The Human Cranial Sutures in Health and Disease

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ABSTRACT

This thesis describes the structure of normal cranial sutures and those which have undergone premature fusion or craniosynostosis. It also reports the results of investigations of human sutures to identify possible underlying aetiologies which result in premature fusion. In addition this thesis also investigates the effects of these abnormally fused sutures on the affected individuals, in particular the secondary effect on intracranial volume both in those with just a single affected suture and those with multiple sutures involved as part of a syndrome. These later individuals often have an identified causative genetic mutation and in these cases genotype/phenotype investigations have been undertaken.

The results of these studies will aid clinicians to make informed decisions regarding the timing and type of surgical intervention in some individuals with the common forms of single suture craniosynostosis. The results of the genotype/phenotype studies have failed to demonstrate clear differences in Apert syndrome, but the study population was small. The continuing search for the aetiology of craniosynostosis remains elusive, but the somatic mutation study and the detailed imaging studies refine the search for the underlying pathological process.

DECLARATION

This thesis contains original material which has been previously published in peer reviewed journals. I have acted as the principal author for the main body of the thesis. I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution. To the best of my knowledge and belief the thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give my consent to this copy of my thesis being made available for photocopy and loan from the University of Adelaide.

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Peter John Anderson

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STATEMENTS OF CONTRIBUTORS

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DEDICATION

This thesis is dedicated to my father

CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

1.1 Introduction

Cranial sutures are important because they are sites of bone growth which impacts on the development of the craniofacial skeleton[1]. They have an unusual combination of functions in that they also provide a firm bond between adjacent calvarial bones, while simultaneously allowing some movement between them.

Cranial sutures have had several definitions but concisely have been defined as "the dense fibrous connections between adjacent intra-membranous calvarial bones which permit minor movement"[2]. The differences in terminology between different authors is often due to the distinction made between the a suture area which consists of adjacent edges of bone together with the soft tissue which separates them and a suture proper which consists solely of the intervening soft tissue[3].

During human embryological development *in utero* the primary ossification centres of the calvarial bones (parietal, frontal occipital) develop. Growing bone radiates out from them and, at about 18 weeks gestation, the growing osteogenic fronts meet and form sutures at the margins of the calvarial bones[4]. The position of the cranial sutures is determined by dural reflections and absence of a specific dural reflection may lead

to failure of a suture to develop with resultant ossification at that site[5]. The sutures are formed from the ectomeninx layer of mesenchyme which is derived from embryological paraxial mesoderm and neural crest cells, but the contribution from each of these sources varies for each type of cranial suture[6] (see Figure 1.1 below). The skull continues to grow *in utero* by appositional bone growth at the suture. To allow for the movement of the skull bones in relation to the developing brain and facial region, the calvarial sutures need to remain as active osteogenic regions but remain unfused until the brain and facial region stop growing post partum.

At birth the fontanelles, dura mater and pericranium overlying the frontal, parietal and occipital bones are all continuous with the sutures. The sutures present in most human populations are the sagittal and metopic (midline structures) and the paired coronal and lambdoid sutures. (Figure 1.1)

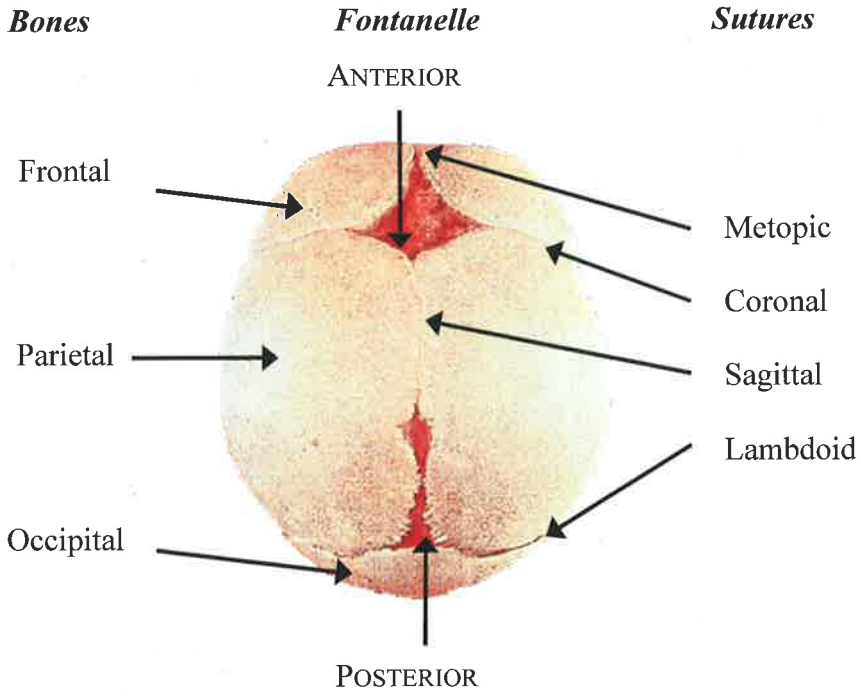

Figure 1.1 The skull of a newborn infant demonstrating the metopic, coronal, sagittal and lambdoid sutures. Note that the small anterolateral and posterolateral fontanelles cannot be seen on this view.

Sutures can occasionally occur in humans in sites other than the common positions shown previously. These include the paramedian longitudinal suture which is parallel to the sagittal suture, and the transverse occipital suture which is a horizontal superior suture joining the lambdoids to the nuchal line[7]. Other common smaller variants may occur connected to one of the main sutures enclosing an accessory bone. These are termed Wormian bones which undergo calcification within a suture and are named after the seventeenth century Danish anatomist who first described them. They occur most commonly the lambdoid suture and are particularly prevalent in cleidocranial dysostosis and osteogenesis imperfecta[8].

The sutures themselves contain undifferentiated mesenchymal derived tissue that provides the resource of cells for the growing bone fronts on either side (Figure 1.2). Within each suture, undifferentiated mesenchymal stem cells produce osteoprogenitor cells, a committed form of stem cell which can undergo further differentiation to produce firstly pre-osteoblasts and finally osteoblasts which secrete a collagen-proteoglycan extracellular matrix (ECM). Mineralization of the ECM traps osteoblasts which become osteocytes, the final maturation phase of the bone cell lineage.

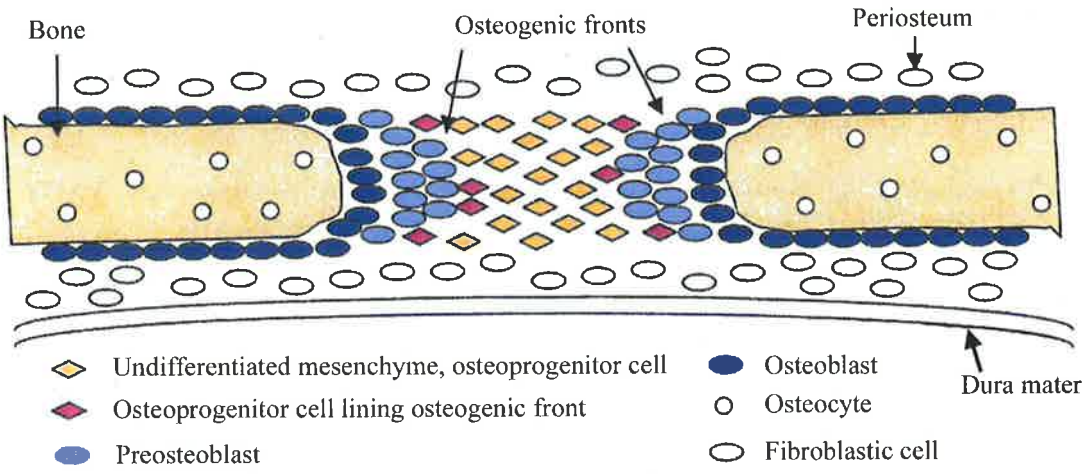

Figure 1.2 Schematic cross section of a sagittal suture. Osteoblasts surround bone and osteo-progenitor cells are present at junction of the bone and the proliferating osteogenic front.

The sutures have important functions in that they permit movement between adjacent calvarial bone allowing moulding of head to occur during childbirth (which reverts during the first week post partum). Later the sutures become important as sites for bone deposition as the calvarial bones increase in size, particularly during infancy during the period of rapid growth of the underlying brain[9]. The growth of the skull, however, slows markedly by the age of four years with most growth occurring during the first 6 months of life. Interestingly, the brain ceases growth before the calvarial sutures stop growing and finally fuse[9]. Although suture fusion may (or may not) occur in later adult life the metopic suture behaves differently from the others and fuses early in life, sometimes *in utero* but usually by three years of age[10].

Normal suture fusion starts with the cessation of proliferation and the differentiation of pre-osteoblasts into osteoblasts at the osteogenic fronts. The margins of the sutures alter and gradually encroach into the intervening space, changing from a flat edge to

one with increasing interdigitation. Bone bridges are formed usually on the endocranial surface and the space becomes obliterated with immature calcified tissue. Final remodelling results in its replacement by a mature bone pattern indistinguishable from adjacent cranial bone.

In addition to the fusion process that normal sutures may undergo as part of their natural history, premature (or pathological) fusion of calvarial sutures may also occur. The pathological process by which a cranial suture undergoes early fusion was termed craniosynostosis by Otto in 1830[11]. This process has been reported in many different human populations, with an incidence of 1 in 2500 live births in the Western World[12].

Craniosynostosis can be classified as either a primary or secondary event. Most cases are primary and in many cases the cause remains uncertain but some cases are due to an identified underlying genetic mutation. A much smaller number of individuals have craniosynostosis secondary to an identifiable cause (including metabolic, haematological, or growth disturbances, mucopolysaccharidoses or pharmacological agents[12]).

Craniosynostosis has a variety of clinical presentations. It may occur at a single suture as an isolated anomaly or may occur at multiple sutures sites and may have limb and visceral anomalies as part of a syndrome. These two types are often distinguished as non-syndromic and syndromic craniosynostosis. Presentation of all craniosynostosis usually occurs *post partum* but the recent recognition that syndromic cases can be identified before birth has led to increasing antenatal diagnosis[13].

The causes of the majority of cases which are the isolated single suture remains uncertain, but hormonal, mechanical and local factors have all been implicated in the aeitiology[12]. Indeed it has been suggested that the process may be a common end result of a number of different initiating factors[9]. Very recently, underlying genetic factors have also been identified in occasional cases[14].

The syndromic forms often have an identified genetic mutation and while all inheritance patterns are recognised most of the craniosynostosis syndromes have an autosomal dominant pattern of inheritance. There are over 100 syndromes that show craniosynostosis as part of the clinical features[15]. Commonly inherited forms are Apert, Crouzon, Pfeiffer, and Muenke syndromes, which all result from mutations of the *FGFR1-3* genes[16,-19]. Other known causative mutations include Saethre-Chotzen syndrome which results from mutations of the *TWIST* gene[20] and Boston type craniosynostosis which results from mutations of the *Msx* gene[21].

The relationship between genotype and phenotypes of the common syndromes of Crouzon and Pfeiffer syndromes is curious. Different *FGFR2* mutations can produce the same syndrome phenotype but the same mutation can also produce different phenotypes[18]. Within both syndromes there is a range of severity of the phenotypes with some overlap of features between the different syndromes[22]. However, the identification of underlying mutations in syndromic craniosynostosis is highly suggestive that there is likely to be significant genetic control over suture patency and closure in non-syndromic craniosynostosis.

This introduction has outlined the structure, function and pathology of the cranial sutures. These provide an important background to the studies undertaken both to increase understanding of their natural history and also the mechanisms and consequences of early pathological fusion (craniosynostosis).

1.2 Literature Review

1.2.1 Intracranial Volume Measurement

Clinicians and scientists have previously attempted to measure the intracranial volumes in those with craniosynostosis because it has been assumed that a consequence of early suture fusion would result in a reduction in the intracranial volume, with implications for subsequent brain development and the basis for surgical management[23]. Previous attempts at accurately measuring the intracranial volume have been limited both by the methodology employed to measure the abnormal skull volume and also the accuracy of the available normal data.

Previously both direct and indirect methods to measure intracranial volume have been used. Direct measurement was undertaken using dried skulls and nylon models using water, rice or mustard seeds to measure the volume[23]. Indirect methods have been attempted utilising 3D CT scans but historically these had the disadvantage of relatively low resolution[24].

The advent of increasingly higher resolution CT scanners allowed the use an indirect method, utilising the Cavalieri method whereby slices are processed at each time and the area measured. The volume associated with each slice is the area of intersection multiplied by the slice thickness, with the required volume given by the sum of the volumes for each slice[25,26].

Previous studies of intracranial volume of cases of craniosynostosis using 3D CT scans include the investigation of sagittal and metopic craniosynostosis before and after surgery. The methodology employed used thick (5mm) CT slices and found that intracranial volume was not diminished when compared to the derived normal volumes[23,24].

In his invited discussion of the study by Posnick *et al.*, Marsh highlighted the major weakness of all existing CT studies to date which was the use of "normal" values. These had all been derived using different methodology from the techniques used to measure the intracranial volumes of skulls with fused sutures[27]. The subsequent measurement of over 300 normal paediatric intracranial volumes using the same techniques employed to investigate crania affected by craniosynostosis, led to the development of new normal values for both sexes from birth (the Abbott-Netherway normals[28]). This was an essential foundation to address the acknowledged major weakness of all the previous studies.

The study of sagittal synostosis is important because it is the commonest of the non-syndromic single suture craniosynostoses. Metopic synostosis is also an important condition to study in detail because as well as being relatively common it is particularly interesting because previously it has been noted that anthropometric comparisons between the metopic age groups indicate that the trigonocephalic phenotype worsens through time[29] and this raises the possibility that this would impact on the cranial volume.

There have been no previous attempts to study intracranial volumes as part of the investigations into genotype/phenotype correlation in Apert syndrome. However, there have been previous studies into the intracranial volume of a Apert syndrome where the underlying genotypes have not been recorded[23,30]. These found that the intracranial volume is within the normal range at birth but beyond six months of age rises to and then remains above normal values.

The existing genotype/phenotype investigations of other clinical features have produced conflicting results[31-34], but interestingly, a worse mental outcome has been reported overall in cases with a mutation at the 253 position in the *FGFR2* gene[34]. One explanation for this difference could be that there are pre-operative differences in the intracranial volumes of the two genotypes. This possibility was investigated as part of the current study.

The new finding of cases in whom there were simultaneous *TWIST* and *FGFR* mutations inevitably means that there are no previous reports of such cases. In addition to measuring the intracranial volumes the results were compared to the intracranial volumes both with Abbott-Netherway normals and values obtained for each syndrome phenotype with just a single *FGFR2* mutation to investigate if there was any discernable effect attributable to the additional *TWIST* mutation.

1.2.2 Genetic Studies of Sutures

The identification of specific genetic mutations underlying the common syndromes that exhibit craniosynostosis[16-21] is suggestive that there is significant genetic control

over suture patency and closure. This could also extend to single suture non-syndromic craniosynostosis which raises the possibility that genetic mutations could also be present in isolated single suture craniosynostosis. The results of such studies to date have been disappointing, although very recently there has been identification of mutations of the *ENFA41* gene[14] in a small number of cases.

Curiously, there have been clinical reports of somatic mutations occurring in both the *FGFR2* and *FGFR3* genes in humans[35,36]. In both of these genes it is recognised that mutations can produce craniosynostosis.

This observation, and the continuing failure to identify underlying mutations in many cases, led to the hypothesis that non-syndromic craniosynostosis could be due to gene mutations limited to the affected cranial suture cells, thus displaying the phenomenon of somatic mutations. This hypothesis was tested as part of this study.

1.2.3 Imaging studies of sutures

Current knowledge of suture biology has been ascertained as a result of morphological studies of normal cranial sutures (and rarely those undergoing craniosynostosis). However, many of these studies have used cranial sutures from animals (often mouse or rabbit)[37,38], so there is some uncertainty as to how applicable the findings are to humans. These studies were initially undertaken often using histological investigations with simple staining[39]. (Figures 1.3 and 1.4).

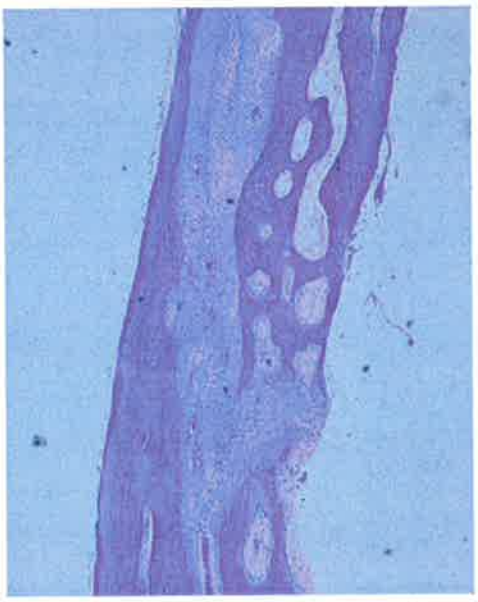

Figure 1.3 Open coronal suture, H&E x100

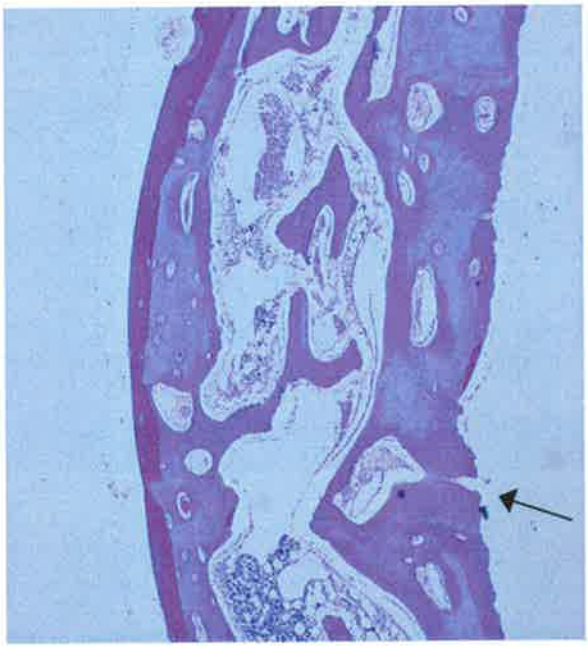

Figure 1.4 Fusing sagittal suture, H&E x100 (arrow marks suture remnant)

More recently, sophisticated investigations using antibodies and autoradiography histological studies have been undertaken to determine the composition of the sutures[37]. (Figure 1.5).

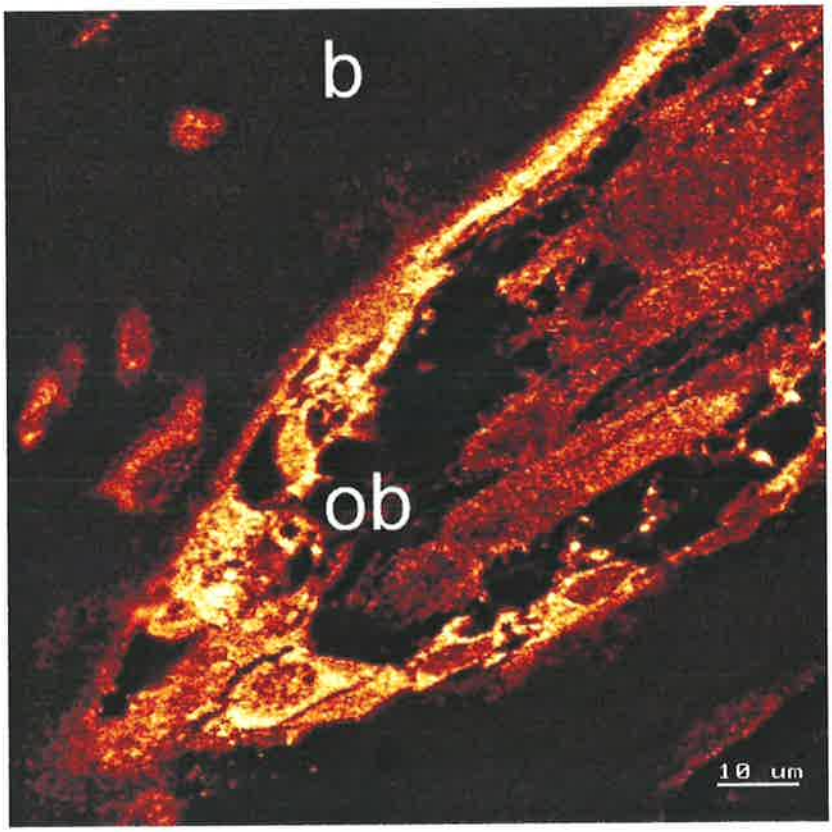

Figure 1.5 Immunohistochemistry to identify Retinol Binding Protein 4 (RBP4) localised to the cytoplasm of osteoblasts (ob) lining the developing bone (b) in an unfused coronal suture

The development of CT scans has led to their use as investigative tools[40,41]. However, very recent technological advances have provided the potential to further refine our understanding of the ultra structure by the use of advanced scanning technology, which offer the possibility of more detailed resolution. This has been used to study the ultra structure of the open suture and also those undergoing fusion.

1.3 Aims of Study

The study of early fusion of a cranial suture, and its consequences on the shape of the growing cranium, have been of interest to scientists since the pioneering work of Virchow in the nineteenth century[42]. These studies of the cranial sutures seek to extend this work but also refine the existing studies utilising new technologies to overcome the recognised limitations of the previous investigations.

The specific aims are:

Firstly, to measure the intracranial volumes of two groups of cases with non-syndromic craniosynostosis, namely sagittal and metopic synostosis, and compare the values with sex and aged matched normal values. The intracranial volume will be determined if it is normal or otherwise, and so review the clinical management of these conditions.

Secondly, to measure the intracranial volumes of cases of Apert syndrome and compare the results both with sex and aged matched normal values but also to compare the two common genotypes with each other.

Thirdly, to measure the intracranial volumes of cases with co-existing *FGFR2* and *TWIST* mutations and to compare the values with sex and aged matched normal values and also the values of syndrome phenotypes with a single *FGFR* mutation to investigate the influence of the additional *TWIST* mutation.

Fourthly, to investigate the possibility of somatic mutations in cranial suture cells which have undergone craniosynostosis.

Fifthly, to investigate the ultra structure of open, fusing and fused cranial sutures using state of the art micro-CT scanning technology.

Lastly, to study the antenatal ultrasound of those born with non-syndromic single suture craniosynostosis to investigate whether the craniosynostosis can be identified *in utero*.

CHAPTER 2

METHODS

CHAPTER 2

METHODS

2.1 Case selection

2.1.1 Intracranial Volume Measurement

The patients with metopic synostosis and non-syndromic sagittal synostosis studied in chapter three were identified from the Australian Craniofacial Unit (ACFU) database. To be included each case had to have a pre-operative high resolution 3D CT scan (which extended above the vertex to below the skull base), available for analysis.

The patients with Apert syndrome studied in chapter four again had the selection criteria of identification from the ACFU database and the availability of a pre-operative high resolution 3D CT scan, but in addition had to have undergone genetic screening and the underlying mutation in the *FGFR2* gene identified.

The double mutation craniosynostosis patients studied in chapter four similarly had undergone the same inclusion criteria but had undergone screening of the *TWIST* gene to identify the position of the mutation in this gene.

