Molecular genetics of craniosynostosis

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Abstract. Tight regulation process and complex interplay occur along the osteogenic interfaces of the cranial sutures in normal growth and development of the skull. Cranial sutures serve as sites of bone growth while maintaining a state of patency to accommodate the developing brain. Cranial sutures are fibro-cellular structures that separate the rigid plates of the skull bones. Premature fusion of one or more cranial sutures leads to a condition known as craniosynostosis. Craniosynostosis is one of the most common craniofacial anomalies with a prevalence of 1 in 2,500 newborns. Several genes have been identified in the pathogenesis of craniosynostosis. Molecular signaling events and the intracellular signal transduction pathways implicated in the suture pathobiology will provide a useful approach for therapeutic targeting.

Keywords: cranial sutures, cranial development, signalling mechanism, sutures fusion,

1. Introduction
Tight regulation process and complex interplay occur along the osteogenic interfaces of the cranial sutures in normal growth and development. Cranial sutures serve as the center for the growth of bones of the skull. The signaling pathway and transcription factors that control the formation of cranial sutures have been identified [1]. The union of one or more cranial sutures before the proper timing is known as craniosynostosis.

Craniosynostosis is occurring in 1 in 2,000 – 2,500 birth in the general population. Classification of craniosynostosis may be based on suture involvement, such as single suture synostosis or multiple suture synostosis. Studies in the last decades have revealed several genes related to the mechanism of suture patency, such as FGFR2, FGFR3, and TWIST1. Here, we will give a brief review of the normal cranial development and further elaboration of craniosynostosis. Molecular biology aspect will be the focus of this review [2].

2. Normal cranial development
Growth may be defined as an increase in size by natural development through cellular proliferation and differentiation. Craniofacial growth is a complex process involving many interactions between different bones that make up the skull and between the hard and soft tissues. The processes that control craniofacial growth are not fully understood and are an area of extremely active research globally [3].
As this review will be focused on craniosynostosis problem, general bone growth mechanisms, the anatomy of the skull and normal growth of the cranial vault (which relates to the synostosis problem) in particular will be discussed briefly.

2.1. Anatomy of the Cranial Vault

The human skull is formed by two main parts, including the neurocranium and the viscerocranium. The neurocranium is the structures that enclose and protect the brain and sensory organs. The viscerocranium, on the other hand, includes the bones of the face and the palatal, temporal, pharyngeal, and auditory bones. Focus in this review would be around the neurocranium. The neurocranium comprises of five bones – a pair of frontal bones, a pair of parietal bones, and occipital bone that are connected by cranial sutures [4].

Cranial sutures are fibro-cellular structures that separate the rigid plates of the skull bones. Later on, sutures would be bridged by a dense network of collagen fibers and become increasingly interdigitated to unite the bone fronts [5]. The cranial sutures include metopic suture, coronal suture, sagittal suture and lambdoid suture. The metopic suture connects the frontal bones, the pair of parietal bones is connected by sagittal suture, coronal suture connects the frontal bones and parietal bones, and lastly, lambdoid suture connects the parietal and supraoccipital bones [2].

Ossification of the cranial vault primarily takes place via intramembranous ossification in which the mesenchymal cells, situated between the mesenchyme and dura mater, will undergo differentiation into osteoblasts. Osteoblasts would then form bone through secretion of an osteoid matrix. The mesenchymal cells in the middle of the suture remain undifferentiated during cranial vault development despite ossification of the cranial vault [2,4].

![Cranial sutures](source: Johnson D, Wilkie AOM, 2011) [6].

The principal function of cranial sutures is to allow the growth of the skull housing the rapidly developing brain, particularly during fetal and infant life. Also, cranial sutures assist in allowing distortion of the skull during delivery, absorb mastication load, and serve as absorbers of shock exerted by external forces [7]. Thus, suture patency is undeniably critical for normal craniofacial development.

2.2. Timing of Craniofacial Sutures Fusion

The earliest suture to fuse is usually metopic suture, closing at three to nine months of age. Coronal suture, sagittal suture, and lambdoid suture fuse later in the third decade of life. Before the fusing of the suture, the equilibrium of sutural elasticity, calvarial osteogenesis, and the development of the brain maintains normal growth of the skull. Intrasutural mesenchymal tissue disruption by genetic mutation and environmental factors might initiate early union of one or more cranial sutures, causing substantial functional damage [8].
3. Craniosynostosis

Normal cranial and facial development is dependent on growth adjustment at the sutures as a response to the growth of the brain and facial soft tissue. Cranial sutures normally close when the children reach 2 to 3 years of age. Synostosis is the term used to describe the abnormal condition where cranial sutures close early in life. Thus, craniosynostosis is described as the fusion of one or more of the cranial sutures earlier than the usual expected time during fetal development. The premature fusion causes a lack of growth to the bones perpendicular to the fused suture. Conversely, as compensation for the lack of bone growth near the fused suture and developing brain, the bones at the non-fused sutures undergo compensatory overgrowth. This compensatory overgrowth causes a progressive distortion in the skull shape. Many complications might be associated with craniosynostosis because of the elevation in intracranial pressure, including sensory, respiratory, and neurological function, making craniosynostosis important to detect and treat [6,7,9].

