

Pathogenesis and Morphogenesis of Craniofacial Developmental Anomalies

G H Sperber,*^{MS, PhD, FICD}

Abstract

A condensed review of the morphogenetic mechanisms of facial fabrication, providing insights into developmental anomalies of clinical concern. Genetics, epigenetics and regulation of molecular, cellular, tissue and organ formation are being elucidated in normal and dysmorphological patterns. The pathogenesis of craniosynostosis is related to fibroblast growth factor expression. Analysis of a 28-month-old child's dysmorphic synostotic skull explores possible mechanisms of malformation and deformation.

Ann Acad Med Singapore 1999; 28:708-13

Key words: *Dysmorphology, Epigenesis, Genetics, Morphogenesis, Teratology*

Introduction

*"...find out the cause of this effect,
or rather say, the cause of this defect,
For this effect defective comes by cause."*

Hamlet, Act II, Scene II

The current explosion of molecular biology encompassing genetics, morphogenesis, teratogenesis and pharmacogenomics for targeted drug therapy is about to have a profound impact upon the practice of medicine in the next century. A new discipline of molecular medicine is becoming a feature of clinical practice.¹ The diagnosis, prognosis, treatment and prevention of disorders will become increasingly based upon the revelations of molecular biology that are expanding exponentially our understanding of the development and maldevelopment of the human organism.

The concomitant coalescence of the current cogent cornucopia of cognitive diagnostic capabilities created by chemicals, immunohistochemistry, computers and clinical conceptualisations of cephalogenesis provides the possibility for prevention and cures for craniofacial maldevelopment. The central theme of dysmorphic patients, around whom rotate therapists and researchers, provides interactive feedback for insights into anomalies (Fig. 1). Advances in clinical practice are becoming increasingly dependent upon discoveries revealed in the basic sciences of biochemistry, genetics, embryology, molecular biology and teratology. A flood of new insights accompany the application of these fields to the aetiology, pathogenesis and therapy of disease proc-

esses. Diseases and maldevelopment have spurred enquiry into their causes that, in turn, have vaulted formerly esoteric academic disciplines into the forefront of modern clinical practice. Witness how the painstaking enquiries into genetics and genealogy now demands karyotyping of all malformations. Discoveries of the mechanisms of morphogenetic activities of cytokines help explain the aberrations of teratogenesis and provides possible chemotherapeutic tools for their cure.

The enormous technological advances in gene identification, electron-, scanning-, transmission-, fluorescence-microscopy, and confocal laser-microscopy, immunohistochemistry, roentgen cephalometry, ultrasonography, CAT scanning, nuclear magnetic resonance imaging, 3-D computer stereology^{2,3} polymerase chain reaction (PCR) technology and experimental cloning have revolutionised the potential for prevention, control and treatment of disorders of development as never before. The revelations of the human genome project, whereby the entire human genetic lexicon encoded in some 100,000 genes determines the basic patterns of development will enable identification of disablement-producing anomalies and possible manipulation for their treatment. The boundaries of surgical intervention are being ever-expanded with new technologies of intra-uterine repair,⁵ distraction osteogenesis^{6,7} and growth factor acceleration of healing.⁸ The heroic therapeutic intervention of formerly lethal disorders by these new technologies pose an ever-increasing clinical challenge for the survival of afflicted infants. The delineation of dysmorphology syndromes, previously based on ge-

* Professor

Department of Dentistry, Faculty of Medicine and Dentistry
University of Alberta, Edmonton, Canada

Address for Reprints: Professor G H Sperber, Department of Dentistry, Faculty of Medicine and Dentistry University of Alberta, Edmonton,
AB T6G 2N8 Canada.

E-mail: gsperber@ualberta.ca.

stalt, phenotypic and clinical descriptions⁹ are now to be identified by their chromosomal, genetic and biochemical defects.^{10,11} Teratological descriptions and therapeutic regimens are increasingly being identified by their molecular constructions.¹² A new taxonomy of diseases is being created using molecular tools to define causative events and aberrant pathways of physiology and development.

