From Phenotype to Genotype And Back Again
Du génotype au phénotype et vice versa

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Abstract Traditionally, anthropologists study evolutionary change through morphological analysis of fossils and comparative primate data. For the analysis of the genotype-phenotype continuum, the current emphasis on genes is misplaced because genes don’t make structure. Developmental processes make structure through the activity of cells that use instructions specified by genes. A critical mechanism underlying any phenotypic trait is the genetically guided change in developmental events that produce the trait. But even when a developmental mechanism is identified, the links between genetically guided instructions and phenotypic outcome are lengthy, complicated, flexible, and sensitive to physical forces of functioning organs. We use the study of craniofacial phenotypes of craniosynostosis (premature closure of sutures) to demonstrate how patterns produced by the covariation of cranial traits cannot always reveal mechanism. Next we turn to encephalization, a critical feature of human evolution that covaries with cranial phenotypes, and show how experimental approaches can be used to analyze mechanism underlying this well-documented pattern in human evolution. With the realization that no single line of evidence can explain the dramatic changes in cranial morphology that characterize human evolution come fundamental changes in the way we conduct anthropological inquiry - collaborative efforts from scientists with diverse expertise will continue to push the field forward.

Keywords Craniosynostosis · Cranial shape · Brain · Encephalization · Complex trait

Résumé Traditionnellement, les anthropologues étudient les changements évolutifs au travers d’analyses morphologiques de fossiles et de données comparatives issues de primates. Dans le cadre de l’analyse du continuum génotype-phenotype, l’accent mis actuellement sur les gènes est disproportionné car les gènes à eux seuls ne font pas les structures. Ce sont les processus développementaux qui font les structures au travers de cellules dont l’activité est dictée par les instructions provenant des gènes. Un mécanisme sous-jacent essentiel à tout trait phénotypique est la modification génétiquement guidée des événements développementaux qui produisent ce trait. Cependant, même lorsqu’un mécanisme développemental est identifié, les liens entre les instructions guidées génétiquement et les conséquences phénotypiques demeurent multiples, complexes, flexibles et sensibles à des forces mécaniques exercées par les organes en fonctionnement. Nous utilisons l’étude des phénotypes craniofaciaux liés aux craniosynostoses (fusion prématurée des sutures) pour démontrer comment les modalités produites par la covariation de différents traits craniens ne peuvent pas toujours révéler un mécanisme. Nous nous tournons ensuite vers l’encéphalisation, une caractéristique cruciale de l’évolution humaine qui covarie avec le phénotype crânien, et nous montrons comment des approches expérimentales peuvent être utilisées pour analyser les mécanismes sous-jacents de cette caractéristique bien documentée de l’évolution humaine. Une unique source de données ne peut à elle seule expliquer les changements majeurs dans la morphologie crânienne qui caractérise l’évolution humaine, par conséquent, il est important pour les chercheurs de changer fondamentalement la manière dont nous menons la recherche en anthropologie. Ce n’est qu’en renforçant les efforts collaboratifs entre des scientifiques aux expertises différentes que nous poursuivrons notre marche en avant.

Mots clés Craniosynostose · Forme crânienne · Le cerveau · Encéphalisation · Trait complexe

Abridged version

Traditionally, anthropologists study evolutionary change through morphological analysis of fossils and comparative primate data. The genetic revolution of the last century
directed the focus of biological inquiry towards genes. For the analysis of the genotype-phenotype continuum, this emphasis on genes is misplaced because genes don’t make structure. Genes make proteins that provide instructions, and cells respond to these instructions by changing size or shape, by differentiating, by proliferating, by dying, or by changing the genes to which the receiving cell responds. In short, developmental processes build phenotypes through the activity of cells that use instructions specified by genes. A domed cranial vault, highly flexed cranial base, and retracted facial skeleton are three phenotypic traits unique to modern humans that accompany the dramatic changes in cranial capacity that characterize human evolution. The evolution of these traits has been documented by observing patterns using fossil and comparative primate data and elucidated by proposed processes (e.g., spatial packing hypothesis), but the mechanism for these changes remains obscure. A critical mechanism underlying any phenotypic trait is the genetically guided change in developmental events that produce the trait, and this can only be determined by an experimental approach. But even when a developmental mechanism is identified, the links between genetically guided instructions and phenotypic outcome are lengthy, complicated, flexible, and sensitive to physical forces produced by developmental events, environmental influences, and mechanical stimulation of functioning organs. We use the study of craniofacial phenotypes of the human condition craniosynostosis (premature closure of sutures) to demonstrate how patterns produced by the covariation of cranial traits cannot always reveal mechanism. Next we turn to encephalization, a critical feature of human evolution that covaries with cranial phenotypes, and show how experimental approaches can be used to analyze mechanism underlying this well-documented pattern in human evolution. With the realization that no single line of evidence can explain the dramatic changes in cranial morphology that characterize human evolution come fundamental changes in the way we conduct anthropological inquiry – collaborative efforts from scientists with diverse expertise will continue to push the field forward.

