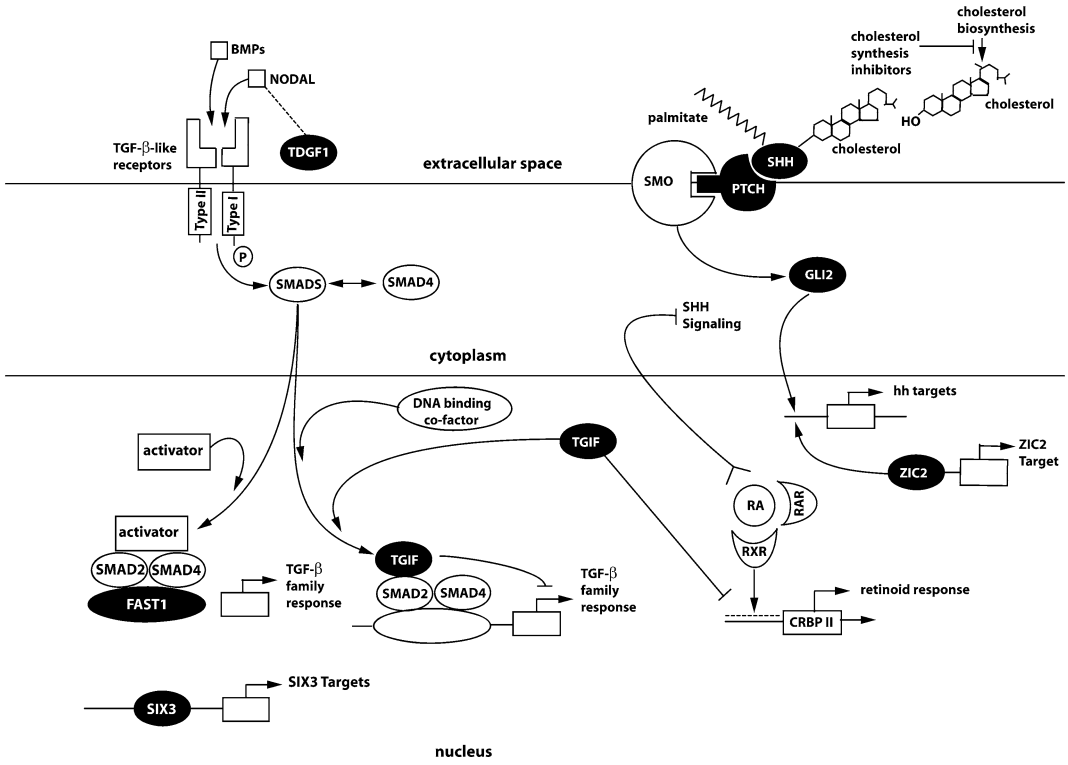

Fig. 14.3 Signaling pathway associated with Holoprosencephaly (HPE). Genes in *black* have been implicated in HPE in humans (after Ming and Muenke 2002)

The systems biology approach stresses the hierarchical nature of biological information and prioritizes the elucidation of gene networks to characterize the system under investigation. With regard to craniofacial biology, a well-developed pathway model has been developed relative to the disorder holoprosencephaly (HPE) (Fig. 14.3). This disorder had been characterized as genetically heterogeneous with at least eight genes being identified as etiologic factors. Additional research has identified the signaling pathways linking these genes, thus identifying the basis for the range of phenotypes seen and the genetic heterogeneity (Gripp et al. 2000; Ming et al. 2002; Ming and Muenke 2002; Orioli et al. 2001; Roessler et al. 2003). Identification of additional such signaling pathways and gene networks is critical for a complete understanding of normal craniofacial development and the etiology of dysmorphologies.

