CRANIOSYNOSTOSIS IN NORWAY – EPIDEMIOLOGY AND GENETIC CAUSES

Dissertation for the degree of Philosophiae Doctor (PhD)

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Oslo, February 2022

Elin Tønne

2. LIST OF PUBLICATIONS

Paper I

Tønne E, Due-Tønnessen BJ, Wiig U, Stadheim BF, Meling TR, Helseth E, Heimdal KR.

"Epidemiology of craniosynostosis in Norway"

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Paper II

Tønne E, Due-Tønnessen BJ, Mero IL, Wiig U, Kulseth MA, Vigeland MD, Sheng Y, Lippe CVD, Tveten K, Meling TR, Helseth E, Heimdal KR.

"Benefits of clinical criteria and high-throughput sequencing for diagnosing children with syndromic craniosynostosis"

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Paper III

Tønne E, Due-Tønnessen BJ, Vigeland MD, Amundsen SS, Ribarska T, Åsten PM, Sheng Y, Helseth E, Gilfillan GD, Mero IL, Heimdal KR.

"Whole-exome sequencing in syndromic craniosynostosis increases diagnostic yield and identifies candidate genes in osteogenic signaling pathways"

Am J Med Genet A. 2022 Jan;25:1-12, doi: 10.1002/ajmg.a.62663. Online ahead of print. PMID: 35080095

3. ABBREVIATIONS

aCGH	Array comparative genomic hybridization
ALX4	Aristaless homeobox 4
BMP	Bone morphogenetic protein
CADD	Combined annotation dependent depletion
CS	Craniosynostosis
CT	Computed tomography
DNA	Deoxyribonucleic acid
EACS	Endoscopy-assisted craniosynostosis surgery
EFNB1	Ephrin-B1
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
Hh	Hedgehog
HTS	High-throughput sequencing
MLPA	Multiplex ligation-dependent probe amplification
MSCs	Mesenchymal stem cells
MSX2	Muscle segment homeobox drosophila homologue 2
SMAD6	SMAD family member 6
TCF12	Transcription factor 12
TGFβ	Transforming growth factor β
TWIST1	Twist homologue drosophila 1

VUS	Variants of uncertain significance
RUNX2	Runt-related transcription factor 2
WES	Whole exome sequencing
WGS	Whole genome sequencing
Wnt	Wingless-type integration site

4. THESIS SUMMARY

4.1 Sammendrag (Norwegian)

Kraniet består av flere benplater som er holdt sammen av bindevevsstrukturer. Disse bindevevsstrukturene kalles sømmer eller suturer. Suturene gir fleksibilitet til kraniet som er nødvendig både for at hodet skal kunne passere ukomplisert gjennom fødselskanalen, og for at hjernen skal ha tilstrekkelig plass til å vokse. I tillegg til å gi fleksibilitet, utgjør suturene benplatenes vekstsoner som sikrer normal vekst av kraniet. Hos noen barn vokser suturene sammen (forbenes) for tidlig, og dette kalles kraniosynostose (KS). KS er en av de vanligste medfødte misdannelsene hos barn. Dersom KS ikke behandles kirurgisk vil skallen ofte få en uvanlig fasong og i noen tilfeller kan det oppstå et forhøyet intrakranielt trykk, som igjen kan gi alvorlige komplikasjoner.

KS klassifiseres i to hovedgrupper: syndromal og ikke-syndromal. Syndromal KS er forbundet med misdannelser i andre organ og/eller andre funn som for eksempel forsinket utvikling eller redusert hørsel. Ved ikke-syndromal KS foreligger det vanligvis ingen andre funn eller vansker. Syndromal og ikke-syndromal KS har ulik prognose, årsak, implikasjoner for familien og ofte behandling. Det er derfor viktig å skille disse to undergruppene.

Insidensen av KS er ikke tidligere rapportert fra Skandinavia, og tallene som er publisert fra andre land viser sprikende forekomst. Nasjonal behandlingstjeneste for kirurgisk behandling ved kraniofaciale misdannelser utreder, behandler og gir oppfølging til alle barn i Norge med KS og har etablert et register. Dette populasjonsbaserte registeret dannet et godt utgangspunkt for en epidemiologisk studie.

En relativ ny teknologi for undersøkelse av gener, High-throughput sequencing (HTS), har resultert i økt kunnskap om de genetiske årsakene til KS. Ved starten av studien (2019) var det etablert omtrent 80 monogenetiske årsaker til KS. En genetisk årsaksdiagnose sier ofte noe om prognose, sannsynlighet for annen sykdom, sannsynlighet for at andre i familien har eller vil kunne få samme tilstand, og påvirker behandling. For barnet og familien, har en genetisk årsak derfor stor betydning. Ved å utvide de HTS baserte analysene, ønsket vi å se om vi kunne finne en genetisk årsak hos flere barn med KS, i tillegg til å avdekke nye genetiske årsaker til KS.

Behandlingstjenestens register over alle samtykkende barn (foresatte) med KS i Norge, født 2003-2017 (Studie I, n=328) og 2002-2019 (Studie II og III, n=381) dannet utgangspunktet for studien. Syndromal KS ble definert ut i fra kliniske kriterier.

Vi fant en av de høyeste insidensene av KS som er rapportert, på 5.5 per 10 000 levende fødte. Vi fant også en av de høyeste andelene av syndromal KS (27%) som er rapportert fra en definert populasjon. I gruppen syndromal KS avdekket vi en genetisk årsaksdiagnose hos 92%, inkludert 10 nye genetiske årsaker. I tillegg avdekket vi to kandidatgener for KS, hvor ytterligere undersøkelser er nødvendig for å kunne stadfeste en eventuell årsakssammenheng. De genetiske årsakene var primært lokalisert i gener som er kjent til å inngå i signalveier viktige for skallebenets vekst og opprettholdelse av suturer.

Omtrent halvparten av de genetiske årsakene befant seg i noen få gener som er relativt hyppig forbundet med syndromal KS. Resten av de genetiske årsakene fordelte seg mellom en rekke sjeldne og ultra-sjeldne syndromer, forenlig med at syndromal KS er svært heterogent. En bred genetisk test-strategi er derfor ofte nødvendig for å avdekke den genetiske årsaken til KS.

Funnene i studien medførte en utvidelse av analysetilbudet som rutinemessig gis til barn med syndromal KS i Norge.

4.2 Summary

Craniosynostosis (CS) is caused by the premature fusion of one or several cranial sutures and is one of the most common inborn anomalies in children. CS is divided into syndromic and nonsyndromic. Syndromic CS is associated with additional anomalies and difficulties, while nonsyndromic CS usually implies no additional findings. Syndromic and nonsyndromic CS have different prognoses, causes, implications for families and often treatments. It is therefore important to separate these two subtypes.

The epidemiology of CS has not previously been reported from any Scandinavian country, and the numbers published from other parts of the world are divergent. The Norwegian National Unit for Craniofacial Surgery has a large population-based cohort of individuals with CS and is well suited for epidemiological research. The introduction of high-throughput sequencing (HTS), enabling a large number of genes to be investigated simultaneously, has resulted in increased knowledge of the genetic causes of CS over the last decade. At the start of this study (2019), there were approximately 80 established monogenic causes of CS. Determining the exact cause of a child's disorder is important, as this often impacts treatment, prognosis, implications for the family and social care. We expected that expanding the genetic analyses provided to children with syndromic CS using HTS would increase the diagnostic yield in the cohort and detect novel genetic causes.

All children and families with CS in Norway receive diagnostics, treatment and follow-up from the Norwegian National Unit for Craniofacial Surgery located at Oslo University Hospital. The Unit`s prospective registry was used to retrieve information from all consenting individuals born 2003-2017 (Study I, n=328) and 2002-2019 (Study II and III, n=381). Syndromic CS was defined by a set of clinical criteria.

We detected one of the highest incidences of CS reported: 5.5 per 10 000 live births. In addition, the incidence increased significantly during the study period. We also found one of the highest numbers of syndromic CS cases (27%) reported from a defined population. In the group of syndromic CS, an established genetic diagnosis was confirmed in 92% of the investigated children, including 10 novel monogenic causes of syndromic CS. In addition, two candidate genes for syndromic CS were revealed. The genetic causes were clustered in genes known to affect the signalling pathways involved in cranial growth and suture patency.

Approximately half of the genetic causes were involved in a few and previously well-described syndromes, almost always including CS. The rest of the genetic diagnoses were distributed between a large number of different genetic causes, including CS, in only a fraction of the cases, confirming that syndromic CS is highly heterogeneous. Thus, a broad genetic test strategy is needed to detect the rare and ultrarare genetic causes of syndromic CS. This knowledge expanded the molecular diagnostics routinely provided to children with syndromic CS in Norway.

5. INTRODUCTION

5.1 Anatomy and embryology of the cranium

The eight bones of the cranium form the vault that encloses the brain. They include the frontal, parietal (left and right), occipital, temporal (left and right), sphenoid and ethmoid bones (1) (Figure 1).

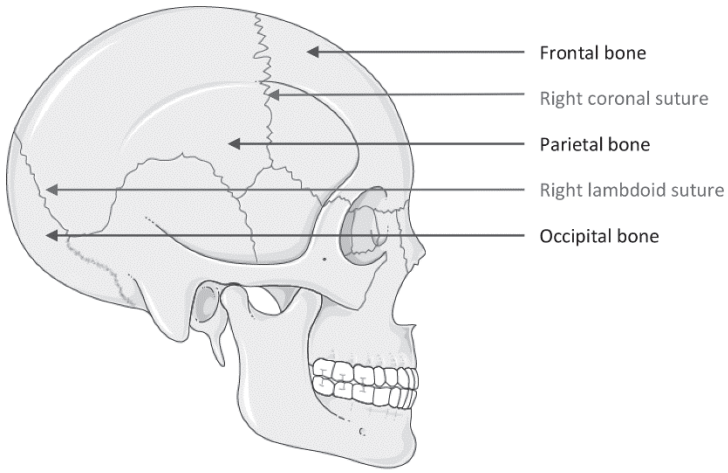

Figure 1. Illustration of a normally developed neurocranium seen from the right. The figure was created using Servier Medical Art images (smart.servier.com), free of use (Elin Tønne)

The bone plates are held together by highly elastic fibrous joints named cranial sutures (2). The main sutures are the metopic, sagittal, coronal (left and right) and lambdoid (left and right) sutures. Metopic and sagittal sutures may be referred to as midline sutures (Figure 2).

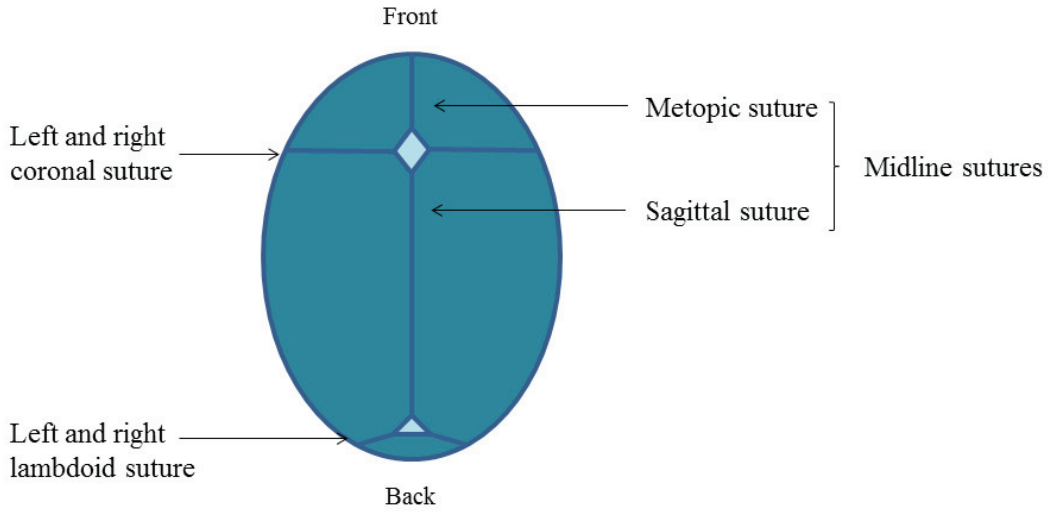

Figure 2. Illustration of the cranial sutures in a healthy newborn seen from above (Elin Tønne).

The sutures have two main tasks. They give flexibility to the scull, thereby enabling the safe passage of the head through the birth canal, and their elasticity secures the growing brain minimal resistance during the rapid growth that occurs during the first years of life. In addition, they act as growth sites, stimulating the growth of the bone plates, while the brain is expanding. The metopic suture closes normally by the age of 18 months, while the other sutures are usually retrievable throughout life (1).

The cranium is made from mesenchymal stem cells (MSCs) that migrate from the embryonic epithelium. These MSCs are pluripotent stem cells that have the ability to differentiate into osteoblasts, myoblasts, chondrocytes and adipocytes (2, 3). The cranial sutures consist of mesenchymal fibrous tissue, the underlining dura mater and pericranium, and the osteogenic bone fronts (2). The craniofacial mesenchymal tissues originate from both the neural crest and mesoderm. The neural crest gives rise to the frontal bone and mesoderm to the parietal and occipital bones (4-6). Metopic sutures are the only sutures that are solely derived from the neural crest. The osteogenic bone fronts act as growth centres and are comprised of proliferative cells expressing osteogenic markers, such as Runx2 (7). Signals from the underlining membranes (dura mater in particular) have been shown to play a part in the growth and patency of sutures, both promoting and inhibiting suture fusion (7, 8). In addition, mechanical strain results in the secretion of paracrine substances affecting suture patency (5). The factors regulating suture patency may be dependent on suture maturation and thus change with time (7). In response to signals, both from the bone fronts, suture mesenchyme and

surrounding tissue, a number of different receptors and pathways are activated and inhibited, all contributing to a fine-tuned interaction of growth in the scull and patency of the sutures. The central signalling pathways involved are the fibroblast growth factor (FGF), transforming growth factors β (TGFβ), wingless-type integration site (Wnt), Hedgehog (Hh), Eph/ephrin, bone morphogenetic protein (BMP) and their downstream targets (2, 9). These signalling pathways are again regulated by a number of genes. This intricate network of signalling pathways and interfering mechanisms involved in the growth of the cranium and maintenance of the sutures have not been completely elucidated.

5.2 Definition of craniosynostosis

Craniosynostosis (CS) is defined by the premature closure of one or more cranial sutures (1). CS often results in an abnormally shaped cranium if not surgically treated and, in some cases, elevated intracranial pressure due to the restricted intracranial volume secondary to the premature closure of the cranial sutures. In a few cases, this leads to high intracranial pressure (10, 11).

5.2.1 Classification of craniosynostosis

The classification of CS has evolved in recent decades. One of the first classifications of CS from 1949 was based on the affected suture and the associated head shape (12). The head shape is the result of the compensatory growth of the regions with open sutures. Synostosis of the sagittal suture often results in scaphocephaly and a long and narrow head shape with frontal bossing (Figure 3), while synostosis of the metopic suture usually results in trigonocephaly, with a triangular shape of the forehead. Synostosis of both coronal sutures often gives brachycephaly and a broad and short head shape, while unilateral coronal sutures may give an uneven head shape, as in plagiocephaly (Figure 4).

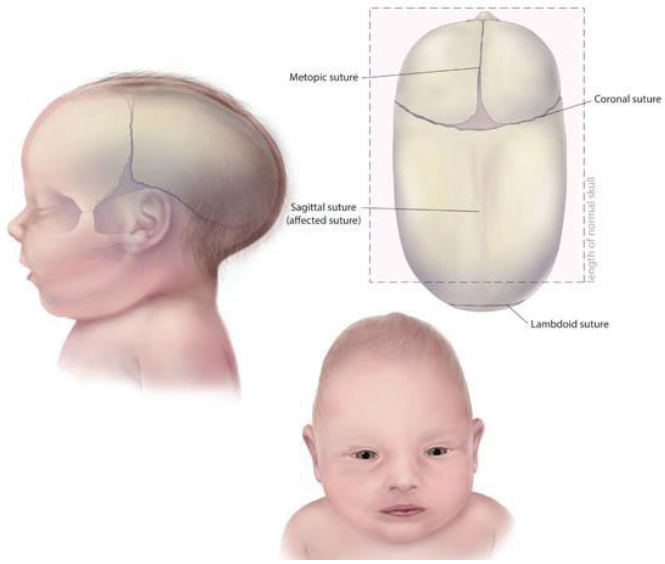

Figure 3. Illustration of a child with scaphocephaly caused by premature fusion of the sagittal suture. The illustration is free of copyright. The illustration was developed and used with permission from the Centers for Disease Control (CDC) and Prevention, National Center on Birth Defects and Developmental Disabilities, and they were notified of the use of the pictures as requested. The use of the illustration does not constitute endorsement by the CDC.

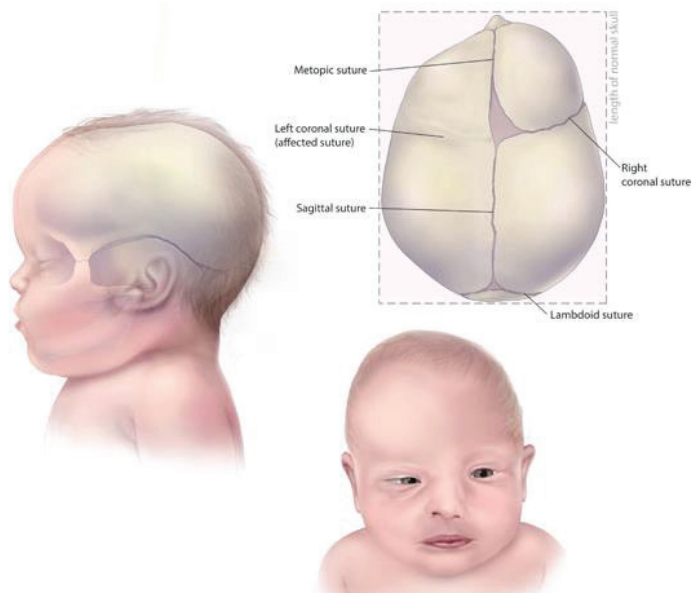

Figure 4. Illustration of a child with premature fusion of the left coronal suture. The illustration is free of copyright. The illustration was developed and used with permission from the Centers for Disease Control and Prevention (CDC), National Center on Birth Defects and Developmental Disabilities, and they were notified of the use of the pictures as requested. The use of the illustration does not constitute endorsement by the CDC.

Later, the clinical classification of the first CS syndromes, such as by Apert, Crouzon, Pfeiffer and Muenke, began (13). From the mid-1990s, the molecular causes of these CS syndromes were elucidated. Since then, a number of studies have defined syndromic CS by the existence of a confirmed genetic diagnosis, often limited to one of the frequently and first described

syndromes (14, 15). Some studies also classify syndromic and nonsyndromic CS based on the affected sutures, as the coronal and complex sutures are more often fused in the frequently reported CS syndromes (16-18).

Today, a combination of clinical criteria to classify CS is often applied based on general principles in syndromology (19, 20). A syndrome is usually defined by a pattern of multiple features, often from different organ systems, that tend to occur together and thus suspected to have the same aetiology. The word "syndrome" is derived from the Greek "run together" (21, 22). The definition thereby relates to the clinical presentation, and syndromic CS may be defined by the addition of other organ malformations and/or intellectual disability (19, 20). However, not all researchers or clinicians apply this definition. The different ways to classify syndromic and nonsyndromic CS, in the sense that some use multiple clinical criteria, others use the affected sutures and others again use molecular findings, or a combination of those, can make it difficult to compare research results.

The classification into syndromic and nonsyndromic is, however, important, as the two groups usually have different prognoses and different causes. Individuals with syndromic CS are often in need of more complex and often multiple craniofacial surgeries and have a higher risk of additional complications than nonsyndromic cases (23). Several syndromic CS cases, in particular those with multiple suture synostoses, develop associated findings or difficulties later in life, while this is uncommon for nonsyndromic cases. In addition, many syndromic CS cases have a genetic cause, while this is much less common in the nonsyndromic group (19, 20, 24).

5.3 Epidemiology of craniosynostosis

CS is one of the most common inborn malformations in children and has a reported incidence that varies between 3.1 and 7.2 per 10 000 live births (14, 16, 25-28). There are several reports indicating an increase in the incidence, particularly concerning nonsyndromic CS. There has not been a cause established for this increase (14, 16).

CS epidemiology is primarily based on reports from single or multiple centres or regions within a country, with a few exceptions of population-based studies (14, 26, 29-32). CS epidemiology has not previously been presented from any Scandinavian country.

The reported proportion of syndromic CS cases varies from 12 to 31% (25, 26, 33, 34). The different numbers may reflect the different definitions, as a restriction of syndromic CS to the

confirmation of a few preselected genetic diagnoses is likely to result in a lower number of syndromic CS than a broad clinical definition.

The sagittal suture is the overall most common fused suture (29, 30, 33, 35). The midline sutures (metopic and sagittal) are reported to be more often affected in nonsyndromic CS, while in syndromic CS, the coronal and complex sutures are the most commonly affected (16, 17).

There are far more males than females born with CS. The number reported is approximately 3:1, but some studies show an almost 5:1 male predominance (14, 26). The male predominance is related to the affected suture, as the midline sutures are more often fused in males. In contrast, the coronal sutures are most often fused in females.

CS is reported as one of the most common inborn anomalies from all continents. However, the number of reports differs, with a higher number of reports from Western countries, which might contribute to explaining some suggestions of differences in the incidence and affected sutures between countries (14, 25, 28, 36-38).

5.4 Aetiology of craniosynostosis

5.4.1 Environmental exposure and mechanical force during development

There is no single environmental exposure known to cause CS, but some risk factors have been described. These include maternal smoking in late pregnancy (39), drug exposure (e.g., nitrofurantoin, valproate) during pregnancy and hyperthyroidism (40-42). Twin pregnancies, high birthweight and macrocephaly have also been suggested as risk factors (25, 43). Mechanical strain on the sutures transmitted by the developing brain as well as applied extrinsic forces to the scull are known to cause cellular signalling changes within the suture, referred to as suture mechanobiology. Suture mechanobiology is proposed to contribute to CS (2, 44). As males are much more likely to develop CS than females, androgens have been suggested to be a contributing factor, but this has not been solidly confirmed (45).

5.4.2 Molecular genetics and monogenic causes

The human exome consists of approximately 20 000 different genes. Often, but not always, one gene makes the code for creation of a specific protein. The protein may have one or many biological functions. A gene consists of a stretch of a deoxyribonucleic acid (DNA). The DNA consists of nucleotides made up of a sugar molecule, a phosphate group and a nitrogen-containing base. There are four different bases: adenine (A), cytosine (C), guanine (G) and

thymine (T), and the order of the bases makes up the code (46). If the base order is disrupted in a damaging way (pathogenic variant), there will be changes in the protein, for instance, no protein (loss-of-function) or an alteration in protein function (e.g., gain-of-function) (47). The nucleotides are organized as a double helix that is thoroughly packed together to form a chromosome. The genes are distributed on 23 different chromosomes, numbered from 1 to 22, where the last one constitutes the sex chromosome (X/Y). Usually, humans have two of each chromosome. Sometimes small or larger parts of a chromosome are missing (deletion) or doubled (duplication), and in some cases, this happens to a whole chromosome. In other cases the structure of the chromosome is changed. Such chromosome aberrations may also disrupt protein(s) functions (47).

Since the first discovery in the 1990s of pathogenic variants in the genes Muscle segment homeobox drosophila homologue 2 (*MSX2*), Fibroblast growth factor receptor (*FGFR*) 1, 2, 3 and Twist homologue drosophila 1 (*TWIST1*) causing Craniosynostosis Boston type, Pfeiffer, Apert, Crouzon, Muenke and Saethre-Chotzen syndrome, respectively (48-51), the discovery of new monogenic causes of CS has exploded. This is essentially due to the introduction of the high-throughput sequencing (HTS) technique, enabling rapid investigation of a large number of genes. In 2015, 57 monogenic causes of CS were described, while in 2019, the number had increased to nearly 80 (5, 52) (Table 1).

Syndromic CS usually has a monogenic cause (19). The most common CS syndromes are Pfeiffer, Apert, Crouzon, Muenke, Saethre-Chotzen and craniofrontonasal dysplasia, caused by pathogenic variants in the *FGFR1, FGFR2, FGFR3, TWIST1* and Ephrin-B1 (*EFNB1*) genes, respectively (23). These syndromes often include multiple suture synostoses, moderate or severe dysmorphic features such as midface hypoplasia, proptosis and hyperthelorism, and in some cases intellectual disability. Some of the CS syndromes, such as those caused by pathogenic variants in *FGFR*s, present with CS in nearly all cases, while with other syndromes, usually rare syndromes, CS is present in only a small fraction of cases, suggesting different pathophysiologic mechanisms (5) (Table 1). Pathogenic variants in *FGFR*s result in a gain of function of the protein, while loss of function is the most common mechanism for pathogenic variants in the other genes associated with CS. This might be a contributing factor for the different frequencies of CS displayed in these syndromes (5, 7, 52).

Nonsyndromic CS is considered to be a multifactorial condition dependent on both environmental and genetic components (20). A small number of individuals with

nonsyndromic CS (5-10%) have a monogenic cause but with reduced penetrance. This seems to be connected to the affected sutures, as individuals with coronal (in particular bicoronal) or multiple synostosis more often are described to have a genetic cause than individuals with midline synostosis (23). Some of the most commonly reported genes associated with nonsyndromic CS are Transcription factor 12 (*TCF12*), SMAD family member 6 (*SMAD6*), *TWIST1* and Aristaless homeobox 4 (*ALX4*) (17, 20, 24, 53). The majority of the genes described to cause nonsyndromic CS are also described to cause syndromic CS (Table 1).

Table 1. Established genetic causes of CS by 2019 (Twigg et al. 2015 (5), Goos et al. 2019 (52))

Gene[a]	Syndromic CS	Nonsyndromic CS	Frequency of CS[b]
ADAMTSL4	Yes	-	Rare
ALPL	Yes	-	Rare
ALX4	Yes	Yes	Rare
ASXL1	Yes	-	Common
ATR	Yes	-	Rare
B3GAT3	Yes	-	Rare
BRAF	Yes	-	Rare
CD96	Yes	-	Rare
CDC45	Yes	-	Common
COLEC11	Yes	-	Common
CTSK	Yes	-	Rare
CYP26B1	Yes	-	Rare
DPH1	Yes	-	Rare
EFNA4	-	Yes	Rare
EFNB1	Yes	Yes	Common
ERF	Yes	Yes	Common
ESCO2	Yes	-	Rare
FAM20C	Yes	-	Rare
FBN1	Yes	-	Rare
FGF9	Yes	-	Rare
FGFR1	Yes	Yes	Common
FGFR2	Yes	Yes	Common
FGFR3	Yes	Yes	Common
FLNA	Yes	-	Rare
FREM1	Yes	Yes	Rare
FTO	Yes	-	Rare
GLI3	Yes	-	Rare
GNAS	Yes	-	Rare
GNPTAB	Yes	-	Rare

GPC3	Yes	-	Rare
HNRNPK	Yes	-	Rare
HUWE1	Yes	-	Rare
IDS	Yes	-	Rare
IDUA	Yes	-	Rare
IFT122	Yes	-	Rare
IFT140	Yes	-	Rare
IGF1R	-	Yes	Rare
IHH	Yes	-	Common
IL11RA	Yes	-	Common
IRX5	Yes	-	Rare
JAG1	Yes	-	Rare
KAT6A	Yes	-	Rare
KAT6B	Yes	-	Rare
KMT2D	Yes	-	Rare
KRAS	Yes	-	Rare
LMX1B	Yes	-	Rare
LRP5	Yes	-	Rare
MASP1	Yes	-	Rare
MEGF8	Yes	-	Common
MSX2	Yes	-	Common
NFIA	Yes	-	Rare
P4HB	Yes	-	Rare
PHEX	Yes	-	Rare
POR	Yes	-	Common
PTPN11	Yes	-	Rare
RAB23	Yes	-	Common
RECQL4	Yes	-	Rare
RSPRY	Yes	-	Rare
RUNX2	Yes	Yes	Common
SCARF2	Yes	-	Rare
SCN4A	Yes	-	Rare
SH3PXD2B	Yes	-	Rare
SKI	Yes	-	Common
SLC25A24	Yes	-	Rare
SMAD6	Yes	Yes	Rare
SMO	Yes	-	Rare
SOX6	Yes	-	Rare
SPECC1 L	Yes	-	Rare
STAT3	Yes	-	Rare
TCF12	Yes	Yes	Common
TGFBR1	Yes	-	Rare
TGFBR2	Yes	-	Rare

TMCO1	Yes	-	Rare
TWIST1	Yes	Yes	Common
WDR35	Yes	-	Common
ZEB2	Yes	-	Rare
ZIC1	Yes	-	Common
ZNF462	Yes	-	Rare

[a] Two or more cases with CS described (5, 52) [b]Common refers to the occurrence of CS in more than 50% of the cases (5) Rare refers to the occurrence of CS in fewer than 50% of the cases, or unknown

5.4.3 Chromosomal aberrations

Chromosomal aberrations are known to cause syndromic CS and are reported to account for approximately 14% of the genetic causes (23, 33). Both rearrangements, duplications and deletions have been reported (20, 23). There are some recurrent aberrations reported (e.g., 9p deletion), but often the chromosomal causes detected are diverse (23, 33). In some cases, the chromosome aberration include a deletion or disruption of a gene associated with CS (e.g., *FREM1*, *RUNX2* or *TWIST1) (23, 54)*. In other cases, the chromosome aberration do not include a candidate gene for CS, and the mechanism is less clear.

5.4.4 Epigenetics

Epigenetic mechanisms enable organisms to respond differently and adjust to environmental conditions. The definition of epigenetics has evolved since it was first presented in 1942 (55). One of the latest definitions, "the study of molecules and mechanisms that can perpetuate alternative gene activity states in the context of the same DNA sequence" (56), embodies the statement of both transgenerational and mitotic inheritance, in addition to stable gene activity or chromatin states over time. Phenotypes are thus considered the product of the interaction between DNA, epigenetic mechanisms and environmental factors (Figure 5) (56). In some disorders, especially monogenic disorders, a change in the DNA sequence is the main pathological mechanism, epigenetic systems and the environment play a smaller role. Some of the *FGFR*-related syndromes would be an example, as almost every individual with a certain variant develops the associated syndrome. For other disorders, such as cancer, the environment and epigenetic mechanisms are known to play a large role (56). However, many of the monogenic conditions (including *FGFR*-related syndromes) have different expression and severity, and epigenetic mechanisms and environments are likely to contribute to this variability.

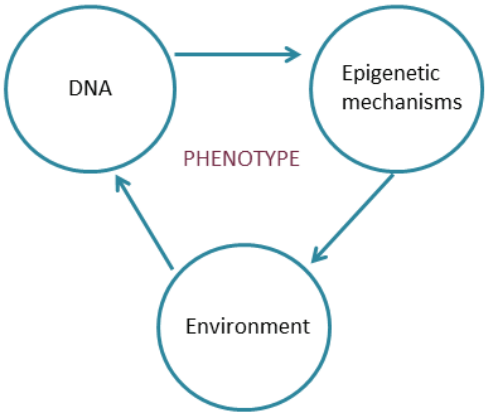

Figure 5. The creation of a specific phenotype often depends on epigenetic mechanisms and environmental factors in addition to specific DNA sequence(s) (Elin Tønne).

5.5 Central signalling pathways and pathophysiological mechanisms of craniosynostosis

5.5.1 *FGFR1, FGFR2* and *FGFR3*

Fibroblast growth factors (FGFs) are positive regulators of osteogenic differentiation and function by activating different FGF receptors (FGFRs) (40). Activating pathogenic variants in *FGFR1*, *FGFR2*, and *FGFR3* cause CS and are associated with the most frequent CS syndromes: Pfeiffer, Apert, Crouzon and Muenke syndrome, respectively. FGFRs consist of three extracellular immunoglobulin-like domains, a single-pass transmembrane domain and an intracellular tyrosine kinase domain (57). *FGFR1-3* uses alternative splicing of two exons to encode the C-terminal half of the third domain, resulting in two isoforms (IIIb and IIIc). The isoforms show different affinities for ligands and localization, as isoform IIIb is mainly expressed in the epithelium and isoform IIIc is expressed in the mesenchyme (57-59). The majority of pathogenic variants reported are missense or splice variants, and a few in-frame deletions and insertions have been described. The variants mainly affect the exons encoding the IgIII domain or the linker region between IgII and IgIII, but some variants in IgI and IgII have also been reported (57). Fibroblast growth factors (FGFs) facilitate cranial growth by activating FGFR tyrosine kinases and through several steps and downstream cross-signalling pathways, including the Ras/MAPK/Erk, protein kinase C (PKC), TGFβ, Eph/ephrin and P13k/Akt pathways, thus stimulating osteoblast proliferation and differentiation (9, 57, 60-63)

(Figure 6). Different pathological variants have different activating effects, such as greater affinity for FGF proteins, loss of FGF-binding specificity, ectopic FGFR splice form expression or enhanced activation of FGFR (57). The pathological variants in the *FGFR1-3* genes cause different syndromes. Interestingly, several of the variants in these three genes have analogous positions: p.Pro252Arg in *FGFR1*, p.Pro253Arg in *FGFR2* and p.Pro250Arg in *FGFR3*, causing Pfeiffer, Apert and Muenke syndrome, respectively (57). For the majority of cases, there is a genotype-phenotype correlation between the variant in the *FGFR* gene and the respective syndrome. For instance, specific variants in the *FGFR2* gene cause Crouzon syndrome (e.g., p. Phe267) and Pfeiffer syndrome (e.g., Trp290), and a few specific variants located in the linker region between IgIII and the transmembrane domain have been reported in Beare-Stevenson cutis gyrata syndrome (64). In *FGFR3*, specific variants are known to cause Muenke syndrome, and a recurrent pathogenic variant in the transmembrane region (p. Ala391Glu) causes Crouzon syndrome with acanthosis nigricans (65). There are, however, exceptions of this genotype-phenotype correlation, and for some pathogenic variants in *FGFR*s, individuals are diagnosed with completely different syndromes, even within the same family (66). These syndromes also demonstrate a variable phenotype within the described spectrum of the syndrome, from mild to severe, not always dependent on the specific pathogenic variant. The complexity of the signalling system and downstream targets, dependent on several factors, might explain some of this variability.

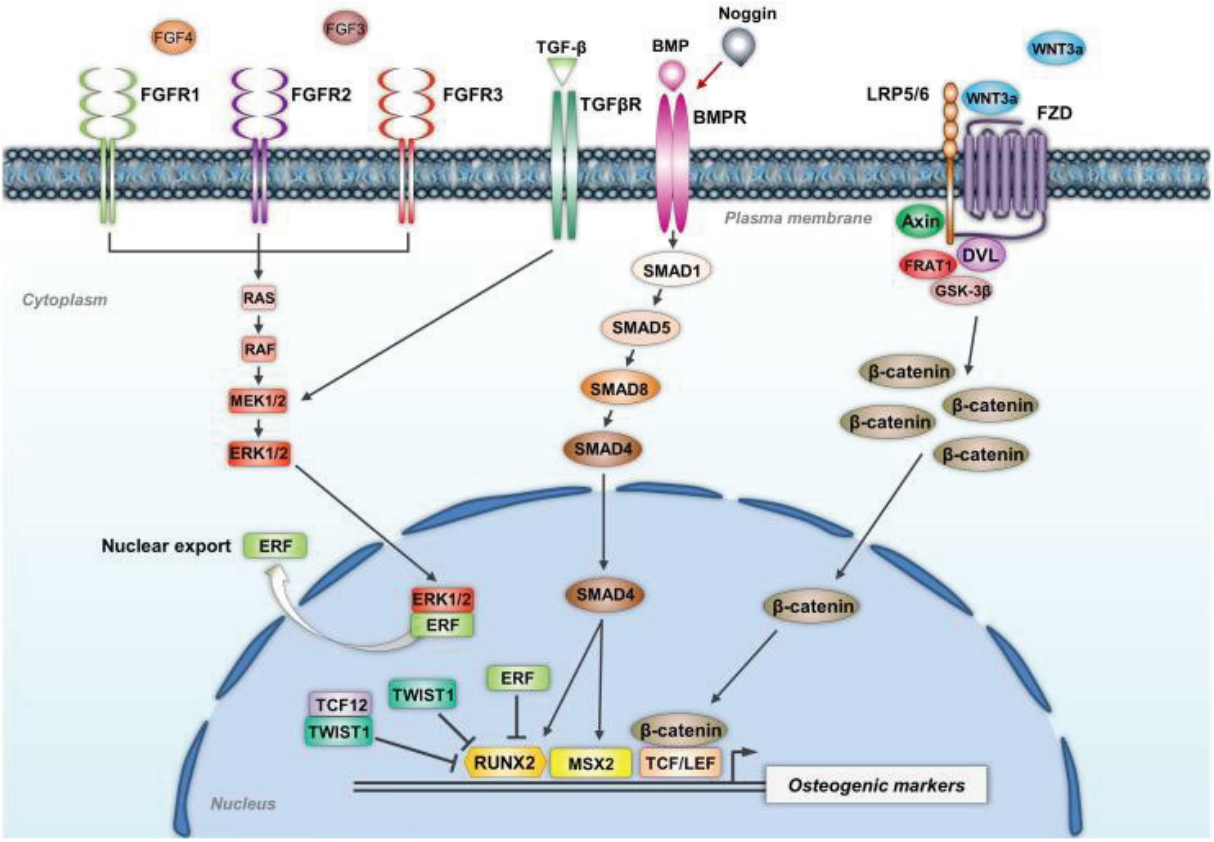

Figure 6. Central signal transduction pathways in cranial development. The figure is extracted from Katsianou et al, Signalling mechanisms implicated in cranial sutures*, BBA Clin 2016 with permission. BBA Clin 2016: https://dx.doi.org/10.1016%2Fj.bbacli.2016.04.006 in accordance with the terms of the Creative Commons user licence: http://creativecommons.org/licenses/by-nc-nd/4.0/ (for noncommercial use).*

5.5.2 *TWIST1, RUNX2* and *MSX2*

The transcription factor Runx2, encoded by Runt-related transcription factor 2 (*RUNX2),* is essential for bone formation in a dose-dependent manner and is regulated by a number of different pathways, such as the Ras/MAPK/Erk pathway, and directly by the protein Twist1, encoded by *TWIST1* (9). The Twist1 protein is a basic helix-loop-helix transcription factor consisting of a DNA binding domain and a bHLH motif of an alpha helix connected by a loop to a second helix. The loop is essential for the protein structure and for the function of the two alpha helixes. The majority of the pathogenic variants in *TWIST1* are localized in the bHLH motif-encoding region (67). In the coronal suture, Twist1 binds to Runx2, acting as a negative regulator of osteogenic differentiation and thus inhibiting bone formation (40, 68). Studies have also implied a synergetic effect of *TCF12* and *TWIST1* in the coronal suture (69) (Figure 6). In contrast to the activating variants in *FGFRs*, heterozygous loss-of-function is the mechanism for variants in the *TWIST1* gene, known to cause Saethre-Chotzen syndrome, presumably due to increased osteogenesis (70). However, a few cases of Saethre-Chotzen

syndrome caused by missense variants in the *FGFR2* or *FGFR3* genes have been reported, indicating that loss-of function variants in *TWIST1* might give a similar phenotype as activation of FGFR signalling (7, 57). The severity of Saethre-Chotzen syndrome varies greatly between affected individuals, and there is no clear genotype-phenotype association for specific variants in *TWIST1*, with the exception of intellectual disability being mostly associated with large deletions (71). Another protein, Msx2, indirectly controls Runx2 by stimulating Runx2 and thus bone formation (72). The transcription of *MSX2* is mainly controlled by BMP and TGF-β (2). Interestingly, studies have shown that gain-of-function variants in *MSX2* result in CS (craniosynostosis, Boston type), while loss-of-function variants have the opposite effect and result in enlarged foramina (73).

5.5.3 *EFNB1*

Ephrin receptors are one of the largest receptor tyrosine kinase (RTK) families and are important for cell migration in addition to establishing tissue segregation and cell boundaries during embryonic development (74). The *EFNB1* gene encodes ephrin-B1, which is a transmembrane ligand for ephrin RTK, and pathogenic variants are known to cause craniofrontonasal dysplasia. The mechanism for CS is hypothesized to be caused by disruption of the establishment of cellular compartments during suture formation (75). Females are more severely affected than males. The *EFNB1* gene is situated on the X chromosome, of which females have two, while men only have one. Loss-of-function variants in the *EFNB1* gene are presumed to cause craniofrontonasal dysplasia through a phenomenon named cellular interference. It seems that cells with different expression of *EFNB1* generate abnormal sorting of cells and ectopic tissue boundaries during development. Random X inactivation is assumed to cause the severe phenotype in females in the sense that some cells express ephrin-B1, while others do not. Males with pathological variants in *EFNB1* express no ephrin-B1 and thus avoid cellular interference (76-78). A few males being mosaics for *EFNB1* variants have been reported, further strengthening the pathophysiology of cellular interference (76).

5.5.4 TGF-β pathway

TGF-β presents in three different forms, TGF-β1, 2 and 3, and is essential in controlling growth, differentiation and apoptosis (79, 80). Studies have demonstrated that TGF-β is expressed in cranial sutures and more specifically indicated that TGF-β2 induces suture fusion, while TGF-β3 ensures patency (81). There have been no reported pathological variants

in the genes encoding TGFs associated with CS, but pathogenic variants in two of their receptors, *TFGBR1* and *TGFBR2*, have been reported (Table 1), thus indicating a role in suture patency (40, 82). In addition, studies have shown that TGFs interact with downstream ERK1/2 and FGF pathways (Figure 6) (83).

5.5.5 BMP pathway

Bone morphogenic proteins (BMPs) are a group of growth factors involved in a number of processes and conditions, such as cancer and vascular diseases, in addition to skeletal conditions. BMPs are known to be important for osteogenesis and implicated in suture patency and closure. BMPs act by binding to transmembrane bone morphogenic protein receptors (BMPRs), thus initiating phosphorylation of downstream targets (Smad1, Smad5 and Smad8 proteins) that interact with Smad4 and translocate into the nucleus, stimulating the expression of targeted genes (2, 40). BMP signalling is regulated by intra- and extracellular modifiers; extracellular modifiers include noggin, which is a BMP antagonist essential for suture patency and has been demonstrated to be suppressed by *FGFR2* and syndromic fgfr-associated CS (84). The *SMAD6* gene is known to act as an inhibitor of TGFβ/BMP signalling. Pathogenic variants in *SMAD6* have been reported in a number of nonsyndromic cases with incomplete penetrance (53). The mechanism is demonstrated to be haploinsufficiency, and recently, *SMAD6* has also been reported to be involved in syndromic CS (24). Increased BMP signalling is thus likely to be one of the mechanisms involved in the occurrence of CS.

5.5.6 Wnt pathway

Wnt signalling pathways are essential in embryological development, as they are involved in cell proliferation, differentiation and migration. The Wnt signalling pathway also ensures the pattering of cranial neural cells (85). In osteogenesis, it promotes the differentiation of precursor cells and ensures them to the osteoblast lineage. There are three different Wnt signalling pathways, and Wnt/β-catenin (canonical Wnt pathway) is known to be involved in the specification of intramembranous endochondral ossification (86, 87). This pathway is activated by a Wnt-protein ligand binding to a Frizzled receptor (Frp) that releases β-catenin into the cytoplasm, where the accumulated β-catenin translocates into the nucleus and stimulates the expression of its target genes, such as *TWIST1/2* (88, 89). Deletion of β-catenin has been shown to result in a reduction in osteogenic factors, such as *RUNX2* (2, 90). The two other Wnt pathways are also assumed to interact and contribute to regulating cranial growth and suture patency (40). Pathogenic variants in several genes proposed to downregulate the

Wnt canonical pathway have been demonstrated in CS patients (40, 53, 91, 92). There has also been a connection to FGFR, as Cetinkaya et al. (92) demonstrated different expression of genes from the Wnt pathway (*FRZB, SFRP2* and *WNT2*) in fibroblasts from individuals with Apert syndrome compared to healthy controls, supporting their contribution to CS. In addition, the interaction between FGF and Wnt signalling is known to be important for normal limb development (93). However, further knowledge on how the Wnt pathway interacts and contributes to CS is needed.

5.5.7 Hedgehog pathways

The Hedgehog signalling pathways are important for mesodermal cell differentiation and tissue patterning during craniofacial development. There are three different HH protein ligands, two of which are expressed in the craniofacial complex (Indian hedgehog (IHH) and Sonic hedgehog (SHH)). These ligands are essential for the formation and fusion of cranial sutures and calvarial ossification (94). Pathogenic variants in *GLI3*, a downstream target of HH, are known to cause CS (95). Hedgehog proteins are also closely related to primary cilia, as they are crucial to HH signalling. Pathogenic variants in *IFT140* and *IFT122* disrupt the transport function of primary cilia, thus causing abnormal skeletal development, such as CS (40).

5.5.8 Mendelian disorders of chromatin modification (Chromatinopathies)

Chromatinopathies, also named Mendelian disorders of chromatin modification, are a heterogeneous group of conditions, and the mechanism is thought to be a direct disruption of the epigenetic machinery. The disruption may be done by targeting the DNA through aberrant methylation or by targeting the associated histone proteins in chromatin. Enzymes acting as writers, readers, remodellers or erasers may all be victims of this disruption (96, 97). Each of the chromatinopathies seems to harbour a greater variability of clinical features than most Mendelian disorders (97). Loss of function is often the mechanism, and it is suggested that dosage sensitivity for epigenetic components in specific cell populations at specific times in early development might explain the variability in clinical presentation (97, 98). CS has been reported in seven of the 44 chromationopathies described today, including Kabuki syndrome, Koolen-de-Vries/KANSL1 haploinsufficiency syndrome, Bohring-Opitz syndrome, KAT6B-related disorders, Charge syndrome, Floating-Harbour syndrome and Kleefstra syndrome. (96, 97, 99, 100).

5.6 Diagnostic workup

5.6.1 Clinical assessment

CS is primarily a clinical diagnosis diagnosed after an assessment of the scull shape, craniometric measurements and sutures by a neurosurgeon (101). It is usually diagnosed during the first year of life. Often, a prominent ridge over the prematurely fused sutures is present, and an abnormal head shape in concordance with the affected suture can be observed (Figures 3 and 4). Evaluation of whether an associated high intracranial pressure (ICP) is present is important and includes an ophthalmological examination and, in a few cases, invasive ICP measurement.

