CRANIOSYNOSTOSIS

A MEDICAL DICTIONARY, BIBLIOGRAPHY,
AND ANNOTATED RESEARCH GUIDE TO
INTERNET REFERENCES

JAMES N. PARKER, M.D.
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Table of Contents

FORWARD

In March 2001, the National Institutes of Health issued the following warning: "The number of Web sites offering health-related resources grows every day. Many sites provide valuable information, while others may have information that is unreliable or misleading."[1] Furthermore, because of the rapid increase in Internet-based information, many hours can be wasted searching, selecting, and printing. Since only the smallest fraction of information dealing with craniosynostosis is indexed in search engines, such as **www.google.com** or others, a non-systematic approach to Internet research can be not only time consuming, but also incomplete. This book was created for medical professionals, students, and members of the general public who want to know as much as possible about craniosynostosis, using the most advanced research tools available and spending the least amount of time doing so.

In addition to offering a structured and comprehensive bibliography, the pages that follow will tell you where and how to find reliable information covering virtually all topics related to craniosynostosis, from the essentials to the most advanced areas of research. Public, academic, government, and peer-reviewed research studies are emphasized. Various abstracts are reproduced to give you some of the latest official information available to date on craniosynostosis. Abundant guidance is given on how to obtain free-of-charge primary research results via the Internet. **While this book focuses on the field of medicine, when some sources provide access to non-medical information relating to craniosynostosis, these are noted in the text.**

E-book and electronic versions of this book are fully interactive with each of the Internet sites mentioned (clicking on a hyperlink automatically opens your browser to the site indicated). If you are using the hard copy version of this book, you can access a cited Web site by typing the provided Web address directly into your Internet browser. You may find it useful to refer to synonyms or related terms when accessing these Internet databases. **NOTE:** At the time of publication, the Web addresses were functional. However, some links may fail due to URL address changes, which is a common occurrence on the Internet.

For readers unfamiliar with the Internet, detailed instructions are offered on how to access electronic resources. For readers unfamiliar with medical terminology, a comprehensive glossary is provided. For readers without access to Internet resources, a directory of medical libraries, that have or can locate references cited here, is given. We hope these resources will prove useful to the widest possible audience seeking information on craniosynostosis.

The Editors

[1] From the NIH, National Cancer Institute (NCI): **http://www.cancer.gov/cancerinfo/ten-things-to-know**.

CHAPTER 1. STUDIES ON CRANIOSYNOSTOSIS

Overview

In this chapter, we will show you how to locate peer-reviewed references and studies on craniosynostosis.

The Combined Health Information Database

The Combined Health Information Database summarizes studies across numerous federal agencies. To limit your investigation to research studies and craniosynostosis, you will need to use the advanced search options. First, go to **http://chid.nih.gov/index.html**. From there, select the "Detailed Search" option (or go directly to that page with the following hyperlink: **http://chid.nih.gov/detail/detail.html**). The trick in extracting studies is found in the drop boxes at the bottom of the search page where "You may refine your search by." Select the dates and language you prefer, and the format option "Journal Article." At the top of the search form, select the number of records you would like to see (we recommend 100) and check the box to display "whole records." We recommend that you type "craniosynostosis" (or synonyms) into the "For these words:" box. Consider using the option "anywhere in record" to make your search as broad as possible. If you want to limit the search to only a particular field, such as the title of the journal, then select this option in the "Search in these fields" drop box. The following is what you can expect from this type of search:

- **Craniosynostosis: Part I: Sagittal Synostosis**

  Source: FACES. 7(1): 5. Winter 1993.

  Contact: Available from National Association for the Craniofacially Handicapped. P.O. Box 11082, Chattanooga, TN 37401. (615) 266-1632; (800) 332-2373.

  Summary: Craniosynostosis can be defined as the premature closing of one or more of the normally present bony gaps between the different bones of the skull. This brief article discusses the diagnosis of **craniosynostosis,** saggital synostosis, and the surgical treatment of infants with isolated **craniosynostosis.** The author emphasizes that these infants are best evaluated and treated by a craniofacial center that utilizes a multidisciplinary team approach.