Introduction

“If we had the complete DNA sequence and unlimited computing power, we could not compute the organism because the organism does not compute itself from genes”. Lewontin (2000).

A goal of Anthropology is to understand the ecological, behavioral, cultural, socioeconomic, and biological changes that occurred during the evolution of human beings and their living and fossil relatives. Historically, biological anthropology deals with the phenotypic aspects of human and non-human primates and depends on the analysis of fossils and of morphological variation across extant primates to provide insight into how our species evolved. Our findings are consequently limited by the nature of the fossil record and realized variation among extant species.

Biological anthropology is traditionally a descriptive, morphological science that uses quantitative studies of observed traits and their variation across populations to understand how phenotypic variation is produced. Within these confines, anthropologists have used the available phenotypic data in unique and original ways to define patterns. Typically, these patterns are then used to propose processes that might produce those patterns, their variation, and their evolution. These are necessary steps in the study of the evolution of traits but often produces a series of “after the fact” explanations that are difficult to test and do not bring us closer to identifying mechanism that connects genotype to phenotype.

Mechanism refers to a system of causally interacting components that produce an effect – in our case, the construction of a phenotype. Patterns and processes are explained by identification of mechanisms that produce the phenotype. The human genome project and the ever-increasing ability to characterize ancient DNA has turned the attention of many biological anthropologists towards identifying genetic variants that underlie complex traits. But genes are not mechanism, as genes do not make phenotypes. Genetic programs provide information to cells that trigger physiological changes that result in cells changing their behaviours. In response to genetic programs cells can, for example, differentiate, die, respond to external stimuli, divide, proliferate, coalesce, or change their ability to respond to signals - and for much of our lives these behaviours take place within a complex network of developmental processes. With input from environmental influences like mechanical forces, genes provide instructions for cells to build tissues and organs that make morphology. The connection between genotype and phenotype then, is largely accomplished by embryonic development and ontogeny [1]. Developmental processes are robust to perturbations that can interfere with normal development and flexible enough to allow adaptations to changing environments and the evolution of new forms.

The significance of determining mechanism underlying trait distribution, dispersal, variation, and evolution is clear, but mechanism rarely reveals itself naturally. Mechanism is most successfully determined through the direct analysis of testable hypotheses. Given the history of our discipline and the nature of our data sets, this has not been possible until recently. To identify and understand mechanism underlying the genotype to phenotype transition that is responsible for complex trait variation, anthropologists can borrow from other disciplines that focus on developmental processes that make morphology and produce the phenotypes that we study.
Our goal in this essay is to demonstrate how biological anthropologists might bridge the genotype-phenotype gap by testing hypotheses related to mechanisms responsible for the production, variation, and evolution of complex traits relevant to human evolution. We do this first with an example of how an experimental approach can be used to determine whether co-variation between phenotypic features is causative or correlative. After presenting this example, we discuss encephalization, a critical feature of human evolution that covaries with cranial phenotypes, and show how experimental approaches can be used to analyze mechanism underlying this well-documented morphological pattern in human evolution.