To classify CS into syndromic and nonsyndromic, a multidisciplinary assessment is necessary, including a clinical geneticist. The assessment includes a detailed history and clinical examination looking for additional anomalies, dysmorphic features or developmental delays. If abnormalities in other organs are suspected, referral to other specialists, for instance, a cardiologist or a paediatrician, might be needed, as well as additional imaging. In some children, additional findings or difficulties are revealed later in life. In those cases, a reassessment is performed, sometimes resulting in a reclassification from nonsyndromic to syndromic CS.

5.6.2 Imaging

In some CS cases, confirmation of synostosis by imaging is needed. Skull X-ray will provide some information; however, the preferred imaging for CS today is cerebral computed tomography (CT), which can be reconstructed in 2- and 3-D (101). CT exposes the child to ionizing radiation and is only used when needed, as the growing brain is sensitive to ionizing radiation (101, 102). For the evaluation of associated intracranial anomalies, MRI is used.

Imaging of other organs is in some cases recommended. This may include ultrasound of the heart or abdomen or radiology of the total skeleton if abnormalities in these organs are suspected or if a confirmed genetic diagnosis implies a high risk of disease or abnormalities in that specific organ.

5.6.3 Molecular diagnostics

The molecular diagnostics of CS have evolved in recent decades due to the enormous technological advances in DNA sequencing. Today, there are several different techniques available.

In Sanger sequencing, the DNA sequence of interest is used as a template for a specific polymerase chain reaction (PCR), named chain termination. The sequence is read by adding modified chain-terminating nucleotides in addition to normal nucleotides before facilitating transcription. Each of the four modified nucleotides has a unique fluorescent label for visualization. This technique is accurate but time-consuming and allows investigation of only one gene at a time (47).

High-throughput sequencing (HTS), also named next-generation sequencing or massively parallel sequencing, has changed molecular diagnostics by enabling the investigation of a high number of genes simultaneously. This technique fragments the target DNA into small pieces/sequences. Each sequence is read several times, some a few and others many, resulting in a variable degree of coverage. HTS is less time-consuming and much more efficient than Sanger sequencing, however also more prone to error, in which is overcome by reading each sequence many times (high coverage) (103).

HTS can be used to investigate a preselected number of genes (gene panel) known to be associated with the clinical findings in question. The gene panel may contain a small, or a high, number of genes. This is often an efficient method to detect a genetic cause. The disadvantage of gene panels is that it is difficult to obtain up-to-date panels, and hence some genetic causes may not be detected. The exome refers to the protein-coding regions of the genome. By investigating the entire exome (whole exome sequencing - WES), the risk of missing a genetic cause is reduced, however deep intronic variants and small deletions/insertions/duplications that affect protein function, might not be detected. Whole genome sequencing (WGS) thus enables the detection of even more genetic causes. However, WES/WGS will increase the likelihood of detecting variants of uncertain significance and the risk of incidental findings. HTS often result in a large number of variants, for instance WES might detect 100 000 different variants. Thus, a high quality downstream filtering and analysis is required in order to single out the variant(s) of clinical interest (104).

In cases with healthy parents and a single affected child, large panel analyses or WES/WGS is often applied to both parents and the child simultaneously (trio analysis), in order to detect variants that are only present in the affected child. This approach increases the power to detect *de novo* variants, and also facilitates the detection of autosomal recessive inherited variants.

Array comparative genomic hybridization (aCGH) is a molecular method used for the identification of chromosome aberrations or copy number variations (CNVs). This method

compares isolated DNA from a test and a reference sample using different fluorescent molecules (red/green) after competitive hybridization on a microarray. Small deletions or duplications will, however, not be visualized by this method (47). For this, a multiplex ligation-dependent probe amplification (MLPA) analysis might be useful.

A common weakness of molecular diagnostics is that not all variants or copy number variations are previously described and thereby difficult to interpret. The standards and guidelines for the interpretation of sequence variants (ACMG guidelines) seek to standardize and avoid differences in interpretation (105). The variants are classified into five categories: benign or likely benign (classes 1 and 2, respectively), pathological and likely pathological (classes 5 and 4, respectively), and variants of uncertain significance (VUS/class 3). Despite these guidelines, variants are often classified differently (106).

When suspecting syndromic CS, some diagnostic laboratories investigate a few of the most frequent genes associated with CS by Sanger sequencing, and some add aCGH. Other laboratories perform custom designed gene panel analyses, investigating preselected genes previously known to cause CS. This might not capture all of the genetic causes, especially when investigating only a small number of genes.

5.6.4 Multidisciplinary team

Diagnostics, treatment and follow-up of children with CS are recommended to be centralized into specialized units consisting of a multidisciplinary team (19, 102). This is especially useful for children with syndromic CS, as they often have multiple organ anomalies and are in need of treatment and follow-up by several specialists (107). A multidisciplinary team should preferably include neurosurgeons, plastic-reconstructive surgeons, ophthalmologists, ear-nose-throat specialists, geneticists, radiologists, specialized dentists, orthodontists and advisers on rare disorders. A multidisciplinary team enables standardized and coordinated treatment and follow-up, fewer appointments and ensures high-quality care (108). Specialized units also ensure highly experienced clinicians, which is particularly useful concerning rare and ultrarare conditions.

5.7 Treatment

CS is surgically treated to avoid cranial deformity, or worse, an elevated intracranial pressure due to a compromised intracranial volume. CS that is not surgically treated may lead to cranial or facial anomalies. If CS leads to raised intracranial pressure, it may cause neurological dysfunction, such as difficulty breathing or impaired vision (10, 109-112). Early

detection is of the utmost importance, as early surgery in many cases can relive restricted intracranial volume and thereby constrain the early fused suture. In some cases, early surgery is not needed, and the child is treated conservatively until intervention is indicated. There are several operative techniques required to surgically treat CS that are tailored depending on age and symptoms. One is open craniotomy with reconstruction, and another is endoscopy-assisted CS surgery (EACS) with the use of postoperative remodelling helmet- or spring-assisted techniques. The different methods have advantages and disadvantages.

Open craniotomy is a time-consuming procedure that involves keeping the child under anaesthesia for a long period of time and is often associated with significant blood loss. EACS is less invasive and involves less blood loss and anaesthesia for a shorter period of time (113). There is a shift from time-consuming open cranioplastics towards more minimally invasive procedures, and EACS is in most cases the preferred first choice of treatment (114). However, the timeframe for when EACS may be performed is shorter than that for open craniotomy. EACS is preferably performed within the age of 6 months, as the cranial bones are more flexible and easier to manipulate, and open craniotomy is performed after the age of 6 months (115). The need for repeat craniofacial surgeries due to progressive disturbances in facial growth is much higher in syndromic CS than in nonsyndromic CS (23, 116). Children with syndromic CS therefore need to be closely monitored.

Surgical treatment of CS is beyond the scope of this thesis, thus providing a brief description of treatment.

5.8 Genetic counselling

Genetic counselling is performed before, after, and sometimes during genetic testing. Before genetic testing, genetic counselling seeks to provide enough information for the individual and family to make an informed choice on whether to perform genetic testing or not. It is also important to prepare the family for the finding of a potential genetic cause.
After the genetic test, counselling seeks to inform the implications of the result for both the affected individual and the family, in addition to providing support (117). Many CS syndromes are caused by dominant, *de novo* variants. In these cases, the recurrence risk is low, since the parents do not have the variant. However, in some cases, one of the parents has the same variant in a low-grade mosaic state that the analysis could not detect (118). In those cases, the recurrence risk for siblings may be high. In regard to the affected child (when becoming an adult), there will be a 50% change in their children inheriting the variant. In CS

syndromes with autosomal recessive inheritance, both parents are usually healthy carriers of a pathogenic variant, and the affected child inherits the variants from both parents. In these cases, the recurrence risk for a sibling to inherit both variants is 25%. CS syndromes caused by genes located on the X chromosome follow X-linked inheritance. In those cases, the mother is usually a healthy carrier, and the children have a 50% risk of inheriting the variant. Usually, only boys become affected, but exceptions do occur, for instance, in regard to craniofrontonasal dysplasia (77). The different genes associated with syndromic CS do in some cases show different degrees of penetrance and expression. These different mechanisms of inheritance and penetrance thus affect the recurrence risk in the family.

5.9 Ethical considerations

There are many advantages of detecting a specific genetic diagnosis. Often, the diagnosis determines the cause, and other possible causes the family may have considered (e.g., alcohol or other events during early pregnancy) may be ruled out. The fact that the genetic cause happened by coincidence and that the parents could not have prevented it might give relief. In addition, the exact genetic cause usually determines the mode of inheritance in the family and thereby can be used to predict recurrence risk. This also enables the parents, and later on the affected individual, to apply for prenatal diagnosis in the next pregnancy if they wish to.

The exact genetic cause may in many cases predict the prognosis and possible later onset diseases. For some syndromes, epilepsy or difficulties such as reduced hearing appear later in childhood or in life. For some syndromes, repeat craniofacial surgeries are common, and they need to be especially closely monitored (23, 116). A genetic diagnosis also enables support from patient organizations and affects social care.

In some cases, in particular after the development of HTS technology, a genetic diagnosis is detected at a very early age and before the family has come to an acceptance of their child having a disorder. In those cases, the detection of a genetic syndrome might cause distress, as the severity of their child’s condition has not yet been elucidated. This is especially relevant to ultrarare conditions, where there is sparse knowledge about the whole spectrum of the condition, and often the most severely affected individuals are the ones that are described. Most genetic disorders have different expressivity, and some have reduced penetrance, which may also cause confusion and insecurity. When a child has a condition with variable expressivity and is mildly affected, the diagnosis may be experienced as a disadvantage for the child as the surroundings expect less than the child`s actual potential.

To avoid unnecessary distress, it is therefore important that the family receives genetic guidance before and after genetic testing to be prepared for what might be found and to understand the implications (117).

6. THESIS AIMS

1. Describe the epidemiology of CS in Norway.

2. Detect and describe the genetic causes within a proposed clinical definition of syndromic CS, including novel genetic causes.

7. MATERIALS AND METHODS

7.1 Study population

In 2019, the number of inhabitants in Norway was 5.3 million, and the number of live births was 54 495 (119).

All children with CS in Norway were referred to the Norwegian National Unit for Craniofacial Surgery at Oslo University Hospital for diagnosis, treatment and follow-up. The children are registered in the Unit`s prospective quality register, and those consenting to research (85%) are registered with supplemental information.

7.2 Participants

The study was a population-based study, including all consenting children with CS born from 2002 to 2019 (Figure 7).

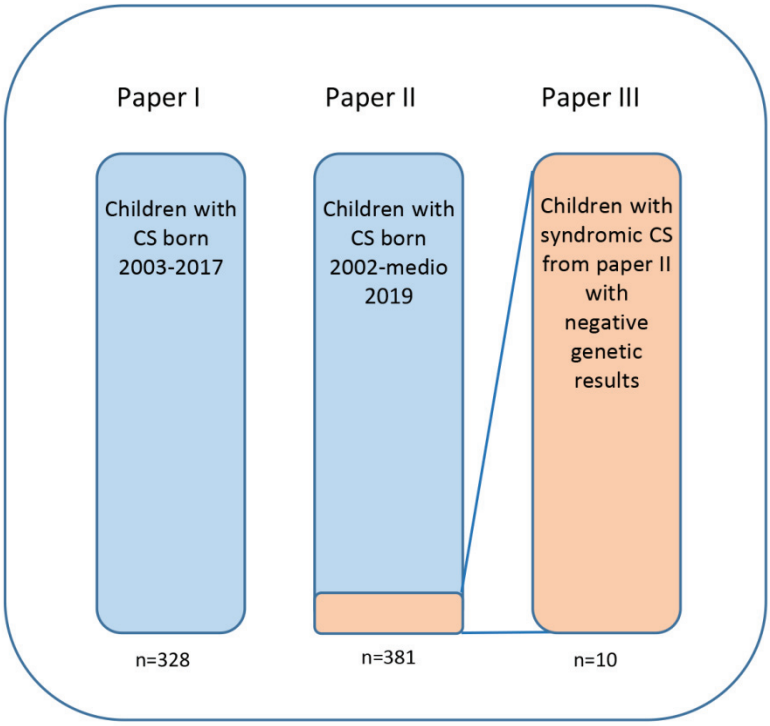

Figure 7. Included individuals in papers I, II and III (Elin Tønne).

7.3 Data collection

The Norwegian National Unit for Craniofacial Surgery is a multidisciplinary team consisting of neurosurgeons, plastic reconstructive surgeons, maxillo-facial surgeons, ophthalmologists, geneticists, radiologists, ear-nose-throat specialists, dentists, orthodontists, nurses and advisors on rare disorders. The data were collected prospectively from all children referred to the National Unit for Craniofacial Surgery and confirmed to have CS.

All children were assessed by an experienced neurosurgeon for the confirmation of CS, and supplemental imaging to confirm the diagnosis was performed if needed. If additional abnormalities or difficulties were suspected, the children were referred to one of the Units' geneticists specializing in craniofacial conditions (Ketil Heimdal or Elin Tønne). All children suspected to have syndromic CS were followed regularly with appointments on the multidisciplinary team. This is the standard of care for all children with CS in Norway.

In the epidemiological study (Paper I), all data were collected prospectively. For the last two studies (Papers II and III), the extended genetic analyses performed in the individuals without a verified genetic diagnosis were collected retrospectively.

7.4 Data categorization

The children were categorized by sex, age at diagnosis, affected sutures or whether there were affected family members (first- or second-degree relatives).

7.4.1 Syndromic and nonsyndromic craniosynostosis

All individuals were classified as syndromic or nonsyndromic CS using a combination of clinical criteria defined by MD Ketil R. Heimdal and MD Elin Tønne, inspired by general principles in syndromology (21, 22). The classification consisted of minor and major criteria, where at least one major or two minor criteria were needed for the classification into syndromic CS (Figure 8).

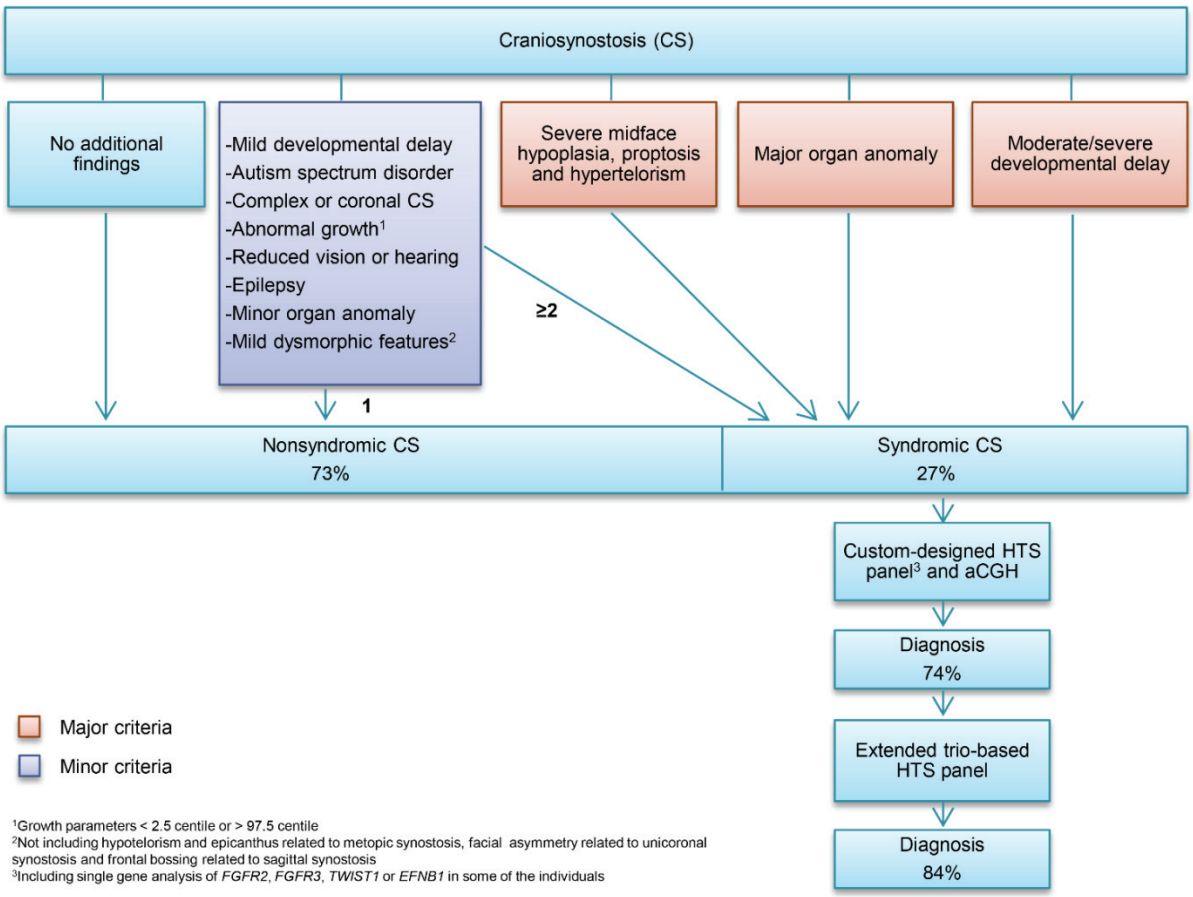

Figure 8. Flowchart of the clinical classification of syndromic and nonsyndromic CS. Extracted from Tønne et al, 2020 (120). Reproduced with permission from Springer Nature. Creative common licence Creative Commons — Attribution 4.0 International — CC BY 4.0

7.5 Genetic analyses

The genetic analyses described in Paper I were offered as part of standard diagnostic care. In regard to Papers II and III, the analyses performed were not part of the standard diagnostics provided at that time. The ACMG guidelines were used to classify all variants detected in Papers I, II and III (105).

7.5.1 Paper I

Single-gene analysis of *FGFR2*, *FGFR3*, *TWIST1* and *EFNB1* by Sanger sequencing and MLPA (*TWIST1*) in addition to aCGH were routinely offered to individuals with syndromic CS until 2016. After 2016, the single gene analyses were replaced by a custom-designed HTS panel consisting of 72 genes associated with CS (Appendix 1). All individuals with syndromic CS born before 2016 were seen regularly by the Unit and offered the HTS panel in addition to the last version of the aCGH (180k) as part of the standard diagnostic care. Of the 89 individuals with syndromic CS in this cohort, 80 accepted routine genetic testing by the custom-designed HTS panel and aCGH (180k).

7.5.2 Paper II

Individuals with syndromic CS who did not receive a genetic diagnosis after the analysis performed in Paper I (custom-designed HTS panel and aCGH) were offered HTS of an extended trio-based panel consisting of 1570 genes associated with inborn anomalies and intellectual disability, informed by the Deciphering Developmental Delay study (DDG2P) (121) (Appendix 2). Of the 104 individuals with syndromic CS in this cohort, a total of 94 individuals accepted the first step of routine genetic testing by the custom-designed HTS panel and aCGH (180k). Of the patients who did not receive a diagnosis by these methods (n=25), 22 accepted the extended trio-based HTS panel. The analysis was performed in a diagnostic laboratory.

7.5.3 Paper III

Of the individuals in Paper II investigated by the extended trio-based HTS panel, 15 did not receive a genetic diagnosis. Of those, 10 accepted reanalysis of the sequence data using our research pipeline. We performed WES, analysing close to the entire exome (18 500 genes), including predicted splice sites. Downstream filtering and analysis were performed using Filtus (104). We searched for rare *de novo*, autosomal recessive and X-linked variants. In one child, the mother also had syndromic CS. In this family, we searched for autosomal dominantly inherited variants. As a result of the large number of inherited variants, only transmitted loss-of function variants with a Combined Annotation Dependent Depletion

(CADD) score of 20 or more were filtered in this specific family. A CADD of 20 or more means that the variant is predicted to be among the 1% most damaging variants in the human genome (122). The variants detected were investigated by a literature search in regard to the respective gene biological functions, including acting and interacting pathways, and their association with normal development and disease. All variants discussed in Paper III were confirmed by Sanger sequencing.

7.6 Statistics

IBM SPSS Statistics version 25 (IBM Corp.) were used to perform the statistical analyses in Paper I. The level of statistical significance was set at $p \leq 0.05$. Chi-square values for the categorical variables were calculated using The OpenEpi Collection of Epidemiological Calculators (123). Mean and median values were used to present continuous variables and absolute and relative frequencies to present categorical variables. To compare categorical variables, the chi-square test was used. To compare the mean (age at diagnosis), one-way ANOVA, including a Tukey post hoc test, was used.

There were no statistical analyses performed in Papers II and III, except for the presentation of absolute and relative frequency (%) of syndromic CS in Paper II.

7.7 Ethics

Ethical approval was obtained from the Regional Committee for Medical Research Ethics (REK_2018/797) and by Oslo University Hospital (permit number P360:18/06246 and 18/24246/(05374)).

All included individuals were children (below the age of 18 years). Consent forms were thereby signed by their parents or legal guardians. It was emphasized that participation was voluntary, without consequence for treatment or follow-up, and that withdrawal was permitted at any time during the study. Contact information of the researchers was included on the consent forms. Photos were not included in any of the papers due to the risk of recognition and the fact that the children were unable to consent themselves.

The parents or legal guardians of all included individuals in Papers I, II and III signed the Norwegian National Unit for Craniofacial Surgery research consent form prior to inclusion. All genetic testing was performed in a clinical setting for diagnostic purposes. The genetic analyses presented in Paper I were a part of the routinely offered diagnostics by the Unit.

7.7.1. Paper II

In Paper II, several rare and ultrarare genetic causes were described. For individuals with these conditions, a second consent form was obtained. This consent form emphasized that with fewer than five individuals harbouring a condition, there was a low probability of identification. The parents or legal guardians of all included individuals with a genetic diagnosis involving fewer than five individuals in the study signed the consent form prior to publication, in addition to the Unit`s consent form obtained prior to inclusion. Some of the children were deceased. It was a difficult ethical dilemma whether to include those. After careful considerations and discussion with one of the patient research partners, we decided to contact the parents, with only positive responses.

The individuals who did not receive a genetic diagnosis after the routinely offered genetic analysis described in Paper I were offered the extended trio-based HTS analysis. All of these families received genetic counselling before and after the analysis and signed the second consent form prior to the analysis. The second consent form was essentially obtained by Elin Tønne and not by the neurosurgeon in charge of treatment or follow-up to prevent the families from feeling obliged to participate.

7.7.2. Paper III

In Paper III, the individuals who did not receive a genetic diagnosis from the analyses performed in Paper II were offered a new analysis including the entire exome. As this analysis included a risk of detecting incidental findings, all families (n=10) received a third genetic counselling (by MD Elin Tønne), and both parents signed a third consent form (obtained by ET), in addition to the other two, prior to the analysis, that included information about incidental findings. The families also received genetic guidance after the analysis and were encouraged to reach out if they had questions during the analysis. To make the probability of incidental findings less likely, some genes associated with severe late onset disease (e.g., *BRCA1/2*) were excluded from the analysis. In addition, we decided and informed prior to the analysis that incidental findings would be reported only if treatment or preventive actions were available.

8. RESULTS

In Paper I, the epidemiology of CS in Norway was presented, including the number of verified genetic diagnoses by routinely offered diagnostics. In Papers II and III, the genetic causes of syndromic CS in Norway were described after extended genetic testing, including novel genetic causes of CS (Table 2). The relative frequencies of a genetically verified

diagnosis by the stepwise analysis strategy described in papers I, II and III are presented in Figure 9.

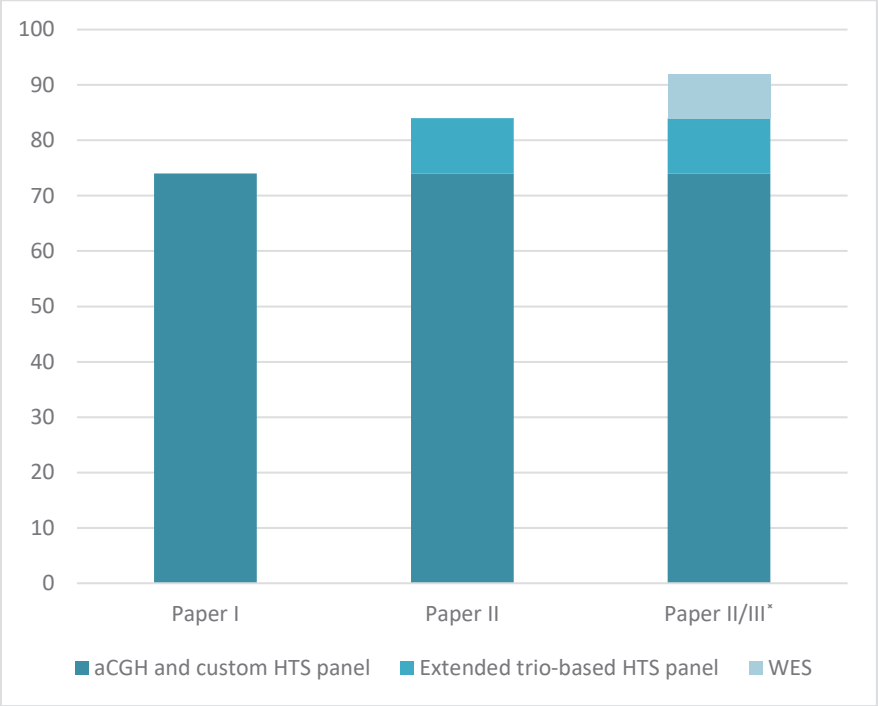

*Figure 9. The relative frequencies (percentage) of a genetically verified diagnosis by the different methods described in Papers I, II and III. *Paper III describes 10 individuals without a genetic finding from the same cohort as described in Paper II. (Elin Tønne).*

8.1 Paper I

Tønne E, Due-Tønnessen BJ, Wiig U, Stadheim BF, Meling TR, Helseth E, Heimdal KR. Epidemiology of craniosynostosis in Norway. *J Neurosurg Pediatr. 2020 Apr 3:1-8. doi: 10.3171/2020.1.PEDS2051. Online ahead of print.*

We detected a high incidence of CS: 5.5 per 10 000 live births. We divided the children into three groups based on year of birth (2003-2007, 2008-2012, 2013-2017) and observed a significant increase in incidence. The increase was mainly restricted to nonsyndromic and sagittal CS.

The relative frequency of syndromic CS was 27%. Of the analysed individuals with syndromic CS, 74% received a genetic diagnosis after routinely offered testing of aCGH and the custom-based HTS panel.

The sagittal suture was the overall most commonly affected suture (49% of the cases), and together with the metopic suture, it constituted most of the nonsyndromic cases (87%). Syndromic CS demonstrated a larger diversity in affected sutures than nonsyndromic CS. Multiple sutures were most commonly affected (including bicoronal); however, 37% of the syndromic CS cases were affected by a single midline suture (metopic or sagittal), and the types of single sutures were distributed almost equally (Figure 10). The absolute numbers of unicoronal synostosis were equally distributed between syndromic and nonsyndromic CS.

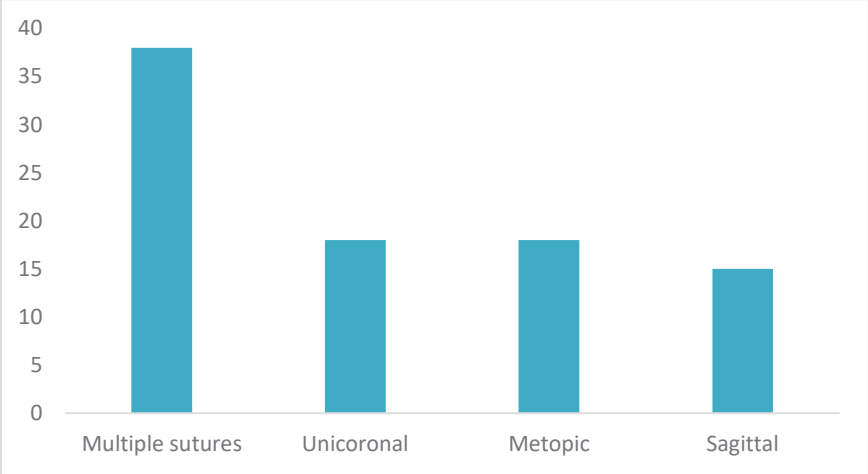

Figure 10. Affected sutures in syndromic CS in absolute numbers (Elin Tønne).

We detected a male dominance of 67% overall. Male dominance was mainly restricted to nonsyndromic CS (70%), as the relative frequency of males in syndromic CS was 58%. However, in regard to unicoronal CS, there was an overload of females (62%). Registered index individuals with familial CS constituted approximately 10% in regard to both syndromic and nonsyndromic CS.

8.2 Paper II

Tønne E, Due-Tønnessen BJ, Mero IL, Wiig U, Kulseth MA, Vigeland MD, Sheng Y, Lippe CVD, Tveten K, Meling TR, Helseth E, Heimdal KR. Benefits of clinical criteria and high-throughput sequencing for diagnosing children with syndromic craniosynostosis. *Eur J Hum Genet. 2021 Jun;29(6):920-929. doi: 10.1038/s41431-020-00788-4. Epub 2020 Dec 7.*

The aim of Paper II was to describe the distribution of genetic causes within the clinical definition of syndromic CS (Figure 8). In addition, we suspected that not all genetic causes were detected by the routinely offered testing of array CGH and custom-based HTS panel, described in Paper I. We therefore expanded the analysis provided to include a trio-based HTS panel of 1570 genes informed by the Dechipher Dysmorphology Study for negative cases. This increased the diagnostic yield in the cohort to 84% (Figure 9).

The frequently described CS syndromes, Crouzon, Pfeiffer, Saethre-Chotzen, Muenke, Apert and Craniofrontonasal dysplasia, caused by variants in *FGFR2, FGFR3, TWIST1* and *EFNB1,* respectively, constituted 56% of the genetically verified syndromes. Approximately 11% had a copy number variation associated with a known microdeletion or duplication syndrome, in most cases including a gene previously associated with CS (*MSX2*, *TWIST1*, *FREM1*(x2), *HDAC4*, *NFIA*). The rest of the genetic causes were distributed between a large number of different and often ultrarare syndromes (Figure 11), including novel causes of syndromic CS (Table 2). The monogenic causes were clustered in genes known to be involved in the pathways of osteogenesis and suture patency (Figure 6) or in genes known to cause Mendelian disorders of chromatin modification (chromatinopathies).

Table 2. Ten novel genetic causes of syndromic CS detected in the study. Described in Paper II and III.

Gene[a]	Number of cases	Syndrome (OMIM)	References
AHDC1	2	Xia-Gibbs syndrome	Gumus et al. 2020 (124)
ASXL3	1	Bainbridge-Ropers syndrome	Dinwiddie et al. 2013 (125)
CHD7	1	CHARGE syndrome	Siakallis et al. 2019 (99)
CDK13[b]	1	CHDFIDD	Bostwick et al. 2017 (126)
EHMT1	1	Kleefstra syndrome	Davis et al. 2019 (127)
EXTL3	1	ISDNA	Volpi et al. 2017 (128)
MAN2B1	1	Alpha-mannosidosis	Grabb et al. 1995 (129)
NFIX	1	Malan syndrome	Klaassens et al. 2015 (130)
POLR2A	1	NEDHIB	Haijes et al. 2019 (131)
SRCAP	1	Floating-Harbor syndrome	Hersh et al. 1998 (100)

[a] At least one other case reported with CS or brachyplagiocephaly (*POLR2A n=4*), [b] Previously reported (132)

Abbreviations: CHARGE, coloboma, congenital heart defects, choanal atresia, retardation of growth, developmental delay, genital abnormalities, ear abnormalities and deafness; CHDFIDD, congenital heart defects,

dysmorphic facial features, and intellectual developmental disorder; ISDNA, immunoskeletal dysplasia with neurodevelopmental abnormalities; NEDHIB, neurodevelopmental disorder with hypotonia and variable intellectual and behavioural abnormalities.

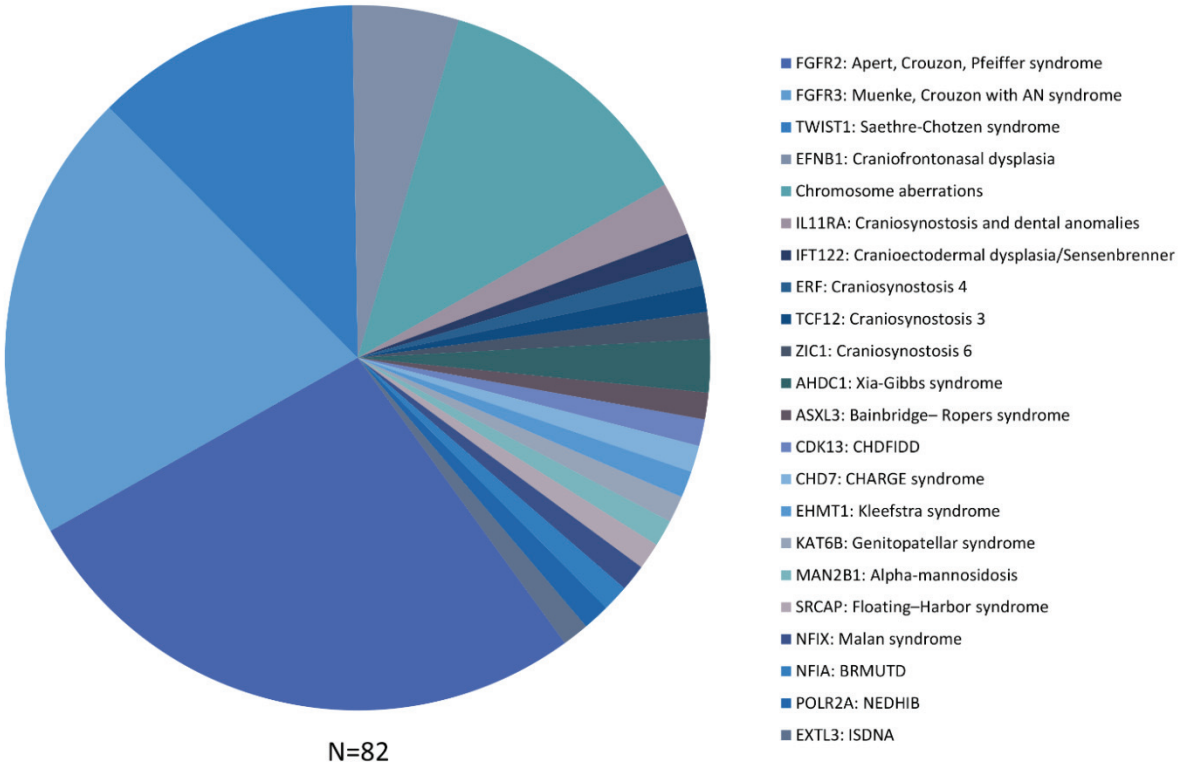

Figure 11. The diversity of genetic causes of syndromic CS described in Papers II and III (Elin Tønne).

*Abbreviations: BRMUTD, BRain Malformations with or without Urinary **T**ract Defects; CHFIDD, Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder; ISDNA, Immunoskeletal dysplasia with neurodevelopmental abnormalities; NEDHIB, Neurodevelopmental disorder with hypotonia and variable intellectual and behavioural abnormalities.*

8.3 Paper III

Tønne E, Due-Tønnessen BJ, Vigeland MD, Amundsen SS, Ribarska T, Åsten PM, Sheng Y, Helseth E, Gilfillan GD, Mero IL, Heimdal KR. Whole-exome sequencing in syndromic craniosynostosis increases diagnostic yield and identifies candidate genes in osteogenic signaling pathways. *Am J Med Genet A. 2022 Jan;25:1-12, doi: 10.1002/ajmg.a.62663. Online ahead of print. PMID: 35080095*

We suspected that WES would increase the diagnostic yield in the cohort even more and wanted to investigate whether the cohort contained more novel causes of syndromic CS. Of the 15 individuals without a confirmed genetic diagnosis after the stepwise diagnostic analyses presented in Paper II, 10 were eligible and agreed to WES. In these, we detected a pathogenic or likely pathogenic variant likely to cause their clinical findings in four individuals. In two of the findings (*POLR2A* and *FOXP2*), CS was not previously reported. However, several individuals with pathogenic *POLR2A* variants are reported to have brachyplagiocephaly (131). Brachyplagiocephaly is often the result of CS. A number of functional studies, including mouse studies, have confirmed that *FOXP2* is involved in cranial development and suture patency. In a fifth individual, we detected a candidate gene (*SH3BP4*) for CS but without confirmative functional studies. In the same individual, combined heterozygosity for two variants of unknown significance in *KMT2D* was detected. Of the filtered variants, where the respective gene was associated with a condition with an overlapping phenotype (*POLR2A, FOXP2, EXTL3, NFIA* and *KMT2D),* all findings turned out to be in a gene involved in the pathways of cranial development and suture patency (Figure 12).

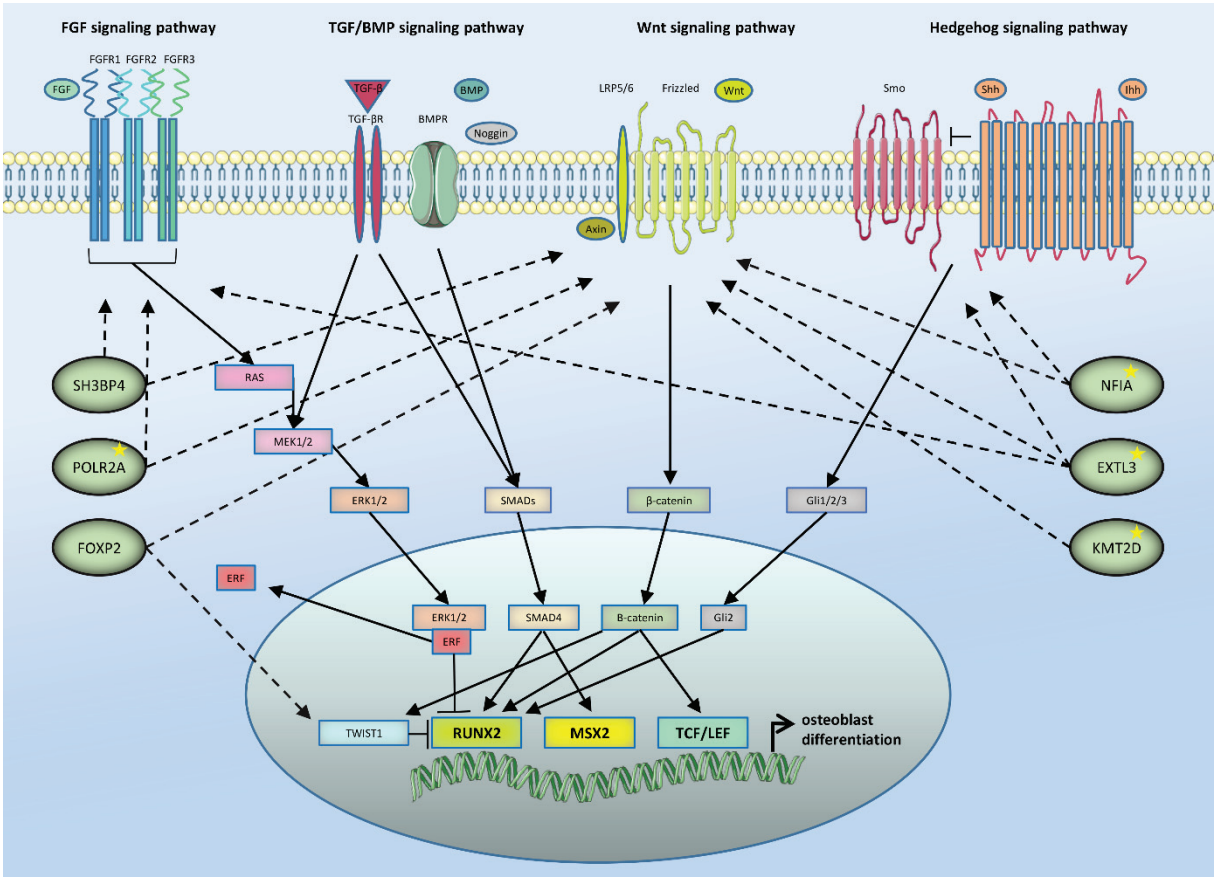

Figure 12. Figure extracted from Tønne et al, AJMG, with permission (133). The figure illustrates the finding`s relation to the most important signalling pathways of cranial development and suture patency. The figure was inspired by Figure 6. The Hedgehog signalling pathway and some interactions between the signalling pathways and osteogenic markers were added.

The total number of individuals in the cohort who accepted the stepwise analyses in papers II and III was 89. Of these, 82 (92%) received a confirmed genetic diagnosis (Figure 11) (excluding *FOXP2*, *SH3BP4* and *KMT2D*), including 10 novel causes of syndromic CS (Table 2). The findings were distributed between a large number of different genetic diagnoses, confirming that syndromic CS is highly heterogeneous (Figure 11).

9. DISCUSSION

The first aim of this thesis was to investigate the epidemiology of CS in Norway, as this has not previously been reported from a Scandinavian country. We detected one of the highest incidences of 5.5 per 10 000 live births, in addition to one of the highest numbers of syndromic cases (27%) reported. The study also revealed a number of children with syndromic CS having affected a single midline suture only (37%), in contradiction to the general assumption that syndromic CS is mainly associated with complex or coronal CS.

The next aim of this thesis was to detect and describe the genetic causes of syndromic CS within a proposed clinical definition. We detected an established genetic syndrome in accordance with the child's phenotype in 92% of the analysed children, including 10 novel syndromes associated with CS (Table 2). Approximately half of the genetic causes were in one of the frequently and first described genes associated with syndromic CS. The rest were distributed between a large number of different genetic causes, further confirming that syndromic CS is highly heterogeneous. The genetic causes were clustered in genes known to participate in the signalling pathways of cranial growth and suture patency or to modify chromatin (Chromatinopathies) (Table 3).

9.1 Study design and sample representability

The study was a population-based observational cohort study. The epidemiological part was prospective, while the genetic analyses were a combination of prospective and retrospective. Observational studies are well suited to detect and describe the occurrence of a condition in a population. The disadvantages are the need for a large sample size and that the method is time-consuming. Common selection bias in cohort studies is nonconsenting/responding individuals and individuals who withdraw from the study (134). As all children with CS in Norway are referred to the Norwegian National Unit for Craniofacial Surgery, we were able to calculate the relative frequency of individuals who did not want to participate, which was 15%. Nonconsenting individuals might result in a bias, as they may have a more severe or less severe disease than consenting individuals. To the best of our knowledge, the nonconsenting individuals in our cohort were not biased concerning sex, age, affected suture or syndromic/nonsyndromic CS. There were no individuals who withdrew from the study.

Norway has an equal access health care system, resulting in most individuals seeking medical care if needed. A paediatrician assessed all newborns in Norway within the first week after birth. All families were also contacted by the public health care centre municipality within the first week and received 14 follow-up visits until the age of five (www.helsedirektoratet.no). If any malformations or difficulties were suspected, they were referred to the local or specialized paediatric ward. This precaution ensured a high diagnostic rate and reduced the risk of socioeconomic differences in the diagnostics.

The advantage of a population-based study is that it reduces the risk of sample bias, as all individuals are included. Even though the Unit is expected to see all children with CS in Norway, we cannot be completely sure that some were not referred elsewhere.

9.2 Craniosynostosis epidemiology

The observed significant increase in the incidence of nonsyndromic and sagittal CS was in concordance with other reports (16, 29, 30, 135). There is no determined cause for this increase, and we attribute the increase to higher awareness amongst health professionals. The incidence of CS of 5.5 per 10 000 live births, however, was higher than most reports, often presenting an incidence between 3.1 and 5 per 10 000 live births (25-28). However, a study from the Netherlands, detected an even higher incidence of 7.2 per 10 000 live births (16). The study from the Netherlands was one of few other population-based studies. One reason for the lower incidence (and large variability) reported elsewhere might be that most of these reports were from single hospitals or regions. This observation might bias the result, compared to the population-based studies, towards the most severe cases being referred, in particular in regard to the highly specialized centres, and hence towards a lower incidence.

Syndromic and nonsyndromic CS demonstrated a suture-specific pattern to some degree, particularly concerning nonsyndromic CS, where most children had affected a single midline suture. This is in concordance with other reports (29, 135-137). However, the pattern was not conclusive for syndromic CS. Interestingly, only 43% of the syndromic CS cases had multiple suture synostoses, including bicoronal synostosis, often associated with syndromic CS (18, 19). The rest of the syndromic cases had affected a single suture only, and these were distributed almost equally between the metopic, sagittal and coronal sutures (Figure 10). This distribution is somehow contradictory to previous reports demonstrating that syndromic CS is dominated by multiple suture effects, including bicoronal synostoses (17, 21, 138). This contradiction indicates that the effect of a single midline suture is not synonymous with nonsyndromic CS and that the affected suture is not a suitable feature on its own for the classification of CS.

Epidemiological studies are not suitable to determine causes but may present associations and potential causes (134). It has been suggested that the previous detection of a high genetic load associated with coronal synostosis may reflect the different embryological origin of the sutures, as the coronal suture lies in the boundaries between the frontal (neural crest) and parietal (cephalic mesenchyme) bone (6, 19), thus suggesting different pathophysiological

mechanisms between the sutures. This suggestion is also in concordance with some genetic syndromes being highly suture specific to coronal synostosis (5). In regard to this, the fact that midline synostoses account for most nonsyndromic cases but also a significant part of syndromic CS cases is interesting. In particular, the finding of unilateral coronal synostosis being equally distributed between syndromic and nonsyndromic CS is rather surprising and might question this hypothesis, as syndromic CS usually has a monogenic cause, while this is rather uncommon for nonsyndromic CS (33). On the other hand, our study did not include genetic analysis of nonsyndromic (coronal) CS, which is a large limitation, as we do not know what they might have of genetic variants. In addition, we did not separate the bicoronal CS cases from the multiple sutures, which is another limitation. The reason for combining the groups was that we had a low number of isolated bilateral coronal sutures in the cohort, but in retrospect, the relative frequency of bilateral coronal synostosis in both syndromic and nonsyndromic CS would be interesting, particularly in regard to reports of a high genetic load in bicoronal CS (33). The sex differences demonstrated, in which coronal synostosis is more common in females, while midline synostoses and CS overall are much more common in males, are in accordance with other reports (139, 140) and suggest sex as a suture-specific contributing factor. We found a proportion of familial cases in nonsyndromic CS at approximately 10%. This percentage is in accordance with other reports, varying between 5.6 and 14.7% (34, 42, 141), further indicating that genetic variants impact the formation of nonsyndromic CS to some extent (142). Thus, these epidemiological findings might lead to further information and understanding of the mechanisms and contributing factors involved in the development of CS.