2.1.2 Genetic Studies of Sutures

The consecutive patients in chapter five with single suture craniosynostosis who had no underlying mutation of the *FGFR* or *TWIST* genes in leukocyte derived DNA were eligible for entry into the study.

2.1.3 Imaging studies of sutures

The six patients whose sutures were studied in chapter six using micro-CT and scanning electron microscopy had their sutures collected as part of their transcranial corrective surgery during a six month period in 2004. One further infant had a suture specimen collected as part of a craniotomy for intracranial tumour.

The patients with the four different types of craniosynostosis studied in chapter six who had antenatal ultrasound examinations suitable for examination, all presented to the Australian Craniofacial Unit over a four month period in 2003.

2.2 Methods

2.2.1 Intracranial Volume Measurement

3D CT MEASUREMENT

Intracranial volume measurement was undertaken using the Silicon Graphics Computer workstations of the ACFU. Each CT slice was processed in turn to obtain the area of intersection. The Persona software package developed by the research unit at the ACFU, Women's and Children's Hospital, Adelaide was used to outline the bone in each CT slice at the specified soft tissue/bone threshold and to edit the contours in a clear and detailed fashion[25]. A threshold of 150 Hounsfield units was selected for determination of the bone surface for the children in this study. The software package enabled simultaneous display of coronal, sagittal and axial cuts and had a magnification facility to precisely identify relevant landmarks on the studies.

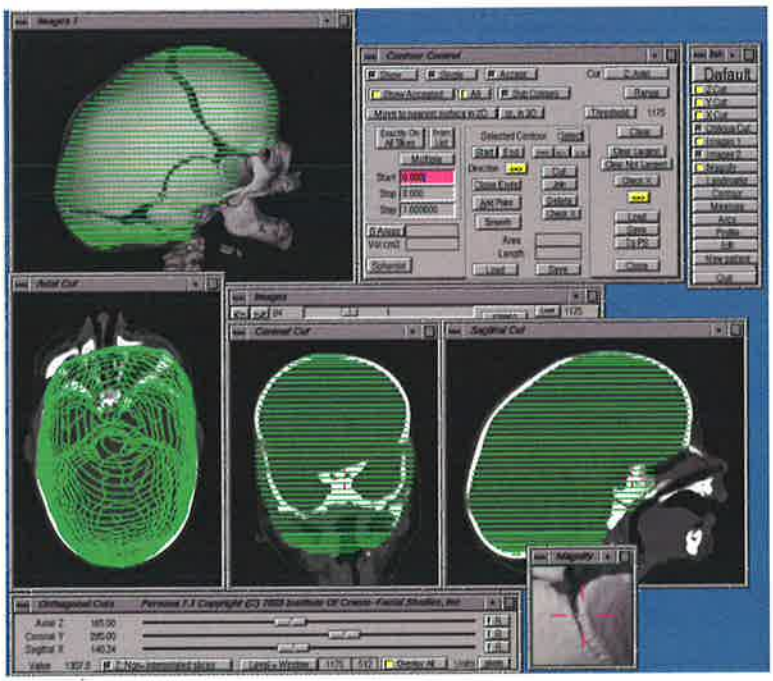

Figure 2.1 Simultaneous display of coronal, sagittal and axial cuts showing the contours used for intracranial volume measurement

Measurements were undertaken using slices ranging from 1 to 5mm, (the thinnest possible), to calculate the area and then these values summated to establish the intracranial volume for each case. An intracranial volume score along with the standard deviation was determined as the difference between the natural logarithm of the patient's intracranial volume and the sex-matched normal curve evaluated at the patient's age, divided by the standard deviation.

The results for each case were compared to the sex and age corrected normal using the Abbott-Netherway curves which are based on normal CT scans measured in the same manner as the cases with craniosynostosis. This is different to the other "normal" values of Lichtenberg and Dekaban which are derived values from two dimensional radiological data[43,44]. Sample t-tests were then undertaken between the mean standard deviation scores between the sagittal and metopic synostosis group and the sex-matched normal group to look for differences.

2.2.2 Genetic Studies of Sutures

Suture cells were harvested at the time of transcranial correction in all cases. Approximately 1cm^2 region from the fused and, where possible a patent suture, was removed during surgical correction. Each sample included approximately 2mm of bone either side of the suture which was cut into chips for culture according to protocol[45].

The osteoprogenitor cells from each site were cultured separately *in vitro* and after the third passage their DNA was extracted and the comprehensive screen of the *FGFR1-3* and *TWIST* genes was undertaken on the DNA of each suture cell sample.

DNA samples from the cases were subjected to a comprehensive screen using denaturing high performance liquid chromatography (DHPLC) for mutations within the *FGFR1-3* and *TWIST* genes (Figure 2.2). The screening protocol consisted of examination of all exons previously shown to harbour mutations known to be responsible for the common craniosynostosis syndromes. These exons included the *FGFR1* exon 7 (Pfeiffer syndrome), the *FGFR2* mutation hot spots associated with Apert, Pfeiffer, Crouzon and Beare-Stevenson syndromes (exons 8, 10 and 11), *FGFR3* exons 7 (Muenke syndrome) and 10 (Crouzon syndrome with Acanthosis Nigricans) and the single coding exon of *TWIST* that is associated with Saethre-Chotzen syndrome. The screen also included the six additional exons of *FGFR2* that have previously been shown to contain mutations associated with Crouzon, Pfeiffer or familial sagittal craniosynostosis. Genomic PCR products showing anomalous denaturing DHPLC patterns were sequenced to identify any nucleotide alteration.

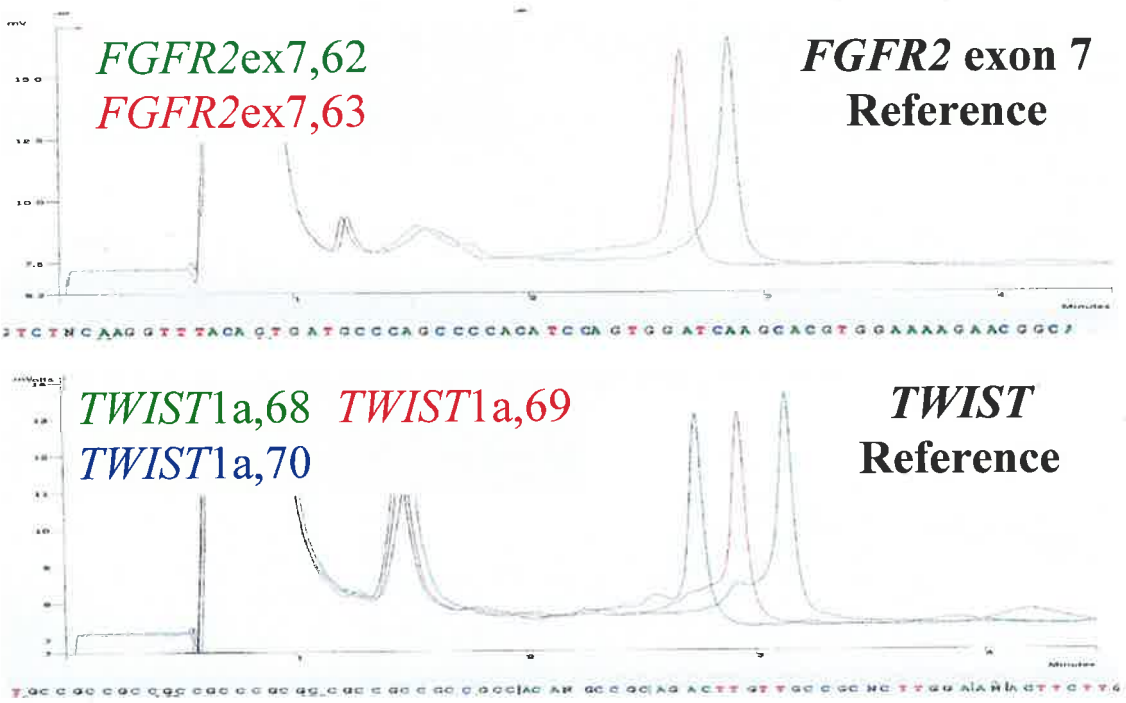

Figure 2.2 Reference DNA analysis screening for known *FGFR2* mutations in exon 7 and *TWIST* mutations.

2.2.3 Imaging studies of sutures

MICRO-CT

Specimens selected to undergo micro-CT analysis and following harvest were immediately placed into a polyethylene container filled with RNA later solution (Ambion). The position of the specimen in the container was maintained using polystyrene blocks. Using a Skyscan micro-CT 1172 scanner (Figure 2.3) sutures at different stages of fusion were scanned at the highest possible resolution. The limit of the available resolution was inversely proportional to the specimen size. Digital images were stored and then used both to demonstrate the surface features and to undertake 3D reconstructions of the microstructure in three planes.

Figure 2.3 The Skyscan micro-CT 1172 scanner

SCANNING ELECTRON MICROSCOPE

Specimens selected to undergo scanning electron microscopy were stored in alcohol and dried before being coated with carbon prior to mounting (as previously described)[46]. These were loaded into a Philips XL30 multi-function microanalytical scanning electron microscope (Figure 2.4).

Figure 2.4 The Philips XL30 multi-function microanalytical Scanning Electron Microscope

The specimens were scanned using 10kV field emission electron gun and both back scattered images and secondary electron images were obtained. The incorporated Energy Dispersive Spectrometer combined with an EDAX multi-channel analyser enabled elemental analysis of increasing distances from the centre of the suture of the sample of lambdoid synostosis. The sample size for each analysis was approximately 1 micrometer diameter of a flat surface.

All images and the elemental analysis results were all stored on the computer and reviewed.

CHAPTER 3

INTRACRANIAL VOLUME MEASUREMENT: NON-SYNDROMIC CRANIOSYNOSTOSIS

CHAPTER 3

INTRACRANIAL VOLUME MEASUREMENT: NON-SYNDROMIC CRANIOSYNOSTOSIS

3.1 Introduction

The consequences of early suture fusion of a single cranial suture and its effect on brain development remains unclear. It has however been recognised by Virchow over a century ago, that a resulting predictable characteristic head shape occurs.

The impact of the resulting cranial anomaly increases the risk of development of raised intracranial pressure, which if produced, can affect brain development and function. One mechanism thought to be important is that the synostosis and the local absence of calvarial growth result not just in cranial distortion but also in an overall reduction in the intracranial volume. To investigate this hypothesis, studies of the intracranial volume have been undertaken to accurately determine this in an affected population and compare these values to values obtained from age and sex matched normal population.

Previous attempts to determine the intracranial volume have had the weakness that different techniques have been used to determine the volumes of the study and normal populations respectively. Previously a normal population data has been established

studying over 300 normal CT scans using the same technique as employed in the present studies. Values from this study have been used to make a comparison with our study population. This is the first study which overcomes an acknowledged weakness of all the previous studies.

3.2 Sagittal Craniosynostosis

Intracranial volume measurement of sagittal craniosynostosis

Anderson PJ, Netherway DJ, McGlaughlin K, David DJ.

J Clin Neurosci. 2007; Vol 14(5): 455-458.

3.2.1 Hypothesis and Aims

Sagittal synostosis is the commonest of the single suture craniosynostoses, occurring in 1:2500 live births. With the advent of more widespread genetic screening it has become evident that there is a significant sub-population of those with sagittal synostosis who have the polymorphism in the *FGFR3* gene 294C>T (Asn294Asn).

This study was designed to test the null hypothesis that the intracranial volumes of those with non-syndromic sagittal synostosis are the same as a sex and aged matched normal population. A secondary aim was to test the null hypothesis that there is no difference between the intracranial volumes between individuals with non-syndromic sagittal synostosis and those who had non-syndromic sagittal synostosis but who had the common polymorphism in the *FGFR3* gene, 294C>T (Asn294Asn) identified on screening the *FGFR* genes.

3.2.2 Outcome

The study identified that those with non-syndromic sagittal synostosis had a statistically significant increased intracranial volume when compared to sex and aged

matched normal population. This difference existed for both males and females, but was of greater significance in the female study population.

The study identified that there was no discernable differences between the intracranial volumes of those with non-syndromic sagittal synostosis and those who had the polymorphism in *FGFR3* gene, 294C>T (Asn294Asn) and sex and aged matched individuals with non-syndromic sagittal synostosis.

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Clinical study

Intracranial volume measurement of sagittal craniosynostosis

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Abstract

We report 41 cases of non-syndromic isolated sagittal synostosis in which evaluation of intracranial volumes was undertaken. Twenty-six were male and fifteen were female. The measured intracranial volumes were then compared with normal age-corrected values. We have found that intracranial volumes were significantly larger than the normal population intracranial volumes in both sexes. However the statistical significance of this finding was much greater in females, ($p < 0.00002$), than males ($p < 0.040$), which was only of borderline significance. The results confirm smaller, earlier studies that intracranial volumes in sagittal synostosis patients are larger than average for age-corrected normal values. Analysis of a sub-set of six patients with sagittal synostosis who were found to have a common polymorphism 294C > T (Asn294Asn) in FGFR3 (fibroblast growth factor Receptor 3) on genetic testing were compared to age and sex matched cases of non-syndromic sagittal synostosis (without an underlying mutation) which confirmed that there were no discernable differences in intracranial volumes between the two groups. We conclude that this investigation supports the role of cranial re-shaping to improve cosmesis as the primary aim of surgical correction in this condition, in the absence of raised intracranial pressure.

Keywords: Craniosynostosis; Sagittal suture

1. Introduction

The relationship between the early fusion of a cranial suture and resulting characteristic head shape was first recognised by Virchow in the nineteenth century.[1] The goals of sagittal synostosis treatment have been cosmetic improvement of a scaphocephalic head shape; however, there is also a well recognised risk of development of raised intracranial pressure.[2] In such cases volume expansion is also important.

Previous attempts have been made to determine the intracranial volumes of patients with non-syndromic isolated sagittal craniosynostosis to provide clear aims for undertaking surgical correction. However, these have had the weakness that they used different investigative techniques to obtain experimental and normal data.[3–6] Indeed, it has been previously suggested that any progress in the study of intracranial volumes will only occur once normal values have been established.[7] To address this problem the Australian Craniofacial Unit (ACFU), Women's and Children's Hospital, Adelaide, Australia, has studied over 300 computed tomography (CT) scans of normal individuals, which led to the development of normal age-related values for males and females (Abbott-Netherway normal values).[8] We have used these normal values (derived by the same method as our study population), to compare our results from those with non-syndromic isolated sagittal craniosynostosis in an attempt to clarify the relationship between intracranial volume and the resulting scaphocephaly. This study of intracranial volume measurement in sagittal craniosynostosis is the first to address an acknowledged weakness in all previous studies.

Additionally, as a result of genetic testing becoming more widely available, we have found a significant subpopulation of patients who have sagittal synostosis and the common polymorphism 294C > T (Asn294Asn) in FGFR3 (fibroblast growth factor Receptor 3). Currently

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the clinical significance of this finding in relation to sagittal synostosis is unknown, although the clinical impression is that it is of minimal significance.

We also aimed to determine the intracranial volume of this sub-group of patients and compare the results to a sub-group of age and sex matched controls to identify if there were any discernable difference which could be attributed to the presence of the polymorphism.

2. Methods

All patients with a diagnosis of non-syndromic sagittal synostosis on the ACFU data base with pre-operative high resolution 3-dimensional (3D) CT scans were reviewed. The inclusion criteria for the study were clinical scaphocephaly and a fused sagittal suture on the CT scans.[9] Intracranial volume measurement was undertaken using 3D CT scans. The horizontal CT slices of each patient were processed in turn to obtain the area of intersection of the region of interest with each slice. The ACFU Persona software package was used to outline the bone margin in each slice.

Triangulation of the contours was undertaken to detect errors. A threshold of 150 Hounsfield units was selected for these children. Intracranial volume was calculated as the sum of the cross-sectional areas that intersected the region of interest multiplied by the slice separation (the Cavalieri estimator). A bias correction term was applied to compensate for the effects of partial voluming, dependent on slice thickness and separation. This allowed archived CT scan data acquired from different scanners to be used.

The intracranial volumes were then compared to the normal volumes, determined in the ACFU from CT scans of unaffected individuals. The results were then plotted as shown (Figs. 1 and 2).

Two sample Student *t*-tests were used to assess differences between the intracranial volume (ICV) measurements

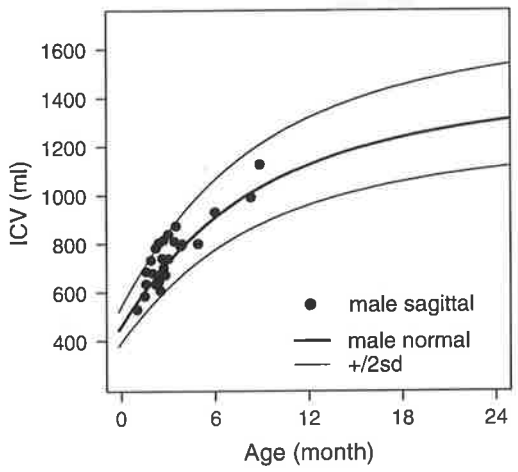

Fig. 1. The intracranial volume (ICV) of 26 males aged 1–9 months with isolated non-syndromic sagittal synostosis (SS).

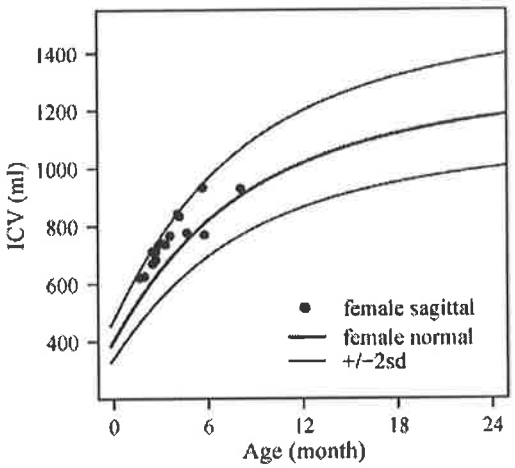

Fig. 2. The intracranial volume (ICV) of 15 females aged 1–9 months with isolated non-syndromic sagittal synostosis (SS).

of the patients with sagittal synostosis and Abbott-Netherway ICV normals.[8] Before applying the statistical tests, the ICV measurements of both the sagittal and normal patient groups were expressed in terms of 'standard deviation scores' to remove the effect of age variation.

The Abbott-Netherway ICV normal curve for each gender has a constant coefficient of variation with age, and formulation is expressed in terms of the logarithm of the ICV and the logarithm of the age from conception. The intracranial volume standard deviation score for each patient was determined as the difference between the natural logarithm of each patient's ICV and the gender-appropriate Abbott-Netherway normal curve, evaluated at the logarithm of the patient's age from conception, divided by the standard deviation.

A subgroup of males who had the commonly occurring polymorphism 294C > T (Asn294Asn) in FGFR3 were also studied and compared to age and sex-matched controls to examine if there were any discernable differences between these two groups.

3. Results

Forty-one patients were identified from the departmental database. Twenty-six were male and fifteen were female. The patients' ages at the time of their CT scans ranged from 1–9 months. Isolated patients aged over 9 months were excluded as most were clustered below this age.

The intracranial volumes of patients with isolated non-syndromic sagittal craniosynostosis ranged from 483 cm^3 in a 1-month-old boy to 1127 cm^3 in a 9-month-old boy. There was a wide range of values when compared with the normal curves. Overall, there were more values below the fiftieth centile than above, which was particularly evident in the female sample (Figs. 1 and 2).

The two-sample Student's *t*-test between the standard deviation scores of male and female patients with sagittal

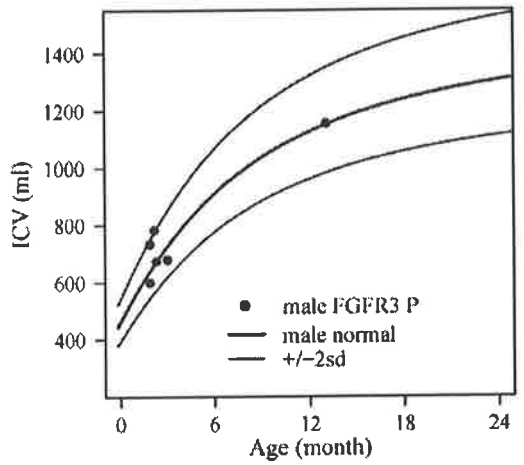

Fig. 3. The intracranial volume (ICV) of six males with isolated sagittal synostosis but with the common polymorphism Asn294Asn in FGFR3.

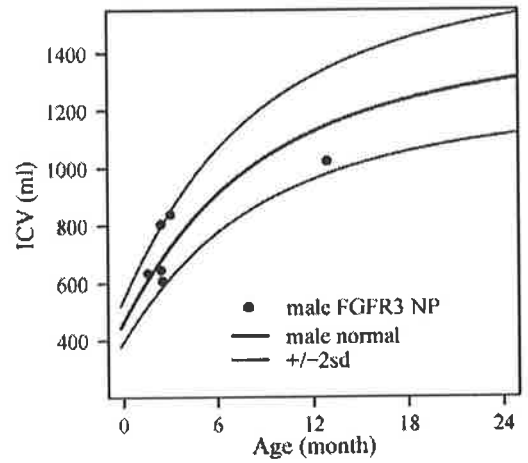

Fig. 4. The intracranial volume (ICV) of six age matched males with isolated non-syndromic sagittal synostosis but without the common polymorphism Asn294Asn in FGFR3.

synostosis and the male and female patients used in the Abbott-Netherway normal, gave the probability that the measurements were from the same distribution as 0.040 and 0.00002 respectively. This is indicative that all of these patients with sagittal synostosis have a larger intracranial volume than normal, but this association is much stronger in the female group.

The subgroup of six males with sagittal synostosis and an underlying FGFR3 polymorphism had their intracranial volumes assessed and compared with Abbott-Netherway normals (Fig. 3). However, there was no statistical difference between this subgroup and six age and sex-matched non-syndromic sagittal synostosis controls (Fig. 4).