3.1. Classification and Clinical Features

Classification of craniosynostosis may be based on suture involvement, such as single suture synostosis or multiple suture synostosis. Single suture synostosis can be further classified according to the specific suture involvement, such as sagittal synostosis, coronal synostosis, metopic synostosis, and lambdoid synostosis. The sagittal synostosis is also called scaphocephaly, derived from the Greek word scaphos for boat and cephalos for head. Coronal synostosis can occur on one side of the skull or both sides of the skull. Bilateral coronal synostosis caused a shortening of anteroposterior dimension and thus lead to a brachycephalic deformity. Metopic synostosis is connected to trigonocephaly in which compensatory growth take place at the posterior portion of the head. Lastly, lambdoid synostosis cause flattening of the occipital bone and contralateral growth in the parietal bone [10].

![Classification of cranial synostosis according to involved suture](Source: Senarath-Yapa K, Chung MT, McArdle A, et al., 2012) [10].
3.2. Epidemiology
Craniosynostosis is occurring in 1 in 2,000 – 2,500 birth in the general population. Out of the reported cases, 25% cases are syndromic craniosynostosis. The remaining 75% account for nonsyndromic cases, that can be further subdivided according to the cranial suture involvement. Sagittal craniosynostosis is the most commonly seen type of nonsyndromic craniosynostosis, comprise of 40 – 50% of nonsyndromic cases, occurring in 1 in 5,000 births. Coronal synostosis occurred in 20 – 25% cases or 1 in every 1,000 births. Metopic synostosis and lambdoid synostosis account for 14% cases (1 in 15,000 births) and 3 – 5% cases (1 in 33,000 births) of nonsyndromic cases respectively [11].

In case of syndromic craniosynostosis, the most common syndromes are Pfeiffer syndrome affecting one every 25,000 births, Crouzon syndrome in about one every 100,000 births, and Apert syndrome in respective manner.

According to a retrospective case report by Anderson et al. in 2014, multiple sutures in nonsyndromic craniosynostosis is more prevalent in Asian community than in the white community. On the other hand, sagittal synostosis is significantly less common in Asian community than white community [12].

The prevalence rate of craniosynostosis in Indonesia is yet to be identified by the time this review was made. However, there is a first clinical and molecular report of Indonesian patients conveyed by Mundhofir et al. in 2013, published in Singapore Medical Journal [13].

3.3. Molecular Genetics of Craniosynostosis
Wilkie et al. (2010) did a 10-year prospective cohort of craniosynostosis on 326 children with craniosynostosis to identify the prevalence of single genes and chromosomal abnormalities associated with craniosynostosis. After a series of genetic analysis, those subjects are arranged into three genetic diagnosis categories: proven genetic, syndromic, and nonsyndromic. The proven genetic cases can be seen in 21% of the subjects with the major contribution of 86% are single gene disorders and the other 15% are chromosomal abnormalities (one patient had both). Mutations in FGFR2 are the most frequent cases account for 32% out of all the genetic cases, 25% of cases are FGFR3 mutations, followed by 19% account for TWIST1 mutations and 7% of EFNB1 mutations respectively [7,14].

Regulation of the formation and patency of cranial sutures by signaling pathways and transcription factors have been identified. The documentation of genes involved mostly is known by identification of mutagenic genes in syndromic forms of craniosynostosis [1]. Signaling molecules determined the fate of cells. The signaling molecules are chemical substances including hormones and neurotransmitters. These molecules are synthesized and secreted by cells to promote extracellular communication with other cells. The complex morphogenetic processes are arranged by FGF, TGF-β, BMP, and Wnt signaling pathway that respond to extracellular factors and environmental signals. Initiation of molecular events and activation of transcription factors take place when signaling molecules bind to their respective surface receptors. Cell behavior is highly dependent on the binding of transcription factors that would either express or repress the related genes [15,16].