9.3 Refining the classification of craniosynostosis

The classification of CS into syndromic and nonsyndromic is important, as the two groups are known to have different causes, prognoses, frequencies of late-onset diseases and often treatments (19, 20, 23). The relative frequency of syndromic CS varies between 12 and 31% in published studies (25, 26, 33, 34). Today, a combination of additional anomalies and affected sutures is recommended for the classification of CS (33). However, no clear definition has been established, and the large variation in the relative frequencies of syndromic CS might reflect this. The absence of a common definition makes it difficult to compare research results and might explain different and contradictory findings, for instance, in regard to neurodevelopmental deficits and additional anomalies in nonsyndromic CS (143), as well as affected sutures. Our study, detecting a relative frequency of syndromic CS of 27%,

is in line with another large study using clinical criteria for the classification, showing a relative frequency of syndromic CS of 31% (33). Other studies report significantly lower relative frequencies of syndromic CS (25, 26, 34). This lower relative frequency might be due to a narrower classification, which again could bias the results, for instance, towards a higher degree of neurodevelopmental difficulties in the nonsyndromic group, in addition to perhaps a higher number of multiple suture synostoses.

When presenting the genetic causes of syndromic CS, we wanted to demonstrate the clinical criteria used to make the research transparent (Figure 8). We also wanted to present the criteria as a contribution to the discussion of the classification of CS. We used general principles in syndromology (as outlined in the introduction section), in addition to specific characteristics of CS (e.g., multiple synostosis being associated with syndromic CS, typical dysmorphic features) to create the criteria (Figure 8). The minor criteria are relatively common findings; therefore, at least two of those needed to be classified as syndromic to avoid a coincidental event being misinterpreted as a syndrome. As these criteria resulted in one of the highest relative frequencies of syndromic CS reported, one could argue that the criteria were too wide. As we were able to detect an established genetic diagnosis in 92% of the tested individuals (including *POLR2A*, excluding *FOXP2*, *SH3BP4* and *KMT2D*), we would argue that the criteria were not too wide. On the other hand, genetic analysis of the nonsyndromic CS group was not included in this study, and we do not know what they may have of genetic variants.

The criteria could be too narrow. Some children develop associated findings or difficulties later in life, and some of these might not have been recognized. Hence, the relative frequency of syndromic CS might be even higher. This is especially relevant, as we included newborns in the study that might not yet have developed additional findings. In addition, we did not reevaluate children with nonsyndromic CS later in life in a routine manner. The children were reassessed only if they were referred to or contacting the Unit themselves if difficulties or findings in organs developed (in which they were encouraged to).

9.4 Genetic results and causality

9.4.1 Diagnostic yield and genetic heterogeneity

We detected an established genetic syndrome in accordance with the child's phenotype in 92% of the investigated children with syndromic CS. This included 10 novel monogenic causes of CS, defined as those not established to be associated with CS at the start of the study

(2019) (52). Approximately half of the genetic causes were frequently reported genes (*FGFR2, FGFR3, TWIST1* and *EFNB1*) (Figure 11). The rest were distributed between a number of different monogenic and chromosomal causes, detected in one or two individuals each, supporting that syndromic CS is highly heterogeneous (Figure 11). The relative frequency of the copy number variations (11%) was in accordance with other reports (23) and in most cases included a predicted loss-of-function effect involving a gene previously associated with CS, suggesting a monogenic cause in these. A few individuals refused genetic analyses (n=15). One could argue that individuals with a mild degree of difficulties or anomalies would be less concerned and thereby less prone to genetic analysis. If so, this might have biased the results towards a higher diagnostic yield. However, the reason for not detecting a genetic cause in the rest of the syndromic CS cases might be related to weaknesses in the analyses performed. For instance, we did not perform MLPA of *EFNB1* and *TCF12*, in which microdeletions/duplications are reported (144, 145). In addition, we performed aCGH (180k), when one million arrays would have been more sensitive. Thus, some small copy number changes might have been overlooked. In addition, other variants, such as noncoding or regulatory variants, may not have been captured by the analysis methods used. This means that the diagnostic yield could have been even higher.

9.4.2 Novel genetic causes

Causality is a term often used in science to describe an event that leads to an outcome. Causality may be used to explain what has happened, predict what will happen, and intervene to improve or prevent outcomes (146). In the context of causation, the event of interest does not need to lead to the outcome in all cases but may be a sufficient cause. Other events are necessary for an outcome to occur and may be termed a necessary cause (134). For a pathogenic variant to be likely causative of a child`s condition, the general perception is that you need several unrelated individuals with a similar phenotype and a pathogenic variant in the same gene, in addition to supportive experimental data, in particular if the cases are few (147, 148). In regard to CS being associated with a known syndrome, the presence of at least two unrelated affected individuals with similar phenotypes, including CS, is needed to establish an association (5, 52). In the novel genetic causes detected (Table 2), there was one gene included (*POLR2A*) that has not previously been reported to be associated with CS. However, brachyplagiocephaply was described in four individuals with the associated condition NEDHIB. Brachyplagiocephaply is in some cases the result of CS but may also be the result of hypotonia, which is common in NEDHIB. The *POLR2A* gene is involved in two

signalling pathways usually affected in CS (FGF and Wnt signalling pathways) (149, 150). However, in regard to *POLR2A,* the evidence is limited, and it might be unclear if this is enough to claim *POLR2A* to be associated with CS at present. There were potential novel monogenic candidate genes also included in the chromosomal aberrations (e.g., *HDAC4,* published once in an individual with CS). These are, however, not included in the list of novel genetic causes, as we cannot be sure that the rest of the deletion did not have a significant impact on the formation of CS. Neither is the candidate genes *FOXP2* and *SH3BP4*, as information about their potential involvement in CS is limited at present.

9.4.3 Genetic causes indicating different pathophysiological mechanisms

The finding of 92% of the investigated individuals with syndromic CS having a monogenic or chromosomal cause strongly argues for a genetic cause, mostly a monogenic cause, being the primary mechanism and possibly a necessary event for syndromic CS. This leaves other cofactors, such as mechanical forces from the growing brain, extrinsic forces acting on the skull (e.g., twin pregnancies, macrocephaly), polygenetic inheritance and environmental factors, less important in syndromic CS. However, these and other contributing factors, including epigenetic changes, may contribute to explaining why some individuals develop CS and others do not, despite the same genetic cause.

In addition to the commonly known CS syndromes that include CS in nearly all cases, we found a large diversity of rare and ultrarare genetic syndromes (Figure 11), including CS in only a small fraction of the cases (Table 3). A classification framework for the genetic causes of CS has previously been established based on how frequent pathogenic variants in a gene are associated with CS. This framework classifies 20 genes as core genes (>50% of the cases associated with CS), and the rest are causally associated (CS in a minority of cases) (5). Using this framework to classify the 21 different monogenic causes detected in the study, the majority of the causes (n=13) were causally associated genes, while eight causes were among the core genes (Table 3). However, the number of cases in those two groups demonstrated opposite patterns, as 14 individuals had affected a causally associated gene, while 58 had affected a core gene. The high number of different causally associated genes detected, further points out the large heterogeneity of syndromic CS and actualizes the question of why the frequency of CS is so different between the syndromes associated with CS.

The genetic findings were primarily in genes known to act in the signalling pathways of cranial growth and suture patency. In addition, there were several findings in genes involved

in chromatin remodelling (Table 3). The definitely most common genetic findings were in the core genes *FGFR2* and *FGFR3*, causing Apert, Crouzon, Pfeiffer and Muenke syndrome (Figure 11). The mechanism described for pathogenic variants in these genes is gain-of-function. This is in accordance with our findings, as we detected only missense or in-frame deletions or duplications and no predicted loss-of-function variants. Detected variants in the other genes acting in the signalling pathways of cranial growth and suture patency were mainly predicted loss-of-function variants, with some exceptions. The overload of gain-of-function variants in the genes most frequently associated with CS suggests different pathophysiological mechanisms and might contribute to explaining the differences in frequency. One possible explanation might be that gain-of-function variants are less affected by interfering signalling mechanisms than loss-of-function variants. Another explanation might be related to the essential role of the *FGFR* genes in the formation of the sutures at crucial times during development (in particular in the coronal sutures) (5). On the other hand, the Wnt and BMP pathways are also demonstrated to be important in the same tissue at the same time (5), in which pathogenic variants in genes interfering with these pathways only demonstrate CS in a fraction of the cases. It could be that *FGFR*s are more essential in this process or lack compensatory mechanisms that the other may have; however, expression or knockout studies have not been able to disentangle these different events or signalling pathway contributions (5).

The next common finding was in another core gene: *TWIST1*, known to cause Saethre-Chotzen syndrome, usually (but not always) associated with CS. *TWIST1* was previously demonstrated to inhibit one of the key targets in cranial osteogenesis (Runx2) (Figure 6). We detected mainly predicted loss-of-function variants in *TWIST1*, supporting loss of inhibition of Runx2 as the key mechanism, but a number of missense variants were also detected, suggesting additional mechanisms.

We detected several variants in the *EFNB1* gene, located at the X chromosome, causing craniofrontonasal dysplasia in females. The mechanism of this condition is hypothesized to be cellular interference, where the cells do not tolerate different expression of *EFNB1*, in which haploinsufficient females are most severely affected (two copies of the X chromosome). We detected a likely pathogenic variant in a mosaic state in a male with classic features of craniofrontonasal dysplasia, supporting the theory of cellular interference as the primary mechanism for this condition.

We detected several disorders of chromatin modification (chromatinopathies) in our cohort (Table 3), including novel disorders associated with CS (Table 2). Chromatinopathies demonstrate a large variability in their clinical presentation, and the mechanism is proposed to be dosage sensitivity to the different epigenetic components due to loss of function (97). This mechanism is in accordance with our findings, as all detected findings were predicted loss-of-function variants. It has been suggested that pathogenic variants disrupt chromatin modification in specific cell populations at specific times during early development and that this is one of the causes of the large variability in clinical presentation (97, 98). This could contribute to explaining the low and different frequencies of CS in these conditions, as the formation of the sutures is known to be dependent on critical events at specific times during embryogenesis (5). It has also been suggested that variants in these genes not only disrupt the dosage of the epigenetic components but also the molecular structure of the targeted genes (151). If so, this makes the effect of the pathogenic variants further complex and might contribute to explaining the large clinical variability in these conditions, including CS. However, as these disruptions do not result in CS more frequently, these processes are most likely dependent on several other cofactors, not yet elucidated.

The large complexity in regulating cranial growth and suture patency may explain some of the observed phenotypic variability in the syndromes caused by genetic alterations involving these signalling pathways, including variation between individuals with the exact same pathogenic variant (66).

Table 3. Monogenic causes of syndromic CS detected in the study and their relation to signalling pathways and frequency of CS

Gene	**Condition**	**Pathways**	**Frequency of CS**	**Reference**
AHDC1	Xia-Gibbs syndrome		Rare	Gumus et al. 2020 (124)
ASXL3	Bainbridge-Ropers syndrome	Chromatin modification and BMP signalling	Rare	Srivastava et al. 2016 (152), Szczepanski et al. 2020 (153), Lichtig et al. 2020 (154)
CHD7	CHARGE syndrome	Chromatin modification	Rare	Bjornsson 2015 (97)
CDK13	CHDFIDD		Rare	Bostwick et al. 2017 (126)
EFNB1	Craniofrontonasal dysplasia	Eph/Ephrin signalling	Common (core gene)	Twigg et al. 2015 (5)
EHMT1	Kleefstra syndrome	Chromatin modification	Rare	Bjornsson 2015 (97)

ERF	Craniosynostosis 4	FGFR signalling	Common (core gene)	Twigg et al. 2015 (5)
EXTL3	ISDNA	Wnt, Hedgehog and FGFR signalling	Rare	Venero et al. 2015 (155) Venero et al. 2016 (156)
FGFR2	Crouzon-, Apert-, Pfeiffer, Beare-Stevenson syndrome	FGFR signalling	Common (core gene)	Twigg et al. 2015 (5)
FGFR3	Muenke syndrome Crouzon with acanthosis nigricans syndrome	FGFR signalling	Common (core gene)	Twigg et al. 2015 (5)
IFT122	Cranioectodermal dysplasia	Hedgehog signalling	Rare	Twigg et al. 2015 (5)
IL11RA	Craniosynostosis and dental anomalies	STAT3; osteogenesis	Common (core gene)	Twigg et al. 2015 (5)
KAT6B	KAT6B-related disorders	Chromatin modification	Rare	Bjornsson 2015 (97)
MAN2B1	Alpha-mannosidosis		Rare	Grabb et al. 1995 (129)
NFIA	BRMUTD	Wnt and Hedgehog signalling	Rare	Singh et al. 2018 (157), Xie et al. 2015 (158)
NFIX	Malan syndrome	Wnt signalling	Rare	Wu et al. 2021 (159)
POLR2A	NEDHIB	Wnt and FGFR signalling	Rare	Clark et al. 2016 (149), Schoch et al. 2020 (150)
SRCAP	Floating-Harbor syndrome	Chromatin modification	Rare	Bjornsson 2015 (97)
TCF12	Craniosynostosis 3	RUNX2, BMP and FGFR signalling	Common (core gene)	Twigg et al. 2015 (5)
TWIST1	Saethre-Chotzen syndrome	RUNX2, BMP and FGFR signalling	Common (core gene)	Twigg et al. 2015 (5)
ZIC1	Craniosynostosis 6	Wnt signalling	Common (core gene)	Twigg et al. 2015 (5)

The high detection rate of monogenic or chromosomal causes in syndromic CS highlights the need to distinguish syndromic and nonsyndromic CS, as the causes and mechanisms seem to be very different. In particular, a monogenic or chromosomal cause seems to be almost a necessary cause in syndromic CS, while this is not the case for nonsyndromic CS. However, we did not genetically investigate individuals with nonsyndromic CS. This is a large limitation of the study, as we do not know what this group might have of genetic findings. Interestingly, some of the detected genetic causes were previously demonstrated to result in nonsyndromic CS, such as *TWIST1*, *TCF12* and *EFNB1*, in addition to other causes not detected in our study (e.g., *SMAD6*) (Table 1). The reason that alterations in some genes may

result in both syndromic and nonsyndromic CS is not known (24, 142). However, the variants in these genes are often unique (24), and one could hypothesize that some of the explanation is related to the specific characteristics of each variant. The ability of some genes to demonstrate both syndromic and nonsyndromic CS when disrupted might also be related to the pathophysiological mechanism by which each gene causes CS in the first place, in addition to other contribution factors previously mentioned. On the other hand, some studies of the genetic causes of nonsyndromic CS report a high relative frequency of nonsyndromic CS of 85% (142) compared to our results (73%). This higher relative frequency might suggest that these studies have included a number of syndromic CS cases in their presumably nonsyndromic cohort, thus biasing the result towards a higher frequency of genetic causes in the nonsyndromic group. This is only speculation, but the divergent numbers in the relative frequency of syndromic CS, most likely caused by different classifications, do question these results and highlight the need for a common consensus.

9.5 Proposal for genetic diagnostic workup

The finding of a monogenic or chromosomal cause in near-all individuals with syndromic CS highlights the importance of clinically detecting syndromic CS cases to provide sufficient genetic analyses. A precise genetic diagnosis is important, as it may affect treatment, suggest additional investigations (e.g., EEG, cerebral MRI), predict later onset diseases and prognosis, affect risk assessment, and give answers to the families.

In addition to a few genes (*FGFR2, FGFR3, TWIST1* and *EFNB1*), the genetic findings in the study were highly heterogeneous. This heterogeneity argues for extensive genetic analyses to be provided to children with syndromic CS. This may be performed stepwise, as in this study, or one may go directly to an extensive analysis, such as WES/WGS.

9.5.1 Genetic analyses in the transition between diagnostics and research

The most efficient test strategy would be to perform aCGH and WES/WGS directly due to the heterogeneity demonstrated. However, this would most likely result in a number of variants of unknown significance in addition to incidental findings, perhaps causing distress and concern for the families. In addition, the costs would be high, as this is a time-consuming method. To avoid incidental findings in the clinic, we advocate for a targeted panel to start, in addition to aCGH. If the panel is up to date, this will capture most of the cases. However, the number of new genes associated with CS increases rapidly, often leaving diagnostics in the transition between diagnostics and research and making it difficult to maintain up-to-date panels. For negative cases, we therefore recommend WES/WGS. In the clinic, the detection of variants in

genes of unknown significance is not appreciated, as the implications are uncertain, thus causing distress without much to offer. In research, however, the discovery of candidate genes is desired, thus making WES or WGS the preferred choice of method, demonstrated in this and similar studies (160), to be fruitful.

The knowledge acquired in this study resulted in an expansion of the molecular diagnostics routinely provided to children with syndromic CS in Norway.

10. CONCLUSION

We detected an incidence of CS in Norway, of 5.5 per 10 000 live births. The incidence increased significantly during the study period.

Using clinical criteria to define syndromic CS, we identified one of the highest numbers of syndromic CS reported. We further detected an established genetic diagnosis in 92% of the syndromic CS cases, including 10 novel genetic causes. Approximately half of the genetic diagnosis were one of the frequently reported and well-known CS syndromes. The rest of the diagnoses were distributed between a large number of rare and ultrarare syndromes, compatible with syndromic CS being highly heterogeneous.

The genetic causes were clustered in genes known to act in the signalling pathways of cranial growth and suture patency, in addition to a number of genes involved in chromatin remodelling, suggesting related pathophysiological mechanisms.

11. FUTURE PERSPECTIVES

Due to the rapid expansion of novel genetic causes associated with syndromic CS in recent years, including this study, we suspect that there are still undiscovered genetic causes. The continuation of performing WES/WGS in the group of syndromic CS is therefore needed to detect all associated causes and to create up-to-date targeted panels.

An interesting study for the future would be to explore the genetic causes of nonsyndromic CS using the same cohort. Another interesting study would be to evaluate the long-term outcome of individuals with nonsyndromic CS. A number of parents of children with nonsyndromic CS express concerns, as there are reports of children with nonsyndromic CS having severe neurocognitive deficits (161-164). However, some of these studies have included individuals with additional anomalies (164). In addition, most publications report a significantly lower relative frequency of syndromic CS than we detected. Thus, our

hypothesis of these studies having included a number of syndromic CS cases, thus biasing the results towards a higher degree of neurocognitive deficits, would be interesting to test.

There are interesting studies on pharmacological treatment of syndromic CS targeting the signalling pathways and genes involved (165, 166). There are also ongoing studies on pharmacological inhibitors of FGFRs in cancer (167). However, further studies and insight into the pathophysiological mechanisms and the pathway's individual contribution in addition to interfering signalling mechanisms are needed to provide targeted and personalized treatment and prevention of CS.

References

1. Opperman LA. Cranial sutures as intramembranous bone growth sites. Developmental dynamics : an official publication of the American Association of Anatomists. 2000;219(4):472-85.
2. Katsianou MA, Adamopoulos C, Vastardis H, Basdra EK. Signaling mechanisms implicated in cranial sutures pathophysiology: Craniosynostosis. BBA clinical. 2016;6:165-76.
3. Mishina Y, Snider TN. Neural crest cell signaling pathways critical to cranial bone development and pathology. Experimental cell research. 2014;325(2):138-47.
4. Noden DM, Trainor PA. Relations and interactions between cranial mesoderm and neural crest populations. Journal of anatomy. 2005;207(5):575-601.
5. Twigg SR, Wilkie AO. A Genetic-Pathophysiological Framework for Craniosynostosis. American journal of human genetics. 2015;97(3):359-77.
6. Morriss-Kay GM, Wilkie AO. Growth of the normal skull vault and its alteration in craniosynostosis: insights from human genetics and experimental studies. Journal of anatomy. 2005;207(5):637-53.
7. Connerney JJ, Spicer DB. Signal Transduction Pathways and Their Impairment in Syndromic Craniosynostosis.
8. Roth DA, Bradley JP, Levine JP, McMullen HF, McCarthy JG, Longaker MT. Studies in cranial suture biology: part II. Role of the dura in cranial suture fusion. Plastic and reconstructive surgery. 1996;97(4):693-9.
9. Benson MD, Opperman LA. Regulation of Calvarial Bone Growth by Molecules Involved in the Craniosynostoses.
10. Thompson DN, Harkness W, Jones BM, Hayward RD. Aetiology of herniation of the hindbrain in craniosynostosis. An investigation incorporating intracranial pressure monitoring and magnetic resonance imaging. Pediatric neurosurgery. 1997;26(6):288-95.
11. Renier D, Sainte-Rose C, Marchac D, Hirsch JF. Intracranial pressure in craniostenosis. Journal of neurosurgery. 1982;57(3):370-7.
12. Fairman D, Horrax G. Classification of craniostenosis. Journal of neurosurgery. 1949;6(4):307-13.
13. Escobar V, Bixler D. On the classification of the acrocephalosyndactyly syndromes. Clin Genet. 1977;12(3):169-78.
14. Kweldam CF, van der Vlugt JJ, van der Meulen JJ. The incidence of craniosynostosis in the Netherlands, 1997-2007. Journal of plastic, reconstructive & aesthetic surgery : JPRAS. 2011;64(5):583-8.

15. Heuze Y, Holmes G, Peter I, Richtsmeier JT, Jabs EW. Closing the Gap: Genetic and Genomic Continuum from Syndromic to Nonsyndromic Craniosynostoses. Current genetic medicine reports. 2014;2(3):135-45.
16. Cornelissen M, Ottelander B, Rizopoulos D, van der Hulst R, Mink van der Molen A, van der Horst C, et al. Increase of prevalence of craniosynostosis. Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-Facial Surgery. 2016;44(9):1273-9.
17. Topa A, Rohlin A, Andersson MK, Fehr A, Lovmar L, Stenman G, et al. NGS targeted screening of 100 Scandinavian patients with coronal synostosis. American journal of medical genetics Part A. 2020;182(2):348-56.
18. Kimonis V, Gold JA, Hoffman TL, Panchal J, Boyadjiev SA. Genetics of craniosynostosis. Semin Pediatr Neurol. 2007;14(3):150-61.
19. Johnson D, Wilkie AO. Craniosynostosis. European journal of human genetics : EJHG. 2011;19(4):369-76.
20. Lattanzi W, Barba M, Di Pietro L, Boyadjiev SA. Genetic advances in craniosynostosis. American journal of medical genetics Part A. 2017;173(5):1406-29.
21. Firth HVHJA. Oxford Desk Reference: Clinical Genetics and Genomics. 2 ed: Oxford University Press; 2017.
22. Rimoin Ea. Emery and Rimoin`s principles and practice of medical genetics. 5 ed2008 September 2008.
23. Wilkie AO, Byren JC, Hurst JA, Jayamohan J, Johnson D, Knight SJ, et al. Prevalence and complications of single-gene and chromosomal disorders in craniosynostosis. Pediatrics. 2010;126(2):e391-400.
24. Calpena E, Cuellar A, Bala K, Swagemakers SMA, Koelling N, McGowan SJ, et al. SMAD6 variants in craniosynostosis: genotype and phenotype evaluation. Genetics in medicine : official journal of the American College of Medical Genetics. 2020;22(9):1498-506.
25. Boulet SL, Rasmussen SA, Honein MA. A population-based study of craniosynostosis in metropolitan Atlanta, 1989-2003. American journal of medical genetics Part A. 2008;146a(8):984-91.
26. Neusel C, Class D, Eckert AW, Firsching R, Gobel P, Gotz D, et al. Multicentre approach to epidemiological aspects of craniosynostosis in Germany. The British journal of oral & maxillofacial surgery. 2018;56(9):881-6.
27. French LR, Jackson IT, Melton LJ, 3rd. A population-based study of craniosynostosis. J Clin Epidemiol. 1990;43(1):69-73.
28. Singer S, Bower C, Southall P, Goldblatt J. Craniosynostosis in Western Australia, 1980-1994: a population-based study. American journal of medical genetics. 1999;83(5):382-7.

29. Selber J, Reid RR, Chike-Obi CJ, Sutton LN, Zackai EH, McDonald-McGinn D, et al. The changing epidemiologic spectrum of single-suture synostoses. Plastic and reconstructive surgery. 2008;122(2):527-33.
30. van der Meulen J, van der Hulst R, van Adrichem L, Arnaud E, Chin-Shong D, Duncan C, et al. The increase of metopic synostosis: a pan-European observation. The Journal of craniofacial surgery. 2009;20(2):283-6.
31. Schraw JM, Woodhouse JP, Langlois PH, Canfield MA, Scheuerle AE, Agopian AJ, et al. Risk factors and time trends for isolated craniosynostosis. Birth defects research. 2021;113(1):43-54.
32. Lee HQ, Hutson JM, Wray AC, Lo PA, Chong DK, Holmes AD, et al. Changing epidemiology of nonsyndromic craniosynostosis and revisiting the risk factors. The Journal of craniofacial surgery. 2012;23(5):1245-51.
33. Wilkie AOM, Johnson D, Wall SA. Clinical genetics of craniosynostosis. Current opinion in pediatrics. 2017;29(6):622-8.
34. Garrocho-Rangel A, Manriquez-Olmos L, Flores-Velazquez J, Rosales-Berber MA, Martinez-Rider R, Pozos-Guillen A. Non-syndromic craniosynostosis in children: Scoping review. Medicina oral, patologia oral y cirugia bucal. 2018;23(4):e421-e8.
35. Hunter AG, Rudd NL. Craniosynostosis. I. Sagittal synostosis: its genetics and associated clinical findings in 214 patients who lacked involvement of the coronal suture(s). Teratology. 1976;14(2):185-93.
36. Izuora GI, Iloeje SO. A review of neurological disorders seen at the Paediatric Neurology Clinic of the University of Nigeria Teaching Hospital, Enugu. Ann Trop Paediatr. 1989;9(4):185-90.
37. Tamada I, Hikosaka M, Sakamoto Y, Tsuda K, Ihara S, Kaneko T, et al. Clinical Features of Bilambdoid and Sagittal Synostosis (BLSS): A Retrospective Multicenter Study in Japan. The Journal of craniofacial surgery. 2021;32(6):2134-8.
38. Shweikeh F, Foulad D, Nuño M, Drazin D, Adamo MA. Differences in surgical outcomes for patients with craniosynostosis in the US: impact of socioeconomic variables and race. Journal of neurosurgery Pediatrics. 2016;17(1):27-33.
39. Carmichael SL, Ma C, Rasmussen SA, Honein MA, Lammer EJ, Shaw GM. Craniosynostosis and maternal smoking. Birth Defects Res A Clin Mol Teratol. 2008;82(2):78-85.
40. Wu X, Gu Y. Signaling Mechanisms Underlying Genetic Pathophysiology of Craniosynostosis. International journal of biological sciences. 2019;15(2):298-311.
41. Gardner JS, Guyard-Boileau B, Alderman BW, Fernbach SK, Greene C, Mangione EJ. Maternal exposure to prescription and non-prescription

pharmaceuticals or drugs of abuse and risk of craniosynostosis. Int J Epidemiol. 1998;27(1):64-7.
42. Lajeunie E, Le Merrer M, Marchac D, Renier D. Syndromal and nonsyndromal primary trigonocephaly: analysis of a series of 237 patients. American journal of medical genetics. 1998;75(2):211-5.
43. Sanchez-Lara PA, Carmichael SL, Graham JM, Jr., Lammer EJ, Shaw GM, Ma C, et al. Fetal constraint as a potential risk factor for craniosynostosis. American journal of medical genetics Part A. 2010;152a(2):394-400.
44. Mao JJ. Mechanobiology of craniofacial sutures. J Dent Res. 2002;81(12):810-6.
45. Lin IC, Slemp AE, Hwang C, Karmacharya J, Gordon AD, Kirschner RE. Immunolocalization of androgen receptor in the developing craniofacial skeleton. The Journal of craniofacial surgery. 2004;15(6):922-7; discussion 8-9.
46. E P. Color atlas of genetics. 5 ed. Stuttgart, Germany, New York, USA: Thieme Medical Publishers; 2018.
47. Read A DD. New Clinical Genetics. 2 ed. Banbury, UK: Scion Publishing Ltd; 2011.
48. Howard TD, Paznekas WA, Green ED, Chiang LC, Ma N, Ortiz de Luna RI, et al. Mutations in TWIST, a basic helix-loop-helix transcription factor, in Saethre-Chotzen syndrome. Nature genetics. 1997;15(1):36-41.
49. Muenke M, Schell U, Hehr A, Robin NH, Losken HW, Schinzel A, et al. A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. Nature genetics. 1994;8(3):269-74.
50. Meyers GA, Orlow SJ, Munro IR, Przylepa KA, Jabs EW. Fibroblast growth factor receptor 3 (FGFR3) transmembrane mutation in Crouzon syndrome with acanthosis nigricans. Nature genetics. 1995;11(4):462-4.
51. Reardon W, Winter RM, Rutland P, Pulleyn LJ, Jones BM, Malcolm S. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. Nature genetics. 1994;8(1):98-103.
52. Goos JAC, Mathijssen IMJ. Genetic Causes of Craniosynostosis: An Update. Molecular syndromology. 2019;10(1-2):6-23.
53. Timberlake AT, Furey CG, Choi J, Nelson-Williams C, Loring E, Galm A, et al. De novo mutations in inhibitors of Wnt, BMP, and Ras/ERK signaling pathways in non-syndromic midline craniosynostosis. Proceedings of the National Academy of Sciences of the United States of America. 2017;114(35):E7341-e7.
54. Vissers LE, Cox TC, Maga AM, Short KM, Wiradjaja F, Janssen IM, et al. Heterozygous mutations of FREM1 are associated with an increased risk of isolated metopic craniosynostosis in humans and mice. PLoS Genet. 2011;7(9):e1002278.

55. Nicoglou A, Merlin F. Epigenetics: A way to bridge the gap between biological fields. Studies in history and philosophy of biological and biomedical sciences. 2017;66:73-82.
56. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. Nature. 2019;571(7766):489-99.
57. Passos-Bueno MR, Sertié AL, Jehee FS, Fanganiello R, Yeh E. Genetics of craniosynostosis: genes, syndromes, mutations and genotype-phenotype correlations. Frontiers of oral biology. 2008;12:107-43.
58. Ornitz DM, Xu J, Colvin JS, McEwen DG, MacArthur CA, Coulier F, et al. Receptor specificity of the fibroblast growth factor family. The Journal of biological chemistry. 1996;271(25):15292-7.
59. Eswarakumar VP, Horowitz MC, Locklin R, Morriss-Kay GM, Lonai P. A gain-of-function mutation of Fgfr2c demonstrates the roles of this receptor variant in osteogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(34):12555-60.
60. Gotoh N. Regulation of growth factor signaling by FRS2 family docking/scaffold adaptor proteins. Cancer science. 2008;99(7):1319-25.
61. Kouhara H, Hadari YR, Spivak-Kroizman T, Schilling J, Bar-Sagi D, Lax I, et al. A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. Cell. 1997;89(5):693-702.
62. Miraoui H, Oudina K, Petite H, Tanimoto Y, Moriyama K, Marie PJ. Fibroblast growth factor receptor 2 promotes osteogenic differentiation in mesenchymal cells via ERK1/2 and protein kinase C signaling. The Journal of biological chemistry. 2009;284(8):4897-904.
63. Raucci A, Bellosta P, Grassi R, Basilico C, Mansukhani A. Osteoblast proliferation or differentiation is regulated by relative strengths of opposing signaling pathways. Journal of cellular physiology. 2008;215(2):442-51.
64. Przylepa KA, Paznekas W, Zhang M, Golabi M, Bias W, Bamshad MJ, et al. Fibroblast growth factor receptor 2 mutations in Beare–Stevenson cutis gyrata syndrome. Nature genetics. 1996;13(4):492-4.
65. Wilkes D, Rutland P, Pulleyn LJ, Reardon W, Moss C, Ellis JP, et al. A recurrent mutation, ala391glu, in the transmembrane region of FGFR3 causes Crouzon syndrome and acanthosis nigricans. Journal of medical genetics. 1996;33(9):744-8.
66. Hollway GE, Suthers GK, Haan EA, Thompson E, David DJ, Gecz J, et al. Mutation detection in FGFR2 craniosynostosis syndromes. Human genetics. 1997;99(2):251-5.
67. Murre C, Bain G, van Dijk MA, Engel I, Furnari BA, Massari ME, et al. Structure and function of helix-loop-helix proteins. Biochimica et biophysica acta. 1994;1218(2):129-35.

68. Bialek P, Kern B, Yang X, Schrock M, Sosic D, Hong N, et al. A twist code determines the onset of osteoblast differentiation. Developmental cell. 2004;6(3):423-35.
69. Sharma VP, Fenwick AL, Brockop MS, McGowan SJ, Goos JA, Hoogeboom AJ, et al. Mutations in TCF12, encoding a basic helix-loop-helix partner of TWIST1, are a frequent cause of coronal craniosynostosis. Nature genetics. 2013;45(3):304-7.
70. el Ghouzzi V, Le Merrer M, Perrin-Schmitt F, Lajeunie E, Benit P, Renier D, et al. Mutations of the TWIST gene in the Saethre-Chotzen syndrome. Nature genetics. 1997;15(1):42-6.
71. Cai J, Goodman BK, Patel AS, Mulliken JB, Van Maldergem L, Hoganson GE, et al. Increased risk for developmental delay in Saethre-Chotzen syndrome is associated with TWIST deletions: an improved strategy for TWIST mutation screening. Human genetics. 2003;114(1):68-76.
72. Satokata I, Ma L, Ohshima H, Bei M, Woo I, Nishizawa K, et al. Msx2 deficiency in mice causes pleiotropic defects in bone growth and ectodermal organ formation. Nature genetics. 2000;24(4):391-5.
73. Wilkie AOM, Tang Z, Elanko N, Walsh S, Twigg SRF, Hurst JA, et al. Functional haploinsufficiency of the human homeobox gene MSX2 causes defects in skull ossification. Nature genetics. 2000;24(4):387-90.
74. Klein R. Eph/ephrin signaling in morphogenesis, neural development and plasticity. Current opinion in cell biology. 2004;16(5):580-9.
75. Twigg SR, Kan R, Babbs C, Bochukova EG, Robertson SP, Wall SA, et al. Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(23):8652-7.
76. van den Elzen ME, Twigg SR, Goos JA, Hoogeboom AJ, van den Ouweland AM, Wilkie AO, et al. Phenotypes of craniofrontonasal syndrome in patients with a pathogenic mutation in EFNB1. European journal of human genetics : EJHG. 2014;22(8):995-1001.
77. Twigg SR, Babbs C, van den Elzen ME, Goriely A, Taylor S, McGowan SJ, et al. Cellular interference in craniofrontonasal syndrome: males mosaic for mutations in the X-linked EFNB1 gene are more severely affected than true hemizygotes. Human molecular genetics. 2013;22(8):1654-62.
78. Wieacker P, Wieland I. Clinical and genetic aspects of craniofrontonasal syndrome: towards resolving a genetic paradox. Molecular genetics and metabolism. 2005;86(1-2):110-6.
79. Moustakas A, Heldin CH. The regulation of TGFbeta signal transduction. Development (Cambridge, England). 2009;136(22):3699-714.
80. Wharton K, Derynck R. TGFbeta family signaling: novel insights in development and disease. Development (Cambridge, England). 2009;136(22):3691-7.

81. Opperman LA, Adab K, Gakunga PT. Transforming growth factor-beta 2 and TGF-beta 3 regulate fetal rat cranial suture morphogenesis by regulating rates of cell proliferation and apoptosis. Developmental dynamics : an official publication of the American Association of Anatomists. 2000;219(2):237-47.
82. Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nature genetics. 2005;37(3):275-81.
83. Opperman LA, Fernandez CR, So S, Rawlins JT. Erk1/2 signaling is required for Tgf-beta 2-induced suture closure. Developmental dynamics : an official publication of the American Association of Anatomists. 2006;235(5):1292-9.
84. Warren SM, Brunet LJ, Harland RM, Economides AN, Longaker MT. The BMP antagonist noggin regulates cranial suture fusion. Nature. 2003;422(6932):625-9.
85. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annual review of cell and developmental biology. 2004;20:781-810.
86. Karsenty G. Transcriptional Control of Skeletogenesis. 2008;9(1):183-96.
87. Behr B, Longaker MT, Quarto N. Differential activation of canonical Wnt signaling determines cranial sutures fate: A novel mechanism for sagittal suture craniosynostosis. Developmental Biology. 2010;344(2):922-40.
88. Howe LR, Watanabe O, Leonard J, Brown AM. Twist is up-regulated in response to Wnt1 and inhibits mouse mammary cell differentiation. Cancer research. 2003;63(8):1906-13.
89. Reinhold MI, Kapadia RM, Liao Z, Naski MC. The Wnt-inducible transcription factor Twist1 inhibits chondrogenesis. The Journal of biological chemistry. 2006;281(3):1381-8.
90. Hill TP, Später D, Taketo MM, Birchmeier W, Hartmann C. Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Developmental cell. 2005;8(5):727-38.
91. Potter AB, Rhodes JL, Vega RA, Ridder T, Shiang R. Gene expression changes between patent and fused cranial sutures in a nonsyndromic craniosynostosis population. Eplasty. 2015;15:e12.
92. Çetinkaya A, Taşkıran E, Soyer T, Şimşek-Kiper P, Utine GE, Tunçbilek G, et al. Dermal fibroblast transcriptome indicates contribution of WNT signaling pathways in the pathogenesis of Apert syndrome. The Turkish journal of pediatrics. 2017;59(6):619-24.

93. ten Berge D, Brugmann SA, Helms JA, Nusse R. Wnt and FGF signals interact to coordinate growth with cell fate specification during limb development. Development (Cambridge, England). 2008;135(19):3247-57.
94. Pan A, Chang L, Nguyen A, James AW. A review of hedgehog signaling in cranial bone development. Front Physiol. 2013;4:61-.
95. McDonald-McGinn DM, Feret H, Nah HD, Bartlett SP, Whitaker LA, Zackai EH. Metopic craniosynostosis due to mutations in GLI3: A novel association. American journal of medical genetics Part A. 2010;152a(7):1654-60.
96. Zollino M, Lattante S, Orteschi D, Frangella S, Doronzio PN, Contaldo I, et al. Syndromic Craniosynostosis Can Define New Candidate Genes for Suture Development or Result from the Non-specifc Effects of Pleiotropic Genes: Rasopathies and Chromatinopathies as Examples. Frontiers in neuroscience. 2017;11:587.
97. Bjornsson HT. The Mendelian disorders of the epigenetic machinery. Genome research. 2015;25(10):1473-81.
98. Mossink B, Negwer M, Schubert D, Nadif Kasri N. The emerging role of chromatin remodelers in neurodevelopmental disorders: a developmental perspective. Cell Mol Life Sci. 2021;78(6):2517-63.
99. Siakallis L, Tan AP, Chorbachi R, Mankad K. A unique case of CHARGE syndrome with craniosynostosis. Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2019;35(1):11-3.
100. Hersh JH, Groom KR, Yen FF, Verdi GD. Changing phenotype in Floating-Harbor syndrome. American journal of medical genetics. 1998;76(1):58-61.
101. Massimi L, Bianchi F, Frassanito P, Calandrelli R, Tamburrini G, Caldarelli M. Imaging in craniosynostosis: when and what? Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2019;35(11):2055-69.
102. Mathijssen IM. Guideline for Care of Patients With the Diagnoses of Craniosynostosis: Working Group on Craniosynostosis. The Journal of craniofacial surgery. 2015;26(6):1735-807.
103. Koboldt DC, Ding L, Mardis ER, Wilson RK. Challenges of sequencing human genomes. Brief Bioinform. 2010;11(5):484-98.
104. Vigeland MD, Gjotterud KS, Selmer KK. FILTUS: a desktop GUI for fast and efficient detection of disease-causing variants, including a novel autozygosity detector. Bioinformatics (Oxford, England). 2016;32(10):1592-4.
105. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics

and Genomics and the Association for Molecular Pathology. Genetics in medicine : official journal of the American College of Medical Genetics. 2015;17(5):405-24.
106. Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. American journal of human genetics. 2017;100(2):267-80.
107. Sakamoto H, Matsusaka Y, Kunihiro N, Imai K. Physiological Changes and Clinical Implications of Syndromic Craniosynostosis. J Korean Neurosurg Soc. 2016;59(3):204-13.
108. Ndoro S. Effective multidisciplinary working: the key to high-quality care. Br J Nurs. 2014;23(13):724-7.
109. Eide PK, Helseth E, Due-Tonnessen B, Lundar T. Assessment of continuous intracranial pressure recordings in childhood craniosynostosis. Pediatric neurosurgery. 2002;37(6):310-20.
110. Judy BF, Swanson JW, Yang W, Storm PB, Bartlett SP, Taylor JA, et al. Intraoperative intracranial pressure monitoring in the pediatric craniosynostosis population. Journal of neurosurgery Pediatrics. 2018;22(5):475-80.
111. Proctor MR, Meara JG. A review of the management of single-suture craniosynostosis, past, present, and future. Journal of neurosurgery Pediatrics. 2019;24(6):622-31.
112. Kajdic N, Spazzapan P, Velnar T. Craniosynostosis - Recognition, clinical characteristics, and treatment. Bosn J Basic Med Sci. 2018;18(2):110-6.
113. Esparza J, Hinojosa J. Complications in the surgical treatment of craniosynostosis and craniofacial syndromes: apropos of 306 transcranial procedures. Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2008;24(12):1421-30.
114. Delye HHK, Borstlap WA, van Lindert EJ. Endoscopy-assisted craniosynostosis surgery followed by helmet therapy. Surgical neurology international. 2018;9:59.
115. Chong S, Wang KC, Phi JH, Lee JY, Kim SK. Minimally Invasive Suturectomy and Postoperative Helmet Therapy : Advantages and Limitations. J Korean Neurosurg Soc. 2016;59(3):227-32.
116. Meling TR, Due-Tonnessen BJ, Hogevold HE, Skjelbred P, Arctander K. Monobloc distraction osteogenesis in pediatric patients with severe syndromal craniosynostosis. The Journal of craniofacial surgery. 2004;15(6):990-1000; discussion 1.
117. Elliott AM, Friedman JM. The importance of genetic counselling in genome-wide sequencing. Nature reviews Genetics. 2018;19(12):735-6.
118. Goriely A, Lord H, Lim J, Johnson D, Lester T, Firth HV, et al. Germline and somatic mosaicism for FGFR2 mutation in the mother of a child with

Crouzon syndrome: Implications for genetic testing in "paternal age-effect" syndromes. American journal of medical genetics Part A. 2010;152a(8):2067-73.
119. Norway S. Oslo: Statisktisk sentralbyrå/Statistics Norway; [Available from: https://www.ssb.no/befolkning/statistikker/fodte.
120. Tønne E, Due-Tønnessen BJ, Mero IL, Wiig US, Kulseth MA, Vigeland MD, et al. Benefits of clinical criteria and high-throughput sequencing for diagnosing children with syndromic craniosynostosis. European journal of human genetics : EJHG. 2020.
121. Large-scale discovery of novel genetic causes of developmental disorders. Nature. 2015;519(7542):223-8.
122. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic acids research. 2019;47(D1):D886-d94.
123. Dean A, Sullivan K, Soe M. R by C table. OpenEpi: Open Source Epidemiologic Statistics for Public Health [
124. Gumus E. Extending the phenotype of Xia-Gibbs syndrome in a two-year-old patient with craniosynostosis with a novel de novo AHDC1 missense mutation. European journal of medical genetics. 2020;63(1):103637.
125. Dinwiddie DL, Soden SE, Saunders CJ, Miller NA, Farrow EG, Smith LD, et al. De novo frameshift mutation in ASXL3 in a patient with global developmental delay, microcephaly, and craniofacial anomalies. BMC medical genomics. 2013;6:32.
126. Bostwick BL, McLean S, Posey JE, Streff HE, Gripp KW, Blesson A, et al. Phenotypic and molecular characterisation of CDK13-related congenital heart defects, dysmorphic facial features and intellectual developmental disorders. Genome medicine. 2017;9(1):73.
127. Davis AA, Haredy MM, Huey J, Scanga H, Zuccoli G, Pollack IF, et al. Syndromic and Systemic Diagnoses Associated With Isolated Sagittal Synostosis. Plast Reconstr Surg Glob Open. 2019;7(12):e2540.
128. Volpi S, Yamazaki Y, Brauer PM, van Rooijen E, Hayashida A, Slavotinek A, et al. EXTL3 mutations cause skeletal dysplasia, immune deficiency, and developmental delay. J Exp Med. 2017;214(3):623-37.
129. Grabb PA, Albright AL, Zitelli BJ. Multiple suture synostosis, macrocephaly, and intracranial hypertension in a child with alpha-D-mannosidase deficiency. Case report. Journal of neurosurgery. 1995;82(4):647-9.
130. Klaassens M, Morrogh D, Rosser EM, Jaffer F, Vreeburg M, Bok LA, et al. Malan syndrome: Sotos-like overgrowth with de novo NFIX sequence variants and deletions in six new patients and a review of the literature. European journal of human genetics : EJHG. 2015;23(5):610-5.

131. Haijes HA, Koster MJE, Rehmann H, Li D, Hakonarson H, Cappuccio G, et al. De Novo Heterozygous POLR2A Variants Cause a Neurodevelopmental Syndrome with Profound Infantile-Onset Hypotonia. American journal of human genetics. 2019;105(2):283-301.
132. Hamilton MJ, Caswell RC, Canham N, Cole T, Firth HV, Foulds N, et al. Heterozygous mutations affecting the protein kinase domain of CDK13 cause a syndromic form of developmental delay and intellectual disability. Journal of medical genetics. 2018;55(1):28-38.
133. Tønne E, Due-Tønnessen BJ, Vigeland MD, Amundsen SS, Ribarska T, Åsten PM, et al. Whole-exome sequencing in syndromic craniosynostosis increases diagnostic yield and identifies candidate genes in osteogenic signaling pathways. American journal of medical genetics Part A. 2022.
134. Magnus P BL. Epidemiologi (3.ed). Oslo: Gyldendal Norsk Forlag AS; 2003.
135. Di Rocco F, Arnaud E, Meyer P, Sainte-Rose C, Renier D. Focus session on the changing "epidemiology" of craniosynostosis (comparing two quinquennia: 1985-1989 and 2003-2007) and its impact on the daily clinical practice: a review from Necker Enfants Malades. Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2009;25(7):807-11.
136. Lajeunie E, Le Merrer M, Bonaiti-Pellie C, Marchac D, Renier D. Genetic study of scaphocephaly. American journal of medical genetics. 1996;62(3):282-5.
137. Lattanzi W, Bukvic N, Barba M, Tamburrini G, Bernardini C, Michetti F, et al. Genetic basis of single-suture synostoses: genes, chromosomes and clinical implications. Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2012;28(9):1301-10.
138. Wang JC, Nagy L, Demke JC. Syndromic Craniosynostosis. Facial Plast Surg Clin North Am. 2016;24(4):531-43.
139. Lajeunie E, Le Merrer M, Bonaiti-Pellie C, Marchac D, Renier D. Genetic study of nonsyndromic coronal craniosynostosis. American journal of medical genetics. 1995;55(4):500-4.
140. Greenwood J, Flodman P, Osann K, Boyadjiev SA, Kimonis V. Familial incidence and associated symptoms in a population of individuals with nonsyndromic craniosynostosis. Genetics in medicine : official journal of the American College of Medical Genetics. 2014;16(4):302-10.
141. Kalantar-Hormozi H, Abbaszadeh-Kasbi A, Sharifi G, Davai NR, Kalantar-Hormozi A. Incidence of Familial Craniosynostosis Among Patients with Nonsyndromic Craniosynostosis. The Journal of craniofacial surgery. 2019.
142. Timberlake AT, Persing JA. Genetics of Nonsyndromic Craniosynostosis. Plastic and reconstructive surgery. 2018;141(6):1508-16.