Lessons from craniofacial anomalies: genotypes and phenotypes of craniosynostosis

Craniosynostosis is a condition of complex etiology that always involves the premature fusion of one or multiple cranial sutures and can include additional anomalies of the soft and hard tissues of the head [2,3]. Cranial sutures are fibrous joints located between bones of the skull that consist of two opposing osteogenic bone fronts separated by a mass of undifferentiated mesenchymal cells that allow temporary deformation during the birth process and during trauma, inhibit bone separation for the protection of underlying soft tissues, and serve as growth sites for developing cranial bones [4,5]. In typically developing individuals, most cranial vault sutures close (ossify) post-natally. When sutures close prematurely, osseous unification of the two bones prevents growth that normally occurs perpendicular to the fused suture and redirects growth to other patent sutures, altering the global shape of the skull in predictable ways (Figure 1) [6].

The relationship between premature closure of a suture and the resulting skull shape is so strong that knowledge of which suture is closed provides enough information to predict the general shape of the skull. Furthermore, a description or picture of the cranial vault shape of a child with a prematurely closed suture provides enough information to identify the suture that was prematurely closed. This means that the covariation of two prominent phenotypes, premature closure of a specific suture and cranial vault shape, is nearly perfect. Depending upon what else is known of the phenotypes, this could signal a cause and effect relationship and be interpreted as such. The implicit assumption of this cause and effect relationship is evident in that the only available treatment is reconstructive surgery designed to open the suture and reshape the cranial vault to a more typical configuration. However, the success of these surgeries is mixed, and there are no informed methods that can be used to predict the craniofacial shape of a patient post-operatively as the child continues to grow into adolescence.

Before the identification of associated genetic mutations, the primary data sources used to understand the disease process were pedigree information and the study of phenotypes using clinical observation, anthropometrics, and clinical images. Craniosynostosis conditions were defined and patients were diagnosed (classified) according to phenotypic features. In the 1980s, craniofacial geneticists began to identify mutations causative for craniosynostosis conditions. Some of the mutations associated with craniosynostosis are located on genes that are members of a family called the fibroblast growth factor receptors (FGFRs) [7,8]. FGFRs are transmembrane molecules that extend from the cell membrane where they bind with their ligands, the fibroblast growth factors (FGFs). FGFs are secreted signaling molecules that function through activation of their receptors (FGFRs). After binding, FGFRs transduce a signal by activating pathways that are important in cell functions that are critical to many developmental processes and to the construction of tissues and morphology. However, when a mutation is present in the receptor, these activating pathways malfunction and can increase, decrease, change, or stop intracellular functions contributing to changes in cell behavior that in turn can change how tissues and morphology are constructed.

Once these mutations associated with craniosynostosis conditions were known, it became possible to insert them into experimental animals to create animal models of craniosynostosis [9] (Figure 2). Many of these mouse models mimic the gross phenotype of craniosynostosis – premature closure of the coronal suture – either bilaterally or unilaterally (see Figure 1). However, the prenatal morphology of the skulls of mice carrying specific Fgfr2 mutations (e.g., Fgfr2S252W, Fgfr2cC342Y) shows that although the coronal suture is rarely closed at embryonic day 17.5 (E17.5), the skulls of these mice are significantly different in shape and size from their unaffected littermates at this age [10,11]. What this reveals is that the skulls of mice carrying these mutations are different from their respective littermates prior to coronal suture closure. Although the prematurely closed suture undoubtedly contributes to postnatal skull dysmorphogenesis, it cannot be the cause of skull dysmorphology prior to suture closure. What other mechanism might contribute to early dysmorphogenesis of the skull?

To answer this question we further investigated the 3D morphology of embryonic cranial tissues using magnetic resonance microscopy (MRM) to visualize and quantify differences in shape between mice carrying Fgfr mutations associated with craniosynostosis and their unaffected littermates. Using landmark data collected from MRM and morphometric methods, we found that the prenatal morphology of cranial soft tissues (i.e., brain, globe of the eye) and some negative spaces (i.e., air-filled nasal passages, fluid filled cochlea and semicircular canals) were different in mice carrying these mutations relative to typically developing
littermates [10,12,13]. We reasoned that the development of these soft tissue structures and negative spaces was influenced by the Fgfr2 mutations.