14.4 Genetics of the Craniofacial Complex

14.4.1 Developmental Genetics of the Craniofacial Complex

The genetic contributions to early craniofacial development have been the subject of study for many decades and significant findings are frequent. Not surprisingly, as much of craniofacial development relies upon the proper formation of the underlying skeletal substructure, many of the genes involved in craniofacial development are those that contribute to general skeletal development throughout the body. These include a number of fibroblast growth factors or their receptors (*Fgf* or *Fgfr*), bone morphogenetic proteins (*Bmp*), or signaling molecules such as sonic hedgehog (*Shh*) or the *Wnt* family (Handrigan et al. 2007;

Havens et al. 2008; Helms and Schneider 2003; Hu and Helms 1999). Craniofacial anomalies associated with mutations in these genes frequently occur alongside other skeletal anomalies. For instance, mutations in fibroblast growth factor receptors are the cause of several craniosynostotic disorders (Apert syndrome, Crouzon syndrome), which along with the craniofacial symptoms of premature suture closure, are also characterized by limb anomalies such as syndactyly. Even a relatively discrete craniofacial disorder, such as cleft lip and palate, may be part of a syndrome with multiple postcranial skeletal and soft-tissue symptoms. Phenotypes within the cleft lip and palate spectrum have been associated with *Bmp* signaling, specifically *Bmp4* deficiency, which is also linked to other alterations in facial form (particularly in mandibular morphology) and postcranial dysmorphologies such as syndactyly and polydactyly (Bonilla-Claudio et al. 2012, Murray and Schutte 2004; Naruse et al. 2007; Zhang et al. 2002).

The question then becomes, if the genes above are responsible for large-scale skeletal morphogenesis, what are the factors dictating the intricate details of craniofacial morphogenesis? Part of the answer lies in the action of these genes along spatial or temporal gradients. For instance, variation in *Bmp4* expression has been shown to correlate with variation in beak morphology in *Geospiza* as noted above, and also with differences in cichlid jaw morphology (Albertson et al. 2003; Albertson and Kocher 2006, reviewed in Helms et al. 2005) and in tooth and palate development in mice (Feng et al. 2002; Gong and Guo 2003). The other part of the answer may lie in additional, currently unknown, genes with smaller, more localized effects.

14.4.2 Genetic Heterogeneity in Dysmorphic Syndromes

When examining the current literature one cannot help but be impressed by the wealth of detailed genetic information that is rapidly becoming available for cranial disorders (e.g., Cohen 2002; Hennekam et al. 2010; Mulliken 2002). It is also clear, however, that the advances made in the genetics of craniofacial disorders do not provide unambiguous answers to questions of causation. For instance, Cohen (2002) lists at least six disorders resulting from mutations in the *FGFR3* gene, at least five disorders associated with mutations in *FGFR2*, and at least nine separate mutations associated with holoprosencephaly (Ming and Muenke 2002). In other words, disorders such as Crouzon or Pfeiffer syndromes, along with other craniosynostotic syndromes, are not distinct entities but rather variable manifestations along a continuous scale. This heterogeneity has made some researchers suggest that, instead of numerous individual distinct syndromes, there are only a handful of syndromes each with considerable variation along a continuum. This idea has largely been rejected, as syndromes do tend to present a definable set of symptoms that breed true in families. Cohen and MacLean (1999) suggest several ways to integrate phenotypic and genotypic nomenclature that are likely to become standard practice as we continue to elucidate these relationships. While their system may be a bit cumbersome (e.g., the simple Crouzon syndrome would be replaced by "Crouzon syndrome, FGFR2, Cys278Phe"), such a system may become necessary for clarity.

In discussing the problems associated with this genetic heterogeneity, Cohen (2002, p. 9) states that "other factors are involved that are not understood at the present time." There are two clear candidates for these other factors: (1) the environment; or (2) other, currently unknown, genes. Environmental insults resulting in growth perturbation or gross anatomical deformities are relatively commonly encountered in utero and range from mechanical disruptions, such as amniotic bands, to complications based on placental-cord insufficiencies, to the introduction of teratogenic substances (Cohen 1990; Cox 2004; Moss 1997b; Sherwood et al. 1992, 1997). The subtle effects of a "normal" environment (acknowledging the extreme heterogeneity of any individual's environment) on variability are less easily characterized.