4. Discussion

Non-syndromic sagittal craniosynostosis can have a range of severity of appearance at presentation and this series contains cases that were judged to be of sufficient severity to require transcranial corrective surgery. We have attempted to address weaknesses of previous studies, investigating the intracranial volume by comparing our results in the sagittal synostosis patients with values from an unaffected (normal) population derived from CT data using the same measurement techniques.[8]

All previous studies[6,7] have compared their experimental results with derived normal values, including those of Lichtenberg[10] and Dekaban,[11] which were derived by mathematical formulae from two-dimensional skull radiographs. This is crucially important since there are significant differences between the Abbott-Netherway normal curves for both sexes and the values previously derived by other authors, which impact on the findings. For example, Gault et al. found that ICVs in males were similar and were borderline normal values, but in females the intracranial volumes were smaller than their 'normal' values, which is the exact opposite to this study.[5] Close inspection reveals that the apparent difference is mainly due to differences in the normal values. It remains uncertain as to why there should be such a difference in the statistical significance between the male and female groups but we have noted differences between the sexes in other types of single-suture craniosynostoses, particularly metopic craniosynostosis.[12,13]

There is a recognised risk of developing raised intracranial pressure in sagittal synostosis, which if present alters the goal of surgical correction. There has been a reported incidence of 15–17%,[14–16] but in our series no cases had evidence of raised intracranial pressure pre-operatively, and only one case was found to have developed it subsequently, at age 5 years. This low incidence of raised pressure is similar to the other cases of sagittal synostosis treated at the ACFU over the last 30 years where the incidence of identified signs of raised intracranial pressure in this condition is restricted to just a few isolated cases. We speculate this may be due at least in part to the younger age at assessment than the previous published studies.[14–16]

The mechanism regarding the development of raised intracranial pressure in craniosynostosis remains uncertain in many cases, although it has been proposed that it can result from excessive brain growth relative to skull volume as a direct result of the craniosynostosis.[17] If this is a common mechanism then the results of this study are particularly significant because of the higher-than-normal values found for the ICV, especially in females in this study. However there are other recognised risk factors for the development of raised pressure and the exact relationship between raised pressure and ICV remains unclear, not least because the symptoms may be absent or subtle, and fundamentally there is no normative intracranial pressure data.[17]

The common polymorphism 294C > T (Asn294Asn) in FGFR3 has been identified in a subgroup of patients with features of non-syndromic sagittal synostosis. Our results of a limited morphological assessment of these patients found no identifiable differences, which lends support to

the anecdotal clinical impression that there are no discernable differences due to the polymorphism.

In conclusion we have found in our study that both males and females with sagittal synostosis had an increased intracranial volume when compared to a normal population, but that this is more pronounced in the female population. This leads us to propose that this data on ICVs supports surgical treatment for cranial vault remodelling rather than suture excision, as previously recommended.[18] However, there remains the low risk of raised intracranial pressure, and if suspected or identified pre-operatively, then additional volume expansion should also be undertaken as part of the surgical correction. Finally, there is no evidence that the presence of the polymorphism 294C > T (Asn294Asn) should lead to any alterations to the surgical management of the sagittal synostosis.

Acknowledgement

The genetic screening for mutations of FGFR and TWIST genes was undertaken by South Eastern Area Laboratory Services, Sydney.

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3.3 Metopic Craniosynostosis

Intracranial Volume Measurement of Metopic Craniosynostosis.

Anderson PJ, Netherway DJ, Abbott AH, David DJ.

J Craniofac. Surg. 2004; Vol 15(6): 1014 – 1016.

3.3.1 Hypothesis and Aims

The incidence of non-syndromic metopic synostosis is uncertain; it is less common than sagittal synostosis but it is now the second commonest single suture craniosynostosis seen in the Australian Craniofacial Unit. Metopic synostosis appears to have become increasingly common with a 200% increase in referred cases to the Australian Craniofacial Unit in the last five years. It is well recognised that there may be associated developmental delay particularly in speech and language development, although the mechanisms producing these remains uncertain. One possibility could be that any reduction in the intracranial volume may predispose to raised intracranial pressure resulting in developmental delay.

This investigation tested the null hypothesis that there is no difference between the intracranial volumes of individuals with non-syndromic metopic synostosis and sex and aged matched normal population.

3.3.2 Outcome

The results of the study found that in the larger male population that the intracranial volumes were significantly smaller than those found in the normal male population ($P<0.05$). There was no statistical difference between the intracranial volumes of the smaller female metopic population and the normal female population.

Intracranial Volume Measurement of Metopic Craniosynostosis

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From the Australian Craniofacial Unit, Women's and Children's Hospital (Associated with the University of Adelaide), North Adelaide, South Australia, Australia.

The authors report 32 patients with nonsyndromic isolated metopic synostosis who have undergone evaluation of their intracranial volumes. Twenty-five were male and seven were female. The measured intracranial volumes were compared with normal age-corrected values established in the authors' unit, and any differences were noted.

The authors found that, although there was a range of intracranial volumes, in the male patients, intracranial volumes were significantly smaller than those found in the normal population ($P < 0.05$). However, this result was not found in the smaller female sample.

These results contrast with those of smaller earlier studies, but the authors conclude that intracranial volumes are smaller than average for age-corrected normal values; this finding highlights the need for volume expansion in conjunction with cranial reshaping.

Key Words: Craniosynostosis, intracranial volume, metopic suture

The relationship between the early fusion of a cranial suture and resulting characteristic head shape was first recognized by Virchow.[1] Although improvement in cosmesis of trigonocephaly is important, it is recognized that craniosynostosis may result in raised intracranial pressure, and although the incidence is low, this can occur even when an isolated suture is affected.[2]

Previous studies of the intracranial volume measurement of cases of nonsyndromic isolated metopic craniosynostosis have used different techniques to obtain values.[3–6] These results (when compared with normal population values), have suggested that the intracranial volume is elevated above a derived "normal" value. Clearly, any study relying on comparison with a normal volume, will be influenced by how that normal value has been determined. Indeed, it has been previously suggested that any progress in the study of intracranial volumes will occur only after normal values have been established using the same method to determine the intracranial volume as the cases under study.[7]

To address this problem, the authors' unit has studied more than 300 computed tomography (CT) scans of normal individuals (75 male and 82 female in this population age range), which led to the development of normal age-related values for males and females.[8] We have used these normal values (derived by the same method as our study population), to compare our results from those with nonsyndromic isolated metopic craniosynostosis in an attempt to clarify the relationship between intracranial volume and the resulting trigonocephaly. Thus, this study into intracranial volume measurement in metopic craniosynostosis attempts to address an acknowledged weakness of previous studies.

Methods

All cases of nonsyndromic metopic synostosis on the Australian Craniofacial Unit (ACFU) data base with preoperative high-resolution three-dimensional (3D) CT scans were reviewed. The inclusion criteria for the study were clinical trigonocephaly with cortical ridging of the fused metopic suture on the CT scans.[9] Thirty-eight cases were identified. However, six cases in which other sutures could possibly be affected were excluded from this study, leaving 32 cases available for study. Twenty-five of the patients were male and seven were female. The patient's age at the time of their CT scans ranged from 1 month to 21 months.

Intracranial volume measurement was undertaken using 3D CT scans. The horizontal CT slices were processed in turn to obtain the area of intersection of the region of interest with each slice. The

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ACFU Persona software package was used to outline the bone margin in each slice.

Triangulation of the contours was undertaken to detect errors. A threshold of 150 Hounsfield units was selected for these patients. Intracranial volume was calculated as the sum of the cross-sectional areas that intersected the region of interest multiplied by the slice separation (the Cavalieri estimator). A bias correction term was applied to compensate for the effects of partial voluming, dependent on slice thickness and separation. This allowed archived CT scan data acquired from different scanners to be used.

The intracranial volumes were then compared with the normal volumes, determined in this unit from CT scans of unaffected individuals. The results were then plotted as shown in Figs 1 and 2.

Two sample Student *t* tests were used to assess differences between the intracranial volume (ICV) measurements of the patients with metopic synostosis and the Abbott-Netherway ICV normal subjects. [8] Before applying the statistical tests, the ICV measurements of both the patient and normal groups were expressed in terms of standard deviation scores to remove the effect of age variation.

The Abbott-Netherway ICV normal curve for each gender has a constant coefficient of variation with age, and the formulation is expressed in terms of the logarithm of the ICV and the logarithm of the age from conception. The intracranial volume standard deviation score for each patient was determined as the difference between the natural logarithm of the patient's intracranial volume and the gender-appropriate Abbott-Netherway normal curve, evaluated at the logarithm of the patient's age from conception, divided by the standard deviation.

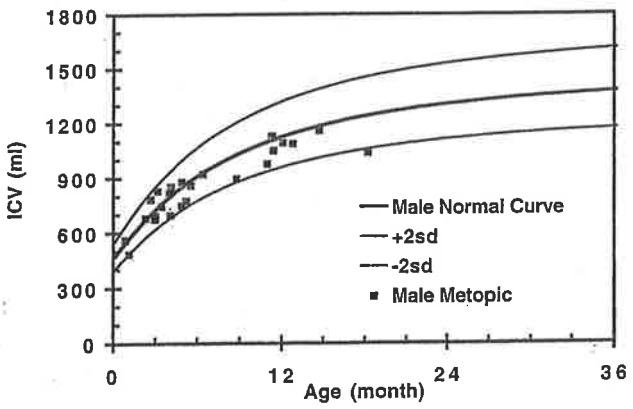

Fig 1 The intracranial volumes of 25 males with isolated nonsyndromic metopic craniosynostosis.

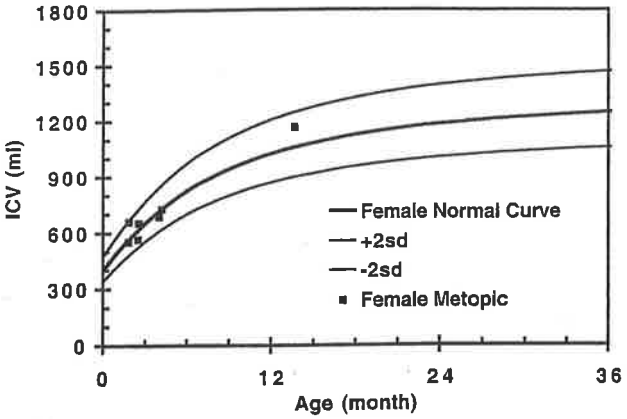

Fig 2 The intracranial volumes of seven females with isolated nonsyndromic metopic craniosynostosis.

Results

The intracranial volumes of males and females with isolated nonsyndromic metopic craniosynostosis ranged from 551 cm^3 in a 1-month-old girl to 1,294 cm^3 in a 21-month-old boy. There was a wide range of values when compared with the normal curves, with values both above and below the 50th centile. There was only a single value outside ± 2 standard deviations of the 50th centile. Overall, there were more values below the 50th centile than above, which was particularly evident in the larger male sample (see Figs 1 and 2).

The two-sample Student *t* test between the standard deviation scores of male patients with metopic synostosis and the male patients used in the Abbott-Netherway normal gave the probability that the measurements were from the same distribution as 0.039, with a mean standard deviation score of –0.48 (see Table 1). This is indicative that patients with metopic synostosis have smaller intracranial volume than normal.

The (smaller) female sample did not give a statistically significant difference between the intracranial volume and normal value.

Discussion

Nonsyndromic metopic craniosynostosis can have a range of severity of presentation. This series contains examples of a range of clinical severity, but all of these cases were judged to be of sufficient severity to require subsequent surgery. Sometimes cases of subtle simple ridging are noted in clinical practice,[10] but none were available for inclusion in this study.

In previous studies[3–6] comparing the intracrani-

Table 1. Statistics of the Standard Deviation Scores of the Intracranial Volume of Patients With Metopic Synostosis

	Gender	*No*	*Mean*	*Median*	*SD*	*Minimum*	*Maximum*	P
Metopic	M	25	−0.48	−0.39	1.01	−2.27	1.46	0.039
Normal	M	75	0.00	−0.14	0.99	−1.82	2.11	
Metopic	F	7	0.27	0.00	1.02	−1.00	1.90	0.428
Normal	F	82	−0.04	0.05	0.99	−2.38	2.64	

al volumes of patients with craniosynostosis and a reference normal, the validity of the reference normal, particularly when it has been derived using techniques different from those used to determine the intracranial volume in craniosynostosis, has been questioned.[7]

We have attempted to satisfy this concern by comparing our results in the patients with metopic synostosis with values from an unaffected (normal) population derived from CT data using the same measurement techniques.[8] This is different from previous studies,[6,7] which have compared their results with derived normal values, including those of Lichtenberg[11] and Dekaban,[12] which were derived by mathematical formulae from skull radiographs.

Clearly, there are differences between our results and those of earlier studies.[3–6] We propose that this may be attributable at least in part to the age- and gender-dependent differences between the Abbott-Netherway normals used in this study and the Lichtenberg normals used in previous studies. There are differences between the normal curves for both sexes, which we have previously highlighted,[8] but of particular importance is that Lichtenberg's male curve is substantially lower than the Abbott-Netherway normal curve between 8 months and 4 years.

In conclusion, we have found in our study that males with metopic synostosis had a decreased intracranial volume when compared with a normal population. This result was not found in the female population, but our female sample was much smaller, and additional studies will be undertaken to clarify if there is a true gender difference in this condition.

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CHAPTER 4:

INTRACRANIAL VOLUME MEASUREMENT: SYNDROMIC CRANIOSYNOSTOSIS

CHAPTER 4

INTRACRANIAL VOLUME MEASUREMENT: SYNDROMIC CRANIOSYNOSTOSIS

4.1 Apert syndrome

Analysis of Intracranial Volume in Apert Syndrome Genotypes

Anderson PJ, Netherway DJ, Abbott AH, Cox T, Roscioli T, David DJ.

Pediatr Neurosurg. 2004; 40: 161 -164.

4.1.1 Hypothesis and Aims

Apert syndrome is caused by a mutation of the *FGFR2* gene, with 99% cases the mutation occurring in one of two adjacent positions on the gene. There have been conflicting reports that a worse neurosurgical outcome is associated in the sub-group who have the mutation at the 253 position. This study investigated whether clinically subtle morphological differences could result in differences in the intracranial volumes which could account for the reported differences in surgical outcomes.

The first null hypothesis is that there is no difference between the intracranial volumes of Apert syndrome individuals and sex and aged matched normal population.

The second null hypothesis is that there is no difference between the intracranial volumes of Apert syndrome individuals who have the Ser252Trp mutation in *FGFR2* compared to those with the Pro253Arg mutation in *FGFR2*.

4.1.2 Outcome

All individuals with Apert syndrome in the study population (except two) had greater than their sex and age adjusted mean intracranial volumes. Comparison of the two genotype sub-populations demonstrated no discernable differences between the two groups.

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Analysis of Intracranial Volume in Apert Syndrome Genotypes

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Key Words
Craniosynostosis · Apert syndrome · Genotype · Tomography

Abstract
Objective: Apert syndrome is caused by a mutation of the fibroblastic growth factor type 2 gene and in nearly all of the cases where the mutation has been identified it occurs in one of two adjacent sites of the gene, either position 252 or position 253. There is currently uncertainty whether a worse neurosurgical outcome occurs in association with a particular genotype. We investigated whether there were clinically subtle (but relevant) morphological differences in the craniofacial skeleton, which would result in differences in the intracranial volume, which might account for apparent differences in surgical outcome. ***Method:*** Three-dimensional CT scans of preoperative Apert syndrome whose genotype had been identified had the intracranial volume measured using the Cavalieri estimator with correction for partial voluming effects. The values were compared to age and sex normals and then the two genotypes compared. ***Results:*** Intracranial volumes were measured for 22 cases, 16 with the 252 mutation and 6 with the 253 mutation. ***Conclusions:*** All cases except two had greater than their sex- and age-adjusted mean normal intracranial volumes. For the 252 and 253 genotypes there were no discernible differences in intracranial volumes between the two genotypes.

Introduction

Apert syndrome is characterised by craniosynostosis and limb anomalies [1]. The cause of the condition is a mutation of the type 2 fibroblastic growth factor gene, and in nearly all of the cases where the mutation has been identified it occurs in one of two adjacent sites of the gene, either position 252 or position 253 in exon 7 [2]. There have been previous investigations as to whether there are subtle phenotypic differences between the two genotypes but the results to date have been conflicting [3, 4]. There are also conflicting reports regarding outcome of cranial surgery, with a report that those with the mutation in the 253 position have a better neurosurgical outcome following cranial surgery [5], while mental outcome has been reported to be poorer by a separate study in the same genotype [6].

We decided to investigate whether the differences in genotype might result in differences in cranial morphology with consequent differences in intracranial volume in our cohort Apert syndrome cases. If differences exist then

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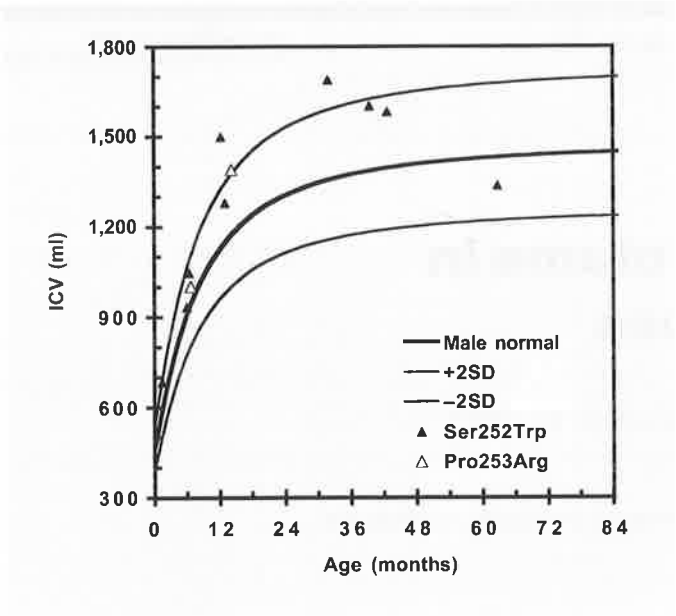


Fig. 1. Graphs to show the pre-operative intracranial volume (ICV) in males with the different genotypes.

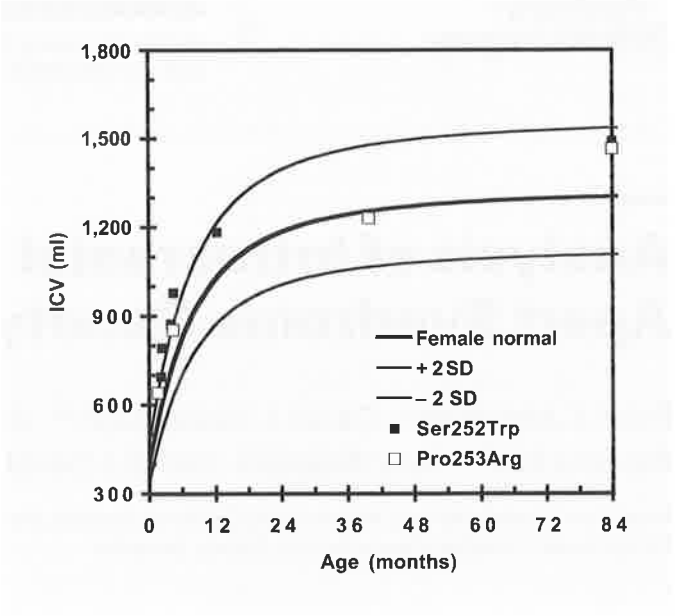


Fig. 2. Graphs to show the pre-operative intracranial volume (ICV) in females with the different genotypes.

this may impact on the neurosurgical outcome of the two groups. To evaluate each case we measured and compared the pre-operative intracranial volumes with each other and normal values.

Method

Cases of Apert syndrome were identified from departmental records. Only those cases who had their genotype identified and who had a high-quality CT scan prior to any surgical intervention were included in the study.

To measure the intracranial volume, the intracranial region was outlined on each slice in turn to obtain the cross-sectional area using the 'Persona' software package developed in the department. Triangulation of these outlines between slices to produce a three-dimensional surface was used to facilitate error detection. A threshold of 150 Hounsfield units was selected for the children. Intracranial volume was calculated as the sum of the cross-sectional areas that intersected the region of interest multiplied by the separation (the Cavalieri estimator). A bias correction was applied to compensate for the effects of partial voluming, dependent on slice thickness and separation. This allowed archived CT scan data acquired using different scanners, with different thickness and spacing protocols, to be used.

The results of the intracranial volume measurement were firstly compared to normal sex- and age-adjusted values (Abbott-Netherway normal values), and secondly, the results from the two genotypes were compared with each other.

Results

Twenty-two cases of Apert syndrome were available for study. This included 11 males and 11 females. The genotypes were 16 cases of the 252 mutation and 6 cases of mutation at the 253 position.

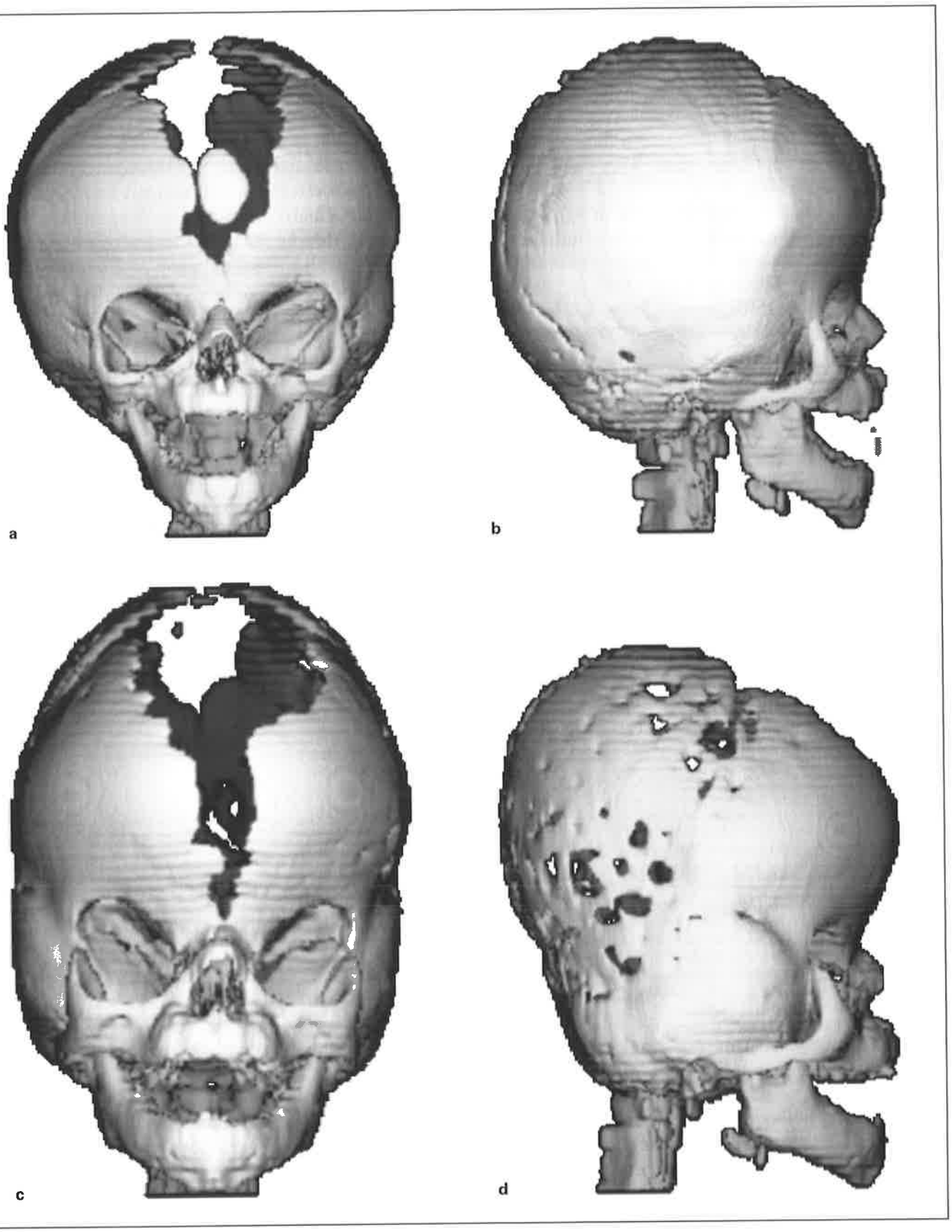
The intracranial volumes were then compared with the normal curves (fig. 1, 2). These confirm that the intracranial volumes are significantly greater than the normal intracranial volumes for both males and females.