Next we turned our attention to the embryonic endoskeleton because we wanted to know if these mutations affect the development of cranial cartilages. The cranial endoskeleton is composed of cartilage and consists of the chondrocranium, which supports the early developing brain and sense organs, and the pharyngeal skeleton that supports the nasal, oral, and pharyngeal structures. In all vertebrates, the cranial endoskeleton develops before the dermal bones of the skull, called the dermatocranium [14]. In experimental C57BL/6J mice, the first signs of the pharyngeal skeleton and chondrocranium are visible by E12, and the cranial endoskeleton is

Fig. 1 Three-dimensional (3D) reconstructions of computed tomography (CT) images of human infants depicting different types of single suture craniosynostoses and the shape associated with premature closure of that suture. When sutures close prematurely, growth cannot occur local to that suture, and compensatory growth occurs at other sutures resulting in predictable head shapes. A typically developing infant skull is shown at center (in yellow). (A) Premature closure of the metopic suture results in trigonocephaly; (B) premature closure of both lambdoidal sutures results in brachycephaly while (C) premature closure of either the right or the left lambdoid suture results in posterior plagiocephaly; (D) premature closure of the sagittal suture results in scaphocephaly; (E) premature closure of either the right or the left coronal suture results in anterior plagiocephaly while (F) bilateral closure of the coronal suture results in brachycephaly. Specific aspects of skull shape differentiate plagiocephaly due to unicoronal or unilambdoidal suture closure. Specific aspects of skull shape differentiate brachycephaly due to bilateral closure of either the coronal or lambdoid sutures. Views are superior (left) and inferior (right) with face toward the top and occiput toward the bottom. Figure 1 is adapted from [2] / Modèles 3D obtenus à partir d’images tomographiques (CT) de crânes humains immatures présentant différents types de craniosynostoses affectant une suture unique ainsi que la conformation du crâne associée à la fermeture de cette suture. Lorsqu’une suture se ferme de façon permanente, la croissance ne peut pas se faire localement autour de cette suture, et une croissance compensatoire se produit au niveau des autres sutures affectant la forme du crâne d’une façon prédictible. Le crâne d’un enfant se développant normalement est présenté au centre de la figure (en jaune). (A) La fermeture prématurée de la suture métopique produit une trigonocéphalie ; (B) la fermeture prématurée des 2 sutures lambdoïdes produit une brachycéphalie alors que (C) la fermeture prématurée de la suture lambdoïde droite ou gauche produit une plagiocéphalie postérieure ; (D) la fermeture prématurée de la suture sagittale produit une scaphocéphalie ; (E) la fermeture prématurée de la suture coronaire droite ou gauche produit une plagiocéphalie antérieure alors que (F) la fermeture prématurée des 2 sutures coronales produit une brachycéphalie. La forme d’un crâne plagiocéphale est différente selon qu’il s’agisse d’une plagiocéphalie due à une soudure unicoronaire ou unilambdoïdale. La forme d’un crâne brachycéphale est différente selon qu’il s’agisse d’une brachycéphalie due à une soudure bilatérale des sutures coronales ou lambdoïdes. Les crânes sont montrés en vues supérieure (gauche) et inférieure (droite) avec le massif facial orienté vers le haut et l’occiput orienté vers le bas. Figure 1 d’après [2].
fully formed by E13.5 [15]. The dermatocranium begins to mineralize at about E14.5. Some elements of the cranial endoskeleton are eventually mineralized through endochondral ossification (e.g., basioccipital bone), but large parts of the cranial endoskeleton are transient and fully replaced by dermal bone.

To quantitatively study the morphology of the chondrocranium, we have developed protocols for staining mice between embryonic day 13.5 (E13.5) and postnatal day 7 (P7) with phosphotungstic acid (PTA) [16] prior to acquiring images by micro computed tomography (μCT). μCT images of stained mice allow for the visualization, segmentation, quantification, and analysis of embryonic soft tissue structures (Figure 3) [16], enabling the quantitative study of the effects of mutations on developing hard and soft tissues. Our preliminary analyses reveal that the chondrocrania of mice carrying Fgfr2 mutations associated with craniosynostosis conditions are markedly different from the chondrocrania of normal littermates [17] indicating that the cartilaginous skull is different from normal before cranial dermal bone forms.