Fibroblast Growth Factors (FGFs) belong to the family of polypeptides controlling several cellular processes. FGF signaling has an important role in prenatal and postnatal skeletal development. FGFs role in chondrogenesis and osteoblastogenesis control is through recruitment of the cells in the osteoblast lineage [17]. FGFs bind to high-affinity fibroblast growth factor receptors (FGFRs) that cause FGFR dimerization. These events lead to autophosphorylation on tyrosine residues initiating activation of transduction pathways as the signal transduction cascade is ligand-dependent. The activation of targets, later on, will transmit the signals to the nucleus in which cell differentiation or proliferation took place [4]. FGFRS comprised of 4 receptors of tyrosine kinase. The FGFRs structure consists of a tyrosine kinase domain, a hydrophobic transmembrane domain, and three immunoglobulin-like repeats (D1 – D3) of the extracellular domain [4,18]. Mutations either in FGF or their receptors (FGFR) may cause early
sutural fusion, hence craniosynostosis, which indicates FGF signaling taking an active part in bone formation, including osteogenesis and chondrogenesis.

When FGFs bind to FGFRs, the intracellular receptor kinase comes into proximity. Hence phosphorylation and activation of the kinases occur. The activated kinase then activate intracellular substrates, such as phospholipase Cγ1 (PLCγ1) and FGFR substrate 2a (FRS2a). The activated FRS2a substrate causes an initiation of the RAS-MAPK pathway. The RAS-MAPK pathway affects cellular proliferation and differentiation. The phosphorylated extracellular signal-regulated kinase 1/2 (ERK 1/2) enter the nucleus and interact with transcription factors ERF, which is then exported from the nucleus. In the nucleus, ERF binds to RUNX2 and repress RUNX2 expression [4,17]. RUNX2 gene is essential for maturation of osteoblast.

FGFR mutations disturb the affinity of ligand binding site that affects the receptor activity, either decreasing the receptor specificity or increasing the receptor activity. In case of craniosynostosis, mutations of FGFRs result in overexpression of FGFRs thus trigger overproduction of ERK1/2. As a result, the number of ERF exported increased, and intranucleus ERF is not enough to suppress RUNX2 expression, causing osteogenesis to occur, hence, premature fusion of the suture (Figure 4) [4,17,19].

Several point mutations in FGFRs have been known and related to craniofacial malformations. Mutations in FGFR1 (Pro252Arg) are related to Pfeiffer syndrome. Mutations in FGFR2 (Ser252Trp and Pro253Arg) are linked to Apert, Pfeiffer, and Crouzon syndrome cases. Mutations in FGFR3 (Pro250Arg) are related to Muenke syndrome [4,13,18].

![Figure 3. Common point mutations in FGFR gene related to syndromic craniosynostosis](Source: Muenke M, Wilkie A.O)

**TGF-β / BMP Signaling**

TGF-β family proteins consist of TGF-βs, BMPs, activins, and growth factors act through receptor kinases. TGF-β signaling is correlated with ERK1/2. Bone Morphogenic Proteins (BMPs) bind to type I/II transmembrane receptors. The activated receptors will cause signal initiation by phosphorylation of target proteins – Smad1, Smad5, Smad8 and Smad4 where Smad4 enter the nucleus and interact with RUNX2 and MSX2 gene expression. Overexpression of BMP signaling cause increased expression of RUNX2, thus lead to fusion of cranial suture. BMP signaling is regulated by extracellular modifiers such as noggin and glypicans. Noggin is BMP antagonist. Thus, overexpression of noggin relates to prevention of sutural fusion [4,16].
**Wnt Signaling**

The wingless-type integration sites (Wnts) molecules act through FZD (Frizzled 1–10) receptors to initiate intracellular signals and to trigger the accumulation of β−catenin that rule a variety of cell actions. β−catenin enter the nuclei and binds to T cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors. Several reports have been made in relation to the importance of Wnt signaling for the proliferation of the neural crest mesenchyme. β−catenin is important in determining the differentiation of neural crest mesenchyme to osteoblast or chondrocytes [4,14].

![Diagram of Wnt signaling](image)

**Figure 4.** Summary of signal transduction pathways in cranial development including FGF signaling, TGF-β/BMP signaling, and Wnt signaling (Source: Katsianou MA, Adamopoulos C, 2016) [4].

### 3.4. Epigenetics of craniosynostosis

Nowadays, the term epigenetics referred to the study of changes in heritable gene function without changes in the sequence of the DNA. Environmental factors might bring an effect to epigenetic factors such as histone modification, DNA methylation, and post-transcriptional silencing by RNA interference. Epigenetics processes might cause gene expression or gene repression [20].

