Craniosynostosis in a South African population.

By Dr. Ilse Crous

CRSILS001

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Supervisor: A/Prof Karen Fieggen

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Table of Contents

DECLARATION I

ABSTRACT II

ACKNOWLEDGEMENTS AND CONTRIBUTIONS IV

LIST OF TABLES V

LIST OF FIGURES VI

ABBREVIATIONS VII

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW 1

History and overview of craniosynostosis 1

Epidemiology and Prevalence 1

Sutures involved in craniosynostosis 1

Craniosynostosis versus positional plagiocephaly 6

Classification of Craniosynostosis 9
Non-syndromic craniosynostosis (NSCS) 9
Syndromic craniosynostosis (SCS) 10
Complex Craniosynostosis 12

Genetics causes of craniosynostosis 12

Epigenetics and other factors 16
Mechanical factors 17
Maternal factors 17
Paternal age effect 17

Associated pathology/complications 18
Raised Intracranial Pressure 18
Hydrocephalus 20
Chiari I malformations 21
Obstructive Sleep Apnoea 22
Ophthalmological complications 23
Hearing impairments 24
Facial and dentofacial deformities 25
Malformation of the extremities 26
Cognitive functioning and behaviour 26

Role of Imaging in the diagnosis and treatment of craniosynostosis 27
Plain X-ray 27

Computed Tomography (CT) Scan 28
Cranial ultrasound (CUS) 28
Magnetic resonance imaging (MRI) 29

Surgical management **30**
1) Fronto -orbital advancement and remodelling 31
2) Barrel stave osteotomies and cranial remodelling 31
3) Unilateral cranioplasty 31
4) Cranial expansion with distraction osteogenesis 31
5) Monobloc advancement and cranioplasty 31
6) Endoscopic suturectomy 32

Craniosynostosis in Africa and specifically South Africa **33**

Purpose of the study **34**
Aim 34
Objectives 34

CHAPTER 2: METHODS 36

Literature review: **36**

Ethics approval: **36**

Recruitment and enrolment: **36**
Inclusion criteria 37

Research procedures and data collection methods **37**

Data Analysis **37**

CHAPTER 3: RESULTS 39

Enrolment **39**

Demographics **43**

Diagnosis confirmation with CT scan **43**

Antenatal History **46**

Birth History **46**

Development **47**

Clinical Features **48**
Craniofacial findings 49
Skeletal abnormalities 51
Other congenital abnormalities 52

Surgery **53**

Genetic Testing **55**

CHAPTER 4: DISCUSSION AND CONCLUSIONS 56

REFERENCES 63

APPENDICES 75

Appendix 1: Data sheet **75**

Appendix 2: Information sheet for participants/ parents of participants **81**

Appendix 3: Informed consent for research project: English version **83**

Appendix 4: Assent for research project: English version **84**

Appendix 5: Ethics approval documents **85**

Declaration

I, ……Ilse Crous……, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date……11 March 2021…….

Abstract

Background:

Craniosynostosis refers to the premature fusion of calvarial bones which lead to restricted growth potential. Compensatory growth occurs in the dimensions not restricted by fusion and causes progressive distortion in the skull shape. The majority of craniosynostosis cases occur in isolation and are so called non-syndromic craniosynostosis. In about 30 % of all cases, anomalies are noted along with the craniosynostosis, often defining a described and recognised syndrome. The aim is to delineate the phenotype observed in a South African population.

Methods:

In this descriptive study, hospital records for the preceding five years were retrospectively reviewed to describe the profile of patients with craniosynostosis seen at the Red Cross War Memorial Children's Hospital in Cape Town. In addition to the retrospective review, a sub cohort of patients were prospectively phenotyped. The patients were subdivided into three groups namely: non-syndromic craniosynostosis, syndromic craniosynostosis and craniosynostosis with additional features. The last group included patients who had additional malformations or clinical findings without a syndromic diagnosis. The prevalence of phenotypic findings, teratogen exposure, birth complications, congenital malformations, surgical interventions and results of genetic testing in this cohort is described. Descriptive statistical analysis was used.

Results:

A total of 47 children with craniosynostosis were included in this study. Twenty-five individuals of the cohort were male, and one patient has a disorder of sexual development. Eighteen patients had non-syndromic synostosis. Twelve of these had sagittal type synostosis and five had metopic type synostosis with one unspecified. Thirteen had syndromic synostosis. Eight were clinically diagnosed with Crouzon syndrome of which three were molecularly confirmed. Four patients had Apert

syndrome and one had Pfeiffer syndrome, these were clinically diagnosed without molecular confirmation. Sixteen patients had craniosynostosis with some additional findings but no syndromic diagnosis. The suture involved in the majority of patients was the sagittal suture. Ten patients had an additional structural brain abnormality and 13 had signs of raised intracranial pressure. The average age at confirmation of diagnosis of craniosynostosis by CT scan was 22.5 months (*SD* = 31.4, range: 0.1 – 140.9). Thirty of the 47 patients had craniosynostosis surgery. The average age of surgery was 22.4 months (*SD* = 19; range: 5-79). The anthropometric, phenotype and developmental features indicate that this is a highly heterogenous group of disorders.

Conclusion:

Craniosynostosis has been widely reported worldwide, especially in individuals of European descent with only a few reports on craniosynostosis in South African or African populations. Knowledge of the phenotypic spectrum will aid in understanding and documenting this group of disorders in our local population. This study also highlights that this is a complex condition best managed by a multidisciplinary team that should include a medical geneticist. The recognition of specific craniosynostosis syndromes together with appropriate molecular testing can be cost effective even in a limited resource setting and aid in accurate prognosis and recurrence risk information for families.

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Phoebe, "Thank you for always being my rainbow after the storm"- unknown.

List of Tables

Table 1. Clinical Features of Common Craniosynostosis syndromes. 10

Table 2. FGFR-Related Craniosynostosis syndromes. 13

Table 3. Additional molecular aetiology in non-syndromic and syndromic craniosynostosis. 14

Table 4. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to demographics. 43

Table 5. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to CT scan results. 45

Table 6. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to development. 48

Table 7. Comparing the two groups, syndromic and craniosynostosis with additional features with regard to clinical features. 52

Table 8. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to surgery. 54

List of Figures

Figure 1. Different major suture involvement described in craniosynostosis (Buchanan et al., 2017).....4

Figure 2. Illustration of minor sutures/skull base sutures (Calandrelli et al., 2014). 5

Figure 3. Flow chart in use for triage of new referral via telephone in a craniofacial clinic in the Netherlands (Bredero-Boelhouwer et al., 2009). 7

Figure 4. Clinical characteristics between positional plagiocephaly and lambdoid synostosis from vertex view (Ridgway and Weiner, 2004).....8

Figure 5. Diagnosis confirmation with CT scan: sutures involved. 44

Figure 6. Age distribution in months according to age at confirming diagnosis with CT scan. 45

Figure 7. Birth weight, height and head circumference per centile. 47

Figure 8. Average weight, height and head circumference per centiles. 49

Figure 9. Age distribution in months at the time of craniosynostosis surgery. 54

Abbreviations

3D	Three dimensional
ALX4	Homeobox protein aristaless-like 4 gene
CNP	Craniofacial nurse practitioner
CPAP	Continuous positive airway pressure
CSF	Cerebrospinal fluid
CS	Craniosynostosis
CT	Computed tomography
CTH	Chronic tonsillar herniation
CUS	Cranial ultrasound
DNA	Deoxyribonucleic acid
DSD	Disorder of sexual development
EFNA4	Ephrin-A4 gene
EFNB1	Ephrin- B1 gene
ENT	Ear, nose and throat
EPO	Erythropoietin
ERF	ETS repressor factor gene
Fe	Iron
FGF	Fibroblast growth factor gene
FGFR1	Fibroblast growth factor receptor 1 gene
FGFR2	Fibroblast growth factor receptor 2 gene
FGFR3	Fibroblast growth factor receptor 3 gene
ICP	Intracranial pressure
ICU	Intensive care unit
IL11RA	Interleukin 11 receptor subunit alpha gene
IV	Intravenous
MEGF8	Multiple EGF like domain 8 gene
MRI	Magnetic resonance imaging
MSX2	Muscle segment homeobox 2 gene
NSAIDs	Nonsteroidal anti-inflammatory drugs

NSCS	Non- syndromic craniosynostosis
OME	Otitis media with effusion
OMIM	Online Mendelian Inheritance in Man
OSAS	Obstructive sleep apnoea syndrome
PAE	Paternal age effect
PICU	Paediatric Intensive Care Unit
PSG	Polysomnography
RAB23	Member RAS oncogene family gene
RCWMCH	Red Cross War Memorial Children's Hospital
REDCap	Research Electronic Data Capture
SIDS	Sudden infant death syndrome
SMAD6	SMAD family member 6 gene
TCF12	Transcription factor 12 gene
TWIST1	Twist-related protein1 gene
VEP	Visual Evoked Potential
ZIC1	Zic family member 1 gene

Chapter 1: Introduction and Literature review

History and overview of craniosynostosis

The term craniosynostosis is derived from the three words *cranio* or cranium, *syn* which signifies together and *ostosis* referring to the formation of bone. The term was first introduced by Adolph Wilhelm Otto in 1830 (Otto, 1830).

Craniosynostosis is the premature fusion of calvarial bones which leads to restricted growth potential. Compensatory growth occurs in the dimensions not restricted by fusion and causes progressive distortion in the skull shape. This concept is known as Virchow's law (Virchow, 1851).

Epidemiology and Prevalence

Craniosynostosis is most often noted in the new-born period, however occasionally it is identified prenatally by ultrasound investigation or may only be identified at a later stage in infancy or even in early childhood. The incidence for all forms of craniosynostosis is estimated at 1: 2,000 to 1: 2,500 live births (Johnson and Wilkie, 2011; Lajeunie et al., 1995). The majority of craniosynostosis cases occur in isolation. In about 30% of all cases, additional anomalies are noted along with the craniosynostosis, often defining a described and recognised syndrome. Over 150 syndromes are presently described where craniosynostosis is an identified feature (Ciurea and Toader, 2009; Kimonis et al., 2007; O'Hara et al., 2019).

Sutures involved in craniosynostosis

Cranial sutures is the term used to indicate the junctions that play an important role in the differentiation and interaction of the cranial/ calvarial bones (Opperman, 2000). These sutures are made up of fibrous tissue and are flexible to allow moulding

during passage through the birth canal, to permit growth of the expanding brain, and to reduce the impact of mechanical trauma in childhood (Levi et al., 2012).
To function as bone growth sites, the sutures need to remain patent while allowing rapid bone formation at the bone edges (Opperman, 2000). Normal development of cranial sutures depends on multiple factors of genetic and/ or environmental nature.

Current theories of craniosynostosis aetiology aim to include primary defects that occur due to altered suture biology and secondary defects such as can occur with abnormal intrauterine compression. Growth of the skull bones relies on complex interactions between the brain, dura mater, suture mesenchyme and bone plates, together referred to as the "functional matrix".

The four major sutures involved in craniosynostosis are the sagittal, coronal, metopic and lambdoid sutures (See Figure 1) . Minor sutures/skull base sutures are also implicated in craniosynostosis, although less frequently. These include the ethmoido-frontal, the fronto-sphenoidal, the ethmoido-sphenoidal, the spheno-squamous, the spheno-parietal, the spheno-petrosal, the occipitomastoid, the spheno-occipital, parieto-squamous and the parietomastoid sutures (See Figure 2) (Calandrelli et al., 2014). Extension of the synostotic process to minor sutures is commonly found in patients with syndromic craniosynostosis.

Sagittal suture synostosis is the most frequently observed craniosynostosis. Premature fusion of the sagittal suture results in abnormal growth at both the coronal and lambdoid sutures. This results in the calvarium having an elongated shape, in medical terms referred to as scaphocephaly, with an increased anteroposterior length and a decrease in width. A decrease in anterior intraorbital distance (hypotelorism), a prominent occipital protuberance and frontal bossing may be present (Kotrikova et al., 2007; Levi et al., 2012; Massimi et al., 2019; Nagaraja et al., 2013).

Premature coronal suture fusion can either occur unilaterally or bilaterally. If only on one side, there is flattening of the ipsilateral fused frontoparietal bone that results in plagiocephaly. Ventral bowing of the sphenoid bone and shortening of the lateral

orbital wall on the ipsilateral side of the fused suture also result in the well-recognised "harlequin" deformity (Levi et al., 2012; Massimi et al., 2019). In bicoronal craniosynostosis, the forehead appears flatter and the head is shortened in the anteroposterior dimension and taller than average. The frontal and maxillary shortening result in shallow orbits (Dias et al., 2020).

Metopic suture synostosis restricts the growth in the region of the frontal bone, resulting in an increase in the bilateral parietal expansion. The resulting head shape is described as trigonocephaly with parietal flaring and associated temporal narrowing. Hypoplastic ethmoid sinuses, deficient supraorbital ridges and orbital roofs that slant upward medially can also be observed (Kotrikova et al., 2007; Nagaraja et al., 2013).

Lambdoid suture synostosis produces an asymmetrically flattened occiput with bulging of the mastoid and posterior cranial base deformities. Lambdoid craniosynostosis is the least frequently seen craniosynostosis of all the major suture synostoses.

Figure 1. Different major suture involvement described in craniosynostosis (Buchanan et al., 2017).

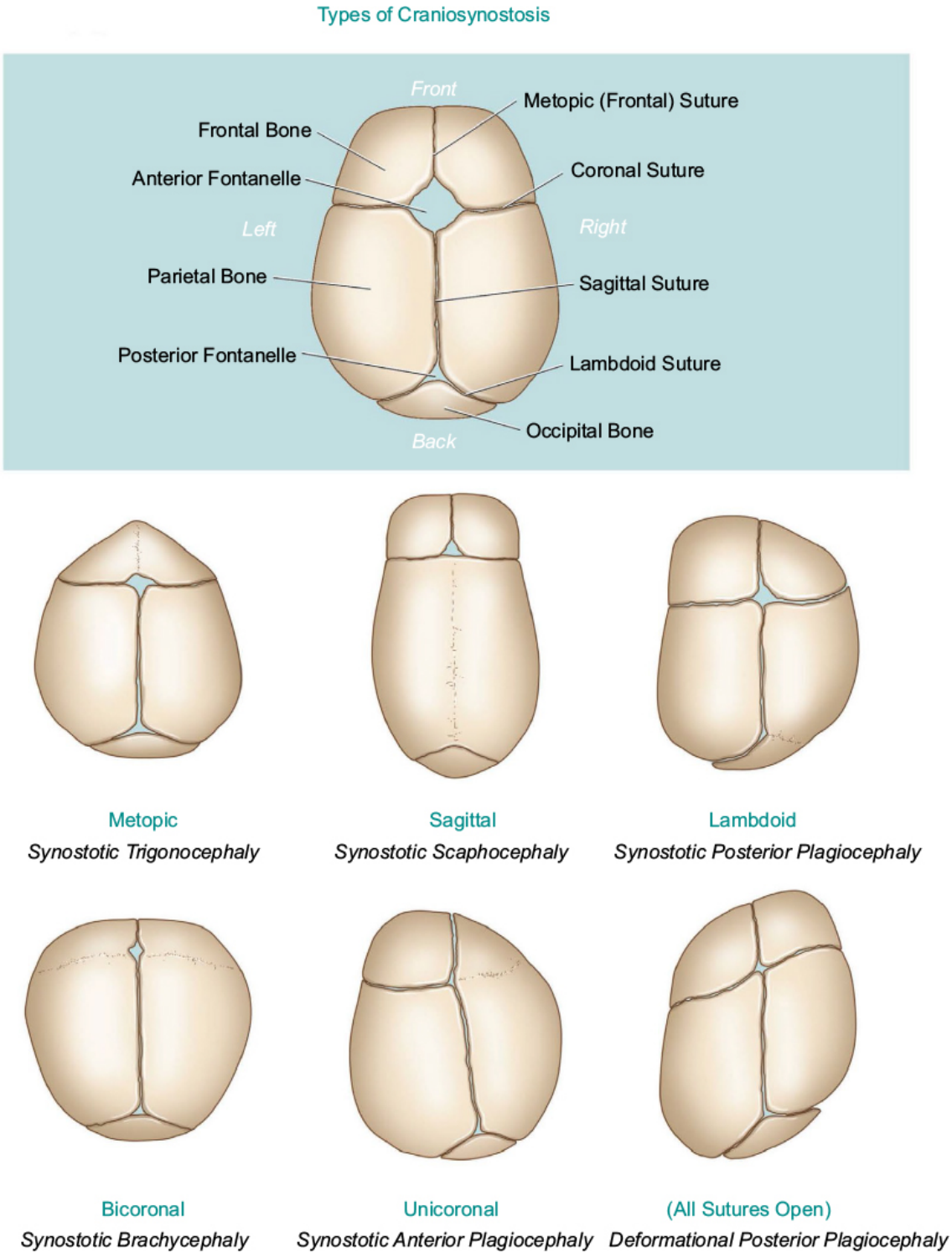

Figure 2. Illustration of minor sutures/skull base sutures (Calandrelli et al., 2014).