The traditional emphasis on covariation of the suture and cranial vault shape resulted in investigations that focused exclusively on the role of bone in the production of skull phenotypes but have not provided an explanation of how these phenotypes are produced. Analysis of non-osseous tissues during prenatal development revealed that they are also affected by mutations associated with craniosynostosis. Some of these tissues form before mineralization is initiated and persist postnatally but others are transient (e.g., chondrocranium). Regardless, their impact on skull morphogenesis cannot be realized through the study of postnatal skulls.

Premature suture closure is a complex trait that strongly co-varies with skull shape. Identification of the genetic mutations associated with the disease provided information critical to genetic counselors but did not reveal the mechanism for early shape change of the skull in craniosynostosis conditions. Without a developmental perspective our focus remains exclusively on the tissue that expresses the obvious phenotype – in this case, bone.

How is this information about craniosynostosis useful to the study of human and primate evolution?

Encephalization in human evolution: phenotypes without genotypes

Phenotypes: Studying patterns and processes

The previous example of craniosynostosis demonstrated how the analysis of postnatal phenotypes is unable to explain mechanism. This applies equally to the study of human evolution, and we provide the specific example of encephalization and its covariation with characteristics of the human skull. Encephalization is an increase in a species’ total brain size in relation to body size and has occurred throughout human evolution. From some of our earliest australopithecine ancestors to modern humans, body size has undergone a 2-fold increase, while brain size has experienced a 3.6 fold increase [18]. This makes the modern human head unique among mammals in several respects:

1) humans are a highly encephalized species and
2) humans have a distinctive suite of cranial morphological traits that are hypothesized to have resulted from encephalization.

The distinctive suite of human cranial traits include: a domed cranial vault, highly flexed cranial base, and retracted facial skeleton. Anthropologists have long hypothesized that this morphological pattern arose as a result of developmental
shifts in brain ontogeny, as well as changes in the complex interactions between the embryonic brain and skull that occurred over evolutionary time with encephalization [19–21]. Throughout the history of anthropology, the assumption has been that encephalization acted as the primary driver for the evolution and development of these cranial traits. However, while there have been significant advances in research supporting the influence of the growing brain on skull morphology, we lack a definitive mechanistic explanation. At the most basic level, we do not yet understand the fundamental physical and/or biological processes involved in evolutionary changes of the primate skull that occurred in parallel with increase in brain size.

Anthropologists have most often used the fossil record and comparative studies of extant nonhuman primates to study the relationship between changes in brain size and differing skull morphology. These limitations on encephalization datasets contribute to the disconnect between understanding the phenotype of the modern human skull and the underlying mechanism(s) that produced it. Cranial base flexion provides an instructive example (Figure 4). Foundational work examined adult specimens of extant nonhuman primate species and fossil hominins and identified modern humans as having an unusually high degree of cranial base flexion. It was hypothesized that flexion could be due in part to increasing brain size, but brain size alone could not fully explain the modern human phenotype [22,23]. In these studies, the human head was viewed as a closed box; the enclosed brain could only become larger if more room could be made within that box. Increasing cranial base flexion was put forth as one option to solve this spatial packing problem. Anthropology had identified the pattern of increasing cranial base flexion as hominin brain size increased and suggested the process of spatial packing to explain that pattern.

While these and many other studies examining the characteristic features of the modern human skull have focused on adult specimens, more recent studies of pre- and early postnatal development revealed additional patterns of growth related to encephalization and skull form. The human cranial base undergoes several distinct phases of both flexion and retroflexion during pre- and early postnatal