Federally Funded Research on Craniosynostosis

The U.S. Government supports a variety of research studies relating to craniosynostosis. These studies are tracked by the Office of Extramural Research at the National Institutes of Health.[2] CRISP (Computerized Retrieval of Information on Scientific Projects) is a searchable database of federally funded biomedical research projects conducted at universities, hospitals, and other institutions.

Search the CRISP Web site at **http://crisp.cit.nih.gov/crisp/crisp_query.generate_screen**. You will have the option to perform targeted searches by various criteria, including geography, date, and topics related to craniosynostosis.

For most of the studies, the agencies reporting into CRISP provide summaries or abstracts. As opposed to clinical trial research using patients, many federally funded studies use animals or simulated models to explore craniosynostosis. The following is typical of the type of information found when searching the CRISP database for craniosynostosis:

- **Project Title: ANIMAL MODELS FOR SAETHRE CHOTZEN SYNDROME**

  Principal Investigator & Institution: Gridley, Thomas H.; Senior Staff Scientist; Jackson Laboratory 600 Main St Bar Harbor, Me 04609

  Timing: Fiscal Year 2002

  Summary: The long term goals of this project are to generate and characterize mouse models of human craniofacial disease syndromes, and to understand the genetic and biochemical pathways underlying these disease syndromes. **Craniosynostosis,** the premature fusion of the calvarial bones of the skull, is a significant medical problem, occurring in 1 in 3000 live births. The abnormal skull growth associated with **craniosynostosis** may result in raised intracranial pressure, impaired cerebral blood flow, airway obstruction, impaired vision and hearing learning difficulties and adverse psychological effects. In this proposal, we will study a mouse model for Saethre-Chotzen Syndrome, one of the most common autosomal dominant disorders of **craniosynostosis** in humans. Haploinsufficiency for the human TWIST gene, which encodes a bHLH-type transcription factor, has been demonstrated to be one of the major causes of Saethre-Chotzen Syndrome. Other familial cases of Saethre-Chotzen Syndrome are caused by mutations in some of the genes encoding fibroblast growth factor receptors (FGR2 and FGR3). A null mutation in the mouse Twist gene results in early embryonic death in homozygotes, and in heterozygotes results in partially penetrant skeletal defects that replicate certain features of Saethre-Chotzen syndrome. The human TWIST gene is a homolog of the Twist gene of Drosophila. Genetic evidence in Drosophila has demonstrated that mutations in the Twist gene interact with mutations in another transcription factor encoded by the Snail gene. We have constructed targeted mutations in two mouse homologs of Snail (termed Sna and Slug). We will test the hypothesis that, as in Drosophila, genes of the Snail and Twist family both function in the same genetic pathway in mice. The specific aims of this proposal are to: 1. Further characterize Twist mutant embryos by testing fro altered expression of the Snail family genes Sna and Slug. Also examine whether expression of other genes responsible for inherited **craniosynostosis** syndromes (the fibroblast growth factor receptors and the

[2] Healthcare projects are funded by the National Institutes of Health (NIH), Substance Abuse and Mental Health Services (SAMHSA), Health Resources and Services Administration (HRSA), Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDCP), Agency for Healthcare Research and Quality (AHRQ), and Office of Assistant Secretary of Health (OASH).

Msx genes) are altered in Twist mutant embryos. 2. Test the hypothesis that, as in Drosophila, genes of the Twist and Snail families will function in the same genetic pathway by generating and analyzing Sna/Twist double mutants. 3. Generate and analyze double mutants with the mother mouse Snail family gene Slug (Slug/Twist double mutants).

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: CEPHALOMETER FOR RAPID, SAFE MEASUREMENT OF INFANT HEAD**

  Principal Investigator & Institution: Goldie, James H.; Foster-Miller, Inc. 350 2Nd Ave Waltham, Ma 02451