Embryology

Comprehension of congenital craniofacial anomalies is based upon an understanding of normal developmental anatomy, to which many texts and articles have been devoted.¹³ The conundrum of the complex choreography of the chromosomes and their contained genome in directing development has not yet been fully elucidated. The identification of genes responsible for craniofacial syndromes constitutes a major source of information on normal development. The differentiation of the complex multicellular organism from a single unicellular ovum is the central challenge of contemporary molecular biology (Fig. 2). Cells proliferate rapidly to create layers and blocks that are folded and shaped into tissues that form embryonic structures. Combining proliferation with cell migration involves a high degree of precision in specifying both the fates of individual cells and their spatial relationships to each other. Even minor changes in this ballet can result in malformations. Yet, developmental defects are rare, suggesting that a powerful suite of control mechanisms exists. These mechanisms of ontogenesis are genetically conserved and employed in postnatal wound healing, regeneration and neoplasia, thus projecting their significance into clinical practice. Knowing the details of these mechanisms might allow their employment in clinically controlling disease conditions.

The identification of the precise genes involved in craniofacial development is still in its infancy due to the complicated interaction of numerous genes at different stages of development. There are genes that initiate the primary germ layers of ectoderm, mesoderm and endoderm, followed by neural folding and subdivision, creating the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain). The segmentation of the rhombencephalon into eight rhombomeres, under the direction of Hox genes, is central to the subsequent development of neural crest ectomesenchyme¹⁴ (Fig. 3). The genetically determined neural crest migrates from its dorsal origin to its ventral destination to create the six pharyngeal arches and the five facial prominences—the median frontonasal prominence and the paired maxillary and mandibular prominences (Fig. 4). These transformations are dependent upon gene expressions at precise and critical times to effect normal morphogenesis. Mutations of any of the patterning genes will be reflected in phenotypic malformations, recognised as syndromes. Affected neural crest derivatives result in neurocristopathies, with defective development of facial primordia. However, when abnormalities occur due to gene mutation it is frequently not known which stages of facial development have been disturbed. The mutation may have a direct effect (a malformation) or an indirect sequential consequence (a deformation).

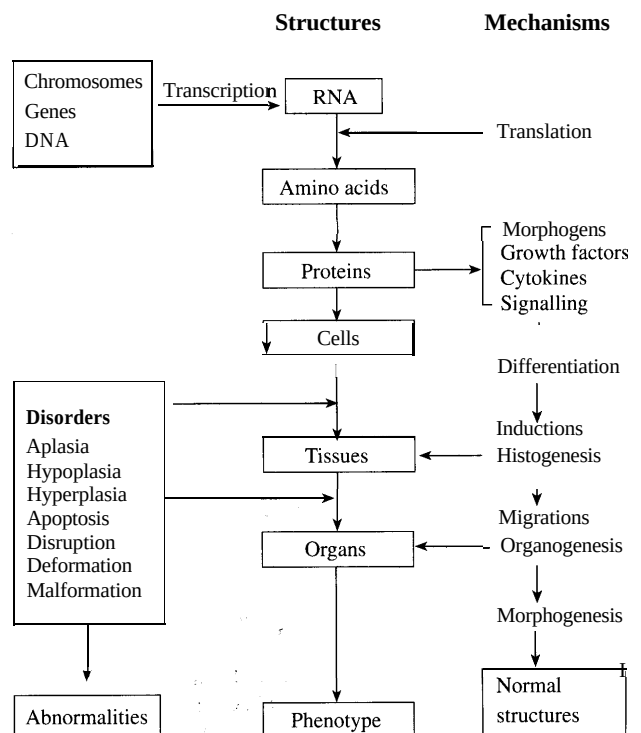
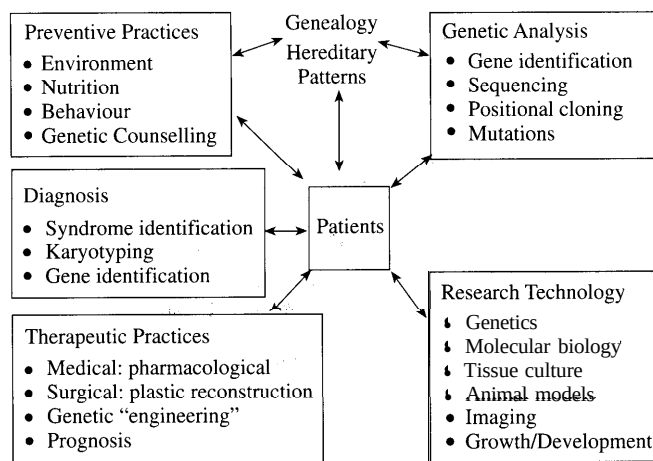


Fig. 1. Schema of patient-centred procedures.

