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Prevalence and complications of single gene and chromosomal disorders in craniosynostosis

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Abstract

OBJECTIVE—We describe the first cohort-based analysis of the impact of genetic disorders in craniosynostosis. We aimed to refine understanding of prognosis and pathogenesis, and to provide rational criteria for clinical genetic testing.

METHODS—We undertook targeted molecular genetic and cytogenetic testing of 326 children requiring surgery for craniosynostosis, born in the years 1993-2002, presenting to a single craniofacial unit, and followed up until the end of 2007.

RESULTS—Eighty-four children (and 64 relatives) had a pathological genetic alteration, comprised of 86% single gene mutations and 14% chromosome abnormalities. The *FGFR3* P250R mutation was the single largest contributor to the genetic cases (24%). Genetic diagnoses accounted for 21% of all craniosynostosis and were associated with increased rates of many complications. Children with an initial clinical diagnosis of non-syndromic craniosynostosis were more likely to have a causative mutation if the synostosis was unicoronal or bicoronal (10 of 48 cases) than if it was sagittal or metopic (none of 55; $P=0.0003$). Repeat craniofacial surgery was required in 58% of children with single gene mutations but only 17% of those with chromosome abnormalities ($P=0.01$).

CONCLUSIONS—Clinical genetic assessment is critical for the management of children with craniosynostosis. Genetic testing of non-syndromic patients (as a minimum, for *FGFR3* P250R and *FGFR2* exons IIIa/c), should be targeted to those with coronal or multisuture synostosis. Single gene disorders that disrupt physiological signalling in the cranial sutures often require re-operation, whereas chromosome abnormalities follow a more indolent course suggesting a different, secondary origin of the associated craniosynostosis.

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CONFLICT OF INTEREST: None.

Keywords

Craniosynostosis; coronal synostosis; FGFR2; FGFR3; TWIST1; Muenke syndrome

The skull vault grows at narrow seams of unossified tissue, termed cranial sutures, which separate the individual bones. Craniosynostosis, the premature fusion of one or more cranial sutures, affects one in 2,100-2,500 children^{1,2} and has diverse presentations and causes. It can involve a single suture (most commonly the sagittal, followed in decreasing frequency by the coronal, metopic and lambdoid sutures) or multiple sutures; can have a mechanical contribution (for example external pressure on the fetal skull as a result of malposition, or reduced internal expansive force from suboptimal signalling of brain growth) or genetic predisposition; and may be isolated (non-syndromic), or associated with additional clinical signs as part of a syndrome.³⁻⁵ Although a single corrective surgical procedure is usually sufficient in non-syndromic, single suture synostosis, complex cases are challenging to manage. In the UK a multidisciplinary team approach is used.⁶ Here we explore the impact that a genetic diagnosis associated with craniosynostosis has on clinical outcome, evaluated at a single unit over a 15-year period starting in 1993.

During this same period, advances in molecular genetics have transformed diagnostic precision in the common craniosynostosis syndromes. Heterozygous mutations in the genes *FGFR2* (fibroblast growth factor receptor 2, causing Crouzon, Apert and Pfeiffer syndromes), *TWIST1* (twist homolog 1, causing Saethre-Chotzen syndrome) and *EFNB1* (ephrin-B1, causing craniofrontonasal syndrome) are characteristic of their respective disorders (clinical features are summarized in Table 5, supporting information),^{4,5} and in the case of *FGFR2* and *TWIST1*, can occasionally occur in individuals with an apparently non-syndromic clinical presentation.⁷⁻⁹ Most important clinically has been the discovery of a previously poorly delineated condition, associated with a heterozygous P250R mutation encoded by *FGFR3* (fibroblast growth factor receptor 3), that is a common cause of coronal synostosis.¹⁰⁻¹² This entity (now termed Muenke syndrome), has rather non-specific clinical features, craniosynostosis does not always occur, and the diagnosis can only be confirmed by molecular genetic testing.¹³

Although there have been previous surveys of the genetic contribution to craniosynostosis,^{12,14-17} none has comprehensively evaluated a large prospectively ascertained cohort of unselected patients. We had four major aims in undertaking this work. First, to quantify the contribution of identifiable genetic lesions to craniosynostosis; second, to derive objective criteria to guide a cost-efficient strategy for genetic testing of this heterogeneous entity; third, to examine the implications of a genetic diagnosis for management and prognosis; and finally, to gain insights into pathogenesis.