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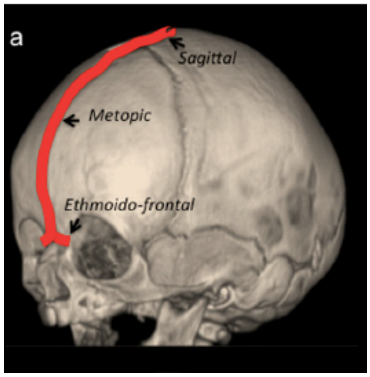
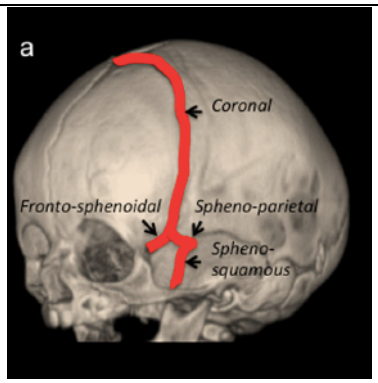
<tr>
<td> 

a) Sagittal arch: ethmoido-frontal minor suture</td>
<td> 

a) Coronal arch: fronto-sphenoidal, spheno-parietal and spheno-squamous minor sutures</td>
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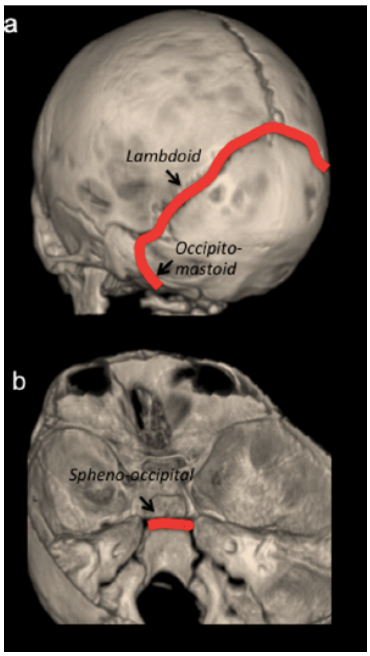
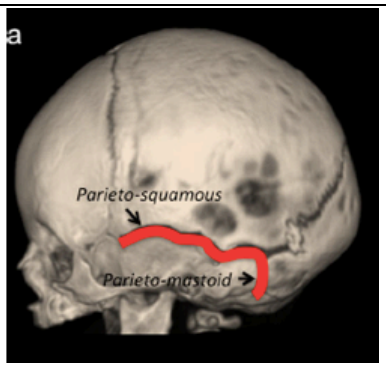
<td> 

a) Lambdoid arch: occipito-mastoid minor suture
b) Spheno-occipital minor suture</td>
<td> 

a) Parieto-squamosal arch: parieto-squamosous and parieto-mastoid minor sutures</td>
</tr>
</table>

Craniosynostosis versus positional plagiocephaly

The recognition of craniosynostosis is complicated by an increasing prevalence in positional plagiocephaly, estimated to occur in between 20% to 48 % of infants. The increase in prevalence observed is due to the recommended supine sleeping position of infants to prevent sudden infant death syndrome (SIDS) (Argenta et al., 1996; Robinson and Proctor, 2009).

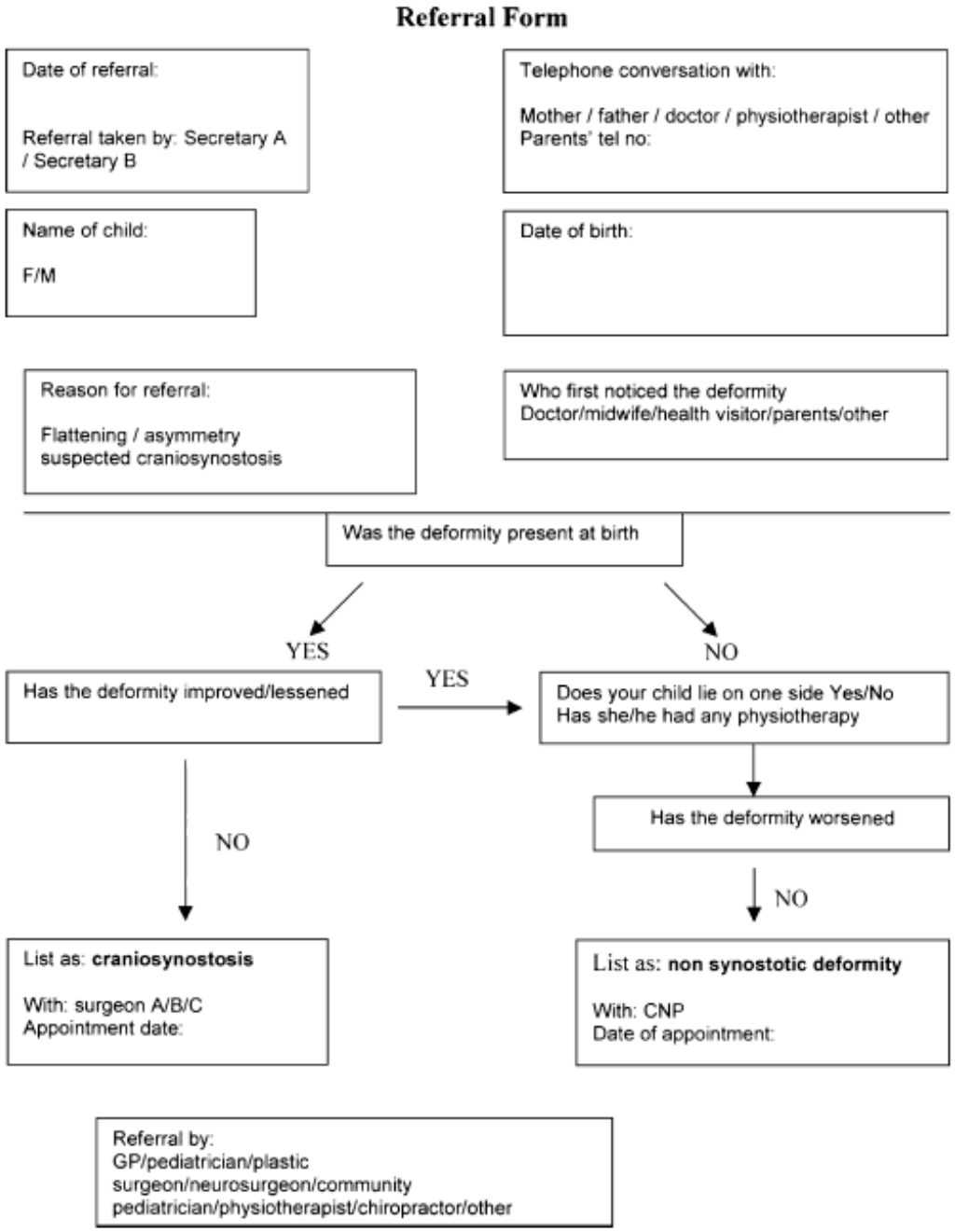
Positional plagiocephaly is characterised by a deformation of the skull bones in the absence of abnormal fusion of sutures (Alden et al., 1999). Ridgway and Weiner advises that history taking and physical examination yield the most important information to distinguish between craniosynostosis and positional plagiocephaly (Ridgway and Weiner, 2004). Diagnostic steps in the form of a flow chart (See Figure 3) appears to simplify this distinction and aid in timely referrals (Bredero-Boelhouwer et al., 2009; Mathijssen, 2015).

Three essential questions can assist to distinguish between craniosynostosis and positional plagiocephaly.

1) Was the deformity already present at birth?
2) Is there a preferred sleep position?
3) Is there improvement of the deformity?

Craniosynostosis can be present at birth whereas positional plagiocephaly usually is not. Improvement of head shape can be observed in positional plagiocephaly, whereas improvement is not observed in craniosynostosis. Lastly the presence of a preferred sleep position is essential when diagnosing positional head shape deformities (Bredero-Boelhouwer et al., 2009; Mathijssen, 2015; Ridgway and Weiner, 2004).

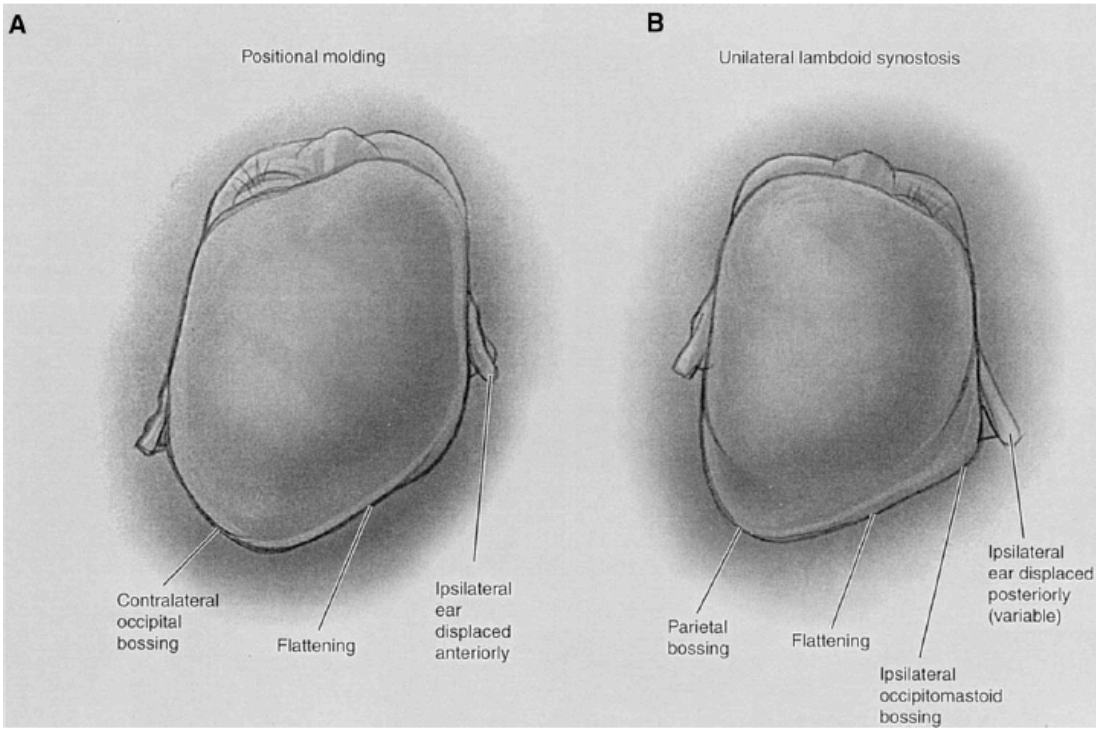
Figure 3. Flow chart in use for triage of new referral via telephone in a craniofacial clinic in the Netherlands (Bredero-Boelhouwer et al., 2009).



Characteristics clinical findings when observing the head from above might aid in the distinction between these two conditions as illustrated in Figure 4. In positional plagiocephaly, seen from above, the head shape appears like a parallelogram with ipsilateral flattening, curved forehead and ipsilateral flattening more anterior. In the case of unilateral lambdoid craniosynostosis the head shape appears trapezium shaped with ipsilateral flattening of the forehead, cranial part of orbit and occiput.

Figure 4. Clinical characteristics between positional plagiocephaly and lambdoid synostosis from vertex view (Ridgway and Weiner, 2004).

Photo A demonstrates positional plagiocephaly. Photo B shows unilateral lambdoid synostosis.



If the clinical diagnosis of positional plagiocephaly is made, additional imaging in the form of x-rays and ultrasound is rarely indicated or performed (Mathijssen, 2015)

Classification of Craniosynostosis

A number of factors should be considered when classifying craniosynostosis. These factors include the following:

The number of sutures involved which can range from simple craniosynostosis when one suture is involved to complex craniosynostosis when multiple sutures are involved.

The aetiology is usually divided into either primary or secondary. Primary aetiology refers to craniosynostosis caused by an intrinsic defect in the suture, and secondary aetiology refers to other medical conditions/systemic disorders affecting the developing suture. Examples of secondary aetiology include: deficient growth of the brain, skeletal dysplasia, hypophosphatasia, mucopolysaccharidoses, and ciliopathies.

Isolated/ non-syndromic craniosynostosis versus syndromic craniosynostosis. Syndromic cases are accompanied by other dysmorphic features and/or developmental abnormalities. Non-syndromic craniosynostosis accounts for approximately 85% of all cases of craniosynostosis, seven percent of cases of craniosynostosis have additional clinical features and nine percent of cases are classified as syndromic (Dempsey et al., 2019)

Non-syndromic craniosynostosis (NSCS)

In non-syndromic craniosynostosis the most common clinical presentation is an abnormal head shape that might be present at birth or develop shortly afterwards. With sagittal suture synostosis, a long and narrow head is observed (scaphocephaly and/or dolichocephaly). With metopic suture synostosis the head is triangular shaped at the front (trigonocephaly). Head shape in coronal suture synostosis depends on unilateral or bilateral involvement. In coronal unilateral synostosis a skewed frontal side (frontal plagiocephaly) is observed and when both the coronal sutures are involved a broad and flattened (frontal brachycephaly) head shape is observed.

Lambdoid suture synostosis results in a skewed posterior side of the head (posterior plagiocephaly) (Garrocho-Rangel et al., 2018).

A diagnosis of NSCS should always be followed by careful clinical examination to exclude the presence of additional malformations or abnormalities.

Syndromic craniosynostosis (SCS)

Syndromic craniosynostosis is predominantly inherited in an autosomal dominant pattern with 50% of cases without a family history and therefore due to the result of a de novo variant. Muenke syndrome (incidence estimated at 1 in 10,000 -30,000 live births) is seen most frequently, followed by Crouzon syndrome (incidence estimated at 1 in 25,000 live births), Saethre-Chotzen syndrome (incidence estimated at 1 in 25,000-50,000 live births), Pfeiffer syndrome (incidence estimated at 1 in 100,000 live births) and Apert syndrome (Incidence estimated at 1 in 100,000 live births) (Buchanan et al., 2014; Doherty et al., 2007; Johnson and Wilkie, 2011; O'Hara et al., 2019; Wilkie et al., 2017).

The table below describes the clinical features of some well described syndromic craniosynostosis disorders.

Table 1. Clinical Features of Common Craniosynostosis syndromes.

Condition	**Clinical Features**
Apert syndrome (OMIM 101200)	Bicoronal craniosynostosis leading to turribrachycephalic skull shape with symmetrical syndactyly of hands and feet. Other malformations and defects can include: midface hypoplasia, soft palate cleft or bifid uvula, fusion of cervical vertebrae, cardiovascular defects, genitourinary, gastrointestinal and respiratory abnormalities. Varying degrees of developmental delay.

Crouzon syndrome (OMIM 123500)	Craniosynostosis (ranging from single suture to pansynostosis), maxillary hypoplasia, mandibular prognathism, shallow orbits, exorbitism and strabismus. No hand and foot abnormalities. Normal intellect.
Muenke syndrome (OMIM 602849)	Unilateral or bilateral coronal synostosis with absent or minimal hand/ foot abnormalities. If present, changes such as carpal fusion of hands, broad hallux, and tarsal fusion of feet. Sensorineural hearing loss, midface hypoplasia, high arched palate, and down slanting palpebral fissures. Normal to mild intellectual disability.
Pfeiffer syndrome (OMIM 101600)	Craniosynostosis of the coronal, lambdoid and sagittal sutures, midface hypoplasia, exorbitism, hypertelorism, high forehead, down slanting palpebral fissures, choanal atresia or stenosis, mild syndactyly of hands and/ or feet, variable brachydactyly of hands and feet, broad thumbs and/ or hallux. Intellect range from normal to developmental delay.
Saethre-Chotzen syndrome (OMIM 101400)	Coronal craniosynostosis, high forehead, facial asymmetry, maxillary hypoplasia, strabismus, ptosis, prominent ear crus. Brachydactyly, partial cutaneous syndactyly, thumb/ hallux abnormalities, and hearing loss.
Beare-Stevenson cutis gyrata syndrome (OMIM 123790)	Craniosynostosis (cloverleaf skull in more than 50% of patients), moderate to severe midface hypoplasia, choanal atresia or stenosis, abnormal ears, natal teeth. Normal extremities with widespread cutis gyrate. Can have genital abnormalities including bifid scrotum.
Jackson-Weiss syndrome (OMIM 123150)	Craniosynostosis, mandibular prognathism, hypertelorism, proptosis, midface hypoplasia, foot abnormalities (broad and medially deviated hallux, broad metatarsals, broad proximal phalanges, partial cutaneous syndactyly of second and third toes), and normal thumbs.

Craniofrontonasal dysplasia (OMIM 304110)	Craniosynostosis of the coronal sutures, hypertelorism, abnormal facial proportions, facial asymmetry, bifid tip of nose, longitudinal ridging and/or splitting of nails, webbed neck, clinodactyly of toes.
Crouzon syndrome with acanthosis nigricans (OMIM 612247)	Clinical features of Crouzon syndrome and acanthosis nigricans (verrucous hyperplasia and hypertrophy of the skin with hyperpigmentation and accentuation of skin markings especially in flexure areas) . Notably more frequent choanal atresia or stenosis, and hydrocephalus.

Complex Craniosynostosis

This group refers to cases of craniosynostosis where there is multiple suture synostosis present , but without a clear clinically identified syndrome and without a proven genetic diagnosis. In the majority of cases, sagittal suture synostosis occur with metopic synostosis but other combinations are also described and not all studies report the same results. In most cases the sutures are however anatomically adjacent. Complex craniosynostosis are mostly managed as syndromic craniosynostosis (Czerwinski et al., 2011).

Genetics causes of craniosynostosis

Current literature suggests that genetic causes are found in approximately 20% of all craniosynostosis cases (Armand et al., 2019). Genetic characterisation of a patient is important for allowing appropriate genetic counselling and providing accurate information concerning co-morbidities, prognosis and recurrence risk.

