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Flat bones and sutures formation in the human cranial vault during prenatal development and infancy: A computational model

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HIGHLIGHTS

- We developed a mathematical model of flat bones and sutures morphogenesis.
- We employ a system of reaction diffusion equations that controls bone remodeling.
- The model predicts flat bone formation and growth from ossification centers.
- The results show how sutures form and develop interdigitated patterns.

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ABSTRACT

The processes of flat bones growth, sutures formation and interdigitation in the human calvaria are controlled by a complex interaction between genetic, biochemical and environmental factors that regulate bone formation and resorption during prenatal development and infancy. Despite previous experimental evidence accounting for the role of the main biochemical factors acting on these processes, the underlying mechanisms controlling them are still unknown. Therefore, we propose a mathematical model of the processes of flat bone and suture formation, taking into account several biological events. First, we model the growth of the flat bones and the formation of sutures and fontanels as a reaction diffusion system between two proteins: TGF-β2 and TGF-β3. The former is expressed by osteoblasts and allows adjacent mesenchymal cells differentiation on the bone fronts of each flat bone. The latter is expressed by mesenchymal cells at the sutures and inhibits their differentiation into osteoblasts at the bone fronts. Suture interdigitation is modelled using a system of reaction diffusion equations that develops spatio-temporal patterns of bone formation and resorption by means of two molecules (Wnt and Sclerostin) which control mesenchymal cells differentiation into osteoblasts at these sites. The results of the computer simulations predict flat bone growth from ossification centers, sutures and fontanels formation as well as bone formation and resorption events along the sutures, giving rise to interdigitated patterns. These stages were modelled and solved by the finite elements method. The simulation results agree with the morphological characteristics of calvarial bones and sutures throughout human prenatal development and infancy.

1. Introduction

The flat bones that make up the human cranial vault (frontal, parietal, temporal and occipital) begin their formation between eighth and ninth week of gestation, growing from ossification centers through intramembranous ossification (Shapiro and Robinson, 1980). In this process, mesenchymal cells located inside the fibrous connective tissue membrane, covering the brain, proliferate and differentiate into osteoblasts, which synthesize osteoid, the organic portion of bone. Mineralization of osteoid will result in new bone tissue (Bronner et al., 2010). The continuous growth of the flat bones of the calvaria ensures a normal morphology of the head and allows a rapid expansion of the brain (Pattisapu et al., 2010), which increases its size at high speed during embryonic development and reaches 80% of its final volume in adulthood after two years of life (Pattisapu et al., 2010).

At the end of the embryonic stage, the ossification fronts of the flat bones of the calvaria are separated by non-ossified tissue barriers, known as sutures and fontanels (see Fig. 1**a**). The former

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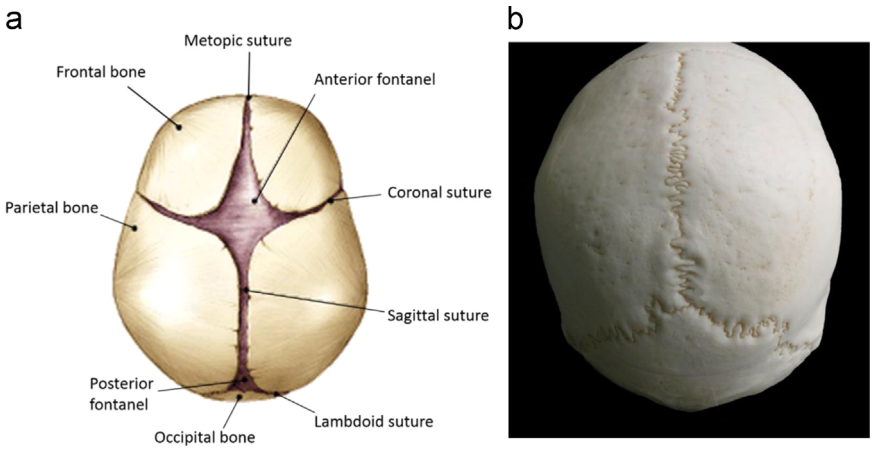

Fig. 1. (a) Coronal view of the neonatal calvaria. Modified from Kiesler and Ricer (2003). (b) Coronal view of an adult calvaria with lambdoid and sagittal sutures showing interdigitations. Modified from Week 121: Skull, the temporal region, (n.d.).

are joints composed by bands of fibrous connective tissue that unite the ossifications fronts of the flat bones, and include: coronal sutures (space between the two frontal and parietal bones), lambdoid sutures (between the two parietal and the occipital bones), metopic sutures (between the frontal bones) and sagittal sutures (between the parietal bones) (Raam et al., 2010). In addition, the sutures serve as the main sites of bone formation in the skull (Raam et al., 2010). Therefore, the overall shape of it is determined by the processes of bone formation along the suture margins (Ogle et al., 2004). Fontanels are membranous sites in the developing cranial vault that haven't ossified yet and work as high deformation areas where the brain can expand. They consist of the anterior fontanel (diamond-shape space located between the two frontal and two parietal bones at the junction of the coronal, sagittal and metopic suture) and the posterior fontanel (triangle-shaped space between the two parietal bones and the occipital bone at the junction of the sagittal and lambdoid suture).