PATIENTS AND METHODS

The study was approved by the Oxfordshire Research Ethics Committee B (C02.143) and written informed consent was obtained from the child's parent or guardian. We included all 326 children born in the years 1993-2002, presenting to Oxford from anywhere in the UK, who underwent at least one major craniofacial surgical procedure (at Oxford or elsewhere) for craniosynostosis. The diagnosis was confirmed by either standard or 3-dimensional computed tomography of the skull. From January 1995 all procedures were undertaken according to uniform surgical protocols by a single team; 22 children (6.7%) had their first surgical procedure elsewhere (more information on surgical protocols¹⁸⁻²⁰ is provided in Additional Methods, supporting information). *Major craniofacial procedures* included all transcranial surgery and major subcranial osteotomies (Le Fort III), but not ventricular

shunting or additional procedures related to surgical complications. The first craniofacial procedure was performed at less than 5 years in 306 (93.9%) cases, indicating that our minimum 5 year follow-up from birth will have captured most children who eventually required surgery.

All children were assessed by a multispecialty team including a clinical geneticist. Most were followed up at least biennially, but 26 were lost to follow-up and 6 died; hence 294 (90.2%) continued to be reviewed at Oxford unit when the study ended (31 December 2007). Medical records were examined for significant events (Additional Methods). The average duration of follow-up from the first craniofacial procedure was 7.04 ± 3.30 (SD) years.

Blood was obtained from 244 children for genetic analysis, including G-banded karyotype and/or DNA extraction. Molecular genetic testing included previously documented mutation hotspots¹⁶ in the *FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, *EFNB1* and several additional genes, and array comparative genomic hybridization (CGH) in selected individuals (Additional Methods).

We developed two diagnostic classifications, one based on *initial diagnosis* (before genetic testing), the other on *final diagnosis* (after genetic testing). Children in whom a genetic diagnosis was considered to be readily apparent clinically (based either on the referral diagnosis, or after assessment of the family history and phenotype at Oxford) were excluded from analysis of the diagnostic utility of genetic testing; in these cases the proposed diagnosis was confirmed by appropriate molecular or cytogenetic testing. After all testing had been completed, children were classified into three major categories of final diagnosis: (1) *proven genetic*, where a causative *chromosome abnormality* or pathogenic *single gene mutation* was identified; (2) *other syndrome*, where additional malformations and/or a developmental quotient <70 (without obvious secondary cause) and/or a positive family history of craniosynostosis were present, but no specific gene mutation, chromosome abnormality or other predisposition (such as prenatal exposure or extreme prematurity) was identified; and (3) *non-syndromic*, where neither (1) nor (2) was applicable. The relationship between the initial and final diagnoses is summarized in Fig 4, supporting information.

Statistical testing employed χ^2 tests for data on annual case recruitment, heteroscedastic *t*-tests (2-tailed) for comparison of surgical outcomes and Fisher's exact tests (2-tailed) for comparison of 2×2 numerical data.

RESULTS

Referral Rates and Genetic Classification of Craniosynostosis in the Cohort The cohort comprises 326 children with radiologically proven craniosynostosis. These were classified (after genetic analysis) into proven genetic ($n = 84$, including 3 sib pairs), other syndrome ($n = 49$, including 5 sib pairs) and non-syndromic ($n = 193$) categories, as defined above. In the last category, a predisposing factor was identified in 20 children (see Additional Methods).