Recent advances in molecular techniques, for example, whole exome and whole genome sequencing, have led to the identification of numerous genes causing craniosynostosis.

Many diverse chromosomal abnormalities are associated with craniosynostosis. Currently chromosomal imbalances account for approximately 13%-20% of the observed genetic causes (Armand et al., 2019). Chromosomal loss or gain is more frequently identified with metopic synostosis (Kini et al., 2010). Children with chromosomal abnormalities have been shown in one study to present later and often with significant learning disability (Wilkie et al., 2013).

Major contributions to the genetic aetiology of craniosynostosis are found in single gene mutations. Many of the genes identified, are involved in the biology of cranial suture development. Examples include the Sonic hedgehog pathway, *WNT*-signalling, *NOTCH/EPH* pathway, the *RAS/MAPK* pathway, Retinoic acid and the *STAT3* pathway (Goos and Mathijssen, 2019). The fibroblast growth factor (*FGF*) signal pathway, has been proven to play an essential role in sutural closure (Connerney and Spicer, 2011). Pathogenic variants in genes in this *FGF* pathway were found to be the cause of the most common craniosynostosis syndromes (See Table 2). Pathogenic variants, which are either de novo or autosomal dominantly inherited, usually lead to a gain- of -function effect. The *FGFR3* P250R variant causing Muenke syndrome is the most prevalent of these (Kruszka et al., 2016; Wilkie et al., 2013).

Table 2. FGFR-Related Craniosynostosis syndromes.

FGFR 1 related craniosynostosis	• Pfeiffer syndrome type 1 to 3
FGFR 2 related craniosynostosis	• Apert syndrome • Bear-Stevenson syndrome • Crouzon syndrome • Isolated coronal synostosis • Jackson-Weiss syndrome • Pfeiffer syndrome type 1 to 3
FGFR 3 related craniosynostosis	• Crouzon syndrome with Acanthosis nigricans (AN)[p.(Ala391Glu)]

	• Isolated coronal synostosis • Muenke syndrome [p.(Pro250Arg)]

Other important molecular aetiologies in both non-syndromic and syndromic craniosynostosis are summarised in Table 3 (Armand et al., 2019; Goos and Mathijssen, 2019; Twigg et al., 2006; Wilkie et al., 2017, 2013):

Table 3. Additional molecular aetiology in non-syndromic and syndromic craniosynostosis.

Gene:	**Condition:** (Unique inheritance features listed)
Ephrin-B1 (*EFNB1*)	Craniofrontonasal dysplasia (OMIM 304110) - X-linked inheritance - Loss of function variant. - Heterozygous females more severely affected due to cellular interference mechanism caused by X inactivation in females.
ALX homeobox 4 (*ALX4*)	Craniosynostosis 5 (OMIM 615529)
Ephrin-A4 (*EFNA4*)	Unicoronal NSCS
ETS repressor factor (*ERF*)	Crouzon like Craniosynostosis/*ERF* related craniosynostosis (OMIM 600775); NSCS
Muscle segment homeobox 2 (*MSX2*)	Craniosynostosis 2/ Boston type (OMIM 604757)
SMAD family member 6 (*SMAD6*)	Craniosynostosis 7 (OMIM 617439); NSCS (metopic and sagittal sutures)
Transcription factor 12 (*TCF12*)	Craniosynostosis 3/ *TCF12*- related craniosynostosis (OMIM 615314); NSCS

Twist-related protein 1 (*TWIST1)*	Saethre-Chotzen syndrome (OMIM 101400) - Loss of function mutation
Zic family member 1 (*ZIC1*)	Craniosynostosis 6 (OMIM 616602); NSCS
Interleukin 11 receptor subunit alpha (*IL11RA*)	Craniosynostosis with dental anomalies/Kreiborg-Pakistani syndrome (OMIM 614188) - Autosomal recessive inheritance
Member RAS oncogene family (*RAB23*)	Type 1 Carpenter syndrome (OMIM 201000) - Autosomal recessive inheritance
Multiple EGF like domain 8 (*MEGF8*)	Metopic syndromic craniosynostosis phenotype - Autosomal recessive inheritance

In some cases non-syndromic craniosynostosis (NSCS) is probably a complex trait, caused by a combination of polygenic influences as well as epigenetic factors. Non-syndromic craniosynostosis is sporadic in more than 95% of affected families. These factors can complicate genetic testing for NSCS. Timberlake and Persing (2018) made the following genetic testing recommendations when dealing with non- syndromic craniosynostosis.

a) All NSCS cases of sagittal and/or metopic craniosynostosis should be screened for *SMAD6* mutations, due to the fact that it is the most frequent cause of NSCS and confers a risk of recurrence.
b) Non-syndromic coronal craniosynostosis cases should be screened for *TCF12* and *TWIST1* mutations in addition to the *FGFR3 P250R* variant associated with Muenke syndrome. Mutations in these 3 genes confer the greater risk of recurrence in future offspring.

c) If screening results are negative for mutations in the genes listed above in a and b, consider participating in ongoing research to identify genetic causes of NSCS. Collection of DNA in trio (case and both parents) is recommended.
d) For familial cases of midline NSCS, families should be screened for rare mutations in *SMAD6* and, if negative, followed by testing for *TWIST1*, *TCF12*, *MSX2* and *ERF*.
e) In cases of single suture craniosynostosis which present with other congenital abnormalities that are not consistent with known syndromes, it is recommended to perform a comparative genomic hybridization array to identify copy number gains or losses. If no copy number variation is found, consider exome sequencing of the case-parent trio (Timberlake and Persing, 2018).

The clinical phenotypes and genetic cause of various syndromic craniosynostosis conditions are very well described and this can aid in clinical diagnosis and targeted genetic testing. Difficulties in genotype phenotype correlation can also be encountered. For example a wide phenotypic range is described in individuals with identical pathogenic variants in *FGFR2* gene (Ito et al., 2005). There are however some genotype phenotype correlations described, for example, midface procedures, ventricular shunting, and tracheostomy are predominantly associated with craniosynostoses caused by mutations in the *FGFR2* gene (Wilkie et al., 2013). Ptosis correction, hearing aids, and upper limb surgery were most common in those with Saethre-Chotzen, Muenke or Apert syndromes. Strabismus is found to be associated with *FGFR2* and *FGFR 3* mutations (MacIntosh et al., 2007).

Epigenetics and other factors

Epigenetics is defined as the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence (Dupont et al., 2009). Epigenetic modifications are especially important for normal development and normal biological processes. Epigenetic regulation is well known to

be modified by environmental conditions, dietary components, chemicals and pollutants (Feil and Fraga, 2012).

To show the effects of environmental and epigenetic factors, twin studies , especially monozygotic twins, have been very useful.

Mechanical factors

Intrauterine compression caused by multiple pregnancies, high birth weight, low pelvic station, and oligohydramnios, is a type of mechanical force that is implicated in craniosynostosis. Intrauterine constraints also possibly play a role in craniosynostosis pathogenesis through force-induced altered gene expression (Borke et al., 2003).

Maternal factors

Maternal risk factors for craniosynostosis have been described and discussed in the literature. Maternal smoking, staying at a high altitude antenatally, alcohol use, substance abuse, Vitamin D deficiency or receptor insensitivity, chronic renal failure, hypophosphatemia, and hyperthyroidism have been recognised as risk factors. Maternal drug use of especially phenytoin, retinoids, valproate, aminopterin/methotrexate, fluconazole, cyclophosphamide, and folic acid nitrosates have also been identified as risk factors (Boulet et al., 2008; Carmichael et al., 2015; Honein and Rasmussen, 2000; Mathijssen, 2015; Ridgway and Weiner, 2004; Selber et al., 2008; Van Der Meulen et al., 2009).

Paternal age effect

Recent literature has shown that paternal age can be correlated with an increased risk for congenital craniofacial malformations. An increase in de novo mutations were found in the sperm from men of an older age. This is termed the paternal age effect (PAE) disorders (Goriely and Wilkie, 2012).

In Apert syndrome, two specific mutations in the *FGFR2* gene c.755C>G at a CpG dinucleotide repeat and c.758C>G transversion in a non CpG nucleotide are accountable for 99% of cases. The c.755C>G accounts for 66% and the c.758C>G for 33% of cases (Glaser et al., 2000; Maher et al., 2014). A reason for the high mutation rate at the CpG dinucleotide has been suggested to be due to an escape from normal methylation processes (Yilmaz et al., 2019). A relationship between advanced paternal age and methylation abnormalities has suggested that epigenetic changes contribute to a high de novo mutation rate in sperm (Milekic et al., 2015).

Associated pathology/complications

Many complications are associated with craniosynostosis, including sensory, respiratory and neurological functional impairment. It is therefore an important condition to detect early and treat appropriately (Johnson and Wilkie, 2011).

Raised Intracranial Pressure

Raised intracranial pressure (ICP) is defined as a baseline above 15 mmHg during slow wave sleep or more than three plateau waves (Mathijssen, 2015). The risk of raised ICP varies between syndromic and non-syndromic craniosynostosis and between the different types of craniosynostosis in each category. Increased ICP after skull remodelling can be a dangerous complication.

The risk of pre- operative raised ICP in the syndromic group according to literature is: 40%-50% in Apert syndrome, 50%-70% in Crouzon and Pfeiffer syndrome, 35%-45% in Saethre-Chotzen syndrome and 50%-80% in complex craniosynostosis but does not occur in Muenke syndrome (Hayward and Gonsalez, 2005; Kress *et al.*, 2006; Greene *et al.*, 2008; Marucci *et al.*, 2008).

Prevalence of raised ICP pre-operatively in non-syndromic craniosynostosis reported is: 5%-24% in scaphocephaly, 0%-33% in trigonocephaly, 0%-22% in plagiocephaly and

31%-50% in bilateral coronal (Florisson et al., 2010; Mathijssen et al., 2006; Renier et al., 2000; Thompson et al., 1995).

Various studies have looked into the reliability of clinical signs or symptoms and screening methods to monitor raised ICP. Clinical symptoms associated with a raised ICP include: headaches, behavioural changes and worsening of vision. None of these were found to be reliable for diagnosis of raised ICP in craniosynostosis patients. A deviating cranial circumference curve was also not proven to be helpful when screening for ICP in this cohort of patients. CT scan findings such as ventricle size and skull impressions, for example, the copper beaten appearance, were also found to be unreliable in screening for raised ICP (Bannink et al., 2008; Mathijssen, 2015).

Raised ICP screening methods described so far include:

1) Invasive techniques: Epidural sensors are placed or a lumbar puncture is performed. Invasive techniques are the so called "gold standard" of screening methods but the need for anaesthesia and risk of complications make it a procedure that needs admission to an intensive care unit (ICU) or high care unit (Marucci et al., 2008; Tamburrini et al., 2005).
2) Fundoscopy: Specifically looking for papilledema. This procedure requires an experienced clinician, with reliability depending on the age of the patient. The absence of papilledema also does not exclude ICP (Bannink et al., 2008; Eide et al., 2002; Tuite et al., 1996; Woods et al., 2009).
3) Visual Evoked Potential (VEP): VEP scans have the ability to show signs of increased ICP or the onset of optical nerve injury at an early stage even before papilledema occurs (Liasis et al., 2006). Unfortunately this technique has not often been directly compared to invasive ICP measurements. In a study mainly comprising non- syndromic craniosynostosis patients, a higher percentage (24%) of abnormal VEP scans were found when compared to invasive ICP measurements (Liasis et al., 2006).
4) Endocortical erosion computed tomography (CT) scan: Although still used, radiologic findings such as impressions are unreliable as a screening method (Mathijssen, 2015).

In summary, the published literature therefore recommends that screening for raised ICP for non-syndromic- and syndromic craniosynostosis be done by means of fundoscopy. Fundoscopy in the case of non-syndromic children should be done before skull remodelling and followed up at the age of two and four years. In syndromic craniosynostosis, fundoscopy should be performed before skull remodelling and once a year until the age of 6 years. (Muenke syndrome, with a decreased risk of raised ICP might be the exception). Findings of papilledema should be followed by a CT- or magnetic resonance imaging (MRI) scan to assess change in the ventricle size. Invasive ICP monitoring should be done in cases of unexplained reduction of vision or where there is uncertainty about the degree of raised ICP (Mathijssen, 2015).

Hydrocephalus

Hydrocephalus is defined as a progressive increase of ventricle size associated with signs of ICP (Cinalli et al., 1998). A distinction should be made between ventriculomegaly with or without ICP. Craniosynostosis related hydrocephalus seems to develop slowly over time and therefore the classical signs of hydrocephalus are mostly not observed. Non-communicating hydrocephalus is a serious complication that can result in neurologic impairment or death.

The cause of hydrocephalus in craniosynostosis patients is not fully understood. Impaired venous drainage and a small posterior cranial fossa with poor cerebrospinal fluid (CSF) drainage from the fourth ventricle has been suggested. Hydrocephalus is often present together with Chiari I malformations. Hydrocephalus can also be present in the absence of Chiari I malformations. The reasons stated above still do not explain all cases (Cinalli et al., 1998; Collmann et al., 2005).

Hydrocephalus is rarely found in non-syndromic craniosynostosis, and if present, the incidence is not higher that in the general population.

In syndromic craniosynostosis, hydrocephalus is seen in one third of the patients and is syndrome dependant. In Apert syndrome, ventricular enlargement is less likely to

be associated with increased ICP (Collmann et al., 2005; Renier et al., 1996). In Crouzon and Pfeiffer syndrome the expanded brain ventricles are associated with raised ICP (Cinalli et al., 1998). In these cases shunt placement or cranial decompression is indicated to prevent complications.

When surgical intervention is needed, performing a cranial vault expansion first, and only proceeding with shunt placement if the ICP and papilledema persists for more than two months after adequate cranial vault expansion is suggested (Collmann et al., 2005; Mathijssen, 2015). Ventriculomegaly and hydrocephalus should be monitored using MRI scanning as well as 6 monthly fundoscopy.

Chiari I malformations

Chiari I malformations result from the overcrowding of a relatively small and shallow posterior fossa. It results in the downward herniation of neural tissue through the foramen magnum (Cinalli et al., 2005). In most cases of craniosynostosis, tonsillar herniation is not present at birth, but develops secondary to premature closure of lambdoid and cranial base sutures. These processes usually takes place at 3 to 6 months of age.

The risk of Chiari I malformations varies widely between the different syndromic craniosynostosis types. Cinalli et al. (2005) reported prevalence rates of: 70% in Crouzon syndrome, 75% in oxycephaly/turricephaly, 50% in Pfeiffer syndrome, 100% in cloverleaf skull (severe craniosynostosis, mostly involving the coronal and lambdoid sutures, with enlargement of the head and a trilobed configuration of the frontal view). Chiari I malformation is rarely observed in patients with Apert syndrome.

Chiari I malformation was found to be present in 88% of syndromic craniosynostosis patients that presented with hydrocephalus but 53% of children with a Chiari I malformation do not have hydrocephalus. Studies seem to suggest that Chiari I malformations develop earlier than hydrocephalus and therefore may be a

prerequisite but there are possibly other contributing factors that leads to the development of hydrocephalus (Mathijssen, 2015).

The majority of Chiari I malformations in syndromic craniosynostosis remain asymptomatic and are only established once the radiologic modality of choice, a MRI scan is done (Fearon et al., 2001). Clinical symptoms caused by chronic tonsillar herniation (CTH) range from suboccipital pain to life threatening brainstem dysfunction. In young children specifically, respiratory problems, for example central apnoea, ventilatory control abnormalities, and persistent cyanosis may be observed together with a bulbar palsy. Any of these symptoms should warrant a neurologic examination and possible MRI scan (Cinalli et al., 2005).

In conclusion, screening for the presence of Chiari I malformations in patients with Crouzon/ Pfeiffer is recommended at the age of diagnosis, at age 4 years and on any clinical suspicion of Chiari I malformations. Surgical treatment of asymptomatic Chiari I malformations is not recommended (Cinalli et al., 1998). Surgical treatment is only recommended with the presence of symptoms and occipital decompression for Chiari I may be indicated. It is important to investigate the presence of abnormal occipital venous drainage preoperatively (Mathijssen, 2015).

Obstructive Sleep Apnoea

Obstructive sleep apnoea syndrome (OSAS) is characterised by partial and/or complete airway obstruction during sleep. It leads to hypercapnia and hypoxemia and can result in pulmonary hypertension and heart disease. OSAS has a high prevalence in syndromic craniosynostosis. Apert, Crouzon and Pfeiffer syndrome have the highest prevalence. (De Jong et al., 2010; Fearon et al., 2009; Järund and Lauritzen, 1996; Kakitsuba et al., 1994; Pijpers et al., 2004).

Clinical symptoms are usually divided into night and day symptoms and may include: troubled sleeping, snoring, apnoea, bedwetting, and perspiration at night and dry

mouth, fatigue, impaired cognitive functioning, poor school performance and behavioural disorders. Growth disturbance can also occur.

Polysomnography (PSG) is the gold standard to detect and confirm OSAS. It measures oronasal airflow, thoracic movements, abdominal movements, transcutaneous saturation and heart rate (Brietzke et al., 2004). Inspection of the upper airway should also be done by an ear, nose and throat (ENT) surgeon on diagnosis of OSAS as there may be multiple locations where obstruction can be found. Possible causes can include: narrow nose, deviated septum, allergic or non-allergic rhinitis, choanal atresia, adenoid hypertrophy, midface hypoplasia, abnormal skull base, tonsillar hypertrophy, macroglossia, abnormal palate, retro- and or micrognathia, laryngeal stenosis, and fused tracheal rings. A cardiology examination is also suggested after diagnosis of OSAS to evaluate right ventricular hypertrophy or pulmonary hypertension (Mathijssen, 2015).