143. Boyadjiev SA. Genetic analysis of non-syndromic craniosynostosis. Orthod Craniofac Res. 2007;10(3):129-37.
144. Wieland I, Weidner C, Ciccone R, Lapi E, McDonald-McGinn D, Kress W, et al. Contiguous gene deletions involving EFNB1, OPHN1, PJA1 and EDA in patients with craniofrontonasal syndrome. Clin Genet. 2007;72(6):506-16.
145. Goos JA, Fenwick AL, Swagemakers SM, McGowan SJ, Knight SJ, Twigg SR, et al. Identification of Intragenic Exon Deletions and Duplication of TCF12 by Whole Genome or Targeted Sequencing as a Cause of TCF12-Related Craniosynostosis. Human mutation. 2016;37(8):732-6.
146. Faust HS. A cause without an effect? Primary prevention and causation. J Med Philos. 2013;38(5):539-58.
147. Strande NT, Riggs ER, Buchanan AH, Ceyhan-Birsoy O, DiStefano M, Dwight SS, et al. Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource. American journal of human genetics. 2017;100(6):895-906.
148. Posey JE, O'Donnell-Luria AH, Chong JX, Harel T, Jhangiani SN, Coban Akdemir ZH, et al. Insights into genetics, human biology and disease gleaned from family based genomic studies. Genetics in medicine : official journal of the American College of Medical Genetics. 2019;21(4):798-812.
149. Clark VE, Harmancı AS, Bai H, Youngblood MW, Lee TI, Baranoski JF, et al. Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. Nature genetics. 2016;48(10):1253-9.
150. Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, et al. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database : the journal of biological databases and curation. 2020;2020.
151. Law MJ, Lower KM, Voon HP, Hughes JR, Garrick D, Viprakasit V, et al. ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size-dependent manner. Cell. 2010;143(3):367-78.
152. Srivastava A, Ritesh KC, Tsan YC, Liao R, Su F, Cao X, et al. De novo dominant ASXL3 mutations alter H2A deubiquitination and transcription in Bainbridge-Ropers syndrome. Human molecular genetics. 2016;25(3):597-608.
153. Szczepanski AP, Zhao Z, Sosnowski T, Goo YA, Bartom ET, Wang L. ASXL3 bridges BRD4 to BAP1 complex and governs enhancer activity in small cell lung cancer. Genome medicine. 2020;12(1):63.
154. Lichtig H, Artamonov A, Polevoy H, Reid CD, Bielas SL, Frank D. Modeling Bainbridge-Ropers Syndrome in Xenopus laevis Embryos. Front Physiol. 2020;11:75.

155. Venero Galanternik M, Kramer KL, Piotrowski T. Heparan Sulfate Proteoglycans Regulate Fgf Signaling and Cell Polarity during Collective Cell Migration. Cell Rep. 2015;10(3):414-28.
156. Venero Galanternik M, Lush ME, Piotrowski T. Glypican4 modulates lateral line collective cell migration non cell-autonomously. Dev Biol. 2016;419(2):321-35.
157. Singh PNP, Yadav US, Azad K, Goswami P, Kinare V, Bandyopadhyay A. NFIA and GATA3 are crucial regulators of embryonic articular cartilage differentiation. Development (Cambridge, England). 2018;145(2).
158. Xie Y, Zhang L, Gao Y, Ge W, Tang P. The Multiple Roles of Microrna-223 in Regulating Bone Metabolism. Molecules (Basel, Switzerland). 2015;20(10):19433-48.
159. Wu X, Wang X, Shan L, Zhou J, Zhang X, Zhu E, et al. High-mobility group AT-Hook 1 mediates the role of nuclear factor I/X in osteogenic differentiation through activating canonical Wnt signaling. Stem Cells. 2021;39(10):1349-61.
160. Timberlake AT, Jin SC, Nelson-Williams C, Wu R, Furey CG, Islam B, et al. Mutations in TFAP2B and previously unimplicated genes of the BMP, Wnt, and Hedgehog pathways in syndromic craniosynostosis. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(30):15116-21.
161. Osborn AJ, Roberts RM, Mathias JL, Anderson PJ, Flapper WJ. Cognitive, behavioral and psychological functioning in children with metopic synostosis: a meta-analysis examining the impact of surgical status. Child Neuropsychol. 2019;25(2):263-77.
162. Da Costa AC, Anderson VA, Savarirayan R, Wrennall JA, Chong DK, Holmes AD, et al. Neurodevelopmental functioning of infants with untreated single-suture craniosynostosis during early infancy. Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery. 2012;28(6):869-77.
163. Garza RM, Khosla RK. Nonsyndromic craniosynostosis. Semin Plast Surg. 2012;26(2):53-63.
164. Becker DB, Petersen JD, Kane AA, Cradock MM, Pilgram TK, Marsh JL. Speech, cognitive, and behavioral outcomes in nonsyndromic craniosynostosis. Plastic and reconstructive surgery. 2005;116(2):400-7.
165. Al-Namnam NM, Jayash SN, Hariri F, Rahman ZAA, Alshawsh MA. Insights and future directions of potential genetic therapy for Apert syndrome: A systematic review. Gene Ther. 2021;28(10-11):620-33.
166. Rachwalski M, Khonsari RH, Paternoster G. Current Approaches in the Development of Molecular and Pharmacological Therapies in Craniosynostosis Utilizing Animal Models. Molecular syndromology. 2019;10(1-2):115-23.

167. Kommalapati A, Tella SH, Borad M, Javle M, Mahipal A. FGFR Inhibitors in Oncology: Insight on the Management of Toxicities in Clinical Practice. Cancers (Basel). 2021;13(12).

Appendices

I Custom designed gene panel

II Extended trio-based gene panel

Custom designed gene panel

#Number of genes: 72

#Gene	Transcript	Omim (phenotype)			
ALPL	NM_000478.4	241510	MSX2	NM_002449.4	604757
ALX3	NM_006492.2	136760	MYH3	NM_002470.3	178110
ALX4	NM_021926.3	615529	P4HB	NM_000918.3	112240
ASXL1	NM_015338.5	605039	PHEX	NM_000444.5	307800
ATR	NM_001184.3	210600	POR	NM_000941.2	201750
BMP4	NM_001202.3	600625	RAB23	NM_183227.2	201000
CCBE1	NM_133459.3	235510	RECQL4	NM_004260.3	266280
CDC45	NM_001178010.2	617063	RSPRY1	NM_133368.2	616723
CEP120	NM_153223.3	616300	RUNX2	NM_001024630.3	119600
COLEC11	NM_024027.4	265050	SCARF2	NM_153334.6	600920
CTSK	NM_000396.3	265800	SH3PXD2B	NM_001017995.2	249420
CYP26B1	NM_019885.3	614416	SKI	NM_003036.3	182212
DPH1	NM_001383.3	616901	SMO	NM_005631.4	601707
EFNA4	NM_005227.2		SON	NM_032195.2	617140
EFNB1	NM_004429.4	304110	SPECC1L	NM_015330.4	145410
ERF	NM_006494.3	600775	STAT3	NM_139276.2	147060
ESCO2	NM_001017420.2	268300	TCF12	NM_207036.1	615314
FAM20C	NM_020223.3	259775	TGFBR1	NM_004612.2	609192
FAM58A	NM_152274.4	300707	TGFBR2	NM_003242.5	610168
FBN1	NM_000138.4	608328	TMCO1	NM_019026.4	213980
FGFR1	NM_023110.2	190440	TWIST1	NM_000474.3	123100
FGFR2	NM_000141.4	609579	WDR19	NM_025132.3	616307
FGFR3	NM_000142.4	602849	WDR35	NM_001006657.1	613610
FREM1	NM_144966.5	614485	ZEB2	NM_014795.3	235730
GLI3	NM_000168.5	175700	ZIC1	NM_003412.3	616602
GNAS	NM_000516.4	612462			
GPC3	NM_004484.3	312870			
GTF2E2	NM_002095.4	616943			
HNRNPK	NM_031262.2	616580			
HUWE1	NM_031407.6	300706			
IDS	NM_000202.6	309900			
IDUA	NM_000203.4	607015			
IFT122	NM_052985.3	218330			
IFT140	NM_014714.3	266920			
IFT43	NM_052873.2	614099			
IHH	NM_002181.3	607778			
IL11RA	NM_001142784.2	614188			
IMPAD1	NM_017813.4	614078			
IRX5	NM_005853.5	611174			
JAG1	NM_000214.2	118450			
KAT6A	NM_006766.3	616268			
KMT2D	NM_003482.3	147920			
KRAS	NM_004985.4	615278			
LMX1B	NM_002316.3	161200			
LRP5	NM_002335.3	607636			
MASP1	NM_139125.3	257920			
MEGF8	NM_001410.2	614976			

Extended trio-based gene panel

n=1570			AIRE	NM_000383.3	607358	ARHGAP31	NM_020754.3	610911
Gene	**Transcript**	**Omim**	AK2	NM_001625.3	103020	ARHGEF6	NM_004840.2	300267
AAAS	NM_015665.5	605378	AKR1D1	NM_005989.3	604741	ARHGEF9	NM_015185.2	300429
AARS	NM_001605.2	601065	AKT1	NM_005163.2	164730	ARID1A	NM_006015.4	603024
AASS	NM_005763.3	605113	AKT3	NM_005465.4	611223	ARID1B	NM_020732.3	614556
ABAT	NM_020686.5	137150	ALAD	NM_000031.5	125270	ARID2	NM_152641.3	609539
ABCB11	NM_003742.2	603201	ALDH18A1	NM_002860.3	138250	ARL6	NM_177976.3	608845
ABCB7	NM_004299.5	300135	ALDH1A3	NM_000693.3	600463	ARMC4	NM_018076.4	615408
ABCC6	NM_001171.5	603234	ALDH3A2	NM_000382.2	609523	ARMC9	NM_025139.5	617612
ABCC9	NM_005691.3	601439	ALDH4A1	NM_003748.3	606811	ARSA	NM_000487.5	607574
ABCD1	NM_000033.3	300371	ALDH5A1	NM_001080.3	610045	ARSB	NM_000046.3	611542
ABCD4	NM_005050.3	603214	ALDH7A1	NM_001182.4	107323	ARSE	NM_000047.2	300180
ABHD5	NM_016006.4	604780	ALDOA	NM_000034.3	103850	ARX	NM_139058.2	300382
ABL1	NM_005157.5	189980	ALDOB	NM_000035.3	612724	ASAH1	NM_177924.4	613468
ACAD9	NM_014049.4	611103	ALG1	NM_019109.4	605907	ASL	NM_000048.3	608310
ACADM	NM_000016.5	607008	ALG11	NM_001004127.2	613666	ASPA	NM_000049.2	608034
ACADS	NM_000017.3	606885	ALG12	NM_024105.3	607144	ASPH	NM_004318.3	600582
ACADVL	NM_000018.3	609575	ALG13	NM_001099922.2	300776	ASPM	NM_018136.4	605481
ACAN	NM_013227.3	155760	ALG2	NM_033087.3	607905	ASS1	NM_000050.4	603470
ACAT1	NM_000019.3	607809	ALG3	NM_005787.5	608750	ASXL1	NM_015338.5	612990
ACO2	NM_001098.2	100850	ALG6	NM_013339.3	604566	ASXL2	NM_018263.5	612991
ACOX1	NM_004035.6	609751	ALG8	NM_024079.4	608103	ASXL3	NM_030632.2	615115
ACP5	NM_001111035.2	171640	ALG9	NM_024740.2	606941	ATIC	NM_004044.6	601731
ACSL4	NM_004458.2	300157	ALMS1	NM_015120.4	606844	ATM	NM_000051.3	607585
ACTA1	NM_001100.3	102610	ALPL	NM_000478.5	171760	ATP13A2	NM_022089.3	610513
ACTA2	NM_001613.2	102620	ALS2	NM_020919.3	606352	ATP1A3	NM_152296.4	182350
ACVR1	NM_001105.4	102576	ALX1	NM_006982.2	601527	ATP6AP2	NM_005765.2	300556
ACVR2B	NM_001106.3	602730	ALX3	NM_006492.2	606014	ATP6V0A2	NM_012463.3	611716
ACY1	NM_000666.2	104620	ALX4	NM_021926.3	605420	ATP6V1B1	NM_001692.3	
ADA	NM_000022.3	608958	AMER1	NM_152424.3	300647	ATP6V1B2	NM_001693.3	606939
ADAR	NM_001111.4	146920	AMPD2	NM_001257360.1	102771	ATP7A	NM_000052.6	300011
ADCK3	NM_020247.4	606980	AMT	NM_000481.3	238310	ATP8B1	NM_005603.4	602397
ADK	NM_001123.3	102750	ANKH	NM_054027.4	605145	ATR	NM_001184.3	601215
ADNP	NM_015339.4	611386	ANKRD11	NM_013275.5	611192	ATRX	NM_000489.4	300032
ADRA2B	NM_000682.6	104260	ANKRD26	NM_014915.2	610855	AUH	NM_001698.2	617887
ADSL	NM_000026.3	608222	ANO5	NM_213599.2	608662	AUTS2	NM_015570.3	
AFF2	NM_002025.3	300806	ANTXR1	NM_032208.2	606410	B3GALNT2	NM_152490.4	610194
AFF3	NM_002285.2		AP1S2	NM_003916.4	300629	B3GALT6	NM_080605.3	615291
AFF4	NM_014423.3	604417	AP3B2	NM_004644.4	602166	B3GNT1	NM_006876.2	605517
AFG3L2	NM_006796.2	604581	AP4B1	NM_006594.4	607245	B4GALT7	NM_007255.2	604327
AGA	NM_000027.3	613228	AP4E1	NM_007347.4	607244	B9D1	NM_015681.4	614144
AGK	NM_018238.3	610345	AP4M1	NM_004722.3	602296	BANF1	NM_001143985.1	603811
AGL	NM_000642.2	610860	AP4S1	NM_007077.4	607243	BBS1	NM_024649.4	209901
AGPS	NM_003659.3	603051	APOA1BP	NM_144772.2	608862	BBS10	NM_024685.3	610148
AGXT	NM_000030.2	604285	APOPT1	NM_032374.4	616003	BBS12	NM_152618.2	610683
AHDC1	NM_001029882.3	615790	APTX	NM_175073.2	606350	BBS2	NM_031885.3	606151
AHI1	NM_017651.4	608894	AR	NM_000044.4	313700	BBS4	NM_033028.4	600374
AIFM1	NM_004208.3	300169	ARCN1	NM_001655.4	600820	BBS5	NM_152384.2	603650
AIMP1	NM_004757.3	603605	ARFGEF2	NM_006420.2	605371	BBS7	NM_176824.2	607590
AIPL1	NM_014336.4	604392	ARG1	NM_000045.3	608313	BBS9	NM_198428.2	607968

Extended trio-based gene panel

BCAP31	NM_001139441.1	300398	CASC5	NM_170589.4	609173	CHM	NM_000390.3	300390
BCKDHA	NM_000709.3	608348	CASK	NM_003688.3	300172	CHMP1A	NM_002768.4	164010
BCKDHB	NM_183050.3	248611	CBL	NM_005188.3	165360	CHRDL1	NM_001143981.1	300350
BCL11A	NM_022893.3	606557	CBS	NM_000071.2	613381	CHRNA1	NM_000079.3	100690
BCOR	NM_017745.5	300485	CC2D1A	NM_017721.4	610055	CHRNA4	NM_000744.6	118504
BCS1L	NM_004328.4	603647	CC2D2A	NM_001080522.2	612013	CHRNB2	NM_000748.2	118507
BFSP2	NM_003571.3	603212	CCBE1	NM_133459.3	612753	CHRNG	NM_005199.4	100730
BGN	NM_001711.5	301870	CCDC103	NM_213607.2	614677	CHST14	NM_130468.3	608429
BHLHA9	NM_001164405.1	615416	CCDC114	NM_144577.3	615038	CHST3	NM_004273.4	603799
BICD2	NM_001003800.1	609797	CCDC115	NM_032357.3	613734	CHSY1	NM_014918.4	608183
BIN1	NM_139343.2	601248	CCDC151	NM_145045.4	615956	CHUK	NM_001278.4	600664
BLM	NM_000057.3	604610	CCDC22	NM_014008.4	300859	CIB2	NM_006383.3	605564
BLOC1S6	NM_012388.3	604310	CCDC39	NM_181426.1	613798	CISD2	NM_001008388.4	611507
BMP2	NM_001200.3	112261	CCDC40	NM_017950.3	613799	CIT	NM_001206999.1	605629
BMP4	NM_001202.5	112262	CCDC41	NM_016122.2	615847	CKAP2L	NM_152515.4	616174
BMPER	NM_133468.4	608699	CCDC65	NM_033124.4	611088	CLCN4	NM_001830.3	302910
BMPR1B	NM_001203.2	603248	CCDC78	NM_001031737.2	614666	CLCN7	NM_001287.5	602727
BOLA3	NM_212552.2	613183	CCDC8	NM_032040.4	614145	CLDN19	NM_148960.2	610036
BPTF	NM_004459.6	601819	CCDC88C	NM_001080414.3	611204	CLMP	NM_024769.3	611693
BRAF	NM_004333.4	164757	CCND2	NM_001759.3	123833	CLN3	NM_001042432.1	607042
BRAT1	NM_152743.3	614506	CCNO	NM_021147.4	607752	CLN5	NM_006493.2	608102
BRCA1	NM_007294.3	113705	CD151	NM_004357.4	602243	CLN6	NM_017882.2	606725
BRCA2	NM_000059.3	600185	CD96	NM_198196.2	606037	CLN8	NM_018941.3	607837
BRIP1	NM_032043.2	605882	CDC45	NM_001178010.2		CLP1	NM_006831.2	607328
BRPF1	NM_001003694.1	602410	CDC6	NM_001254.3	602627	CLPB	NM_001258394.2	616254
BRWD3	NM_153252.4	300553	CDH1	NM_004360.4	192090	CLPP	NM_006012.2	601119
BSND	NM_057176.2	606412	CDH15	NM_004933.2	114019	CLTC	NM_001288653.1	118955
BTD	NM_000060.4	608306	CDH23	NM_022124.5	605516	CNKSR2	NM_001168647.2	300724
BUB1B	NM_001211.5	602860	CDH3	NM_001793.5	114021	CNOT3	NM_014516.3	604910
C12orf57	NM_138425.3	615140	CDK13	NM_031267.3	603309	CNTNAP1	NM_003632.2	602346
C12orf65	NM_152269.4	613541	CDK5	NM_004935.3	123831	CNTNAP2	NM_014141.5	604569
C1QBP	NM_001212.3	601269	CDK5RAP2	NM_018249.5	608201	COASY	NM_025233.6	609855
C1QTNF5	NM_015645.4	608752	CDKL5	NM_003159.2	300203	COG1	NM_018714.2	606973
C21orf2	NM_004928.2	603191	CDKN1C	NM_000076.2	600856	COG4	NM_015386.2	606976
C21orf59	NM_021254.3	615494	CDON	NM_016952.4	608707	COG5	NM_006348.3	606821
C2CD3	NM_015531.5	615944	CDT1	NM_030928.3	605525	COG7	NM_153603.3	606978
C2orf71	NM_001029883.2	613425	CENPJ	NM_018451.4	609279	COG8	NM_032382.4	606979
C4orf26	NM_178497.3	614829	CEP104	NM_014704.3	616690	COL10A1	NM_000493.3	120110
C5orf42	NM_023073.3	614571	CEP135	NM_025009.4	611423	COL11A1	NM_001854.3	120280
C8orf37	NM_177965.3	614477	CEP152	NM_014985.3	613529	COL11A2	NM_080680.2	120290
CA2	NM_000067.2	611492	CEP290	NM_025114.3	610142	COL13A1	NM_001130103.1	120350
CA8	NM_004056.5	114815	CEP41	NM_018718.2	610523	COL18A1	NM_130445.3	120328
CACNA1A	NM_001127221.1	601011	CEP57	NM_014679.4	607951	COL1A1	NM_000088.3	120150
CACNA1C	NM_000719.6	114205	CEP63	NM_025180.3	614724	COL25A1	NM_198721.3	610004
CACNA1D	NM_000720.3	114206	CFL2	NM_021914.7	601443	COL2A1	NM_001844.4	120140
CAD	NM_004341.4	601883	CHAMP1	NM_001164144.2	616327	COL4A1	NM_001845.5	120130
CAMK2A	NM_015981.3	114078	CHD2	NM_001271.3	602119	COL4A2	NM_001846.3	120090
CAMK2B	NM_001220.4	607707	CHD4	NM_001273.3	603277	COL4A3	NM_000091.4	120070
CAMTA1	NM_015215.3	611501	CHD7	NM_017780.3	608892	COL4A3BP	NM_001130105.1	604677
CARS2	NM_024537.3	612800	CHD8	NM_001170629.1	610528	COL4A4	NM_000092.4	120131

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COL6A1	NM_001848.2	120220	CYC1	NM_001916.4	123980	DOCK6	NM_020812.3	614194
COL6A3	NM_004369.3	120250	CYP1B1	NM_000104.3	601771	DOCK7	NM_001271999.1	615730
COL9A1	NM_001851.4	120210	CYP2U1	NM_183075.2	610670	DOCK8	NM_203447.3	611432
COL9A2	NM_001852.3	120260	DAG1	NM_004393.5	128239	DOLK	NM_014908.3	610746
COL9A3	NM_001853.3	120270	DARS	NM_001349.3	603084	DPAGT1	NM_001382.3	191350
COLEC10	NM_006438.4	607620	DARS2	NM_018122.4	610956	DPM1	NM_003859.2	603503
COLEC11	NM_024027.4	612502	DBT	NM_001918.3	248610	DPM3	NM_153741.1	605951
COMP	NM_000095.2	600310	DCAF17	NM_025000.3	612515	DRC1	NM_145038.4	615288
COQ2	NM_015697.7	609825	DCC	NM_005215.3	120470	DSG1	NM_001942.3	125670
COQ4	NM_016035.4	612898	DCDC2	NM_016356.4	605755	DSPP	NM_014208.3	125485
COQ9	NM_020312.3	612837	DCHS1	NM_003737.3	603057	DSTYK	NM_015375.2	612666
COX10	NM_001303.3	602125	DCX	NM_178153.2	300121	DVL1	NM_004421.2	601365
COX15	NM_004376.6	603646	DDB2	NM_000107.2	600811	DVL3	NM_004423.3	601368
COX6B1	NM_001863.4	124089	DDC	NM_000790.3	107930	DYM	NM_017653.3	607461
COX7B	NM_001866.2	300885	DDHD1	NM_001160147.1	614603	DYNC1H1	NM_001376.4	600112
CPAMD8	NM_015692.3	608841	DDHD2	NM_015214.2	615003	DYNC2H1	NM_001080463.1	603297
CPS1	NM_001875.4	608307	DDOST	NM_005216.4	602202	DYRK1A	NM_001396.4	600855
CRADD	NM_003805.4	603454	DDR2	NM_006182.2	191311	DYX1C1	NM_130810.3	608706
CRB1	NM_201253.2	604210	DDX3X	NM_001193416.2	300160	EBF3	NM_001005463.2	605788
CRB2	NM_173689.6	609720	DDX59	NM_001031725.5	615464	EBP	NM_006579.2	300205
CRBN	NM_016302.3	609262	DEAF1	NM_021008.3	602635	ECEL1	NM_004826.3	605896
CREBBP	NM_004380.2	600140	DENND5A	NM_015213.3	617278	EDA	NM_001399.4	300451
CRELD1	NM_015513.4	607170	DEPDC5	NM_001242896.1	614191	EDAR	NM_022336.3	604095
CRX	NM_000554.5	602225	DHCR24	NM_014762.3	606418	EDN1	NM_001955.4	131240
CRYAA	NM_000394.3	123580	DHCR7	NM_001360.2	602858	EDNRA	NM_001957.3	131243
CRYBA1	NM_005208.4	123610	DHDDS	NM_024887.3	608172	EDNRB	NM_000115.4	131244
CRYBA4	NM_001886.2	123631	DHFR	NM_000791.3	126060	EED	NM_003797.4	605984
CRYBB1	NM_001887.3	600929	DHODH	NM_001361.4	126064	EEF1A2	NM_001958.3	602959
CRYBB2	NM_000496.2	123620	DHTKD1	NM_018706.6	614984	EFNB1	NM_004429.4	300035
CRYBB3	NM_004076.4	123630	DHX30	NM_014966.3	616423	EFTUD2	NM_004247.3	603892
CRYGC	NM_020989.3	123680	DIS3L2	NM_152383.4	614184	EGR2	NM_000399.4	129010
CRYGD	NM_006891.3	123690	DISP1	NM_032890.4		EHMT1	NM_024757.4	607001
CSNK2A1	NM_001895.3	115440	DKC1	NM_001363.4	300126	EIF2AK3	NM_004836.6	604032
CSPP1	NM_024790.6	611654	DLAT	NM_001931.4	608770	EIF2S3	NM_001415.3	300161
CSTA	NM_005213.3	184600	DLD	NM_000108.4	238331	EIF4A3	NM_014740.3	608546
CSTB	NM_000100.3	601145	DLG3	NM_021120.3	601114	ELAC2	NM_018127.6	605367
CTC1	NM_025099.5	613129	DLG4	NM_001365.4	600966	ELMO2	NM_182764.2	606421
CTCF	NM_006565.3	604167	DLL1	NM_005618.3		ELN	NM_001278939.1	130160
CTDP1	NM_004715.4	604927	DLL3	NM_016941.3	602768	ELOVL4	NM_022726.3	605512
CTNNB1	NM_001904.3	116806	DLL4	NM_019074.3	605185	EMC1	NM_015047.2	616846
CTNND1	NM_001206885.1	601045	DMD	NM_004006.2	300377	EMG1	NM_006331.7	611531
CTNS	NM_004937.2	606272	DMP1	NM_004407.3	600980	EML1	NM_004434.2	602033
CTSA	NM_000308.3	613111	DMPK	NM_004409.4	605377	EMX2	NM_004098.3	600035
CTSD	NM_001909.4	116840	DNA2	NM_001080449.2	601810	ENPP1	NM_006208.2	173335
CTSK	NM_000396.3	601105	DNAAF3	NM_001256714.1	614566	EOGT	NM_173654.2	614789
CUL4B	NM_003588.3	300304	DNAH5	NM_001369.2	603335	EP300	NM_001429.3	602700
CUL7	NM_014780.4	609577	DNAJC12	NM_021800.2	606060	EPG5	NM_020964.2	615068
CUX2	NM_015267.3		DNM1	NM_004408.3	602377	ERCC1	NM_202001.2	126380
CWC27	NM_005869.3	617170	DNMT3A	NM_175629.2	602769	ERCC2	NM_000400.3	126340
CYB5R3	NM_000398.6	613213	DNMT3B	NM_006892.3	602900	ERCC3	NM_000122.1	133510

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ERCC4	NM_005236.2	133520	FGF12	NM_004113.5	601513	GABRA1	NM_000806.5	137160
ERCC5	NM_000123.3	133530	FGF3	NM_005247.2	164950	GABRB2	NM_000813.2	600232
ERCC6	NM_000124.3	609413	FGF8	NM_033163.3	600483	GABRB3	NM_000814.5	137192
ERCC6L2	NM_001010895.2	615667	FGF9	NM_002010.2	600921	GABRG2	NM_000816.3	137164
ERCC8	NM_000082.3	609412	FGFR1	NM_023110.2	136350	GAD1	NM_000817.2	605363
ERF	NM_006494.3	611888	FGFR2	NM_000141.4	176943	GALC	NM_000153.3	606890
ERLIN2	NM_007175.6	611605	FGFR3	NM_000142.4	134934	GALE	NM_000403.3	606953
ERMARD	NM_018341.2	615532	FH	NM_000143.3	606945	GALK1	NM_000154.1	604313
ESCO2	NM_001017420.2	609353	FHL1	NM_001449.4	300163	GALNS	NM_000512.4	612222
ETFA	NM_000126.3	608053	FIG4	NM_014845.5	609390	GALT	NM_000155.3	606999
ETFB	NM_001985.2	130410	FKBP14	NM_017946.3	614505	GAMT	NM_000156.5	601240
ETFDH	NM_004453.3	231675	FKRP	NM_024301.4	606596	GAS1	NM_002048.2	139185
ETHE1	NM_014297.4	608451	FKTN	NM_001079802.1	607440	GAS8	NM_001286209.1	605179
EVC	NM_153717.2	604831	FLAD1	NM_025207.4	610595	GATA2	NM_032638.4	137295
EVC2	NM_147127.4	607261	FLNA	NM_001456.3	300017	GATA4	NM_002052.4	600576
EXOSC3	NM_016042.3	606489	FLNB	NM_001457.3	603381	GATA6	NM_005257.5	601656
EXOSC8	NM_181503.2	606019	FLT4	NM_002020.4	136352	GATAD2B	NM_020699.3	614998
EXPH5	NM_015065.2	612878	FLVCR1	NM_014053.3	609144	GATM	NM_001482.2	602360
EXT1	NM_000127.2	608177	FLVCR2	NM_017791.2	610865	GBA2	NM_020944.2	609471
EXT2	NM_207122.1	608210	FMN2	NM_020066.4	606373	GCDH	NM_000159.3	608801
EYA1	NM_000503.5	601653	FMR1	NM_002024.5	309550	GCH1	NM_000161.2	600225
EZH2	NM_004456.4	601573	FN1	NM_212482.2	135600	GCSH	NM_004483.4	238330
FAH	NM_000137.2	613871	FOLR1	NM_016725.2	136430	GDF5	NM_000557.4	601146
FAM105B	NM_138348.5	615712	FOXC1	NM_001453.2	601090	GDF6	NM_001001557.3	601147
FAM111A	NM_022074.3	615292	FOXC2	NM_005251.2	602402	GDI1	NM_001493.2	300104
FAM126A	NM_032581.3	610531	FOXE1	NM_004473.3	602617	GFAP	NM_002055.4	137780
FAM134B	NM_001034850.2	613114	FOXE3	NM_012186.2	601094	GFER	NM_005262.2	600924
FAM161A	NM_032180.2	613596	FOXF1	NM_001451.2	601089	GFM1	NM_024996.5	606639
FAM20A	NM_017565.3	611062	FOXG1	NM_005249.4	164874	GHR	NM_000163.4	600946
FAM20C	NM_020223.3	611061	FOXL2	NM_023067.3	605597	GJA3	NM_021954.3	121015
FAM58A	NM_152274.4	300708	FOXN1	NM_003593.2	600838	GJA8	NM_005267.4	600897
FANCA	NM_000135.3	607139	FOXP1	NM_032682.5	605515	GJB2	NM_004004.5	121011
FANCB	NM_001018113.2	300515	FOXP2	NM_014491.3	605317	GJB3	NM_024009.2	603324
FANCC	NM_000136.2	613899	FOXP3	NM_014009.3	300292	GJC2	NM_020435.3	608803
FANCD2	NM_033084.4	613984	FOXRED1	NM_017547.3	613622	GK	NM_000167.5	300474
FANCE	NM_021922.2	613976	FRAS1	NM_025074.6	607830	GLB1	NM_000404.3	611458
FANCF	NM_022725.3	613897	FREM1	NM_144966.5	608944	GLDC	NM_000170.2	238300
FANCG	NM_004629.1	602956	FREM2	NM_207361.5	608945	GLDN	NM_181789.3	608603
FANCI	NM_001113378.1	611360	FRMD7	NM_194277.2	300628	GLE1	NM_001003722.1	603371
FANCL	NM_018062.3	608111	FRMPD4	NM_014728.3	300838	GLI2	NM_005270.4	165230
FANCM	NM_020937.3	609644	FRRS1L	NM_014334.3	604574	GLI3	NM_000168.5	165240
FAR1	NM_032228.5	616107	FTCD	NM_006657.2	606806	GLIS2	NM_032575.2	608539
FAT4	NM_024582.4	612411	FTL	NM_000146.3	134790	GLIS3	NM_152629.3	610192
FBN1	NM_000138.4	134797	FTO	NM_001080432.2	610966	GLMN	NM_053274.2	
FBN2	NM_001999.3	612570	FTSJ1	NM_012280.3	300499	GLUD1	NM_005271.4	138130
FBP1	NM_000507.3	611570	FUCA1	NM_000147.4	612280	GLUL	NM_002065.6	138290
FBXL4	NM_012160.4	605654	FYCO1	NM_024513.3	607182	GM2A	NM_000405.4	613109
FEZF1	NM_001160264.2	613301	FZD5	NM_003468.3	601723	GMNN	NM_001251989.1	602842
FGD1	NM_004463.2	300546	FZD6	NM_003506.3	603409	GMPPA	NM_205847.2	615495
FGF10	NM_004465.1	602115	GAA	NM_000152.4	606800	GMPPB	NM_021971.2	615320

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GNA11	NM_002067.4	139313	HEPACAM	NM_152722.4	611642	IFT80	NM_020800.2	611177
GNA14	NM_004297.3	604397	HESX1	NM_003865.2	601802	IGBP1	NM_001551.2	300139
GNAI1	NM_002069.5	139310	HEXA	NM_000520.5	606869	IGF1	NM_000618.4	147440
GNAI3	NM_006496.3	139370	HEXB	NM_000521.3	606873	IGF1R	NM_000875.4	147370
GNAO1	NM_020988.2	139311	HGSNAT	NM_152419.2	610453	IGF2	NM_000612.5	147470
GNAQ	NM_002072.4	600998	HIBCH	NM_014362.3	610690	IGFBP7	NM_001553.2	602867
GNAS	NM_000516.5	139320	HINT1	NM_005340.6	601314	IGHMBP2	NM_002180.2	600502
GNB1	NM_002074.4	139380	HIST1H1E	NM_005321.2	142220	IGSF1	NM_001170961.1	300137
GNB5	NM_016194.3	604447	HIST1H4C	NM_003542.3	602827	IHH	NM_002181.3	600726
GNPAT	NM_014236.3	602744	HIVEP2	NM_006734.3	143054	IKBKG	NM_001099857.2	300248
GNPTAB	NM_024312.4	607840	HLCS	NM_000411.6	609018	IL11RA	NM_001142784.2	600939
GNPTG	NM_032520.4		HMGCL	NM_000191.2	613898	IL1RAPL1	NM_014271.3	300206
GNS	NM_002076.3	607664	HMGCS2	NM_005518.3	600234	IMPAD1	NM_017813.4	614010
GORAB	NM_152281.2	607983	HMX1	NM_018942.2	142992	INPP5E	NM_019892.5	613037
GPAA1	NM_003801.3	603048	HNF1B	NM_000458.3	189907	INPP5K	NM_016532.3	607875
GPC3	NM_004484.3	300037	HNF4A	NM_175914.4	600281	INPPL1	NM_001567.3	600829
GPC6	NM_005708.4	604404	HNRNPH2	NM_001199974.1	300610	IQSEC2	NM_001111125.2	300522
GPR126	NM_020455.5		HNRNPU	NM_031844.2	602869	IRF6	NM_006147.3	607199
GPR179	NM_001004334.3	614515	HOXA1	NM_005522.4	142955	IRX5	NM_005853.5	606195
GPR56	NM_005682.6	604110	HOXA13	NM_000522.4	142959	ISPD	NM_001101426.3	614631
GPSM2	NM_013296.4	609245	HOXB1	NM_002144.3	142968	ITCH	NM_031483.6	606409
GPX4	NM_001039847.2	138322	HOXC13	NM_017410.2	142976	ITGA3	NM_002204.3	605025
GRHL2	NM_024915.3	608576	HOXD13	NM_000523.3	142989	ITGA7	NM_002206.2	600536
GRHL3	NM_198174.2	608317	HPD	NM_002150.2	609695	ITGA8	NM_003638.2	604063
GRIA3	NM_000828.4	305915	HPGD	NM_000860.5	601688	ITPR1	NM_002222.5	147265
GRIK2	NM_021956.4	138244	HPRT1	NM_000194.2	308000	IVD	NM_002225.3	607036
GRIN1	NM_007327.3	138249	HPS1	NM_000195.4	604982	JAG1	NM_000214.2	601920
GRIN2A	NM_000833.4	138253	HPSE2	NM_021828.4	613469	JAGN1	NM_032492.3	616012
GRIN2B	NM_000834.3	138252	HR	NM_005144.4	602302	JAK3	NM_000215.3	600173
GRIN2D	NM_000836.2	602717	HRAS	NM_005343.3	190020	JAM3	NM_032801.4	606871
GRM1	NM_001278066.1	604473	HSD17B10	NM_004493.2	300256	KANSL1	NM_001193466.1	612452
GRM6	NM_000843.3	604096	HSD17B4	NM_000414.3	601860	KARS	NM_001130089.1	601421
GSPT2	NM_018094.4	300418	HSD3B7	NM_025193.3	607764	KAT6A	NM_006766.4	601408
GTF2E2	NM_002095.5	189964	HSF4	NM_001538.3	602438	KAT6B	NM_012330.3	605880
GTF2H5	NM_207118.2	608780	HSPD1	NM_002156.4	118190	KATNB1	NM_005886.2	602703
GTPBP3	NM_133644.3	608536	HSPG2	NM_005529.6	142461	KBTBD13	NM_001101362.2	613727
GUCY2C	NM_004963.3	601330	HUWE1	NM_031407.6	300697	KCNA2	NM_001204269.1	176262
GUSB	NM_000181.3	611499	HYAL1	NM_153281.1	607071	KCNB1	NM_004975.3	600397
GZF1	NM_022482.4	613842	HYDIN	NM_001270974.2	610812	KCNC1	NM_001112741.1	176258
HACE1	NM_020771.3	610876	HYLS1	NM_145014.2	610693	KCNC3	NM_004977.2	176264
HADH	NM_005327.4		IARS	NM_002161.5	600709	KCNE1	NM_000219.5	176261
HADHA	NM_000182.4	600890	IDS	NM_000202.7	300823	KCNH1	NM_172362.2	603305
HAX1	NM_006118.3	605998	IDUA	NM_000203.4	252800	KCNJ10	NM_002241.4	602208
HCCS	NM_005333.4	300056	IER3IP1	NM_016097.4	609382	KCNJ11	NM_000525.3	600937
HCFC1	NM_005334.2	300019	IFIH1	NM_022168.3	606951	KCNJ6	NM_002240.4	600877
HCN1	NM_021072.3	602780	IFITM5	NM_001025295.2	614757	KCNMA1	NM_002247.3	600150
HDAC4	NM_006037.3	605314	IFT122	NM_052985.3	606045	KCNQ1	NM_000218.2	607542
HDAC8	NM_018486.2	300269	IFT140	NM_014714.3	614620	KCNQ2	NM_172107.3	602235
HEATR2	NM_017802.3	614864	IFT172	NM_015662.2	607386	KCNQ3	NM_004519.3	602232
HECW2	NM_020760.3	617245	IFT43	NM_052873.2	614068	KCNQ5	NM_001160133.1	607357

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KCNT1	NM_020822.2	608167	LIAS	NM_006859.3	607031	MECR	NM_001024732.3	608205
KCTD1	NM_001258221.1	613420	LIG4	NM_002312.3	601837	MED12	NM_005120.2	300188
KCTD7	NM_153033.4	611725	LINS	NM_001040616.2		MED13L	NM_015335.4	608771
KDM1A	NM_001009999.2	609132	LIPN	NM_001102469.1	613924	MED17	NM_004268.4	603810
KDM5B	NM_006618.4	605393	LIPT1	NM_145199.2	610284	MED23	NM_015979.3	605042
KDM5C	NM_004187.3	314690	LIPT2	NM_001144869.2	617659	MEF2C	NM_002397.4	600662
KDM6A	NM_021140.3	300128	LMBRD1	NM_018368.3	612625	MEGF10	NM_032446.2	612453
KIAA0196	NM_014846.3	610657	LMNA	NM_170707.3	150330	MEGF8	NM_001410.2	604267
KIAA0226	NM_001145642.4	613516	LMX1B	NM_002316.3	602575	MEOX1	NM_004527.3	600147
KIAA0586	NM_001244192.1	610178	LONP1	NM_001276480.1	605490	MESP2	NM_001039958.1	605195
KIAA1109	NM_015312.3	611565	LRAT	NM_004744.4	604863	MFSD2A	NM_001136493.2	614397
KIAA1279	NM_015634.3	609367	LRBA	NM_006726.4	606453	MFSD8	NM_152778.2	611124
KIAA2022	NM_001008537.2	300524	LRIG2	NM_014813.2	608869	MGAT2	NM_002408.3	602616
KIDINS220	NM_020738.3	615759	LRIT3	NM_198506.4	615004	MGP	NM_000900.4	154870
KIF11	NM_004523.3	148760	LRP2	NM_004525.2	600073	MICU1	NM_006077.3	605084
KIF14	NM_014875.2	611279	LRP4	NM_002334.3	604270	MID1	NM_000381.3	300552
KIF1A	NM_004321.7	601255	LRP5	NM_002335.3	603506	MITF	NM_000248.3	156845
KIF22	NM_007317.2	603213	LRPPRC	NM_133259.3	607544	MKKS	NM_018848.3	604896
KIF2A	NM_001098511.2	602591	LRRC6	NM_012472.5	614930	MKS1	NM_017777.3	609883
KIF4A	NM_012310.4	300521	LTBP2	NM_000428.2		MLC1	NM_015166.3	605908
KIF5C	NM_004522.2	604593	LTBP3	NM_001130144.2		MLYCD	NM_012213.2	606761
KIF7	NM_198525.2	611254	LYST	NM_000081.3	606897	MMAA	NM_172250.2	607481
KIT	NM_000222.2	164920	MAB21L2	NM_006439.4	604357	MMAB	NM_052845.3	607568
KLF1	NM_006563.4	600599	MAF	NM_005360.4	177075	MMACHC	NM_015506.2	609831
KLHL40	NM_152393.3	615340	MAFB	NM_005461.4	608968	MMADHC	NM_015702.2	611935
KLHL7	NM_001031710.2	611119	MAGEL2	NM_019066.4	605283	MMP13	NM_002427.3	600108
KMT2A	NM_001197104.1	159555	MAMLD1	NM_005491.4	300120	MMP21	NM_147191.1	608416
KMT2D	NM_003482.3	602113	MAN1B1	NM_016219.4	604346	MNX1	NM_005515.3	142994
KPTN	NM_007059.3	615620	MAN2B1	NM_000528.3	609458	MOCS1	NM_005943.5	603707
KRAS	NM_004985.4	190070	MANBA	NM_005908.3	609489	MOCS2	NM_176806.3	603708
KRIT1	NM_194456.1	604214	MAOA	NM_000240.3	309850	MOGS	NM_006302.2	601336
KRT74	NM_175053.3	608248	MAP2K1	NM_002755.3	176872	MORC2	NM_014941.3	616661
L1CAM	NM_000425.4	308840	MAP2K2	NM_030662.3	601263	MPDU1	NM_004870.3	604041
L2HGDH	NM_024884.2	609584	MAP3K1	NM_005921.1	600982	MPI	NM_002435.2	154550
LAMA1	NM_005559.3	150320	MAP3K7	NM_003188.3	602614	MPLKIP	NM_138701.3	609188
LAMA2	NM_000426.3	156225	MAPRE2	NM_001143826.2	605789	MPV17	NM_002437.4	137960
LAMB1	NM_002291.2	150240	MASP1	NM_139125.3	600521	MRE11A	NM_005591.3	600814
LAMC3	NM_006059.3	604349	MAT1A	NM_000429.2	610550	MRPS22	NM_020191.2	605810
LAMP2	NM_002294.2	309060	MATN3	NM_002381.4	602109	MRPS34	NM_023936.1	611994
LARGE	NM_004737.5	603590	MBD5	NM_018328.4	611472	MSL3	NM_078629.3	
LARP7	NM_016648.3	612026	MBOAT7	NM_001146083.2	606048	MSX1	NM_002448.3	142983
LARS2	NM_015340.3	604544	MC2R	NM_000529.2	607397	MSX2	NM_002449.4	123101
LBR	NM_002296.3	600024	MCCC1	NM_020166.4	609010	MTHFR	NM_005957.4	607093
LDB3	NM_001080116.1	605906	MCCC2	NM_022132.4	609014	MTM1	NM_000252.2	300415
LEMD3	NM_014319.4	607844	MCEE	NM_032601.3	608419	MTO1	NM_012123.3	614667
LEPRE1	NM_022356.3	610339	MCOLN1	NM_020533.2	605248	MTOR	NM_004958.3	601231
LFNG	NM_001040167.1	602576	MCPH1	NM_024596.4	607117	MTR	NM_000254.2	156570
LGI4	NM_139284.2	608303	MDH2	NM_005918.3	154100	MTRR	NM_002454.2	602568
LHX3	NM_014564.4	600577	MECOM	NM_004991.3	165215	MUT	NM_000255.3	609058
LHX4	NM_033343.3	602146	MECP2	NM_004992.3	300005	MYCN	NM_005378.5	164840