The number of children in each birth year by final diagnosis is shown in Fig 1. Whereas there was no long-term change in the annual number of referrals in the proven genetic and other syndrome categories, the non-syndromic cases showed a marked step-change increase ($P < 0.001$) in children born in 1998 onwards. This coincided with a revised definition of the craniofacial surgery service (operative from 1 April 1998), which stated that the designated craniofacial units were funded for the assessment of all patients having a suspected congenital craniofacial condition;²¹ hence, the lower number of non-syndromic cases prior to 1998 is likely to reflect under-referral (confirmed by known changes in referral criteria used by specific surgical colleagues). To estimate the proportions of children in the three

major diagnostic categories, and those with different non-syndromic synostoses, we used only data for those born 1998-2002 ($n = 215$; Fig 2A and Table 6, supporting information). The relative prevalence of different single suture synostoses, including marked sex biases in presentation, broadly accords with previous findings.^{1,2,22,23} However to sub-classify the proven genetic cases we included children born in all years (Fig 2B), as there was no evidence for a change in these referrals over time (Fig 1 and Table 6).

Twenty-one percent (46/215; 95% CI 16-27%) of children born 1998-2002 had a proven genetic cause for their craniosynostosis (Fig 2A), and, based on all birth years, the major contributions to the proven genetic cases ($n = 84$) were made by chromosome abnormalities (15%) and mutations in *FGFR2* (32%), *FGFR3* (25%), *TWIST1* (19%) and *EFNB1* (7%) (Fig 2B). Single mutations in *FAM20C* and *LMX1B* were identified, which represent novel associations with craniosynostosis.^{24,25} There were no clearly pathogenic mutations in several other genes previously implicated in craniosynostosis (Additional Methods), including *FGFR1*, *MSX2*, *EFNA4* or in the Twist box encoded by *TWIST1*.

The 13 chromosome abnormalities identified were very diverse (Table 1). The t(7p,8q) translocation was associated with deletion of *TWIST1* at 7p21.1²⁶ and the deletions of 9p and 11q represent known associations with metopic synostosis.^{3,5} Multiple suture synostosis in the child with trisomy 6p21→pter might be attributable to excess dosage of the key osteogenic regulator *RUNX2*.¹⁶ Two of the chromosome abnormalities were submicroscopic, being detected by array CGH: both rearrangements (deletion of 17q21.31; duplication of 22q11.21-11.23) have been described previously in multiple individuals, but no previous case has had craniosynostosis.^{27,28} Molecular analysis of the inv(22), present as a *de novo* change in two brothers, revealed a more complex rearrangement with at least 4 breaks (C. Babbs, unpublished data). A causal relationship to the craniosynostosis (as a low frequency complication) was judged likely in these last 3 cases, because of the relatively large extent of the rearrangements and associated clinical problems, including learning disability. Overall, synostosis of the midline (metopic/sagittal) sutures predominated in association with chromosome abnormalities (11/13 cases), and was exclusively present in 7 cases. None of the aneuploid chromosomal regions overlapped between cases, indicating marked causal heterogeneity. The frequent association of metopic synostosis with chromosomal abnormalities was demonstrated in a separate study from our unit.²⁹

In contrast to the rarity of individual chromosome abnormalities, some single gene mutations were present in multiple patients (Table 2); of these, the *FGFR3* P250R substitution was the most common (24% of the genetic cases, 95% CI 15-34%; and 5.6% of all cases born 1998-2002, 95% CI 2.9-9.6%). In addition to the 20 children with this mutation who had craniosynostosis, a further 5 (counting only those born in 1993-2002) with *FGFR3* P250R were first degree relatives of affected individuals, but did not themselves have clinically significant craniosynostosis. Assuming an overall prevalence of craniosynostosis in childhood¹ of 1 in 2,100 and 80% penetrance, we estimate from these data that the population prevalence of the *FGFR3* P250R mutation is ~1 in 30,000.