Fig. 3 Example of μCT reconstructions of embryonic mouse (E14.5) stained with PTA prior to scanning and visualized using Avizo 9.4 (ThermoFisher Scientific, Waltham, MA) workspace. Left image: sagittal section of mouse embryo showing the variety of tissues that can be detected and segmented by μCT. Right image: coronal section through the head of the same specimen showing the variety of cranial tissues and organs visualized by this method. Exemple d’une reconstruction à partir de micro-tomographie (μCT) d’un embryon de souris (E14.5) coloré au PTA avant la numérisation et visualisé à l’aide d’Avizo 9.4 (ThermoFisher Scientific, Waltham, MA). Gauche : section sagittale de l’embryon de souris montrant les différents tissus qui peuvent être détectés et segmentés par μCT. Droit : section coronale de la tête du même spécimen montrant les différents tissus crâniens et les organes visibles grâce à cette méthode.
development [24,25], indicating that the relationship between brain size and cranial base flexion may not be as tightly correlated as once believed. This potential conflict can more easily be resolved by taking the developmental context of the brain and skull into consideration. Cranial sutures play a critical role in skull growth and therefore contribute significantly in the production of skull morphology. By examining adult skulls which have fully closed sutures, changing the angle of cranial base flexion appears as an optimal way to increase endocranial capacity as a response to an enlarging brain. However, as the brain undergoes exponential growth during pre- and early postnatal growth, cranial sutures are patent, offering alternate spatial accommodations for the growing brain [26]. This change in perspective redirects the focus of inquiry away from postnatal morphological patterns and towards prenatal growth processes.

With a shift in focus to earlier periods of development, research has found that several of the characteristic features of the Neanderthal facial skeleton and cranial vault are present in perinatal specimens and likely the result of differential rates of prenatal growth or changes in early postnatal growth [27–31]. Studies of extant nonhuman primates provide additional evidence that modern humans have distinct prenatal cranial morphologies and growth trajectories [32–34]. While prenatal and early postnatal cranial growth and development has been understudied by anthropologists, this time period is likely critical to understanding how the growth of a larger brain might affect the developing skull.

**When genes aren’t enough: from genotype to mechanism**

Expanding the anthropological and evolutionary perspective to encompass early stages of development has already resulted in novel hypotheses regarding how encephalization may have contributed to modern human skull morphology [e.g., 26–32,35]. Yet, none of these observed patterns or proposed processes can establish mechanism. Understanding that hominins became more encephalized or that the hominin skull changed in response to a larger brain cannot provide an explanation for how one structure changed in response to another or changed in parallel. This requires yet another fundamental shift of perspective, from the observation, identification, and characterization of a phenotype to the mechanistic understanding of how that phenotype is produced by the processes directed by the underlying genotype. Funding agencies echo this shift, placing emphasis on identifying mechanism underlying the production of phenotypic variation and disease states. Mechanism is often interpreted as identifying the genetic variant or gene network responsible for the processes that result in the observed patterns, but as we found in the case of craniosynostosis, knowing the gene or the specific genetic variant associated with a phenotype does not always provide the mechanism, nor does it explain the phenotype. This is because genes don’t make structures—cells make structures using the information provided by genes [6]. In the case of encephalization, even the identification of specific genes, mutations, or genetic networks that drive increased brain size in the hominin lineage will not provide a full explanation of an encephalized phenotype.

In the most general terms, anthropologists recognize the impact of genes and environment in the production of the phenotype, but we often think of environment in terms of external environment: temperature, diet, precipitation, etc. As an example, previous studies have shown a strong correlation of midfacial morphology and extreme cold, interpreting these correlations as representative of adaptations of...
facial morphology to extreme climates [37-39], but these studies have yet to propose how these changes would come about.

In addition to the external environment, we need to consider the internal environment of growing cells and tissues. Determining the nature of the relationship between encephalization and changes in skull morphology will require an experimental approach focused on the relational principles of organismal development. Using an experimental approach, we can propose data-based generalizations that go beyond enumerating specific cases and focus instead on higher order emergent results of structure produced through interaction. We recognize the tight correspondence between brain and skull across ontogenetic and evolutionary time, and we hypothesize that this signals a consistent but labile functional and structural association of brain and skull, and that this association may be cooperative rather than causative. For example, as tissues of the head grow, mechanical properties of cells and tissues change. The changes in shape and arrangement of parts of the head are due to the way that genes make cells communicate with each other. When cells detect the signal sent by genes, they respond by changing size or shape, differentiating, dying, dividing, or changing the genes the receiving cell uses. Over time, these signals cause local areas of cells to change. They may then grow and communicate with signals to different types of cells. So that for certain contexts these signals are making cells ready for one function which might be different in another context. This approach recognizes that although bones of the skull might change in response to a changing brain, developing skull bones might also influence the growing brain [40,41], underscoring the integrative or cooperative nature of development [42].