Treatment depends on initial diagnosis and may include pharmacological agents, surgery, nocturnal O_2 administration and continuous /bi-level positive airway pressure (CPAP/BiPAP). In severe cases, a tracheostomy might be necessary, especially as an interim procedure before craniofacial surgery is done. Le Fort III or monobloc procedures improve upper airway problems (Arnaud et al., 2007).

Ophthalmological complications

Ocular complications occur frequently and can include: optic neuropathy, strabismus, refractive errors, corneal injury (due to exposure keratopathy) and amblyopia. In the case of amblyopia early diagnosis and treatment is essential as treatment is only possible during childhood (Khong et al., 2006; Lehman, 2006). Eye complications depend on the type of craniosynostosis and occur more frequently with greater severity in syndromic craniosynostosis. Studies have also detected a higher incidence of ocular abnormalities in patients with unicoronal non-syndromic craniosynostosis (MacKinnon et al., 2009; Tarczy-Hornoch et al., 2008).

The *FGFR2* gene, known to cause certain syndromic craniosynostoses, has also been shown to be expressed during the development of the foetal orbit (Khan et al., 2005). Spontaneous dislocation of the eye ball, mainly encountered in Crouzon syndrome, is a medical and surgical emergency as the cornea is damaged and the optic nerve is distended (Touzé et al., 2019).

Due to the high incidence of ocular abnormalities in syndromic craniosynostosis and non-syndromic unicoronal craniosynostosis, referral for ophthalmic evaluation should be made at the first consultation.

Hearing impairments

Hearing loss is rarely seen in children with non-syndromic craniosynostosis, but in children with syndromic craniosynostosis there are several reasons for hearing impairment or loss. A high incidence of congenital middle-, inner- and outer ear abnormalities is reported, especially in Apert syndrome. Otitis media with effusion (OME) is also regularly reported in children with syndromic craniosynostosis.

In Muenke syndrome, perceptive hearing loss was found in patients (Doherty et al., 2007). Auditory processing disorder has also been described in children with syndromic craniosynostosis (Church et al., 2007).

Hearing loss can be an additional factor in delay in development, especially speech delay. It is therefore very important to diagnose at an early stage. Many countries currently have neonatal hearing screening programs in place which contributes to early detection. For syndromic craniosynostosis patients, an annual hearing screen is indicated for at least the first four years of life. Speech or language monitoring with standard assessment tools is also recommended.

A skull CT scan including adequate sections of the petrous parts of the temporal bone is recommended in Apert syndrome (Mathijssen, 2015).

Therapy or management depends on the type of hearing loss and can include tympanostomy tubes, hearing aids or cochlear implants.

Facial and dentofacial deformities

Dentofacial deformities occur in most of the syndromic craniosynostosis syndromes. Facial features found are syndrome dependant. Commonly seen facial features include ocular hypertelorism, a beaked nose, prognathism, and a high arched palate. More rarely a cleft palate is associated. Sutural synostosis can lead to premature fusion of the skull base causing midface hypoplasia, shallow orbits, maxillary hypoplasia and potential upper airway obstruction (O'Hara et al., 2019).

Orthodontic and dental problems were found to be related to abnormal growth that results in a hypoplastic maxilla. Clinical problems identified include a narrow and high arched palate, large gingival swellings, unilateral or bilateral cross bite, delayed eruption and retention of teeth, crowding, especially in the upper dental arch, hypodontia and overall delayed dental development (Kaloust et al., 1997; Letra et al., 2007; Mathijssen, 2015).

In syndromic patients it might be difficult to maintain oral hygiene especially with orthodontic treatment due not only to developmental and behavioural problems, but also due to physical disabilities such as hand deformities. Regular oral hygiene and orthodontic monitoring is thus essential.

Presurgical orthodontic treatment usually focus on alignment of dental arches. Orthognathic surgery is usually needed to achieve normal alignment of the jaw and occlusion. A first consultation is recommended soon after birth to plan future check-ups and possible interventions.

Malformation of the extremities

Deformities of the extremities are commonly seen with syndromic craniosynostosis. These can however range from mild with minimal or no functional impairment to severe with very significant functional impairment. Apert syndrome has the most severe deformities (Wilkie et al., 1995).

The aim of surgery is to increase functionality. Separation of the syndactylous thumb and index finger to create a pincer grip is important for hand function. Surgical intervention for severe hand deformity in Apert syndrome usually starts at a very young age, between 3 and 6 months (Guero, 2005).

Cognitive functioning and behaviour

Studies that investigated the cognitive function of non-syndromic craniosynostosis have differed extensively. Some studies report minimal or no cognitive impairment or behavioural problems while other studies report significant cognitive impairment with associated behavioural difficulties (Mathijssen, 2015).

Children with syndromic craniosynostosis have a significantly higher risk of intellectual disability (Lajeunie et al., 1998). A large variation in outcome is however seen between different syndromes as well as within a syndrome (Mathijssen, 2015; Renier et al., 1996). Children with intellectual disability also have an increased risk for developing behavioural problems (Dekker et al., 2002). As hearing loss can be significant in syndromic craniosynostosis especially in Apert and Muenke syndrome, language and speech development should be closely monitored (Shipster et al., 2002).

Routine screening for developmental delay and behavioural problems is therefore recommended.

Role of Imaging in the diagnosis and treatment of craniosynostosis

The discovery of or the suspicion of an abnormal skull configuration, needs careful radiological evaluation. The radiological evaluation plays an important role in characterising the deformity, confirming the diagnosis and formulating the correct prognosis. It may also guide the search for possible associated anomalies, direct corrective surgery if indicated and monitor for post -operative complications. (Ginat et al., 2018; Massimi et al., 2019).

A working group from the Netherlands used their considerable experience and information obtained from published literature to draw up guidelines regarding imaging in the case of patients with craniosynostosis (Mathijssen, 2015). These authors from a tertiary craniofacial clinic suggest that timely referral of patients should not be hindered by extended waiting times for imaging and that a four view plain X-ray (anterior- posterior view , lateral view, Towne view for the back of the head and Tschebull view for the forehead) should usually be adequate. Unnecessary medical imaging that results in high cost, placing a burden on both patients and parents, and that results in unnecessary radiation exposure, should be avoided.

Four modalities currently described in literature are:

Plain X-ray: a four view skull X-ray is usually considered the first radiologic diagnostic test for craniosynostosis. It is a fast, cost effective method with a relatively low radiation exposure. Poor sensitivity due to the low mineralisation of the skull has been reported in infants under the age of 3 months. X-rays do have the ability to exclude craniosynostosis if all sutures are evidently open (Massimi et al., 2019). Partial suture closure can be difficult to diagnose and it is essential that X-rays are performed and evaluated by experienced clinicians/radiographers.

Normal unfused sutures appear lucent, serrated and non-linear, while prematurely fused sutures can show peri-sutural sclerosis, linearity, bony bridging or show complete non-visualization of the suture and loss of suture clarity (Massimi et al., 2019). Secondary factors such as altered cranium shape, changes in shape and timing

of closure of fontanelles and facial anomalies might also be observed. X-rays are suboptimal for the evaluation of associated brain anomalies.

Computed Tomography (CT) Scan: a three dimensional CT scan is more reliable in diagnosing craniosynostosis than a plain X-ray. CT scans are seen as the current gold standard in diagnosing craniosynostosis. If an X-ray confirms or does not exclude craniosynostosis, it is recommended that a three dimensional reconstruction CT scan be performed (Medina et al., 2002).

CT scans are also the method of choice for surgery planning and can aid in additional information regarding brain and cerebrospinal fluid (CSF) space morphology and skull base hypoplasia. CT scans can also be advantageous in evaluating minor suture synostosis. CT scans are also a useful tool in monitoring post-operative complications and to guide appropriate follow up. CT scans, however do expose a child to radiation. As the average time for a CT scan is 30-45 minutes, most infants or children will need sedation or anaesthesia with associated risk.

Cranial ultrasound (CUS): has been shown to visualise craniosynostosis effectively in children under the age of 1 year and has consequently been suggested as an alternative imaging modality (Rozovsky et al., 2016; Simanovsky et al., 2009). By utilising a trans fontanelle approach, a hypoechoic gap between two hyperechoic bony plates can be demonstrated to exclude craniosynostosis. Rozovsky *et al*.(2016) demonstrated that CUS can identify craniosynostosis with a sensitivity of 100% and specificity of 98% (95% confidence, interval 94%-100%) in infants ranging from birth to age 12 months. In a study by Simanovsky *et al*. (2009) there was a 100% reader agreement for determining synostosis in the sagittal, coronal and lambdoid sutures when comparing CUS to 4 view skull radiography. Disagreement was however found for three cases of metopic sutures (Simanovsky et al., 2009). This can probably be explained by the fact that the metopic suture is the first suture to close. Metopic suture closure normally takes place as early as 3 months with 100% closed by the age of 9 months (Vu et al., 2001; Weinzweig et al., 2003). CUS has also proven helpful in

distinguishing craniosynostosis from positional plagiocephaly when uncertainty exists after history taking and clinical examination alone (Linz et al., 2015).

The average CUS study takes approximately 15 minutes, which is half the time for a CT scan, is radiation free and needs no sedation. Infants can be sleeping or feeding during examination, making it a comfortable procedure. Cranial ultrasound investigations also have the ability to give more information on associated pathology for instance midline abnormalities, ventricle abnormalities and calcifications. CUS is operator dependant and therefore requires training in identifying craniosynostosis.

Prenatal ultrasound also has the ability to diagnose craniosynostosis from the third semester in pregnancy (Miller et al., 2002). Currently syndromic craniosynostosis patients may be diagnosed antenatally due to the associated skull, facial and brain abnormalities. Prenatal diagnosis has the advantage of allowing correct information on prognosis and therapeutic plans including early surgery to be made before the birth of a child.

Magnetic resonance imaging (MRI): eliminates the risk of ionising radiation but needs the patient to remain still during a long examination. This generally would necessitate general anaesthesia for an infant or small child. In the context of craniosynostosis, MRI imaging is historically considered a complementary technique to evaluate cerebral and craniofacial soft tissue anomalies. For MRI technique to be considered a first-line investigation, it will have to involve a shorter acquisition time and provide accurate diagnostic information including 3D reconstructed bone anatomy. Several techniques are being investigated and currently the 'black bone' MRI, which takes about 4 minutes to produce an image, shows promise. It utilizes novel gradient echo parameters to minimise soft tissue contrast to enhance the bone soft tissue boundary. There are however difficulties in evaluating areas with air bone interface such as the mastoid and paranasal regions (Eley et al., 2014).

Surgical management

The management of craniosynostosis usually consists of various surgical interventions. The goal of surgery is twofold, to increase the intracranial volume (functional) and restore the altered craniofacial appearance (aesthetic). The majority of surgery is done between the ages of 3 to 12 months, when the bone is still malleable and the child is old enough to endure surgery.

Indications for surgical treatment of non-syndromic craniosynostosis are: increased intracranial pressure (ICP), abnormal skull and facial morphology, and the prevention or restriction of associated neuropathology. Surgery is not usually indicated for mild types such as metopic craniosynostosis or partial craniosynostosis.

In children with syndromic craniosynostosis especially Apert, Crouzon and Pfeiffer syndrome, posterior decompression is usually performed in the first year of life to expand the cranial vault. This protects the posterior fossa contents and prevents cerebellar tonsil herniation and results in fewer associated complications. A monobloc advancement or Le Fort III can then be successfully done at a later stage (Arnaud et al., 2007; De Jong et al., 2010). A midface advancement procedure is usually executed to address ocular proptosis and sleep apnoea syndrome. In the case of Saethre-Chotzen and Muenke syndrome, a fronto-orbital advancement usually normalise the profile and a monobloc or Le Fort III is hardly ever indicated (Honnebier et al., 2008).

The surgical techniques used in craniosynostosis are broadly divided in two groups, namely, osteoclastic techniques and remodelling techniques. In osteoclastic techniques, the bone is removed to enable the developing and expanding brain to change the shape of the skull. Remodelling techniques are developed from the realisation that the self-correcting capabilities of the skull and the brain cannot be solely relied on. In remodelling surgery the aim is therefore to directly achieve the desired skull shape by reconstruction (Mathijssen, 2015).

The six most widely used procedures that address the various kinds of craniosynostosis are listed here (Barone and Jimenez, 1999; Aryan *et al.*, 2005; Mathijssen, 2015; Ginat *et al.*, 2018):

1) Fronto -orbital advancement and remodelling: This procedure is widely used in correction of metopic, unicoronal and bicoronal craniosynostosis. This procedure involves a bifrontal craniotomy and superolateral wall osteotomies to temporarily remove a strip of bone known as the supraorbital bandeau/bar. A newly shaped supraorbital bandeau is then secured to the frontal skull base in a more advanced position. This results in the frontal ridge at the metopic suture being less prominent, and deepening of the orbital cavities.
2) Barrel stave osteotomies and cranial remodelling: A procedure used mainly for treating scaphocephaly. It both addresses the closed suture as in a strip craniectomy and corrects for compensatory growth that has occurred. The surgery involves the removal of parts of the frontal and parietal bones at the base of the skull in a series of radial osteotomies known as barrel staves.
3) Unilateral cranioplasty: Mostly used to correct plagiocephaly and any associated orbital deformity and intracranial hypertension. This involves unilateral fronto-orbital advancement and cranial vault remodelling.
4) Cranial expansion with distraction osteogenesis: During this procedure the cranial vault is enlarged by distraction osteogenesis. Semi-buried internal distractor devices or external devices are used. This is typically a staged procedure consisting of different phases. During the initial phase osteotomies are performed. In the latency phase primordial bone healing takes place at the bony gaps that were made during the osteotomies. In the distraction phase bone development and formation takes place. The last phase, the consolidation phase, is where immature bone develops into mature bone.
5) Monobloc advancement and cranioplasty: This is the surgical procedure of choice for craniosynostosis complicated by elevated intracranial pressure, upper airway obstruction and corneal exposure. It involves the mobilisation and advancement of both the frontal and facial bones with or without the use of distraction osteogenesis. When compared to conventional advancement it

has greater advancement potential, decreased morbidity, decreased blood loss and decreased operative time.

6) Endoscopic suturectomy: During this procedure one or two small incisions are made for endoscopic visualisation of a suture. The fused suture is then cut out within a 1 cm area of non-suture containing bone on either side. Typically this procedure is followed up with helmet wearing therapy to aid in reshaping the calvarium. Most advantageous results have been obtained in children below the age of 6 months.

The main complication of craniosynostosis surgery is blood loss. A loss of anything from 20% to 500% of circulating volume has been described. It is estimated that around 80% of these patients require intraoperative blood transfusions (Kearney et al., 1989; Meara et al., 2005). Risk factors for massive blood loss have been found to be associated with surgery for syndromic craniosynostosis, pansynostosis, age below 18 months, and long duration of the surgery (White et al., 2009). Other complications include cerebrospinal fluid leaks, surgical site infection, bone resorption, venous air embolism with subsequent cardiovascular collapse, consumption coagulopathy due to depletion of clotting factors and cerebral salt wasting syndrome .

The main preoperative factors to be address include the optimalisation of haematological conditions due to anticipated blood loss and review of possible anaesthetic challenges. Although different preventative techniques have been describe, for example, the administration of erythropoietin (EPO), iron (Fe) supplements and the preoperative blood sampling for autologous transfusion has been described, this has many disadvantages and are often advised against (Di Rocco et al., 2004; Mathijssen, 2015; Meara et al., 2005). These disadvantages include multiple instances of blood withdrawal which can require anaesthesia, limited cost effectiveness and uncertainty about optimal drug dosing. This cohort of patients, especially syndromic cases have frequent anaesthesia related comorbidities which include hypoplasia of the midface and compromised airways with or without obstructive sleep apnoea syndrome (OSAS). There should be a low threshold to postpone surgery following recent upper airway infection as this is strongly associated

with complications during surgery. Preoperative planning of surgery by using three dimensional (3D) models seems to reduce surgery time and therefore blood loss.

Intraoperative monitoring of blood loss and monitoring of temperature is crucial in the prevention and treatment of depleted clotting factors. Optimal positioning of the patient during surgery is also important such as avoiding venous cerebral congestion by hyperflexion of the head. Placing the patient in a moderate anti-Trendelenburg position reduces blood loss. Children with exorbitism should have measures taken to protect exposed eyes. Administration of a single dose of antibiotic is recommended. Postoperative admission to a Paediatric Intensive Care Unit (PICU) is necessary in most cases. Adequate pain treatment has been studied extensively and most centres suggest intravenous (IV) paracetamol and nonsteroidal anti-inflammatory drugs (NSAIDs). Sedation in the extubated patient should be avoided (Mathijssen, 2015).

Outcomes of surgery are reported as morbidity and mortality. Mortality in craniosynostosis surgery is reported as very low at 0% to 1% (Fearon et al., 2009). Morbidity is usually reflected in reporting of complications ,length of hospitalisation and post operation infection rates. Infection rate is reported as very low. In general, the complication rate in non-syndromic craniosynostosis (3,5%) is lower than that seen in syndromic craniosynostosis (39%). Risk of relapse was found to be higher after limited early intervention than after later complete skull remodelling (Fearon et al., 2009). It was also found that resection of the synostosis suture (strip craniectomy) alone provides unsatisfactory results compared with remodelling procedures (Aryan et al., 2005; Selber et al., 2008). Orbital deformity also does not normalise after resection of the synostotic suture only (Aryan et al., 2005; Selber et al., 2008; Stelnicki et al., 2009). Timing of surgery differs depending on the technique or procedure used and at which centre the surgery is performed.