The other potential confounding factor in understanding the genetics of dysmorphology is

the relationship of mutated genes with other genes. While it is readily acknowledged that complex traits are often oligogenic in nature (i.e., a few genes with pronounced and identifiable effects of varying degrees are together responsible for most of the genetic contribution to the phenotypic variance of a trait), there still persists an expectation that a given mutation will produce a singular outcome. Even if the (nongenetic) environment were held constant, this expectation would not be warranted. The cumulative pleiotropic effects of genes and gene-by-gene interactions would be expected to produce a wide range of phenotypes proportional to the number of genes involved. In other words, variability among normal genes would be expected to produce variable phenotypes when acting in concert with a mutated gene. The basic genetics underlying normal variation of the craniofacial complex are not well defined but clearly important for continued progress.

14.4.3 Animal Models for Human Craniofacial Genetics

A number of animal models have been used to explore the genetic underpinnings of craniofacial structures. Zebrafish and chicks have been used extensively to study the genetics influencing early development of important structures such as the pharyngeal arch system (Helms and Schneider 2003; Yelick et al. 1996; Yelick and Schilling 2002). Murine models have also proven important especially for understanding the genetics of the dentition and palate (Jernvall et al. 1998; Jernvall and Thesleff 2000; Miettinen et al. 1999; Vaahtokari et al. 1996). Mammalian models are important for understanding aspects of human craniofacial genetics such as the integration or modularity of the cranium (e.g., Cheverud 1995).

Nonhuman primates, given their phylogenetic proximity to humans, would serve as the best model. The craniofacial complex of nonhuman primates has been the subject of numerous anatomical studies (e.g., Hylander 1979, 1986; Ravosa et al. 2000; Ross and Hylander 2000; Ross 2001; Vinyard et al. 2003; Washburn 1947). Much of this research has been aimed at elucidating the evolutionary history of the order by understanding how craniofacial components, the basicranium, neurocranium, and splanchnocranium, are integrated in both a developmental and evolutionary sense. Within primates, a number of associations between the basicranium and other structures have been suggested. As the basicranium serves as the floor to the neurocranium, the most obvious association is between the skeletal elements of the base and the brain. Scientists have long considered brain size and the extent of basicranial flexion to be related in primates. Humans possess both a large brain (relative to body mass) and a strongly flexed cranial base (Lieberman et al. 2000). Within non-human primates, a significant correlation between relative encephalization and cranial base angle has also been demonstrated (Ross and Ravosa 1993). However, not all brain/base relationships are consistent throughout primates. For example, Lieberman et al. (2000) report significant correlations between brain stem volume and cranial base flexion in strepsirrhines (lemurs and lorises) but not in haplorhines (tarsiers, monkeys, apes, and humans).

Associations have also been suggested between basicranial and facial structures such as the orientation of the orbits and the anterior cranial base (Ravosa 1991; Ross and Ravosa 1993). Again, a difference exists in correlations between haplorhines and strepsirrhines with the former being characterized by significant correlations between orbit orientation and the anterior cranial base, most likely due to the close approximation of the orbits below the olfactory tract (Lieberman et al. 2000); McCarthy and Lieberman (2001) have also identified an integrated region they term the "facial block" defined by the superoposterior portions of the face. The facial block is said to rotate about an axis loosely defined by the greater wings of the sphenoid bone during ontogeny. In haplorhines, the orientation of the block is correlated with the cranial base angle. Strepsirrhines do not show this correlation.

14.4.4 Quantitative Genetic Studies of the Craniofacial Complex in Animals

Despite these acknowledged correlations between craniofacial components, it is not clear what elements are the primary determinants driving craniofacial variation in primates. While experimental approaches to primate craniofacial morphology are not practical, quantitative genetic techniques are proving fruitful in elucidating the genetic architecture underlying craniofacial variation. Quantitative genetic analysis of craniofacial traits has primarily focused on two families of primates: *Callitrichidae* represented by the saddle-back tamarin (*Saguinus fuscicollis*) (Cheverud 1995), and *Cercopithecidae* represented by the rhesus macaque (*Macaca mulatta*) (Cheverud and Buikstra 1981a, b, 1982; Cheverud 1982; Cheverud et al. 1990a, b; McGrath et al. 1984) and baboon (*Papio hamadryas ssp.*) (Hlusko et al. 2002; Hlusko and Mahaney 2003). These studies focused on facial, mandibular, and dental traits. Sherwood et al. (2006a, b, c, 2008c, d, 2011) broadened this perspective and included internal measures of the basicranium along with neurocranial and splanchnocranial phenotypes in the baboon.