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MYH3	NM_002470.3	160720	NODAL	NM_018055.4	601265	PALB2	NM_024675.3	610355
MYH6	NM_002471.3	160710	NOG	NM_005450.4	602991	PAPSS2	NM_001015880.1	603005
MYH8	NM_002472.2	160741	NONO	NM_001145410.1	300084	PARN	NM_002582.3	604212
MYH9	NM_002473.5	160775	NOP10	NM_018648.3	606471	PAX2	NM_003987.4	167409
MYLK	NM_053025.3	600922	NOTCH1	NM_017617.4	190198	PAX3	NM_181457.3	606597
MYO5A	NM_000259.3	160777	NOTCH2	NM_024408.3	600275	PAX6	NM_000280.4	607108
MYO5B	NM_001080467.2	606540	NPC1	NM_000271.4	607623	PAX8	NM_003466.3	167415
MYO7A	NM_000260.3	276903	NPC2	NM_006432.3	601015	PAX9	NM_006194.3	167416
MYT1L	NM_015025.3	613084	NPHP1	NM_000272.3	607100	PC	NM_000920.3	608786
NAA10	NM_003491.3	300013	NPHP3	NM_153240.4	608002	PCBD1	NM_000281.3	126090
NAA15	NM_057175.4	608000	NPHP4	NM_015102.4	607215	PCCA	NM_000282.3	232000
NACC1	NM_052876.3	610672	NPHS1	NM_004646.3	602716	PCCB	NM_000532.4	232050
NAGA	NM_000262.2	104170	NPHS2	NM_014625.3	604766	PCDH19	NM_001184880.1	300460
NAGLU	NM_000263.3	609701	NPR2	NM_003995.3	108961	PCGF2	NM_007144.2	
NAGS	NM_153006.2	608300	NR2F1	NM_005654.5	132890	PCNT	NM_006031.5	605925
NALCN	NM_052867.3	611549	NR2F2	NM_021005.3	107773	PCYT1A	NM_005017.3	123695
NANS	NM_018946.3	605202	NR5A1	NM_004959.4	184757	PDCD10	NM_145860.1	609118
NBAS	NM_015909.3	608025	NRAS	NM_002524.4	164790	PDE10A	NM_001130690.2	610652
NBN	NM_002485.4	602667	NRXN1	NM_001135659.2	600565	PDE4D	NM_001104631.1	600129
NDE1	NM_001143979.1	609449	NRXN2	NM_138732.2	600566	PDE6G	NM_002602.3	180073
NDP	NM_000266.3	300658	NSD1	NM_022455.4	606681	PDE6H	NM_006205.2	601190
NDST1	NM_001543.4	600853	NSDHL	NM_015922.2	300275	PDGFRB	NM_002609.3	173410
NDUFA1	NM_004541.3	300078	NSUN2	NM_017755.5	610916	PDHA1	NM_000284.3	300502
NDUFA10	NM_004544.3	603835	NT5C3A	NM_016489.12	606224	PDHX	NM_003477.2	608769
NDUFAF2	NM_174889.4	609653	NTRK1	NM_001012331.1	191315	PDSS1	NM_014317.4	607429
NDUFB11	NM_001135998.2	300403	NTRK2	NM_006180.4	600456	PDSS2	NM_020381.3	610564
NDUFS1	NM_005006.6	157655	NUBPL	NM_025152.2	613621	PEPD	NM_000285.3	613230
NDUFS4	NM_002495.3	602694	NUP107	NM_020401.3	607617	PET100	NM_001171155.1	614770
NDUFS7	NM_024407.4	601825	NUP62	NM_001193357.1	605815	PEX1	NM_000466.2	602136
NDUFS8	NM_002496.3	602141	NUS1	NM_138459.4	610463	PEX10	NM_153818.1	602859
NDUFV1	NM_007103.3	161015	NYX	NM_022567.2	300278	PEX11B	NM_003846.2	603867
NEB	NM_004543.4	161650	OBSL1	NM_015311.2	610991	PEX12	NM_000286.2	601758
NEDD4L	NM_015277.5	606384	OCLN	NM_002538.3	602876	PEX13	NM_002618.3	601789
NEK1	NM_012224.2	604588	OCRL	NM_000276.3	300535	PEX14	NM_004565.2	601791
NEK8	NM_178170.2	609799	OFD1	NM_003611.2	300170	PEX16	NM_004813.2	603360
NEU1	NM_000434.3	608272	OPHN1	NM_002547.2	300127	PEX19	NM_002857.3	600279
NF1	NM_000267.3	613113	ORC1	NM_004153.3	601902	PEX2	NM_000318.2	170993
NFIX	NM_002501.3	164005	ORC4	NM_002552.4	603056	PEX26	NM_017929.5	608666
NFU1	NM_001002755.2	608100	ORC6	NM_014321.3	607213	PEX3	NM_003630.2	603164
NGLY1	NM_018297.3	610661	OSGEP	NM_017807.3	610107	PEX5	NM_001131025.1	600414
NHP2	NM_017838.3	606470	OTC	NM_000531.5	300461	PEX6	NM_000287.3	601498
NHS	NM_198270.3	300457	OTOGL	NM_173591.3	614925	PEX7	NM_000288.3	601757
NIPBL	NM_133433.3	608667	OTUD6B	NM_016023.3	612021	PGAP2	NM_001256240.1	615187
NKX2-1	NM_001079668.2		OTX2	NM_001270524.1	600037	PGAP3	NM_033419.4	611801
NKX2-5	NM_004387.3		OXCT1	NM_000436.3	601424	PGK1	NM_000291.3	311800
NKX3-2	NM_001189.3		P4HB	NM_000918.3	176790	PGM1	NM_002633.2	171900
NKX6-2	NM_177400.2		PACS1	NM_018026.3	607492	PGM3	NM_001199917.1	172100
NLGN3	NM_018977.3	300336	PAFAH1B1	NM_000430.3	601545	PHC1	NM_004426.2	602978
NME1	NM_000269.2	156490	PAH	NM_000277.1	612349	PHF21A	NM_001101802.1	608325
NMNAT1	NM_022787.3	608700	PAK3	NM_002578.4	300142	PHF6	NM_032458.2	300414

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PHF8	NM_015107.2	300560	POMT2	NM_013382.5	607439	RAB11A	NM_004663.4	605570
PHGDH	NM_006623.3	606879	PORCN	NM_203475.2	300651	RAB11B	NM_004218.3	604198
PHOX2B	NM_003924.3	603851	POU1F1	NM_000306.3	173110	RAB18	NM_021252.4	602207
PIEZO1	NM_001142864.3	611184	PPA2	NM_176869.2	609988	RAB23	NM_183227.2	606144
PIEZO2	NM_022068.3	613629	PPM1D	NM_003620.3	605100	RAB39B	NM_171998.3	300774
PIGA	NM_002641.3	311770	PPP1CB	NM_206876.1	600590	RAB3GAP1	NM_012233.2	602536
PIGG	NM_017733.4	616918	PPP2R1A	NM_014225.5	605983	RAB3GAP2	NM_012414.3	609275
PIGL	NM_004278.3	605947	PPP2R5D	NM_006245.3	601646	RAC1	NM_018890.3	602048
PIGN	NM_176787.4	606097	PPP3CA	NM_000944.4	114105	RAD21	NM_006265.2	606462
PIGO	NM_032634.3	614730	PPT1	NM_000310.3	600722	RAD50	NM_005732.3	604040
PIGT	NM_015937.5	610272	PQBP1	NM_005710.2	300463	RAD51	NM_002875.4	
PIGV	NM_017837.3	610274	PRDM12	NM_021619.2	616458	RAD51C	NM_058216.2	602774
PIK3CA	NM_006218.3	171834	PREPL	NM_006036.4	609557	RAF1	NM_002880.3	164760
PIK3R1	NM_181523.2	171833	PRKAR1A	NM_002734.4	188830	RAI1	NM_030665.3	607642
PIK3R2	NM_005027.3	603157	PRKD1	NM_002742.2	605435	RAPSN	NM_005055.4	601592
PITX1	NM_002653.4	602149	PRMT7	NM_019023.3	610087	RARB	NM_000965.4	180220
PITX2	NM_153427.2	601542	PROP1	NM_006261.4	601538	RARS2	NM_020320.4	611524
PITX3	NM_005029.3	602669	PROSC	NM_007198.3	604436	RASA1	NM_002890.2	139150
PKD1L1	NM_138295.4	609721	PRPS1	NM_002764.3	311850	RAX	NM_013435.2	601881
PKHD1	NM_138694.3	606702	PRRT2	NM_145239.2	614386	RBBP8	NM_002894.2	604124
PLA2G6	NM_003560.3	603604	PRRX1	NM_022716.3	167420	RBM10	NM_005676.4	300080
PLAA	NM_001031689.2	603873	PRSS12	NM_003619.3	606709	RBM28	NM_018077.2	612074
PLCB1	NM_015192.3	607120	PRSS56	NM_001195129.1	613858	RBPJ	NM_005349.3	147183
PLCB4	NM_000933.3	600810	PRUNE	NM_021222.2		RECQL4	NM_004260.3	603780
PLCE1	NM_016341.3	608414	PSAP	NM_002778.3	176801	RELN	NM_005045.3	600514
PLK4	NM_014264.4	605031	PSAT1	NM_058179.3	610936	RERE	NM_012102.3	605226
PLOD1	NM_000302.3	153454	PSMB8	NM_148919.3	177046	RET	NM_020975.4	164761
PLOD2	NM_182943.2	601865	PSPH	NM_004577.3	172480	RFT1	NM_052859.3	611908
PLOD3	NM_001084.4	603066	PTCH1	NM_000264.3	601309	RFX6	NM_173560.3	612659
PLP1	NM_000533.4	300401	PTCHD1	NM_173495.2	300828	RIN2	NM_018993.3	610222
PMM2	NM_000303.2	601785	PTDSS1	NM_014754.2	612792	RIPK4	NM_020639.2	605706
PNKP	NM_007254.3	605610	PTEN	NM_000314.6	601728	RIT1	NM_006912.5	609591
PNPLA1	NM_001145717.1	612121	PTF1A	NM_178161.2	607194	RLIM	NM_016120.3	300379
PNPLA2	NM_020376.3	609059	PTH	NM_000315.3	168450	RMND1	NM_017909.3	614917
PNPO	NM_018129.3	603287	PTH1R	NM_000316.2		RNASEH2A	NM_006397.2	606034
PNPT1	NM_033109.4	610316	PTHLH	NM_198965.1	168470	RNASEH2B	NM_024570.3	610326
POC1A	NM_015426.4	614783	PTPN11	NM_002834.4	176876	RNASEH2C	NM_032193.3	610330
POC1B	NM_172240.2	614784	PTPN14	NM_005401.4	603155	RNASET2	NM_003730.4	612944
POGZ	NM_015100.3	614787	PTS	NM_000317.2	612719	RNF135	NM_032322.3	611358
POLD1	NM_002691.3	174761	PUF60	NM_078480.2	604819	RNF168	NM_152617.3	612688
POLG	NM_002693.2	174763	PURA	NM_005859.4	600473	ROBO3	NM_022370.3	608630
POLR1A	NM_015425.4	616404	PVRL4	NM_030916.2	609607	ROGDI	NM_024589.2	614574
POLR1C	NM_203290.3	610060	PXDN	NM_012293.2	605158	ROR2	NM_004560.3	602337
POLR1D	NM_015972.3	613715	PYCR1	NM_006907.3	179035	RPE65	NM_000329.2	180069
POLR3A	NM_007055.3	614258	PYCR2	NM_013328.3	616406	RPGRIP1	NM_020366.3	605446
POLR3B	NM_018082.5	614366	PYGL	NM_002863.4	613741	RPGRIP1L	NM_015272.4	610937
POMGNT1	NM_017739.3	606822	PYROXD1	NM_024854.4	617220	RPL11	NM_000975.3	604175
POMGNT2	NM_032806.5	614828	QARS	NM_005051.2	603727	RPS19	NM_001022.3	603474
POMK	NM_032237.4	615247	QDPR	NM_000320.2	612676	RPS23	NM_001025.4	603683
POMT1	NM_007171.3	607423	QRICH1	NM_198880.2	617387	RPS6KA3	NM_004586.2	300075

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RRAS	NM_006270.4	165090	SIN3A	NM_001145357.1	607776	SMAD4	NM_005359.5	600993
RRM2B	NM_015713.4	604712	SIX1	NM_005982.3	601205	SMARCA2	NM_003070.4	600014
RSPH1	NM_080860.3	609314	SIX3	NM_005413.3	603714	SMARCA4	NM_001128849.1	603254
RSPH3	NM_031924.5	615876	SIX5	NM_175875.4	600963	SMARCAL1	NM_014140.3	606622
RSPO4	NM_001029871.3	610573	SKI	NM_003036.3	164780	SMARCB1	NM_003073.4	601607
RSPRY1	NM_133368.2	616585	SKIV2L	NM_006929.4	600478	SMARCE1	NM_003079.4	603111
RTEL1	NM_032957.4	608833	SLC12A6	NM_133647.1	604878	SMC1A	NM_006306.3	300040
RTN4IP1	NM_032730.5	610502	SLC13A5	NM_177550.4	608305	SMC3	NM_005445.3	606062
RTTN	NM_173630.3	610436	SLC16A2	NM_006517.4	300095	SMCHD1	NM_015295.2	614982
RUNX2	NM_001024630.3	600211	SLC17A5	NM_012434.4	604322	SMG9	NM_019108.3	613176
RYR1	NM_000540.2	180901	SLC19A3	NM_025243.3	606152	SMO	NM_005631.4	615854
SACS	NM_014363.5	604490	SLC1A2	NM_004171.3	600300	SMOC1	NM_001034852.2	608488
SALL1	NM_002968.2	602218	SLC22A5	NM_003060.3	603377	SMOC2	NM_022138.2	607223
SALL4	NM_020436.4	607343	SLC24A4	NM_153646.3	609840	SMPD1	NM_000543.4	607608
SAMHD1	NM_015474.3	606754	SLC25A15	NM_014252.3	603861	SMS	NM_004595.4	607642
SATB2	NM_015265.3	608148	SLC25A19	NM_021734.4	606521	SNAP25	NM_130811.3	600322
SC5D	NM_006918.4		SLC25A20	NM_000387.5	613698	SNAP29	NM_004782.3	604202
SCARF2	NM_153334.6	613619	SLC25A22	NM_024698.5	609302	SNIP1	NM_024700.3	608241
SCN11A	NM_014139.2	604385	SLC25A24	NM_013386.4	608744	SNRPB	NM_003091.3	182282
SCN1A	NM_001165963.1	182389	SLC25A26	NM_173471.3	611037	SNRPE	NM_003094.3	128260
SCN1B	NM_001037.4	600235	SLC25A38	NM_017875.2	610819	SNX14	NM_020468.5	616105
SCN2A	NM_021007.2	182390	SLC25A4	NM_001151.3	103220	SOBP	NM_018013.3	613667
SCN3A	NM_006922.3	182391	SLC26A2	NM_000112.3	606718	SON	NM_032195.2	182465
SCN4A	NM_000334.4	603967	SLC27A4	NM_005094.3	604194	SOS1	NM_005633.3	182530
SCN8A	NM_014191.3	600702	SLC2A1	NM_006516.2	138140	SOX10	NM_006941.3	602229
SCO1	NM_004589.3	603644	SLC2A10	NM_030777.3	606145	SOX11	NM_003108.3	600898
SCO2	NM_005138.2	604272	SLC2A2	NM_000340.1	138160	SOX17	NM_022454.3	610928
SCYL1	NM_020680.3	607982	SLC33A1	NM_004733.3	603690	SOX2	NM_003106.3	184429
SDCCAG8	NM_006642.4	613524	SLC35A1	NM_006416.4	605634	SOX3	NM_005634.2	313430
SDHAF1	NM_001042631.2	612848	SLC35A2	NM_001042498.2	314375	SOX5	NM_006940.5	604975
SEC23B	NM_006363.4	610512	SLC35C1	NM_018389.4	605881	SOX9	NM_000346.3	608160
SEC24D	NM_014822.3	607186	SLC35D1	NM_015139.2	610804	SPAG1	NM_172218.2	603395
SECISBP2	NM_024077.4	607693	SLC39A13	NM_152264.4	608735	SPARC	NM_003118.3	182120
SEPSECS	NM_016955.3	613009	SLC39A8	NM_001135147.1	608732	SPATA5	NM_145207.2	613940
SET	NM_001122821.1	600960	SLC45A1	NM_001080397.2	605763	SPECC1L	NM_015330.4	614140
SETBP1	NM_015559.2	611060	SLC46A1	NM_080669.5	611672	SPEG	NM_005876.4	615950
SETD1A	NM_014712.2	611052	SLC4A1	NM_000342.3	109270	SPG11	NM_025137.3	610844
SETD2	NM_014159.6	612778	SLC4A11	NM_032034.3	610206	SPR	NM_003124.4	182125
SETD5	NM_001080517.2	615743	SLC4A4	NM_003759.3	603345	SPRED1	NM_152594.2	609291
SF3B4	NM_005850.4	605593	SLC52A3	NM_033409.3	613350	SPTAN1	NM_001130438.2	182810
SGSH	NM_000199.3	605270	SLC5A5	NM_000453.2	601843	SPTLC2	NM_004863.3	605713
SH3PXD2B	NM_001017995.2	613293	SLC5A7	NM_021815.4	608761	SRCAP	NM_006662.2	611421
SHANK1	NM_016148.3	604999	SLC6A1	NM_003042.3	137165	SRD5A3	NM_024592.4	611715
SHANK2	NM_133266.4	603290	SLC6A17	NM_001010898.3	610299	SRP54	NM_003136.3	604857
SHANK3	NM_033517.1	606230	SLC6A3	NM_001044.4	126455	SRPX2	NM_014467.2	300642
SHH	NM_000193.3	600725	SLC6A5	NM_004211.4	604159	SRY	NM_003140.2	480000
SHOC2	NM_007373.3	602775	SLC6A9	NM_001024845.2	601019	ST14	NM_021978.3	606797
SHROOM3	NM_020859.3	604570	SLC9A6	NM_006359.2	300231	ST3GAL3	NM_006279.4	606494
SIK1	NM_173354.4	605705	SLX4	NM_032444.3	613278	ST3GAL5	NM_003896.3	
SIL1	NM_022464.4	608005	SMAD3	NM_005902.3	603109	STAG1	NM_005862.2	604358

Extended trio-based gene panel

STAMBP	NM_006463.4	606247	TBXAS1	NM_001061.4	274180	TRAPPC12	NM_016030.5	614139
STAR	NM_000349.2	300708	TCF12	NM_207036.1	600480	TRAPPC2	NM_001011658.3	300202
STAT1	NM_007315.3	600555	TCF20	NM_005650.3	603107	TRAPPC9	NM_031466.7	611966
STAT5B	NM_012448.3	604260	TCF4	NM_001083962.1	602272	TREX1	NM_033629.4	606609
STIL	NM_003035.2	181590	TCN2	NM_000355.3	613441	TRIM32	NM_012210.3	602290
STRA6	NM_022369.3	610745	TCOF1	NM_001135243.1	606847	TRIM37	NM_015294.4	605073
STRADA	NM_153335.5	608626	TCTN1	NM_001082538.2		TRIO	NM_007118.3	601893
STS	NM_000351.5	300747	TCTN2	NM_024809.4	613846	TRIP11	NM_004239.4	604505
STT3A	NM_001278503.1	601134	TCTN3	NM_015631.5	613847	TRIP12	NM_004238.2	604506
STT3B	NM_178862.2	608605	TECPR2	NM_014844.4	615000	TRIP13	NM_004237.3	604507
STX1B	NM_052874.4	601485	TEK	NM_000459.4	600221	TRIP4	NM_016213.4	604501
STXBP1	NM_003165.3	602926	TELO2	NM_016111.3	611140	TRMT10C	NM_017819.3	615423
SUCLG1	NM_003849.3	611224	TERT	NM_198253.2	187270	TRPM1	NM_002420.5	603576
SUFU	NM_016169.3	607035	TFAP2A	NM_003220.2	107580	TRPS1	NM_014112.4	604386
SUMF1	NM_182760.3	607939	TFAP2B	NM_003221.3	601601	TRPV3	NM_145068.3	607066
SURF1	NM_003172.3	185620	TGDS	NM_014305.3	616146	TRPV4	NM_021625.4	605427
SUV420H1	NM_017635.4	610881	TGFB1	NM_000660.6	190180	TSC1	NM_000368.4	605284
SYN1	NM_133499.2	313440	TGFB2	NM_003238.4	190220	TSC2	NM_000548.4	191092
SYNE1	NM_033071.3	608441	TGFB3	NM_003239.4	190230	TSEN15	NM_052965.3	608756
SYNGAP1	NM_006772.2	603384	TGFBR1	NM_004612.3	190181	TSEN2	NM_025265.3	608753
SYP	NM_003179.2	313475	TGFBR2	NM_003242.5	190182	TSEN34	NM_024075.4	608754
SZT2	NM_015284.3	615463	TGIF1	NM_173208.2	602630	TSEN54	NM_207346.2	608755
TAB2	NM_015093.5	605101	TH	NM_199292.2	191290	TSHB	NM_000549.4	188540
TAC3	NM_013251.3	162330	THAP1	NM_018105.2	609520	TSHR	NM_000369.2	603372
TACO1	NM_016360.3	612958	THOC2	NM_001081550.1	300395	TSPAN7	NM_004615.3	300096
TACR3	NM_001059.2	162332	THOC6	NM_024339.4	615403	TTC19	NM_017775.3	613814
TAF1	NM_004606.4	313650	THRA	NM_199334.3	190120	TTC37	NM_014639.3	614589
TAF13	NM_005645.3	600774	TIMM8A	NM_004085.3	300356	TTC7A	NM_020458.3	609332
TAF2	NM_003184.3	604912	TINF2	NM_001099274.1	604319	TTC8	NM_198309.3	608132
TANGO2	NM_001283186.2	616830	TK2	NM_004614.4	188250	TTI2	NM_001102401.2	614426
TAPT1	NM_153365.2	612758	TKT	NM_001135055.2	606781	TUBA8	NM_018943.2	605742
TAT	NM_000353.2	613018	TM4SF20	NM_024795.4	615404	TUBB	NM_178014.3	191130
TAZ	NM_000116.4	300394	TMCO1	NM_019026.4	614123	TUBB2A	NM_001069.2	615101
TBC1D20	NM_144628.3	611663	TMEM126B	NM_018480.5	615533	TUBB2B	NM_178012.4	612850
TBC1D23	NM_001199198.2	617687	TMEM165	NM_018475.4	614726	TUBB4A	NM_006087.3	602662
TBC1D24	NM_001199107.1	613577	TMEM216	NM_001173990.2	613277	TUBG1	NM_001070.4	191135
TBC1D7	NM_001143965.3	612655	TMEM237	NM_001044385.2	614423	TUBGCP4	NM_014444.4	609610
TBCD	NM_005993.4	604649	TMEM260	NM_017799.3	617449	TUBGCP6	NM_020461.3	610053
TBCE	NM_003193.4	604934	TMEM5	NM_014254.2	605862	TUFM	NM_003321.4	602389
TBCK	NM_001163436.2	616899	TMEM67	NM_153704.5	609884	TUSC3	NM_006765.3	601385
TBL1XR1	NM_024665.5	608628	TMEM70	NM_017866.5	612418	TWIST1	NM_000474.3	601622
TBR1	NM_006593.3	604616	TMPRSS6	NM_153609.3	609862	TWIST2	NM_057179.2	607556
TBX1	NM_080647.1	602054	TMTC3	NM_181783.3	617218	TXNL4A	NM_006701.4	611595
TBX15	NM_152380.2	604127	TNFRSF13B	NM_012452.2	604907	TYR	NM_000372.4	606933
TBX18	NM_001080508.2	604613	TOE1	NM_025077.3	613931	TYRP1	NM_000550.2	115501
TBX20	NM_001077653.2	606061	TP63	NM_003722.4	603273	UBA5	NM_198329.3	610552
TBX22	NM_001109878.1	300307	TPM2	NM_003289.3	190990	UBE2A	NM_003336.3	312180
TBX3	NM_005996.3	601621	TPP1	NM_000391.3	607998	UBE2T	NM_014176.3	610538
TBX4	NM_018488.3	601719	TRAIP	NM_005879.2	605958	UBE3A	NM_130838.1	601623
TBX5	NM_000192.3	601620	TRAPPC11	NM_021942.5	614138	UBE3B	NM_130466.3	608047

Extended trio-based gene panel

UBR1	NM_174916.2	605981	YWHAG	NM_012479.3	605356
UBTF	NM_001076683.1	600673	YY1	NM_003403.4	600013
UGT1A1	NM_000463.2	191740	ZBTB16	NM_006006.4	176797
UMPS	NM_000373.3	613891	ZBTB18	NM_205768.2	608433
UNC80	NM_032504.1	612636	ZBTB20	NM_001164342.2	606025
UPF3B	NM_080632.2	300298	ZC4H2	NM_018684.3	300897
UQCRB	NM_006294.4	191330	ZDHHC15	NM_001146256.1	300576
UQCRQ	NM_014402.4	612080	ZDHHC9	NM_016032.3	300646
UROC1	NM_144639.2	613012	ZEB2	NM_014795.3	605802
UROS	NM_000375.2	606938	ZFP57	NM_001109809.2	612192
USB1	NM_024598.3	613276	ZFYVE26	NM_015346.3	612012
USP18	NM_017414.3	607057	ZIC1	NM_003412.3	600470
USP27X	NM_001145073.2	300975	ZIC2	NM_007129.4	603073
USP9X	NM_001039590.2	300072	ZIC3	NM_003413.3	300265
UVSSA	NM_020894.3	614632	ZMPSTE24	NM_005857.4	606480
VDR	NM_001017535.1	601769	ZMYND10	NM_015896.3	607070
VIPAS39	NM_022067.3	613401	ZMYND11	NM_006624.5	608668
VLDLR	NM_003383.4	192977	ZNF462	NM_021224.5	617371
VPS13B	NM_017890.4	607817	ZNF711	NM_021998.4	314990
VPS33B	NM_018668.4	608552	ZNF750	NM_024702.2	610226
VPS53	NM_001128159.2	615850	ZNHIT3	NM_001281432.1	604500
VRK1	NM_003384.2	602168	ZSWIM6	NM_020928.1	615951
VSX2	NM_182894.2				
WAC	NM_016628.4	615049			
WDPCP	NM_015910.5	613580			
WDR11	NM_018117.11	606417			
WDR19	NM_025132.3	608151			
WDR26	NM_001115113.2	617424			
WDR34	NM_052844.3	613363			
WDR35	NM_001006657.1	613602			
WDR45	NM_007075.3	300526			
WDR60	NM_018051.4	615462			
WDR62	NM_001083961.1	613583			
WDR73	NM_032856.3	616144			
WNT1	NM_005430.3	164820			
WNT10B	NM_003394.3	601906			
WNT3	NM_030753.4	165330			
WNT4	NM_030761.4	603490			
WNT5A	NM_003392.4	164975			
WNT7A	NM_004625.3	601570			
WRAP53	NM_018081.2	612661			
WT1	NM_024426.4	607102			
WWOX	NM_016373.3	605131			
XPA	NM_000380.3	611153			
XPC	NM_004628.4	613208			
XPNPEP3	NM_022098.3	613553			
XRCC4	NM_022406.3	194363			
XYLT1	NM_022166.3	608124			
XYLT2	NM_022167.3	608125			
YAP1	NM_001130145.2	606608			

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PAPER I-III

JNS PEDIATRICS

CLINICAL ARTICLE

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Epidemiology of craniosynostosis in Norway

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OBJECTIVE The authors present population-based epidemiological data for craniosynostosis regarding incidence, age at diagnosis, sex differences, and frequency of syndromic and familial cases.

METHODS The prospective registry of the Norwegian National Unit for Craniofacial Surgery was used to retrieve data on all individuals with craniosynostosis treated between 2003 and 2017. The cohort was divided into three 5-year groups based on year of birth: 2003–2007, 2008–2012, and 2013–2017.

RESULTS The authors identified 386 individuals with craniosynostosis. Of these, 328 (85%) consented to be registered with further information. The incidence increased significantly during the study period and was 5.5 per 10,000 live births (1/1800) in the last 5-year period. The increase was seen almost exclusively in the nonsyndromic group. Syndromic craniosynostosis accounted for 27% of the cases, and the incidence remained stable throughout the three 5-year periods. Both syndromic and nonsyndromic craniosynostosis were highly suture specific. There was a male preponderance (male/female ratio 2:1), and males accounted for 75% of the individuals with midline synostosis. Overall, 9.5% were index individuals in families with more than one affected member; of these, 73% were nonsyndromic cases.

CONCLUSIONS The incidence of craniosynostosis increased during the study period, and the observed incidence is among the highest reported. The authors attribute this to increasing awareness among healthcare professionals. The number of syndromic cases was high, likely due to a broader definition compared to the majority of earlier reports. The study revealed a high number of familial cases in both syndromic and nonsyndromic craniosynostosis, thus highlighting the importance of genetics as an underlying cause of craniosynostosis.

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KEYWORDS craniosynostosis; epidemiology; incidence; syndromic; nonsyndromic; Norway; craniofacial

Craniosynostosis is one of the most common malformations in children, usually presenting during the 1st year of life.[1] In the healthy population, the metopic suture closes between 6 and 12 months of age, while the other cranial sutures remain open until adulthood.[2] Premature closure of one or more sutures often results in cranial deformity and compromised intracranial volume.[3–6] The latter may result in raised intracranial pressure and secondary brain injury.[6–9]

Clinically, craniosynostosis is divided into syndromic and nonsyndromic craniosynostosis. Syndromic craniosynostosis may be characterized by the coexistence of associated malformations, a dysmorphic appearance, or intellectual disability.[10] The coronal and/or lambdoid sutures are usually affected, often in combination with other sutures. Individuals with nonsyndromic craniosynostosis have no other major findings, and the midline sutures are most commonly affected.

The reported incidence of craniosynostosis varies between 1 in 1600 and 1 in 4000 live births, and it seems to be increasing.[11–15] Syndromic craniosynostosis constitutes between 12% and 31%[10,11,15,16] of all cases. The sagittal suture is the most commonly affected suture, the proportion varying between 41% and 68%.[10,17–19] The male/female ratio ranges from 1.8:1 to 4.7:1,[13,15,20–22] and the proportion of familial craniosynostosis is reported to be between 5.6% and 14.7%.[14,22,23]

The epidemiology of craniosynostosis in the literature varies significantly between countries, regions, and medical centers. To our knowledge, the epidemiology of craniosynostosis has not previously been reported from any Scandinavian country. The objective of the current study

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was to present population-based epidemiological data for craniosynostosis with regard to incidence, age at diagnosis, sex differences, and frequency of syndromic and familial cases.

Methods

Since 2001, all individuals in Norway with suspected craniosynostosis have been referred to the Norwegian National Unit for Craniofacial Surgery at Oslo University Hospital for diagnostic workup, treatment, and follow-up. The registry of the unit is prospective and includes all consenting individuals diagnosed with craniosynostosis.

This population-based study includes individuals with craniosynostosis born between 1/1/2003 and 12/31/2017. From the registry, the following data were extracted on 4/5/2019: date of birth, sex, date of first consultation with a neurosurgeon, affected suture(s), associated findings (other congenital malformations, intellectual disability/developmental delay, dysmorphic features), genetically confirmed diagnosis, and affected family members.

A clinical assessment of all individuals referred to the Norwegian National Unit for Craniofacial Surgery was performed by one of two pediatric neurosurgeons (B.J.D.T., U.W.). All individuals meeting the criteria for surgical treatment were included in the study. The decision to recommend surgery was based on the risk of the child developing compromised brain growth or progressive deformity of the skull if untreated. Individuals with craniosynostosis who met the criteria, but who were not surgically treated for some reason (diagnosed at an older age, died before surgery, parents resisted), were also included (n = 47). The date of the first consultation with the neurosurgeon was defined as age at diagnosis. The affected suture was grouped into metopic, sagittal, unicoronal, or multiple, with the latter group including bicoronal synostosis. All individuals with suspected syndromic craniosynostosis were seen by a clinical geneticist. Syndromic craniosynostosis was defined by the presence of one or more of the following additional findings: 1) malformations (one major or one minor in combination with unusual growth or dysmorphic features), 2) intellectual disability/severe developmental delay, and 3) a genetically confirmed craniosynostosis syndrome. All individuals with syndromic craniosynostosis had been offered genetic analysis, including array comparative genomic hybridization (180K) and high-throughput-sequencing, with a panel of 70 known genes associated with craniosynostosis.

Familial craniosynostosis was defined as having more than one family member with craniosynostosis (first- or second-degree relative).

Based on year of birth, we divided the individuals into three 5-year cohorts (2003–2007, 2008–2012, and 2013–2017) in order to investigate possible changes regarding the incidence and the age at diagnosis.

Statistical Analysis

Live birth statistics in the 2003–2017 period were extracted from Statistics Norway.[24] IBM SPSS Statistics version 25 (IBM Corp.) was used to perform statistical analysis. The OpenEpi Collection of Epidemiological Calculators, version 3.01,[25] was used to calculate chi-square values for the categorical variables. The data are presented as the median and mean values for continuous variables and as the number (%) for discrete variables. The chi-square test was used to compare categorical variables, and one-way ANOVA was used to compare mean differences in age at diagnosis between the three cohorts, including a Tukey post hoc test. Standard deviations and p values were calculated. Statistical significance was set at $p \leq 0.05$.

Ethics

The study was approved by the Norwegian Regional Committees for Medical and Health Research Ethics and by Oslo University Hospital.

Results

Incidence

A total of 386 individuals born between 2003 and 2017 were confirmed to have craniosynostosis requiring surgery. Of these, 328 individuals (85%) consented to be registered with further information. Norway had a population of 5.3 million in 2017.

The mean annual incidence of craniosynostosis in Norway during the study period was 4.4 cases per 10,000 live births (1/2250). In the 2013–2017 cohort, the incidence was 5.5 cases/10,000 live births (1/1800), increasing from 3.3 cases/10,000 live births (1/3000) in the 2003–2007 cohort and 4.3 cases/10,000 live births (1/2300) in the 2008–2012 cohort (Fig. 1).

The difference in incidence in the first and last 5-year period constitutes a 70% increase in registered individuals with craniosynostosis. The chi-square analysis showed this to be a significant difference within all three cohorts (Table 1).

The increasing incidence was almost exclusively seen in nonsyndromic craniosynostosis, while the incidence in syndromic craniosynostosis remained stable over time (Table 1 and Fig. 2). However, within the syndromic group, the incidence of rare genetic syndromes increased significantly. With regard to suture involvement, there was a significant increase in incidence of sagittal craniosynostosis.

Age at Diagnosis

The median age at diagnosis went down from 204 days in the first 5-year period to 122 days in the last period (Fig. 3 and Table 2). The age at diagnosis went down in both syndromic and nonsyndromic craniosynostosis cases (Fig. 3). The largest reduction in age at diagnosis occurred in individuals with midline craniosynostosis. These individuals were diagnosed at the age of 125 days in the last 5-year period, compared to 220 days in the first 5-year period. Individuals with syndromic craniosynostosis were diagnosed at a younger age than individuals with nonsyndromic craniosynostosis. The age at diagnosis for individuals with syndromic craniosynostosis was almost equal to the age at diagnosis for individuals with coronal and complex craniosynostosis in the last 5-year period.

Post hoc Tukey analysis showed a significant difference in age at diagnosis between the 2003–2007 and 2013–2017 cohorts only (Table 2). The one-way ANOVA showed a

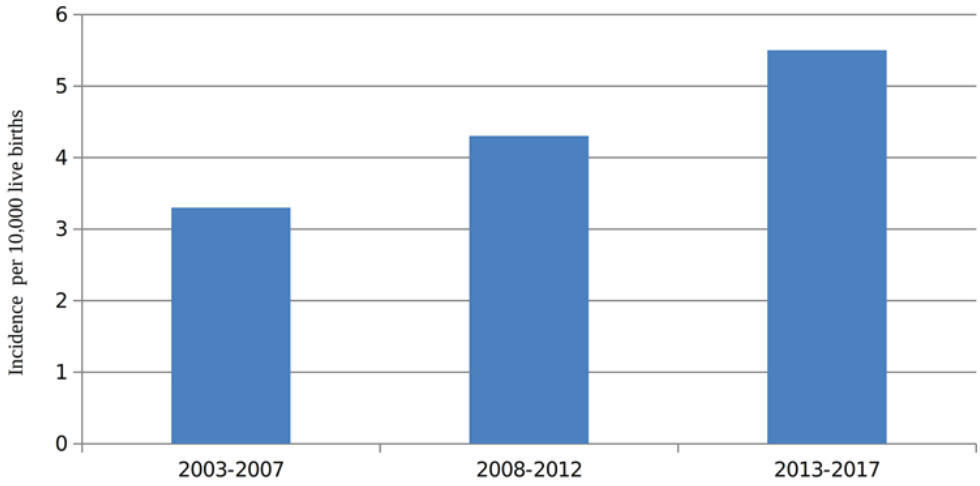

FIG. 1. Period incidence of craniosynostosis in Norway, 2003–2017. Figure is available in color online only.

significant difference between groups (p = 0.011), an F-value of 4.5, and a degree of freedom of 2.

Syndromic Craniosynostosis

A total of 89 individuals (27%) had syndromic craniosynostosis. Of these, 80 individuals accepted routine genetic testing. Of the analyzed individuals, 59 (74%) were confirmed by genetic analysis. Of the individuals with a confirmed genetic diagnosis, 39 individuals (66%) had one of the common syndromes associated with craniosynostosis (Apert, Crouzon, Muenke, Pfeiffer, or Saethre-Chotzen syndrome), and 20 (34%) had a rare genetic syndrome (Table 1).

Affected Suture(s)

Overall, the sagittal suture was the most commonly affected suture, constituting 49% of the cases (Table 3). In individuals with syndromic craniosynostosis, multiple affected sutures (including bicoronal synostosis) were the most common finding (43%). For the remaining syndromic cases, the affected suture was nearly equally distributed among the metopic, sagittal, and unicoronal suture.

TABLE 1. Absolute numbers and period incidence of craniosynostosis per 10,000 live births from 2003 to 2017

	2003–2007		2008–2012		2013–2017		Total		
Craniosynostosis	Abs	Incid	Abs	Incid	Abs	Incid	Abs	Incid	p Value
Total	94	3.3	132	4.3	160	5.5	386	4.4	0.0003
Consenting	70	2.4	116	3.8	142	4.9	328	3.7	0.00001
Syndromic	25	0.9	31	1.0	33	1.1	89	1.0	0.62
Apert	3	0.1	7	0.2	2	0.1	12	0.1	0.20
Muenke	6	0.2	3	0.1	2	0.1	11	0.1	0.28
Crouzon/Pfeiffer	2	0.1	4	0.1	1	0.03	7	0.08	0.40
Saethre-Chotzen	3	0.1	4	0.1	2	0.1	9	0.1	0.75
Rare genetic*	5	0.2	3	0.1	12	0.4	20	0.2	0.03
Negative test	5	0.2	7	0.2	9	0.3	21	0.2	0.58
Not tested	1	0.03	3	0.1	5	0.2	9	0.1	0.27
Nonsyndromic	45	1.2	85	2.8	109	3.7	239	2.7	0.00004
Suture									
Sagittal	25	0.9	52	1.7	85	2.9	162	1.8	0.0000001
Metopic	16	0.6	31	1.0	31	1.1	78	0.9	0.077
Unicoronal	10	0.3	13	0.4	14	0.5	37	0.4	0.74
Multiple†	19	0.7	20	0.7	12	0.4	51	0.6	0.35

Abs = absolute number; Incid = incidence.
* Rare genetic syndrome, confirmed by genetic analysis.
† Multiple sutures including bicoronal synostosis.

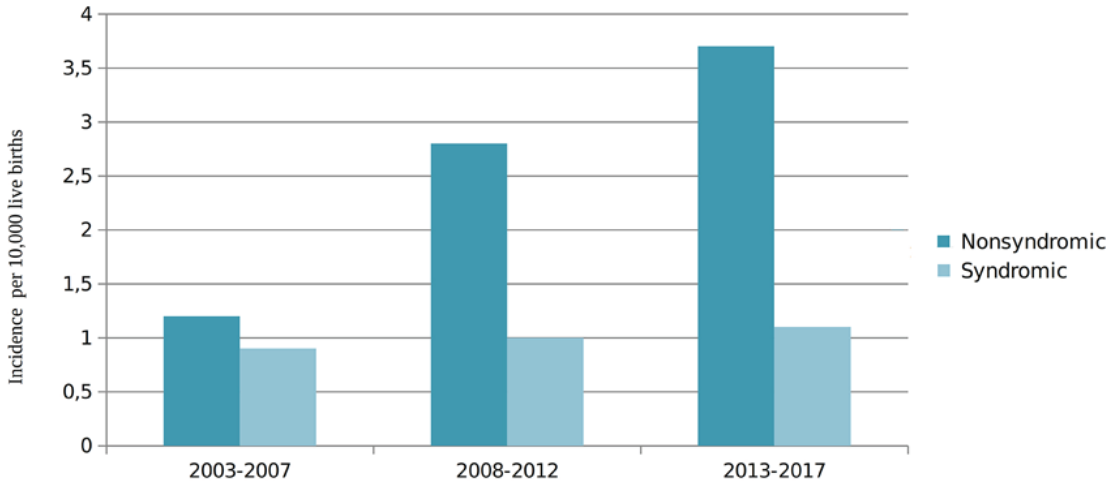

FIG. 2. Period incidence of nonsyndromic and syndromic craniosynostosis, 2003–2017. Figure is available in color online only.

However, in nonsyndromic craniosynostosis the midline sutures, particularly the sagittal suture, were most often affected (Table 3 and Fig. 4).

Sex Differences

Overall, 67% of individuals with craniosynostosis were male (Table 4). For metopic and sagittal craniosynostosis, males accounted for 78% and 73%, respectively. Conversely, for unicoronal synostosis, 62% were females. In nonsyndromic craniosynostosis, 70% were male, compared to 58% in syndromic craniosynostosis.

Familial Cases

The 328 individuals in our cohort represent 314 different families. Thirty index individuals (9.5%) had one affected family member, and 22 (73%) of these had nonsyndromic craniosynostosis.

Registered index individuals in familial craniosynostosis constituted about 10% of the cases in both syndromic and nonsyndromic craniosynostosis (10% and 9.5%, respectively).

Discussion

In this study, we present population-based epidemiological data on the incidence, age at diagnosis, sex differences, and frequency of syndromic and familial cases of craniosynostosis. We found a high incidence—5.5 per 10,000 live births—in the last 5-year period, increasing from 3.3 per 10,000 live births in the first period. The study not only reveals a high proportion of syndromic cases but also demonstrates that syndromic and nonsyndromic craniosynostosis are highly suture specific.

Incidence

The increase over the three 5-year periods is significant for individuals with nonsyndromic craniosynostosis and/or midline synostosis in addition to individuals with rare genetic syndromes.

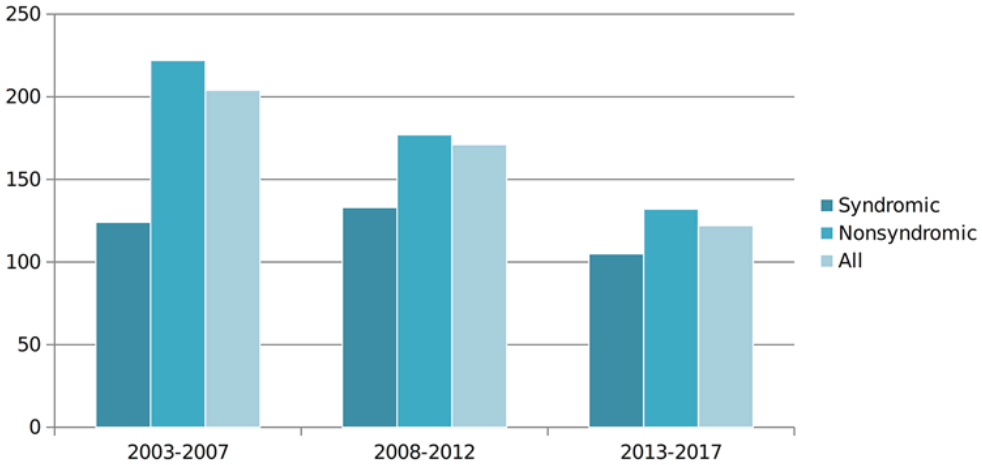

FIG. 3. Median age (in days; y-axis) at diagnosis for syndromic and nonsyndromic craniosynostosis, 2003–2017. Figure is available in color online only.

TABLE 2. Median age (in days) at diagnosis for nonsyndromic and syndromic craniosynostosis, affected midline and complex suture(s), and total numbers with multiple comparisons

5-Yr Period	Syndromic	Nonsyndromic	Midline Suture	Complex Suture*	Total	Multiple Comparisons†	p Value
2003–2007	124 (866)	222 (311)	220 (323)	199 (775)	204 (575)	2008–2012	0.134
						2013–2017	0.008
2008–2012	133 (475)	177 (182)	177 (267)	148 (347)	171 (290)	2003–2007	0.134
						2013–2017	0.451
2013–2017	105 (325)	132 (160)	125 (182)	108 (307)	122 (210)	2003–2007	0.008
						2008–2012	0.451
Total	111 (571)	170 (206)	162 (242)	148 (520)	162 (348)		

Values are presented as the median (SD).
* Including uni- and bicoronal synostosis.
† Multiple comparisons between total numbers.

We believe that higher awareness and better diagnostic routines by health professionals are the most likely explanation of the increasing incidence of craniosynostosis.[12,26] Other factors such as higher paternal age,[14,19] higher maternal age,[11] birth weight,[11] increasing use of antidepressant medication during pregnancy,[27] and more have been discussed without any firm associations being established. There may also be an actual increase in the incidence for reasons not yet understood. In particular, nonsyndromic craniosynostosis is most likely the result of multiple factors, both environmental and genetic, the details of which are yet to be elucidated.

The increasing number of reported cases of nonsyndromic midline synostosis has been thoroughly debated. New recommendations that babies should sleep on their back were introduced in Norway in the 1990s, inspired by the international Back to Sleep campaign[26] in order to prevent sudden infant death syndrome (SIDS). This change in sleeping position brought about a large number of babies seen by health professionals with plagiocephaly,[28] this again may have contributed to higher awareness of skull development in general and may have contributed to a higher diagnostic rate of craniosynostosis in recent years.[26]

The increasing incidence of rare genetic syndromes is interesting. It may reflect an increasing awareness of rare syndromes with multiorgan affection also manifesting with craniosynostosis, hence an increase in referral. De novo variants in genes involved in growth and development are known to be important mechanisms for these syndromes.[10,29–31] High paternal age is suggested to increase the risk of de novo variants in general.[32,33] We have not accounted for paternal age in our study, but this could be a topic for further investigations. The fact that the incidence of syndromic craniosynostosis was stable over the three time periods in addition to our calculation being based on very low numbers might indicate that this finding is a coincidence that should not be emphasized.