To evaluate critically the added diagnostic value provided by genetic testing, we assessed in each case whether the clinical features and/or a family history of a known mutation implied an obvious diagnosis. This was so for 45/84 children with a proven genetic cause (Table 3, footnote a; Fig 4). We then analysed how the remaining 39 children with a positive genetic test result (11 with chromosome abnormalities, 27 with single gene mutations, and 1 with both) had presented (Table 3). All children with chromosome abnormalities had a syndrome, comprising 12/77 (16%) of cases initially placed in this diagnostic group. A further 16/77 (21%) children with a syndrome had a single gene mutation; these included 9 *FGFR3* P250R mutations (5 had originally been misdiagnosed as Crouzon or Saethre-Chotzen syndrome

and the remainder had a family history of unusual head shapes). Single gene mutations were also present in 11/204 (5.4%) of cases originally classified as having non-syndromic synostosis (Table 3); again *FGFR3* P250R was the most common individual mutation (7 cases). In contrast to the predominance of midline suture synostosis in children with chromosome abnormalities, 10/11 of the apparently non-syndromic cases with single gene mutations had unicoronal or bicoronal synostosis (Table 3). Children with non-syndromic unicoronal synostosis and a single gene mutation were more likely than mutation-negative children to require squint correction (4/7 versus 5/35; $P=0.005$) and hearing aids (2/7 versus 0/35; $P=0.025$). The high prevalence of strabismus associated with *FGFR2* and *FGFR3* mutations was noted previously.³⁰

Indications for Cytogenetic and Molecular Genetic Testing

The data presented in Table 3 may be used to formulate rational criteria for cytogenetic and molecular genetic testing in craniosynostosis. Cytogenetic analysis should clearly be considered for all syndromic cases (although it may be deferred when a single gene mutation is suspected and subsequently confirmed), but no clinically significant chromosome abnormalities were identified in 92 mutation-negative, non-syndromic children known to have been tested; these figures include negative array CGH studies on 14 individuals with sagittal synostosis and 13 with metopic synostosis. Array CGH was performed on 30 syndromic individuals without a known genetic basis, and identified microdeletions judged to be clinically contributory in 2 (6.7%; 95% CI 1-22%), a rate significantly lower ($P=0.05$) than a recently published figure of 28% for the prevalence of submicroscopic abnormalities in syndromic craniosynostosis of unknown cause.³¹

Molecular genetic analysis is also indicated in syndromic cases; in non-syndromic cases, all children with sagittal ($n=27$), metopic ($n=28$) or lambdoid ($n=7$) synostosis had negative tests, in contrast to 3/8 (37.5%) bicoronal cases and 7/40 (17.5%) unicoronal cases, in whom a single gene mutation was identified. The majority of the non-syndromic cases with mutations had *FGFR3* P250R ($n=7$), but mutations in *FGFR2* ($n=3$) and *TWIST1* ($n=1$) were also identified. Hence the positive diagnostic yield from molecular genetic testing in non-syndromic synostosis is substantially higher in children with fusion of the coronal suture(s) only, compared to those with fusion of the midline sutures ($P=0.0003$).

Surgical Prognosis and Genetic Aetiology

The number of children who had undergone 1, 2, 3 or 4 major craniofacial procedures (as defined above) was 246, 62, 13 and 5, respectively; the timing of these procedures is shown in Fig 5, supporting information. There are several reasons why a child with craniosynostosis might require more than one corrective procedure (Additional Methods). In severe turribrachycephaly our operative protocol involves an initial 2-stage procedure comprising posterior release followed by definitive fronto-orbital advancement and remodelling.^{18,20} Additional unplanned revisions may be necessary because of secondarily raised intracranial pressure or progressive calvarial deformity.¹⁸⁻²⁰ Later on, children with significant midface hypoplasia and exorbitism may require midface advancement,³² and those with craniofrontonasal syndrome may require hypertelorism correction.³³

We tabulated the number of major craniofacial procedures that each child had undergone for each completed half-year (up to 5 years) or year of life. Using the average number of procedures as an index of craniofacial severity, Fig 3A shows that the proven genetic cases consistently had a more severe surgical course than the other two groups (significant at $P<0.05$ from ages 2-7 years). We were interested to determine whether this greater severity was consistent for different underlying genetic diagnoses. Figure 3B shows that children with mutations in *FGFR2*, *FGFR3* (P250R) and *TWIST1* all exhibited more severe surgical

courses than those without a genetic diagnosis ($P<0.05$ for each gene from ages 4.5-7 years). By contrast, children with chromosome abnormalities had surgical courses similar to those without a genetic diagnosis, repeat surgery being required in only 17% compared to 58% with a single gene mutation ($P=0.01$).