The first step in quantitative genetic analysis of complex traits is to establish the relative genetic influence on traits. Narrow-sense heritability provides such a measure. Narrow-sense heritability is expressed as

$$h^2 = \sigma_A^2/\sigma_P^2 \qquad (14.1)$$

where σ_A^2 refers to the additive genetic variance and σ_P^2 refers to the total phenotypic variance. In a study by Cheverud (1982) of macaque facial metrics, heritability estimates, calculated using mother-offspring pairs, were moderate ($\sim$0.33). The sample available for this study was drawn from 297 positively identified individuals containing 51 mother–offspring sets with a total of 134 mother–offspring pairs. While the analysis resulted in a number of significant heritabilities, the small sample size may explain why approximately 52 % of the estimates were not significant. A similar study of craniofacial traits in tamarins found heritabilities averaging 0.37 with a range of 0.04–0.94. While the number of related individuals, 134 animals, was slightly less than in the macaque study, extended genealogies were available and the heritabilities were calculated using a maximum-likelihood approach with pedigree data. With this methodology, the number of significant heritabilities increased to 67 %. In a study of dental metrics, using the pedigreed population of baboons at the Texas Biomedical Research Institute (formerly the Southwest Foundation for Biomedical Research), Hlusko and colleagues (Hlusko et al. 2002; Hlusko and Mahaney 2003) report heritabilities ranging from 0.38 to 0.85 for dental metrics of baboons (*Papio*) with all heritabilities significant.

Genetic correlations (ρ_G) provide a means to examine the shared effects of genes on traits. As noted, a number of associations have been described for the primate craniofacial complex at the phenotypic level and these have been further explored at the genetic level using the concept of morphological integration. The concept of morphological integration was formalized in 1958 (Olson and Miller 1958) and is used to describe how the interdependent nature of traits relates to the total complex form of an organism.

In several classic papers Cheverud explored the integration of the primate cranium from phenotypic and genetic perspectives (Cheverud 1982, 1995). In an analysis of the macaque skull, 56 measures were partitioned into function sets (F-sets) based on existing research. Two primary functional matrices, neurocranial and orofacial, were identified with three and four submatrices, respectively (frontal, parietal, occipital in the neurocranial matrix, orbital, nasal, oral, and masticatory in the orofacial matrix). Theoretically, there would be a hierarchical pattern of correlations with the measures in each submatrix and matrix being more correlated than measures spanning submatrices or matrices.

Phenotypically, the expectation of a hierarchical relationship is met. That is, Cheverud reports that the average coefficient of determination (r^2) for traits within the same F-set is more than five times higher than average r^2 values

among traits from different F-sets (Cheverud 1982). The relationship was somewhat different, however, when the genetic correlations were examined. The average r^2 for traits within and among F-sets was more similar than that seen with phenotypic correlations, indicating that "F-sets are not necessarily independently evolving entities" (Cheverud 1982, p. 508). When analyzed separately there was a difference between the neurocranial and orofacial sets. Neurocranial traits showed greater correlations within submatrices than between neurocranial submatrices. In contrast, traits within orofacial submatrices tended to show roughly equivalent correlations independent of whether they were within or among other orofacial submatrices.

Using a slightly different design where traits were assigned to one of six sets (oral, nasal, cranial vault, orbit, zygomatic, cranial base), a similar study was conducted on a small New World monkey, the saddle-back tamarin (Cheverud 1995). In this study, cranial vault and oral traits showed higher average levels of genetic correlation (0.49 and 0.66, respectively) to traits within their respective sets than with traits in other sets. Nasal, orbital, cranial base, and zygomatic traits showed no tendency for higher genetic correlations within sets relative to those between sets.