However, it can also be seen that when the results for the two genotypes are compared there are no discernible differences between the two genotype groups.

Discussion

Apert syndrome with its range of clinical severity is usually the result of one of two adjacent point mutations of the FGFR2 molecule [2], the other causative mutations being exceptionally rare [7]. The existence of two geno-

Fig. 3. Three-dimensional CT scans of the craniofacial skeleton of 2 females aged 4 months with Apert syndrome with 252 (**a**, **b**) and 253 (**c**, **d**) mutation, demonstrating subtle morphological differences.


a
b
c
d

types has allowed investigation as to whether there are significant differences in the clinical presentation in the two main genotypes, and the evidence is conflicting [3, 4]. We have previously found from our studies of the range of craniofacial morphology of Apert cases using three-dimensional CT scans, that there is some suggestion that there may be morphological differences in age- and sex-matched genotypes (fig. 3).

Although possible differences related to genotype in various phenotype parameters (including the incidence of cleft palate and severity of the syndactyly of the hands) have been investigated, it is the previous attempts to relate the neurosurgical outcome to genotype which are of particular concern to neurosurgeons. However, this too has produced conflicting results with one study finding that the neurosurgical outcome was better in the 253 cohort [5], and the other study where the mental outcome was worse in the 253 cohort [6]. The findings of our study demonstrating that comparison of the two groups showed no obvious differences in the intracranial volumes is perhaps not surprising. This does require some caution given the numbers of cases available for study, but review of our clinical records also failed to find any obvious differences in outcome between the two genotypes with regard to surgical re-operation or psychological development.

This study is notable in that the method of determining the intracranial volume from the three-dimensional CT is the same method that has been used to produce the normal intracranial volume values to which our sample cases have been compared [8]. Previous studies of intracranial volumes in Apert syndrome have used either cases where the genotype is not known and normal values derived using different techniques including radiographic measurements [9].

All of the cases (except 2) in this series had an enlarged intracranial volume when compared to age- and sex-matched normals. This is similar to an earlier study from this unit which did not distinguish between the genotypes [9], and this confirms the finding of an earlier study [10].

In conclusion the results from this study demonstrate that there is no discernible difference in intracranial volumes between the two genotypes. So if there are morphological differences (and this is the subject of further study), they do not significantly affect the intracranial volume and so this does not explain any difference in neurosurgical outcome (if it truly exists at all). If in the future it becomes established that there are differences in the neurosurgical outcome attributable to genotype, then they must be due to factors other than differences in the preoperative intracranial volume.

Acknowledgements

We would like to acknowledge the SEALS Laboratories in Sydney and their director Micheal Buckley, who undertook the genotype identification.

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4.2 Double mutation

Do Craniosynostosis syndrome phenotypes with both *FGFR2* and *TWIST* mutations have a worse clinical outcome?

Anderson PJ, Netherway DJ, Cox T, Roscioli T, David DJ.

J Craniofac. Surg. 2006; 17(1): 166 -172.

4.2.1 Hypothesis and Aims

It has become recognised that the commonly occurring craniosynostosis syndromes of Crouzon, Pfeiffer, Apert and Saethre-Chotzen can result from mutations of either the *FGFR* or *TWIST* genes. We have recently identified three patients who uniquely have mutations in both genes. These appear on clinical examination to have phenotypes consistent those in the spectrum of Crouzon, Pfeiffer and Apert syndromes. The natural history is unclear because there has been no previous study of such individuals. The possibility that there might be morphological consequences resulting from two genetic mutations prompted the investigation of their intracranial volumes as part of the assessment into their clinical examination and review of their history.

The null hypothesis is that the intracranial volumes of individuals with co-existing *FGFR* and *TWIST* mutations are the same as those sex matched with the same phenotype due to a single *FGFR* mutation.

4.2.2 Outcome

The study consisted of just three cases, but there was no clear difference between these exceptional cases and the Crouzon and Pfeiffer phenotypes with just a single *FGFR* mutation. However, the Apert phenotype had a smaller intracranial volume than the normal value unlike any single *FGFR* mutation phenotype.

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Case Report

Do Craniosynostosis Syndrome Phenotypes with Both FGFR2 and TWIST Mutations have a Worse Clinical Outcome?

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The recent discovery of underlying multiple genetic mutations in some patients with syndromic craniosynostosis phenotypes after genetic analysis has raised questions as to the clinical significance of each mutation. We review the clinical outcome in three such cases; each case has a different syndrome phenotype, and two patients have reached skeletal maturity.

Key Words: Craniosynostosis, mutation, Apert, Crouzon, Pfeiffer

The underlying mutations commonly found in Apert, Crouzon, and Pfeiffer syndromes are different mutations affecting the FGFR2 gene.[1–3] Another of the common craniosynostosis syndromes, Saethre-Chotzen syndrome, has been found to result from mutations affecting the TWIST gene.[4]

We have recently identified three craniosynostosis patients who had mutations of both the FGFR2 and the TWIST genes (i.e., double mutations).[5] These cases have Pfeiffer, Crouzon, and Apert syndrome phenotypes, respectively. These individuals have been treated according to our protocols for a minimum of 10 years, and two have reached skeletal maturity. We retrospectively review their clinical course to assess whether their phenotype and clinical outcome is different from the cohort of craniosynostosis patients with phenotypes resulting from a single mutation treated in this unit.

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Case Reports

Case 1

An Indonesian female age 16 years with a clinical diagnosis of Pfeiffer syndrome was referred for assessment (Fig 1). She had a history of breathing difficulties and was noted to have mid-face retrusion and proptosis with corneal exposure and a divergent squint. Her other notable feature was hearing loss of −50 dB bilaterally. She had no previous cranial surgery. Clinical genetic assessment highlighted broad big toes (Fig 2), but her thumbs were unremarkable. She was given a diagnosis of a type 1 Pfeiffer phenotype. However, the formal DNA testing identified that she had mutations in FGFR2 exon 9 resulting in Cys342Arg substitution and an insertion TWIST c.603-604 Ins21bp.

After multidisciplinary assessment, it was decided to manage her with simultaneous fronto-orbital and midface osteotomies with insertion of distractors to maximize the facial advancement and to allow differential movement (a technique we have previously described).[6] The fronto-orbital bar was advanced 12 mm horizontally, whereas the mid-face was advanced using a vector with both inferior and anterior components a total of 30 mm. After 8 weeks consolidation, the distractors were removed and a craniomaxillary fixation placed. Apart from a cerebrospinal fluid (CSF) leak postoperatively, which was managed conservatively and resolved spontaneously after 5 days, her recovery was otherwise unremarkable.

This skeletal position has remained stable, and she is shown at her last review, 3 years after surgery in Figure 3. This figures shows that she has some

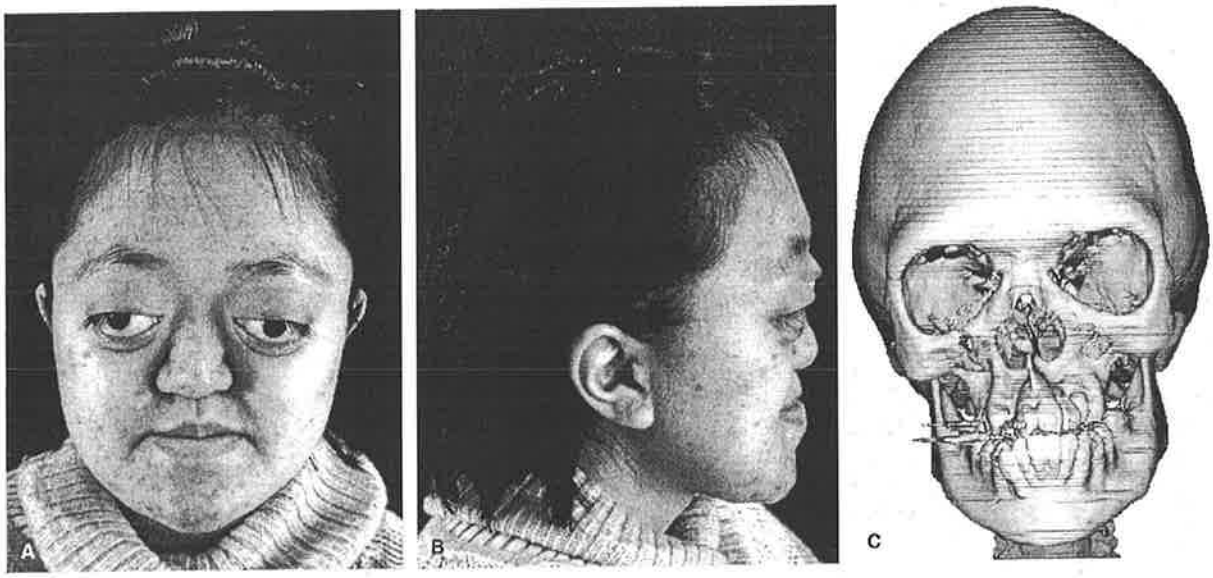

Fig 1 Case 1: preoperative age 15 years. AP and Lat, and three-dimensional computed tomography scan.

lower lid fullness, which was confirmed clinically, and she is currently awaiting bilateral blepharoplasties.

Case 2

A Chinese female, age 4 years, was referred for assessment and further management of orbitostenosis (Fig. 4). She had no extracranial anomalies, and her development was above normal; a clinical diagnosis of Crouzon syndrome was made. At the time of referral, she had already undergone a limited coronal craniectomy by local neurosurgeons for craniosynostosis at age 6 months. Subsequent DNA testing revealed her to have mutations in the FGFR2 exon 9 leading

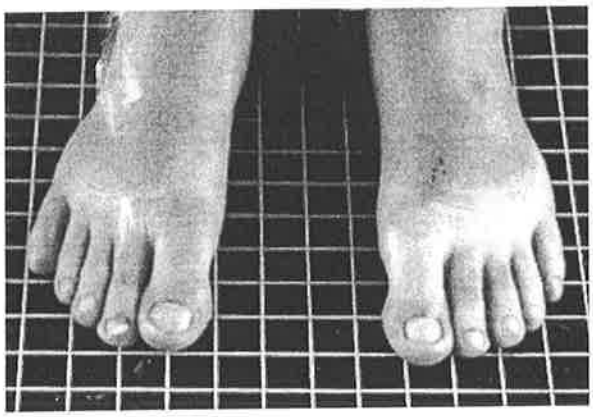

Fig 2 Case 1: both feet. Note the wide big toes.

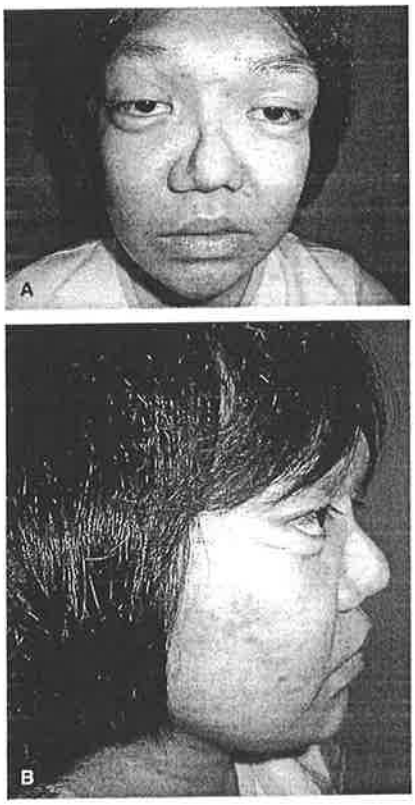

Fig 3 Case 1: postoperative age 18 years. AP and Lat.

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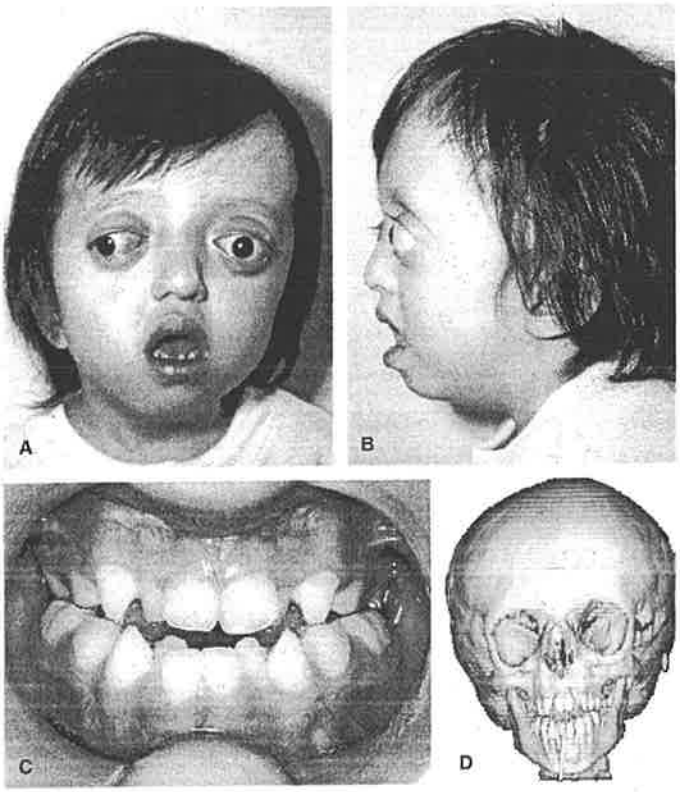

Fig 4 Case 2: age 5 years preoperative. AP and Lat. Intraoral view showing malocclusion and three-dimensional computed tomography scan.

to a Cys342Ser substitution and a deletion mutation in TWIST c.604-621 del18.

After multidisciplinary assessment a fronto-orbital advancement to provide ocular protection was undertaken. After an otherwise unremarkable postoperative course, she was kept under outpatient review. By the age of 8 years, she was noted to have worsening mid-face hypoplasia, although she was still above average in psychologic testing. She underwent a Le Fort III mid-face advancement osteotomy, which was complicated by a dural tear and CSF leak. One month later, with no improvement, re-operation and dural repair combined with a repeat fronto-orbital advancement for persisting proptosis was undertaken. Her postoperative course was unremarkable, and she remained under review.

On review 11 years later, she was completing high school before starting university. On clinical examination, it was found that the frontal advance had been maintained and mandibular growth was normal (Fig 5), but once again, there was marked mid-face retrusion. She subsequently underwent a Le Fort III mid-face advancement osteotomy and placement of distractors. Distraction of 28 mm bilaterally was

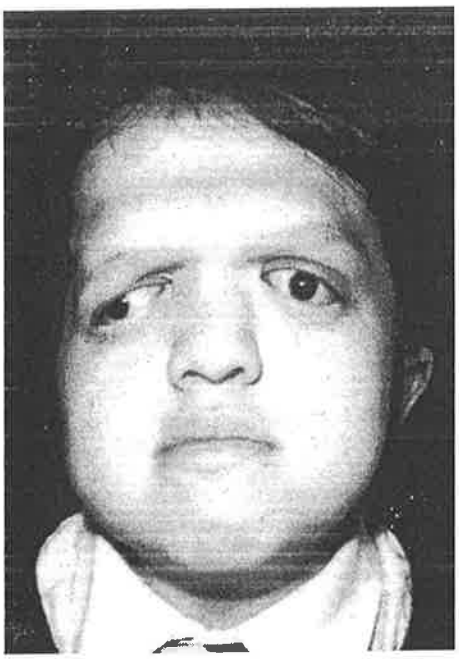

Fig 5 Case 2: AP view, age 17 years.

undertaken to skeletal class II position before removal, along with onlay bone graft to both molars, browlift, canthopexies, and right entropion correction. Postoperatively, she has remained under regular review, and 3 years later, the result is skeletally stable (Fig 6).

Case 3

A Chinese male, aged 5 years, was referred for assessment and treatment. He had the clinical appearance of Apert syndrome with turribrachycephaly and marked retrusion of the forehead (Fig 7). Subsequent analysis revealed mutation in FGFR2 exon 7 resulting in Ser252Trp substitution and a deletion in TWIST c244-262 del 18.

However, radiology of spine showed no obvious fusion, his hands were well formed with both his thumbs and little fingers free (Upton type 1),[7] and he had a submucous cleft palate. However, speech and language development were both severely delayed.

Previously, it was recorded that he had been born by emergency caesarian section at term. A few days later, he had respiratory distress and was noted to have choanal atresia, which was operated on at age 1 month with improvement in his airway. At age 3 months, he developed recurrent epilepsy for which he has been maintained on long-term valproate. His outpatient follow-up was noted to be satisfactory, apart from the persisting developmental delay, until his last review, when he was noted to have developed snoring.

After multidisciplinary assessment, he was managed by fronto-orbital advance with palatal split to improve his airway and mid-face osteotomy, and placement of distractors was undertaken at age 6 years. Syndactyly, release was undertaken 1 month later. Two months later, the mid-face distractors were removed.

He has remained under regular review and is shown at age 10 years . Currently, he has noisy breathing at night but has not required any further surgical intervention, although further mid-face surgery at skeletal maturity is considered inevitable in his case.

Discussion

The existence of craniosynostosis cases with both FGFR2 and TWIST mutations has only recently been reported.[5] These three cases not only have very unusual double mutations of both the FGFR2 gene and the TWIST gene but have different phenotypes on clinical examination. None of these cases have features of Saethre-Chotzen syndrome (low hairline, beaked nose),[8] and significantly, this diagnosis was not considered for any of these cases, suggesting that the FGFR2 mutation has a bigger influence on the resulting phenotype than the TWIST mutation. All cases have until recently been managed without the identification of their underlying mutations, which suggests that their clinical behavior has been similar to phenotypes resulting from a single FGFR2 mutation. Each case will be scrutinized in more detail in turn.

In patient, 1 the Pfeiffer phenotype is perhaps unusual from other Pfeiffer phenotypes in that she did not seek treatment until she had almost reached skeletal maturity. Although she underwent an extensive procedure, with advancement of both the forehead and the mid-face, overall, her treatment is indistinguishable from a Pfeiffer syndrome who

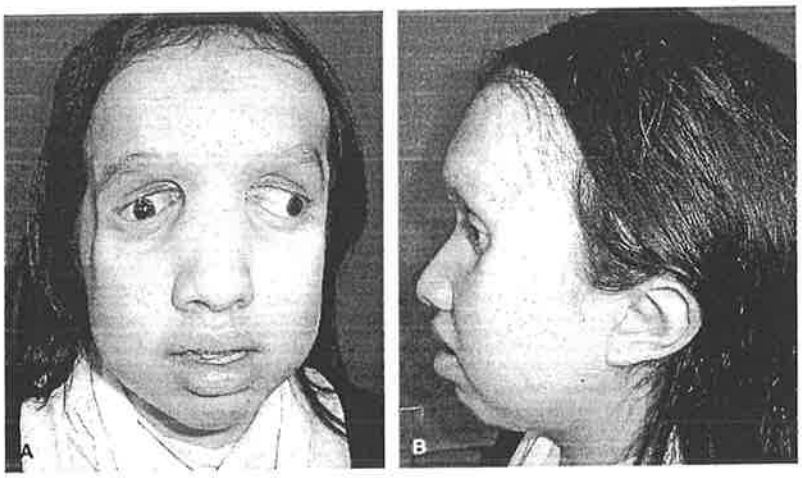

Fig 6 Case 2: Postoperative age 19 years. AP and Lat.

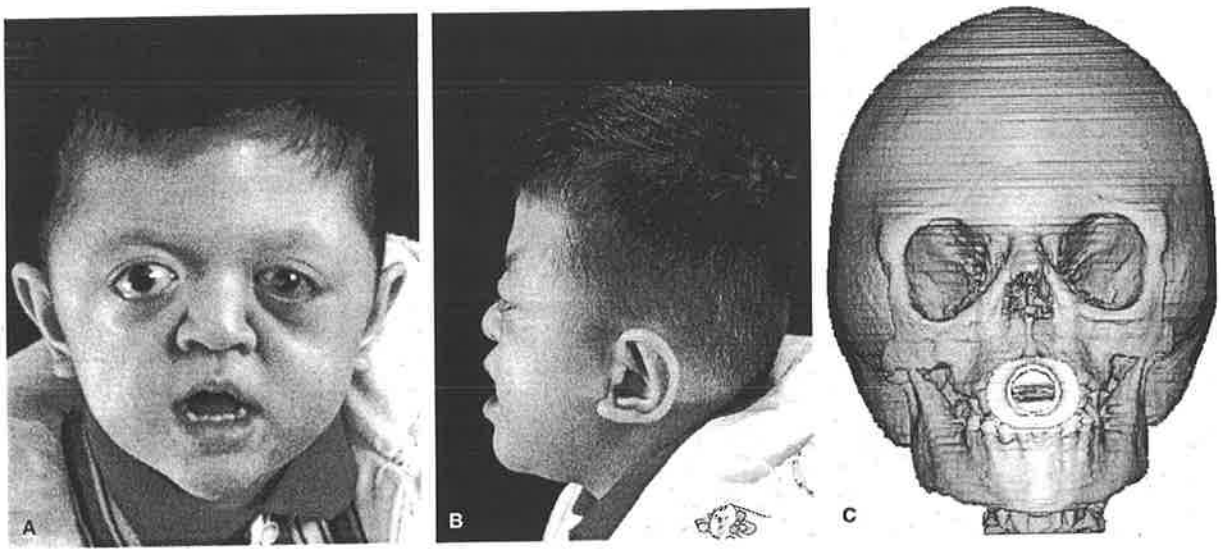

Fig 7 Case 3: age 5 years preoperative. AP and Lat and three-dimensional computed tomography scan.

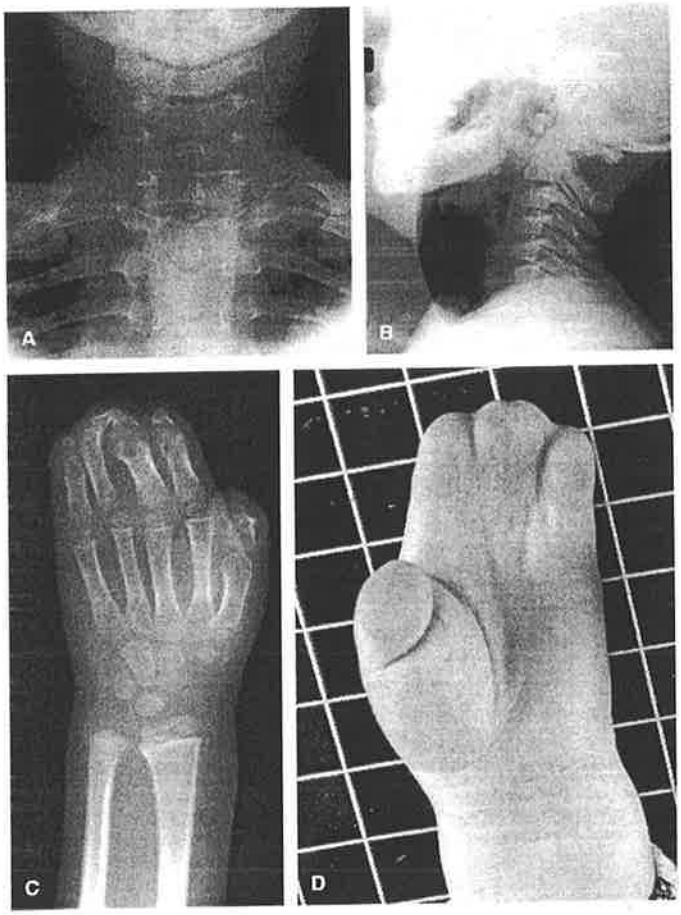

Fig 8 Case 3: Radiographs of spine. AP and Lat, right hand with photograph.