In addition to major craniofacial procedures, children may have additional problems related either to the presence of craniosynostosis, or to the underlying syndrome. We tabulated the more common complications and found that, for every complication measured, children with a proven genetic diagnosis fared worse than those in the two other diagnostic categories combined (summarized in Table 4 for those measures reaching statistical significance, see Table 7, supporting information, for detailed data). Requirements for midface procedures, ventricular shunting, and tracheostomy were predominantly associated with *FGFR2* mutations, whilst the need for ptosis correction, fitting of hearing aids and upper limb surgery largely represent associations with particular syndromes (Saethre-Chotzen, Muenke and Apert syndromes, respectively). Significant learning disability (developmental quotient <70), present in 21/81 (26%) of the proven genetic cases, was most commonly associated with chromosome abnormalities (8/11) and Apert syndrome (5/9). The six children in the cohort who died (1.8%) all had syndromes: either Pfeiffer syndrome with an identified *FGFR2* mutation ($n=2$), chromosome abnormality ($n=1$) (Tables 1 and 2) or of unknown genetic basis ($n=3$). Four deaths (at ages 0.5, 1.2, 2.1 and 3.9 years) were directly attributable to complications of craniosynostosis, which involved multiple sutures in all cases. This included one perioperative death at 0.5 years, in a child with an unbalanced t(6;7) chromosome translocation and uncorrected tetralogy of Fallot, who required emergency intervention for severe raised intracranial pressure.

DISCUSSION

This series provides the most accurate demographic data available to estimate the relative prevalence of proven genetic causes in craniosynostosis (Fig 2) and demonstrates the substantial additional medical and surgical burden implied by a genetic diagnosis (Fig 3, Table 4). As well as the 84 proven genetic cases, additional mutation positive individuals were identified in 36 families and totalled 64 affected relatives (Tables 1 and 2), indicating the need for extended genetic counselling in many families.

For clinical management, a particularly important category comprises those cases in whom the initial clinical diagnosis was uncertain. By combined cytogenetic and molecular genetic analysis we achieved a new and specific diagnosis in 39/326 (12%) of the cohort (Table 3). About two-fifths ($n=16$) of these children had just one mutation (*FGFR3* P250R) and 7 of these cases presented with apparently non-syndromic coronal craniosynostosis. A further 6 children with uncertain initial diagnoses harboured mutations in *FGFR2* (Table 3). The identification of causative mutations is important for clinical management, as it indicates a higher risk for repeat craniofacial procedures (Fig 3),^{18-20,34,35} as well as having implications for genetic counselling of the extended family, and future strategies for targeted pharmacological inhibition of genetically determined craniosynostosis.³⁶ By contrast the evidence does not support routine testing of children with non-syndromic sagittal, metopic or (with less confidence) lambdoid synostosis (Table 3), which together comprise 48% of cases (Fig 2A). The striking difference in molecular epidemiology of coronal synostosis, compared to synostosis of other cranial sutures, probably reflects the distinctive origin of coronal sutures at the boundary between two different embryonic tissues, the neural crest and the cephalic mesoderm.^{37,38}

Our data provide trajectories for the surgical outcome of different classes of craniosynostosis. Whilst variations occur in the surgical management of children with

FGFR2, *FGFR3*, and *TWIST1* mutations (Table 7), all are associated with a greater number of repeat craniofacial procedures at most ages compared to cases without identified genetic cause (Fig 3B). This reflects the important biological roles of these genes in the development and maintenance of the cranial sutures.³⁷ Surprisingly, children with chromosome abnormalities tended to present later and with a different pattern of suture involvement (metopic or sagittal), compared to those with single gene mutations. They rarely required repeat craniofacial surgery (Fig 3B, Table 7), suggesting that intrinsic signalling abnormalities in the cranial sutures were less likely to be present. As the associated abnormal karyotypes are very diverse (Table 1)^{3,31} and impaired cognitive development is frequent, an alternative possibility in these chromosomal cases is that the craniosynostosis has a biomechanical origin (reduced transduction of stretch forces exerted by the growing brain). We conclude that although a few recurrent chromosome abnormalities [such as deletions in 7p21 (*TWIST1*), 9p22-p24 and 11q23-q24, and duplication of 5q35 (*MSX2*)] clearly predispose genetically to craniosynostosis, attempts to identify specific craniosynostosis “genes” at many other putative loci will fail.