Dudacovic et al. (2015) conduct a study focusing on expression profiles of epigenetic regulators that influence osteogenesis. Molecular analysis revealed suppression of Enhancer of Zeste Homolog 2 (EZH2) during differentiation stage of adipose – tissue derived mesenchymal cells. EZH2 gene is located on the long arm (q) of chromosome 7, region 3, band 6, sub-band 1 (7q36.1). EZH2 gene is responsible for making histone methyltransferase. Histone methyltransferase modify structural proteins that bind to DNA and shape chromosomes, called histone [21].
Figure 5. Location of EZH2 gene – 7q36.1 (Source: https://ghr.nlm.nih.gov/gene/EZH2)

EZH2 catalyzes tri-methylation of histone 3 lysine 27 (H3K27me3) that stop differentiation of osteogenic cells. EZH2 mediated H3K27 methylation assists chromatin condensation and heterochromatin formation. Heterochromatin limits the target DNA transcription. mRNA sequence analysis reveals that EZH2 gene inhibition encourages the production of bone extracellular matrix protein needed for a mineralized matrix. The study concluded that depletion of functional EZH2 in the mesenchyme leads to craniosynostosis [21].

MicroRNAs (miRNAs) are part of the non-coding RNAs, serve as regulators of gene expression after transcription process. MicroRNAs are involved in biological processes including cell differentiation, cell proliferation, and metabolism. FGF signaling pathway can be regulated by microRNAs. Loss of miRNAs regulation might cause disease progression. Regulation of FGF or FGFR expression by microRNAs can directly affect differentiation of a cell. For example, miR-338 in osteoblastic cells was found to unswervingly affect the regulation of 3' untranslated region (UTR) of FGFR2 that result in suppression of FGFR2 expression. Down regulation of miR-338 cause an increase in FGFR2 expression thus enhancing osteoblast differentiation [22].

3.5. Orthodontic perspective of craniosynostosis management

Craniosynostosis should be managed by a multispecialty team providing interdisciplinary care because of the complexity of the case. The interdisciplinary team comprises of professionals from several disciplines including: anesthesiologist, craniofacial surgeon, genetician, neurosurgeon, ophthalmologist, oral and maxillofacial surgeon, orthodontist, otolaryngologist, pediatricians, pedodontist, prosthodontist, psychologist, radiologist, and speech and language therapist [23].

Dental care for the patients with craniosynostosis yields special care because they have unique oral health and craniofacial growth problems. Common problems including malformed or missing teeth, delayed and ectopic eruptions, crowding, carries, and difficulties on performing oral hygiene procedure due to lack of motivation and limited physical ability. Orthodontic care facilitates better oral hygiene procedures by creating well-aligned and coordinated dental arches. Orthodontist serves as manager of growth data that plan the timing of jaw reconstruction and facial surgery [23].

The orthodontist might need to make dental casts, serial photographs, dental and cephalometric radiographs. Hand wrist radiographs, in some patients, might be needed to plan the interventions. The active role of the orthodontist in preparing the dentition at each stage is required, including: prediction tracings for surgery and provides treatment as indicated. The following table summarizes the dental, orthodontic, and surgical interventions according to child’s age:

| Table 1. Summary of dental, orthodontic, and surgical interventions [23]. |
|-----------------------------|-----------------------------|
| Age                        | Intervention                                      |
| Infancy onward             | Pedodontist provides oral hygiene instruction, prophylactic and restorative treatment as needed |
|                            | Orthodontist takes serial photographs and radiographs as needed at regular intervals. Growth monitoring and coordination with pedodontist regarding dental extractions and with surgeons regarding midface and orthognathic procedures. |
| 7 – 12 years               | Phase 1 orthodontic treatment, including extractions |
|                            | Midface advancement planning                      |
4. Conclusions

Craniosynostosis, the premature suture fusion of the skull, can be classified in many ways. It can involve one or more sutures. It can also be isolated nonsyndromic without other medical complications or syndromic if there are any other medical conditions involved. Studies in molecular biology of the cell suggest that a correct balance between cellular proliferation and differentiation is the key factor in the development of the skull. Regulation of cranial suture formation and patency by signaling pathways and transcription factors have been identified. The complex morphogenetic processes are arranged by FGF, TGF-β, BMP, and Wnt signaling pathway that respond to extracellular factors and environmental signals.

Many complications might be associated with craniosynostosis because of the increase in intracranial pressure, including sensory, respiratory, and neurological function, making craniosynostosis important to detect and treat. Craniosynostosis management involves a multidisciplinary team including professionals from many disciplines. Among them are a craniofacial surgeon, geneticist and orthodontist. Orthodontist’s knowledge of craniofacial growth and the nature of the various craniosynostosis conditions is valuable in the timing and planning of jaw and facial surgery.

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