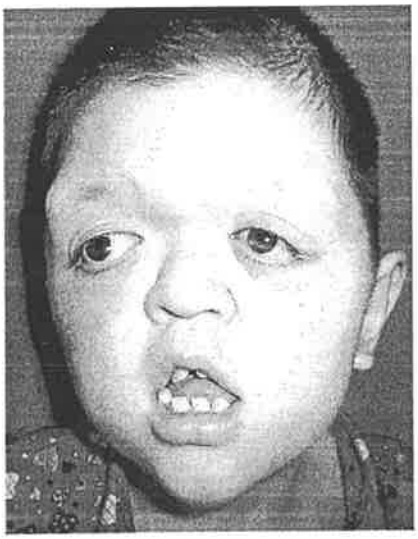

Fig 9 Case 3: postoperative AP view, age 10 years.

had a single mutation. Her phenotype demonstrated moderate to severe craniofacial manifestations at presentation with marked proptosis and mid-face retrusion. However, it is notable that there was no developmental delay on psychologic assessment, and she was in mainstream high school and has subsequently completed tertiary education, an achievement few of the remaining cohort of Pfeiffer cases in this unit have achieved. The only extracranial manifestation is the enlarged big toes, and curiously, she has with normal sized thumbs, which has been recognized in Pfeiffer syndrome.[9]

Case 2 history is more unusual in that it suggests that the condition was apparent shortly after birth with the undertaking of a limited craniectomy. Certainly, the history of recurrent forehead advancement and two episodes of mid-face advancement represents a greater number of surgical interventions than many Crouzon cases under the care of the unit. However, the forehead and mandibular growth continued during skeletal maturation with just the mid-face requiring surgery at skeletal maturity. The absence of any extracranial manifestations again is notable, with even the elbow movements noted to be normal; these have been reported to be restricted in almost 50% of cases.[10]

Case 3 is different from the two previous cases in that the patient has not yet reached skeletal maturity. However, he has been managed since shortly after birth, and his operative history is again indistinguishable from other Apert cases. However, he remains significantly developmentally delayed, even when compared with other Apert children, but whether this is as a result of his double mutation is unclear. The craniofacial anomalies have so far had a similar operative history, repeat forehead advancement and mid-face advancement, to other Apert cases. Curiously, his extracranial anomalies are relatively mild with an absence of fusions in his cervical spine, relatively well-developed hands, and just a submucous cleft palate. It is interesting that the FGFR2 mutation in his case is at the 252 position, which has been reported to be more commonly associated with cleft palate and less severe forms of hand syndactyly,[11] although another study failed to find this.[12] Despite this, there can be no doubt that the absence of cervical fusions at age 5 years in Apert syndrome is unusual.[13]

Studying all three cases to look for common features is difficult. An objective assessment was attempted by measuring the intracranial volume before fronto-orbital advancement surgery and comparing with age and sex-matched normals[14] and single mutation syndrome genotypes to investigate whether there was a more severe phenotype. The results are shown in Figures 10 and 11. Cases 1 and 2, the Pfeiffer and Crouzon phenotypes, show no discernible difference from Crouzon and Pfeiffer cases with single FGFR2 mutations. However, Case 3, the Apert phenotype, has a smaller intracranial volume than either the single 252 or 253 mutation Apert genotypes. There is no sex and age comparison, but, given his significant developmental delay, the possibility is raised that his case is a more severe craniofacial manifestation, and this may be related to his marked developmental delay. It is noteworthy that

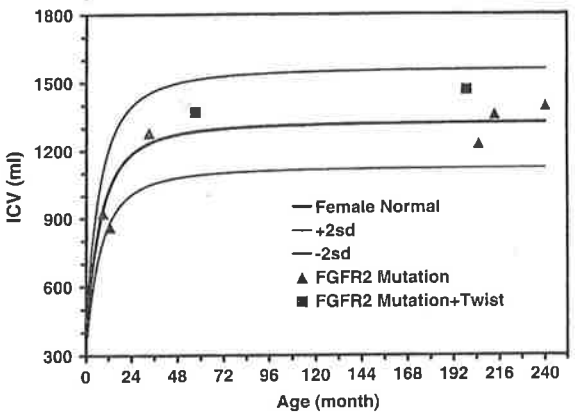

Fig 10 Normal intracranial volume for females. Single FGFR2 mutation (triangles); double mutations with FGFR2 and TWIST mutations (squares).

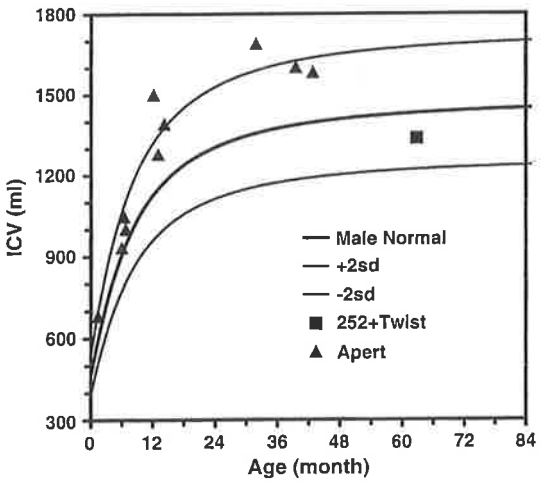

Fig 11 The normal Intracranial volume for males. Single FGFR2 mutations (triangles). Double mutation with FGFR2 and TWIST mutations (square).

it has been reported that outcome of the craniofacial surgery in Apert syndrome can be related to genotype,[15] although our earlier study of preoperative intracranial volumes as a factor in this failed to identify any difference between 252 and 253 genotypes.[16]

The extracranial anomalies in all three cases are significant in that all three have only mild (and none in case 2) extracranial anomalies. Because both cases who have reached skeletal maturity (nos. 1 and 2) have completed formal education, this suggests that this phenotype is no worse than phenotypes with just FGFR2 mutations.

In conclusion, we have reported three cases of syndromic craniosynostosis who had unusual double mutations in both FGFR2 and TWIST genes. Assessment of their phenotype and clinical course does not suggest that they have a more severely affected phenotype and have a poorer clinical outcome, and any future cases will continue to be treated using the existing craniosynostosis syndromes protocol management in this unit.

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CHAPTER 5:

GENETIC STUDIES

CHAPTER 5

GENETIC STUDIES

5.1 Somatic Mutations

Somatic *FGFR* and *TWIST* mutations are not a common cause of isolated non-syndromic single suture craniosynostosis.

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J Craniofac. Surg. 2007; Vol 18(2): 312-314.

5.1.1 Hypothesis and Aims

The phenomenon of somatic mosaicism for *FGFR* gene mutations has been reported to underlie a variety of clinical presentations, including epidermal mosaicism for *FGFR2* mutations in acne[7], and a less severe skeletal dysplasia variant distinct from thanatrophic dysplasia associated with the *FGFR3* R248C mutation (which usually results in lethal Thanatrophic Dysplasia)[8]. Given this background the null hypothesis is that there is no evidence to suggest that somatic mutations occurring within either the *FGFR1-3* or *TWIST* genes might be identified within cells of, and thus be responsible for, the abnormally fusing sutures in individuals with the more common single suture craniosynostosis.

5.1.2 Outcome

There is no evidence to suggest that the phenomenon of somatic mutations of the *FGFR1-3* genes or the *TWIST* genes is common in non-syndromic craniosynostosis.

Scientific Foundations

Somatic FGFR and TWIST Mutations are not a Common Cause of Isolated Nonsyndromic Single Suture Craniosynostosis

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Pathogenic mutations in FGFR2 and TWIST genes are detected in the majority of individuals with Crouzon, Pfeiffer, Apert, and Saethre-Chotzen syndromes. In contrast, mutations have been identified rarely in cases of nonsyndromic, single suture craniosynostosis. Recently, two studies confirming somatic mosaicism with local expression of an FGFR mutation have been reported. This study investigates whether somatic mosaicism could account for nonsyndromic, single suture craniosynostosis. Eight individuals with single suture craniosynostosis who were negative for known mutations in FGFR1-3 and TWIST after screening in their leucocyte DNA were tested for the presence of pathogenic mutations in suture cell-derived DNA. Five had sagittal synostosis, two had metopic synostosis, and the other unicoronal synostosis. Osteoprogenitor cells from surgically excised fusing sutures and an adjacent open suture were cultured. DNA from the cultured cells grown to passage 3 was then examined for underlying FGFR and TWIST mutations. No mutations within the exons of the FGFR or TWIST genes studied were identified in any suture cells. This study found no evidence to support the notion that mosaicism for *FGFR* or *TWIST* mutations, normally associated with syndromal forms of craniosynostosis, occur in single suture craniosynostosis. Thus, any underlying genetic defects must occur in regions outside those normally implicated in syndromal craniosynostosis, or this disorder could arise as a consequence of some other epigenetic modification.

Key Words: Craniosynostosis, mutation, gene

Craniosynostosisis the end product of premature cranial suture fusion occurring as an isolated anomaly affecting a single suture or as part of a syndrome, with an incidence of 1: 2,500 live births.[1] The development of craniosynostosis can be a consequence of a number of different molecular factors. The most common syndromal craniosynostoses, Crouzon, Pfeiffer, Apert, and Saethre-Chotzen syndromes, are associated with mutations within one of three *FGFR* (*FGFR1-3*) genes[2–4] or the *TWIST* gene.[5] In total, more than 50 mutations have been reported. This contrasts with the vast majority of cases of single suture craniosynostosis in which mutations have been occasionally identified.[6]

The phenomenon of somatic mosaicism for *FGFR* gene mutations has been reported to underlie a variety of clinical presentations, including epidermal mosaicism for *FGFR2* mutations in acne[7] and a less severe skeletal dysplasia variant distinct from thanatophoric dysplasia associated with the *FGFR3* R248C mutation (which usually results in lethal thanatophoric dysplasia).[8] In addition, an achondroplasia phenotype, which commonly is the result of the *FGFR3* G380R mutation,[9] was caused by possible mosaicism for the *FGFR3* R248C mutation.[10] Given this background, we hypothesized that somatic mutations within either the *FGFR1-3* or *TWIST* genes might be identified within cells of, and thus

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be responsible for, the abnormally fusing sutures in individuals with the more common single suture craniosynostosis.

METHODS

Leucocyte-derived samples from a large cohort of craniosynostosis cases were subjected to a comprehensive screen using denaturing high-performance liquid chromatography (DHPLC) for mutations within the *FGFR 1–3* and *TWIST* genes. The screening protocol (primers available upon request) consisted of examination of all exons previously shown to harbor mutations known to be responsible for the common craniosynostosis syndromes. These exons included the *FGFR1* exon 7 (Pfeiffer syndrome), the *FGFR2* mutation hot spots associated with Apert, Pfeiffer, Crouzon, and Beare-Stevenson syndromes (exons 8, 10, and 11), *FGFR3* exons 7 (Muenke syndrome) and 10 (Crouzon syndrome with acanthosis nigricans), and the single coding exon of *TWIST* that is associated with Saethre-Chotzen syndrome. The screen also included the six additional exons of *FGFR2* that have previously been shown to contain mutations associated with Crouzon, Pfeiffer, or familial sagittal craniosynostosis. Genomic polymerase chain reaction products showing anomalous denaturing DHPLC patterns were sequenced to identify any nucleotide alteration. Eight cases of single suture craniosynostosis that were scheduled for surgical repair and that were found to have no underlying mutation of the *FGFR* or *TWIST* genes in leucocyte derived DNA were entered into the study once ethical committee guidelines were completed. Suture cells were harvested at the time of transcranial correction in all cases. An approximately 1 cm^2 region from the fused and, where possible, patent suture were removed during surgical correction according to established protocols.[11] The osteoprogenitor cells from each site were cultured separately in vitro, and after the third passage, their DNA was extracted, and the comprehensive screen (as outlined above) was repeated on each DNA sample.

RESULTS

Eight cases of single suture craniosynostosis were selected for inclusion in this study because previous screening of white cell DNA failed to identify any mutations in the regions of *FGFR1-3* or *TWIST* previously associated with the syndromal forms of craniosynostosis. Three cases had the common polymorphism 294C > T (Asn294Asn) in the *FGFR3* gene (Table 1) in their leucocyte DNA. Four of the patients were females and four males. Five patients had sagittal synostosis, two patients had metopic synostosis, and the remaining had unicoronal synostosis (Table 1). The five cases of sagittal synostosis also had suture cells cultured from patent cranial sutures. In each case, microscopy assessment of cell morphology and growth patterns revealed no discernible differences between osteoprogenitor cells from fused and patent sutures from all patients and controls. DHPLC screening of all gene regions revealed no sequence abnormalities in the *FGFR* or *TWIST* genes in any of the DNA samples from the cultured suture cells (either from fused or open sutures). Furthermore, the three patients with the *FGFR3* polymorphism in their leucocyte DNA also had this polymorphism in their osteoprogenitor cell DNA consistent with the lack of mosaicism.

DISCUSSION

The underlying cause of most cases of single suture craniosynostosis remains unknown despite its prevalence and social impact. The reports that somatic mosaicism has been identified in a syndrome with craniosynostosis[8–10] prompted us to investigate whether this phenomenon could also occur as an isolated anomaly in the suture cells undergoing premature fusion in cases of nonsyndromal, single suture craniosynostosis, the most commonly encountered craniosynostosis type. Under this scenario, such changes would be irrespective of the suture involved because this would depend simply on the timing or site at which the somatic mutation arose.

The finding of the *FGFR3* c.882T > C, Asn294Asn polymorphism in three cases in both

Table 1. Genotypes in single suture craniosynostoses.

Sex	*Synostosis*	*Blood Genotype*	*Suture Genotype*
F	*Sagittal*	*Negative*	*Negative*
F	*Sagittal*	*Negative*	*Negative*
F	*Sagittal*	*Negative*	*Negative*
M	*Sagittal*	*FGFR3 c.882T>C, Asn294Asn polymorphism*	*FGFR3 c.882T>C, Asn294Asn polymorphism*
M	*Sagittal*	*Negative*	*Negative*
M	*Metopic*	*FGFR3 c.882T>C, Asn294Asn polymorphism*	*FGFR3 c.882T>C, Asn294Asn polymorphism*
F	*Metopic*	*Negative*	*Negative*
M	*Coronal*	*FGFR3 c.882T>C, Asn294Asn polymorphism*	*FGFR3 c.882T>C, Asn294Asn polymorphism*

circulating leucocyte DNA and suture cells is not statistically significant when compared with a larger cohort of 39 mutation negative individuals with unisutural synostosis ($\chi^2 = 0.8$, $P = 0.37$). No clinical consequences have been recognized, and no difference in craniofacial morphology has been identified in those patients with sagittal synostosis when compared with age- and sex-matched patients of sagittal synostosis without the polymorphism (K. McGlaughlin, personal communication).

The results of our extensive screening protocol demonstrate the absence of mutations in all exons known to harbor mutations in syndromal forms of craniosynostosis in the cranial suture cells of individuals with single suture craniosynostosis. These data suggest that somatic change in regions of the *FGFR1-3* or *TWIST* genes known to be mutational hotspots in syndromal forms of craniosynostosis are unlikely to be a major cause of isolated single suture craniosynostosis.

Conclusions

We conclude that single suture craniosynostosis may be caused by gene sequence variants present outside all regions implicated in the syndromal forms of craniosynostosis. These studies therefore highlight the need to focus on either regulatory regions of the *FGFR1-3* and *TWIST* genes, those genes known to underlie less common syndromal craniosynostoses, for example, involving the *MSX2* gene,[12] or even possibly those genes implicated from murine studies such as the *ALX4* gene.[13] Another possibility worthy of future investigations is that epigenetic or environmental factors contribute to the high incidence of this common form of craniosynostosis.

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CHAPTER 6:

IMAGING STUDIES

CHAPTER 6

IMAGING STUDIES

6.1 Micro-CT/ Scanning Electron Microscope

Scanning Electron Microscope and micro-CT evaluation of cranial sutures in health and disease.

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Imaging studies can be used to study both the structure of the cranial sutures themselves and the resultant effects on the morphology of the cranial vault cranial occurring as a result of normal or abnormal function. Recent advances in technology with enhanced resolution provide the possibility of more detailed studies.

6.1.1 Hypothesis and Aims

Micro-CT examination of human sutures has been undertaken using a Skyscan micro-CT 1172 scanner. The specimens were harvested at corrective transcranial surgery and immediately placed in RNAlater (Ambion). The resolution was inversely proportional to the specimen size, so the specimens were trimmed so that they only had 2mm of bone adjacent to each suture. However due to the suture conformation

this resulted in different sized samples and that the highest possible resolution was inconsistent. The aim of the study was in all cases to undertake scanning at the highest possible resolution.

Scanning electron microscopy has been undertaken on human cranial sutures using a Philips XL30 multifunction microanalytical scanning electron microscope. Scanning was undertaken which allowed the production of both back scattered images and secondary electron images. This machine also had an incorporated EDAX multi-channel analyser which enabled elemental analysis to be undertaken of the suture and the surrounding bone at different distances away form the suture. The sample size for each analysis was 1μm diameter of a flat surface. The aim of the study was to investigate different human cranial sutures at different stages of fusion at the highest possible magnification. The second aim was to undertake elemental analysis of the suture at increasing distances from it to assess the composition of the structures.

6.1.2 Outcome

The high resolution images from the micro-CT scanner helped assess the architecture of the trabeculae in the bone adjacent to the suture. The scanning electron microscope images aided the understanding of the structure of the unfused suture, while the elemental analysis demonstrated an increase in calcium composition of the bone the further away from the suture the samples were tested.

Scientific Studies

Scanning Electron Microscope and Micro-CT Evaluation of Cranial Sutures in Health and Disease

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Current knowledge of suture biology has been ascertained as a result of morphological studies of normal cranial sutures (and rarely those undergoing craniosynostosis). These were initially undertaken often using histological investigations, or more recently using CT scans, as investigative tools, but have often used animal models. However, recent technological advances have provided the potential to refine our understanding of the ultrastructure by the use of new advanced scanning technology, which offers the possibility of more detailed resolution.

Our aim was to undertake detailed scans of normal, fusing and fused sutures from patients with craniosynosotosis affecting different sutures, to study the detailed structure at different stages of the fusion process using a modern micro-CT scanner and a microanalytical scanning electron microscope. We wished to include in our study all the human sutures because previous studies have mostly been undertaken using the sagittal suture.

Ten sutures from seven patients have revealed a complex ultra-structural arrangement. The different patterns of bone ridging seen on the ectocranial and endocranial surfaces of the fused sagittal suture were not repeated on closer inspection of either fused coronal or lambdoid sutures. Elemental analysis confirmed that the amount of calcium increased and the amount of carbon decreased as sampled areas moved away from the suture margin.

We conclude that scanning allowed detailed assessment and revealed the complex arrangement of the structure of the human cranial sutures and those undergoing the process of craniosynostosis, with some differences in final structure depending on the affected suture.

Key Words: Scanning electron microscope, micro-CT scan, cranial suture, craniosynostosis

Cranial sutures are articulations in which the margins of adjacent bones are united by a thin layer of fibrous tissue. Their functions include contributing to craniofacial growth as well as allowing movement between the adjacent cranial bones during childbirth. Current understanding of the structure of the normal cranial suture is still largely based as a result of histological investigations.[1] Since then, investigations into structure and function have been undertaken using immunohistochemistry,[2] autoradiography,[3] and more recently conventional CT[4] and micro-CT scans.[5] However, most of these earlier studies had used animal models including mice, rats and rabbits.[2–4]

The underlying causes of both normal suture fusion in adulthood and premature fusion in craniosynostosis remain unclear. The study of craniosynostosis remains important because it provides a model for the study of suture fusion and the factors maintaining normal suture patency and function. While underlying genetic mutations have been identified in the common craniosynostosis syndromes[6] the causes of non-syndromic craniosynostosis remain uncertain but are thought to be multi-factorial with mechanical, genetic and hormonal factors all implicated.[2]

Since the initial recognition that the cranial sutures could be studied using micro-CT,[5] there have been no further published reports. This is regardless of the development of improved technology

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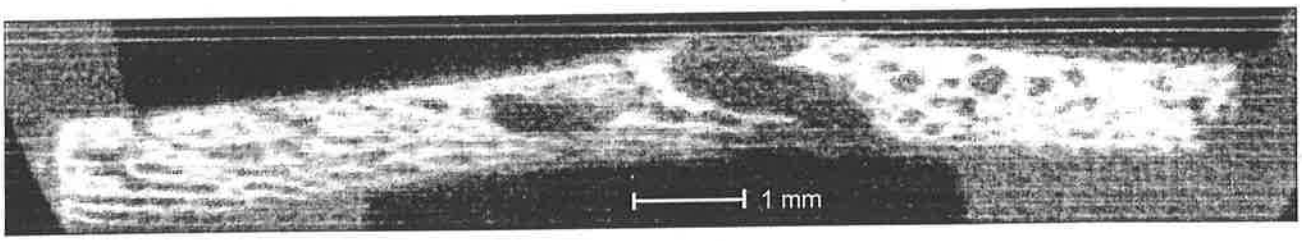

Fig 1 Low-power view of a micro-CT scan of an open coronal suture, demonstrating interdigitation of the bone margins.

producing enhanced image resolution with the current generation of scanners.

Our aim was to investigate the morphology of all the human cranial sutures (both open, fused and undergoing the pathological process of craniosynostosis), using a modern micro-CT scanner and micro-analytical scanning electron microscope (which enables elemental analysis of the sample), to evaluate the ultra-structural morphological changes associated with craniosynostosis. In particular, we wished to study the appearances of the endocranial and ectocranial surfaces of fused coronal and lambdoid sutures to investigate whether the ridging patterns associated with a fused sagittal suture[7] occur with other sutures.

Methods

The sutures from non-syndromic sagittal synostosis (three patients), metopic synostosis (one patient), unicoronal synostosis (one patient), unilambdoid synostosis (one patient) and approximately 6 mm of adjacent bone were harvested during corrective transcranial surgery. In three cases an adjacent open suture was harvested along with the abnormally fused or fusing suture providing nine sutures for study. The patients were aged from 6–25 months, with 3 female and 3 male. One further suture sample (normal lambdoid) was obtained from an infant male with an intracranial tumor.