Our data also provide insight into the etiology of craniosynostosis in children with other syndromes. This group is highly heterogeneous; amongst proposed diagnoses, only Kabuki syndrome³⁹ was present recurrently (2 cases). The cumulative craniofacial procedures were significantly fewer in the other syndrome group than in the subgroups with identified single gene disorders, and similar overall to the non-syndromic children (Fig 3A,B). This relatively benign surgical course suggests that only a minority have intrinsic abnormalities of biogenesis or signalling in the cranial sutures.

CONCLUSIONS

This study highlights the added value that a clinical genetic assessment and, where indicated, cytogenetic and molecular genetic testing, can bring to the management of a child with craniosynostosis – informing both the surgical prognosis and the estimation of recurrence risks – and provides a benchmark for future surgical series. We propose that molecular genetic testing, as a minimum for mutations in *FGFR3* (P250R) and *FGFR2* exons IIIa and IIIc, should be essential in children with bicoronal, unicoronal or multisuture synostosis. Research aimed at identifying new genes mutated in craniosynostosis requires careful choice of patients, as many with chromosomal abnormalities or other syndromes may have secondary causes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATION

CGH comparative genomic hybridization

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FIGURE 1. Referrals of craniosynostosis to the Oxford Craniofacial Unit by year of birth and final diagnostic category. Note the increased referral of non-syndromic craniosynostosis after the revised service definition issued by the National Specialist Commissioning Advisory Group in 1998 (arrow).

FIGURE 2.

The proportion of children with different types of craniosynostosis analysed by final diagnosis. A, Non-syndromic cases (left) and all cases (right) were analysed for children born in the years 1998-2002, to minimise bias from under-ascertainment of non-syndromic cases. B, Proven genetic cases, analysed for all birth years (1993-2002). Dashed line indicates categorical overlap in patient with combined *TWIST1* deletion and chromosome translocation. Numbers in square brackets after gene names indicate the number of different mutations identified in that gene.

FIGURE 3.

Number of major craniofacial procedures for children followed up for completed half years (up to age 5 years) or whole years (above age 5 years), for ages with $n \geq 5$ children in a given category. A, all children, analysed by final diagnosis. B, selected children in the proven genetic category have been subdivided according to whether they had mutations in *FGFR2*, *FGFR3* (P250R), *TWIST1* or a chromosome abnormality, and compared to all cases without a laboratory genetic diagnosis (other syndrome and non-syndromic categories combined). Error bars indicate standard error of the mean. Note the change in the age scale at 5 years.

Table 1

Chromosome abnormalities in children with craniosynostosis

Karyotype from peripheral blood ^a	Sutures involved
47,XX,+mar.ish der(2)(wcp2+)[4]/46,XX[30] ^b	Metopic
46,XY,der(12)t(3;12)(p26.3;p13.33)mat	Metopic
46,XY,der(10)t(3;10)(q27;q26.1)	R+L Squamo-temporal
46,XY,del(4)(q25q28.2)	Sagittal→Multiple
46,XY,add(4)(q32.3q33)	Sagittal
46,XX,der(7),t(6;7)(p21;q36) ^{c,d}	Multiple
46,XY,t(7;8)(p21;q13)	L Coronal
46,XX,del(9)(p22.1)	Metopic
46,XY,del(11)(q23.3)	Metopic
46,XX,del(17)(q21.31) ^e	Sagittal
47,XX,+21	Sagittal, R Coronal
46,XX,dup(22)(q11.21q11.23) ^f	Sagittal, R Coronal
46,XY,inv(22)(p1?1.2q13?1.1) ^g	Metopic, bicoronal