14.4.4.1 Dentition

Within comparative and evolutionary anatomy, the dentition has frequently served as the focus of much research. The reasons for this are multiple. First, teeth are essential to the procurement and mastication of food, as well as for inter- and intraspecific communication. Teeth are discrete elements that are relatively easy to examine in living animals (high-resolution dental casting methods are readily available). The morphology of the teeth varies greatly within primates. Finally, teeth are among the most durable of biological structures and are, therefore, more prone to fossilization than many other elements. As a result, the dentition and jaws provide an excellent source of information regarding adaptations to a given environment and may even provide detailed information on the niche occupied by an animal or even behavioral aspects. It is true that many primate taxa are known largely, if not entirely, by dentition alone.

Primates are heterodontic animals with up to four different tooth types with each type having been described as evolving as "largely independent units" (Weiss et al. 1998, p. 369). Primitive mammalian dental formulas, seen in early primates, consisted of three incisors, one canine, four premolars, and three molars in each quadrant of the jaw. Most modern mammals have reduced the number of teeth within each jaw, with many eliminating some types (e.g., the lack of canines and premolars in rodents).

The development of the dentition is complex with precursors derived from ectoderm (ameloblasts) and neural crest cells (odontoblasts, cementum). The dentition begins development as a series of epithelial ingrowths into the subjacent ectomesenchyme. The presumptive tooth progresses through three well-characterized phases, the bud, cap, and bell stages. It is during the last of these stages, the bell stage, where substantial histo- and morphodifferentiation occurs. By late bell stage, the hard tissue components of the tooth, dentin, and enamel have begun to form and the nerve and vascular supply are beginning to develop (Ten Cate 1989). Permanent dentition begins as successional tooth germs arising from the dental lamina adjacent to the dental organ of the incisors through premolars. Permanent molars have no deciduous precursors and arise from a posterior extension of the dental lamina. Within each stage a number of genes have been identified, which, when disrupted, can result in dental agenesis (e.g., *PAX9* or *MSX1*), dentin dysgenesis (e.g., *COL1A1*, *COL1A2*), or amelogenesis imperfecta (e.g., *AMELX*, *ENAM*) (Hu and Simmer 2007).

Morphogenesis of individual teeth has been studied in the mouse and is largely directed by two signaling centers, the primary and secondary enamel knots (Jernvall et al. 1994; Jernvall and Thesleff 2000; Vaahtokari et al. 1996). The primary enamel knot develops during the transition from the bud to cap stage at the point where epithelial folding begins to define tooth shape

(Cho et al. 2007; Jernvall and Thesleff 2000). In multicusped teeth, the primary enamel knot is removed apoptotically and the secondary enamel knots appear at the site of individual cusps. As noted, the enamel knots are signaling centers and Jernvall and Thesleff (2000) have identified reiterative patterns of expression, particularly in reciprocal *FGF* signaling between the primary and secondary knots and the underlying mesenchyme.

14.4.4.2 Quantitative Genetic Studies of Primate Dentition

In an effort to elucidate the genetic architecture of primate dentition, Hlusko and colleagues have explored the quantitative genetics of dentition in the baboon (Hlusko et al. 2004a, b, 2006). In an analysis of genetic correlations among dental traits there is an expectation of hierarchical relationships similar to those discussed for cranial components. For the dentition, it is hypothesized that antimeric teeth (e.g., left and right first molars) will show a high degree of genetic correlation (with ρ_G approaching or equaling 1.00 indicating complete pleiotropy). Because of the developmental relationship, serial pairs of teeth (e.g., M_1, M_2, etc.) would also be expected to exhibit high levels of genetic correlation, followed by occluding pairs of teeth with slightly lower expectations for genetic correlations.