  Timing: Fiscal Year 2004; Project Start 15-MAY-2004; Project End 14-NOV-2004

  Summary: (provided by applicant): A cranial measurement device, or cephalometer is proposed that will provide a safe and convenient means for screening for and determining the severity and nature of plagiocephaly in an infant. Currently, initial screening by pediatricians is entirely subjective. The first quantitative evaluation is typically done with large calipers by a neurosurgeon or by radiographic means. However, there is need for an inexpensive device that either clinicians or parents can use to rapidly obtain accurate and repeatable data, both for screening and during the course of treatment. The proposed measurement device offers a practical method for obtaining frequent measurements, allowing the treating physician to evaluate the progress and effectiveness of treatment. This will benefit patients with conditions ranging in significance from deformational plagiocephaly, treated with orthotic devices such as helmets, to sagittal synostosis, a congenital skull abnormality requiring surgery. Although not a requirement, the proposed device can be upgraded to include sensors, data acquisition and a computer, in order to create a complete system in which measurements are displayed, evaluated, and diagnostic and record-keeping tasks undertaken. The Phase I will focus on development and test of several cephalometer prototypes. Testing will be done with infant head models that represent both normal head shapes and deformities of interest, in order to evaluate rapidity of measurement, measurement accuracy and repeatability (as compared with calipers and other accepted methods), measurement quality as a function of user's level of training (again, compared with existing methods), and device cost. Testing with infants will be deferred until the Phase II, in which measurements will be taken on many infants with a wide range of head shapes by parents, doctors, and other clinicians.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: COPING WITH CRANIOFACIAL DISORDERS**

  Principal Investigator & Institution: Slifer, Keith J.; Kennedy Krieger Institute, Inc. 707 N Broadway Baltimore, Md 21205

  Timing: Fiscal Year 2002

  Summary: Children with craniofacial disorders have increased incidence of anxiety, withdrawal and social competence problems, but they tend to rate their own appearance more positivity than it is rated by others. Some may learn to cope with facial impairment by minimizing it an developing compensatory behavior. Direct observations of these children reveal social interactions that differ significantly from peers. Other data suggest they have impaired ability to communicate emotion through facial expressions. This study will employ: (1) microanalytic direct observation methods, and (2) computer-automated recognition of facial expressions to quantify the social and facial behavior of

8 to 16 year-old subjects videotaped during: (1) an analogue social interaction with a confederate peer, and (2) a structured facial encoding task. Results will be compared across matched Craniofacial (oral cleft and craniosynostosis). Non-facial (short stature), and Normal Control groups. Within-group comparisons will examine ow subjects with positive self-perceptions differ from those with comparable physical impairments and average or negative self-perceptions. Differences in self ratings of appearance and self-concept are expected to be significantly correlated with observable differences in social behavior. Craniofacial subjects (particularly those with oral clefts) are expected to evidence impaired ability to encode emotion through facial expressions. Some differences in social and facial behavior may reflect compensatory behavior developed by those who are better-adjusted and more socially competent, which could be taught those who are less competent. If differences in ability to generate facial expression are found in subjects with craniofacial disorders, and associated with impaired social competence, the results may lead to assessment techniques for detecting subtle but clinically significant facial dysfunction. Some may be amenable to surgical correction, others to behavioral intervention (e.g. response shaping with reinforcement contingencies or biofeedback training of facial muscles). Such intervention could be tested by subsequent demonstration projects. The combined results will provide an empirical basis for selecting specific skills to be taught in future prospective interventions for children with craniofacial disorders.

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: CORE--CLINICAL**

  Principal Investigator & Institution: Vander Kolk, Craig; Johns Hopkins University 3400 N Charles St Baltimore, Md 21218