As postnatal development progresses, the cranial sutures exhibit morphological changes, going from straight lines to an interdigitated pattern, with a corresponding increase in suture length (Rice, 2008) (see Fig. 1**b**). It is considered that interdigitation arises from a continuous interplay between bone formation and resorption events taking place at the sutures convexities and concavities, respectively (Byron, 2006). The bone formation processes, at the bone fronts of the flat bones, progressively decrease the width of the sutures, until these fully ossify. For the metopic suture, suture fusion is usually completed before nine months of age (Vu et al., 2001), while coronal, sagittal and lambdoid sutures will fuse around the third decade of life (Kumar et al., 2012).

Numerous studies have focused on determining the mechanisms underlying the processes of bone growth and suture formation and interdigitation. In general, it is believed that a complex interaction among different genetic, biochemical and environmental factors exists, where local spatio-temporal variations in both cellular signaling and mechanotransduction mechanisms might play a crucial role (Ogle et al., 2004; Alaqeel et al., 2006; Enlow, 1986; Greenwald et al., 2000; Herring, 2008; Herring and Teng, 2000; Opperman, 2000). Several *in vitro* and *in vivo* studies have tried to establish the role of the main molecular factors acting during these developmental processes. Amongst them, regional variations in the concentrations of transforming growth factor beta three (TGF-β3) and transforming growth factor beta two (TGF-β2) have been found between patent and prematurely fused sutures (Roth et al., 1997; Opperman et al., 2002a, 1999, 2002b, 2000), implying an osteoinhibitory role for TGF-β3 and an osteoinductive role for TGF-β2 during suture formation and maintenance. These findings are in concordance with a previous hypothesis from Opperman (2000), which suggest that suture phenotypic maintenance is dependent on the spatial concentrations of both osteogenic inhibitors and promoters coming from the endocranium, a membrane which is part of the dura mater and is in contact with the skull. The subsequent interdigitation of sutures during infancy has been related to linked bone formation and resorption events along their length controlled by osteoblast and osteoclast function (Byron, 2006). Recently, the Wnt family of glycoproteins, expressed predominantly by osteocytes, have been associated to bone homeostasis, where its canonical pathway, the Wnt/-β Catenin signaling pathway, has been experimentally shown to regulate mesenchymal cells differentiation into osteoblasts at the bone fronts (Kramer et al., 2010; Issack et al., 2008; Bonewald and Johnson, 2008). On the other hand, Sclerostin, a protein also secreted by osteocytes, have been shown to inhibit bone formation by antagonizing the Wnt/beta-catenin signaling pathway (Poole et al., 2005; Lin et al., 2009; Ten et al., 2008). In turn, resorption events have been associated with the concentration of receptor activator of nuclear factor kappa-B ligand (RANKL), a protein required for osteoclast differentiation, shown to be expressed by both osteocytes alone and active osteoblasts through the Wnt/beta-catenin signaling pathway (Beederman et al., 2014; Karsenty and Wagner, 2002; Glass et al., 2005; Nakashima et al., 2011; Xiong et al., 2012).

However, despite previous experimental evidence accounting for the role of different biochemical factors on the processes of suture formation and interdigitation, the underlying biological mechanisms controlling these processes are still unknown. Additionally, the intrinsic difficulty of live experimentation has hindered the quantification of the way these molecules interact and regulate bone growth along the calvaria. Hence, no consensus exists about the ways sutures are formed during prenatal development and change their morphology during infancy.

As a result, the use of computational techniques has emerged as an alternative to conventional experimentation, resulting in the development of mathematical models and computer simulations focused on establishing the biological mechanisms driving flat bone formation and suture formation and interdigitation. Using a biochemical framework, Garzón-Alvarado et al. (2013) (Garzón-Alvarado, 2013) formulated a computational model of the process of flat bone formation and growth during embryonic development using a system of reaction diffusion equations between BMP2 and Noggin. The model simulated the appearance of the primary ossification centers of each of the cranial bones, which were regulated by spatio-temporal patterns developed from a Turing