Age at Diagnosis

The median age at which craniosynostosis was diagnosed went down overall during our study period. In the last 5-year period, the median age was 122 days for all individuals and 105 days for individuals with syndromic craniosynostosis. The younger age at diagnosis was most pronounced in cases of syndromic and/or coronal and complex craniosynostosis. This differs from other studies in which a higher age at diagnosis of complex craniosyn-

TABLE 3. Affected sutures in nonsyndromic and syndromic craniosynostosis presented in absolute numbers and percentages

	Sagittal		Metopic		Unicoronal		Multiple*		Total	
Craniosynostosis	Abs	%	Abs	%	Abs	%	Abs	%	Abs	%
Syndromic	15	17	18	20	18	20	38	43	89	100
Apert	0		0		1		11		12	
Muenke	0		0		4		7		11	
Crouzon/Pfeiffer	1		0		0		6		7	
Saethre-Chotzen	0		0		6		3		9	
Rare syndrome	4		5		5		6		20	
Unknown	10		13		2		5		30	
Nonsyndromic	147	62	60	25	19	8	13	5	239	100
Total	162	49	78	24	37	11	51	16	328	100

* Multiple synostosis including bicoronal synostosis.

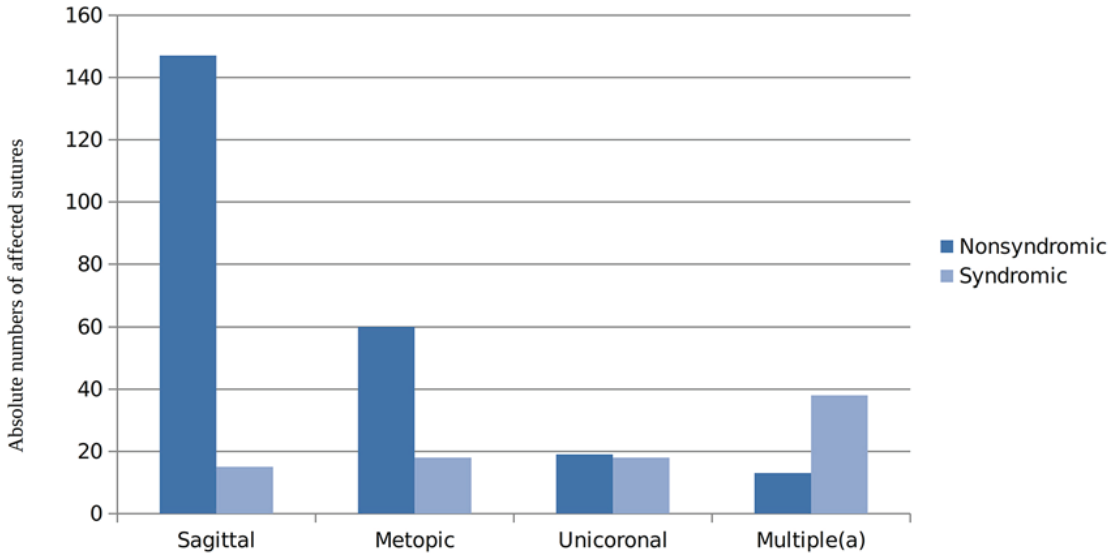

FIG. 4. Absolute numbers of affected suture in syndromic and nonsyndromic craniosynostosis. a = multiple sutures including bicoronal sutures. Figure is available in color online only.

ostosis has been reported.[26,28,34] The younger age at diagnosis of syndromic and/or complex craniosynostosis might also be explained by greater awareness among health professionals. The fact that nonsyndromic craniosynostosis is diagnosed at a later age may be explained by a higher number of mild cases in which delayed referral occurs due to health professionals' beliefs that there will be improvement in skull shape with time.

Syndromic Cases and Affected Suture(s)

We report a high proportion of syndromic cases compared to the majority of other published reports. This may be due to previous publications including only the known and common craniosynostosis syndromes (Apert, Crouzon, Muenke, Pfeiffer, and Saethre-Chotzen syndrome) in their syndromic cohorts. There is currently no established consensus for the definition of syndromic craniosynostosis. We believe the grouping of syndromic and nonsyndromic craniosynostosis needs to include a detailed genetic workup. We were able to establish a genetic cause in the majority of the syndromic cases (74% of the analyzed individuals), supporting the need for a broader definition. We believe this to be important, as a misclassification could potentially impact and bias the research on craniosynostosis beyond epidemiology. This could be exemplified by studies on neuropsychological outcomes of individuals with nonsyndromic craniosynostosis, where a presumably nonsyndromic cohort actually includes a number of syndromic cases, thus biasing the results toward a higher proportion of neuropsychological deficits.

We found a higher proportion of sagittal and metopic suture involvement in our nonsyndromic cohort than has previously been reported in the literature. In addition, we found a high proportion of bicoronal and multiple-suture involvement in syndromic craniosynostosis, with a low proportion of sagittal synostosis in this group. This emphasizes the argument that syndromic and nonsyndromic craniosynostosis are highly suture specific, probably related to different embryological mechanisms and signaling pathways in part regulated by genetics.[1,20]

TABLE 4. Affected sutures and sex differences presented in absolute numbers and percentages

	Female		Male		Total	
Affected Suture	Abs	%	Abs	%	Abs	%
Sagittal	44	27	118	73	162	100
Metopic	17	22	61	78	78	100
Unicoronal	23	62	14	38	37	100
Multiple*	24	47	27	53	51	100
Total	108	33	220	67	328	100

* Multiple sutures including bicoronal sutures.

Sex Differences

Many reports have shown a male preponderance in midline synostosis, and our data support this finding. This does not extend to unicoronal synostosis, in which the majority of cases are female, supporting earlier conclusions that the overall male predominance of craniosynostosis does not include the coronal suture.[14,20] The cause for this sex bias is still to be established. Whether male dominance is partly due to genetic variants on the X chromosome or the cause of circulating androgens in early craniofacial development[35] is yet to be established.

Familial Cases

The high number of familial cases in nonsyndromic craniosynostosis suggests a genetic cause, in addition to the more established genetic involvement in syndromic craniosynostosis. The genes involved in nonsyndromic craniosynostosis are in the beginning of their discovery, and inheritance seems to be complex.[36]

Study Strengths and Limitations

One of the major strengths of this study is that the epidemiological data are population based and collected prospectively. Previous reports are mainly based on data from single hospitals or regions (with a few exceptions[13]), and this might explain the broad range found with regard to the incidence, proportion of syndromic to nonsyndromic cases, affected suture(s), sex distribution, and frequency of familial cases. All individuals with suspected syndromic or familial craniosynostosis were seen by one of the team's clinical geneticists, thus allowing more accurate figures for syndromic and familial cases. We believe Norway to be a suitable country for studying the epidemiology of craniosynostosis because of the equal-access healthcare system that ensures a high inclusion rate and because of the organization of the unit in a centralized multidisciplinary team diagnosing and treating all individuals with craniosynostosis.

The main limitation in any study of the incidence of craniosynostosis is the lack of objective diagnostic criteria. All published classification systems have an element of subjective expert opinion. Our series is based on diagnosis by the same experienced pediatric neurosurgeons. Mild cases/partial synostoses not requiring surgery were excluded. A full radiological workup was not routinely performed. Partly fused single-suture cases with minimal stigmata would therefore not have been registered. We have included cases judged by the unit's experienced pediatric neurosurgeons to be surgical candidates but in which surgery was deferred for different reasons. Thus, our series may not be directly comparable to others.

Another limitation of the study is that we cannot be entirely sure that all individuals in need of surgery were referred to the National Unit for Craniofacial Surgery as recommended and may have been treated at a local/regional hospital. We made an inquiry to the Norwegian Directorate of Health, which in turn provided summary statistics of all individuals surgically treated in 2008–2016. This revealed a small number of individuals who had undergone relevant surgery at one of the regional hospitals outside Oslo. Unfortunately, we do not have all relevant information regarding these patients, as the data provided are anonymous, do not include age, and are only linked to the procedure performed. Furthermore, we believe that some of the individuals operated on elsewhere were included in our database, having been referred to the National Unit for further treatment and follow-up. In effect, this means that the overall incidence may be slightly higher than presented in this series. The surgeries performed outside Oslo are equally distributed over the study period, and therefore the impact on the increase in incidence is negligible.

With the exception of overall incidence, we have included only consenting individuals (85%) in the study. This could potentially bias the results. However, to the best of our knowledge, the nonconsenting individuals were distributed equally for all parameters.

We also included as a limitation the possibility that there may be children born in the last few years of the study yet to be diagnosed. However, we expect this number to be low, as most children are diagnosed by 1 year of age.

Conclusions

This population-based epidemiological study confirms previous reports of an increasing incidence of craniosynostosis. In addition, we present one of the highest incidence rates reported in the literature. The increase is primarily found in nonsyndromic, midline craniosynostosis. Children are diagnosed at a younger age in the latter cohort, and this is more pronounced for individuals with syndromic or complex craniosynostosis. The high number of syndromic cases presented in this study is probably related to a broader definition when compared to other studies. We demonstrate that syndromic craniosynostosis is highly suture specific. The study revealed a high occurrence of familial cases in both syndromic and nonsyndromic craniosynostosis, further emphasizing the importance of genetics in craniosynostosis.

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References

1. Twigg SR, Wilkie AO. A genetic-pathophysiological framework for craniosynostosis. *Am J Hum Genet*. 2015;97(3):359–377.
2. Weinzweig J, Kirschner RE, Farley A, et al. Metopic synostosis: defining the temporal sequence of normal suture fusion and differentiating it from synostosis on the basis of computed tomography images. *Plast Reconstr Surg*. 2003;112(5):1211–1218.
3. Eide PK, Helseth E, Due-Tønnessen B, Lundar T. Assessment of continuous intracranial pressure recordings in childhood craniosynostosis. *Pediatr Neurosurg*. 2002;37(6):310–320.
4. Judy BF, Swanson JW, Yang W, et al. Intraoperative intracranial pressure monitoring in the pediatric craniosynostosis population. *J Neurosurg Pediatr*. 2018;22(5):475–480.
5. Proctor MR, Meara JG. A review of the management of single-suture craniosynostosis, past, present, and future. *J Neurosurg Pediatr*. 2019;24(6):622–631.
6. Thompson DN, Harkness W, Jones BM, Hayward RD. Aetiology of herniation of the hindbrain in craniosynostosis. An investigation incorporating intracranial pressure monitoring and magnetic resonance imaging. *Pediatr Neurosurg*. 1997;26(6):288–295.
7. Nguyen JQN, Resnick CM, Chang YH, et al. Impact of obstructive sleep apnea on optic nerve function in patients with craniosynostosis and recurrent intracranial hypertension. *Am J Ophthalmol*. 2019;207:356–362.
8. Renier D, Sainte-Rose C, Marchac D, Hirsch JF. Intracranial pressure in craniostenosis. *J Neurosurg*. 1982;57(3):370–377.
9. Thompson DN, Harkness W, Jones B, et al. Subdural intracranial pressure monitoring in craniosynostosis: its role in surgical management. *Childs Nerv Syst*. 1995;11(5):269–275.
10. Wilkie AOM, Johnson D, Wall SA. Clinical genetics of craniosynostosis. *Curr Opin Pediatr*. 2017;29(6):622–628.
11. Boulet SL, Rasmussen SA, Honein MA. A population-based study of craniosynostosis in metropolitan Atlanta, 1989–2003. *Am J Med Genet A*. 2008;146A(8):984–991.
12. Cornelissen M, Ottelander Bd, Rizopoulos D, et al. Increase of prevalence of craniosynostosis. *J Craniomaxillofac Surg*. 2016;44(9):1273–1279.
13. Kweldam CF, van der Vlugt JJ, van der Meulen JJ. The inci-

dence of craniosynostosis in the Netherlands, 1997–2007. *J Plast Reconstr Aesthet Surg*. 2011;64(5):583–588.
14. Lajeunie E, Le Merrer M, Bonaïti-Pellie C, et al. Genetic study of nonsyndromic coronal craniosynostosis. *Am J Med Genet*. 1995;55(4):500–504.
15. Neusel C, Class D, Eckert AW, et al. Multicentre approach to epidemiological aspects of craniosynostosis in Germany. *Br J Oral Maxillofac Surg*. 2018;56(9):881–886.
16. Garrocho-Rangel A, Manriquez-Olmos L, Flores-Velazquez J, et al. Non-syndromic craniosynostosis in children: scoping review. *Med Oral Patol Oral Cir Bucal*. 2018;23(4):e421–e428.
17. Hunter AG, Rudd NL. Craniosynostosis. I. Sagittal synostosis: its genetics and associated clinical findings in 214 patients who lacked involvement of the coronal suture(s). *Teratology*. 1976;14(2):185–193.
18. Selber J, Reid RR, Chike-Obi CJ, et al. The changing epidemiologic spectrum of single-suture synostoses. *Plast Reconstr Surg*. 2008;122(2):527–533.
19. van der Meulen J, van der Hulst R, van Adrichem L, et al. The increase of metopic synostosis: a pan-European observation. *J Craniofac Surg*. 2009;20(2):283–286.
20. Greenwood J, Flodman P, Osann K, et al. Familial incidence and associated symptoms in a population of individuals with nonsyndromic craniosynostosis. *Genet Med*. 2014;16(4):302–310.
21. Lajeunie E, Le Merrer M, Bonaïti-Pellie C, et al. Genetic study of scaphocephaly. *Am J Med Genet*. 1996;62(3):282–285.
22. Lajeunie E, Le Merrer M, Marchac D, Renier D. Syndromal and nonsyndromal primary trigonocephaly: analysis of a series of 237 patients. *Am J Med Genet*. 1998;75(2):211–215.
23. Kalantar-Hormozi H, Abbaszadeh-Kasbi A, Sharifi G, et al. Incidence of familial craniosynostosis among patients with nonsyndromic craniosynostosis. *J Craniofac Surg*. 2019;30(6):e514–e517.
24. Statistics Norway (SSB). Population: Births. Updated March 7, 2019. Accessed February 13, 2020. https://www.ssb.no/befolkning/statistikker/fodte
25. Dean AG, Sullivan KM, Soe MM. R by C table. OpenEpi: Open Source Epidemiologic Statistics for Public Health. Accessed February 13, 2020. http://openepi.com/RbyC/RbyC.htm
26. Branch LG, Kesty K, Krebs E, et al. Deformational plagiocephaly and craniosynostosis: trends in diagnosis and treatment after the "back to sleep" campaign. *J Craniofac Surg*. 2015;26(1):147–150.
27. Bérard A, Zhao JP, Sheehy O. Antidepressant use during pregnancy and the risk of major congenital malformations in a cohort of depressed pregnant women: an updated analysis of the Quebec Pregnancy Cohort. *BMJ Open*. 2017;7(1):e013372.
28. Argenta LC, David LR, Wilson JA, Bell WO. An increase in infant cranial deformity with supine sleeping position. *J Craniofac Surg*. 1996;7(1):5–11.
29. Heuzé Y, Holmes G, Peter I, et al. Closing the gap: genetic and genomic continuum from syndromic to nonsyndromic craniosynostoses. *Curr Genet Med Rep*. 2014;2(3):135–145.
30. Miller KA, Twigg SR, McGowan SJ, et al. Diagnostic value of exome and whole genome sequencing in craniosynostosis. *J Med Genet*. 2017;54(4):260–268.
31. Xu Y, Sun S, Li N, et al. Identification and analysis of the genetic causes in nine unrelated probands with syndromic craniosynostosis. *Gene*. 2018;641:144–150.
32. Simard M, Laprise C, Girard SL. Impact of paternal age at conception on human health. *Clin Chem*. 2019;65(1):146–152.
33. Taylor JL, Debost JPG, Morton SU, et al. Paternal-age-related de novo mutations and risk for five disorders. *Nat Commun*. 2019;10(1):3043.
34. Seruya M, Oh AK, Boyajian MJ, et al. Age at initial consultation for craniosynostosis: comparison across different patient characteristics. *J Craniofac Surg*. 2013;24(1):96–98.
35. Lin IC, Slemp AE, Hwang C, et al. Immunolocalization of androgen receptor in the developing craniofacial skeleton. *J Craniofac Surg*. 2004;15(6):922–929.
36. Timberlake AT, Persing JA. Genetics of nonsyndromic craniosynostosis. *Plast Reconstr Surg*. 2018;141(6):1508–1516.

Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Tønne, Due-Tønnessen, Helseth, Heimdal. Acquisition of data: Tønne, Due-Tønnessen, Wiig, Stadheim, Heimdal. Analysis and interpretation of data: Tønne, Due-Tønnessen, Meling, Helseth, Heimdal. Drafting the article: Tønne. Critically revising the article: Due-Tønnessen, Wiig, Stadheim, Meling, Helseth, Heimdal. Statistical analysis: Tønne. Study supervision: Meling, Helseth, Heimdal.

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ARTICLE

Benefits of clinical criteria and high-throughput sequencing for diagnosing children with syndromic craniosynostosis

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Abstract

An accurate diagnosis of syndromic craniosynostosis (CS) is important for personalized treatment, surveillance, and genetic counselling. We describe detailed clinical criteria for syndromic CS and the distribution of genetic diagnoses within the cohort. The prospective registry of the Norwegian National Unit for Craniofacial Surgery was used to retrieve individuals with syndromic CS born between 1 January 2002 and 30 June 2019. All individuals were assessed by a clinical geneticist and classified using defined clinical criteria. A stepwise approach consisting of single-gene analysis, comparative genomic hybridization (aCGH), and exome-based high-throughput sequencing, first filtering for 72 genes associated with syndromic CS, followed by an extended trio-based panel of 1570 genes were offered to all syndromic CS cases. A total of 381 individuals were registered with CS, of whom 104 (27%) were clinically classified as syndromic CS. Using the single-gene analysis, aCGH, and custom-designed panel, a genetic diagnosis was confirmed in 73% of the individuals ($n = 94$). The diagnostic yield increased to 84% after adding the results from the extended trio-based panel. Common causes of syndromic CS were found in 53 individuals (56%), whereas 26 (28%) had other genetic syndromes, including 17 individuals with syndromes not commonly associated with CS. Only 15 individuals (16%) had negative genetic analyses. Using the defined combination of clinical criteria, we detected among the highest numbers of syndromic CS cases reported, confirmed by a high genetic diagnostic yield of 84%. The observed genetic heterogeneity encourages a broad genetic approach in diagnosing syndromic CS.

Supplementary information The online version of this article (https://doi.org/10.1038/s41431-020-00788-4) contains Supplementary Material, which is available to authorized users.

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Introduction

Craniosynostosis (CS) is one of the most common inborn anomalies in children, affecting 1/1600–1/1800 live births [1, 2]. CS is classified into syndromic and nonsyndromic CS, where syndromic CS is reported to constitute 12–31% of all cases [3–5]. Individuals with syndromic CS have an increased risk of additional complications and repeat craniofacial surgery [6, 7], and need to be identified. Hence, an accurate molecular diagnosis is important for personalized treatment and surveillance, in addition to genetic counselling, family planning, social care, and support from patient organizations.

Previously, syndromic CS was defined by the occurrence of one of the frequent and well-known syndromes: Apert, Muenke, Saethre–Chotzen, Pfeiffer, or Crouzon, caused by genetic variants in the *FGFR2*, *FGFR3*, *TWIST1*, *FGFR1/2*, and *FGFR2* genes, respectively [1, 8]. High-throughput sequencing (HTS) has improved and changed the diagnostics of syndromic CS over the last two decades, and

genetic variants in at least 80 genes are known to cause syndromic CS [9, 10].

There is no clear consensus regarding the definition of syndromic CS. Some studies limit their cohort to a defined selection of verified genetic diagnoses [11, 12], whereas others limit their cohort to affected sutures only, as complex or coronal synostoses are more commonly associated with syndromic CS [12, 13], or use a combination of clinical criteria [5, 14]. A recent population-based epidemiological study from our group demonstrated a high proportion of syndromic cases of 27% defined by clinical criteria and a genetic detection rate of 75% after testing with array comparative genomic hybridization (aCGH) and exome-based HTS, filtering for 72 genes associated with syndromic CS [2]. We detected many midline synostoses in individuals with syndromic CS, in particular in individuals with rare genetic syndromes [2], suggesting that an affected suture alone does not provide sufficient evidence to determine whether an individual has syndromic or nonsyndromic CS. We hypothesized that a broader approach to genetic testing would further increase the diagnostic yield.

In this study, all individuals with syndromic CS born between 1 January 2002 and 30 June 2019, selected by clinical criteria, and registered in the registry of the Norwegian National Unit for Craniofacial Surgery were included. Supplemental genetic diagnostics of HTS filtering for a panel of 1570 genes informed by the Deciphering Developmental Delay study (DDG2P) were offered for negative cases. We present a large variety of genetic syndromes and aim to propose a strategy for clinical classification and genetic testing of individuals with syndromic CS.

Materials and methods

The study was approved by the Norwegian Regional Committees for Medical and Health Research Ethics (REK_2018/797) and by Oslo University Hospital (permit number P360:18/05374). Informed consent was obtained from all individuals that participated in the study. Since 2001, all individuals in Norway with suspected CS have been referred to the Norwegian National Unit for Craniofacial Surgery at Oslo University Hospital for diagnostics, treatment, and follow-up [2]. Individuals suspected of having syndromic CS are seen regularly by the unit's multidisciplinary team, including a clinical geneticist. The unit's registry is prospective and includes all consenting individuals diagnosed with CS (85%) [2]. Individuals with CS born between 1 January 2002 and 30 June 2019 and registered by 23 October 2019 were included in the study ($n = 381$). The database was updated January 2020 to include the latest genetic results. Syndromic CS was defined by a combination of clinical criteria, formulated by the authors, with one major criterion or two or more minor criteria; details are presented in Fig. 1. All individuals were classified by the same two clinical geneticists prior to inclusion (ET and KRH).The genetic analyses were offered stepwise. Individuals suspected of having one of the common and well-described CS syndromes were initially tested by single-gene analysis of *FGFR2*, *FGFR3*, *TWIST1* or *EFNB1*. If the results came back negative, aCGH was performed. When the clinical presentation did not resemble one of the common CS syndromes, aCGH was offered initially. From 2016, exome-based HTS filtering for a custom-designed panel of 72 genes associated with syndromic CS (Supplemental Table 1) was performed if the result of the aCGH came back negative. If this did not result in a genetic diagnosis the extended trio-based HTS panel of 1570 genes was offered. A few individuals ($n = 6$) were diagnosed prior to assessment by the unit's team. Their findings are presented in the results section under the diagnostic tool in which they would have been found in the stepwise approach (Tables 2–4). Ten individuals were excluded from the calculations of diagnostic yield, because they did not want genetic testing ($n = 4$), and were analysed with aCGH only ($n = 5$) or with single gene and aCGH only ($n = 1$). Individuals analysed with aCGH and HTS filtering for the custom-designed panel only ($n = 3$) were included in the calculations. All individuals with nonsyndromic CS of the coronal suture (s), or with an affected first-degree relative, were offered the custom-designed HTS panel due to the risk of having a monogenetic cause (e.g., *TCF12*). As genetic causes of nonsyndromic CS is not the scope of this study, these results are not included. Blood samples were obtained from all patients, followed by DNA extraction with QiaSymphony DSP DNA Mini Kit (Qiagen, Cologne, Germany). For Sanger sequencing of *FGFR2*, *FGFR3*, *TWIST1*, and *EFNB1*, primers were designed using primer3 software, sequencing was done on an ABI 3730 sequencer (Applied Biosystems, Life

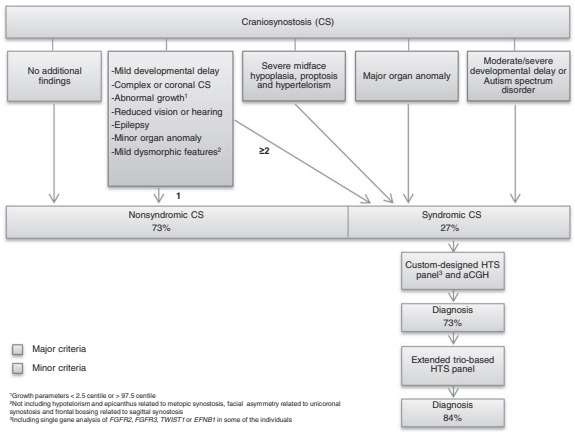

Fig. 1 Flow chart showing clinical criteria and genetic analysis of syndromic CS. Minor criteria are presented in the dark blue panel and major criteria in the red panels. Syndromic CS is defined by the addition of two or more minor criteria or one major criterion.

Table 1 Genetically confirmed diagnoses by single-gene analysis (Sanger sequencing).

Syndrome	Gene	Cases	Male/female	Suture[a]	Familial[b]
Apert	*FGFR2*	15	6/9	BC, LCS, MS	0
Muenke	*FGFR3*	14	7/7	BC, RC	8 (6 index)
Saethre–Chotzen	*TWIST1*	8	4/4	BC, LC, RC,	6 (4 index)
Crouzon/Pfeiffer/Beare–Stevenson syndrome	*FGFR2*	5	2/3	BC, BL, BCBL, P, S	1
Crouzon with acanthosis nigricans	*FGFR3*	3	1/2	BCS, P	0
Craniofrontonasal dysplasia	*EFNB1*	2	0/2	BC, RC	0

BC bicoronal, *BCBL* bicoronal and bilambdoid, *BCS* bicoronal and sagittal, *BL* bilambdoid, *LC* left coronal, *LCS* left coronal and sagittal, *MS* metopic and sagittal, *P* pancynostosis, *RC* right coronal, *S* sagittal.

[a]Affected suture: BC, BCBL, BL, BCS, LC, LCS, MS, P, RC, S.

[b]Individuals with an affected first- or second-degree relative.

Technologies, CA, USA), and sequence data were analysed using SeqScape v2.7 (Life Technologies, CA, USA). For MLPA of *TWIST1*, the Salsa MLPA Probemix P054 (MRC Holland) was used. Array CGH was performed using Agilent 180 K SurePrint G3 Human CGH (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's recommendations. Data were processed with Feature Extraction and DNA Analytics (Agilent Technologies). Exome-based HTS was performed by using Agilent SureSelect^{XT} Target Enrichment 50 Mb Kit (Agilent Technologies, Santa Clara, CA, USA) for library preparation and Illumina HiSeq 2500 in high-output run mode. Bioinformatic handling of the sequencing data followed the practice from Genome Analysis Tool Kit for exome sequencing [15]. Raw reads were mapped to the reference sequence (GRCh37/hg19). Joint variant calling was performed within each trio. Variant annotation was done by Annovar [16]. Downstream filtering and analysis were done with Filtus [17] on the variants within coding regions and intron/exon boundaries of the custom-designed panel or the extended trio-based panel of 1570 genes. The extended trio-based panel was informed by the Deciphering Developmental Disorders study (DDG2P) [18] and was the largest panel available at our laboratory. We selected variants with allele frequency of less than 0.5% (for genes inherited as autosomal dominant) or less than 1% (for other inheritance patterns), as reported in gnomAD [19]. Variants were classified according to the guidelines by the American College of Medical Genetics and Genomics [20], and only class 4 (likely pathogenic) and class 5 (pathogenic) variants were included in the results. All variants were submitted to ClinVar (SCV001437545–SCV001437592).

Results

In total, 381 individuals were registered with CS, of which 104 (27%) were clinically classified as syndromic based on the criteria presented in Fig. 1. A total of 94 individuals

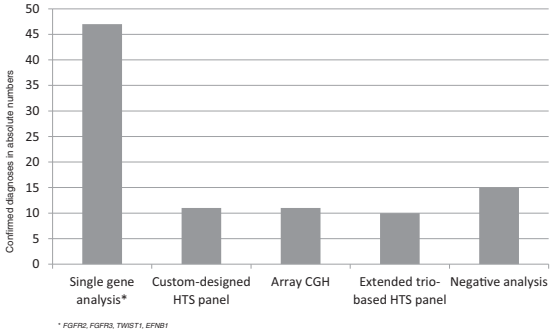

Fig. 2 Confirmed genetic diagnoses by method. The distribution of confirmed diagnoses is given in absolute numbers.

with syndromic CS (90%) accepted the stepwise genetic testing presented in the method section. By single-gene analysis, aCGH and the custom-designed panel, a genetic diagnosis was confirmed in 69 individuals (73%; Figs. 1 and 2). When including the results of the extended trio-based HTS panel, the number of genetically confirmed diagnoses increased to 79 (84%; Figs. 1 and 2, Supplemental Table 2). When excluding the CS syndromes caused by variants in the *FGFR2*, *FGFR3*, *TWIST1*, or *EFNB1* gene, a genetic cause was confirmed in 26 individuals (28%), partitioned into 23 different genetic or chromosomal causes, 16 of these not commonly associated with CS (Tables 1–4). Fifteen individuals (16%) had negative genetic test results (Fig. 2).

Fifty-three individuals (56%) had variants in one of the genes frequently associated with CS syndromes (*FGFR2*, *FGFR3, TWIST1*, and *EFNB1*). Of these, 47 individuals (89%) had a clinical phenotype in concordance with the genetic diagnosis and were diagnosed by single-gene analysis (Table 1).

Ten individuals (11%) had a de novo copy number variation associated with a known microdeletion or duplication syndrome (Fig. 2); seven of these are not commonly associated with CS (Table 2). In addition, one case of

Table 2 Chromosome aberrations in individuals with syndromic CS.

	Chromosome aberration[a]	Position[b]	Size (MB)	Candidate gene	Diagnosis	Male/ female	Suture[c]	Clinical phenotype
Chromosome aberrations not commonly associated with CS	Del 17p13.3	g.84287_2468384del	2.4	*CRK* *YWHAE*	17p13.3 Microdeletion syndrome (without *PAFAH1B1* deletion)	M	P	Development delay, short stature, hypotonia, reduced vision
	Del 1p32.3p31.3	g.53675707_ 66644963del	13	*NFIA*	1p32-p31 Deletion syndrome	M	M	Intrauterine growth restriction, developmental delay, preaxial polydactyly, inguinal hernia, short stature, corpus callosum agenesis, optic nerve hypoplasia, thoracic hypoplasia, hearing loss, microphthalmia, micrognathia, dysplastic ears
	Dup 22q11.1q12.1	g.16888899_26483608dup	9.6	No candidate gene	Cat Eye syndrome and 22q11.1q12.1 microduplication syndrome	M	M	Developmental delay, ASD, reduced vision, torticollis, micrognathia, hypotelorism, epicanthus
	Del 1p22.1	g.92405898_ 94018197del	1.6	*RPL5*	Diamond–Blackfan anaemia, 6	M	S	Developmental delay, AVSD, severe feeding difficulties, anaemia, short stature, long philtrum, thin upper lip, proximal thumb
	Del 2q37.1q37.3 Dup 11p15.5p15.4 mat	g.233110452_ 243028452del g.210300_ 8664358dup	10 8.5	*HDAC4*	2q37 Deletion syndrome and Silver–Russell syndrome	F	RC	Developmental delay, respiratory distress, cardiomegaly, hypotonia, midface hypoplasia, epicanthus, died at 12 months of age
	Del 2q24.2q31.3[d]	g.163078055_ 182119617del	19	No candidate gene	2q24 Deletion syndrome	M	BL, LC, S	Developmental delay, VSD, epilepsy, finger contractures, syndactyly, proptosis, hypertelorism, died at 12 months of age
	Del 6q16.2q21	g.98949950_114533905del	16	No candidate gene	6q15-6q23 deletion syndrome	F	M	Developmental delay, reduced vision, respiratory distress
Chromosome aberrations commonly associated with CS	Del 7p15.3p21.2	g.14470668_20385165del	6	*TWIST1*	Saethre–Chotzen syndrome	F	LC	Normal development, facial asymmetry, low frontal hairline, small rounded ears, brachydactyly, scoliosis, father mosaic
	Del 9pterp22.2	g.204193_ 18073357del	17.8	*FREM1*	9p Deletion syndrome	F	M	Developmental delay, epilepsy, omphalocele, reduced vision
	Del 9p23p22.1	g.13638428_ 17121764del	3.5	*FREM1*	9p Deletion syndrome	M	M	Developmental delay, reduced vision
	Dup 5q35.1q35.3[d]	g.170805664_ 180719789dup	10	*MSX2* *NSD1*	5q35 Duplication syndrome	M	S	Developmental delay, VSD, midface hypoplasia, hypotelorism

BC bicoronal, *BL* bilambdoid, *LC* left coronal, *M* metopic, *P* pancynostosis, *RC* right coronal, *S* sagittal.

[a]NCBI_Build 37 (hg19).

[b]Inner start-stop coordinate.

[c]Affected suture: BC, BL, LC, M, P, RC, S.

[d]Analysis performed at an external laboratory.

Saethre–Chotzen syndrome, caused by a deletion including the *TWIST1* gene, was detected by aCGH (Table 2).

Of the 11 individuals diagnosed by the custom-designed HTS panel, seven had clinical phenotype in concordance with their genetic diagnosis, while four had unexpected clinical presentation (Table 3). A girl with an *EFNB1* variant had a complex heart malformation not associated with craniofrontonasal syndrome. A boy with classic features of craniofrontonasal syndrome was not analysed by single-gene analysis due to his gender. However, HTS revealed that he was mosaic for a variant in the *EFNB1* gene (Table 3) and karyotyping confirmed XY, male. In a boy with Crouzon-like appearance, with negative result of a *FGFR2* analysis, HTS detected a variant in *TWIST1* consistent with Saethre–Chotzen syndrome. A homozygous variant in *IL11RA* consistent with CS and dental anomalies syndrome was detected in a girl with late-occurring pansynostosis (4 years old) and no dental anomalies (Table 3). In addition, we detected two cases of parental mosaicism for variants in *FGFR2* and *ZIC1*, respectively, both associated with autosomal dominant inheritance (Table 3). The individuals with the *FGFR2* variant were siblings and not analysed by single-gene analysis due to the suspicion of autosomal recessive inheritance.

We performed the analysis using the extended trio-based HTS panel on 22 individuals and revealed a diagnosis in 10; these were partitioned into 9 genetic syndromes, none of them commonly reported to include CS (Table 4). We detected two individuals with variants in the *AHDC1* gene, consistent with Xia–Gibbs syndrome. We further confirmed the following diagnoses: **c**oloboma, congenital **h**eart defects, choanal **a**tresia, **r**etardation of growth, developmental delay, **g**enital abnormalities, **e**ar abnormalities and deafness (CHARGE) syndrome, Bainbridge–Ropers syndrome (BRPS), CHDFIDD (Congenital heart defects, dysmorphic facial features, and intellectual developmental disorder, previously published [21]), Kleefstra syndrome, Genitopatellar syndrome, Floating–Harbor syndrome, Alpha-Mannosidosis (previously published [22]), and Malan syndrome (Table 4).

Discussion

In our 18-year population-based cohort of children with CS, 27% fulfilled the presented clinical criteria and were diagnosed with syndromic CS. This is the highest number of syndromic cases reported from a population-based cohort and we believe the high genetic diagnostic yield of 84% supports the clinical criteria. We found a high level of genetic heterogeneity, with variants in common and well-known genes associated with CS accounting for 67% of the solved cases; the remaining cases were distributed across a diverse range of genetic syndromes, many of which are not commonly associated with CS.

We detected mosaicism in four families: one index individual and three healthy parents (Tables 2 and 3). A variant in the *EFNB1* gene was detected in a male with classic features of craniofrontonasal syndrome by HTS analysis. The variant presented as heterozygous in the analysis, suggesting mosaicism. The *EFNB1* gene is located on the X chromosome and loss-of-function variants in the *EFNB1* gene are assumed to cause craniofrontonasal syndrome through a paradoxical gender reversal in severity, where females usually develop typical features of craniofrontonasal syndrome and males usually have hypertelorism as the only feature. Random X-inactivation is assumed to be the cause of the severe phenotype in females, causing cellular interference as the cells have different expressions of EPHRIN-B1, generating abnormal tissue boundaries [23]. It has previously been proposed that males, being mosaic for variants in the *EFNB1* gene, will present with a severe phenotype, similar to females, due to the different expression of EPHRIN-B1, which is not tolerated [23]. Our results support this. We further detected low-grade mosaicism for a variant in the *FGFR2* gene in a healthy parent of two children with Crouzon syndrome and for a deletion (including the *TWIST1* gene) in a healthy father of a child with Saethre–Chotzen syndrome. Parental mosaicism for *FGFR2* and *TWIST1* variants is previously described [24, 25]. Crouzon and Saethre–Chotzen syndrome are inherited in an autosomal dominant manner, and this finding is important for genetic guidance, as it will impact the recurrence risk. Parental mosaicism for a *ZIC1* variant led to the variant initially being missed in the Trio-HTS analysis (filtering for de novo variants) in a boy with CRS6 and was only detected after manual re-evaluation of the gene due to his classical phenotype (Table 3). These cases demonstrate the need for a thorough evaluation of a well-described clinical phenotype, as diagnosis may be missed on trio analysis due to parental mosaicism.

We diagnosed syndromes not commonly associated with CS in 17 individuals, of whom 10 were detected by the extended trio-based panel and 7 by aCGH. We demonstrate an expansion of the clinical phenotype beyond CS in some cases (Table 4). Interestingly, all cases of rare syndromes detected by the extended trio-based panel, with two exceptions, had synostosis of a single midline suture only (Table 4). Likewise, seven out of ten microdeletion syndromes (Table 2) had midline synostosis only. This contrasts with the pattern typically seen in individuals with syndromic CS, where multiple suture synostosis is the most common finding [5, 26], and also with our finding in individuals with the more common CS syndromes (Tables 1 and 3). The most common reported CS syndromes have a high frequency of CS and are caused by genes acting in

Table 3 Genetically confirmed diagnoses by the custom-designed HTS panel, divided by expected and unexpected clinical presentation.

	Syndrome	Gene	Variant	Inheritance	Male/female	Suture[b]	Clinical features
Expected clinical presentation	Craniosynostosis and dental anomalies	*IL11RA* NM_001142784.2	c.781 C > T p.(Arg261Cys)	Recessive	M	P	Chiari I malformation, microcephaly, midface hypoplasia, Crouzon-like appearance
	Cranioectodermal dysplasia/Sensenbrenner	*IFT122* NM_052985.1	c.1118 C > T p.(Ser373Phe)	Recessive	M	S	Renal failure, sensorineural hearing deficit, short statue, telecanthus, micrognathia tooth anomalies, brachydactyly, Tourette syndrome
	Craniosynostosis 4 CRS4	*ERF* NM_006494.2	c.1201_1202del p.(Lys401Glufs*10)	Dominant, de novo	F	S	Midface hypoplasia, hypertelorism, short nose
	Craniosynostosis 3 CRS3	*TCF12* NM_207036.1	c.778_779del p.(Met260Valfs*5)	Dominant, paternal	M	LC	Low anterior hairline, brachydactyly, transverse palmar crease, healthy father
	Craniosynostosis 6 CRS6	*ZIC1*[a] NM_003412.3	c.1153 G > T p.(Glu385*)	Dominant, maternal mosaic	M	BC, LL	Developmental delay, severe speech delay, reduced vision, proptosis, midface hypoplasia, tubular nose, healthy mother
	Crouzon	*FGFR2* NM_000141.4	c.824_829dup p.(Glu275_Phe276dup)	Dominant, paternal mosaic	M/F	BC,S	Two siblings with typical Crouzon phenotype, healthy father
Unexpected clinical presentation	Craniofrontonasal dysplasia	*EFNB1* NM_004429.4	c.182 A > G p.(Asp61Gly)	De novo, mosaic	M	RC	Short and asymmetric skull, hypertelorism, broad and depressed nasal root, asymmetric eyes, widow's peak, pectus excavatum, dysplastic nails
	Craniofrontonasal dysplasia	*EFNB1* NG_008887.1 (NM_004429.4)	c.128 + 5 G > A splice	Dominant, de novo	F	LC	Atrial septal defect (ASD), facial asymmetry, hypertelorism, broad nasal root, bifid nasal tip, widow's peak
	Craniosynostosis and dental anomalies	*IL11RA* NM_001142784.2	c.281 G > T p.(Cys94Phe)	Recessive	F	P	Late pancynostosis (4 y), papilloedema, hydrocephalus, midface hypoplasia, normal teeth
	Saethre–Chotzen	*TWIST1* NM_000474.3	c.309 C > G p.(Tyr103*)	Dominant, maternal	M	P	Crouzon-like appearance

BC coronal, *LC* left coronal, *LL* left lambdoid, *P* pancynostosis, *RC* right coronal, *S* sagittal.

[a]Analysed at an external laboratory, gene included in the custom-designed HTS panel.

[b]Affected suture: BC, LC, LL, P, RC, S.

Table 4 Genetically confirmed diagnoses by the extended trio-based HTS panel.

Syndrome	Gene	Variant	Inheritance	Male/female	Suture[c]	Clinical features in line with the phenotypic description	Extension of phenotype
Xia–Gibbs syndrome	*AHDC1* NM_001029882.2	c.3185_3186del p.(Thr1062Serfs*63)	De novo	F	LC, S	Moderate developmental delay, autism, hypotonia, reduced vision, sleep disturbances	
Xia–Gibbs syndrome	*AHDC1* NM_001029882.3	c.2772del p.(Arg925Glufs*7)	De novo	M	M	Moderate developmental delay, short corpus callosum, hypotonia, short stature, proptosis, midface hypoplasia, long philtrum	Tethered cord, Chiari I malformation, omphalocele
Bainbridge–Ropers syndrome	*ASXL3* NM_030632.1	c.3033dup p.(Leu1012Serfs*23)	De novo	M	M	Moderate developmental delay, autism, reduced vision, feeding difficulties, sleep disturbances, strabismus, telecanthus, long philtrum, full lips, broad and proximally placed thumbs, behaviour difficulties	Craniosynostosis
CHDFIDD	*CDK13*[a,b] NM_003718.4	c.2524 A > G p.(Asn842Asp)	De novo	F	M	Moderate developmental delay, autism, reduced vision, strabismus, proptosis, microcephaly, midface hypoplasia, broad nasal bridge, behaviour difficulties	Craniosynostosis
CHARGE syndrome	*CHD7* NM_017780.3	c.7593dup p.(Thr2532Aspfs*9)	De novo	M	S	Developmental delay, pulmonary atresia, VSD, cleft lip/palate, sensorineural hearing deficit, sleep apnoea, behaviour difficulties, feeding difficulties, scoliosis, micrognathia, hypotelorsim, cup-shaped ears	Late occurrence of craniosynostosis (5 years)
Kleefstra syndrome	*EHMT1* NG_011776.1 (NM_024757.4)	c.2018 + 1 G > C splice	De novo	M	S	Severe developmental delay, microcephaly, missing teeth, and delayed eruption, coarse facies, brachydactyly	Craniosynostosis
Genitopatellar syndrome	*KAT6B* NM_012330.3	c.3769_3772del p.(Lys1258Glyfs*13)	De novo	F	S	Knee flexion deformities, dislocated patella bilaterally, agenesis of corpus callosum, apnoea, hydronephrosis, severe eating difficulties, coarce facies, micrognathia, broad nose, died at 7.5 months of age	Craniosynostosis
Alpha-mannosidosis	*MAN2B1*[a,b] NM_000528.3	c.1055 T > C p.(Leu352Pro)	Recessive	M	P	Intellectual disability, sensorineural hearing deficit	
Floating–Harbor syndrome	*SRCAP*[a] NM_006662.2	c.7303 C > T p.(Arg2435*)	De novo	M	S	Developmental delay, short stature, hypertension, midface hypoplasia, deep-set eyes	Craniosynostosis
Malan syndrome	*NFIX* NM_002501.3	c.143 T > A p.(Met48Lys)	De novo	F	S	Moderate intellectual disability, macrocephaly, reduced vision, strabismus, long narrow face, deep-set eyes	Puberta praecox, craniosynostosis

LC left coronal, *M* metopic, *P* pansynostosis, *S* sagittal.

[a]Analysis performed at an external laboratory, gene included in the extended trio-based HTS panel.

[b]Previously reported.

[c]Affected suture: LC, M, P, S.

signalling pathways important for the development of the cranial sutures, mostly associated with osteogenic differentiation of stem cells (FGF/FGFR, Eph/Ephrin, TGFbeta/BMP, WNT) [27, 28]. The difference in affected sutures between the common CS syndromes and the rare or ultra-rare syndromes, with a low frequency of CS caused by genes acting in other pathways, might indicate that the synostoses in these two groups have different molecular mechanisms. Individuals with rare genetic syndromes which includes macrocephaly (e.g., Malan syndrome) might also be at higher risk of developing CS due to foetal head constraints that are associated with CS, especially regarding coronal premature fusion [27, 29].

Notably, in our cohort we detected several Mendelian disorders of chromatin modification (chromatinopathies), including (with the associated gene in parentheses): CHARGE (*CHD7*), Kleefstra (*EHMT1*), Floating–Harbor syndrome (*SRCAP*), KAT6B-related disorders (*KAT6B*), and 2q37 deletion syndrome (caused by haploinsufficiency of the *HDAC4* gene [30]). These genes influence the epigenetic machinery by targeting the DNA or the DNA-associated histone proteins, and variants that affect function are expected to have widespread epigenetic consequences [31, 32]. Approximately 44 chromatinopathies have been described to date. The most common mechanism is presumed to be haploinsufficiency, as a majority of the individuals have a loss-of-function variant [32]; this concords with our results (Tables 2 and 4). A few of the chromatinopathies have previously been associated with CS: Kabuki syndrome, Bohring–Opitz syndrome (BOS), and two cases of KAT6B-related disorders [31–36]. To our knowledge, only one case of CS in CHARGE syndrome [37], one case in Floating–Harbor syndrome [38], one case in 2q37 deletion syndrome [30], and none in Kleefstra syndrome have been reported. This study confirms CS as a feature of CHARGE syndrome, Floating–Harbor syndrome, KAT6B-related disorders, and suggests CS as a feature in Kleefstra syndrome and 2q37 deletion syndrome. We cannot be certain that haploinsufficiency of the *HDAC4* gene is the cause of CS in this case, as the individual also had a duplication on 11p15 in concordance with Silver–Russell syndrome. However, Silver–Russell syndrome is not associated with CS but rather delayed fontanelle closure. The presence of CS in several chromatinopathies at a low frequency adds to reports of other low-frequent malformations in these disorders. Their presence may be dependent on the molecular characteristics of the targeted genes, in addition to a general disruption of the epigenetic machinery; these are both suggested mechanisms for this phenotypic variability [31, 32, 39, 40]. Clinically, these findings suggest that individuals with chromatinopathies should be monitored for CS, in addition to other organ anomalies.

BRPS has phenotypic overlap with BOS. The former is caused by loss-of-function variants in the *ASXL3* gene and the latter by variants in the *ASXL1* gene. However, metopic synostosis, often seen in BOS, is not commonly reported in BRPS [41, 42]. Our case confirms that metopic synostosis is a rare feature in BRPS. CS has been reported in a very few individuals with CHDFIDD, Xia–Gibbs, Alpha-mannosidosis, and Malan syndrome [10, 43–45]. Individuals with Diamond–Blackfan anaemia have not been reported with CS.