Craniosynostosis in Africa and specifically South Africa

While numerous cases of craniosynostosis have been reported worldwide, especially in those of European descent there are fewer reports of craniosynostosis in South

Africa or the rest of Africa. Ofodile Ferdinand (1982) reported on a rare case of acrocephalosyndactyly with hydrocephalus and dextrocardia in a patient from Nigeria (Ofodile Ferdinand, 1982). Kleintjes (2005) reported on twins with craniofacial abnormalities who received surgery at the craniofacial unit, Tygerberg Hospital (Kleintjes, 2005). Hlongwa (2009) discussed early orthodontic management of a seven year old South African black boy with Crouzon syndrome (Hlongwa, 2009). Christofides and Steinmann (2010) published an anthropometric chart for craniofacial surgery (Christofides and Steinmann, 2010). Lumaka *et al*.(2014) reported on Apert syndrome diagnosed in a Congolese male patient and his mother (Lumaka et al., 2014). Kana *et* al. (2018) reported on a Nigerian women with Apert syndrome (Kana et al., 2018). Neurosurgical techniques, for example the use of acellular dermal matrix in craniosynostosis, and the role of three dimensional reconstruction has also been discussed in the South African setting (Cremin and Zeeman, 1989; Greyvensteyn and Madaree, 2016; Madaree, 2018).

Although both, the genetic services and a dedicated craniofacial clinic have been running at Red Cross War Memorial Children's Hospital (RCWMCH) for around 3 decades, not much has been formally reported on in this group of paediatric craniosynostosis patients. Likewise, with limited access to molecular testing, the genetic contributors to this disorder in the South African population are not well delineated. This study aims, by retrospectively reviewing hospital records for the preceding 5 years ,to describe the profile of patients with craniosynostosis and to carefully prospectively phenotype a sub cohort, while ascertaining the potential contribution of a medical geneticist as part of the multidisciplinary service.

Purpose of the study

Aim

To describe the craniosynostosis phenotype in a South African population.

Objectives

1. To identify, both retrospectively and prospectively, cases of craniosynostosis seen at RCWMCH.

2. To describe the demographic profile, the clinical presentation, intervention performed and outcome of a cohort of children with proven craniosynostosis.
3. To comprehensively phenotype children prospectively presenting to the RCWMCH craniofacial or genetic clinics over a 6 month period.

Chapter 2: Methods

Literature review:

A PubMed and Google Scholar literature search was carried out and appropriate articles presented in English were selected. Search terms included " craniosynostosis", "craniosynostosis genotype-phenotype correlation", "craniosynostosis and South Africa", "craniosynostosis and African population", "craniosynostosis and Africa", "syndromic craniosynostosis" and " non-syndromic craniosynostosis".

Ethics approval:

Approval for this study was granted by the Human Research Ethics Committee of the University of Cape Town. HREC REF 774/2018. Approval was also obtained from the RCWMCH to conduct the study in the hospital (See Appendix 5: Ethics approval documents). The research was conducted in accordance with the Declaration of Helsinki.

Recruitment and enrolment:

A) Retrospectively, a record review was performed collating the available clinical information on patients known with craniosynostosis at RCWMCH. Craniosynostosis was confirmed with an available CT scan result. These patients would have either attended the Genetics clinic at RCWMCH in the five year period February 2014 to April 2019 and were identified using the UCT Genetic Clinic Database or they would have had craniofacial surgery between the period August 2014 and February 2020 at RCWMCH and identified using the RCWMCH Neurosurgery Surgical Database.
B) Prospectively, patients diagnosed with craniosynostosis that attended the genetic clinic at RCWMCH during the 6 month period April 2019 to Sept 2019 were enrolled in the study if they fulfilled the inclusion criteria.

Inclusion criteria

- Children up to the age of 18 years, diagnosed with craniosynostosis.
- Non-syndromic, syndromic, and non-determined cases of craniosynostosis were included in the study. Craniosynostosis had to be confirmed by CT scan.
- Patients who formed part of the prospective descriptive phenotyping had to be available for and agreeable to clinical examination.
- Informed consent and, where appropriate, assent was obtained from individuals and parents for the prospective descriptive study

Research procedures and data collection methods

A) During the retrospective folder review, all available demographic and clinical information were extracted. Information was captured on a standardised data capture sheet and added to a secure electronic database (REDCap).
B) During the prospective part of the study, thorough phenotyping of each patient was performed through a systematic assessment. Information was captured on a standardised data capture sheet and added to an electronic database (REDCap).

Data Analysis

Descriptive statistics were used to characterize patients and outcomes. Continuous variables were described as means and standard deviations (or medians and interquartile ranges). Categorical variables were described as frequencies and percentages. Data were disaggregated for presentation by the following categories; non-syndromic, syndromic, and craniosynostosis with additional findings. Bi-variate associations between the participant collected variables and the craniosynostosis stratifying variable was done using the appropriate parametric/non-parametric tests and the associated p-values reported. The association between numerical data and the stratifying variable were tested using either Kruskal Wallis or One way ANOVA depending on the normality of data. Chi-square or Fisher's exact test

was used to test the association between categorical variables and the latter was used if one of the cells had an expected frequency of less than five or the assumption for a large sample size was not met. A p-value of less than or equal to 0.05 was considered statistically significant.

Chapter 3: Results

Enrolment

In total, 47 patients with craniosynostosis were identified. Of these, 18 had the clinical diagnosis of non-syndromic/ isolated synostosis (12 of the sagittal type and five of the metopic type, in one case the suture was not specified), 13 had the clinical diagnosis of syndromic synostosis (eight were clinically diagnosed with Crouzon syndrome of which three were molecularly confirmed, four patients were clinically diagnosed with Apert syndrome and one with Pfeiffer syndrome). Sixteen patients were diagnosed with craniosynostosis with additional features. The craniosynostosis with additional features group were patients without a syndromic diagnosis but all exhibited additional clinical features that excluded them from the non-syndromic craniosynostosis group.

Of the cohort of 47 patients, 22 individuals were from the genetic dataset, 23 from the neurosurgery subset and two patients were prospectively enrolled.

The genetic subset consisted of patients identified with craniosynostosis from the genetic clinic database. These patients would have attended the genetic clinic at RCWMCH in the period February 2014 to April 2019.

The neurosurgery subset consisted of patients that all had craniofacial surgery at RCWMCH and was therefore on the neurosurgery database. The surgery took place in the period August 2014 to February 2020.

Prospectively enrolled individuals were patients seen at the genetic clinic at RCWMCH in the period April 2019 to September 2019.

Figure 5. Patients identified from the Genetic Clinic Database and enrolled in retrospective record review.

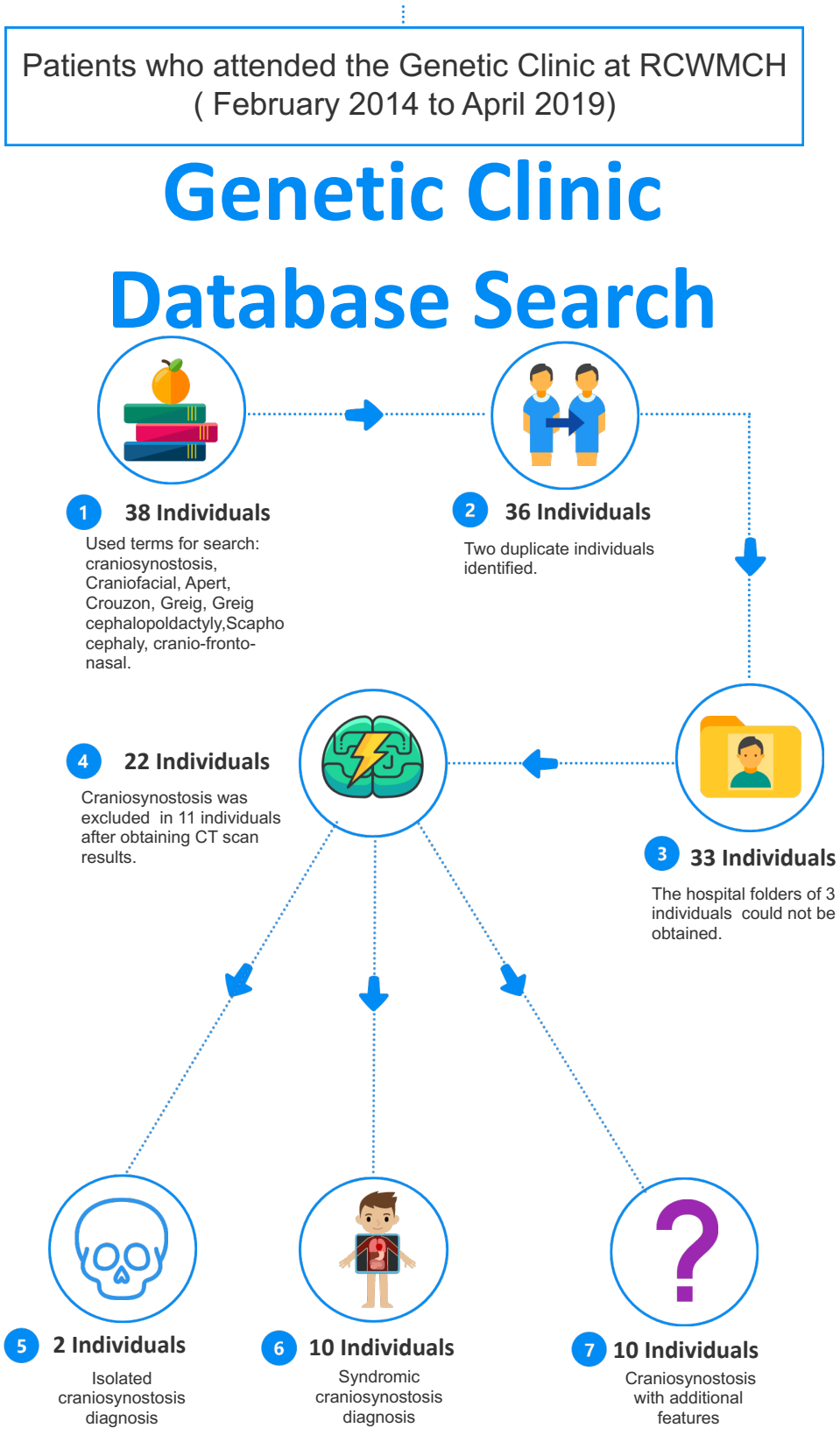


Figure 6. Patients identified from the Neurosurgery Database and enrolled in retrospective record review.

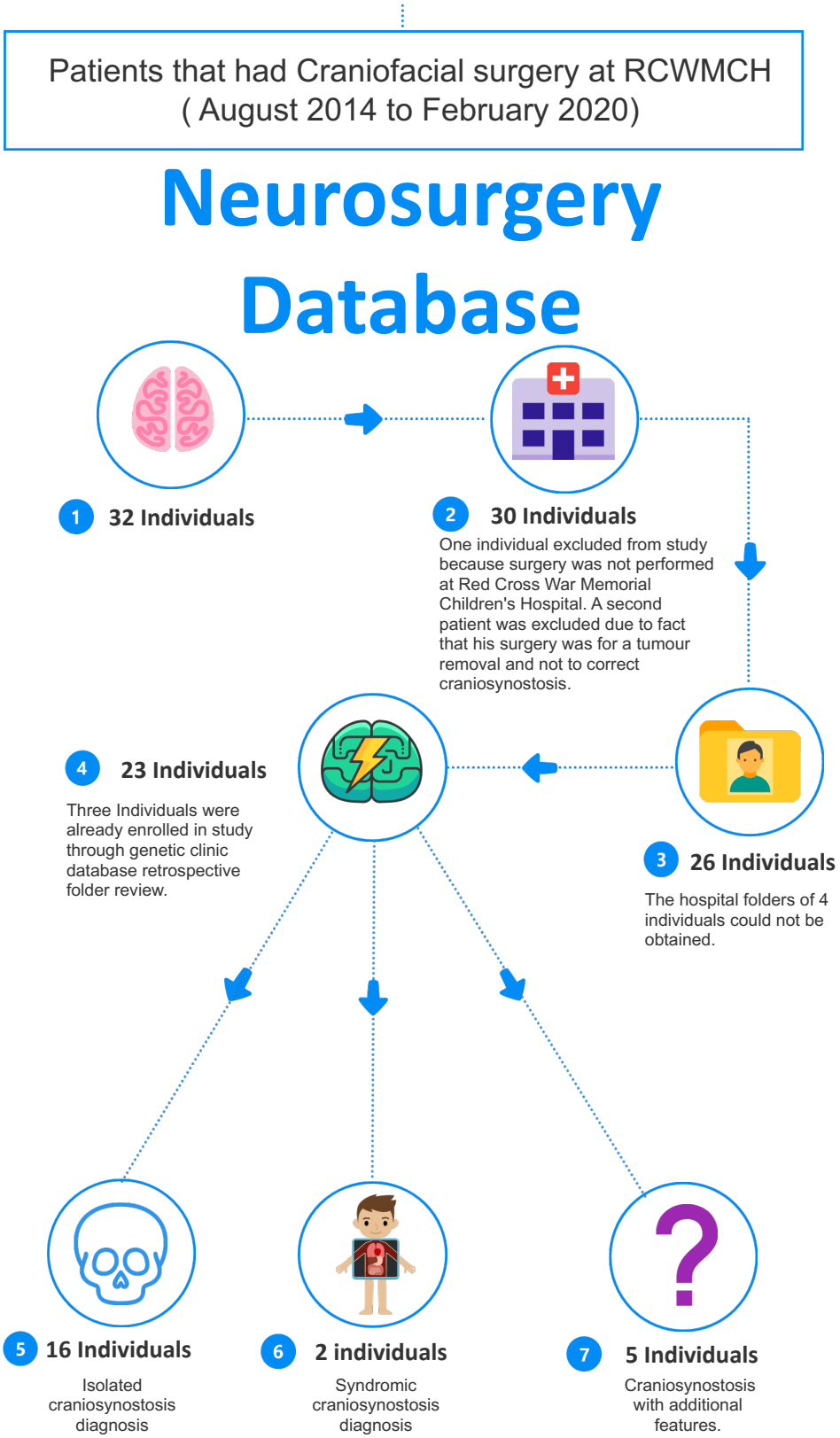
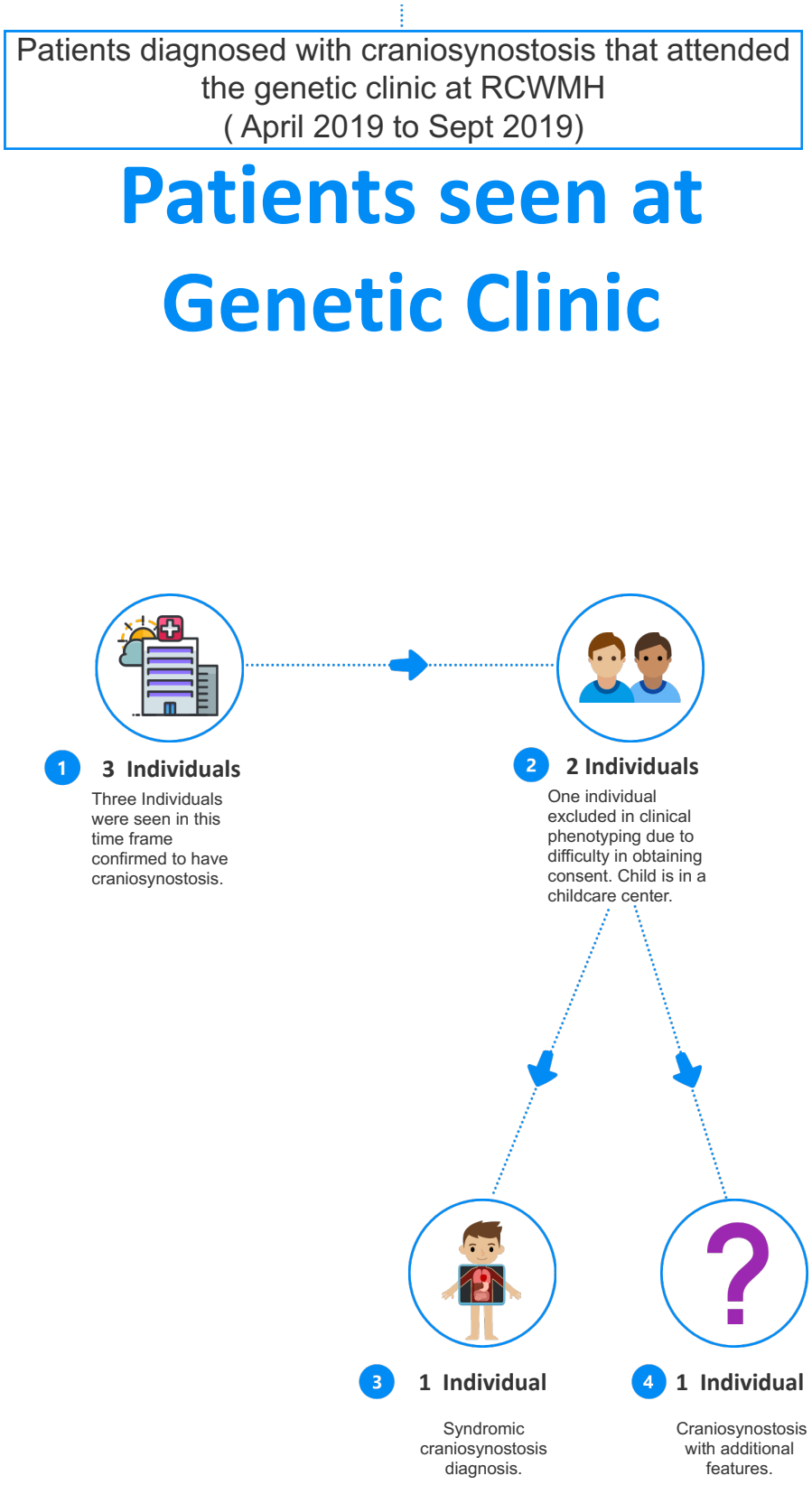


Figure 7. Patients enrolled in prospective phenotyping.