On examination of molar cusp patterning and cingular remnant expression, the expectations of complete pleiotropy for traits from antimeric teeth were met (Hlusko et al. 2004a; Hlusko and Mahaney 2003). Genetic correlations for cingular remnant traits also showed the expected pattern with a reduction in magnitude from antimeric pairs, to serial pairs, to occluding pairs. Molar cusp patterning showed a slight deviation from the expected patterns wherein many of the serially homologous traits in mandibular molars demonstrated genetic correlations equal to one, the same traits in serial maxillary molars demonstrate incomplete pleiotropy (genetic correlations different from one).

14.4.4.3 Current Work on the Quantitative Genetics of the Human and Nonhuman Craniofacial Complex

We have undertaken three studies designed to elucidate the genetic architecture of the craniofacial complex. Two of these studies are designed to be parallel complementary studies: one examining craniofacial structure in humans (Sherwood et al. 2005, 2008a, 2011), the other in a nonhuman primate, the baboon (Sherwood et al. 2006a, 2008b, c). The third study focuses on the dentognathic complex in humans (Duren et al. 2006, Sherwood et al. 2007). In the first studies, each craniofacial developmental component is characterized by a series of metric traits derived from lateral cephalographs, while the third study uses high-resolution dental casts.

The first study involves participants in the Fels Longitudinal Study (Roche 1992), the largest and longest running study of human growth and development. Throughout the study there has been a concentration on aspects of skeletal growth, most notably on methods of assessing skeletal maturation from hand-wrist and knee radiographs (Roche et al. 1988a, b; Roche 1989; Xi and Roche 1990). Cranial radiography of Fels Longitudinal Study participants was conducted between 1931 and 1982. In keeping with the general focus of the study, primary attention has been on growth and development of cranial components in participants. Several key papers focused on the growth of specific bones or anatomical units, for example, early work by Young (1957) on the frontal and parietal bones, or Garn and Lewis' work on the mandible (Garn et al. 1963; Lewis et al. 1982, 1985). A series of papers also detailed growth of cranial base structures (Lewis and Roche 1972; Lewis et al. 1985) including a classic paper investigating changes in basicranial flexion (i.e., saddle angle) (Lewis and Roche 1977). Significant findings from this work include the identification of subtle but distinct pubertal spurts in basicranial dimensions in both males and females. Most growth studies restrict analysis to ages 18 years

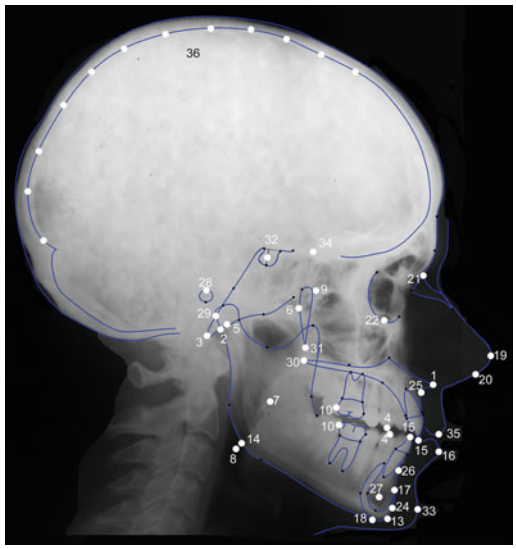

Fig. 14.4 Lateral cephalograph showing the 47 craniometric points used for measurements (For details of methodology, see Sherwood et al. 2011)

or below. Because of the unique quality of the Fels Longitudinal Study data, some studies have investigated the changes that continue throughout the lifetime (Garn et al. 1967; Lewis and Roche 1988). Both of these studies note a small, but significant, growth in skull dimensions past attainment of adulthood.

Recent work using the original craniofacial data collected from the Fels Longitudinal Study examined the genetic architecture of 80 traits based on 47 craniometric points (Fig. 14.4) derived from lateral cephalographs. All traits were significantly heritable (Table 14.1 provides data for basicranial traits as an example). Examination of genetic correlations between traits identified a subset of traits exhibiting shared genetic effects. While our initial work revolved around analysis of the original data collected by Lewis (Sherwood et al. 2008a), subsequent efforts focused on reanalysis of the entire lateral cephalographic collection have recently been published (Sherwood et al. 2011; Sherwood and McNulty 2011). This collection of numerous phenotypes drawn from standard craniometric or orthodontic analyses allows a full characterization of all components of the craniofacial complex.

