Fibroblast growth factor receptor 3 mutation (Pro 250 Arg): A study of its phenotypic- genotypic expression in craniosynostotic patients & their families

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Abbreviations List

3D CT	Three Dimensional Computed Tomography
Alx4	Aristaless-like Homeobox 4
Arg	Arginine
BMP	Bone Morphogenic Protein
bp	Base Pair
BSID-II	Bayley Scales for Infant Development, second version
CAM	Cell Adhesion Molecule
CHD	CAM Homology Domain
CBL	Calculated Blood Loss
CD adherence	Calvarial Dural Adherence
CD14	Cluster of Differentiation 14
CT	Computed Tomography
Cys	Cysteine
DAG	Diacylglycerol
ddNTPs	Dideoxyriboneucleotid
DNA	Deoxyribonucleic Acid
dNTPs	Deoxyriboneucleotid
EBV	Estimated Blood Volume
Hct	Haematocrit
ECM	Extracellular matrix
FGF	Fibroblast Growth Factor
FGFR	Fibroblast Growth Factor Receptor
FGFRb	Fibroblast Growth Factor Receptor b splice form
FGFRc	Fibroblast Growth Factor Receptor c splice form
FoxC1	Fork Head Box c1
FRS2	Fibroblast Growth Factor Receptor Substrate 2
G	Guanine
Glu	Glutamic Acid
GRB2	Fibroblast Growth Factor Receptor Bound Protein
HSPGs	Heparin Sulfate Proteoglycans
ICD	Intercoronal Distance

INTRODUCTION

The modern era of diagnosis, evaluation, and management of the patient with craniosynostosis probably began in the late 1960s with Tessier's dramatic demonstration that the cranial vault and midface could be surgically reconstructed in patients with Apert and Crouzon syndromes. Before then the craniosynostoses had received little attention, though they had been studied to some extent in an almost nineteenth-century taxonomic manner.

Early in this century, Roentgen's discovery of the radiograph permitted documentation and long term follow-up of the associated cranial vault abnormalities. These findings focused attention on the cranial sutures and prompted the development of the strip craniectomy, a surgical technique that was generally unsuccessful in achieving any semblance of normal craniofacial form.

In the late 1940s, Gillies of London performed an unsuccessful le Fort III midface advancement and publicly concluded that this surgical procedure was too dangerous to be performed ever again. He obviously did not anticipate the contributions of Tessier less than two decades later.

Probably the most important result of Tessier's surgical demonstration, aside from offering hope for the first time to the craniosynostosis patient, was the organization of multidisciplinary craniofacial teams around the world. But it should be noted that Pruzansky had previously established his pioneering center in Chicago.

The development of multidisciplinary craniofacial teams represented a renaissance in the study of craniosynostosis, Clinical geneticists began studies that shed light on the inheritance patterns and physical features of the various syndromes and showed that many of those syndromes were highly variable in their phenotypic expression. Their studies permitted the organization of a clinical database that served as the foundation for the clinical investigations of the other members of the team.

Psychologists demonstrated that many of these patients had normal and, in some cases, even above average intelligence. These findings stood in contrast to previous studies indicating that many of the affected patients had below-normal intelligence and were therefore not candidates for rehabilitation. This led to a marked change in attitude and a true commitment by the team members to offer all that was available to the affected patient.

Otolaryngologists documented unique airway and hearing problems. Ophthalmologists documented retinal changes and, perhaps even more importantly, extraocular muscle pathology. They also established criteria for surgical correction of the latter. Speech pathologists treated the unique speech problems in patients with syndromic craniosynostosis.

In the beginning, radiologists on craniofacial teams had only plain films and tomograms for radiographic documentation of the skeletal pathology. Within a few years, however, the CT scan became available. This was one of the most important developments for the craniofacial teams. For the first time, the clinicians were truly able to "see inside the head", the associated

pathology could be documented and treatment could be scientifically planned.

A second generation of craniofacial surgeons emerged and, working with their neurosurgical colleagues, showed that the infant could safely undergo craniofacial surgery. Fronto-orbital advancement with cranial vault remodeling became accepted therapy before the age of one year, Moreover, midface advancements were recommended at some centers for children at the age at which they entered school in an effort to reduce the facial stigmata that resulted in psychosocial trauma.

As the craniofacial teams matured, treatment protocols improved; some operations were abandoned and new ones recommended. Longitudinal studies provided clinical data by which the treatment protocols could be evaluated and from which new treatment recommendations could be made. In more recent years, ultrasonic prenatal diagnoses have become more frequent. A particularly exciting new development has been the documentation by molecular geneticists of fibroblast growth factor receptor (FGFR) mutations in some of the syndromes. Clinical and laboratory studies then followed, documenting these changes in the cranial sutures.