Demographics

Fifty three percent (n=25) of the cohort (n=47) were male, and one patient was diagnosed with a disorder of sexual development (DSD). Nine patients were black African and ten were of Mixed Ancestry. Ancestry data were not available for 28 patients. There was a pedigree available for 30 of the patients. The majority (n=25; 83.3%) did not have an affected family member. Of the five who had a family history, two patients were twin brothers with craniosynostosis (zygosity not stated in folder), two children had mothers who were also known to have Crouzon syndrome (one of whom also had craniosynostosis surgery), and one had a paternal cousin with craniosynostosis.

Table 4. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to demographics.

Variable	Total (N=47)	Non-syndromic (n=18)	Syndromic (n=13)	CS with additional features (n=16)
Sex				
Female	21/47 (44.7)	9/18 (50.0)	9/13 (69.2)	3/16 (18.8)
Male	25/47 (53.2)	9/18 (50.0)	4/13 (30.8)	12/16 (75.0)
DSD	1/47 (2.1)	0	0	1/16 (6.2)
Ethnolinguistic group				
Black African	9/19 (47.4)	1/2 (50.0)	3/8 (37.5)	5/9 (55.6)
Mixed ancestry	10/19 (52.6)	1/2 (50.0)	5/8 (62.5)	4/9 (44.4)
Positive family history	5/30 (16.7)	2/7 (28.6)	3/12 (25.0)	0

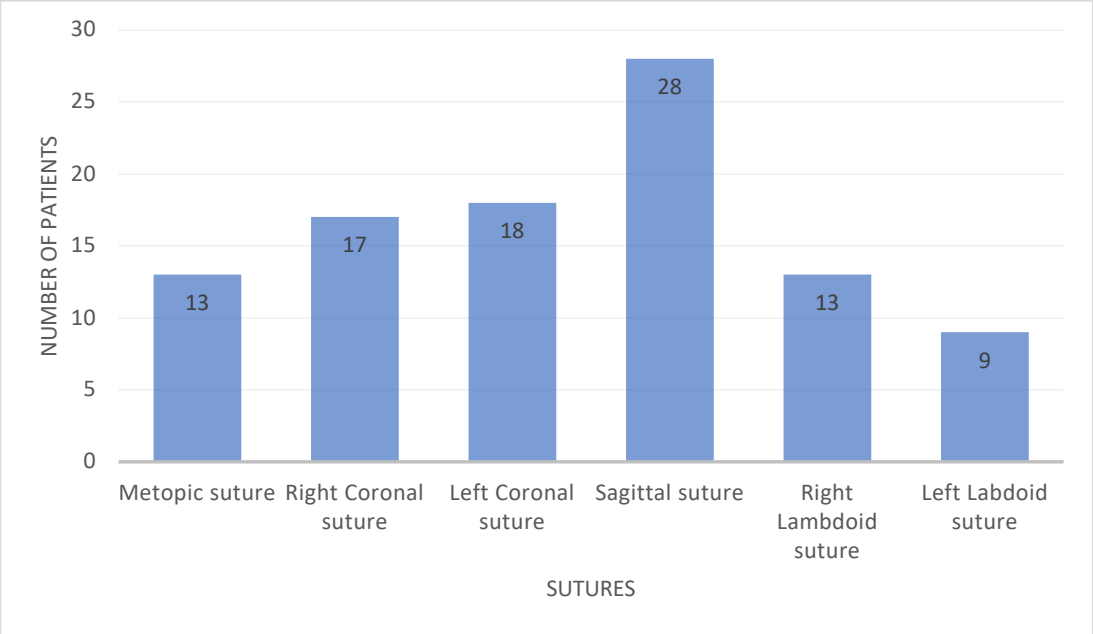
Diagnosis confirmation with CT scan

On CT scan, the majority of patients (59.6%) had sagittal suture involvement, roughly one third had either unicoronal or bicoronal suture synostosis (36.2% on the right) and (38.3% on the left), and just over a quarter had the metopic suture (27.7%) or the right lambdoid suture (27.7%) involved. A number of the children had multiple suture involvement (see Figure 5).

Thirty four patients had information documented on the head shape noted on CT scan, eighteen (38.2%) patients had scaphocephaly, six (12.7%) had anterior brachycephaly, six (12.7%) had trigonocephaly, three (6.3%) had plagiocephaly, and one (2.1%) had turribrachycephaly.

Ten patients (21.3%) had a structural brain abnormality and 13 (27.7%) had signs of raised intracranial pressure. The mean age at diagnosis of craniosynostosis was 22.5 months (*SD* = 31.4, range: 0.1 – 140.9)(see Figure 6).

Figure 5. Diagnosis confirmation with CT scan: sutures involved.



Structural brain abnormalities, noted with CT scan, included: three cases of Chiari I malformation, one case of a Chiari I malformation and ventriculomegaly, one case of Chiari II malformation, one patient with an absent corpus callosum, two cases of ventriculomegaly, one case of cortical dysplasia of the cerebral hemisphere and cerebellum and one case of peritrigonal heterotopia.

Figure 6. Age distribution in months according to age at confirming diagnosis with CT scan.

Total count	Min	Max	Mean	StDev	Sum	Percentile						
						0.05	0.10	0.25	0.50 Median	0.75	0.90	0.95
47	0.10	140.87	22.50	31.37	1057.62	1.21	1.52	2.86	11.73	23.05	57.42	88.57

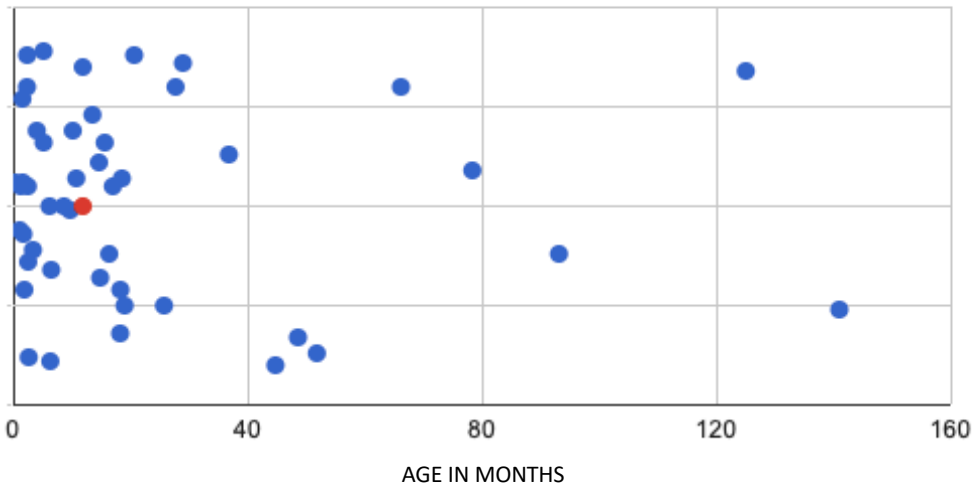


Table 5. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to CT scan results.

Variable	Total (N=47)	Non-syndromic (n=18)	Syndromic (n=13)	CS with additional features (n=16)	p-value
Sutures involved					
Metopic	13 (27.7)	5 (27.8)	3 (23.1)	5 (31.3)	0.89
Right coronal	17 (36.2)	1 (5.6)	10 (76.9)	6 (37.5)	***<0.001***
Left coronal	18 (38.3)	1 (5.6)	10 (76.9)	7 (43.8)	***<0.001***
Sagittal	28 (59.6)	12 (66.7)	6 (46.2)	10 (62.5)	0.50
Right lambdoid	13 (27.7)	1 (5.6)	7 (53.9)	5 (31.3)	***0.01***
Left lambdoid	9 (19.2)	1 (5.6)	5 (38.5)	3 (18.8)	0.07
Structural brain abnormality	10 (21.3)	0	2 (15.4)	8 (50.0)	***0.02***
Raised intracranial pressure	13 (27.7)	3 (16.7)	4 (30.8)	6 (37.5)	0.38
Age at diagnosis confirmation with CT scan (months)	11.7 (2.5-25.6)	10.1 (2.3-18.1)	5.0 (1.4-13.4)	31.1 (10.1-72.1)	

Antenatal History

Antenatal information was documented for 22 mothers of which four had potential exposure to teratogens that included sodium valproate (one), alcohol (one), methamphetamine (one) and cigarette smoking (one).

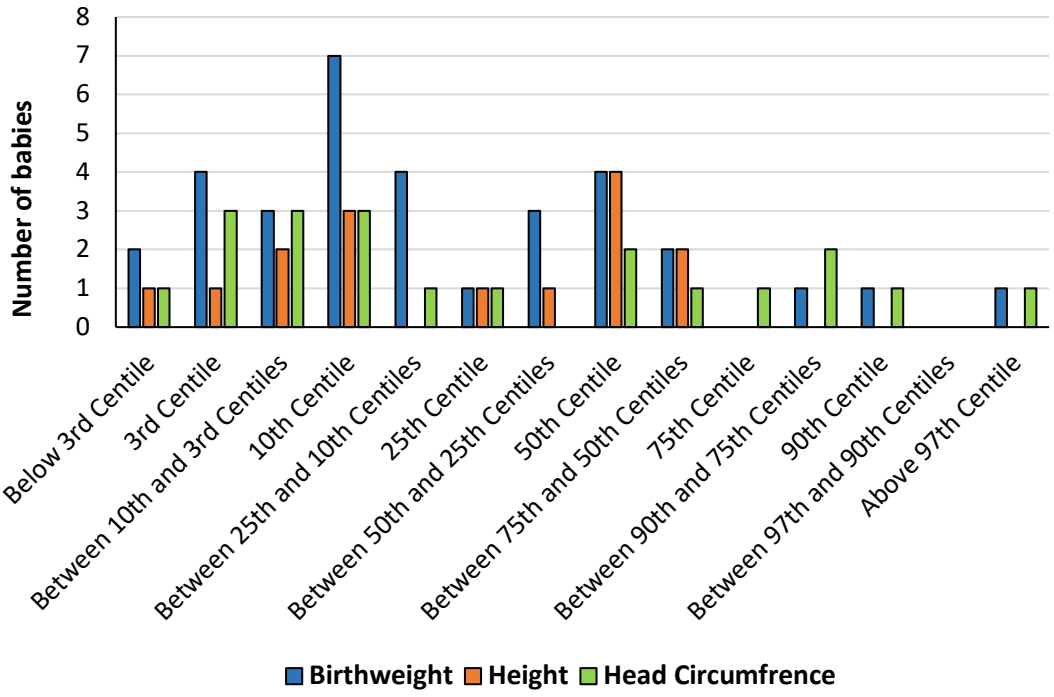
Thirteen of the mothers had been asked about a prenatal ultrasound. Ultrasound abnormalities or markers were identified in six foetuses and included polyhydramnios, oligohydramnios, soft markers for aneuploidy, intrauterine growth restriction, short femurs, and ventriculomegaly. In the foetus with ventriculomegaly a foetal MRI had shown isolated occipital horn dilatation. Only two mothers opted for invasive testing, both in the form of an amniocentesis. Results showed normal karyotypes in both cases.

Birth History

Eleven babies were delivered pre-term (*M* = 31 weeks, *SD* = 3, range: 26 – 36) and 23 at term. Seventeen babies had neonatal complications, and 12 babies presented with congenital abnormalities. The neonatal complications in the cohort included: neonatal jaundice, respiratory distress, anaemia, sepsis, seizures, upper airway obstruction, feeding difficulties , congenital pneumonia and apnoea. The congenital abnormalities included: thoracic myelomeningocele, cleft soft palate, talipes, congenital hip dysplasia, syndactyly of hands and feet, arthrogryposis of elbows, thumb abnormalities, abnormal genitalia and hypospadias.

Twelve babies were delivered by a normal vaginal delivery, 22 by Caesarean section, and two with assisted vaginal delivery. The average birthweight was 2.6kg (*SD* = 1, range: 0.8 – 4.3), the average height was 46.2cm (*SD* = 5.4, range: 33 – 52), and the average head circumference was 32.6cm (*SD* = 4, range: 24 - 38).

Figure 7. Birth weight, height and head circumference per centile.



Development

Of 39 patients for whom details of development were documented, 12 (30.8%) had Global Developmental Delay (GDD). Of these, five were classified as mild, three as moderate, one as severe, and for three severity was not noted (see Table 6).

Data for education and behaviour was available for 13 patients. Five had Intellectual Disability (two mild, two moderate with no data for the other one), these five were also earlier noted to have developmental delay, and two had behavioural difficulties (one was hyperactive and the other was hyperactive and had mild intellectual disability). Four patients had seizures (one had a febrile seizures, one had focal seizures and two had tonic-clonic seizures).

Table 6. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to development.

Variable	Total (N=47)	Non-syndromic (n=18)	Syndromic (n=13)	CS with additional features (n=16)	p-value
Global development delay (GDD)	12/39 (30.8)	1/16 (6.3)	4/10 (40.0)	7/13 (53.9)	***0.01***
Mild	5/12 (41.7)	1/1 (100)	2/4 (50.0)	2/7 (28.6)	0.71
Moderate	3/12 (25.0)	0	1/4 (25.0)	2/7 (28.6)	
Severe	1/12 (8.3)	0	1/4 (25.0)	0	
Unknown	3/12 (25.0)	0	0	3/7 (42.9)	
Additional neuro-behavioural features					
Behaviour difficulties	2/13 (15.4)	0	0	2/7 (28.6)	0.77
Seizures	4/13 (30.7)	0	2/6 (33.3)	2/7 (28.6)	

Clinical Features

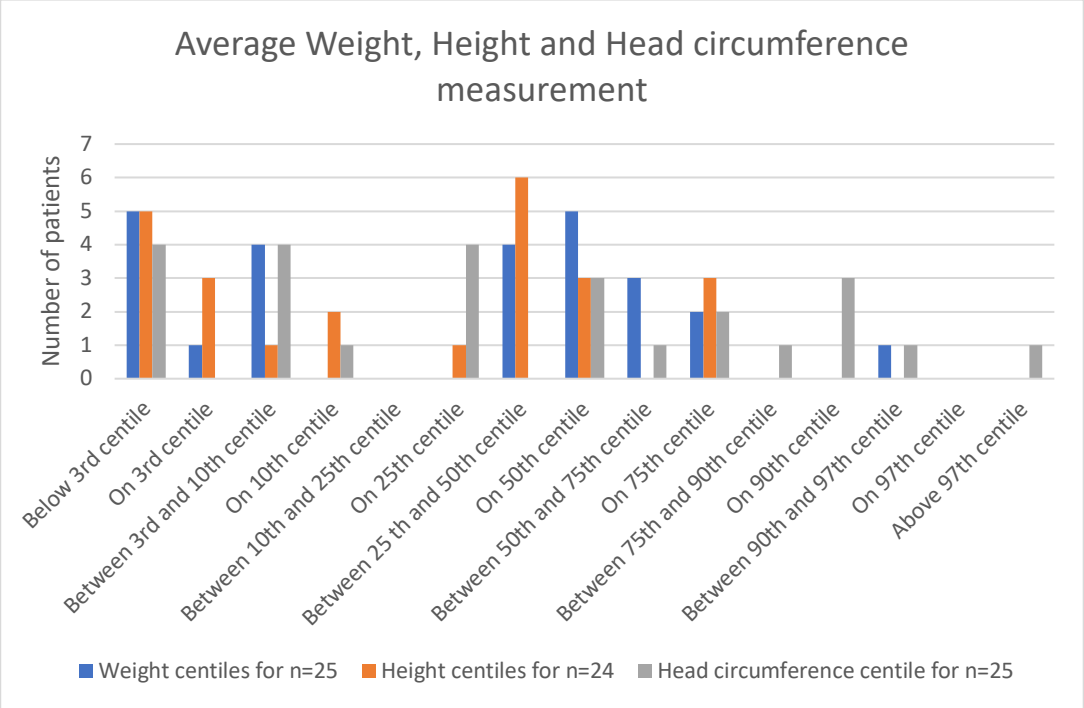
Anthropometric data included average weight, height and head circumference (see Figure 8).

Data on weight was available for 25 patients (n=2 for non-syndromic group; n=12 for syndromic group and n=11 craniosynostosis with additional findings group). Five were below the 3rd centile, one on the 3rd centile, four between the 3rd and 10th centile, four between the 25th and 50th centile, five on the 50th centile, three between the 50th and 75th centile, two on the 75th centile and one between the 90th and 97th centile.

Data on average height was available for 24 patients (n=2 for non-syndromic group; n=12 for syndromic group and n=10 for the craniosynostosis with additional findings group). Five were below the 3rd centile, three were on the 3rd centile, one was between the 3rd and 10th centile, two were on the 10th centile, one was on the 25th centile, six were between the 25th and 50th centile, three were on the 50th centile and three were on the 75th centile.