Syndromic CS may be subdivided into syndromes with high risk of developing CS and a multitude of diverse syndromes usually defined by extracranial features with a low risk of developing CS. Due to the rarity of many syndromes, it is to be expected that the list defining the latter group is incomplete. Our results may point to a greater risk in subgroups of syndromes, such as the chromatinopathies.

Supported by our high diagnostic yield, we argue for the use of the presented clinical criteria, to ensure that all individuals with syndromic CS are identified, and thereby offered a broad genetic approach and assessment in a multidisciplinary team. For research purposes, a common clinical definition of syndromic CS is important to make reliable comparisons across cohorts. For some individuals, the features, indicating syndromic CS will not be present when the CS is evident. This argues for clinical follow-up after surgery for all individuals with CS. We recommend assessment of all individuals with syndromic CS in a multidisciplinary team to identify additional anomalies and progressive disturbances in facial growth, which may require repeat craniofacial surgeries [6, 7]. A high number of the syndromic cases in our cohort had a rare or ultra-rare genetic cause, mostly due to variants in different genes, emphasizing that syndromic CS is highly heterogeneous. This argues for a broad genetic approach. We suggest stepwise testing initiated by a custom-based HTS panel and aCGH, as the majority of the confirmed diagnoses were detected by these two analyses. In addition, our study showed that a number of variants were inherited from parents (including mosaics), all likely to be missed on the extended trio-based HTS panel. We then recommend trio-analyses, applying an extended panel of genes associated with development delay/anomalies in general, for negative cases. If the clinical presentation is highly suspicious of one of the frequent CS syndromes, one might consider testing the *FGFR2, FGFR3, TWIST1*, or *EFNB1* genes first; however, as this and other studies [46] have shown, a number of individuals have atypical presentations.

The main strength of the study is that the data are population-based and prospectively collected. Norway has an equal-access healthcare system that ensures a high inclusion rate. The unit is organized as a centralized multidisciplinary team, including a clinical geneticist. The

clinical geneticist reassesses individuals initially diagnosed with nonsyndromic CS when new findings or difficulties present. A limitation of the study is that individuals diagnosed with CS over the last two or three years may not yet have presented with additional findings; thus, some syndromic cases may have been missed and the true number might be slightly higher. In syndromes not previously associated with CS, we cannot exclude the possibility of an additional genetic diagnosis associated with CS not detected by today's methods (e.g., deep intronic variants). Newly associated genes, such as *SMAD6*, recently documented to be an important cause of CS [47], were not included in the panels. In addition, MLPA of *EFNB1* and *TCF12* were not available at our laboratory. According to this some diagnoses may have been missed. In addition, a few individuals included in the calculations were not analysed with the extended trio-based HTS panel ($n = 3$). This could mean that the genetic detection rate should be even higher.

Conclusion

Using the presented clinical criteria, we identified one of the highest numbers of syndromic CS cases reported, strongly supported by a high genetic detection rate of 84%. The observed genetic heterogeneity and atypical presentations encourage a broad genetic approach in diagnosing syndromic CS. Surveillance for CS is recommended in a variety of genetic syndromes, including syndromes rarely associated with CS, such as the chromatinopathies, for the purpose of early diagnosis and treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Kweldam CF, van der Vlugt JJ, van der Meulen JJ. The incidence of craniosynostosis in the Netherlands, 1997-2007. J Plast Reconstr Aes. 2011;64:583–8.
2. Tønne E, Due-Tonnessen BJ, Wiig U, Stadheim BF, Meling TR, Helseth E, et al. Epidemiology of craniosynostosis in Norway. J Neurosurg Pediatr. 2020. https://doi.org/10.3171/2020.1.PEDS2051.
3. Neusel C, Class D, Eckert AW, Firsching R, Gobel P, Gotz D, et al. Multicentre approach to epidemiological aspects of craniosynostosis in Germany. Br J Oral Max Surg. 2018;56:881–6.
4. Wilkie AOM, Johnson D, Wall SA. Clinical genetics of craniosynostosis. Curr Opin Pediatr. 2017;29:622–8.
5. Lattanzi W, Barba M, Di Pietro L, Boyadjiev SA. Genetic advances in craniosynostosis. Am J Med Genet A 2017;173:1406–29.
6. Wilkie AO, Byren JC, Hurst JA, Jayamohan J, Johnson D, Knight SJ, et al. Prevalence and complications of single-gene and chromosomal disorders in craniosynostosis. Pediatrics 2010;126:e391–400.
7. Meling TR, Due-Tonnessen BJ, Hogevold HE, Skjelbred P, Arctander K. Monobloc distraction osteogenesis in pediatric patients with severe syndromal craniosynostosis. J Craniofac Surg. 2004;15:990–1000.
8. Heuze Y, Holmes G, Peter I, Richtsmeier JT, Jabs EW. Closing the gap: Genetic and genomic continuum from syndromic to nonsyndromic craniosynostoses. Curr Genet Med Rep. 2014;2:135–45.
9. Goos JAC, Mathijssen IMJ. Genetic causes of craniosynostosis: an update. Mol Syndromol. 2019;10:6–23.
10. Gumus E. Extending the phenotype of Xia-Gibbs syndrome in a two-year-old patient with craniosynostosis with a novel de novo AHDC1 missense mutation. Eur J Med Genet. 2020;63:103637.
11. Lee HQ, Hutson JM, Wray AC, Lo PA, Chong DK, Holmes AD, et al. Changing epidemiology of nonsyndromic craniosynostosis and revisiting the risk factors. J Craniofac Surg. 2012;23:1245–51.
12. Cornelissen M, Ottelander B, Rizopoulos D, van der Hulst R, Mink van der Molen A, van der Horst C, et al. Increase of prevalence of craniosynostosis. J Craniomaxillofac Surg. 2016;44:1273–9.
13. Topa A, Rohlin A, Andersson MK, Fehr A, Lovmar L, Stenman G, et al. NGS targeted screening of 100 Scandinavian patients with coronal synostosis. Am J Med Genet A 2020;182:348–56.
14. Johnson D, Wilkie AO. Craniosynostosis. Eur J Hum Genet. 2011;19:369–76.
15. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303.
16. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164.
17. Vigeland MD, Gjotterud KS, Selmer KK. FILTUS: a desktop GUI for fast and efficient detection of disease-causing variants,

including a novel autozygosity detector. Bioinformatics 2016;32:1592–4.
18. Deciphering Developmental Disorders Study. Large-scale discovery of novel genetic causes of developmental disorders. Nature. 2015;519:223–8.
19. Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020;581:434–43.
20. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–24.
21. Hamilton MJ, Caswell RC, Canham N, Cole T, Firth HV, Foulds N, et al. Heterozygous mutations affecting the protein kinase domain of CDK13 cause a syndromic form of developmental delay and intellectual disability. J Med Genet. 2018;55:28–38.
22. Riise Stensland HM, Klenow HB, Van Nguyen L, Hansen GM, Malm D, Nilssen O. Identification of 83 novel alpha-mannosidosis-associated sequence variants: functional analysis of MAN2B1 missense mutations. Hum Mutat. 2012;33:511–20.
23. Twigg SR, Babbs C, van den Elzen ME, Goriely A, Taylor S, McGowan SJ, et al. Cellular interference in craniofrontonasal syndrome: males mosaic for mutations in the X-linked EFNB1 gene are more severely affected than true hemizygotes. Hum Mol Genet. 2013;22:1654–62.
24. Goriely A, Lord H, Lim J, Johnson D, Lester T, Firth HV, et al. Germline and somatic mosaicism for FGFR2 mutation in the mother of a child with Crouzon syndrome: Implications for genetic testing in "paternal age-effect" syndromes. Am J Med Genet A 2010;152a:2067–73.
25. Apostolopoulou D, Kaxira OS, Hatzaki A, Panagopoulos KP, Alexandrou K, Stratoudakis A, et al. Genetic analysis of syndromic and nonsyndromic patients with craniosynostosis identifies novel mutations in the TWIST1 and EFNB1 Genes. Cleft Palate Craniofac J. 2018;55:1092–102.
26. Di Rocco F, Arnaud E, Meyer P, Sainte-Rose C, Renier D. Focus session on the changing "epidemiology" of craniosynostosis (comparing two quinquennia: 1985-1989 and 2003-2007) and its impact on the daily clinical practice: a review from Necker Enfants Malades. Childs Nerv Syst. 2009;25:807–11.
27. Wu X, Gu Y. Signaling mechanisms underlying genetic pathophysiology of craniosynostosis. Int J Biol Sci. 2019;15:298–311.
28. Twigg SR, Wilkie AO. A genetic-pathophysiological framework for craniosynostosis. Am J Hum Genet. 2015;97:359–77.
29. Sanchez-Lara PA, Carmichael SL, Graham JM Jr., Lammer EJ, Shaw GM, Ma C, et al. Fetal constraint as a potential risk factor for craniosynostosis. Am J Med Genet A 2010;152a:394–400.
30. Williams SR, Aldred MA, Der Kaloustian VM, Halal F, Gowans G, McLeod DR, et al. Haploinsufficiency of HDAC4 causes brachydactyly mental retardation syndrome, with brachydactyly type E, developmental delays, and behavioral problems. Am J Hum Genet. 2010;87:219–28.
31. Zollino M, Lattante S, Orteschi D, Frangella S, Doronzio PN, Contaldo I, et al. Syndromic Craniosynostosis can define new candidate genes for suture development or result from the non-specifc effects of pleiotropic genes: rasopathies and chromatinopathies as examples. Front Neurosci. 2017;11:587.
32. Bjornsson HT. The Mendelian disorders of the epigenetic machinery. Genome Res. 2015;25:1473–81.
33. Topa A, Samuelsson L, Lovmar L, Stenman G, Kolby L. On the significance of craniosynostosis in a case of Kabuki syndrome with a concomitant KMT2D mutation and 3.2 Mbp de novo 10q22.3q23.1 deletion. Am J Med Genet A 2017;173:2219–25.
34. Dong C, Umar M, Bartoletti G, Gahankari A, Fidelak L, He F. Expression pattern of Kmt2d in murine craniofacial tissues. Gene Expr Patterns. 2019;34:119060.
35. Dangiolo SB, Wilson A, Jobanputra V, Anyane-Yeboa K. Bohring-Opitz syndrome (BOS) with a new ASXL1 pathogenic variant: Review of the most prevalent molecular and phenotypic features of the syndrome. Am J Med Genet A 2015;167a:3161–6.
36. Bashir RA, Dixit A, Goedhart C, Parboosingh JS, Innes AM, Ferreira P, et al. Lin-Gettig syndrome: craniosynostosis expands the spectrum of the KAT6B related disorders. Am J Med Genet A 2017;173:2596–604.
37. Siakallis L, Tan AP, Chorbachi R, Mankad K. A unique case of CHARGE syndrome with craniosynostosis. Childs Nerv Syst. 2019;35:11–3.
38. Hersh JH, Groom KR, Yen FF, Verdi GD. Changing phenotype in Floating-Harbor syndrome. Am J Med Genet. 1998;76:58–61.
39. Poot M. Structural genome variations related to craniosynostosis. Mol Syndromol. 2019;10:24–39.
40. Law MJ, Lower KM, Voon HP, Hughes JR, Garrick D, Viprakasit V, et al. ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size-dependent manner. Cell 2010;143:367–78.
41. Bacrot S, Mechler C, Talhi N, Martin-Coignard D, Roth P, Michot C, et al. Whole exome sequencing diagnoses the first fetal case of Bainbridge-Ropers syndrome presenting as pontocerebellar hypoplasia type 1. Birth Defects Res. 2018;110:538–42.
42. Chinen Y, Nakamura S, Ganaha A, Hayashi S, Inazawa J, Yanagi K, et al. Mild prominence of the Sylvian fissure in a Bainbridge-Ropers syndrome patient with a novel frameshift variant in ASXL3. Clin Case Rep. 2018;6:330–6.
43. Bostwick BL, McLean S, Posey JE, Streff HE, Gripp KW, Blesson A, et al. Phenotypic and molecular characterisation of CDK13-related congenital heart defects, dysmorphic facial features and intellectual developmental disorders. Genome Med. 2017;9:73.
44. Klaassens M, Morrogh D, Rosser EM, Jaffer F, Vreeburg M, Bok LA, et al. Malan syndrome: Sotos-like overgrowth with de novo NFIX sequence variants and deletions in six new patients and a review of the literature. Eur J Hum Genet. 2015;23:610–5.
45. Grabb PA, Albright AL, Zitelli BJ. Multiple suture synostosis, macrocephaly, and intracranial hypertension in a child with alpha-D-mannosidase deficiency. Case Rep J Neurosurg. 1995;82:647–9.
46. Miller KA, Twigg SR, McGowan SJ, Phipps JM, Fenwick AL, Johnson D, et al. Diagnostic value of exome and whole genome sequencing in craniosynostosis. J Med Genet. 2017;54:260–8.
47. Calpena E, Cuellar A, Bala K, Swagemakers SMA, Koelling N, McGowan SJ, et al. SMAD6 variants in craniosynostosis: genotype and phenotype evaluation. Genet Med. 2020;22:1498–506.

Identified gene variants

Patient	Diagnosis	Gene	Transcript	cDNA	Protein
1	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
2	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
3	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
4	Apert syndrome	FGFR2	000141.4	c.758C>G	p.(Pro253Arg)
5	Apert syndrome	FGFR2	000141.4	c.758C>G	p.(Pro253Arg)
6	Apert syndrome	FGFR2	000141.4	c.758C>G	p.(Pro253Arg)
7	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
8	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
9	Apert syndrome	FGFR2	000141.4	c.758C>G	p.(Pro253Arg)
10	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
11	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
12	Apert syndrome	FGFR2	000141.4	c.758C>G	p.(Pro253Arg)
13	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
14	Apert syndrome	FGFR2	000141.4	c.758C>G	p.(Pro253Arg)
15	Apert syndrome	FGFR2	000141.4	c.755C>G	p.(Ser252Trp)
16	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
17	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
18	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
19	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
20	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
21	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
22	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
23	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
24	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
25	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
26	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
27	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
28	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
29	Muenke syndrome	FGFR3	000142.4	c.749C>G	p.(Pro250Arg)
30	Saethre-Chotzen syndrome	TWIST1	000474.3	c.309C>G	p.(Tyr103*)
31	Saethre-Chotzen syndrome	TWIST1	000474.3	c.475C>G	p.(Leu159Val)
32	Saethre-Chotzen syndrome	TWIST1	000474.3	c.309C>G	p.(Tyr103*)
33	Saethre-Chotzen syndrome	TWIST1	000474.3	c.329G>C	p.(Arg110Pro)
34	Saethre-Chotzen syndrome	TWIST1	000474.3	c.309C>G	p.(Tyr103*)
35	Saethre-Chotzen syndrome	TWIST1	000474.3	c.466A>G	p.(Ile156Val)
36	Saethre-Chotzen syndrome	TWIST1	000474.3	c.309C>G	p.(Tyr103*)
37	Saethre-Chotzen syndrome	TWIST1	000474.3	c.171del	p.(Gly59Alafs*66)
38	Saethre-Chotzen syndrome	TWIST1	000474.3	c.385_405dup	p.(Ala129_Ile135dup)
39	Crouzon/Pfeiffer syndrome	FGFR2	000141.4	c.833G>T	p.(Cys278Phe)
40	Crouzon/Pfeiffer syndrome	FGFR2	000141.4	c.1025G>A	p.(Cys342Tyr)
41	Crouzon/Pfeiffer syndrome	FGFR2	000141.4	c.824_829dup	p.(Glu275_Phe276dup)
42	Crouzon/Pfeiffer syndrome	FGFR2	000141.4	c.824_829dup	p.(Glu275_Phe276dup)
43	Crouzon/Pfeiffer syndrome	FGFR2	000141.4	c.868T>C	p.(Trp290Arg)
44	Crouzon/Pfeiffer syndrome	FGFR2	000141.4	c.870G>T	p.(Trp290Cys)
45	Beare-Stevenson syndrome	FGFR2	000141.4	c.1124A>G	p.(Tyr375Cys)
46	Crouzon with A.N	FGFR3	000142.4	c.1172C>A	p.(Ala391Glu)
47	Crouzon with A.N	FGFR3	000142.4	c.1172C>A	p.(Ala391Glu)

48	Crouzon with A.N	FGFR3	000142.4	c.1172C>A	p.(Ala391Glu)
49	Craniofrontonasal dysplasia	EFNB1	004429.4	c.161C>T	p.(Pro54Leu)
50	Craniofrontonasal dysplasia	EFNB1	004429.4	c.635_636del	p.(Val212fs)
51	Craniofrontonasal dysplasia	EFNB1	NG_008887.1(NM_004429.4)	c.128+5G>A	splice
52	Craniofrontonasal dysplasia	EFNB1	004429.4	c.182A>G	p.(Asp61Gly)
53	CRSDA	IL11RA	001142784.2	c.781C>T	p.(Arg261Cys)
54	CRSDA	IL11RA	001142784.2	c.281G>T	p.(Cys94Phe)
55	Cranioectodermal dysplasia	IFT122	052985.1	c.1118C>T	p.(Ser373Phe)
56	Craniosynostosis 4 CRS4	ERF	006494.2	c.1201_1202del	p.(Lys401Glufs*10)
57	Craniosynostosis 3 CRS3	*TCF12*	207036.1	c.778_779del	p.(Met260Valfs*5)
58	Craniosynostosis 6 CRS6	ZIC1	003412.3	c.1153G>T	p.(Glu385*)
59	Xia-Gibbs syndrome	AHDC1	001029882.2	c.3185_3186del	p.(Thr1062Serfs*63)
60	Xia-Gibbs syndrome	AHDC1	001029882.3	c.2772del	p.(Arg925Glufs*7)
61	Bainbridge Ropers syndrome	ASXL3	030632.1	c.3033dup	p.(Leu1012Serfs*23)
62	CHDFIDD	CDK13	003718.4	c.2524A>G	p.(Asn842Asp)
63	CHARGE syndrome	CHD7	017780.3	c.7593dup	p.(Thr2532Aspfs*9)
64	Kleefstra syndrome	EHMT1	NG_011776.1(NM_024757.4)	c.2018+1G>C	splice
65	Genitopatellar syndrome	KAT6B	012330.3	c.3769_3772del	p.(Lys1258Glyfs*13)
66	Alpha-Mannosidosis	MAN2B1	000528.3	c.1055T>C	p.(Leu352Pro)
67	Floating-Harbor syndrome	SRCAP	006662.2	c.7303C>T	p.(Arg2435*)
68	Malan syndrome	NFIX	002501.3	c.143T>A	p.(Met48Lys)

Identified chromosome aberrations

Patient	Diagnosis	Chromosome aberration	Position_ NCBI Build 37
69	Saethre-Chotzen syndrome	Del 7p15.3p21.2	14470668_20385165 del
70	17p13.3 microdeletion	Del 17p13.3	84287_2468384 del
71	1p32-p31 deletion	Del 1p32.3p31.3	53675707_ 66644963 del
72	22q11.1q12.1 microduplication	Dup 22q11.1q12.1	16888899_26483608 dup
73	Diamond-Blackfan	Del 1p22.1	92405898_ 94018197 del
74	2q37 deletion and Silver-Russell syndrome	Del 2q37.1q37.3 and Dup 11p15.5p15.4 mat	233110452_ 243028452 del 210300_ 8664358 dup
75	2q24 deletion	Del 2q24.2q31.3	163078055_ 182119617 del
76	6q15-6q23 deletion	Del 6q16.2q21	98949950_114533905 del
77	9p deletion	Del 9pterp22.2	204193_ 18073357 del
78	9p deletion	Del 9p23p22.1	13638428_ 17121764 del
79	5q35 duplication	Dup 5q35.1q35.3	170805664_ 180719789 dup

III

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ORIGINAL ARTICLE

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Whole-exome sequencing in syndromic craniosynostosis increases diagnostic yield and identifies candidate genes in osteogenic signaling pathways

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Abstract

Craniosynostosis (CS) is a common congenital anomaly defined by premature fusion of one or more cranial sutures. Syndromic CS involves additional organ anomalies or neurocognitive deficits and accounts for 25%–30% of the cases. In a recent population-based study by our group, 84% of the syndromic CS cases had a genetically verified diagnosis after targeted analyses. A number of different genetic causes were detected, confirming that syndromic CS is highly heterogeneous. In this study, we performed whole-exome sequencing of 10 children and parents from the same cohort where previous genetic results were negative. We detected pathogenic, or likely pathogenic, variants in four additional genes (*NFIA*, *EXTL3*, *POLR2A*, and *FOXP2)* associated with rare conditions. In two of these (*POLR2A* and *FOXP2*), CS has not previously been reported. We further detected a rare predicted damaging variant in *SH3BP4*, which has not previously been related to human disease. All findings were clustered in genes involved in the pathways of osteogenesis and suture patency. We conclude that whole-exome sequencing expands the list of genes associated with syndromic CS, and provides new candidate genes in osteogenic signaling pathways.

KEYWORDS
craniosynostosis, exome, high-throughput sequencing, signaling pathways, syndromic

1 | INTRODUCTION

Craniosynostosis (CS) is a common inborn anomaly, defined by the premature closure of one or more cranial sutures, affecting 1/1600–1/1800 live births (Kweldam et al., 2011; Tønne et al., 2020). Surgical treatment is performed in order to avoid skull deformation or compromised intracranial pressure (Eide et al., 2002; Proctor & Meara, 2019). Syndromic CS is defined by the presence of additional anomalies (e.g., limb malformations, cardiac anomalies), developmental delay, intellectual disability, or other major findings. Approximately 25%–30% of CS cases are syndromic (Lattanzi et al., 2017; Tønne et al., 2021; Wilkie et al., 2017).

CS is genetically heterogeneous, and pathogenic variants in more than 80 different genes have been identified (Goos & Mathijssen, 2019; Tønne et al., 2021). Multiple monogenetic causes have been recognized for nonsyndromic CS (Calpena et al., 2020; Timberlake et al., 2017). Nevertheless, syndromic CS is by far the most genetically diverse group, and the incidence of CS seems to vary greatly between different syndromes. In the well-described CS syndromes, including Pfeiffer, Apert, Crouzon, and Muenke, CS is usually

present. These syndromes are caused by activating pathogenic variants in the *FGFR1*, *FGFR2*, and *FGFR3* genes, acting in the fibroblast growth factor (FGF) pathway. In a number of other CS-associated syndromes, only a proportion of the individuals present with CS and the mechanism is often loss-of-function (partial or complete; Connerney & Spicer, 2011; Goos & Mathijssen, 2019; Twigg & Wilkie, 2015).

The cranial sutures provide flexibility to the skull and act as growth sites for the calvarial bones. The balance of cranial growth and patency of the sutures are maintained through activation and inactivation of several signaling pathways, regulated by a number of different genes (Connerney & Spicer, 2011; Katsianou et al., 2016; Roth et al., 1996; Twigg & Wilkie, 2015). Syndromic CS is often caused by pathogenic variants in genes participating in the pathways of osteogenesis and suture patency (Goos & Mathijssen, 2019; Timberlake et al., 2019; Twigg & Wilkie, 2015). Central pathways are the FGFs, transforming growth factors β (TGFβ), wingless-type integration site (Wnt), Hedgehog (Hh), Eph/ephrin, and bone morphogenetic protein (BMP) signaling pathways. They control the key target genes (*RUNX2*, *MSX2*, and *TCF/LEF*) that promote osteoblast differentiation and are markers of osteogenic differentiation (Katsianou et al., 2016; Figure 1).

A recent population-based study from our group detected a genetic cause in 84% of the syndromic CS cases. The individuals had

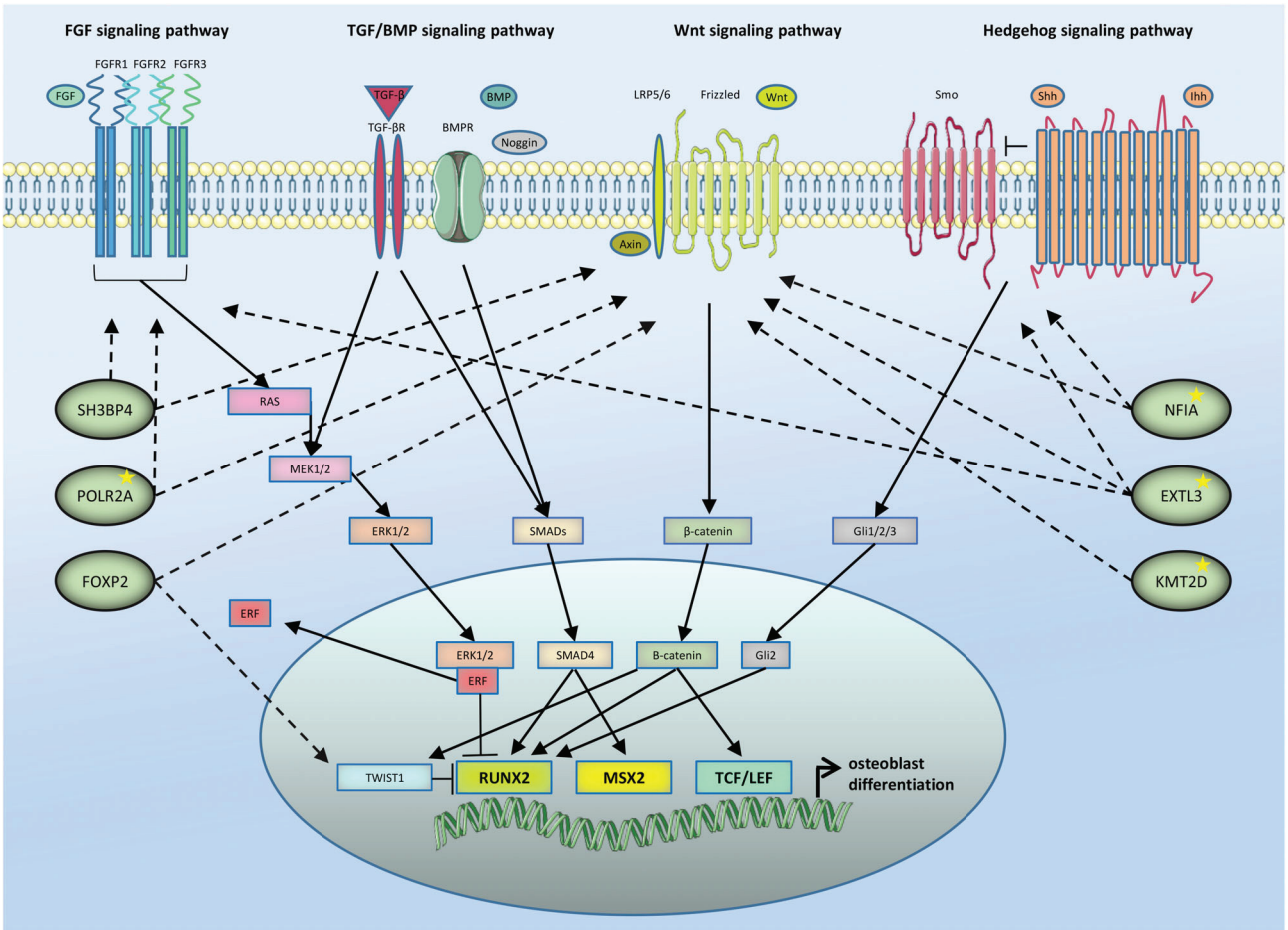

FIGURE 1 Signaling pathways involved in cranial growth and suture patency. The figure illustrates the most important signal transduction pathways in the development of the cranial sutures. Disruptions in one of these pathways may lead to craniosynostosis. The figure is inspired by figure 2 in Katsianou et al. (2016). Compared to the original figure, the pathways have been simplified, the graphic is slightly adjusted, one pathway has been added (hedgehog signaling pathway) and some interactions between the pathways and key target genes have been added. The genes discovered in the study are marked with a dotted line toward the pathways in which functional studies have implicated a regulatory effect. This includes studies on other tissues than the cranium. The conditions associated with the genes *NFIA*, *EXTL3*, *KMT2D*, and *POLR2A* (marked with a star) are previously reported to include craniosynostosis ($n = 3$) or brachy plagiocephaly ($n = 1$). Abbreviations: ERF, ETS domain-containing transcription factor; ERK1/2, extracellular signal-regulated kinase 1/2; *EXTL3*, Exostosin-like glycosyltransferase 3; FGF, fibroblast growth factor; *FOXP2*, Forkhead box P2; GLI, glioma-associated oncogene homolog; Ihh, Indian hedgehog; *KMT2D*, lysine-specific methyltransferase 2D; LRP5/6, low-density lipoprotein receptor-related protein 5/6; MEK, mitogen-activated protein kinase; MSX2, muscle segment homeobox 2; *NFIA*, nuclear factor I/a; *POLR2A*, polymerase 2 RNA subunit a; RUNX2, runt-related transcription factor 2; *SH3BP4*, SH3 domain-binding protein 4; Shh, sonic hedgehog; SMO, smoothened; TCF/LEF, T-cell factor/lymphoid enhancer factor; TGF/BMP, transforming growth factors β/ bone morphogenetic protein; TWIST1, TWIST-related protein 1; Wnt, wingless-type integration site

been investigated by comparative genomic hybridization (aCGH) and exome-based high-throughput sequencing (HTS), filtering for 1570 clinically relevant genes (Tønne et al., 2021). We suspected that some genetic causes were not detected by these methods. In this study, we used WES in order to detect possible unidentified syndromes in addition to novel CS genes.

2 | METHODS

2.1 | Editorial policies and ethical considerations

The study was approved by the Norwegian Regional Committees for Medical and Health Research Ethics (REK_2018/797) and by Oslo University Hospital (permit number P360:18/05374). A signed informed consent form was collected from all included individuals. All families received genetic counseling before and after whole-exome sequencing (WES).

Since 2001, all individuals in Norway with CS have been diagnosed, treated, and received follow-up from the Norwegian National Unit for Craniofacial Surgery at Oslo University Hospital. The result of genetic testing of an 18-year cohort of syndromic CS cases ($n = 104$), retrieved from the unit's perspective registry, were recently published by our group, including clinical criteria for syndromic CS (Tønne et al., 2021). In 15 individuals, the genetic results came back negative. Of these, 10 were available and consented to further genetic analysis. All parents were healthy, except one mother who had a similar phenotype as the child (individual 4).

Collection of EDTA blood, exome-based HTS, and bioinformatic handling were performed and described in our previous study (Tønne et al., 2021). We reanalyzed the sequencing data from the previous study using our research pipeline. Downstream filtering and analysis were done with Filtus (Vigeland et al., 2016) on the variants within coding regions and predicted splice sites close to the entire exome (approximately 18,500 genes). Untranslated regions and noncoding RNA were not included. A few genes associated with severe late-onset disease (e.g., *BRCA1/2*) were excluded. We filtered for de novo, homozygous, compound heterozygous, and hemizygous variants. For individual 4, we also included all maternally transmitted loss-of-function variants and missense variants with a Combined Annotation Dependent Depletion (CADD) score of 20 or more (meaning that the variant is predicted to be among the 1% of most deleterious substitutions in the human genome (Rentzsch et al., 2019). Variants reported with a frequency exceeding 0.0001 in gnomAD, or exceeding 0.05 in our in-house database were excluded, as well as synonymous variants (all located outside predicted splice-sites). The remaining variants were classified in accordance with the American College of Medical Genetics and Genomics (ACMG) and the Association for Clinical Genomics Science (ACGS) criteria (Ellard et al., 2020; Richards et al., 2015). Variants were thereafter investigated by a literature search with regard to their effect on gene function in addition to the respective gene's biological function, acting, and interacting pathways. Databases used included GeneCards (Stelzer et al., 2016), Uniprot (Uniprot Consortium, 2020), Alamut ("Alamut Visual version 2.11,"; Interactive Biosoftware, 2018), UCSC Genome browser (Kent et al., 2002), HGMD (Stenson et al., 2020), gnomAD (Karczewski et al., 2020), ClinVar (Landrum et al., 2020), OMIM (Amberger & Hamosh, 2017), Pubmed (Sayers et al., 2021), and Google Scholar. Sanger sequencing confirmed all selected and discussed variants (Figure S1). The variants in *EXTL3* and *SH3BP4* were submitted to GeneMatcher (Sobreira et al., 2015).

3 | RESULTS

WES revealed likely or possible genetic causes in five of the 10 analyzed individuals (individuals 1–5; Table 1). For the remainder, there were no likely causes detected. Of the individuals without a detected cause, three had a clinical diagnosis. Clinical information for all individuals is presented in Table 1. All detected rare de novo and homozygous/compound heterozygous/hemizygous variants are presented in Table S1.

Individual 1 is a 15-year-old boy with healthy nonconsanguineous parents and two healthy siblings. At birth, his weight was 3660 g (75th centile), his length 49 cm (25th centile), and his head circumference 39 cm (1 cm > 97.5th centile). A metopic CS was surgically treated at the age of 10 months. At the age of 2 years, he developed hydrocephalus and MRI revealed a thin corpus callosum. He exhibited a mild developmental delay, autism, macrocephaly (97.5th centile), supernumerary teeth, and reduced vision (not related to his hydrocephalus). His facial features included a long face, hypotelorism, short nose, long philtrum, micrognathia, and simple low-set ears. Ultrasound of kidneys and urinary tract were normal.

Two de novo variants inducing premature stop codons were detected in cis in exon 2 of the Nuclear factor I/A (*NFIA*) gene, c.124A>T, p.(Lys42*) and c.250C>T, p.(Arg84*). The variants are not reported in gnomAD and are expected to individually result in degradation of NFIA mRNA due to nonsense-mediated mRNA decay. The variant c.124A>T is considered to be the causal variant as the two variants are located in cis, hence c.124A>T will result in degradation of NFIA mRNA. *NFIA* has a high probability of loss-of-function intolerance (pLi = 1). Heterozygous pathogenic loss-of-function variants in *NFIA* are associated with BRain Malformations with or without Urinary Tract Defects (BRMUTD; MIM#613735). The described phenotype is variable and associated features include corpus callosum abnormalities, urinary tract anomalies, developmental delay, macrocephaly, and nonspecific dysmorphic features (Senaratne & Quintero-Rivera, 1993). CS has previously been reported in one single case and in one family with three out of four affected individuals, all associated with microdeletions involving solely *NFIA* (Nyboe et al., 2015; Rao et al., 2014).

Individual 2 is a 13-year-old boy of consanguineous parents, with two healthy siblings. His birthweight was 2600 g (3rd centile). A metopic CS was surgically treated abroad at the age of 5 months. He has short stature (6 cm < 3rd centile), microcephaly (2 cm < 3rd centile), kyphosis, hip dysplasia, and delayed skeletal age. He also has

TABLE 1 Likely and possible genetic causes detected in individuals with syndromic craniosynostosis

ID	Suture[a]	Gene	Variant	ACMG score	Inheritance	Condition (MIM#)	CADD score	gnomAD frequency	Phenotype
1	M	*NFIA* NM_001134673.3	c.124A>T p.(Lys42*) and c.250C>T p.(Arg84*)	Pathogenic (PVS1, PS2, PM2)	Dominant, de novo	Brain malformations with or without urinary defects (613735)	39 38	0 0	Developmental delay, autism, hydrocephalus, CNS anomalies, reduced vision, macrocephaly, dysmorphic features
2	S	*EXTL3* NM_001440.3	c.2392G>A p.(Val798Met)	Likely pathogenic (PM2, PP4 [moderate] PP2, PP3)	Recessive	Immunoskeletal dysplasia with neurodevelopmental abnormalities (617425)	32	0	Intellectual disability, short stature, microcephaly, hip dysplasia, kyphosis, delayed skeletal age. Hypotonic. Immunodeficiency (T-cell). Dysmorphic features
3	M	*POLR2A* NM_000937.4	c.4329_4330delinsAA p.(Ala1444Thr)	Likely pathogenic *(PS2, PM2, PP2, PP3, PP4)*	Dominant, de novo	Neurodevelopmental disorder with hypotonia and intellectual and behavioral abnormalities (618603)	23.4	0	Hypotonic. Impaired motor skills. Hypospadias. Hypermobile joints. Hyperactive behavior. Tics. Dysmorphic features
4	S	*FOXP2* NM_148899.3	c.484del p.(Gln162fs)	Likely pathogenic (PVS1, PM2, PS4)	Dominant, maternal affected mother	Speech-language disorder-1 (602081)	–	0	Developmental delay (speech), orofacial dyspraxia, social difficulties, hypermetropia
5	P	*SH3BP4* NM_014521.2	c.128C>A p.(Pro43His)	Uncertain significance (PM2, PP3, PP4)	Recessive		33	9.6×10^{-5}	Chiari I malformation, exophthalmos, eating difficulties as an infant, microcephaly, recurrent infections, dysmorphic features
		KMT2D NM_003482.3	c.11599C>A p.(Gln3867Lys) and c.7182C>A p.(Ser2394Arg)	Uncertain significance (PM2/BS2, BP4, BP1)	Recessive		22 20	1.2×10^{-5} 0	
6	M	–	–	–	–	Baller–Gerold syndrome (218600)	–	–	Developmental delay. Hypoplasia/aplasia of radius and thumbs. AVSD. Dysplastic ears, internal ear deformities and unilateral deafness. Dysmorphic features
7	S	–	–	–	–	Oculoauriculovertebral spectrum (OAVS) (164210)	–	–	Hemifacial microsomia. Aqueduct stenosis and hydrocephalus. Internal ear anomalies and reduced hearing. Reduced vision and nystagmus. Klippel feil II malformation and scoliosis

TABLE 1 (Continued)

ID	Suture[a]	Gene	Variant	ACMG score	Inheritance	Condition (MIM#)	CADD score	gnomAD frequency	Phenotype
8	M	–	–	–	–	–	–	–	Intellectual disability. ADHD. Hypermobile joints. Sensitivity to heat. Reduced vision. Dysmorphic features
9	LC	–	–	–	-	Cerebral palsy periventricular leukomalacia	–	–	Extremely premature. Bilateral subependymal bleeding and periventricular leukomalacia. Developmental delay. High forehead and epicanthus
10	M	–	–	–	–	–	–	–	Developmental delay. Reduced vision. Inguinal hernia. Preaxial polydactyly. Shallow orbits

Abbreviations: ACMG, The American College of Medical Genetics and Genomics; CADD, Combined Annotation Dependent Depletion; *NFIA*, nuclear factor I/A; *EXTL3*, exostosin-like glycosyltransferase 3; *POLR2A*, polymerase 2 RNA subunit A; *FOXP2*, forkhead box P2; *SH3BP4*, SH3 domain-binding protein 4; *KMT2D*, lysine-specific methyltransferase 2D.

[a]Suture: LC, left coronal; M, metopic; P, pansynostosis; S, sagittal.

a mild intellectual disability, hypotonia, eating difficulties, reflux and recurrent, and long-lasting infections. Immunological investigations revealed a low number of T-cells and a reduced fraction of CD8+ late effector/memory T-cells. His facial features include coarse facies, deep-set eyes, hypotelorism, prominent nose, and a broad nasal tip.

A homozygous missense variant in the Exostosin-like glycosyltransferase 3 (*EXTL3*) gene was detected, c.2392G>A, p.(Val798Met). The variant has not been reported in gnomAD and changes an amino acid that is highly conserved between species and received a CADD score of 32. All other pathogenic or likely pathogenic variants in *EXTL3* reported in the literature are missense variants. The variant is located in a predicted Pfam domain (glycosyltransferase family 64 domain, amino acids 663–904) catalyzing the transfer reaction of N-acetylglucosamine (GlcNac) and N-acetylgalactosamine essential for the formation of heparin sulfate (HS) chains. Pathological biallelic missense variants in *EXTL3* are associated with Immunoskeletal dysplasia with neurodevelopmental abnormalities (ISDNA; MIM#617425). Variable skeletal abnormalities and neurodevelopmental defects are associated with the condition, in addition to immunodeficiency restricted T-cell deficiency. CS has previously been reported in two siblings with ISDNA (Volpi et al., 2017). There has been one likely pathogenic missense variant reported in the same domain as detected in individual 2, hypothesized to cause the condition by disrupting the GlcNac transferase activity (Oud et al., 2017). The variant detected in individual 2 is interpreted to be likely pathogenic.

Individual 3 is a 5-year-old boy with healthy nonconsanguineous parents. A thickened nuchal fold was detected during pregnancy. He was born at term with a weight of 3425 g (50th centile), length 50 cm (25th centile), and head circumferences 37 cm (90th centile). Metopic CS was treated at the age of 2 months. As an infant, he had severe hypotonia and needed tube feeding. Hypospadia was detected shortly after birth. Newborn screening indicated congenital adrenal hyperplasia (CAH), which was confirmed by the detection of a homozygous deletion within the *CYP21A2* gene (exon 1–3). He received extensive physical therapy due to hypotonia from 3 months of age, and at the age of 14 months, he walked independently. He is motorically clumsy, runs slowly, and gets tired easily. He has facial tics, midface hypoplasia, hypotelorism, broad nasal root, dysplastic ears, single palmar fissure, and 2–3 syndactyly bilaterally.

A de novo heterozygous variant was detected in polymerase 2 RNA subunit A (*POLR2A*), c.4329_4330delinsAA, p.(Ala1444Thr). The variant has not been reported in gnomAD and changes a highly conserved amino acid. Pathogenic variants in POLR2A are associated with Neurodevelopmental disorder with hypotonia and variable intellectual and behavioral abnormalities (NEDHIB; MIM#618603). The condition has a variable phenotype with varying severity. There are 27 individuals reported and common features are delayed development (mild to severe), hypotonia, ataxia, epilepsy, feeding difficulties, sleep disturbances, and behavior abnormalities. There are no other individuals reported with CS, but four individuals are reported to have brachy plagiocephaly (Haijes et al., 2019) and two are reported to have prominent supraorbital ridges (Hansen et al., 2021). Missense variants seem to be associated with a more severe phenotype, presumably due to a dominant-negative effect. Four reported cases have in-frame deletions in *POLR2A*, predicted to alter the protein structure (Haijes et al., 2019; Hansen et al., 2021). Individual 3's phenotype is in concordance with the described features, however slightly milder. The variant detected is considered to be likely pathogenic.

Individual 4 is a 6-year-old boy with nonconsanguineous parents. He was born at term with a weight of 3320 g (25th centile), length 51 cm (50th centile), and head circumference 35 cm (25th centile). A sagittal CS was surgically treated at the age of 3 months. He has severe verbal dyspraxia, confirmed by a speech-language pathologist (SLP), and experiences social difficulties. He has reduced vision. His mother was surgically treated for sagittal CS and has verbal dyspraxia with great difficulties in complex speech production, confirmed by the same SLP. He has one healthy sister (9 years old) without CS. The sister had delayed language development until the age of 6. At present, she has normal speech, in particular no verbal dyspraxia or articulation difficulties (confirmed by the same SLP). She is however in need of some special education for reading and writing.

A maternally inherited heterozygous frameshift variant in Forkhead box P2 (*FOXP2*) was detected, c.484del, p.(Gln162Asnfs*100). *FOXP2* has a high pLi of 1. The variant has not been reported in gnomAD and induces a premature stop codon. Both missense and nonsense variants in *FOXP2* are known to cause Speech-language disorder-1/Developmental verbal dyspraxia (MIM#605317). The condition is characterized by severe orofacial dyspraxia, affecting both speech, and expressive language (Morgan et al., 2016). Both mother and son have persisting symptoms of Speech-language disorder, confirmed by a SLP. The variant is considered to be likely pathogenic. The variant was also detected in the healthy sister, with delayed speech up until the age of 6, suggesting variable expressivity. Although *FOXP2* has not previously been associated with syndromic CS, altered facial skeletal morphology has been reported, and the *FOXP2* transcription factor interacts with several pathways involved in osteogenesis (Section 4). No other maternally inherited loss of function variants in genes predicted to be involved in cranial development or which interfere with bone metabolism were detected. A few rare maternally inherited missense variants with CADD >20 were identified, in which four have been demonstrated to be involved in the pathways of osteogenesis and suture patency (*RHOB*, *CTNND2*, *COL27A1*, and *PDILT*). Missense variants are less likely to interfere with the pathways concerning CS, and there were no reports of involvement in cranial development for these genes. All maternally inherited rare variants with CADD >20 are presented in Table S1.

Individual 5 is a 9-year-old boy of healthy nonconsanguineous parents. He was born at term, with a weight of 2900 g (5th centile) and length 48 cm (10th centile). Between the age of 5 and 18 months, he had severe eating difficulties and a significant growth stagnation. Pansynostosis was detected at 18 months. He had no sign of intracranial hypertension or restricted intracranial volume and was treated conservatively. He has had one epileptic seizure and MRI revealed a Chiari I malformation. He has normal growth parameters, except

microcephaly (2 cm < 2.5th centile). His facial features include midface hypoplasia with exophtalmos, hypertelorism, long palpebral fissures and broad arched eyebrows with lateral sparing, a depressed nasal tip, and widely spaced teeth. He has persistent fetal pads. His psychomotor development is normal.

Homozygosity for a missense variant in the SH3 domain-binding protein 4 (*SH3BP4*) was detected, c.128C>A, p.(Pro43His). The variant changes a highly conserved amino acid, is not reported in homozygous state in gnomAD, and received a CADD score of 33. *SH3BP4* has not been previously associated with human disease. The variant is considered to be of uncertain significance.

Compound heterozygosity for two rare missense variants in the lysine-specific methyltransferase 2D (*KMT2D*) gene was also detected, c.11599C>A, p.(Gln3867Lys) and c.7182C>A, p.(Ser2394Arg). Heterozygosity for *KMT2D* pathogenic variants is associated with Kabuki syndrome (MIM#147929). The mechanism is usually loss-of-function, but missense variants are reported (15%–20% of the cases), however with a slightly different phenotype (Baldridge et al., 2020). Individual 5 has typical facial features of Kabuki syndrome (long palpebral fissures, arched eyebrows with lateral sparing). Synostosis of multiple sutures, fetal pads, and early eating difficulties are also in concordance with the described phenotype (Topa et al., 2017). However, he has a normal psychomotor development, which is uncommon in Kabuki syndrome. The parents have no dysmorphic features. Both variants are considered to be of uncertain significance. The variant c.11599C>A is reported in ClinVar as a VUS for Kabuki syndrome in two other individuals.

4 | DISCUSSION

We performed WES in 10 children clinically diagnosed with syndromic CS, where previous genetic analysis did not reveal any causative variants. We detected six likely or possible genetic causes in five of the children. In four of the children (individuals 1–4), a pathogenic or likely pathogenic variant was detected in a gene associated with a condition related to their respective phenotype. This increased the diagnostic yield in the cohort. All findings were in genes participating in one or more pathways known to be involved in osteogenesis or suture patency (Figure 1).