  Timing: Fiscal Year 2002

  Summary: The overall goal of the Clinical Core is to be the organizational structure to collate information and specimens on patients with **craniosynostosis** and oral clefting and associated dental and facial anomalies. Patients will participate in our research projects from the Mid-Atlantic (Maryland, Washington D.C., Virginia) and Midwest (Missouri) REGIONS OF THE United States, Denmark, Czech Republic, Mexico, and Argentina. Databases will include information on phenotype, diagnosis, and classification of these individuals. The condition of the patient will be classified as sporadic or familial, isolated or syndromic (and whether the syndrome is recognized or previously unknown), or environmental in origin. Salient medical, surgical, dental, and family history information will be retained in the database. 3D CAT and brain morphology scans will also be entered into the database for patients with **craniosynostosis.** Using this data, patients will be triaged for future studies, such as chromosomal analysis, molecular DNA analysis, questionnaire of environmental factors, and behavioral analysis. The database will be used by Projects V, VI, VII, and VIII of this Center, in order to correlate genotypes with phenotypes, environmental, and behavioral factors, develop accurate diagnostic criteria, and map and clone genes responsible for isolated, familial, and syndromic **craniosynostosis** and multiplex families with oral clefting. To implement this project 1) patients with **craniosynostosis** or oral clefting and their families will be ascertained and enrolled, 2) medical and family histories and radiology test results will be entered into the database, and 3) blood samples will be obtained for chromosome analysis, DNA isolation, and establishment of a lymphoblast cell line and potential other tissue cell lines from surgical specimens for future analysis. Outpatient clinics through the Johns Hopkins Cleft & Craniofacial Center, the Johns Hopkins Division of Dentistry and Oral & Maxillofacial Surgery, the Center for Medical

Genetics in Rockville, Maryland, the University of Maryland Medical Center and Virginia Commonwealth University's School of Dentistry and Center for Facial Reconstruction will be utilized to recruit patients for these research projects. The collaborative research efforts will identify, isolate and characterize genetic, environmental, and behavioral factors essential in craniofacial development.

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: CRANIOFACIAL DEVELOPMENTAL MOLECULAR BIOLOGY**

  Principal Investigator & Institution: Shuler, Charles F.; Ctr/Craniofacial Molec Biol; University of Southern California 2250 Alcazar Street, Csc-219 Los Angeles, Ca 90033

  Timing: Fiscal Year 2002; Project Start 01-AUG-1999; Project End 31-JUL-2004

  Summary: The Craniofacial Developmental Molecular Biology Program will consist of investigators from the University of Southern California, Schools of Dentistry and Medicine who participate in a multi-disciplinary research team focused on investigations of craniofacial genetics and developmental biology. The faculty collaboration includes investigators from the USC Institute of Genetic Medicine, the USC Comprehensive Cancer Center and the USC Center for Craniofacial Molecular Biology. The overall Program theme will use molecular biology approaches to investigate fundamental mechanisms required for normal development of the craniofacial complex. These findings will be applicable to further the understanding of normal developmental regulation and the molecular etiology of craniofacial birth defects. The Craniofacial Developmental Molecular Biology Program will consist of five Research Projects and one Core Resource. Project 1 (Dr. Yang Chai), "Characterization of Genes Involved in the Specification of Tooth and Meckel's Cartilage Morphogenesis," will characterize the Smad- related intracellular signaling pathway that is activated during mandibular morphogenesis. Project 2 (Dr. Larry Kedes) "Co-Activators and Repressors of Muscle Transcription," will investigate the activity of Twist, p300/CBP and PCAF during the differentiation of muscle cells. Project 3 (Dr. Yi-Hsin Liu) "Genes Involved in Skull Suture Morphogenesis," will examine patters of gene expression unique to the cranial sutures that may be correlated with abnormal suture development resulting in **craniosynostosis.** Project 4 (Dr. Robert Maxon) "Function of MSX2 and Twist in Calvarial Morphogenesis," will examine the regulation and activation of interacting transcription factors Twist and Msx2 during bone cell commitment and differentiation. Project 5 (Dr. Charles Shuler) " Mechanisms of Epithelial-Mesenchymal Transformation during Palatogenesis," will investigate the molecular mechanism underlying the epithelial-mesenchymal transdifferentiation of the medial edge epithelium during palatal fusion. The activities of the Program will be supported by an Administrative Core that will provide support for purchasing, manuscript preparation, scheduling for seminars and collaborative research planning and financial management. The Program will benefit from the availability of several other established cores at the University of Southern California available to support the research activities of all the Projects. This program will continue a long-term series of collaborations that have provided an excellent scientific environment to advanced the understanding of the molecular mechanisms underling craniofacial development.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: CRANIOSYNOSTOSIS AND SUTURE BIOLOGY**

  Principal Investigator & Institution: Cunningham, Michael L.; Associate Professor; University of Washington Grant & Contract Services Seattle, Wa 98105