The parallel study of the baboon craniofacial complex uses the pedigreed population from the Texas Biomedical Research Institute/Southwest National Primate Research Center, San Antonio, Texas. These animals are a mixture of two subspecies, *Papio hamadryas anubis* and *Papio hamadryas cynocephalus* and their hybrids. Following the protocol established in the Fels Longitudinal Cranial Study, lateral cephalographs were taken of 830 baboons. These were phenotyped in the same manner as the human cephalographs although a portion of the phenotypes do not translate onto the shape of the baboon skull; therefore, there are fewer traits measured in this sample. Recent work (Sherwood et al. 2008c, 2011) has shown that the craniofacial traits in the baboon, similar to those in the human study, are all significantly heritable.

Both the human and baboon studies successfully identified QTLs influencing variation in craniofacial traits. Ten significant QTLs were identified for human craniofacial traits (Sherwood et al. 2004, 2011), and 14 QTLs were identified for baboon craniofacial traits (Sherwood et al. 2008c). Many of the regions identified in both species contain genes known to influence craniofacial features (e.g., *SIX3, OTCS, BMP6*, or several members of the WNT family). Future work will seek to systematically interrogate the QTLs, prioritize the genes within, and conduct functional assessment of sequence variation in those candidate genes. The goal of this work is not only to identify genes with a potential to result in dysmorphologies when mutated, but to better characterize the variation in the background genetic matrix with which mutated genes interact.

14.5 Implications

As with *Geospiza*, the Darwin's finches described at the start of this chapter, the diversity of craniofacial forms across primate species raises questions of the interplay between genetic control, functional adaptation, and architectural byproducts of those processes (i.e., spandrels, Gould and Lewontin 1979). The magnitude of

Table 14.1 Heritability estimates (h^2) and standard errors for basicranial traits

Variable	N	h^2 [a]	S.E.	p	Covariates					% Var[b]
					Sex	Age	Sex × Age	Age^2	Sex × Age^2	
Sella to sphenoethmoidal junction (mm)	975	0.32	0.07	2.51E-08	●			●		7.3
Sella to posterior nasal spine (mm)	969	0.42	0.06	9.87E-17	●	●	●	●	●	31.5
Sella to nasion (mm)	974	0.45	0.06	6.49E-19	●			●		27.3
Posterior condylion to S–N (mm)	974	0.47	0.06	1.39E-18	●					4.0
Porion location (mm)	953	0.22	0.07	4.75E-05	●	●				10.9
Nasion to sella to basion (degrees)	964	0.58	0.06	4.47E-25	●					2.2
Cranial deflection (degrees)	946	0.16	0.06	5.43E-04	●	●				2.0
Basion to sella (mm)	965	0.43	0.06	1.30E-13	●					28.7
Basion to posterior nasal spine (mm)	962	0.36	0.06	2.16E-15	●	●				10.6
Basion to nasion (mm)	965	0.42	0.06	5.41E-16	●			●		31.0

Significant covariates, and the percent variance explained by those covariates, are indicated

[a] h_0: $h^2 = 0$

[b] Percent variation of trait explained by significant covariate effects

these interactions, and the effect on evolutionary trajectories, will be increasingly understood as the genetic influences on primate craniofacial variation are revealed. Clinical applications, in the form of tissue engineering and gene therapies, will benefit from detailed analysis of the genetic underpinnings for craniofacial variation in humans and in closely related animal species.

14.5.1 Evolutionary Implications

The evolutionary history of the order *Primates* is of great interest for a variety of reasons, not the least of which is that humans belong to the order. Phylogenetic reconstruction of fossil primates, and notably the *Hominoidea* (apes and humans), have relied almost exclusively on analyses of craniofacial remains. These analyses often incorporate extensive trait lists enumerating hundreds of characters that are frequently treated as independent.