Specimens selected to undergo micro-CT analysis and following harvest were immediately placed into a polyethylene container filled with RNAlater solution (Ambion). The position of the specimen in the container was maintained using polystyrene blocks. Using a Skyscan micro-CT 1172 scanner sutures at different stages of fusion were scanned at the highest possible resolution. The limit of the available resolution was inversely proportional to the specimen size. Digital images were stored and then

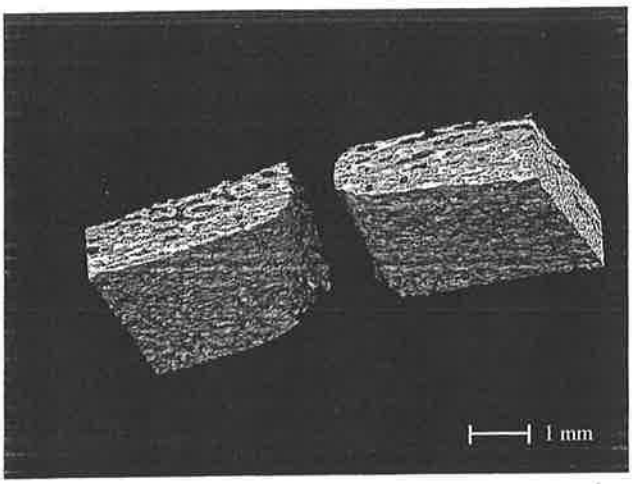

Fig 2 Low-power view of micro-CT scan of a coronal suture starting to undergo craniosynostosis, demonstrating the rounded edges of both bone margins.

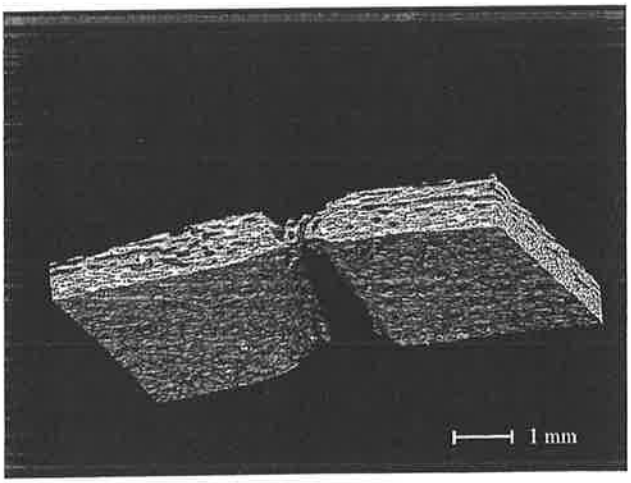

Fig 3 Low-power view of micro-CT scan of the same coronal suture as Figure 2, demonstrating fusion of the endocranial surface (inferiorly) but the ectocranial surface is still undergoing fusion.

shortly the bone margins are rounded with no interdigitation (Fig 2).

The micro-CT images of the fusing suture allow multiple sections to be built up (Figs 2–4) and these confirm that the process of closure arises at different levels along all sutures and occurs at different levels starting at the deep surface next to the dura (Fig 3). The bone micro-architecture matures along the suture and rapidly becomes undistinguishable from the pattern in the adjacent bone (Fig 4).

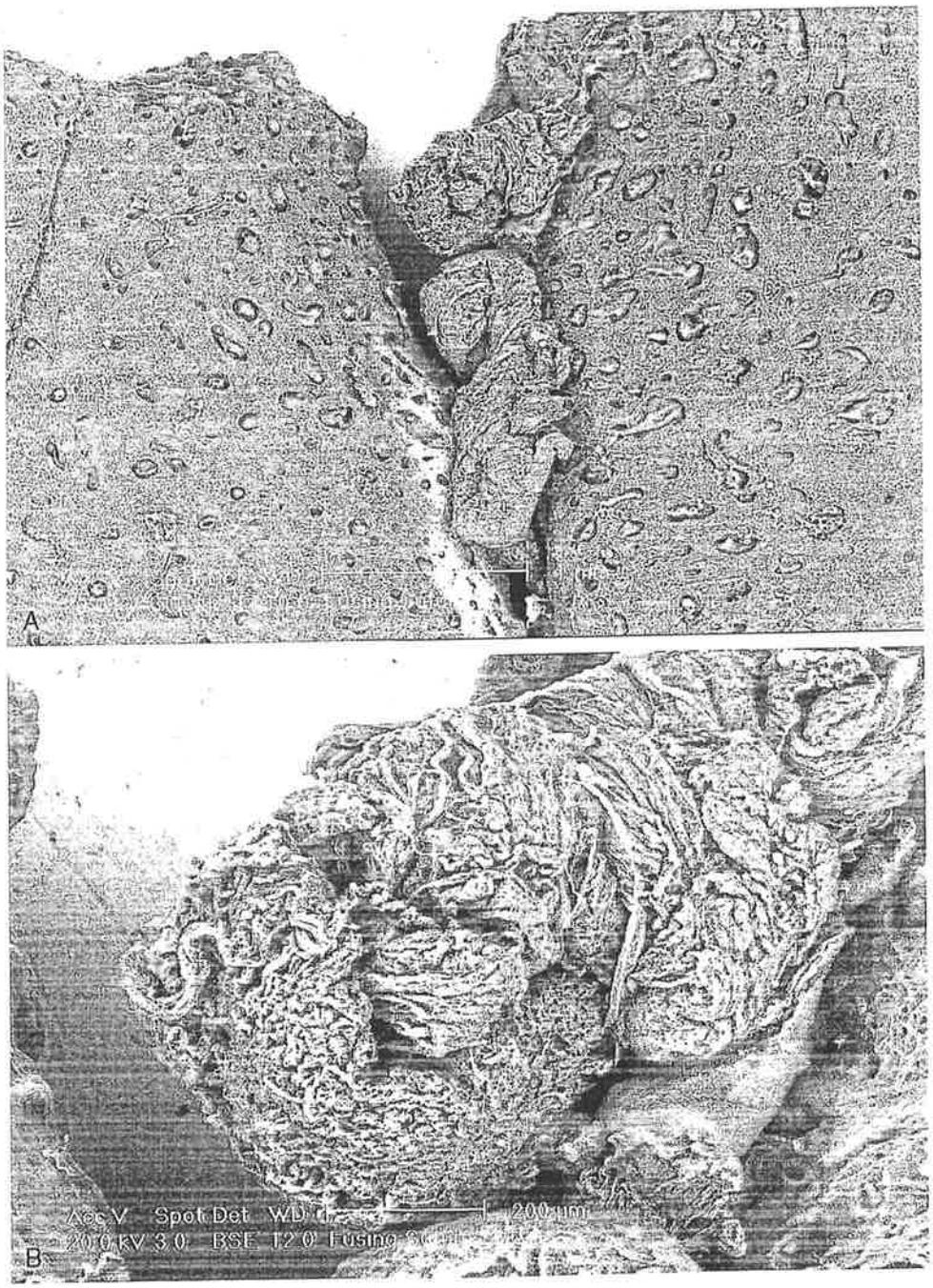

Fig 6 (A) A low-power scanning electron microscope secondary electron image to show the remains of central the suture with the collagen fibers in multiple orientations. (B) A high-power scanning electron microscope secondary electron image view to show the remains of central the suture with the collagen fibersin multiple orientations.

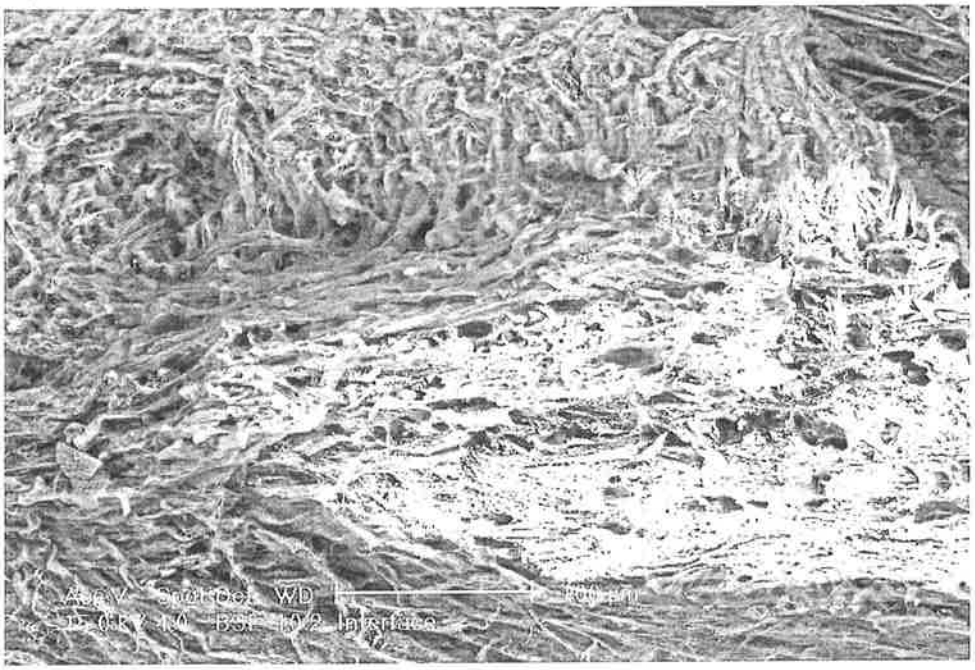

Fig 7 A higher-power scanning electron microscope secondary electron image view to show the bone margin of a suture with newly formed bone shown here in white, with the architecture of the collagen fibers maintained.

The low-power views of the reconstructed scanning electron microscope images of the ectocranial surface revealed the arrangement of the normal open suture showed that the collagen fibers were parallel to each other and the periosteum (Fig 5). In contrast the lambdoid suture undergoing closure due to craniosynostosis the remaining collagen fibers show a variety of orientations (Figs 6A, B).

The higher-power scanning views show that the collagen fibers in the suture become calcified but initially maintain the same architecture as the collagen fibers (Fig 7). In a suture undergoing fusion due to craniosynostosis (Fig 8A) the scanning electron microscope reveals the first connections are fine strands bridging between the adjacent bone margins (Fig 8B). As the fusion process progresses these become calcified by spherical globules (Fig 8C).

Bone further away from the suture interface changes in ultrastructure, gradually converting into the mature highly organized pattern (Fig 9). This arrangement of the bone then becomes identical to that found at the site of a fused metopic suture and is cancellous bone (Fig 10).

The elemental analysis confirms that the center of an open suture is composed largely of carbon (Fig 11). Assessment of the suture margin demonstrates that calcium is now present (Fig 12), but that the amount of calcium increases and the carbon component decreases as the bone matures in samples further away from the suture margin (Fig 13).

Comparison of the fusion patterns of different sutures revealed a clear difference between the ectocranial and endocranial surfaces of a fused sagittal suture (Figs 14A, B). However, studies of the lambdoid and coronal sutures failed to show any ridging at (the site of or adjacent to) either surface with detailed low-power scanning electron microscope examination of the fusing suture (Figs 15A, B). In addition the highest resolution the fusing site on the endocranial surface also had the same appearance as noted on the ectocranial surface, with fine strands with attached calcified masses (Fig 16).

Discussion

Human cranial sutures are formed by dural reflections.[9] The sutures are normally patent until adulthood, and consist of 90% type 1 collagen, cell adhesion proteins (osteopontin, fibronectin), calcium binding proteins osteonectin and bone sialoprotein), proteins involved in mineralization (osteocalcin) and enzymes (collagenase and alkaline phosphatase).[10]

Current understanding of the histology of the normal human cranial suture has identified two

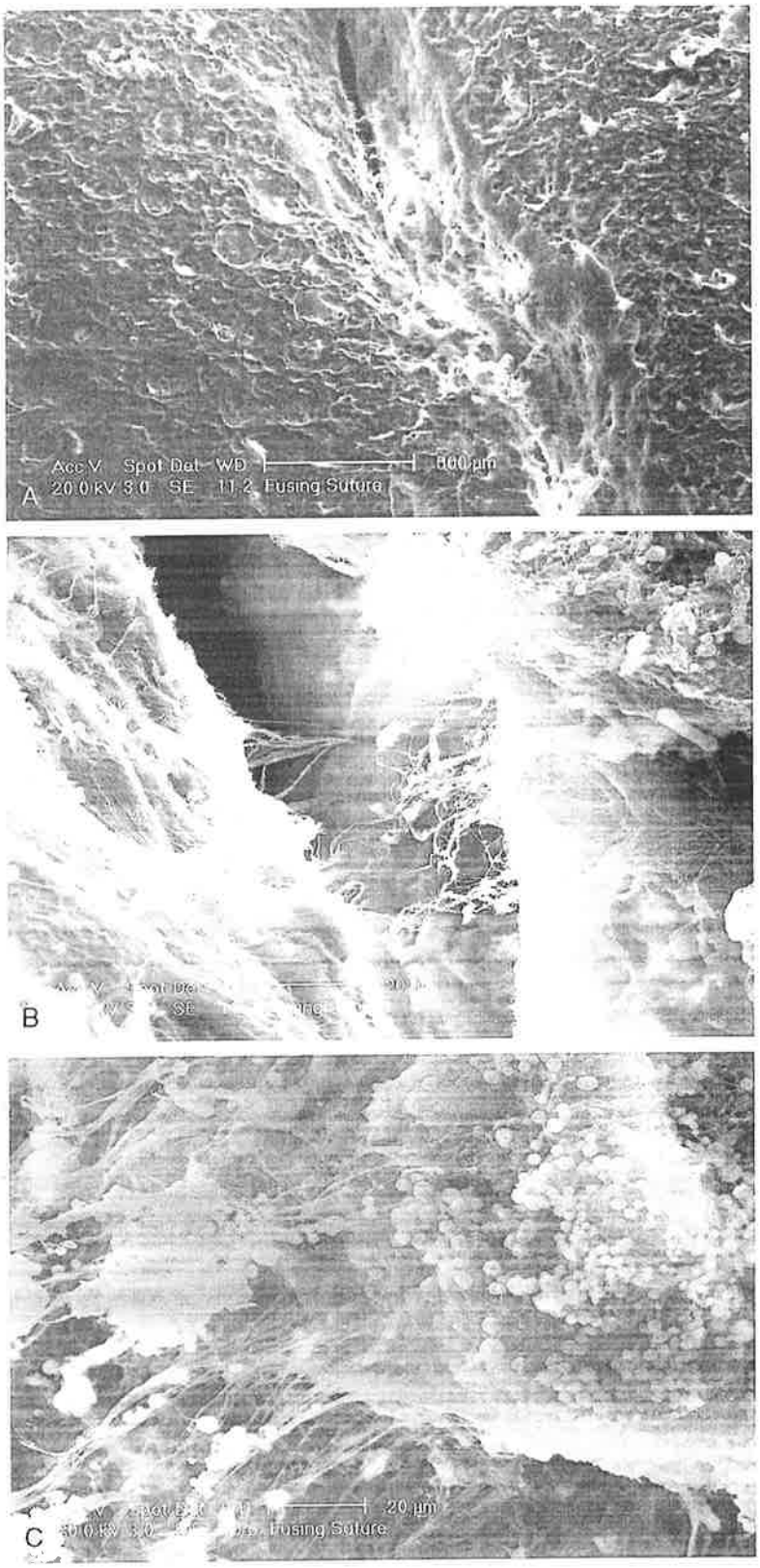

Fig 8 (A) A low-power scanning electron microscope back scattered electron image view to show the fusing lambdoid suture undergoing craniosynostosis. (B) A high-power scanning electron microscope back scattered electron image view to show the fusing lambdoid suture with the fibrous strands bridging between the adjacent bone margins. (C) A high-power scanning electron microscope back scattered electron image view to show the fusing lambdoid suture (closer to the fused region), with the fibrous strands bridging between the adjacent bone margins but now with attached spherical calcified regions.

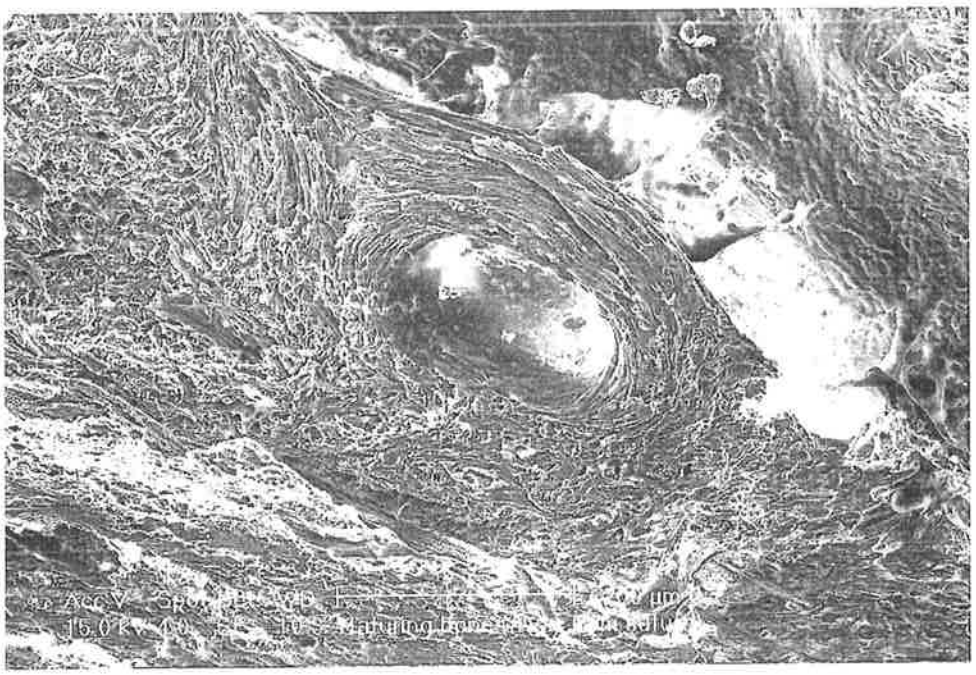

Fig 9 The appearance of the bone 5 mm from the suture interface demonstrating the maturing pattern with the development of cancellous bone.

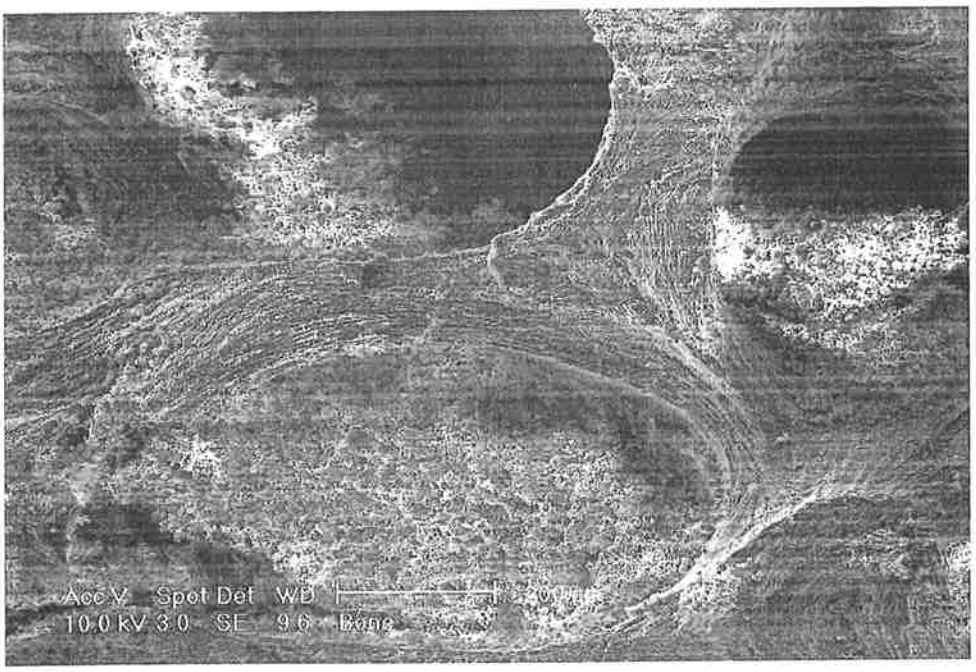

Fig 10 The appearance of the mature bone at the site of a fused metopic suture demonstrating typical cancellous bone.

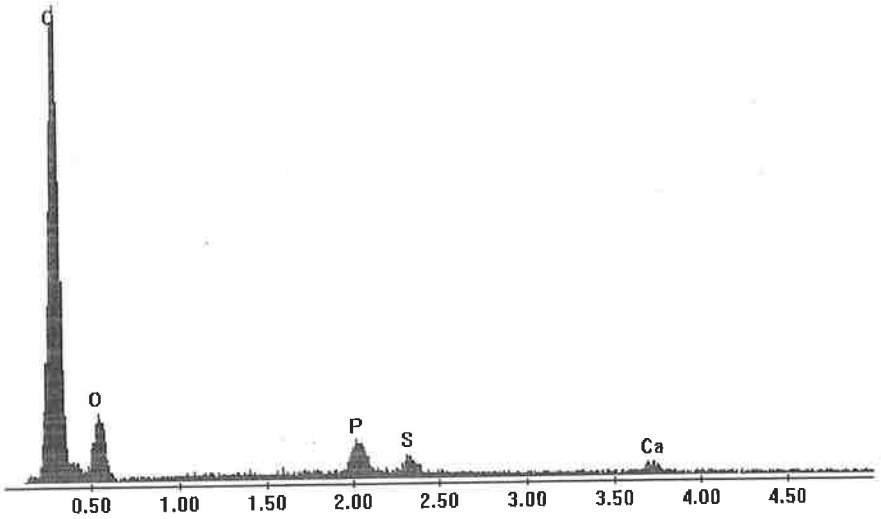

Fig 11 Elemental analysis of the dark central suture revealing the high carbon composition.

structural patterns of collagen fibers between the bone margins. Either parallel to the suture margin or in other cases the fibers were perpendicular.[2] Using our refined technology in normal sutures we too have observed collagen fibers parallel to the overlying periosteum (Fig 5), but not perpendicular. However, we have also noted the collagenfibers to be multi-orientated immediately prior to closure due to craniosynostosis (Fig 6B). We speculate that the perpendicular orientation noted previously could be a two-dimensional representation of the multi-orientated fibers identified by the 3-D scanning electron microscope.

The study of craniosynostosis remains important because it provides a model for the study of suture fusion and the factors maintaining normal suture patency and function. It has previously been noted in histological studies of craniosynostosis that suture closure involves bone spicules extended from the suture margins bridging the gap.[2] The advances in the quality and resolution of scans have enabled more detail study of the process of craniosynostosis. The high magnification images produced by the scanning electron microscope show that the process occurs firstly with fibers crossing the gap (Fig 8B) but these then associate with calcium globules (Fig 8C). This pattern is seen on examination of both the endocranial and ectocranial surfaces. (Fig 15).