Known genetic causes of syndromic CS are rapidly accumulating, primarily due to an increased use of HTS. This sometimes leaves the genetic findings in the transition between research and diagnostics. For the conditions BRMUTD (*NFIA*) and ISDNA (*EXTL3*), these are the fifth and third cases, respectively, which include CS, and confirm CS as a feature in these two rare conditions. Both *NFIA* and *EXTL3* have been demonstrated to be involved in the pathways of osteogenesis and suture patency. *NFIA* is a transcription factor important for normal development of several organ systems (Lu et al., 2007). The protein participates in osteoblast differentiation by interacting with the Wnt and Ihh pathways (Singh et al., 2018; Xie et al., 2015; Figure 1).

EXTL3 is essential during early development and knockout of *EXTL3* in mice results in lethality (Takahashi et al., 2009). EXTL3 is a key protein in the regulation of heparan sulfates (HS) biosynthesis, and thereby the formation of heparan sulfate proteoglycans (HSPGs) known to be important for normal skeletal development (Oud et al., 2017). Extensive studies have shown HSPGs to play an important role in regulating the Wnt, Hh, BMP, and FGFs activity and signaling pathways (Norton et al., 2005). Functional studies performed in zebrafish have shown *EXTL3* to be involved in the regulation of the FGF, Wnt, and Hh signaling pathways in particular (Venero Galanternik et al., 2015, 2016; Figure 1).

NEDHIB (*POLR2A*), detected in individual 3, has not previously been described to cause CS, but brachy plagiocephaly has been reported in several individuals. Brachy plagiocephaly is in some cases the consequence of CS (uni- or bi-coronal CS). *POLR2A* encodes the largest subunit (RPB1) of RNA polymerase II and its function is regulated by methylation and acetylation of particular residues in its C-terminal domain, which again is essential for the regulation of growth factor induced genes (Mita et al., 1995; Schröder et al., 2013). RNA polymerase II is a protein complex responsible for mRNA-synthesis of all human protein-encoding genes and shown to regulate transcription through a number of different mechanisms and interactions, where RPB1 is important for both transcription initiation, elongation, and termination (Haijes et al., 2019; Kecman et al., 2018; X. Liu et al., 2013). *POLR2A* has been demonstrated to be involved in several different pathways, including the Wnt pathway in a study of meningiomas, where mutant *POLR2A* was shown to affect the expression of *WNT6/WNT10*, known to be involved in controlling neural crest cell development (Clark et al., 2016). *POLR2A* has also been predicted to be indirectly involved in alternative splicing of FGFR2 through Epithelial splicing regulatory protein 2 (ESRP2) (Jassal et al., 2019; Figure 1) and directly involved in the regulation of osteoclast genesis by interacting with CREB1 (C. Liu et al., 2021).

Speech-language disorder-1 (*FOXP2*), detected in individual 4 and his affected mother, has not previously been described to include CS. However, several individuals are reported to have dysmorphic features involving the facial skeleton (e.g., prominent forehead; Cesario et al., 2016). In addition, *FOXP2*'s important roles in regulating bone morphogenic genes and ensuring normal cranial development have been demonstrated in several functional studies (Cesario et al., 2016; Xu et al., 2018; Zhao et al., 2015). This supports our hypothesis that the loss-of-function variant detected in *FOXP2* may also cause CS in these two family members. The *FOXP2* gene encodes the FOXP2 transcription factor, one of the most highly conserved proteins in mammals. It is involved in a number of developmental pathways (Richter et al., 2020), one of which is involved in craniofacial development, where FOXP2 has been shown to play an important role in skull shaping and bone remodeling (Cesario et al., 2016; Xu et al., 2018). FOXP2 participates in the Wnt signaling pathway where it negatively regulates several bone morphogenic genes (e.g., *RUNX2*, *AXIN2*, *BMP4*, and *FGF9)* involved in ossification in cranial mesenchyme and CS (Govindarajan & Overbeek, 2006; Katsianou et al., 2016; Richter et al., 2020; Figure 1). Importantly, *FOXP2* has been shown to be one of the suppressors of *RUNX2* in several studies (Cesario et al., 2016; Zhao et al., 2015). RUNX2 is one of the key transcription factors for

bone formation, including the cranium (Benson & Opperman, 2011; Katsianou et al., 2016; Long & Ornitz, 2013). Loss-of-function variants in another RUNX2 suppressor, *TWIST1*, are associated with coronal CS in Saethre–Chotzen syndrome, presumably due to increased osteogenesis, as TWIST1 normally binds to RUNX2 in the coronal suture, acting as a negative regulator of osteogenic differentiation (Bialek et al., 2004; el Ghouzzi et al., 1997; Wu & Gu, 2019). *FOXP2* has also been shown to be significantly downregulated in the sutures of sagittal CS (Potter et al., 2015). In a study of FOXP2's role in cranial base development, *Foxp2* conditional knock-out demonstrated a significant impact on the lambdoid sutures in a dose-dependent manner in mice (Xu et al., 2018). However, the knockout mice exhibited decreased expression of Runx2 with attenuated synostosis of the lambdoid sutures as a result. These different observations might be explained by the large number of cofactors involved in promoting osteoblast differentiation by stimulating *RUNX2* expression (Zhao et al., 2015). In addition, the *FOXP* genes are hypothesized to have context- and time-dependent functions in bone development, in the sense that their impact may vary in time, tissue, and developmental stages (Zhao et al., 2015). Another example of how *FOXP2* affects skeletal development is its role in endochondral ossification in long bones. In a functional study performed in mice, overexpression of *FOXP2* was shown to inhibit endochondral ossification, and compound deficiency of *FOXP1/2* resulted in advanced osteoblast maturation and increased proliferation (Zhao et al., 2015). The study also demonstrated attenuated and reduced growth of the mice skulls when *FOXP2* was both overexpressed and when deficient. Finally, *FOXP2* has been shown to induce growth arrest in osteosarcoma (Gascoyne et al., 2015).

In individual 5, a presumably damaging variant in a gene of unknown significance, *SH3BP4* was detected. *SH3BP4* forms a complex together with FGFR2b and phosphatidylinositol-3-kinase (FGFR2b-P13K-SH3BP4), which ensures FGFR2b recycling and controls cell migration and epithelial branching (Francavilla et al., 2013; Ornitz & Itoh, 2015). Fibroblast growth factors (FGFs) are positive regulators of osteogenic differentiation and function by activating the FGFRs and through several steps and downstream pathways stimulate osteoblast proliferation and differentiation. The FGFRs have however two different isoforms (IIIb and IIIc) with different affinities to ligands and localization. The FGFR2c isoform is the one involved in the osteocyte lineage and normal bone development, while the IIIb isoform is involved in epithelial branching (Eswarakumar et al., 2004). P13K/Akt is a major growth-signaling pathway, known to be crucial for normal bone development, and is suggested to interact with the Wnt pathway, together affecting osteoblast activity (Raucci et al., 2008). In addition, *SH3BP4* has been demonstrated to act as a negative regulator of the Wnt pathway (Antas et al., 2019; Figure 1). The impact of pathogenic variants in *SH3BP4* concerning bone and cranial development is however uncertain and further studies or affected individuals would be needed in order to make such association.

The importance of the two variants in *KMT2D*, detected in the same individual, is also uncertain. Kabuki syndrome has been associated with CS in several cases. The *KMT2D* gene is known to participate in the Wnt pathway (Schwenty-Lara et al., 2020; Figure 1). Autosomal recessive Kabuki syndrome has not previously been reported, and the two variants might represent rare normal variants. As the presumed mechanism of Kabuki syndrome is loss-of-function, it is however possible that compound heterozygosity for two missense variants might result in reduced production of the lysine-specific methyltransferase 2D protein, and in turn reduced total activity of this enzyme, with a similar or milder phenotypic result.

By performing WES in children with syndromic CS we identified genetic causes that were previously undetected, in addition to identifying variants in genes not previously associated with CS. Recently, a similar study discovered other novel genetic causes of CS, also comprising genes involved in the pathways of osteogenesis and suture patency (Timberlake et al., 2019). The small sample sizes studied with this approach suggest that there are still undetected genetic causes of syndromic CS, presumably in genes involved in these pathways.

The ultra-rare genetic syndromes identified in the study, present with CS in only a fraction of the cases, in contradiction to the more common CS syndromes (e.g., *FGFR*s), in which nearly all individuals present with CS. This suggests different pathophysiological mechanisms. The gain-of-function variants in the FGFRs might be less influenced by other factors and interfering mechanisms than the loss-of-function variants, often detected in the rare causes of syndromic CS. Several of the genes detected in this study were also transcription factors (*NFIA*, *POLR2A*, and *FOXP2*). Transcription factors are known to be influenced by a number of different factors, which may explain some of the differences. In addition, poor brain growth and extrinsic forces applied to the skull are known to affect the development of CS (Twigg & Wilkie, 2015). Variants in other genes, environmental and epigenetic factors might also influence and contribute to the result of CS in these ultra-rare syndromes.

The high diagnostic yield when including our previous study, underscores the importance of identifying syndromic CS cases in the clinic, in order to provide genetic analyses to this group. We have demonstrated that the genetic causes of syndromic CS are highly heterogeneous and that targeted analysis is not always sufficient. The most efficient test strategy for syndromic CS would probably be to perform a small targeted panel, followed by WES or whole-genome sequencing (WGS) for negative cases, without the second step of an extended panel. The advantages of performing a targeted analysis initially are that this detects a large number of the genetic causes and reduces the risk of incidental findings.

The strength of this study is that we have investigated close to all human protein-coding genes, limiting the possibility of another undetected cause. Nevertheless, we were unable to detect a genetic cause in five individuals, three of which had a clinical diagnosis. In two cases with a clinical diagnosis, a lack of genetic findings may not be surprising, as these syndromes are usually not associated with a genetic cause (Oculoauriculovertebral spectrum [OAVS] and cerebral palsy). Their CS might be an isolated event, not associated with their other findings. For the remainder, an undetected genetic cause is nonetheless suspected, for instance noncoding, regulatory, or small copy number alterations that our analysis did not capture.

We have not been able to detect a second individual or family to confirm the association with CS in *SH3BP4*, *FOXP2*, and *POLR2A*. We attribute this to the limited number of individuals having these rare disorders. Functional studies or other affected individuals would be needed in order to confirm causation.

5 | CONCLUSION

By performing WES, we identified several rare genetic causes of syndromic CS, previously undetected by panel analysis. In addition, interesting candidate genes for CS were revealed, all participating in the pathways of osteogenesis and suture patency.

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CONFLICT OF INTEREST

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper. The work has received no funding.

AUTHOR CONTRIBUTIONS

All authors have contributed to and approved the final manuscript. All authors have agreed to the order in which their names are listed. Elin Tønne: Conceptualization and design of the study, acquisition, analysis, and interpretation of the data, drafting and revising the manuscript. Bernt J Due-Tønnessen: Conceptualization and design of the study, acquisition, and interpretation of the data, revising the manuscript. Magnus D Vigeland: Design of the study, analysis, and interpretation of the data, revising the manuscript. Silja Amundsen: Analysis and interpretation of the data, revising the manuscript. Teodora Ribarska: Analysis and interpretation of the data, revising the manuscript. Pamela M Åsten: Acquisition and interpretation of the data, revising the manuscript. Ying Sheng: Analysis of the data, revising the manuscript. Eirik Helseth: Design of the study, interpretation of the data, revising the manuscript. Gregor D Gilfillan: Design of the study, analysis, and interpretation of the data, revising the manuscript. Inger-Lise Mero: Design of the study, analysis, and interpretation of the data, revising the manuscript. Ketil Heimdal: Conceptualization and design of the study, acquisition, analysis, and interpretation of the data, revising the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in ClinVar at www.ncbi.nlm.nih.gov/clinvar, accession number SCV001943320–SCV001943321 (Tønne et al., 2021).

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REFERENCES

Amberger, J. S., & Hamosh, A. (2017). Searching online Mendelian inheritance in man (OMIM): A knowledgebase of human genes and genetic phenotypes. *Current Protocols in Bioinformatics*, *58*, 1.2.1–1.2.12. https://doi.org/10.1002/cpbi.27

Antas, P., Novellasdemunt, L., Kucharska, A., Massie, I., Carvalho, J., Oukrif, D., Nye, E., Novelli, M., & Li, V. S. W. (2019). SH3BP4 regulates intestinal stem cells and tumorigenesis by modulating β-catenin nuclear localization. *Cell Reports*, *26*(9), 2266–2273.e4. https://doi.org/10.1016/j.celrep.2019.01.110

Baldridge, D., Spillmann, R. C., Wegner, D. J., Wambach, J. A., White, F. V., Sisco, K., Toler, T. L., Dickson, P. I., Cole, F. S., Shashi, V., & Grange, D. K. (2020). Phenotypic expansion of KMT2D-related disorder: Beyond kabuki syndrome. *American Journal of Medical Genetics. Part A*, *182*(5), 1053–1065. https://doi.org/10.1002/ajmg.a.61518

Benson, M. D., & Opperman, L. A. (2011). *Regulation of calvarial bone growth by molecules involved in the craniosynostoses*. Wiley-Blackwell.

Bialek, P., Kern, B., Yang, X., Schrock, M., Sosic, D., Hong, N., Wu, H., Yu, K., Ornitz, D. M., Olson, E. N., Justice, M. J., & Karsenty, G. (2004). A twist code determines the onset of osteoblast differentiation. *Developmental Cell*, *6*(3), 423–435. https://doi.org/10.1016/s1534-5807(04)00058-9

Calpena, E., Cuellar, A., Bala, K., Swagemakers, S. M. A., Koelling, N., McGowan, S. J., Phipps, J. M., Balasubramanian, M., Cunningham, M. L., Douzgou, S., Lattanzi, W., Morton, J. E. V., Shears, D., Weber, A., Wilson, L. C., Lord, H., Lester, T., Johnson, D., Wall, S. A., … Wilkie, A. O. M. (2020). SMAD6 variants in craniosynostosis: Genotype and phenotype evaluation. *Genetics in Medicine*, *22*(9), 1498–1506. https://doi.org/10.1038/s41436-020-0817-2

Cesario, J. M., Almaidhan, A. A., & Jeong, J. (2016). Expression of forkhead box transcription factor genes Foxp1 and Foxp2 during jaw development. *Gene Expression Patterns*, *20*(2), 111–119. https://doi.org/10.1016/j.gep.2016.03.001

Clark, V. E., Harmancı, A. S., Bai, H., Youngblood, M. W., Lee, T. I., Baranoski, J. F., Ercan-Sencicek, A. G., Abraham, B. J., Weintraub, A. S., Hnisz, D., Simon, M., Krischek, B., Erson-Omay, E. Z., Henegariu, O., Carrión-Grant, G., Mishra-Gorur, K., Durán, D., Goldmann, J. E., Schramm, J., … Günel, M. (2016). Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. *Nature Genetics*, *48*(10), 1253–1259. https://doi.org/10.1038/ng.3651

Connerney, J. J., & Spicer, D. B. (2011). Signal transduction pathways and their impairment in syndromic craniosynostosis. In M. Muenke, W. Kress, H. Collmann, & B. D. Solomon (Eds.), *Craniosynostoses: Molecular genetics, principles of diagnosis, and treatment. Monograph of Human Genetics* (Vol. 19, pp. 28–44). Karger.

Eide, P. K., Helseth, E., Due-Tonnessen, B., & Lundar, T. (2002). Assessment of continuous intracranial pressure recordings in childhood craniosynostosis. *Pediatric Neurosurgery*, *37*(6), 310–320. https://doi.org/10.1159/000066311

el Ghouzzi, V., Le Merrer, M., Perrin-Schmitt, F., Lajeunie, E., Benit, P., Renier, D., Bourgeois, P., Bolcato-Bellemin, A. L., Munnich, A., & Bonaventure, J. (1997). Mutations of the TWIST gene in the Saethre-Chotzen syndrome. *Nature Genetics*, *15*(1), 42–46. https://doi.org/10.1038/ng0197-42

Ellard S, Baple, E. L. Callaway A, Berry I, Forrester N, Turnbull C, Owens M, Eccles D.M, Abbs S, Scott R, Deans Z. C, Lester T, Campbell J, Newman W. G, Ramsden S, & McMullan D. J. (2020). ACGS Best Practice Guidelines for Variant Classification in Rare Disease 2020. Retrieved from https://www.acgs.uk.com/quality/best-practice-guidelines/#VariantGuidelines.

Eswarakumar, V. P., Horowitz, M. C., Locklin, R., Morriss-Kay, G. M., & Lonai, P. (2004). A gain-of-function mutation of Fgfr2c demonstrates the roles of this receptor variant in osteogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(34), 12555–12560. https://doi.org/10.1073/pnas.0405031101

Francavilla, C., Rigbolt, K. T., Emdal, K. B., Carraro, G., Vernet, E., Bekker-Jensen, D. B., Streicher, W., Wikström, M., Sundström, M., Bellusci, S., Cavallaro, U., Blagoev, B., & Olsen, J. V. (2013). Functional proteomics defines the molecular switch underlying FGF receptor trafficking and cellular outputs. *Molecular Cell*, *51*(6), 707–722. https://doi.org/10.1016/j.molcel.2013.08.002

Gascoyne, D. M., Spearman, H., Lyne, L., Puliyadi, R., Perez-Alcantara, M., Coulton, L., Fisher, S. E., Croucher, P. I., & Banham, A. H. (2015). The Forkhead transcription factor FOXP2 is required for regulation of p21WAF1/CIP1 in 143B osteosarcoma cell growth arrest. *PLoS One*, *10*(6), e0128513. https://doi.org/10.1371/journal.pone.0128513

Goos, J. A. C., & Mathijssen, I. M. J. (2019). Genetic causes of Craniosynostosis: An update. *Mol Syndromol*, *10*(1–2), 6–23. https://doi.org/10.1159/000492266

Govindarajan, V., & Overbeek, P. A. (2006). FGF9 can induce endochondral ossification in cranial mesenchyme. *BMC Developmental Biology*, *6*, 7. https://doi.org/10.1186/1471-213x-6-7

Haijes, H. A., Koster, M. J. E., Rehmann, H., Li, D., Hakonarson, H., Cappuccio, G., Hancarova, M., Lehalle, D., Reardon, W., Schaefer, G. B., Lehman, A., van de Laar, I., Tesselaar, C. D., Turner, C., Goldenberg, A., Patrier, S., Thevenon, J., Pinelli, M., Brunetti-Pierri, N., … van Hasselt, P. (2019). De novo heterozygous POLR2A variants cause a neurodevelopmental syndrome with profound infantile-onset Hypotonia. *American Journal of Human Genetics*, *105*(2), 283–301. https://doi.org/10.1016/j.ajhg.2019.06.016

Hansen, A. W., Arora, P., Khayat, M. M., Smith, L. J., Lewis, A. M., Rossetti, L. Z., Jayaseelan, J., Cristian, I., Haynes, D., DiTroia, S., Meeks, N., Delgado, M. R., Rosenfeld, J. A., Pais, L., White, S. M., Meng, Q., Pehlivan, D., Liu, P., Gingras, M. C., … Gibbs, R. A. (2021). Germline mutation in POLR2A: A heterogeneous, multi-systemic developmental disorder characterized by transcriptional dysregulation. *HGG Advances*, *2*(1), 100014. https://doi.org/10.1016/j.xhgg.2020.100014

Interactive Biosoftware. (2018). Alamut Visual version 2.11, Rouen, France. Retrieved from http://www.interactive-biosoftware.com/alamut-visual.

Jassal, B., Matthews, L., Viteri, G., Gong, C., Lorente, P., Fabregat, A., Sidiropoulos, K., Cook, J., Gillespie, M., Haw, R., Loney, F., May, B., Milacic, M., Rothfels, K., Sevilla, C., Shamovsky, V., Shorser, S., Varusai, T., Weiser, J., … D'Eustachio, P. (2019). The reactome pathway knowledgebase. *Nucleic Acids Research*. https://doi.org/10.1093/nar/gkz1031

Karczewski, K. J., Francioli, L. C., Tiao, G., Cummings, B. B., Alföldi, J., Wang, Q., Collins, R. L., Laricchia, K. M., Ganna, A., Birnbaum, D. P., Gauthier, L. D., Brand, H., Solomonson, M., Watts, N. A., Rhodes, D., Singer-Berk, M., England, E. M., Seaby, E. G., Kosmicki, J. A., … MacArthur, D. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*, *581*(7809), 434–443. https://doi.org/10.1038/s41586-020-2308-7

Katsianou, M. A., Adamopoulos, C., Vastardis, H., & Basdra, E. K. (2016). Signaling mechanisms implicated in cranial sutures pathophysiology: Craniosynostosis. *BBA Clinical*, *6*, 165–176. https://doi.org/10.1016/j.bbacli.2016.04.006

Kecman, T., Kuś, K., Heo, D. H., Duckett, K., Birot, A., Liberatori, S., & Vasiljeva, L. (2018). Elongation/termination factor exchange mediated by PP1 phosphatase orchestrates transcription termination. *Cell Reports*, *25*(1), 259–269.e255. https://doi.org/10.1016/j.celrep.2018.09.007

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., & Haussler, D. (2002). The human genome browser at UCSC. *Genome Research*, *12*(6), 996–1006. https://doi.org/10.1101/gr.229102

Kweldam, C. F., van der Vlugt, J. J., & van der Meulen, J. J. (2011). The incidence of craniosynostosis in The Netherlands, 1997-2007. *Journal of Plastic, Reconstructive & Aesthetic Surgery*, *64*(5), 583–588. https://doi.org/10.1016/j.bjps.2010.08.026

Landrum, M. J., Chitipiralla, S., Brown, G. R., Chen, C., Gu, B., Hart, J., & Kattman, B. L. (2020). ClinVar: Improvements to accessing data. *Nucleic Acids Research*, *48*(D1), D835–D844. https://doi.org/10.1093/nar/gkz972

Lattanzi, W., Barba, M., Di Pietro, L., & Boyadjiev, S. A. (2017). Genetic advances in craniosynostosis. *American Journal of Medical Genetics. Part A*, *173*(5), 1406–1429. https://doi.org/10.1002/ajmg.a.38159

Liu, C., Han, Y., Zhao, X., Li, B., Xu, L., Li, D., & Li, G. (2021). POLR2A blocks osteoclastic bone resorption and protects against osteoporosis by interacting with CREB1. *Journal of Cellular Physiology*, *236*(7), 5134–5146. https://doi.org/10.1002/jcp.30220

Liu, X., Bushnell, D. A., & Kornberg, R. D. (2013). RNA polymerase II transcription: Structure and mechanism. *Biochimica et Biophysica Acta*, *1829*(1), 2–8. https://doi.org/10.1016/j.bbagrm.2012.09.003

Long, F., & Ornitz, D. M. (2013). Development of the endochondral skeleton. *Cold Spring Harbor Perspectives in Biology*, *5*(1), a008334. https://doi.org/10.1101/cshperspect.a008334

Lu, W., Quintero-Rivera, F., Fan, Y., Alkuraya, F. S., Donovan, D. J., Xi, Q., Turbe-Doan, A., Li, Q. G., Campbell, C. G., Shanske, A. L., Sherr, E. H., Ahmad, A., Peters, R., Rilliet, B., Parvex, P., Bassuk, A. G., Harris, D. J., Ferguson, H., Kelly, C., … Maas, R. L. (2007). NFIA haploinsufficiency is associated with a CNS malformation syndrome and urinary tract defects. *PLoS Genetics*, *3*(5), e80. https://doi.org/10.1371/journal.pgen.0030080

Mita, K., Tsuji, H., Morimyo, M., Takahashi, E., Nenoi, M., Ichimura, S., Yamauchi, M., Hongo, E., & Hayashi, A. (1995). The human gene encoding the largest subunit of RNA polymerase II. *Gene*, *159*(2), 285–286. https://doi.org/10.1016/0378-1119(95)00081-g

Morgan, A., Fisher, S. E., Scheffer, I., & Hildebrand, M. (2016). FOXP2-related speech and language disorders. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, G. Mirzaa, & A. Amemiya (Eds.), *GeneReviews*(®). University of Washington, Seattle.

Norton, W. H., Ledin, J., Grandel, H., & Neumann, C. J. (2005). HSPG synthesis by zebrafish Ext2 and Extl3 is required for Fgf10 signalling during limb development. *Development*, *132*(22), 4963–4973. https://doi.org/10.1242/dev.02084

Nyboe, D., Kreiborg, S., Kirchhoff, M., & Hove, H. B. (2015). Familial craniosynostosis associated with a microdeletion involving the NFIA gene. *Clinical Dysmorphology*, *24*(3), 109–112. https://doi.org/10.1097/mcd.0000000000000079

Ornitz, D. M., & Itoh, N. (2015). The fibroblast growth factor signaling pathway. *Wiley Interdisciplinary Reviews: Developmental Biology*, *4*(3), 215–266. https://doi.org/10.1002/wdev.176

Oud, M. M., Tuijnenburg, P., Hempel, M., van Vlies, N., Ren, Z., Ferdinandusse, S., Jansen, M. H., Santer, R., Johannsen, J., Bacchelli, C., Alders, M., Li, R., Davies, R., Dupuis, L., Cale, C. M., Wanders, R. J. A., Pals, S. T., Ocaka, L., James, C., … Kuijpers, T. W. (2017). Mutations in EXTL3 cause neuro-immuno-skeletal dysplasia syndrome. *American Journal of Human Genetics*, *100*(2), 281–296. https://doi.org/10.1016/j.ajhg.2017.01.013

Potter, A. B., Rhodes, J. L., Vega, R. A., Ridder, T., & Shiang, R. (2015). Gene expression changes between patent and fused cranial sutures in a non-syndromic craniosynostosis population. *Eplasty*, *15*, e12.

Proctor, M. R., & Meara, J. G. (2019). A review of the management of single-suture craniosynostosis, past, present, and future. *Journal of Neurosurgery. Pediatrics*, *24*(6), 622–631. https://doi.org/10.3171/2019.7.Peds18585

Rao, A., O'Donnell, S., Bain, N., Meldrum, C., Shorter, D., & Goel, H. (2014). An intragenic deletion of the NFIA gene in a patient with a hypoplastic corpus callosum, craniofacial abnormalities and urinary tract defects. *European Journal of Medical Genetics*, *57*(2–3), 65–70. https://doi.org/10.1016/j.ejmg.2013.12.011

Raucci, A., Bellosta, P., Grassi, R., Basilico, C., & Mansukhani, A. (2008). Osteoblast proliferation or differentiation is regulated by relative strengths of opposing signaling pathways. *Journal of Cellular Physiology*, *215*(2), 442–451. https://doi.org/10.1002/jcp.21323

Rentzsch, P., Witten, D., Cooper, G. M., Shendure, J., & Kircher, M. (2019). CADD: Predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Research*, *47*(D1), D886–d894. https://doi.org/10.1093/nar/gky1016

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Richter, G., Gui, T., Bourgeois, B., Koyani, C. N., Ulz, P., Heitzer, E., von Lewinski, D., Burgering, B. M. T., Malle, E., & Madl, T. (2021). β-Catenin regulates FOXP2 transcriptional activity via multiple binding sites. *The FEBS Journal*, *288*, 3261–3284. https://doi.org/10.1111/febs.15656

Roth, D. A., Bradley, J. P., Levine, J. P., McMullen, H. F., McCarthy, J. G., & Longaker, M. T. (1996). Studies in cranial suture biology: Part II. Role of the dura in cranial suture fusion. *Plastic and Reconstructive Surgery*, *97*(4), 693–699. https://doi.org/10.1097/00006534-199604000-00001

Sayers, E. W., Beck, J., Bolton, E. E., Bourexis, D., Brister, J. R., Canese, K., Comeau, D. C., Funk, K., Kim, S., Klimke, W., Marchler-Bauer, A., Landrum, M., Lathrop, S., Lu, Z., Madden, T. L., O'Leary, N., Phan, L., Rangwala, S. H., Schneider, V. A., … Sherry, S. T. (2021). Database resources of the National Center for biotechnology information. *Nucleic Acids Research*, *49*(D1), D10–d17. https://doi.org/10.1093/nar/gkaa892

Schröder, S., Herker, E., Itzen, F., He, D., Thomas, S., Gilchrist, D. A., Kaehlcke, K., Cho, S., Pollard, K. S., Capra, J. A., Schnölzer, M., Cole, P. A., Geyer, M., Bruneau, B. G., Adelman, K., & Ott, M. (2013). Acetylation of RNA polymerase II regulates growth-factor-induced gene transcription in mammalian cells. *Molecular Cell*, *52*(3), 314–324. https://doi.org/10.1016/j.molcel.2013.10.009

Schwenty-Lara, J., Nehl, D., & Borchers, A. (2020). The histone methyltransferase KMT2D, mutated in kabuki syndrome patients, is required for neural crest cell formation and migration. *Human Molecular Genetics*, *29*(2), 305–319. https://doi.org/10.1093/hmg/ddz284

Senaratne, T. N., & Quintero-Rivera, F. (1993). NFIA-related disorder. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews(®)*. University of Washington, Seattle.

Singh, P. N. P., Yadav, U. S., Azad, K., Goswami, P., Kinare, V., & Bandyopadhyay, A. (2018). NFIA and GATA3 are crucial regulators of embryonic articular cartilage differentiation. *Development*, *145*(2), 1–12. https://doi.org/10.1242/dev.156554

Sobreira, N., Schiettecatte, F., Valle, D., & Hamosh, A. (2015). GeneMatcher: A matching tool for connecting investigators with an interest in the same gene. *Human Mutation*, *36*(10), 928–930. https://doi.org/10.1002/humu.22844

Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T. I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., Dahary, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M., & Lancet, D. (2016). The GeneCards suite: From gene data mining to disease genome sequence analyses. *Current Protocols in Bioinformatics*, *54*, 1.30.1. https://doi.org/10.1002/cpbi.5

Stenson, P. D., Mort, M., Ball, E. V., Chapman, M., Evans, K., Azevedo, L., Hayden, M., Heywood, S., Millar, D. S., Phillips, A. D., & Cooper, D. N. (2020). The human gene mutation database (HGMD(®)): Optimizing its use in a clinical diagnostic or research setting. *Human Genetics*, *139*(10), 1197–1207. https://doi.org/10.1007/s00439-020-02199-3

Takahashi, I., Noguchi, N., Nata, K., Yamada, S., Kaneiwa, T., Mizumoto, S., Ikeda, T., Sugihara, K., Asano, M., Yoshikawa, T., Yamauchi, A., Shervani, N. J., Uruno, A., Kato, I., Unno, M., Sugahara, K., Takasawa, S., Okamoto, H., & Sugawara, A. (2009). Important role of heparan sulfate in postnatal islet growth and insulin secretion. *Biochemical and Biophysical Research Communications*, *383*(1), 113–118. https://doi.org/10.1016/j.bbrc.2009.03.140

Timberlake, A. T., Furey, C. G., Choi, J., Nelson-Williams, C., Yale Center for Genome Analysis, Loring, E., Galm, A., Kahle, K. T., Steinbacher, D. M., Larysz, D., Persing, J. A., & Lifton, R. P. (2017). De novo mutations in inhibitors of Wnt, BMP, and Ras/ERK signaling pathways in non-syndromic midline craniosynostosis. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(35), E7341–E7347. https://doi.org/10.1073/pnas.1709255114

Timberlake, A. T., Jin, S. C., Nelson-Williams, C., Wu, R., Furey, C. G., Islam, B., Haider, S., Loring, E., Galm, A., Yale Center for Genome Analysis, Steinbacher, D. M., Larysz, D., Staffenberg, D. A., Flores, R. L., Rodriguez, E. D., Boggon, T. J., Persing, J. A., & Lifton, R. P. (2019). Mutations in TFAP2B and previously unimplicated genes of the BMP, Wnt, and hedgehog pathways in syndromic craniosynostosis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(30), 15116–15121. https://doi.org/10.1073/pnas.1902041116

Tønne, E., Due-Tønnessen, B. J., Mero, I. L., Wiig, U. S., Kulseth, M. A., Vigeland, M. D., Sheng, Y., von der Lippe, C., Tveten, K., Meling, T. R., Helseth, E., & Heimdal, K. R. (2021). Benefits of clinical criteria and high-throughput sequencing for diagnosing children with syndromic craniosynostosis. *European Journal of Human Genetics*, *29*, 920–929. https://doi.org/10.1038/s41431-020-00788-4

Tønne, E., Due-Tønnessen, B. J., Wiig, U., Stadheim, B. F., Meling, T. R., Helseth, E., & Heimdal, K. R. (2020). Epidemiology of craniosynostosis in Norway. *Journal of Neurosurgery. Pediatrics*, *1–8*, 68–75. https://doi.org/10.3171/2020.1.Peds2051

Topa, A., Samuelsson, L., Lovmar, L., Stenman, G., & Kölby, L. (2017). On the significance of craniosynostosis in a case of kabuki syndrome with a concomitant KMT2D mutation and 3.2 Mbp de novo 10q22.3q23.1 deletion. *American Journal of Medical Genetics. Part A*, *173*(8), 2219–2225. https://doi.org/10.1002/ajmg.a.38296

Twigg, S. R., & Wilkie, A. O. (2015). A genetic-pathophysiological framework for Craniosynostosis. *American Journal of Human Genetics*, *97*(3), 359–377. https://doi.org/10.1016/j.ajhg.2015.07.006

UniProt Consortium. (2021). UniProt: The universal protein knowledgebase in 2021. *Nucleic Acids Research*, *49*(D1), D480–D489. https://doi.org/10.1093/nar/gkaa1100

Venero Galanternik, M., Kramer, K. L., & Piotrowski, T. (2015). Heparan sulfate proteoglycans regulate Fgf signaling and cell polarity during collective cell migration. *Cell Reports*, *10*(3), 414–428. https://doi.org/10.1016/j.celrep.2014.12.043

Venero Galanternik, M., Lush, M. E., & Piotrowski, T. (2016). Glypican4 modulates lateral line collective cell migration non cell-autonomously. *Developmental Biology*, *419*(2), 321–335. https://doi.org/10.1016/j.ydbio.2016.09.002

Vigeland, M. D., Gjotterud, K. S., & Selmer, K. K. (2016). FILTUS: A desktop GUI for fast and efficient detection of disease-causing variants, including a novel autozygosity detector. *Bioinformatics*, *32*(10), 1592–1594. https://doi.org/10.1093/bioinformatics/btw046

Volpi, S., Yamazaki, Y., Brauer, P. M., van Rooijen, E., Hayashida, A., Slavotinek, A., Sun Kuehn, H., di Rocco, M., Rivolta, C., Bortolomai, I., du, L., Felgentreff, K., Ott de Bruin, L., Hayashida, K., Freedman, G., Marcovecchio, G. E., Capuder, K., Rath, P., Luche, N., … Notarangelo, L. D. (2017). EXTL3 mutations cause skeletal dysplasia, immune deficiency, and developmental delay. *The Journal of Experimental Medicine*, *214*(3), 623–637. https://doi.org/10.1084/jem.20161525

Wilkie, A. O. M., Johnson, D., & Wall, S. A. (2017). Clinical genetics of craniosynostosis. *Current Opinion in Pediatrics*, *29*(6), 622–628. https://doi.org/10.1097/mop.0000000000000542

Wu, X., & Gu, Y. (2019). Signaling mechanisms underlying genetic pathophysiology of Craniosynostosis. *International Journal of Biological Sciences*, *15*(2), 298–311. https://doi.org/10.7150/ijbs.29183

Xie, Y., Zhang, L., Gao, Y., Ge, W., & Tang, P. (2015). The multiple roles of Microrna-223 in regulating bone metabolism. *Molecules*, *20*(10), 19433–19448. https://doi.org/10.3390/molecules201019433

Xu, S., Liu, P., Chen, Y., Chen, Y., Zhang, W., Zhao, H., Cao, Y., Wang, F., Jiang, N., Lin, S., Li, B., Zhang, Z., Wei, Z., Fan, Y., Jin, Y., He, L., Zhou, R., Dekker, J. D., Tucker, H. O., … Guo, X. (2018). Foxp2 regulates anatomical features that may be relevant for vocal behaviors and bipedal locomotion. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(35), 8799–8804. https://doi.org/10.1073/pnas.1721820115%J

Zhao, H., Zhou, W., Yao, Z., Wan, Y., Cao, J., Zhang, L., Zhao, J., Li, H., Zhou, R., Li, B., Wei, G., Zhang, Z., French, C. A., Dekker, J. D., Yang, Y., Fisher, S. E., Tucker, H. O., & Guo, X. (2015). Foxp1/2/4 regulate endochondral ossification as a suppresser complex. *Developmental Biology*, *398*(2), 242–254. https://doi.org/10.1016/j.ydbio.2014.12.007

SUPPORTING INFORMATION

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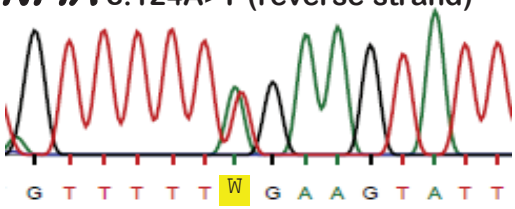
Supportive information Table 1

	Gene/NM_number/Variant	CADD	Frequency	pLi
Individual 1				
De novo (heterozygeous)	NFIA:NM_001134673:exon2:c.A124T:p.K42X	39	0	1
	NFIA:NM_001134673:exon2:c.C250T:p.R84X	38	0	1
Homozygeous/compound heterozygeous/hemizygous	ATRX:NM_138270:exon8:c.G3341T:p.G1114V	24,1	6.14e-06	1
Individual 2				
De novo (heterozygeous)	ABAT:NM_000663:exon10:c.G619A:p.D207N	23.6	2.03e-05	0.01
	DNAH9:NM_001372:exon13:c.A2195G:p.Y732C	25.8	8.952e-06	0
Homozygeous/compound heterozygeous/hemizygous	EXTL3:NM_001440:exon5:c.G2392A:p.V798M	32	0	0
	MALRD1:NM_001142308:exon18:c.C2962T:p.L988F	27.1	6.982e-06	0
	CUBN:NM_001081:exon55:c.G8701A:p.V2901I	24.2	6.459e-05	0
	MON2:NM_001278469:exon26:c.A3452G:p.Q1151R	24.0	8.989e-06	0.59
	TBC1D31:NM_001330606:exon14:c.1841_1842del	<10	3.284e-05	0
	MAP7:NM_001198617:exon7:c.A670G:p.N224D	23.1	6.267e-05	0.42
	OBSCN:NM_001271223:exon20:c.G5926A:p.A1976T	21.9	5.396e-05	0
	OBSCN:NM_001098623:exon48:c.G12845A:p.R4282H	<10	6.468e-05	0
	OBSCN:NM_001098623:exon8:c.C2444T:p.A815V	<10	6.675e-05	0
	CAND2:NM_012298:exon8:c.C2288T:p.P763L	12.5	0	0
	TPRX1:NM_198479:exon2:c.693_694insCCAGGCCCAATC:	<10	2.465e-05	0.36
	RTL3:NM_152694:exon2:c.G504C:p.Q168H	11.36	1.729e-05	0
Individual 3				
De novo (heterozygeous)	POLR2A:NM_000937:exon26:c.4329_4330delinsAA:p.A1444T	23.4	0	1
	SLC2A4RG:NM_020062:exon5:c.C584T:p.P195L	23.0	8.961e-06	0.22
Homozygeous/compound heterozygeous/hemizygous	IDS:NM_000202:exon6:c.G760A:p.E254K	<10	9.53e-05	1
Individual 4				
De novo (heterozygeous)	None			
Homozygeous/compound heterozygeous/hemizygous	MICALCL:NM_032867:exon3:c.1367_1378del	<10	0	0
Maternally transmitted predicted loss-of function	FOXP2:NM_148899:exon4:c.484del:p.Q162fs		0	1
	SSPO:NM_198455:exon66:c.C9418T:p.Q3140X	18,8	8.145e-06	
	C10orf128:NM_001010863:exon3:c.112_113insGTTTCAGATTTCAAGTACGCCC:	<10	8.129e-06	0.01
	FBRS:NM_001105079:exon18:c.2850del:p.P950fs	<10	0	1
Maternally transmitted missense with CADD >20	OTOF:NM_001287489:exon16:c.G1825A:p.E609K	34	0	0
	OTOF:NM_194322:exon5:c.G620A:p.R207Q	26.1	7.264e-05	0
	BSDC1:NM_001143890:exon8:c.C968T:p.S323F	32	6.689e-05	0.02
	SYPL2:NM_001040709:exon5:c.A637G:p.N213D	28.4	9.692e-05	0
	CTNND2:NM_001288716:exon14:c.G1951A:p.E651K	28.2	9.747e-05	1
	HEYL:NM_014571:exon3:c.G231C:p.Q77H	27.6	3.581e-05	0
	COL27A1:NM_032888:exon55:c.C4844T:p.P1615L	26.9	7.242e-05	0.59
	ASAP3:NM_001143778:exon6:c.A623G:p.K208R	26.4	2.883e-05	0

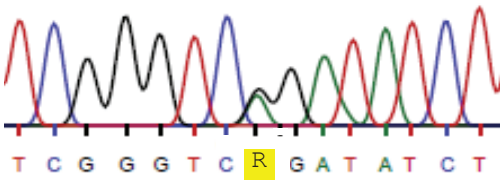
	PDILT:NM_174924:exon9:c.A1138T:p.I380F	26.2	6.268e-05	0
	ATG9A:NM_024085:exon13:c.G2273A:p.R758H	25.9	9.571e-06	0.97
	ST6GALNAC5:NM_001320273:exon2:c.G160T:p.G54C	24	8.507e-05	0.17
	ZMAT4:NM_024645:exon5:c.G385A:p.D129N	23.3	6.667e-05	0.08
	CCDC166:NM_001162914:exon1:c.C395G:p.A132G	23	0	0
	PLA2G4F:NM_213600:exon19:c.C2230A:p.R744S	22.9	0	0
	HMX3:NM_001105574:exon1:c.C17T:p.P6L	22.7	0	0.92
	RHOB:NM_004040:exon1:c.C586A:p.L196I	22.6	4.278e-06	0.12
	ADAM15:NM_001261464:exon6:c.G502A:p.G168R	21.4	8.98e-06	0
	ZFP28:NM_020828:exon8:c.G2020A:p.E674K	20.6	3.583e-05	0
Individual 5				
De novo (heterozygeous)	None			
Homozygeous/compound heterozygeous/hemizygous	SH3BP4:NM_014521:exon4:c.C128A:p.P43H	33	9.769e-05	0
	ARR3:NM_004312:exon9:c.C484T:p.R162W	25,8	9.304e-05	0.1
	KMT2D:NM_003482:exon39:c.C11599A:p.Q3867K	22,6	8.763e-05	1
	KMT2D:NM_003482:exon31:c.C7182A:p.S2394R	20	0	1
	PHKA2:NM_000292:exon32:c.G3502C:p.V1168L	22,7	0	0.38
	HMGN5:NM_030763:exon7:c.G763T:p.D255Y	14,7	7.724e-05	0.31
	ARSF:NM_001201538:exon11:c.C1466T:p.S489L	<10	7.498e-05	0
	RAG1:NM_000448:exon2:c.G1048A:p.V350I	<10	9.353e-05	0
	RAG1:NM_000448:exon2:c.A2721T:p.E907D	<10	8.179e-06	0
Individual 6				
De novo (heterozygeous)	None			
Homozygeous/compound heterozygeous/hemizygous	None			
Individual 7				
De novo (heterozygeous)	None			
Homozygeous/compound heterozygeous	PDIA4:NM_004911:exon9:c.G1496A:p.R499H	25.2	3.249e-05	0.08
	PDIA4:NM_004911:exon6:c.G826A:p.V276I	22.9	7.696e-05	0.08
	TTN:NM_003319:exon69:c.G17347A:p.V5783M	23.4	6.672e-05	0
	TTN:NM_003319:exon154:c.G51206A:p.R17069H	23.4	6.671e-05	0
Individual 8				
De novo (heterozygeous)	None			
Homozygeous/compound heterozygeous/hemizygous	SHROOM2:NM_001649:exon4:c.G1184T:p.G395V	<10	5.999e-06	0
	ASB12:NM_130388:exon2:c.A146C:p.Y49S	<10	0	0
Individual 9				
De novo (heterozygeous)	SDK1:NM_152744:exon6:c.G850A:p.D284N	15.3	0	0
Homozygeous/compound heterozygeous/hemizygous	VSIG1:NM_182607:exon3:c.A314T:p.H105L	12.6	9.489e-05	0
Individual 10				

De novo (heterozygeous)	DNAH9:NM_001372:exon43:c.C8408G:p.A2803G	25.9	0	0
Homozygeous/compound heterozygeous/hemizygous	POF1B:NM_001307940:exon13:c.A1364C:p.E455A	27.4	0	0

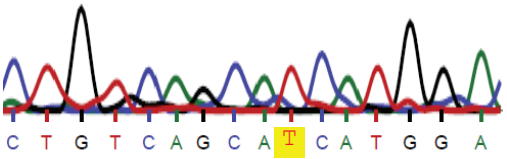
NFIA c.124A>T (reverse strand)



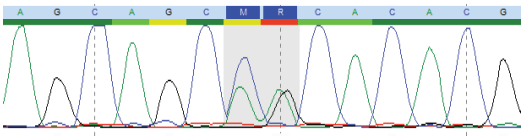
NFIA c.250C>T (reverse strand)



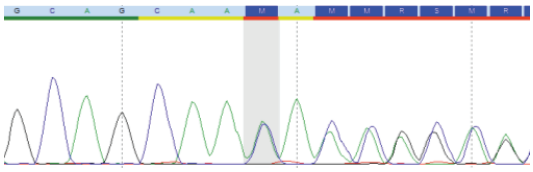
EXTL3 c.2392G>A (reverse strand)



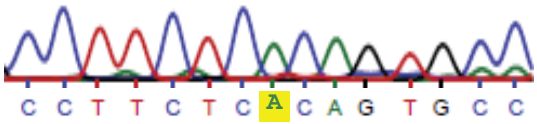
POLR2A c.4329_4330delins



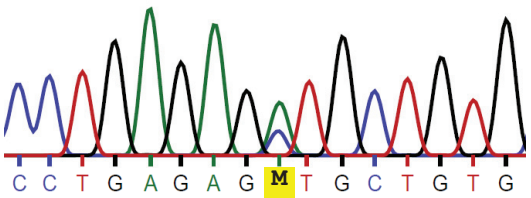
FOXP2 c.484del



SH3BP4 c.128C>A



KMT2D c.7182C>A



KMT2D c.11599C>A