Phylogenetic analyses can benefit from genetic research in three ways. First, identification of a heritable component to craniofacial morphology is necessary to demonstrate that traits have evolutionary relevance. While, to some, it may seem obvious that traits of the craniofacial complex are under genetic influence, it is important to point out that previous studies failed to identify significant heritabilities for craniofacial traits in humans and nonhuman primates. It is also reasonable to suggest that, for some traits, there are significant environmental influences (in the largest sense) that may limit the ability to detect the genetic influences on variation.

Second, characterization of the levels of integration and modularity in the cranium will help determine the levels of independence between traits used in phylogenetic analyses. Given the

complex three-dimensional architecture of the primate skull, it is difficult to imagine that changes in one structure will not be associated with concomitant changes in other structures. Phenotypic integration of the craniofacial complex has been discussed. Numerous studies have also demonstrated levels of pleiotropy between traits, including traits from different developmental components. As phylogenetic analyses of fossil remains essentially employ morphological traits as surrogate measures of underlying genetic similarity and differences, the use of genetically correlated traits may bias phylogenetic assessments by effectively reducing the genetic signal being analyzed (Sherwood et al. 2008a).

Finally, the localization of QTL and genes influencing variation in the craniofacial complex allows us to begin to identify the true traits upon which evolutionary forces act. This enables the expansion of current genetic techniques aimed at determining the timing of evolutionary events and may answer some long-standing questions within paleoanthropology, such as the rapid expansion of the hominin brain approximately 2 million years ago.

14.5.2 Biomedical Implications

Few modern scientific endeavors have enjoyed the publicity, and concomitant controversies, as has the explosion of genetic research in the past two decades. While many people are familiar with the Human Genome Project, they may not realize that genome maps for a wide variety of animals and plants ranging from beetles to pigs to the platypus are becoming available. Harold Slavkin, the former director of the National Institute of Dental and Craniofacial Research, described the potential impact of this research as including "understanding fundamental basics of diseases and disorders, targeting research to the fundamental root causes of disease processes, risk assessment for preclinical interventions, diagnostics, and tailoring treatment and therapeutics to individual risk and responses" (Slavkin 2001, p. 476). In the decade since that statement was written, a number of advances have been made into the research and application of clinically relevant genetic techniques.

The craniofacial and dentognathic complexes comprise one of the primary foci for research into areas of gene therapy and tissue engineering (Wan et al. 2006). The clinical reasons for this focus are numerous; even small craniofacial defects (whether congenital or acquired) can influence multiple aspects of physical and mental health. Additionally, for the dentition, discrete elements such as the teeth provide an easily managed object for manipulation, and the "normality" of the engineered structures is relatively easy to assess. Current approaches to regenerative medicine are examining the potential of restoring specific tissues in the pulp chamber of teeth (Murray et al. 2007; Nakashima 2005), periodontal ligaments (Jin et al. 2004; Nakahara 2006), complete teeth (Duailibi et al. 2006; Hu et al. 2006), or the supporting bone (Dunn et al. 2005; Nussenbaum and Krebsbach 2006, Rutherford et al. 2003; Young et al. 2005a, b). Gene therapy has even been investigated as a means to accelerate orthodontic treatment (Kanzaki et al. 2006). Increased characterization of the genetic architecture of the human craniofacial and dentognathic complexes will facilitate application of gene therapy and tissue engineering approaches.

14.6 Conclusions

Significant advances to understanding craniofacial biology have been made since the days of pure descriptive anatomy. Just as the formalization of functional craniology opened new avenues of research resulting in a new understanding of craniofacial form, the genomic revolution is providing new insights on a regular basis. While bird and rodent models have proven extremely valuable in elucidating developmental determinants, use of an animal in close phylogenetic proximity to humans, such as the baboon or other nonhuman primates, will become increasingly important, most notably in development of new therapeutic techniques. New approaches in quantitative genetics may prove particularly valuable in these endeavors.

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