Fig. 2. Schema of development phenomena.

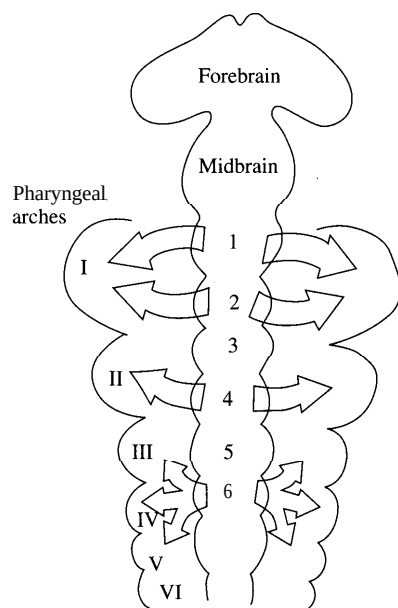


Fig. 3. Schematic depiction of neural crest migration pathways from rhombomeres (labelled 1 to 7) to the pharyngeal arches (labelled I to VI).

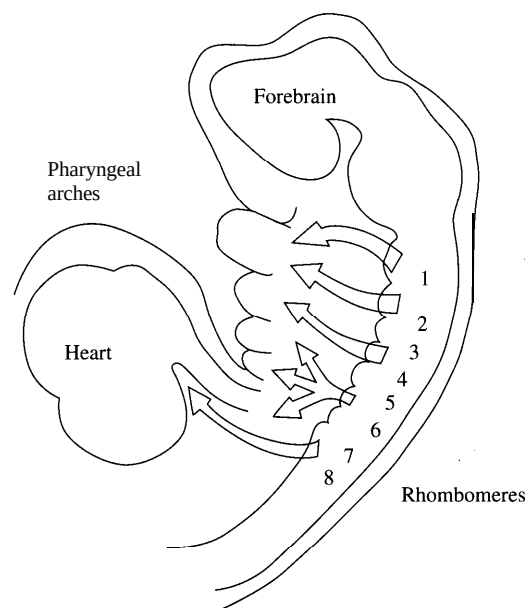


Fig. 4. Lateral perspective of neural crest migration from rhombomeres 1 to 8 into the pharyngeal arches.

The genes' control of development is effected through signalling information,¹⁵ and it is the identification of these products of gene expression that is revealing the complexity of control of cell differentiation, survival, motility and death (apoptosis). An increasing number of signalling factors are being identified during development^{16,17} (Table I).

Malformations, Deformations, Disruptions

Dysmorphology may arise from genetic inheritance (generally syndromes), mutations, or developmental disturbances during embryogenesis.¹⁸⁻²⁰ Such disturbances may be identified as malformations, deformations or disruptions, depending upon their aetiology. Malformations reflect retarded, incomplete or dispro-

TABLE I: GROWTH FACTORS

Factor	Abbreviation	Derivation	Action
Brain-derived neural growth factor	BDNF	Neural tube	Stimulates dorsal root ganglion anlagen
Insulin-like growth factors I and II	IGF-I IGF-II	Sympathetic chain ganglia	Stimulates proliferation of fat and connective tissue cells and metabolism
Nerve growth factor	NGF	Various organs	Promotes axon growth and neuron survival
Transforming growth factor- α	TGF- α	Various organs	Promotes differentiation of certain cells
Transforming growth factor- β (Activin A, Activin B)	TGF- β	Various organs	Mesoderm induction. Potentiates or inhibits response to other growth factors
Fibroblastic growth factor	FGF	Various organs	Mesoderm induction. Stimulates proliferation of fibroblasts, endothelium, myoblasts
Transcriptional factor	TF	Intermediate gene in mesoderm induction cascade	Stimulates transcription of actin gene
Platelet-derived growth factor	PDGF	Platelets	Stimulates proliferation of connective tissue cells and neuroglia
Epidermal growth factor	EGF	Various organs; salivary glands	Stimulates proliferation and differentiation of many cell types
Interleukin-2	IL-2		Stimulates proliferation of T-lymphocytes
Hemopoietic cell growth factors Erythropoietin	IL-3, GM-CSF M-CSF, G-CSF		Stimulates erythropoiesis
Angiogenesis growth factor	AGF		Stimulates angiogenesis