Previous micro-CT suture study of human suture focused on the sagittal suture, and our results

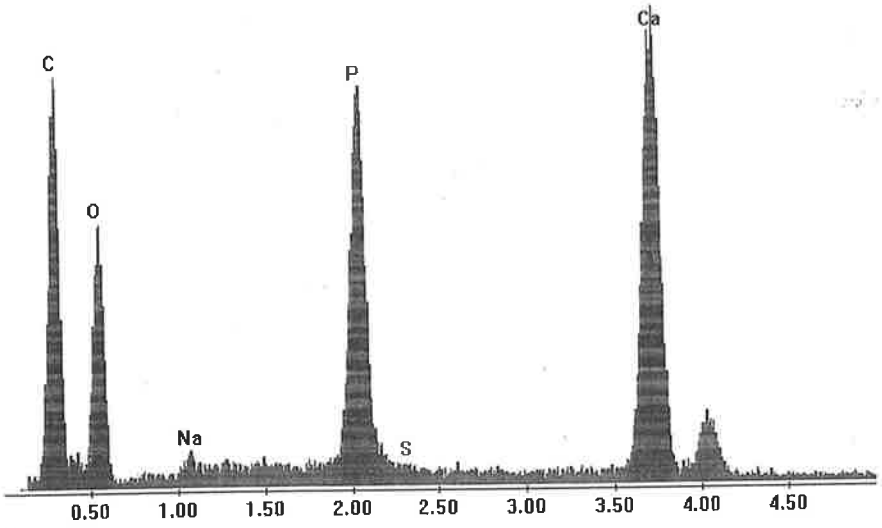

Fig 12 The elemental analysis of the contents of suture margin bone with peaks for calcium, phosphorus and oxygen.

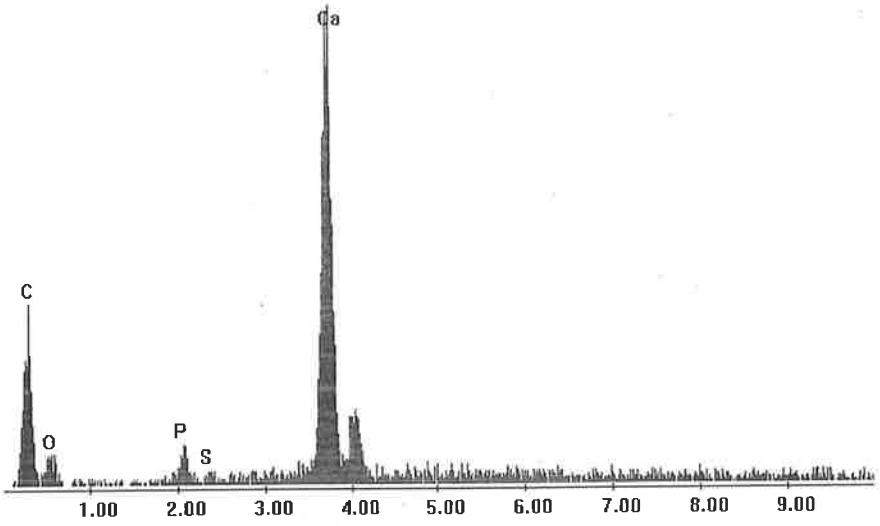

Fig 13 The elemental analysis of the mature cancellous bone trabeculae, with a reduced peak for carbon.

confirm the previous findings at this site, particularly that the craniosynostosis starts at different levels of a suture, (usually the endocranial surface, Figure 3), as well as ridging on both surfaces of a fused suture.[5,7] However, our findings of lambdoid and coronal sutures undergoing craniosynostosis on both surfaces identified that here too craniosynostosis started at the endocranial surface and progressed to the ectocranial surface. This could be explained by the report that paracrine factors in the dura are important initiators of the fusion process.[12]

However, we also identified a marked difference from the previous sagittal suture studies, with no evidence of ridging on either surface of a fused suture (Figs 15A, B). We speculate that these differences may be related to differences between the embryological development of the lambdoid and coronal sutures and sagittal suture.[13]

Elemental analysis has been shown to be a reliable method of undertaking mineralization studies of bone.[14] Our results have confirmed that the center of the suture is composed largely of carbon, which is in keeping with its known content of type 1 collagen. The results at the studies at the suture margin with its new formed bone compared to the more mature bone away from the suture margin demonstrate that the calcium content increases while carbon content proportionally decreases. This finding is in keeping with the report that the degree of mineralization increases with time.[15]

In conclusion, we have shown that the modern scanning technology can advance the understanding

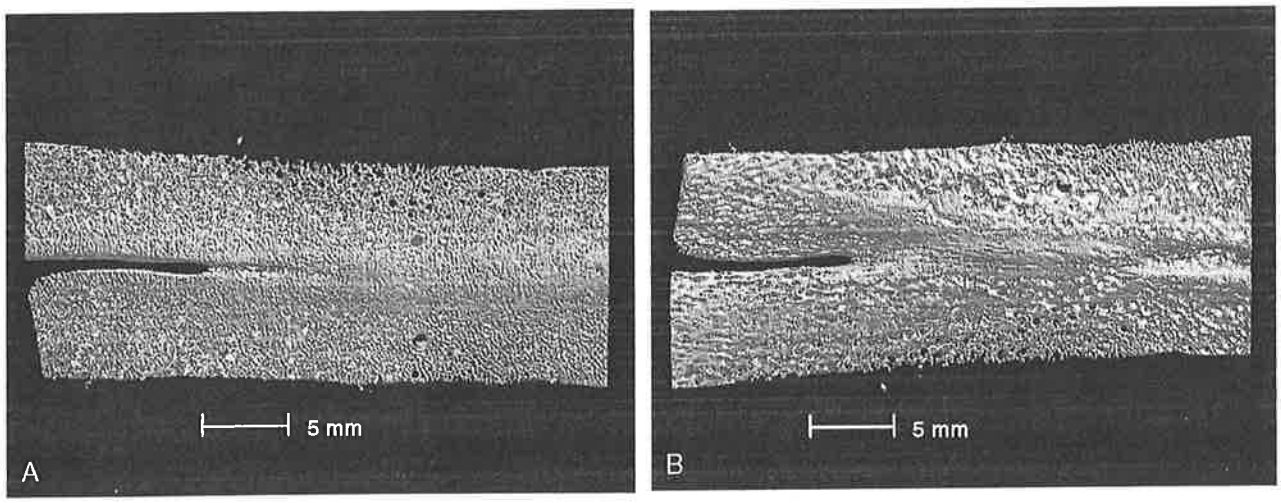

Fig 14 Micro-CT views of endocranium and ectocranium surfaces in sagittal synostosis demonstrating ridging.

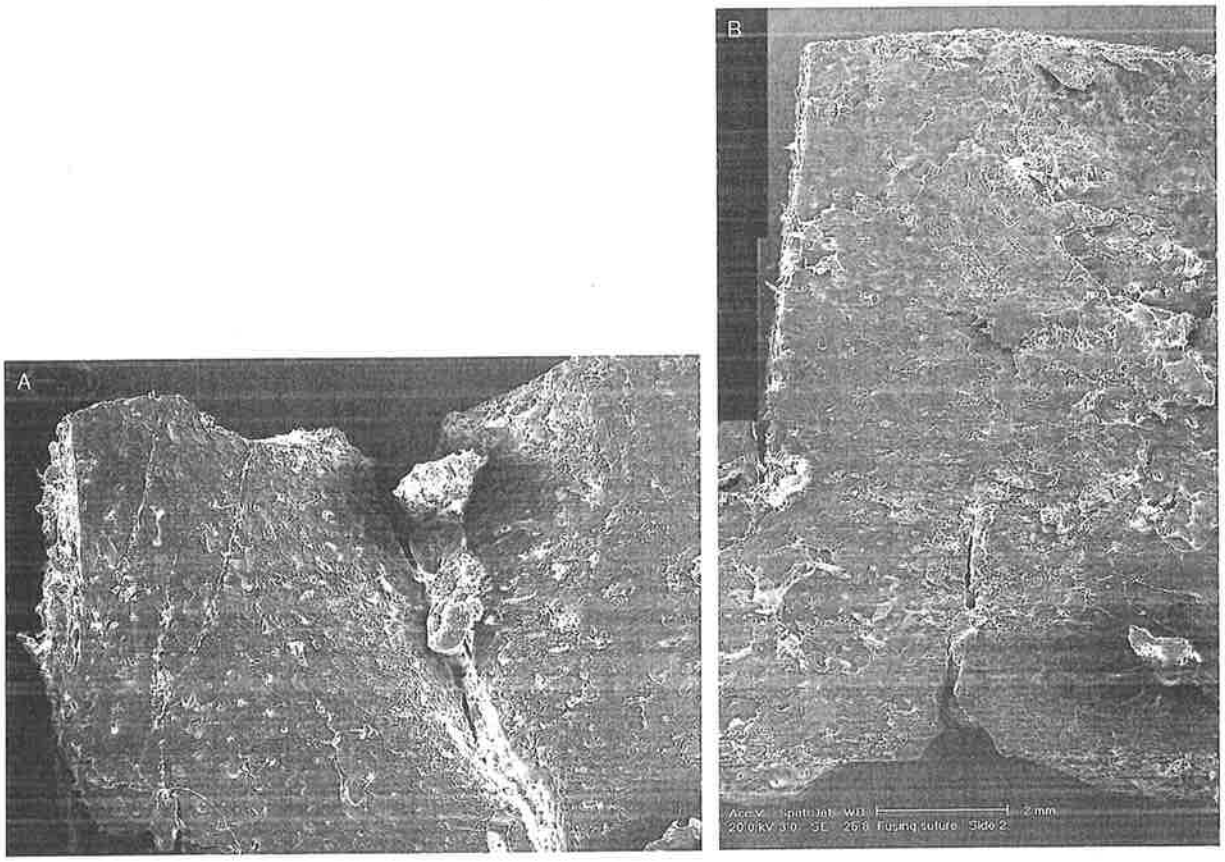

Fig 15 (A) Low-power scanning electron microscope view of the ectocranium of lambdoid synostosis. Note the absence of ridging. (B) Low-power scanning electron microscope view of the endocranium of lambdoid synostosis. Note the absence of ridging.

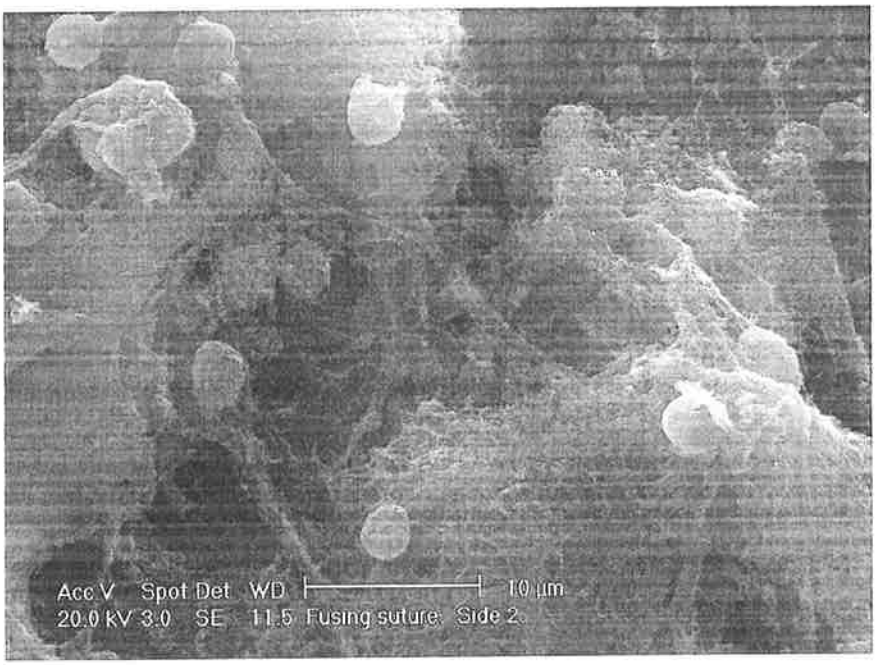

Fig 16 A high-power scanning electron microscope back-scattered electron image view to show the fusing lambdoid suture on the endocranial side, with the fibrous strands bridging between the adjacent bone margins but now with attached spherical calcified regions.

of the process of suture closure. We speculate that further developments in the understanding of human suture fusion may come from studies using specimens stored in RNAlater for micro-CT scanning and subsequent correlation with gene expression patterns of the scanned tissue cells using recombinant DNA technology.

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6.2 Foetal Ultrasound

Anderson PJ, McLean NR, David DJ.

Craniosynostosis and Childbirth.

Eur J Plast. Surg. 2005; 28: 94 -98.

6.2.1 Hypothesis and Aims

Can foetal ultrasound examination be used as an investigative tool to study the timing as to when the process of craniosynostosis starts *in utero*?

This study is a preliminary investigation as to whether different types of non-syndromic single suture craniosynostosis can be identified by antenatal ultrasound examination.

6.2.2 Outcome

This study demonstrates that sagittal, unicoronal, bicoronal and metopic cranio-synostosis can all be identified on antenatal ultrasound examination.

Eur J Plast Surg (2005) 28: 94–98
DOI 10.1007/s00238-005-0753-z

CASE REPORT

P. J. Anderson · N. R. McLean · D. J. David

Craniosynostosis and childbirth

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Abstract The current management of craniosynostosis is focussed on an affected child who presents with abnormality of the head shape shortly after birth. We report four cases of non-syndromic craniosynostosis, recently seen in a 4-month period, presenting to the Australian Craniofacial Unit (ACFU) where all the mothers had prolonged and difficult labour. This included emergency caesarian section in two cases, and perineal repair in the other two cases. Interestingly, all these women had undergone pre-natal ultrasound examination and critical retrospective review highlighted that craniosynostosis could be observed in their children pre-natally. These cases highlight that children with craniosynostosis at birth can be associated with morbidity of both mother and her child, but this may be preventable as careful review of an ante-natal ultrasound examination tends to reveal craniosynostosis.

Keywords Craniosynostosis · Ultrasound examination · Caesarian section

Introduction

The process of labour and delivery of children with craniosynostosis has received little attention with most of the management directed to the care of an affected child only after it is born and the condition diagnosed.

However, the presence of even a single suture affected by craniosynostosis may have consequences during delivery since the physiological "moulding" of the head which occurs during delivery requires normal cranial sutures. The possibility of ante-natal diagnosis of craniosynostosis could influence subsequent obstetric management.

We report four cases where the mothers of children with four different patterns of craniosynostosis have had a prolonged labour and an adverse delivery. We suggest that these might have been avoided with careful review of the ante-natal ultrasound examination.

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Case reports

Case 1

A 3-month-old girl was referred for assessment by ACFU with trigonocephaly (Fig. 1a). She was the second child of parents both aged 24, and was born at term, the other non-surviving twin having been lost in the first trimester.

Induction was performed at 41 weeks and after 22 h of labour, a child weighing 2,975 g and with an abnormal head shape was delivered. However, during delivery the mother sustained a significant perineal tear, which required repair. An ultrasound examination at 18 weeks had identified the abnormal head shape and a subsequent examination at 34 weeks showed flattening of the frontal bones (Fig. 1b).

After 3 months during which she developed satisfactorily, she was referred to ACFU for further assessment. The diagnosis of trigonocephaly due to metopic synostosis was confirmed (Fig. 1c). She subsequently underwent frontal remodelling.

Case 2

A 3-month-old girl was referred for assessment of forehead asymmetry, who was otherwise thriving and developing normally (Fig. 2a).

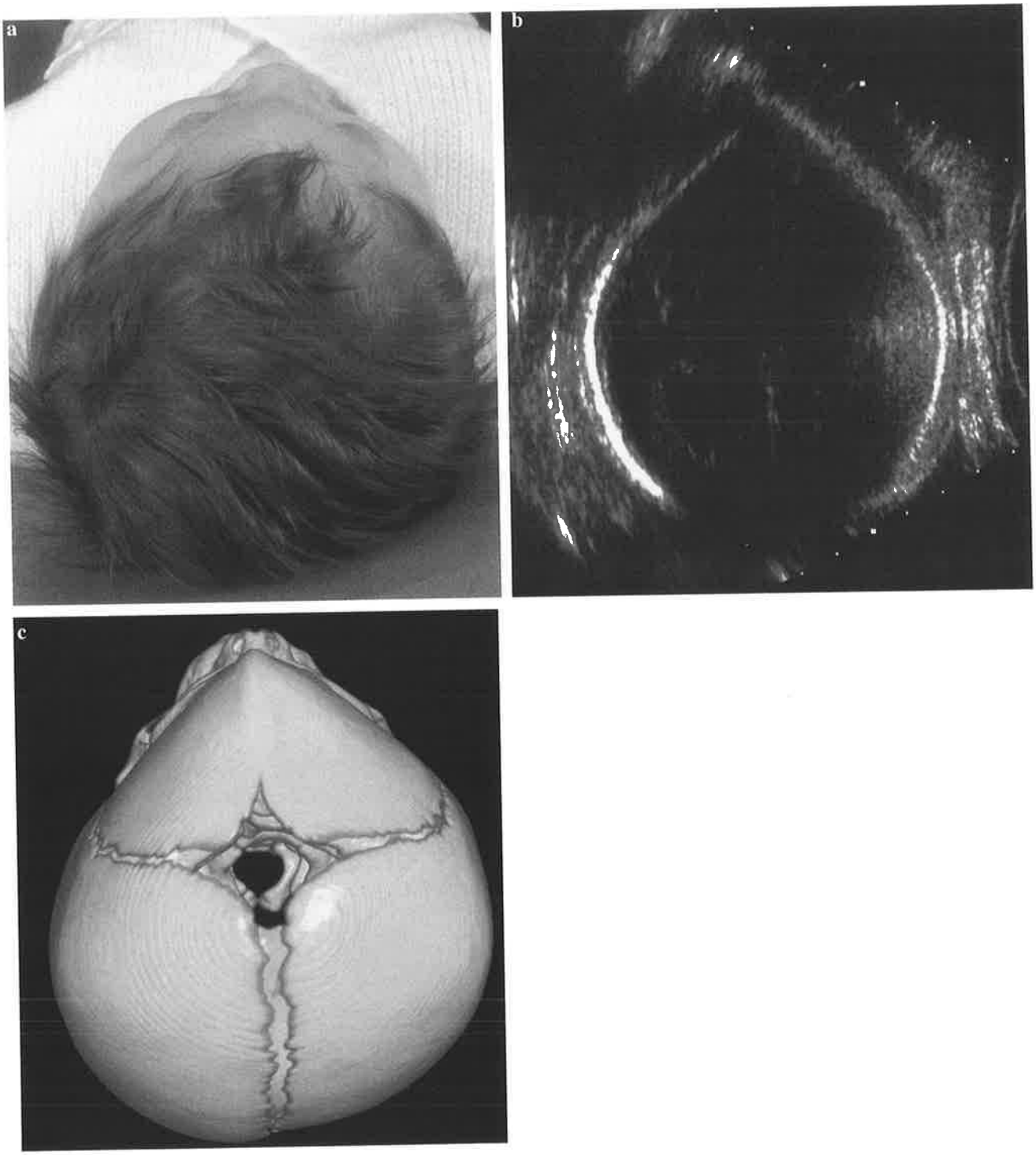

Fig. 1 **a** Case 1, vertical view of the child's head demonstrating the trigonocephaly. **b** The foetal ultrasound examination at 34 weeks, demonstrating the flattening of the frontal bones, suggesting trigonocephaly. **c** Case 1, 3d CT reconstruction, age 3 months

She was the fourth child of a mother aged 34 and a father aged 33, and she was born at term after a problematic pregnancy due to minimal foetal movement during the last 10 weeks. The mother went into labour spontaneously before successfully delivering her baby, who weighed 3,800 g, 24 h later. This was associated with disruption of the perineum which required repair. This labour was much longer than any of her previous three deliveries, and in addition to this was the most difficult to achieve pain control. Ultrasound at 19 weeks was reported as showing no abnormality, although a retrospective review revealed cranial asymmetry (Fig. 2b).

The child was noted to have a similar marked head asymmetry at birth, and when this worsened at 5 weeks,

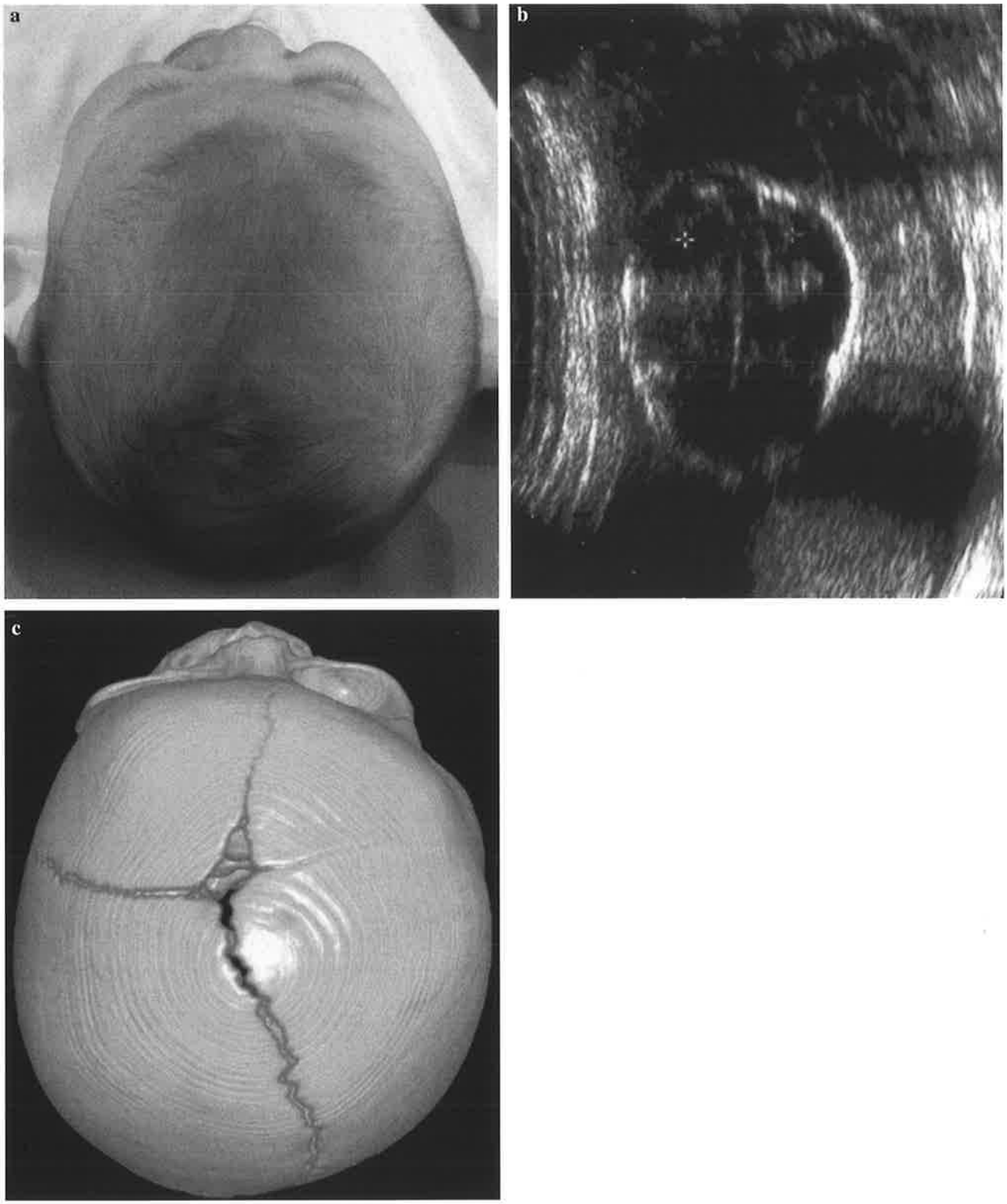

Fig. 2 **a** Case 2, age 3 months, viewed from above. **b** Case 2, foetal ultrasound, aged 18 weeks. **c** Case 2, 3d CT scan demonstrating unicoronal synostosis

radiological examination suggested unicoronal synostosis which was subsequently confirmed (Fig. 2c). Surgical correction was undertaken.