A developmental approach involves expanding our understanding of environment to include the internal environment of the organism and requires that we bring new and unexplored data to questions of the genotype-phenotype continuum in human evolution. Any serious attempt to understand the mechanism underlying encephalization and the associated suite of cranial traits requires experimental alteration of a few key, controlled variables—approaches that are neither ethically nor experimentally possible in humans or primates. However, other vertebrate species, widely used in evolutionary developmental biology, are available to explore these questions.

Mice are a particularly valuable model organism; they have a short generation time, low maintenance requirements, and most importantly, share the same relational principles of organismal design and developmental pathways common across mammalian taxa [43–46]. It is unlikely that science will ever produce a mouse model with the genetic variants causative for human encephalization or the development of human cranial traits. However, we can produce mouse models with genotypes that provide information pertaining to developmental pathways and mechanisms similar to those that contribute to modern human brain and skull phenotypes. Just as the various FGFR mouse models have allowed examination of the mechanisms of craniosynostosis, mouse models will undoubtedly play a significant role in the continued elucidation of the evolution of the human cranial phenotype.

Studies that search for genes that co-occur with specific phenotypes have fallen short in determining how phenotypes are produced because genes do not make structures—developmental processes make structures through the organization and function of cells using instructions provided by genes. The specific genetic variants that initiated phenotypic change during human evolution may differ from those present in modern humans and are certainly different than those in a mouse model, but the participation of genetic variants in conserved developmental pathways ultimately will produce similar phenotypic outcomes valuable for our understanding of the evolutionary process.

Looking forward

Hypotheses regarding human encephalization propose that cranial morphology changed in response to an enlarging brain. This hypothesis has been effectively studied by anthropologists who have used comparative data to investigate patterns and propose processes using fossils and comparative data [18]. These studies lay a solid foundation, but to determine the mechanism for encephalization and changes in the skull that parallel encephalization, we will have to adopt new approaches. An experimental approach is one of the most powerful ways to test hypotheses about mechanism, and we suggest that the study of cells and tissues during development can inform hypotheses about complex traits that characterize human evolution. There are many tools and techniques from molecular biology, developmental biology, genetics, and other fields that can be used to determine the effects of specific genetic variants on developing tissues. Making use of these tools will require that anthropologists continue to train outside of traditional anthropology curricula and increase collaboration with scientists trained in other disciplines. We need the knowledge of scientists from other disciplines to apply these tools effectively, and they need our knowledge to link mechanism to recognized patterns and proposed processes, and in this way link mechanistic properties of developing systems to phenotypes. Without a developmental perspective our focus remains exclusively on the tissue preserved in the archeological record – in this case, bone.

Studying development will require that anthropologists employ new approaches in the study of old problems. First, analysis of single traits may not be the best way to analyze...
genotype-phenotype transitions when the genetic and environmental inputs for the construction of that trait are not understood. The complex trait of interest or “end phenotype”, may represent a secondary outcome of other primary changes, and those primary changes might be impossible to deduce by studying end phenotypes. A developmental perspective requires that one step back to address the question of exactly how a phenotype could arise and be maintained within a population. With few exceptions, the appearance of a phenotype requires changes in the developmental programs that produce that phenotype. Second, pleiotropy is real and probably underappreciated among anthropologists. Our example of FGFRs shows that genes often have many functions and that mutations associated with particular phenotypes are changing many other things about craniofacial development before the emergence of the end phenotype of interest appears [47]. The other side of this coin is that there is no single “gene for” encephalization, or “gene for” a domed vault, a flexed cranial base, or a retracted facial skeleton. These phenotypes are outcomes of complex developmental processes with genetic, environmental, and functional contributions. And finally, the crucial information that we have gained about pattern and process from the study of fossils and comparative data can be greatly enriched by the study of experimental animals. We are aware that many genes associated with complex traits have been identified, but how these traits are made remains largely unknown. This implies that complexity cannot be explained by a list of the genes involved. Identification of specific genes is less important in the study of the evolution of phenotypes because genes change over evolutionary time. However, conserved developmental pathways instructed by genetic networks will most commonly produce similar phenotypic outcomes across species making the study of experimental animals vital to the understanding of human evolution.

Conflict of interest: The authors do not have any conflict of interest to declare.

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