Reproduced with permission from: Sperber G H, Machin G A. The enigma of cephalogenesis. *Cleft Palate Craniofac J* 1994; 31:91-6.

portionate development of tissues or organs. Deformations are the consequence of mechanical displacement of usually previously normal parts. Disruptions are anomalies arising from teratogenic interruption of normal development. Disruptive agents may be metabolic (toxins), radioactive, ischaemic or haemorrhagic or even mechanical when considering intrauterine amniotic bands. The distinction between these aetiological factors in determining the cause of anomalous development is not always clear. As an example, analysis of the dysmorphic skull of a 28-month-old infant reveals craniosynostosis of the sagittal suture (Figs. 5 & 6). The craniosynostosis is a consequence of defects in fibroblast growth factors and their receptors²¹⁻⁴⁴ that led to premature fusion of the sagittal suture. This defective development is a malformation that, in turn, has led to deformation of the skull. The deformation has arisen from the continued growth of the brain, that constrained by the synostosis, has resulted in a bulging of the containing bone in an abnormal direction, producing the distortion. Bone is highly plastic during its formative stages and reflects the shape of the underlying brain that is unaffected by the defective fibroblast growth factor or its receptor that acts upon the sutures. The misshapen bone is thus deformed

by the brain, and not by the prematurely fused sutures. The distinction between malformation and deformation is thus obscured. Nonetheless, it is important to seek distinctions to understand not only the aetiology but also the prognosis and possible therapeutic regimens to be followed. Counteracting or correcting the defective fibroblast growth factor or its receptor in this case would prevent the craniosynostosis and the consequent deformation of the skull.

Genetics

Disorders of development based upon confirmed chromosomal aneuploidies or genetic mutations constitute current and future clinical practice. The complex multifactorial aetiologies of most dysmorphic conditions constitute a limitation to full understanding of their development.

The location of mutant genes determining specific developmental phenomena can be identified by abnormal phenotypes manifesting specific syndromes. Linkage studies using highly polymorphic DNA markers provide a capability of locating candidate genes. Experimentally, one can tag a chromosomal region by insertion

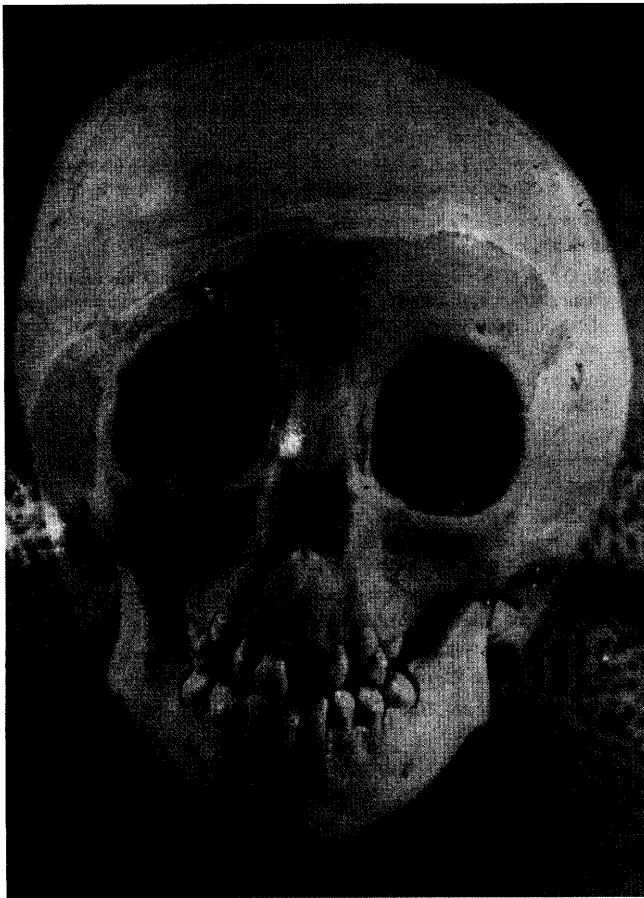


Fig. 5. Frontal view of skull of 28-month-old infant showing hemispheric bulging of right parietal region. Central incisor tooth loss is postmortem.