Case 3

A 4-month-old boy was referred for assessment of scaphocephaly (Fig. 3a). He was the second child of father aged 36, mother aged 32. The pregnancy was complicated by the onset of gestational diabetes and pre-eclampsia at 32 weeks.

The mother was induced at 39 weeks but after a trial of labour lasting for 16 h, she underwent emergency caesarian section. An ante-natal ultrasound examination had been performed but was reported as normal (Fig. 3b). The cranial deformity of scaphocephaly was obvious at birth.

He was subsequently assessed as having sagittal synostosis and underwent calvarial remodelling.

Case 4

A 3-month-old girl was referred for assessment of brachycephaly, who was otherwise thriving and developing normally.

She was the first child of a mother aged 23 and a father aged 39; she was born at term after an uneventful pregnancy. After 24 h of labour and failing to progress, an emergency caesarian section was performed. Birth weight was 3,060 g and it was documented that she had an abnormal skull shape at delivery. An ante-natal ultrasound examination of the foetus at 19 weeks had been carried out, but this was reported as normal.

Skull radiographs were undertaken which suggested bilateral coronal synostosis and this was confirmed at specialist review. She has since undergone calvarial remodelling.

Discussion

These four cases of non-syndromic craniosynostosis having different sutures affected presented within a 5-month period in our department. In each case, the cranial anomaly was clinically apparent either at birth or shortly afterwards, and in all the four, the mothers had undergone prolonged labour and complicated delivery.

All the four cases had undergone ultrasound examination and in each case, the biparietal diameter

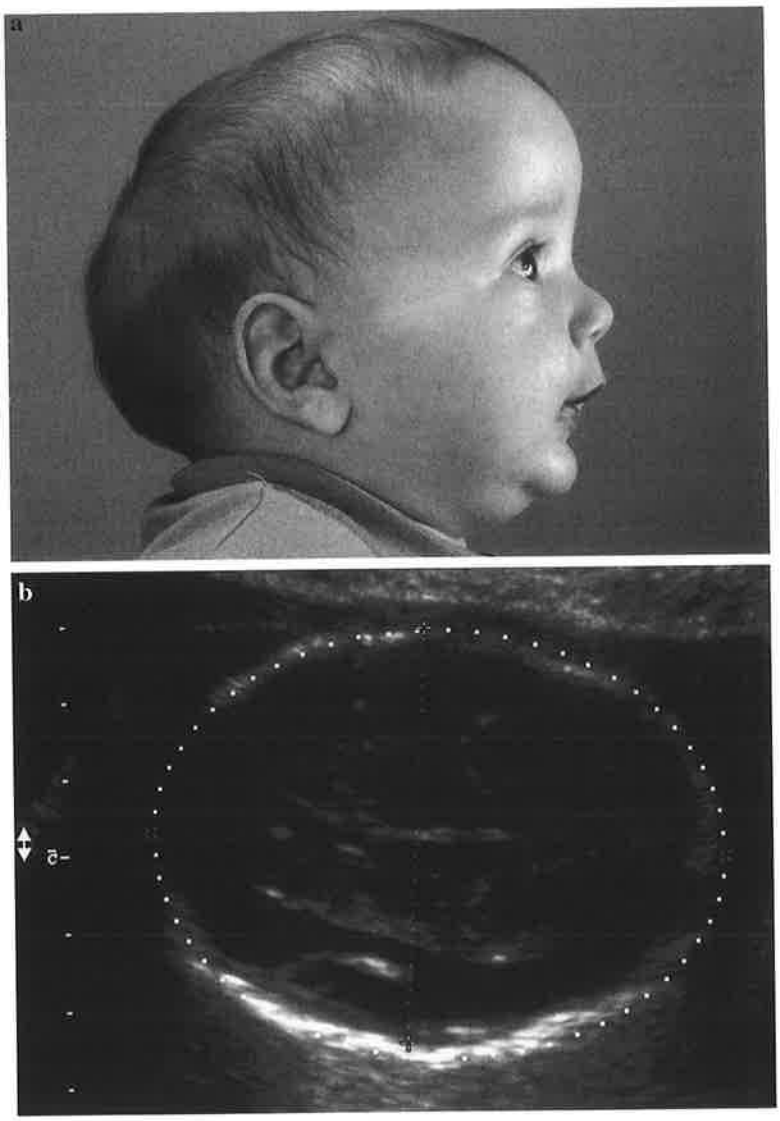

Fig. 3 **a** Case 3, age 5 months, lateral view. **b** Case 3, foetal ultrasound, age 32 weeks

CHAPTER 7:

DISCUSSION

CHAPTER 7

DISCUSSION

7.1 Discussion

These papers describe three different approaches to investigate the structure and behaviour of human cranial sutures and the clinical consequences of their early fusion (craniosynostosis). These three approaches: the investigation of intracranial volumes, the cytological genetic studies, and the suture ultrastructure and timing of craniosynostosis, have each contributed to the overall understanding of the behaviour of the human cranial sutures. The significance of each will be considered in turn during the chapter.

7.2 Intracranial Volume Studies

The pathological premature fusion of the cranial sutures produces disturbances in cranial growth not just at the site of fusion but also compensatory changes in the adjacent unfused sutures leading to the characteristic changes in cranial morphology depending on the affected suture[47]. It has been assumed by clinicians that the local restriction of suture growth following craniosynostosis has an overall effect of a reduced intracranial volume. This has led to the philosophy underlying surgical correction with excision of the affected sutures and simultaneous expansion of the intracranial volume[23].

These studies overcome the recognised weaknesses of the existing published studies which test the underlying hypothesis that the intracranial volume is reduced in cases craniosynostosis. Early attempts at direct measurement were made using water[25] and mustard seeds[48]. These approaches were both found to be unreliable methods and yielded inconsistent results. This was due to air bubbles in the water and the mustard seeds having variable packing density. To overcome these flaws of the direct measurement further studies using indirect measurement became possible with the advent of using 3D CT scans.

The early studies of indirect intracranial volume measurement in cases with single suture craniosynostosis[23,24] have had the disadvantage that they used normal values for intracranial volume derived using a different method of calculation than the method used to calculate the intracranial volume in the individual study. This raised concerns regarding the validity of the results and suggested that this could be improved using

normal values determined in the same manner as the experimental subjects[27]. Comparison of the normal intracranial volume determined by the Abbott-Netherway curves with Lichtenberg values demonstrates significant differences between the two sets of normal data[25] and indeed explains the apparent differences between the conclusions of this study into sagittal synostosis and the earlier study by Gault et al[23].

The findings of the sagittal synostosis study does confirm the findings of the previous study that overall the intracranial volume is increased in both males and females[24]. The clinical significance of these findings is that the goal of surgical correction of isolated non-sagittal synostosis, in the absence of raised intracranial pressure, is calvarial re-modelling alone without volume expansion.

The findings of the study of metopic synostosis is significant because this is the first time that it has been found in any study of children with non-syndromic craniosynostosis that they have a smaller intracranial volume than an unaffected age and sex matched normal population. Although this is new it cannot be completely unexpected given the previous finding that cranial dysmorphology in this condition worsens through time[29]. While the numbers in this study are still small and only the values for the male group have reached statistical significance, further studies of males aged over six months using larger numbers (and all females), will clarify the relationship between trigonocephaly and intracranial volume.

The study of Apert syndrome genotypes is interesting as it suggests that there is no discernable difference in the intracranial volumes of the two genotypes. The study has the disadvantage in that there are relatively few cases and requires some caution

in forming conclusions. It does confirm the results of the earlier studies of Gault and Posnick that the intracranial volumes for Apert syndrome as a whole are greater than normal. However, it is interesting to compare this outcome with the findings of the study by von Gernet et al.[34] which identified differences in the mental outcomes of the two Apert genotypes. If there is a true difference in mental outcome then this must be related to neurological factors other than differences in the pre-operative intracranial volume.

The intracranial volume in the Case reports with both *FGFR* and *TWIST* mutations when compared to the intracranial volumes of the Crouzon and Pfeiffer phenotypes with single *(FGFR)* mutations fails to identify any discernable differences. This suggests that in these cases the consequence of the additional *TWIST* mutation is minimal on the resulting cranial morphology. However, this contrasts with the Apert syndrome where the intracranial volume in the double mutation case is the only value below the age and sex matched normal value when compared to all the other Apert syndrome cases in this unit and indeed in the other previous studies[23,30]. Interestingly, the clinical course has been similar to other Apert cases despite the clear difference in intracranial volume. Clearly, the significance of the additional *TWIST* mutation will become clearer as more cases are available for study but the currently available evidence suggests that the additional effect of this mutation in the presence of a pre-existing *FGFR2* mutation is minimal.

7.3 Somatic mutations

The underlying cause of most cases of non-syndromic single suture craniosynostosis remains unknown. It is established that pathological conditions with somatic mutations affecting the *FGFR2* and *FGFR3* genes occur. The possibility that non-syndromic craniosynostosis could be the result of somatic mutations of genes which, when mutated, are known to be responsible for syndromic craniosynostosis was worthy of investigation. The subsequent finding that there is an absence of *FGFR* and *TWIST* mutations in all exons known to contain mutations in syndromal forms of craniosynostosis in the cultured fused cranial suture cells tested suggests that somatic mutations of these genes are unlikely to be a major cause of single suture non-syndromic craniosynostosis.

However, the possibility of somatic mutations occurring in cranial suture cells hasn't completely been excluded by the study as there are other explanations of the current findings. Firstly, it is possible that mutations of *FGFR* and *TWIST* (if they occur) are in sites that have not been previously recognised. Some support for this comes from the recent finding of the mutation in *FGFR1* gene underlying Kallman syndrome[49] which uniquely occurs in the intracellular component of a fibroblastic growth factor receptor. Secondly, it is possible that somatic mutations could occur in other genes known to be important in craniosynostosis including *Msx2*[21] and *Alx4*[50]. Finally, it is also possible that epigenetic and environmental factors contribute to this common form of craniosynostosis.

Despite the negative finding of this study it remains likely that there will be genetic mutations associated with the process of craniosynostosis. Additional studies of the genes of fused and unfused suture cells will aid the investigation and aid the identification of the genes which control the intracellular processes which are central to the fusion process. The use of microarray technology will be invaluable to this study and preliminary results have already identified some candidate genes[51].

7.4 Ultrastructural Studies

The use of high resolution scanning electron microscopy to study sutures undergoing craniosynostosis has yielded images which reveal the orientation of the collagen fibres at the calcification front. These studies confirm that the process advances at different levels within the same suture and also that it usually appears to commence at the endocranial surface. Further studies may clarify if this is always the case in all sutures. This is important for clarifying the relationship between the dura mater and the cranial sutures.

Micro-CT scans allow accurate assessment of the suture patency and have the advantage that the whole suture can be visualised when compared to its histological evaluation. This is particularly so in cases where the process occurs at different levels between the endocranial and ectocranial surfaces. This technique will become particularly useful to study ex-vivo suture culture studies. The pattern and orientation of the trabeculae can also be studied using this tool, which may provide clues to the mechanical forces present in and around the suture.

It has previously been recognised that cranial anomalies associated with syndromic craniosynostosis can be identified during the second and third trimester of antenatal ultrasound examinations[13]. The small investigation undertaken here identifies that non-syndromic single suture craniosynostosis can also be recognised on foetal ultrasound examinations during the second trimester. This is the case with sagittal, metopic and coronal sutures. Further retrospective studies of the antenatal ultrasound examinations of a large cohort of children with non-syndromic craniosynostosis will

clarify whether the timing of the process is constant for a particular affected suture. Further study of the timing of this fusion process is worthy given that it is also currently recognised that the onset of craniosynostosis may not start until several years after birth[52,53] suggesting that there is a long period during which the fusion process can be initiated.

7.5 Overall

The importance of the cranial sutures is that their development and function plays a critical part of craniofacial development. Abnormalities of this process, particularly craniosynostosis, impact on the developing craniofacial skeleton.

The study of the cranial anomalies resulting from craniosynostosis and accurately determining the intracranial volume are important aids to the considered clinical management of children with craniosynostosis[23]. The finding that the intracranial volume is increased in comparison with age and sex normal values in sagittal synostosis but decreased in males older than six months with metopic synostosis both impact on surgical practice. It also suggests that further study of the other affected sutures in non-syndromic individuals is warranted. Preliminary results on small samples have identified that intracranial volumes are often greater than age and sex normal values in both unicoronal and unilambdoid craniosynostosis but without reaching statistical significance[54].

It has been the working hypothesis of craniofacial surgeons that craniosynostosis results in reduced intracranial volume and surgical correction has included volume expansion to correct this. These studies challenge the validity of the underlying hypothesis and it would appear that calvarial remodelling is the more important surgical goal in cases of non-syndromic sagittal craniosynostosis. In Apert syndrome although the intracranial volume may be above normal the rationale for undertaking a fronto-orbital advancement remains when despite the subsequent increase in

intracranial volume it is undertaken for the exorbitism to provide protection of the corneas.

Further studies using larger populations and a wide range of clinical and morphological parameters will be required to fully elucidate the relationship between genotype and phenotype in Apert syndrome.

At the cellular level there is still much which remains to be discovered particularly regarding the intracellular processes resulting in the process of craniosynostosis. While there has been the discovery of underlying genetic mutations in the more common craniosynostosis syndromes, a cause for underlying factors in the more prevalent single suture craniosynostosis remains largely elusive. It would appear likely from the currently available evidence that the intracellular pathways which control the processes of suture cell differentiation and proliferation are key to the understanding of the pathological process of craniosynostosis in non-syndromic individuals. In this regard an increased understanding of molecular biology will be important to further study.

In the long term, improved understanding of these cellular processes could provide the foundation to develop treatments aimed at slowing or stopping the premature differentiation process of the suture cells and thereby interfere therapeutically with the process of craniosynostosis. This goal would have very significant beneficial clinical consequences to patients and their families, as well as the wider community.

APPENDICES

APPENDICES

APPENDIX ONE Academic Activities

Published Papers

ANDERSON PJ, NETHERWAY DJ, McGLAUGHLIN K, DAVID DJ.

Intracranial volume measurement of sagittal craniosynostosis

J Clinical Neurosci. *In press*. (e-pub 7 Feb, 2007)

ANDERSON PJ, NETHERWAY DJ, ABBOTT A, DAVID DJ.

Intracranial volume measurement of metopic craniosynostosis

J Craniofac Surg. 2004; Vol 15(6): 1014 -1016.

ANDERSON PJ, NETHERWAY DJ, ABBOTT A, COX T, ROSCIOLI T, DAVID DJ.

Intracranial volume in Apert syndrome

Pediatric Neurosurg. 2004; Vol 40(4): 161 -164. Doi: 10.1159/000081933

ANDERSON PJ, NETHERWAY DJ, COX T, ROSCIOLI T, DAVID DJ.

Do craniosynostosis phenotypes with both *FGFR2* and *TWIST* mutations have a worse prognosis?

J Craniofac Surg. 2005; Vol. 17(1): 166 -172.

ANDERSON PJ, COX T, ROSCIOLI T, ELARKIS G, SMITHERS L, DAVID DJ, POWELL B.

Somatic *FGFR* and *TWIST* mutations are not a common cause of isolated non-syndromic single suture craniosynostosis.

J Craniofac Surg. *In press*

ANDERSON PJ, NETHERWAY DJ, DAVID DJ, SELF P.

Scanning electron microscope and Micro-CT evaluation of cranial sutures in health and disease.

J Craniofac Surg. Vol 17(5): 909 - 919.

ANDERSON PJ, McLEAN NR, DAVID DJ.

Craniosynostosis and Childbirth.

Eur J Plast Surg. 2005; Vol 28(2): 94 - 98.

Abstracts

ANDERSON PJ, NETHERWAY DJ, ABBOTT AH, DAVID DJ. (2003)

Intracranial volumes in unoperated metopic craniosynostosis.

J Dent Res. Vol 82, Special Issue C, C-98. P9.

ANDERSON PJ, SMITHERS L, COX T, DAVID DJ, POWELL BC. (2005)

Cytogenetic studies of osteogenic progenitor cells from sutures in craniosynostosis.

ANZ J Surg. Vol 75: suppl p A95

ANDERSON PJ, COX T, ROSCIOLI T, SMITHERS L., POWELL BC, DAVID DJ. (2005)

Somatic mutations in cranial suture cells.

Craniofacial Surgery 11: Medimond International Proceedings, Bologna. p 19 - 20.

ANDERSON PJ, NETHERWAY DJ, COX TC, ROSCIOLI T, DAVID DJ. (2005)

Intracranial volume in different Apert genotypes.

Craniofacial Surgery 11: Medimond International Proceedings, Bologna. p 291 - 292.

ANDERSON P, NETHERWAY DJ, POWELL BC, DAVID DJ. (2005)

Micro-CT assessment of the structure of cranial sutures in health and disease.

Craniofacial Surgery 11: Medimond International Proceedings, Bologna. p 293 - 294.

ANDERSON P, NETHERWAY DJ, ABBOTT AH, DAVID DJ. (2005)

Intracranial Volume in Metopic Craniosynostosis.

Craniofacial Surgery 11: Medimond International Proceedings, Bologna. p 295 - 296.

Oral Presentations

ANDERSON PJ, NETHERWAY DJ, ABBOTT AH, COX T, DAVID DJ. (SEPTEMBER 2003)
Intracranial volumes in craniosynostosis syndromes.
Australian and New Zealand Association of Oral & Maxillofacial Surgeons 20th Biennial Conference, Glenelg, South Australia.

ANDERSON PJ, NETHERWAY DJ, ABBOTT AH, DAVID DJ. (SEPTEMBER 2003)
Intracranial volume measurement of pre-operative children with non-syndromic metopic synostosis
International Association for Dental Research ANZ Division, Melbourne.

ANDERSON PJ, SMITHERS L, COX T, DAVID DJ, POWELL BC. (AUGUST 2004)
Cytogenetic studies of osteogenic progenitor cells from sutures in craniosynostosis.
Australian Dental Research Conference.
Adelaide.

ANDERSON PJ, SMITHERS L, COX T, DAVID DJ, POWELL BC. (OCTOBER 2004)
Cytogenetic studies of osteogenic progenitor cells from sutures in craniosynostosis.
5th Asian Pacific Craniofacial Society Biennial Conference. Seoul, Korea.

ANDERSON PJ, COX T, ROSCIOLI T, SMITHERS L., POWELL BC, DAVID DJ.

(2005)

Somatic mutations in cranial suture cells.

XIth International Craniofacial Surgeons Conference, Gold Coast, Queensland.

ANDERSON PJ. (DECEMBER 2005)

Craniofacial Research using Cranial Suture Cells

10th Asian Research Symposium in Rhinology, Kuala Lumpur, Malaysia.

ANDERSON PJ, NETHERWAY DJ, McGLAUGHLIN K, DAVID DJ.

(NOVEMBER 2006)

Intracranial volume measurement of sagittal craniosynostosis.

6th Asian Pacific Craniofacial Association Conference, Singapore.

ANDERSON PJ. (JANUARY 2007)

The use of imaging in craniofacial research

Research Symposium, 9th People's Hospital, Shanghai, China.

Poster Presentations

ANDERSON PJ, NETHERWAY DJ, ABBOTT AH, DAVID DJ. (SEPTEMBER 2003)

Intracranial volume measurement of pre-operative children with non-syndromic metopic synostosis

International Association for Dental Research ANZ Division, Melbourne.

ANDERSON PJ, McLEAN NR, DAVID DJ. (NOVEMBER 2003)

Foetal ultrasound assessment of craniosynostosis

Australasian Society for Medical Research, 42nd Scientific Conference.

Adelaide University.

ANDERSON PJ, SMITHERS L, COX T, DAVID DJ, POWELL BC. (MAY 2005)

Cytogenetic studies of osteogenic progenitor cells from sutures in craniosynostosis.

Royal Australasian College of Surgeons Annual Scientific Congress, Perth, Western Australia.

ANDERSON PJ, NETHERWAY DJ, SELF P, DAVID DJ. (JUNE 2005)

Micro-CT Studies of Craniosynostosis

Australian Medical Research Society Annual Scientific Meeting, Adelaide.

ANDERSON PJ, NETHERWAY DJ, DAVID DJ. (SEPTEMBER 2005)

Genotype morphology in Apert syndrome

XIth International Craniofacial Surgeons Conference, Gold Coast, Queensland.

ANDERSON PJ, NETHERWAY DJ, DAVID DJ. (SEPTEMBER 2005)

Intracranial volume measurement of children with metopic synostosis

XIth International Craniofacial Surgeons Conference, Gold Coast, Queensland.

ANDERSON PJ, NETHERWAY DJ, SELF P, DAVID DJ. (SEPTEMBER 2005)

Micro-CT assessment of cranial sutures undergoing craniosynostosis

XIth International Craniofacial Surgeons Conference, Gold Coast, Queensland.

ANDERSON PJ, McLEAN NR, DAVID DJ. (SEPTEMBER 2005)

Ultrasound diagnosis of craniosynostosis

XIth International Craniofacial Surgeons Conference, Gold Coast, Queensland.

APPENDIX TWO Ethical Approvals

1. Craniofacial Shape Analysis project REC 1438/3/2006

2. Molecular pathways of Craniosynostosis project REC 1033/10/2005

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30th June 2004

Prof D David
Head, The Australian Craniofacial Unit
WCH

Dear Prof David

Re: Craniofacial Shape Analysis. REC1438/3/2006

Thank you for your letter dated 22nd June 2004 in which you responded to matters raised by the WCH Research Ethics Committee at its March 2003 meeting. All matters have been addressed and final approval is given for the study to proceed.

I remind you approval is given subject to:
- immediate notification of any serious or unexpected adverse events to subjects;
- immediate notification of any unforeseen events that might affect continued ethical acceptability of the project;
- submission of any proposed changes to the original protocol. Changes must be approved by the Committee before they are implemented;
- immediate advice, giving reasons, if the protocol is discontinued before its completion;
- submission of an annual report on the progress of the study, and a final report when it is completed.

Please note it is your responsibility to provide these reports – without reminder from the Ethics Committee.

Approval is given for three years only, and if the study is more prolonged than this, a new submission will be required. Please note the approval number above indicates the month and year in which approval expires and it should be used in any future communication.

Yours sincerely

TAMARA ZUTLEVICS (DR)
CHAIR
WCH RESEARCH ETHICS COMMITTEE

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5 May 2004

Dr Barry Powell
Child Health Research Institute
Women's & Children's Hospital

Dear Dr Powell

Re: The Molecular Pathways of Craniosynostosis. REC1033/10/2005

Thank you for your letter dated 22nd April 2004. As advised in my email of 27th April, all matters have been satisfactorily addressed with the exception of one point pertaining to the Information Sheet. The information regarding the commercial possibilities of the study needs to be contained in a separate paragraph immediately following the general information about the study, rather than embedded in the general question section of the Information Sheet. Please refer to the attached Information Sheet in which I have indicated how this should be done.

Yours sincerely

TAMARA ZUTLEVICS (DR)
CHAIR
WCH RESEARCH ETHICS COMMITTEE

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