Dr Samuel Pruzansky, the late Professor of Pediatrics, and the founder of the Center for Craniofacial Anomalies, University of Illinois, Chicago, summarized his lifetime experience with craniosynostosis in these vivid words;

"Craniosynostosis intrigues me as a drama of nature in which a growing brain and its hydrodynamic forces compete against the rigidities and

sometimes yielding barriers of a brain case derived from dermal placodes and primitive cartilage. In the second act, the drama is intensified by the surgeon's knife that unlocks nature's barriers, stills the storm and brings tranquility in form and size, now the third act is to be written by genes and signaling peptides, how will it turn out over the long term?"

AIM OF THE WORK

Gene mutations causing several craniosynostosis syndromes have been identified in the last decade. A growing body of literature is published on the prevalence of such mutations in specific forms of craniosynostosis as well as their genotype/phenotype correlation.Very few studies have correlated these mutations to specific clinical features or treatment outcomes.

We undertook this prospective, controlled study to evaluate the incidence of FGFR3 P250A mutation in patients with craniosynostosis and their families (parents and sibs). In this mutation the gene coding for Fibroblast growth factor receptor 3 (FGFR3) has a single nucleotide substitution C→G at position 749 of the gene leading to substitution of proline amino acid by arginine at position 250 of the resulting protein receptor (C749G; Pro250Arg). This mutation is proven to enhance the receptor binding to fibroblast growth factors which ultimately lead to exaggeration of the normal function of this receptor in suture morphogenesis and induces its premature closure. The mutation incidence was tested in a cohort of 23 consecutive cases of craniosynostosis treated at the South West Craniofacial Center, Phoenix, Airzona, USA.

Furthermore we aimed to test the hypothesis that the synostotic sutures in patients harboring the mutation have a different biological behavior than those who don't. So we assumed that those patients have a peculiar clinical and radiological presentation, operative findings, and postoperative cosmetic and functional outcomes. We tested the presence or absence of the mutation against several preoperative clinical, radiological features, intraoperative blood loss, degree of calvarial-dural adhesions and postoperative cosmetic

and functional outcome parameters to prove or disprove the original hypothesis.

The expected benefit from correlating this mutation to a specific clinical, radiological, operative and outcome findings is proving or disproving the value of testing for FGFR3 P250A mutation in diagnosis, operative planning, prognosis and counseling of patients with craniosynostosis.

LITERATURE REVIEW

Definition of craniosynostosis

The term craniostenosis is used to indicate premature fusion of one or more sutures. The term craniosynostosis is more widely used. Technically craniosynostosis is the process of premature sutural fusion-craniostenosis is the result. Actually, the terms have been used interchangeably, and craniosynostosis seems to be replacing craniostenosis as the more common term. The word faciostenosis has been used to indicate premature closure of facial sutures, although this feature is uncommon except in some craniosynostosis syndromes on occasion (20).

Incidence

Based on the admissions of children in the French hospitals, the frequency of craniosynostoses, including syndromic cases, has been estimated at 1 in 2,100 (117). Others quote figures around 1 in 2,500 live births (2).

Syndromic forms constitute around 15% of the total [Marchac 2000]. Apert and Crouzon syndromes are the most frequent among syndromic forms; each is approximately 4.5% [Aleck 2004]. As for isolated forms, sagittal synostosis being the commonest occurring in 40-55% of isolated cases. Coronal represent 20-29%, Metopic 10-14% and lambdoid in 2-4% (117).

Regarding gender predilection, sagittal is commoner in males with a male: female ratio of 4:1. Unilateral coronal synostosis is commoner in females with female: male ratio of 3:2. In metopic, lambdoid, and bilateral coronal synostosis, there appears to be no clear gender predilection (103).

Historical Perspectives

Unusual head shape has made a striking impression throughout history and across cultures. Hippocrates described cranial deformations and their relationship to the cranial sutures as follows; “Men's heads are by no means all like to one another, nor are the sutures of the heads of all men constructed in the same form. Thus, whoever has a prominence in the anterior part of the head . . . in him the sutures of the head take the form of a Greek letter tau, T . . . . But whoever has the prominence in the back part of the head, in him the sutures are constructed in quite the opposite form to the former . . . But whoever has a prominence of the head both before and behind, in him the sutures resemble the Greek letter eta, H” (2, 26).

Although the statement cannot be interpreted with certainty, when Hippocrates spoke of different forms of the cranial sutures, he may have been referring to premature synostosis of the coronal or lambdoid sutures in the first two instances, and to closure of all the sutures in the third instance. According to Giinther, the association of arch palate and premature synostosis of the coronal suture may well have been known to Hippocrates (17).

The significance of the cranial sutures was known to Galen, who described headache and exophthalmos in patients with too few sutures. The first scientific investigator of cranial deformities in modern times was Stjmmerring, who in 1800 described the structure of sutures and considered them of primary importance in skull growth. He noted that premature sutural