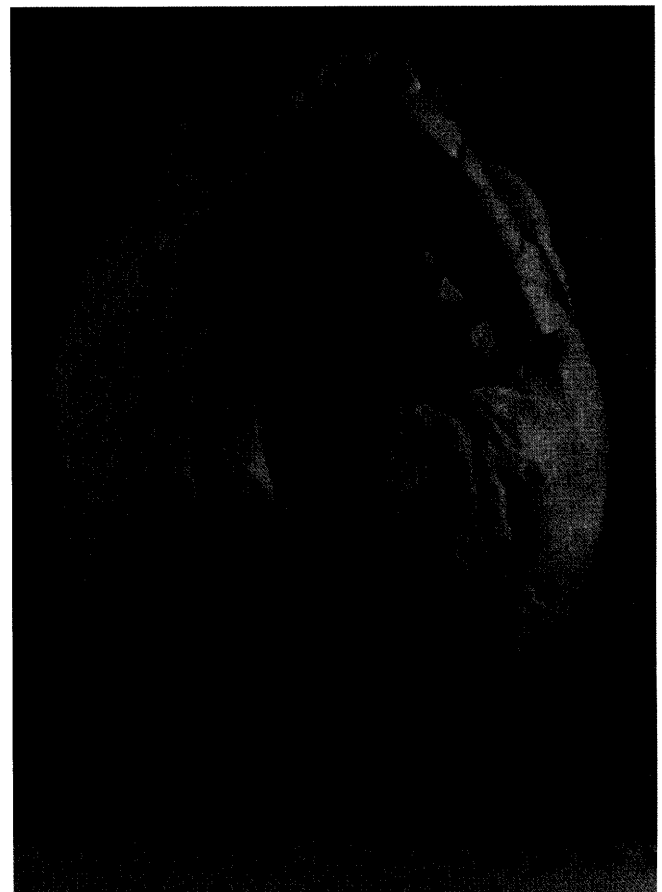


Fig. 6. Basal view of skull depicted in Figure 5. Note the bone distortion around the foramen magnum with the right occipital condyle hyperplastic and adjacent bone "folded" and the bulging of the left occipito-parietal region. The basicranium appears "twisted".

of a transgene or retrovirus into the germline of a mouse to produce targeted disruptions. This approach provides a methodology to identify craniofacial malformations by breeding transgenic mice to homozygosity.

At another level, the identification and characterisation of growth and differentiation factors that signal positional information during development have provided insights into the basis of malformation syndromes. These signalling molecules are present in picomolar or nanomolar concentrations. These factors include Vitamin D metabolites, retinoic acid, polypeptides and receptors, adhesion molecules, molecular signalling transducers, growth factors, cytokines and morphogens.

While identification of the minute amounts of these molecules has become possible with advanced microtechnology, the regulatory pathways that control inductive signalling remain largely unknown. The mechanistic basis of the temporal and spatial expression of genomic organisation may be elicited by targeted gene inactivations ("knockout" technology) and transgenic approaches. Development is regulated at multiple levels.

The continuing identification of genes poses a problem of their nomenclature and a worldwide web site has been established for standardisation and international recognition of newly discovered genes.⁴⁵

Conclusion

The field of craniofacial development is currently the subject of intense research investigation as a consequence of unprecedented funding being devoted to the molecular biology, embryology, syndromology and epidemiology of craniofacial disorders. The National Institute of Health of the United States of America has received the largest funding increase in its history for its 1999 budget of US\$15.652 billion. One consequence of this is that one of its agencies, the 50 year-old National Institute of Dental Research, has expanded to become the National Institute of Dental and Craniofacial Research, with a budget of US\$234,338,000 to spend in 1999. Birth defects of the face have become an area of increased attention, and research grants totalling US\$16.5 million have been awarded in 1999 to six American institutions under a programme entitled "Comprehensive Oral Health Research Centres of Discovery". The multidisciplinary areas of enquiry under this programme range from the genetics of zebrafish and mice through human hereditary patterns of orofacial clefting and craniofacial disorders to the epidemiology and behavioural consequences of subjects suffering cranial congenital anomalies. The explosive growth of knowledge in this area anticipated from this venture will undoubtedly lead to diagnostic, prognostic, therapeutic and preventive capabilities for craniofacial disorders that were impossible to conceive previously. The future for

the advancement of knowledge in this area has never been brighter.