Timing: Fiscal Year 2002

Summary: Craniosynostosis is the morbid condition of premature fusion of calvarial bones. The sequelae of premature calvarial sutural fusion include 1) increased intracranial pressure associated with brain injury, 2) skull malformations requiring extensive surgical correction, and 3) abnormal development of the zygoma and maxilla necessitating orthodontic management and orthognatic surgery. Despite the rapid advance of our understanding of the molecular etiology of hereditary synostosis, the biological basis of **craniosynostosis** has yet to be elucidated. Over the past four years we have learned that mutations of fibroblast growth factor receptor family (FGFRs) and the TWIST are responsible for the majority of cases of syndromic synostosis. In order to understand the biology behind hereditary suture fusion, we propose to: 1) determine whether osteoblasts derived from patients with syndromic synostosis, harboring FGFR2, FGFR3, or TWIST mutations, will induce premature closure of rat coronal sutures 2) establish whether changes in suture development induced by mutant osteoblasts are due to intrinsic differences in mutant osteoblast growth and bone formation (e.g. cell autonomous) or the elaboration of cytokines which effect suture development and 3) correlate changes induced in suture development by mutant osteoblasts with changes in apoptotic cell death, mitogenic activity, and/or rates of matrix formation or mineralization. In order to test the hypothesis that: Syndromic **craniosynostosis** in humans is mediated by osteoblasts and their influence on the microenvironment of the calvarial suture. We propose that changes in osteoblast growth, mitotic rate, resistance to apoptosis and/or elaboration of cytokines results in premature suture fusion and **craniosynostosis.** These studies will lead to important information about the biology behind the development of **craniosynostosis** and may have significant implications for the treatment and primary prevention of synostosis in humans.

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: DYSFUNCTIONAL FGFR SIGNALING IN CRANIOSYNOSTOSIS**

  Principal Investigator & Institution: Friesel, Robert E.; Director; Maine Medical Center 22 Bramhall St Portland, Me 04102

  Timing: Fiscal Year 2002; Project Start 20-SEP-1998; Project End 31-JUL-2004

  Summary: Craniosynostosis, an abnormality of skull development in which the sutures of the growing calvarial bones fuse prematurely, occurs with a frequency of approximately 1 in 2500 live births. Recently, six autosomal dominant craniosynostotic syndromes, Crouzon, Jackson-Weiss, Pfeiffer, Apert, Crouzon with acanthosis nigricans and Beare-Stevenson cutis gyra were shown to be associated with mutations in either fibroblast growth factor receptor (FGFR)-l, FGFR-2 or FGFR-3. In addition, several dwarfing syndromes, achondroplasia, thanatophoric dysplasia types I and II, and hypochondroplasia were shown to be associated with mutations in FGFR-3. The FGFRs consist of a family of four high affinity transmembrane tyrosine kinase receptors. The prototype FGFR is comprised of an extracellular ligand-binding domain made up of three immunoglobulin (Ig)-like domains, a hydrophobic membrane-spanning region and a cytoplasmic tyrosine kinase domain. Mutations in the extracellular ligand-binding domain and the transmembrane domain of FGFR-1, FGFR-2 and FGFR-3 have been associated with craniosynostotic syndromes while mutations in the extracellular, transmembrane and tyrosine kinase domains of FGFR-3 have been associated with dwarfing syndromes. Our recent studies indicate that mutations in the extracellular, transmembrane and tyrosine kinase domains that are associated with **craniosynostosis** and other skeletal dysplasias result in ligand-independent constitutive activation of the mutant receptors. The central hypothesis of this application is that point mutations in

FGFRs that are associated with **craniosynostosis** and other skeletal dysplasias result in constitutive activation of these receptors and that these receptors have altered signal transduction capabilities compared to their wild-type counterparts. This altered signaling capacity may in part be responsible for the phenotypic manifestations of these mutations. Accordingly, the specific aims of the proposal are: 1) to determine whether additional mutations identified in FGFRs that are associated with skeletal dysplasias result in constitutive receptor activation; 2) to determine whether these different mutations impart the mutant receptors with altered signal transduction properties, and 3) to determine whether constitutively activating mutations in FGFRs result in altered stability or intracellular trafficking. Together, these studies should begin to elucidate the role of mutant FGFRs in the pathogenesis of craniosynostotic conditions, as well as the role of FGFRs in normal bone growth and development.