Data on head circumference was available for 25 patients (n=2 for non-syndromic group; n=12 for syndromic group and n=11 for craniosynostosis with additional findings group). Four were below the 3rd centile, one was on the 10th centile, four were on the 25th centile, three were on the 50th centile, one was between the 50th and 75th centile, two were on the 75th centile, one was between the 75th and the 90th centile, three were on the 90th centile, one was between the 90th and 97th centile and one was above the 97th centile.

Figure 8. Average weight, height and head circumference per centiles.



Craniofacial findings

In the case of 11 patients, there were notes on the clinical appearance of the anterior and posterior fontanelles. Three patients were noted to have normal appearing fontanelles. Eight were noted to have an abnormal anterior fontanelle. Two of these also had an abnormal posterior fontanelle noted . In the first patient all sutures were prematurely fused and therefore no fontanelle was clinically palpable. In the second case, a large anterior fontanelle communicated with the posterior fontanelle.

Thirty nine patients were clinically identified as having craniosynostosis. Four were noted to have anterior brachycephaly, three had plagiocephaly, 17 had a scaphocephaly, eight had a trigonocephaly, five had a turribrachycephaly, and in two cases the head shape was not noted although it was stated as being abnormal.

When documenting the examination of the nervous system, one patient had clinical signs of raised intracranial pressure and one was noted to have abnormal tone.

Regarding facial features, four cases were noted to have frontal bossing, one case had facial asymmetry, in two cases the face was described as elongated, one was described as having coarse facial features and one had a triangular face. Two patients had eyebrow abnormalities (one with prominent eyebrows and the other with synophrys). Fourteen patients had midface hypoplasia. Two children were noted to have a low hairline.

Nine of the children had hypertelorism and two had hypotelorism. Five had upslanting palpebral fissures and three cases downslanting palpebral fissures. Seven patients had epicanthic folds. Eleven patients were noted to have shallow orbits with three being described as exorbitism and five proptosis. Two patients were also described as having ptosis. Additional features listed under other eye abnormalities included: one patient with a left orbital haemangioma, one patient with canthus inversus, one patient with pseudoproptosis, one patient with an episode of eyelid eversion and one patient with the inability to close eyelids. Five individuals had strabismus, one was diagnosed with refractive errors, three had fundal abnormalities and two presented with vertical nystagmus. One of the three children with fundal abnormalities, was noted to had bilateral salt and pepper retinopathy with severe myopia and macrophthalmia.

When looking at ear dysmorphology, eight had low set ears, one had posterior rotated ears, one had ear pits and one case had ear tags. Data were available on formal audiometry for 15 patients, one child had sensorineural hearing loss, four had conductive hearing loss, the remaining 10 normal hearing . The child with sensorineural hearing loss was clinically diagnosed with Apert syndrome. Of the four

children with conductive hearing loss, two were diagnosed with Crouzon syndrome and two were in the group craniosynostosis with additional features. No patients had hearing loss secondary to recurrent otitis media.

In nine cases the nasal bridge was described as flat, one had a small nose and two cases were described as having a bulbous nasal tip.

Two patients were described as having a smooth philtrum and three as having a short philtrum. Two were noted to have a soft palate cleft and 6 had a high arched palate. Seven had micrognathia. Other features found included thick philtrum and lips with macroglossia in one patient and relative macroglossia in another patient. Four patients had feeding difficulties. Three patients had a dental abnormality (one patient had an overbite; one patients was diagnosed with dental overcrowding; one patient had both dental crowding and an overbite).

Skeletal abnormalities

Patients with both hand and feet abnormalities include: one patient with both a broad thumb and a broad hallux, four patients with syndactyly of hands and feet and one patient with polydactyly of hands and feet.

Upper limb abnormalities included, one patient with polydactyly of the hands, one patient with syndactyly of hands and two patients with brachydactyly of the hands. Four patients had broad thumbs and one nail hypoplasia. One patient had single palmar creases bilaterally, one had tapered fingers and one patient was noted to have broad and abnormal thumbs.

When describing lower limb abnormalities, two patients were noted to have a broad hallux only. One patient had talipes and in one case the metacarpals of the feet were described as short.

Other limb abnormalities included: one patient with right leg hypertrophy due to popliteal vein abnormality, one patient with congenital bilateral hip dysplasia and dislocation, one patient with arthrogryposis, and one patient with femoral bowing.

Two patients had scoliosis and two patients were noted to have sacral dimples.

Other congenital abnormalities

Only six patients out of the cohort of 47 had an echocardiogram. One patient was identified with pulmonary stenosis (PS) , one had a congenital mitral valve defect, one patient was identified with a patent foramen ovale (PFO),one patient had left ventricular outflow tract obstruction (LVOTO) and the remaining two had no abnormality on echocardiogram.

With regard to spinal abnormalities, one patient was diagnosed with myelomeningocele.

Renal abnormalities included reduced function and pelvic ureteric dilation in one patient and renal calculi and urinary tract infections in another.

One was identified as having a disorder of sexual development (DSD) in keeping with undervirilisation. Two males had undescended testes and one had hypospadias. One female patient had a vaginal tag.

Table 7. Comparing the two groups, syndromic and craniosynostosis with additional features with regard to clinical features.

Variable	Total (N=47)	Syndromic (n=13)	CS with additional features(n=16)	p-value
Hands				
Polydactyly	2/47 (4.3)	0	2/16 (12.5)	0.18
Syndactyly	5/47 (10.6)	4/13 (30.8)	1/16 (6.3)	***0.02***
Brachydactyly	1/47 (2.1)	1/13 (7.7)	0	0.28
Broad Thumbs	5/47 (10.6)	4/13 (30.8)	1/16 (6.3)	***0.02***
Feet				
Polydactyly	1/47 (2.1)	0	1/16 (6.3)	0.62

Syndactyly Broad Hallux	4/47 (8.5) 3/47 (6.4)	4/13 (30.8) 2/13 (15.4)	0 1/16 (6.3)	***0.004*** 0.18
Hypertelorism	9/47 (19.2)	4/13 (30.8)	5/16 (31.3)	***0.02***
Shallow orbits	11/47 (23.4)	10/13 (76.9)	1/16 (6.3)	***<0.001***
Exorbitism	3/47	3/13 (23.1)	0	***0.02***
Proptosis	5/47 (10.6)	4/13 (30.8)	1/16 (6.3)	***0.02***
Nose (flat nasal bridge)	9/47 (19.2)	3/13 (23.1)	6/16 (37.5)	***0.01***
Dental abnormality	3	2	1	-
Renal abnormalities	2	1	1	-
Under weight for age Below 3rd centile for weight	 5/25 (20.0)	 1/12 (8.3)	 4/11 (36.4)	
Short stature Below 3rd centile for height	 5/24 (20.8)	 2/12 (16.7)	 3/10 (30.0)	
Micro- and macrocephaly Below 3rd centile for head circumference Above 97th centile head circumference	 4/25 (16.0) 1/25 (4.0)	 2/12 (16.7) 1/12 (8.3)	 2/11 (18.2) 0	

Surgery

Two-thirds (*n* = 30; 63.8%) of the 47 patients had craniosynostosis surgery. The mean age at surgery was 22.4 months (*SD* = 19; range: 5-79). Surgical procedures included: Bilateral barrel stave osteotomy and cranial vault remodelling, fronto-orbital advancement and cranial vault remodelling, Le Forte III osteotomy and left frontal bone remodelling (unilateral cranioplasty). Two patients were reported to have complications during surgery. In the case of patient 1, a Le Fort III osteotomy was aborted due to cardiac compromise as a result of bleeding and the second patient developed a pressure sore from the endotracheal tube. At a later stage, three patients had prominent plates removed and two had screws removed that were causing pain.

Seventeen patients had other types of surgeries, nine individuals had multiple operations. Surgery likely related to the craniofacial abnormality included tracheostomy, left lateral canthotomy and bilateral tarsorrhaphy, strabismus correction, adenoidectomy, tonsillectomy and ventriculoperitoneal shunt placement.

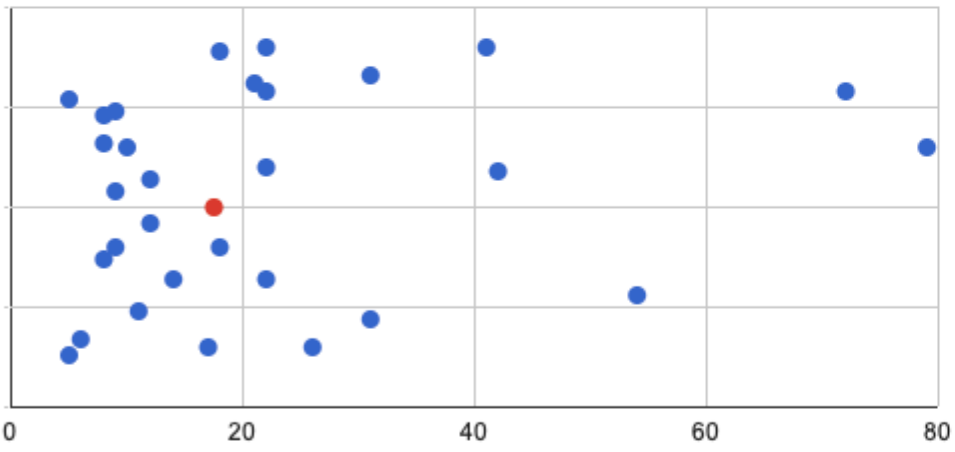
Surgery related to other congenital abnormalities included placement of grommets, syndactyly release, repair of myelomeningocele, soft palate repair, gastrostomy tube placement, surgery for hip dysplasia, tenotomy for talipes, bilateral inguinal hernia repair, left pyeloplasty, Duckett tube repair for hypospadias, urethrocutaneous fistula repair and circumcision.

Table 8. Comparing the three groups, non-syndromic, syndromic and craniosynostosis with additional features with regard to surgery.

Variable	Total (N=47)	Non-syndromic (n=18)	Syndromic (n=13)	CS with additional features (n=16)	p-value
Craniosynostosis surgery	30 (63.8)	16 (88.9)	7 (53.9)	7 (43.8)	***0.01***
Other surgeries	17 (36.2)	0	7 (53.9)	10 (62.5)	***<0.001***
Age of craniosynostosis surgery in months	18 (9-26)	11.5 (8.5-22.0)	18.0 (10.0-42.0)	28.5 (22.0-31.0)	0.14

Figure 9. Age distribution in months at the time of craniosynostosis surgery.

Total count of individuals who had craniosynostosis surgery	Min	Max	Mean	StDev	Sum	Percentile						
						0.05	0.10	0.25	0.50 Median	0.75	0.90	0.95
30	5.0	79.0	22.13	18.78	664.0	5.45	7.80	9.00	17.50	25.00	43.20	63.90



Genetic Testing

Twelve patients had karyotyping done after birth. Eleven of the karyotypes were normal and one was abnormal. The abnormal karyotype was 47,XXY or Klinefelter syndrome and likely an incidental finding.

One patient had full sequencing of the *FGFR2* gene and a pathogenic variant, *FGFR2*, c.799T>C (p.Ser267Pro) was identified. The diagnosis of Crouzon syndrome was therefore molecularly confirmed.

A total of five patients had targeted *FGFR2* testing for Crouzon and Pfeiffer syndrome. In the case of one patient, a pathogenic variant, p.Gly338Glu(c.1013G>A) was identified, confirming Crouzon syndrome. In the other four cases no pathogenic variant was identified. One patient had further testing for the p.Pro250Arg(c.749C>G) variant in *FGFR3* that causes Muenke syndrome and the p.Ala391Glu(c.1172C>A) variant in *FGFR3* that is responsible for Crouzon syndrome with Acanthosis Nigricans. The patient tested negative for both of these specific mutations in *FGFR3.*
One patient had a family history of Crouzon syndrome and was tested for the known family mutation c.1025G>A(p.Cys342Tyr) in the *FGFR2* gene. The patient tested positive for this pathogenic variant.

Other molecular testing requested in this cohort of patients included deletion and duplication screening using Multiplex ligation-dependent Probe Amplification(MLPA) (MRC Holland) in five patients, for common microdeletions/ duplications (two), and subtelomeric gains and losses (three). All tested negative. One patient was tested for an expansion mutation in the *FMR-1* gene causing Fragile X syndrome. The patient tested negative. One patient was tested for a lysosomal storage disorder but tested negative for Mucopolysaccharidosis types I, II and VI and Mucolipidosis types II and III.

Chapter 4: Discussion and Conclusions

Craniosynostosis is an element of a very diverse group of conditions, complicating diagnosis. It is however a condition that needs early diagnosis and intervention as complications can severely impact on the patients and their families. Complications that can occur due to the increase in intracranial pressure include sensory, respiratory and neurological functional impairment (Johnson and Wilkie, 2011). Surgical interventions are indicated in a number of these patients.

In literature no preference for geographic region, ethnic group or socioeconomic status was found (Flaherty et al., 2016; Garrocho-Rangel et al., 2018). There was no suggestion of any specific ancestry bias in this cohort who broadly reflected the ethnicity of patients generally accessing RCWMCH services.

Craniosynostosis is described to occur more commonly in males than in females (Tahiri et al., 2017). In this study, 53% of the patients were male so the male predominance was not convincing in our cohort. When looking at specific sutures, synostosis of the sagittal suture, show a strong male predominance with a male : female ration of 3.5:1. Metopic synostosis also has a male to female ratio of 3.3:1. However in unilateral or bilateral coronal synostosis, 60-75% of those affected are female (Ciurea and Toader, 2009). In the craniosynostosis with additional features group there was a predominance of males at 75% with sagittal suture being involved in most of these patients (62%). In the syndromic group in this study, there was a female predominance of 69,2% with coronal suture involvement in 76,9% of cases. Our sex distribution therefore correlates with previous literature (Ciurea and Toader, 2009; Tahiri et al., 2017).

The syndromic group were most likely to have an affected family member (2 mothers and 1 paternal first cousin) which can aid in early diagnosis. This is probably due to

autosomal dominant inheritance patterns known to occur in most of the syndromic craniosynostosis conditions.

The craniosynostosis with additional features group had no history of affected family members noted. In our cohort, this group also had the highest proportion of children showing developmental delay at 53.9% and these children frequently had coronal synostosis with left coronal suture involvement at 43.8%, right coronal suture involvement at 37.5%. A detectable genetic cause is more likely if coronal suture or multiple suture involvement is observed, if a patient shows growth or developmental restriction and if the patient has other congenital anomalies (Goos and Mathijssen, 2019). It is therefore still likely that the majority of this group have a genetic aetiology for their condition. Reasons for not observing affected family members could be de novo mutations, inheritance patterns other than autosomal dominant, or even complex or polygenic inheritance.

True craniosynostosis should be differentiated from other conditions in the differential diagnosis of abnormal skull shape. It is therefore important to obtain a good history and do a thorough clinical examination according to set guidelines and to ask specific questions to aid in correctly differentiating between craniosynostosis and other conditions such as positional plagiocephaly for example and document additional clinical findings. As children with an abnormal skull shape may first present to a general practitioner or paediatrician it is important that these clinicians be made aware of the condition and have access to diagnostic guidelines and an appropriate referral route to follow, if the diagnosis of craniosynostosis is suspected.

The syndromic synostoses with a typical facial gestalt and associated limb abnormalities are probably the easiest in the group to identify. In support of this, this study found that the syndromic group had the earliest age of diagnostic confirmation with a CT scan at a median age of five months compared to the non-syndromic group with a median age of ten months. The children who had craniosynostosis with additional features but no recognisable syndromic diagnosis were diagnosed even later at a median of 31 months.

Early syndromic recognition is important for timeous referral and consideration of craniosynostosis surgery. Syndromic craniosynostosis is reported as having the highest risk for additional brain abnormalities and complications such as raised intracranial pressure (Mathijssen, 2015; Sawh-Martinez and Steinbacher, 2019). Structural brain abnormalities were identified in 15.4% and raised intracranial pressure in 30.8% of the syndromic cohort but both of these findings were even more common in children with additional features but no syndromic diagnosis. In half of the cases (50.0%) other structural brain abnormalities were also noted and they had the most frequent association with raised intracranial pressure which was noted in 37.5% of cases. This may suggest that the lack of access to genetic testing and possible medical genetic services may make a specific syndromic diagnosis less likely in our setting.

Suture involvement was most often sagittal in non-syndromic cases (66.7%) which is well described (Dempsey et al., 2019). In the children who had craniosynostosis with additional features sagittal suture involvement was still the most prevalent (62.5%) followed by the left coronal suture (43. 8%).

Although prematurity and neonatal complications were common in the cohort, they were infrequent in those with non-syndromic craniosynostosis. No prenatal ultrasound abnormalities were detected and no invasive testing was done. Those who had CS with additional features had highest incidence of preterm deliveries at 66.7% and are also the group with the highest reported neonatal complication rate at 83.3%. The most frequent neonatal complications noted were jaundice, sepsis and apnoea that could be the result of prematurity or other congenital abnormalities. An association between craniosynostosis and preterm delivery has been described (Sanchez-Lara et al., 2010; Singer et al., 1999).