Acknowledgements

The author would like to express his appreciation to Dr Marilyn C Jones for the loan of the skull and to Ms Anne-Marie McLean for her excellent word processing skills.

REFERENCES

1. Bell J I. Clinical research is dead; long live clinical research. *Nat Med* 1999; 5:477-8.
2. Machin G A, Sperber G H. Computerized graphic imaging for three dimensional representation: general principles and applications to embryo/fetal development. *Int Rev Exper Path* 1996; 36:1-30.
3. Machin G A, Sperber G H. Computer graphic three dimensional reconstruction of normal human embryo morphogenesis. *Anat Embryol* 1996; 194:439-44.
4. Burris J, Cook-Deegan R, Alberts B. The Human Genome Project after a decade: Policy issues. *Nat Genet* 1998; 20:333-5.
5. Longacre M T, Whitby D J, Adzick N S, Kaban L B, Harrison M R. Fetal surgery for cleft lip: a plea for caution. *Plast Reconstr Surg* 1991; 88:1087.
6. Cope J B, Samchukow M L. Mandibular distraction osteogenesis: a historic perspective and future directions. *Am J Orthod Dentofac Orthoped* 1999; 115:448-60.
7. Watzinger F, Wanschitz F, Rasse M, Milles W, Schopper CH, Kremer J, et al. Computer aided surgery in distraction osteogenesis of the maxilla and mandible. *Int J Oral Maxillofac Surg* 1999; 28:171-5.
8. Mulliken J B, Warman M L. Molecular genetics and craniofacial surgery. *Plast Reconstr Surg* 1996; 97:666-75.
9. Bolsover S R, Hyams J S, Jones S, Shepard E A, White H A. *From Genes to Cells*. New York: Wiley-Liss, 1997.
10. Nuckolls G H, Shum L, Slavkin H C. Progress towards understanding craniofacial malformations. *Cleft Palate Craniofac J* 1999; 36:12-26.
11. Hibbert S A, Field J K. Molecular basis of familial cleft lip and palate. *Oral Dis* 1996; 2:238-41.
12. Mulder G B, Manley N, Maggio-Price L. Retinoic acid-induced thymic abnormalities in the mouse are associated with altered pharyngeal morphology, thymocyte maturation defects, and altered expression of Hoxa3 and Pax1. *Teratology* 1998; 58:263-75.
13. Wolpert L, Beddington R, Brooks J, Jesse T. *Principles of Development*. London: Oxford Univ Press, 1998.
14. Gale E, Prince V, Lumsden A, Clarke J, Holder N, Maden M. Late effects of retinoic acid on neural crest and aspects of rhombomere identity. *Development* 1996; 122:783-93.
15. Glinka A, Wu W, Delius H, Monaghan A I, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 1998; 391:357-62.
16. Francis-West P H, Ladher R, Barlow A, Graveson A. Signalling interactions during facial development. *Mech Dev* 1998; 75:3-28.
17. Sperber G H, Machin G A. The enigma of cephalogenesis. *Cleft Palate Craniofac J* 1994; 31:91-6.
18. Ming J E, Muenke M. Holoprosencephaly: from Homer to Hedgehog. *Clin Genet* 1998; 53:155-63.
19. Yamagishi H, Garg V, Matsuoka R, Thomas T, Srivastava D. A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. *Science* 1999; 283:1158-61.
20. Lacombe D. Transcription factors in dysmorphology. *Clin Genet* 1999; 55:137-43.
21. Albright A L, Byrd R P. Suture pathology in craniosynostosis. *J Neurosurg* 1981; 54:384-7.
22. Furuya Y, Edwards M S B, Alpers C E, Tress B M, Norman D, Ousterhout