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: FGF SIGNALING IN BONE GROWTH AND DEVELOPMENT**

  Principal Investigator & Institution: Ornitz, David M.; Professor; Washington University Lindell and Skinker Blvd St. Louis, Mo 63130

  Timing: Fiscal Year 2002

  Summary: Fibroblast growth factors (FGFs) are essential molecules for mammalian development. Several human genetic diseases have been identified that are caused by point mutations in the genes encoding FGF receptors (FGFRs), 1, 2 and 3. These disorders result in craniofacial and skeletal dysplasias (craniosynostosis syndromes) and chondrodysplasia syndromes and demonstrate that FGF signaling pathways are essential regulators of chondrogenesis and osteogenesis. FGFR is expressed in proliferating and proliferating and pre-hypertrophic chrondrocytes. FGFR1 is expressed in hypertrophic chrondrocytes in an adjacent domain to that of FGFR3. FGFR2 is expressed in mesenchymal condensations, the perichondrium and in the osteoblast compartment of developing bone. These very defined and non-overlapping expression patterns suggest that different FGFRs have unique signaling properties required for different stages of bone development and/or that different FGFRs are utilized to take advantage of unique responsiveness to specific ligands. Additionally, the identity and function of the FGF ligand(s) that activate three different FGFRs throughout bone growth and development is not known. The experiments proposed here will test the hypotheses that unique signaling and unique ligand binding properties of FGFRs are essential to their function at different stages of bone development. In addressing this hypothesis we will elucidate the specific roles for FGFRs in the development of the proliferating and hypertrophic compartment of the growth plate and the perichondrium/periosteum. These studies will also provide insight into the mechanisms underlying human genetic diseases. To accomplish these goals we will specifically disrupt FGFR signaling in each compartment of developing bone versus tissue-specifically expressed cre recombinase and conditionally targeted FGFRs. To test for signaling differences versus ligand specificity differences we will expand the domain of FGFR1 expression in the growth plate to encompass that of FGFR3. Additionally objectives will be to identify physiologically relevant FGF ligands involved in chondrogenesis, cranial suture growth and perichondrium/periosteum growth and to examine the biochemical function of a mutation in FGFR2 that causes Aperts syndrome.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: FGFR2 IN SKELETOGENESIS--MUTATIONAL ANALYSIS IN MICE**

  Principal Investigator & Institution: Nah-Cederquist, Hyun-Duck; Biochemistry; University of Pennsylvania 3451 Walnut Street Philadelphia, Pa 19104

  Timing: Fiscal Year 2002; Project Start 01-APR-2001; Project End 31-MAR-2003

  Summary: (Provided by Applicant) The development of the craniofacial skeleton with its interposed sutures involves a complex process, for which molecular and cellular regulatory mechanisms are not well understood. Recent genetic studies have linked various activating mutations in the fibroblast growth factor receptor 2 (FGFR2) gene to a subset of **craniosynostosis** syndromes, which have in common craniofacial skeletal deformities associated with the premature fusion of cranial sutures. This suggests that FGFR2 may play important roles in skeletal and sutural development. To further develop this notion, the investigators have introduced bone-targeted mFGFR2 transgene constructs, containing an activating mutation (Pro253Arg; an Apert mutation) or a dominant negative mutation, into the mouse germ line and generated several lines of transgenic mice. Initial analyses of these mice revealed that those with an activating mutation manifested some of the typical craniofacial features of **craniosynostosis** patients. The mice with a dominant negative mutation also displayed a variety of skeletal abnormalities, but they were distinctively different from the **craniosynostosis** phenotype. Based on pro- mitogenic and anti-apoptotic responses to FGF signaling in bone cells, the investigators hypothesize that an activating FGFR2 mutation promotes proliferation of osteoblasts, while suppressing apoptosis in these cells, resulting in uncontrolled bone formation and, ultimately, suture fusion. In contrast, loss of normal FGFR activities may result in reduced bone cell proliferation with an increased rate of apopotosis, culminating in dystrophic bone and wide-open sutures. To test their hypothesis, the investigators will define the skeletal and suture phenotypes of newly generated transgenic mouse lines (Aim 1), investigate how these mutations alter bone cell functions associated with osteogenesis and suture formation (Aim 2), and finally determine whether the mitogene activated protein (MAP) kinase pathway is involved in some of the altered bone cell functions induced by FGFR2 mutations (Aim 3). Data collected from this pilot study will be used to develop hypotheses to be tested in a larger research project.

  Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: FIBRONECTIN ANTAGONISTS IN CRANIAL SUTURE FORMATION**

  Principal Investigator & Institution: Moursi, Amr M.; Pediatric Dentistry; Ohio State University 1960 Kenny Road Columbus, Oh 43210

  Timing: Fiscal Year 2003; Project Start 01-MAY-2003; Project End 31-JAN-2007

  Summary: (provided by applicant): Craniosynostoses are a group of congenital disorders which involve the premature fusion of one or more of the cranial sutures, causing cessation or distortion of craniofacial growth. Increased intracranial pressure is the most critical complication, often resulting in poor intellectual development as well as alterations in vision. The prevalence of these disorders is 1:1000 live births and they can occur alone, or in association with craniofacial syndromes, such as Crouzon or Apert. To allow for adequate craniofacial growth and brain development, excision of the fused suture(s) is the treatment of choice. Unfortunately, re-ossification of the excised suture is common and necessitates several surgical procedures throughout childhood. A therapeutic agent which could prevent re-ossification of the excised sutures by transiently blocking osteogenesis would be an effective adjunct to surgery. Therefore,

this study will investigate the effect on cranial suture ossification of a novel anti-osteogenic agent delivered in a collagen gel. Fibronectin (FN), an extracellular matrix molecule, has been shown to play an essential role in calvarial osteoblast osteogenesis. Anti-FN antibodies, soluble cell-binding FN fragments and anti-FN receptor antibodies are all FN antagonists which have been shown to selectively and reversibly block osteogenesis. Preliminary studies utilizing a well-characterized rat calvarial organ culture system have demonstrated that anti-FN antibodies, in particular, can inhibit cranial suture fusion. Preliminary experiments also demonstrated that the delivery system, a collagen gel vehicle, can transfer the active antibody to cranial suture sites and is retained over time. In Aim 1 we will use this novel approach to identify FN antagonists that can prevent suture fusion in rabbit calvarial organ culture. The candidate reagents will be evaluated to determine their optimal suture-perturbing influence and working concentrations prior to in vivo rabbit studies. In Aim 2 we will determine characteristics of the collagen vehicle which will enhance the retention and delivery of reagents in a normal rabbit animal model. Together, studies in Aims 1 and 2 will provide a cost-efficient and time-saving method to identify and optimize the most effective suture perturbing reagents. In Aim 3 the FN antagonist selected from studies conducted in Aims 1 and 2 will be delivered to craniosynostotic rabbits to assess its ability to prevent cranial suture re-ossification. Together, these studies will 1) produce an effective screening method for potential cranial suture-perturbing reagents, 2) provide valuable information on the biology of cranial suture formation and 3) determine the potential for therapeutic applications of FN antagonists in the treatment of **craniosynostosis.**

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: FUNCTION OF MSX2 AND TWIST IN CALVARIAL MORPHOGENESIS**

  Principal Investigator & Institution: Maxson, Robert E.; Professor; University of Southern California 2250 Alcazar Street, Csc-219 Los Angeles, Ca 90033

  Timing: Fiscal Year 2002

  Summary: This is a proposal to investigate mechanisms of cranial patterning and the pathophysiology of of **craniosynostosis,** a human developmental defect characterized by the premature fusion of calvarial bones. Recent findings in human genetics have demonstrated that mutations in several genes can produce **craniosynostosis** syndromes. We showed that an activating mutation in the homeodomain protein Msx2 causes **craniosynostosis,** Boston type. Several groups have demonstrated that activating mutations in FGF receptors 1-3 cause Crouzon, per, Jackson-Weiss, and Pfeiffer syndromes. Loss of function mutations in the basic HLH protein M-twist are responsible for Saethre-Chotzen syndrome. Despite the identification of specific genetic defects that cause **craniosynostosis,** the cellular an developmental mechanisms underlying this disorder re poorly understood. In this proposal, we focus on these unresolved issues through an investigation of the function of the Msx2 and twist genes. In this proposal, we focus on these unresolved issues through an analysis of the function of the Msx2 and twist genes. We based our proposal on several key findings. First, gain of function and loss of function phenotypes in the mouse suggest a critical and complex role for the Msx2 and twist genes in calvarial morphogenesis, and in the differentiation of calvarial osteogenic cells. Second, a variety of studies suggest that Msx and twist genes are likely to function in growth factor mediated signaling in calvarial development-Msx genes in the BMP and possibly FGF pathways, twist in the FGF pathway. Third, our preliminary data with the Kedes group suggest that the Kedes group suggest that the Msx2 and