Developmental delay and intellectual disability are rarely directly due to craniosynostosis. In a 10 year multicentre study, where the cognitive development of children with single suture/non-syndromic craniosynostosis was looked at, it was found that developmental delay in this subgroup is generally mild. Individuals with

unicoronal and lambdoid synostosis are at the highest risk for GDD and individuals with sagittal synostosis are usually spared (Millichap, 2015). However, a consistent association between neurodevelopmental status, optimal age for surgery, and intracranial pressure (ICP) has not been observed in isolated single suture craniosynostosis (SSC) (Shim et al., 2016). Twelve patients (30.8 %) in our cohort were reported with developmental delay. Only one patient was identified with mild global developmental delay in the non-syndromic group and the highest incidence of global developmental delay was observed in the CS with additional features group (53.9%).

A total of seven children were identified with developmental delay in the subgroup craniosynostosis and additional features. One child with a myelomeningocele, who also had raised ICP and had a VP shunt inserted, had mild GDD and the second child with mild GDD required surgery at 3.8 months due to raised ICP. There were two children with moderate GDD, neither had surgery but one has an absent corpus callosum. Of the remaining three whose severity of developmental delay wasn't documented, two had structural brain abnormalities, one with pachygyria and one a Chiari I malformation.

In the syndromic subgroup, mild developmental delay was noted in a child with Pfeiffer syndrome and a child with Crouzon syndrome. Developmental delay was moderate to severe in the two children who had Apert syndrome. This correlates with literature that states there is a strong increase in the risk of developmental delay in individuals with Apert Syndrome (Mathijssen, 2015).

These findings suggest that the syndromic craniosynostosis, Apert syndrome in particular, and the presence of additional intracranial abnormalities are indicative of a high risk for developmental delay. Those children should be identified early for surveillance and developmental therapies.

In this study syndromic diagnoses clinically identified included eight Crouzon syndrome patients, four patients with Apert syndrome and one with Pfeiffer

syndrome. From prevalence studies it is known that these three conditions have a lower recurrence risk in the populations studied (mostly European and USA based) than conditions such as Muenke syndrome and Saethre-Chotzen syndrome (Buchanan et al., 2014; Doherty et al., 2007; Johnson and Wilkie, 2011; O'Hara et al., 2019; Wilkie et al., 2017). This suggests that Muenke syndrome may have been overlooked as a potential diagnosis, although no African data is available on prevalence of this syndrome which is characterised by variable expression. In the group of patients with unilateral or bilateral coronal synostosis, testing should be considered to aid the identification of Muenke syndrome and allow for early monitoring of hearing loss and appropriate genetic counselling. Mild to moderate low frequency hearing loss occurs in 95% of individuals with Muenke syndrome (Doherty et al., 2007).

Limb abnormalities were also reported in the craniosynostosis with additional features group of children. This included polydactyly of both hands and feet, syndactyly of hands and broad thumbs and broad halluces. These are features well described in craniosynostosis syndromes and a syndromic diagnosis is likely in some of this undiagnosed group but was not made either for lack of recognition or due to doubt in defining a diagnosis that could not be confirmed by molecular testing (Biesecker and Johnston, 2021; Sawh-Martinez and Steinbacher, 2019).

Early syndrome identification is important for early intervention or surgery to create functional limbs, to prevent eye complications, detect hearing loss and timeously identify any developmental delay. These patients should ideally be followed up on a regular basis by a multidisciplinary team consisting of a neurosurgeon, plastic surgeon, maxillary facial surgeon, medical geneticist, developmental paediatrician, audiologist, ENT surgeon and possibly social worker/ psychologist.

Non-syndromic craniosynostosis usually accounts for the majority of craniosynostosis cases at approximately 85 %, while seven percent of cases of craniosynostosis have additional clinical findings and about nine percent of patients with craniosynostosis are found to be syndromic (Dempsey et al., 2019).In our cohort however there were

higher proportions of syndromic patients (27,6%) and those with additional abnormalities (34%) than described. Thirty (63.8%) of the cohort underwent craniofacial surgery. There may however be an enrolment bias as patients were enrolled via the neurosurgery surgical database and the genetic database rather than prospectively ascertained. The non-syndromic group reflected the highest rate of craniofacial surgery. The age of surgery (at date of first surgery) was found the lowest at a mean of 11.5 months in the non-syndromic group compared to a mean of 18 months in the syndromic group and 28.5 months in the craniosynostosis with additional features group. This may in part be explained by the presence of additional abnormalities either delaying the diagnosis or increasing the risk for early surgery. Two thirds of these children had other surgeries reported.

Genetic testing access is limited in the public sector in South Africa and, even where available, is expensive and limited understanding of the value reduces utilization. Molecular testing for FGFR-related craniosynostosis was undertaken in only seven patients with three cases of Crouzon syndrome confirmed.

Early recognition by skilled professionals as part of a multidisciplinary team of specific craniosynostosis syndromes can drive cost effective testing and aid in accurate prognosis and recurrence risk information for families. More than 60% of this cohort had either syndromic craniosynostosis or had additional clinical findings suggesting a more complex condition. A medical geneticist therefore has an important role to play in the multidisciplinary team and can assist in education of primary care providers of the affected individuals.

In conclusion, recognising and understanding the phenotypic spectrum of craniosynostosis will aid in improving the detection rate and allow for earlier diagnosis of complex craniosynostosis which can lead to an improved outcome. While numerous cases of craniosynostosis have been reported worldwide, especially in patients of European descent there are few reports from South Africa or the rest of Africa. Although this was a small cohort with limited molecular genetic confirmation, this study gives us insight into South African children that have presented with

craniosynostosis at the RCWMCH in the past five years. The REDCap database designed as part of this study to facilitate the capture of detailed information on craniosynostosis can be built on to allow comprehensive data collection in larger cohort to improve audit of outcomes and aid in future research.

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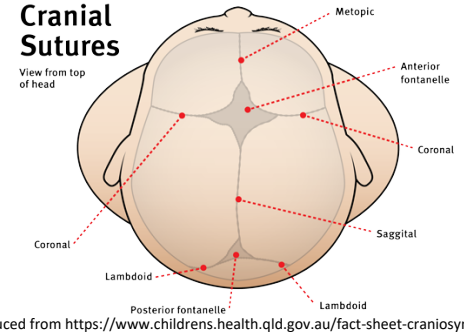
Appendices

Appendix 1: Data sheet



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Craniosynostosis Data Collection Sheet:

Patient code:

Place Seen: Date:

Contact details:

1. Socio demographic data:

DOB:		Age at diagnosis:	
Ethnolinguistic group:			
Country of origin:	RSA	Other:	

2. Family history/ Pedigree:

3. Perinatal history:
   a. Prenatal history

Maternal illness:
Teratogens:
Prenatal ultrasound abnormalities:
Amniocentesis:
Karyotype if applicable:

b. Birth

Gestation:	
Type of delivery:	Where:
APGAR:	Birthweight:
Length:	Head circumference:
Complications:	
Congenital abnormalities noted:	

4. Developmental history:

Smiled:
Sat:
Walked:
Speech:

5. Past Medical history:
   a. Medical

Structural Defects:
Functional Defects:
Metabolic Defects:
Other:

   b. Surgical

History of surgery:
Type of surgery:
Date of surgery:
Complications:

6. Clinical Data
    a. Craniofacial

Fontanelle:			
Clinically craniosynostosis:			
Head shape: ▪ Brachycephaly- Anterior or posterior ▪ Plagiocephaly ▪ Scaphocephaly ▪ Trigonocephaly ▪ Turribrachycephaly			
Head circumference:		Centile:	
▪ Microcephaly		▪ Macrocephaly	
Forehead:		High	
Face:			
▪ Asymmetry:			
Elongated	Coarse	Myopathic	Other
Scoliotic			
Eyebrows:			
Eyes:			
▪ Hypertelorism		▪ Hypotelorism	
▪ Palpebral Fissure		▪ Epicanthic folds	
▪ Shallow orbits		▪ Proptosis	
▪ Exorbitism		▪	
▪ Ptosis		▪ Other:	
Ears:			
▪ Low set		▪ Rotated	
▪ Pits		▪ Tags	
▪ Structural Abnormality			
▪ Other			
Midface:			
▪ Hypoplasia		▪ Other	
Nose:			
▪ Shape		▪ Nasal Bridge	
▪ Upturned tip		▪ Columella	
▪ Beaked shaped		▪ Nasolabial fold	
Other			
Mouth:			
▪ Philtrum		▪ Vermillion	

▪ Cleft lip	
▪ Micrognathia	▪ Retrognathia
▪ Mandibular Prognathism	
▪ Other	
Neck:	
▪ Webbing	▪ Other
Other:	

b. Growth

Weight:	Centile:
Height	Centile:

c. Central Nervous System

Hydrocephalus:
Chiari Malformation:
Structural abnormalities:
Other:

d. Ophthalmology

Coloboma:
Strabismus:
Anterior segment changes:
Refractive errors:
Fundal abnormalities:
Nystagmus:
Other:

e. Auditory

Formal Audiology testing: Yes/No
Sensorineural Hearing loss:
Hearing loss secondary to recurrent otitis media:
Conductive hearing loss:
Other:

f. Oral and Dental

Dental Abnormalities:
Cleft palate:
High arched palate:
Other:

g. Cardiovascular

Congenital heart defects:	
▪ AVSD	▪ ASD
▪ VSD	▪ PS
▪ PDA	▪ Valve defects
▪ Tetralogy of Fallot	▪ Other
Cardiomyopathy:	
ECG abnormalities:	
Other:	

h. Musculoskeletal

Chest shape:	
▪ Pectus Carinatum	
▪ Pectus Excavatum	
Spine:	
▪ Scoliosis	
▪ Other	
▪ Klippel-Feil	
Limbs:	
Hands	▪ Syndactyly
▪ Polydactyly	▪ Brachydactyly
▪ Carpal fusion	▪ Other
▪ Broad thumbs	▪ Nail hypoplasia
Feet	▪ Syndactyly
▪ Polydactyly	▪ Brachydactyly
▪ Tarsal fusion	▪ Other
▪ Broad Hallux	▪ Nail Hypoplasia
▪ Talipes	
Other limb abnormalities:	
Joints:	
▪ Contractures	▪ Synostosis
▪ Hyper extensibility	▪ Ankylosis
Other:	

i. Gastrointestinal

Feeding difficulties:
GORD:
Intestinal problems:
Hepatomegaly:
Other:

j. Genitourinary

Male:	Female:
Structural genital problems:	
▪ Hypospadias	
▪ Undescended testes	
▪ Bifid Scrotum	
Renal Abnormalities:	
Other:	

k. Dermatology

Hair:	Hairline:
Pigmentary changes:	
Café-au-lait:	
Other:	

l. Neurology, education and behaviour

Mild intellectual disability:
Severe intellectual disability:
Learning difficulties:
Seizures:
Epilepsy:
Behavioural Issues:
Other:

m. Other

Appendix 2: Information sheet for participants/ parents of participants

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS
INFORMATION SHEET FOR PARTICIPANTS OF RESEARCH PROJECT:

CRANIOSYNOSTOSIS IN THE SOUTH AFRICAN POPULATION

INTRODUCTION

This study aims to investigate craniosynostosis, the early closure of the skull bones, in our South African Population. You have been approached and asked to take part in this study as you / your child has been diagnosed with craniosynostosis."

In medical research there have been many studies done on craniosynostosis. Most of the information about craniosynostosis was gathered from people living in Europe or America but unfortunately, we do not have a lot of information available on craniosynostosis in Africa and South Africa. We know that the fusion of cranial sutures can occur alone, or it can form part of a syndrome that has other symptoms or signs. These symptoms can range from various hand and foot abnormalities, different facial features and sometimes developmental delay/ intellectual disability.

Craniosynostosis is often caused by a genetic change and it can sometimes be inherited even if it doesn't have all the same features in everyone. This means that there are possibly implications for the family and future children. This study may help identify some of those implications and you will be offered the opportunity of being referred for genetic counselling.

WHY ARE WE DOING THIS STUDY?

Our intention is to gather information on craniosynostosis in the South Africa population. We hope to learn about how patients present, how the problems are addressed and the outcome. We aim to improve our knowledge and provide the best care possible for craniosynostosis patients and their families.

WHAT WILL I NEED TO DO IF I AGREE TO PARTICIPATE IN THIS STUDY?

If you participate you will need to be clinically examined by a doctor working in the Division of Human Genetics of the University of Cape Town.

If you give permission, photos of you / your child may be taken to help other people learn more about craniosynostosis but your / your child's name will not be with the pictures.

WHAT WILL HAPPEN TO MY INFORMATION?

Information obtained from the study may be shared with other health care professionals such as in a journal publication but if so, no identifiable data will be used.

CONFIDENTIALITY

All information provided to the medical doctor and information obtained through the clinical examination is confidential. All the information will be safely stored in locked offices. All information stored on computers will be password protected. Your / your child's participation in this study and any results obtained will not be shared with any individuals not involved with your / your child's medical care.

We might ask you about taking photographs to be used in publications, but it is entirely your decision if you feel comfortable and want to agree to this.

WHAT IS THE RISK OF BEING INVOLVED IN THE STUDY?

The risk of harm or discomfort expected is not greater than what would you encounter in daily life or during routine medical examinations.

ARE THERE ANY BENEFITS FOR ME?

There might not be direct benefit for you at the moment, but it could provide knowledge for future improvement in care.

CAN I DECLINE TO TAKE PART IN STUDY?

Participation is entirely voluntary. You can at any time during the study withdraw if you wish to do so. Withdrawal will not harm your/your child's medical care. You will be treated exactly the same whether you participate in the study or not. Please feel free to ask family members or friends for advice if you want to, before agreeing to participate.

Please do not hesitate to contact the following persons if you have any problem or questions about the research.

Dr Karen Fieggen (Project Supervisor)
Division of Human Genetics, University of Cape Town
Anzio Road, Observatory 7925, Cape Town, South Africa
Tel: +27 (0) 21 404 6298
Email: karen.fieggen@uct.ac.za

Dr Ilse Crous
Division of Human Genetics, University of Cape Town
Anzio Road, Observatory 7925, Cape Town, South Africa
Tel: +27 (0) 21 404 6235
Email: ilse.crous@uct.ac.za

Appendix 3: Informed consent for research project: English version

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS
INFORMED CONSENT FOR RESEARCH PROJECT:

CRANIOSYNOSTOSIS IN THE SOUTH AFRICAN POPULATION

I,……., hereby voluntarily consent to myself / my child……being included in the craniosynostosis study.

In this research study:
Each participant will have:

1. A clinical examination done by one of the medical doctors of the Division of Human Genetics at the Red Cross War Memorial Children Hospital.

By consenting to this study:

☐ I agree that my /my child's data can be stored, shared and used for the present craniosynostosis study and scientific publications after removal of my/ my child's personal identifiers.

☐ I agree that my photographs can be taken and be used in scientific publications after removal of personal identifiers.

The benefits, risks and procedure for this study have been explained to me and I have been given opportunity to ask questions about the research.

Participation in this study is voluntary and may be withdrawn at any stage, without in any way affecting my/ my child's future care.

Signature:……
Date:……

Witness signature:……
Date:……

Appendix 4: Assent for research project: English version

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS
ASSENT FOR RESEARCH PROJECT:

CRANIOSYNOSTOSIS IN SOUTH AFRICAN POPULATIONS

1. This study is being done by the doctors at the University of Cape Town to try and learn more about why your skull bones closed earlier than in other children.
2. The doctors are trying to see if they can learn more about children in whom this happens so they can better help you and others in the future.
3. If you decide to be a part of the study one of the doctors from Genetics will examine you. Nothing that will be done will be harmful to you.
4. If you give permission, photos of you may be taken to help other people learn more about craniosynostosis but your name will not be with the pictures.
5. Everything that is done during the study will be confidential, meaning that no one who is not a part of the study will know what you did.
6. You can decide for yourself whether you want to be a part of this study. If you decide to not be a part of it then no one will be angry with you, and you will still get all the help and care that you need.

I am assenting to participate in the study

Signature: …….. Date: ……

Witness signature: …….. Date: ……

Appendix 5: Ethics approval documents

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6626
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

27 March 2019

HREC REF: 774/2018

A/Prof K Fieggen
Human Genetics
Room 4.23
Falmouth Building

Dear A/Prof Fieggen

PROJECT TITLE: CRANIOSYNOSTOSIS IN THE SOUTH AFRICAN POPULATION (MMed candidate-Dr I Crous)

Thank you for submitting your response to the Faculty of Health Sciences Human Research Ethics Committee dated 1 March 2019.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 March 2020.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

The HREC acknowledge that the student, Dr Ilse Crous will also be involved in this study.

Yours sincerely

Signature Removed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

UNIVERSITY OF CAPE TOWN — FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30.03.2021
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	Signature Removed	Date Signed	8/3/2020

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	06/03/2020		
HREC REF Number	774/2018	Current Ethics Approval was granted until	30/03/2020
Protocol title	Craniosynostosis in the South African population		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	A/Prof Karen Fieggen		
Department / Office Internal Mail Address	Division on Human Genetics, Fourth Floor, Falmouth Building, Falmouth road, Observatory		

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Western Cape Government
Health

DR AN PARBHOO
Manager: Medical Services
Red Cross War Memorial Children's Hospital
Email: Anita.Parbhoo@westerncape.gov.za
Tel: +27 21 658 5430 Fax: +27 21 658 5006/5166

05 June 2019

Dr I Crous
Genetics

Dear Dr Crous,

RESEARCH: RXH: RCC 189

PROJECT TITLE: Craniosynostosis in the South African Population

It is a pleasure to inform you that the hospital Research Review Committee has approved your application to conduct above-mentioned study at Red Cross War Memorial Children's Hospital.

Yours sincerely,

Signature Removed

DR AN PARBHOO
MANAGER: MEDICAL SERVICES