- DK. Computerized tomography of cranial sutures. Part 2: Abnormalities of sutures and skull deformity in craniosynostosis. *J Neurosurg* 1984; 61:59-70.
23. Hall S H, Decker J D. Calvarial suture morphogenesis: Cellular and molecular aspects. In: Persing J A, Edgerton M T, Jane J A, editors. *Scientific Foundations and Surgical Treatment of Craniosynostosis*. Baltimore: Williams and Wilkins, 1989:29-37.
 24. Kokich V G, Moffett B C, Cohen M M Jr. The cloverleaf skull anomaly: An anatomic and histologic study of two specimens. *Cleft Palate J Craniofac* 1982; 19:89-99.
 25. Kokich V G. The biology of sutures. In: Cohen M M Jr, editor. *Craniosynostosis: Diagnosis, Evaluation and Management*. New York: Raven Press, 1986:81-103.
 26. Koskinen-Moffett L, Moffett B C, Graham J M. Cranial synostosis and intrauterine compression: A developmental study of human sutures. In: Dixon A D, Sarnat B G, editors. *Factors and Mechanisms Influencing Bone Growth*. New York: Alan R Liss, 1982:365-78.
 27. Koskinen-Moffett L, Moffett B C. Sutures and intrauterine deformation. In: Persing J A, Edgerton M T, Jane J A, editors. *Scientific Foundations and Surgical Treatment of Craniosynostosis*. Baltimore: Williams and Wilkins, 1989:96-106.
 28. Liu Y H, Ramendra K, Wu L, Luo W, Ignelzi M, Snead M, et al. Premature suture closing and ectopic cranial bone in mice expressing *MSX2* transgenes in the developing skull. *Proc Natl Acad Sci U S A* 1995; 92:6137-41.
 29. Markens I S. Embryonic development of the coronal suture in man and rat. *Acta Anat (Basel)* 1975; 93:257-73.
 30. Moss M L. The pathogenesis of premature cranial synostosis in man. *Acta Anat (Basel)* 1959; 37:351-70.
 31. Moss M L. Functional anatomy of craniosynostosis. *Child's Brain* 1975; 1:22-33.
 32. Opperman L A, Passarelli R, Morgan E P, Reintjes M, Ogle R C. Cranial sutures require tissue interactions with dura mater to resist osseous obliteration in vitro. *J Bone Miner Res* 1995; 10:1978-87.
 33. Opperman L A, Nolen A A, Ogle R C. TGF- β 1, TGF- β 2 and TGF- β 3 exhibit distinct patterns of expression during cranial suture formation and obliteration in vivo and in vitro. *J Bone Miner Res* 1997; 12:301-10.
 34. Ousterhout D K, Eskenazi L, Golabi M, Edwards M S. Histological evaluation of the coronal sutures in trigonocephaly. *J Craniofac Surg* 1990; 1:15-17.
 35. Persson K M. Regulating factors in suture development, growth, and closure. In: Persing J A, Edgerton M T, Jane J A, editors. *Scientific Foundations and Surgical Treatment of Craniosynostosis*. Baltimore: Williams and Wilkins, 1989:45-9.
 36. Reddy K, Hoffman H, Armstrong D. Delayed and progressive multiple suture craniosynostosis. *Neurosurgery* 1990; 26:442-8.
 37. Smith D V, Tondury G. Origin of the calvaria and its sutures. *Am J Dis Child* 1978; 132:662-6.
 38. Wilkie A O, Wall S A. Craniosynostosis: novel insights into pathogenesis and treatment. *Curr Opin Neurol* 1996; 9:146-52.
 39. Lewanda A F, Meyers G A, Jabs E W. Craniosynostosis and skeletal dysplasias: fibroblast growth factor receptor defects. *Proc Assoc Am Physicians* 1996; 108:19-24.
 40. Malcolm S, Reardon W. Fibroblast growth factor receptor-2 mutations in craniosynostosis. *Ann N Y Acad Sci* 1996; 785:164-70.
 41. Wilkie A O. Craniosynostosis: genes and mechanisms. *Hum Mol Genet* 1997; 6:1647-56.
 42. Gorlin R J. Fibroblast growth factors, their receptors and receptor disorders. *J Craniomaxillofac Surg* 1997; 25:69-79.
 43. Muller U, Steinberger D, Kunze S. Molecular genetics of craniosynostotic syndromes. *Graefes Arch Clin Exp Ophthalmol* 1997; 235:545-50.
 44. Jabs E W. Toward understanding the pathogenesis of craniosynostosis through clinical and molecular correlates. *Clin Genet* 1998; 53:79-86.
 45. White J, Wain H, Bruford E, Povey S. Human gene nomenclature database search, 1999; www.gene.ucl.ac.uk/nomenclature/