twist proteins can interact physically and functionally (Project 2). These data are the foundation of our overall hypothesis that Msx2 and regulate calvarial morphogenesis through effects on specific cell populations in the calvarial plates and sutures, that these effects re mediated by BMP and FGF signaling, and that a synergistic interaction between Msx2 and twist is a key aspect of this regulation. These are our specific aims: First, we will document in greater detail now normal cranial development is altered in Msx and twist mutant mice, and we will use chimera analysis to identify the tissues in which Msx2 and twist are required for calvarial development Third, we will develop structure-function assays for Msx2 and twist in calvarial development. Third, we will develop structure-function assays for Msx2 and twist in calvarial development, and ultimately test the hypothesis that a synergistic interaction between Msx2 and twist is required for calvarial development. This work will provide fundamental information about the molecular genetics calvarial development and may explain how mutations in three different classes of genes, Msx, fgfr, and twist-produce **craniosynostosis** in humans.

Website: http://crisp.cit.nih.gov/crisp/Crisp_Query.Generate_Screen

- **Project Title: GENES INVOLVED IN EAR DEVELOPMENT**

  Principal Investigator & Institution: Mansour, Suzanne L.; Assistant Professor; Institute of Human Genetics; University of Utah Salt Lake City, Ut 84102

  Timing: Fiscal Year 2004; Project Start 01-JUL-1993; Project End 28-FEB-2007

  Summary: (provided by applicant): The main objective of this research is to determine the identities, interactions and regulation of genes that participate in the development and/or function of the mouse peripheral auditory and vestibular systems. Previously, a gene trap screening strategy was employed to identify and mutate genes expressed in or adjacent to the developing inner ear. One of the genes found in the screen, Dusp6 is expressed in otic mesenchyme and encodes a dual-specificity protein phosphate that inactivates the mitogen-activated protein kinase, ERK, a downstream effectors of Fibroblast Growth Factor (FGF) signaling. FGFs play critical roles in many aspects of otic development and Dusp6 mRNA is not only expressed in many sites of FGF signaling, including otic sites, its expression also depends on FGF signaling. Furthermore, mice that lacks Dusp6 have partially penetrate postnatal lethality associated with small size and **craniosynostosis.** Affected animals also have ossicle and otic capsule abnormalities. The size and cranial phenotypes are characteristic to different extents of humans and mice with dominant activating mutations in FGF receptors. Hearing impairment is variably associated with the human mutations, but has not been evaluated in the mouse models. Taken together, these data suggest the hypothesis that DUSP6 is a partially redundant negative feedback regulator of FGF signaling during the development of otic and other tissues. Three Specific Aims are proposed to test the hypothesis. First, the ontogeny and cellular basis of the otic phenotypes of Dusp6 null mutants will be characterized and the status of the inner ear will be determined. Next, expression analysis performed during the critical period for development of the Dusp6 phenotypes will be used to identify candidate FGF signaling pathways mediating those phenotypes. The otic phenotypes of mouse models of Pfeiffer (FGFR1), Apert (FGFR2) and Muenke (FGFR3) Syndromes will be compared with those of Dusp6 mutants and genetic interaction studies will be used to determine which of the FGFR signaling pathways are regulated by DUSP6. Finally, expression analysis will be used to evaluate other ERK phosphates for potential redundancy with Dusp6 and their roles in otic development will be defined genetically. As the mouse ear is very similar to that of humans, we expect that our studies will apply to human ear development and shed