^aAbnormalities arose *de novo* unless otherwise indicated.^b24/30 cells examined from skin contained the der(2) marker.^cThe translocation includes a deletion of the *TWIST1* gene at the 7p21 breakpoint (case CP)²⁶ and is primarily classed as a single gene mutation.^dDied perioperatively aged 0.5 years.^eSubmicroscopic deletion detected by array comparative genomic hybridization (case 11).²⁷^fSubmicroscopic duplication detected by array comparative genomic hybridization; mother with apparently identical duplication did not have craniosynostosis. Similar to LCR22D-22G rearrangements.²⁸^gSibling with apparently identical inversion has autistic spectrum disorder but not craniosynostosis; both parents cytogenetically normal.

Table 2 Pathogenic single gene mutations in children with craniosynostosis^a

Gene	Nucleotide change	Amino acid change	Diagnosis	Number of cases	De novo (d)/familial (f)/not tested (nt) ^b	Additional family members with mutation	Previous mutation report relating to this case
<i>EFNB1</i>	-95T>G ^c	-	craniofrontonasal (male)	1	d		
	1A>G	M1V	craniofrontonasal	1	d		40
	406+1G>A	-	craniofrontonasal	1	nt		40
	463A>C	T155P	craniofrontonasal	1	d		40
	472A>G	M158V	craniofrontonasal	1	f	1	40
	474G>T	M158I	craniofrontonasal	1	f	1	40
<i>FAM20C</i>	1309G>A	D437N	Raine	1	N/A ^d		24
<i>FGFR2</i>	755C>G	S252W	Apert	4	4d		41
	755_756CG>TC	S252F	Apert	1	d ^e		42
	758C>G	P253R	Apert	5	5d		41
	833G>A	C278Y	multisuture	1	f	3	16
	940-2A>G	-	Pfeiffer ^f	1	d		43
	940-2A>T	-	Pfeiffer	1	d		43
	943G>T	A315S	right coronal	1	f	2	7
	1009G>A	A337T	left coronal	1	f	6	16
	1012G>C	G338R	Crouzon	1	f	1	
	1021A>C	T341P	Pfeiffer ^f	1	nt		41
	1024T>C	C342R	Pfeiffer ^f	1	d		16
	1025G>A	C342Y	Crouzon	3	3d		41
	"	"	multisuture	1	d		41
	1025G>C	C342S	Pfeiffer	1	d		41
	1032G>A	A344A ^g	sagittal	2	2f	5	16,41
	1040C>G	S347C	Crouzon	1	d		41

Gene	Nucleotide change	Amino acid change	Diagnosis	Number of cases	De novo (d)/familial (f)/not tested (nt) ^b	Additional family members with mutation	Previous mutation report relating to this case
	1988G>A	G663E	Pfeiffer	1	nt		
<i>FGFR3</i>	749C>G	P250R	Muenke	20 ^b	4d, 14f, 1nt	27	18
	1620C>A	N540K	hypochondroplasia	1	d		
<i>LMX1B</i>	607C>T	L203F	nail-patella	1	f	1	
<i>TWIST1</i>	115C>G	R39G	right coronal	1	f	2	8
	230delA; 232T>C	K77SfsX48	Saethre-Chotzen	2 ^j	f	1	26
	310G>T	E104X	Saethre-Chotzen	1	d		26
	331delG	V111SfsX14	Saethre-Chotzen	1	f	2	20
	355delC	Q119fsX6	Saethre-Chotzen	1	f	1	44
	368C>G	S123W	Saethre-Chotzen	1	f	1	26
	376G>T	E126X	Saethre-Chotzen	1	f	1	20
	379_381dup	A127dup	Saethre-Chotzen	1	d		44
	396_416dup	K133_P139dup	Saethre-Chotzen	2 ^j	f	1	16
	406C>T	P136S	Saethre-Chotzen	1	f	2	20
	465C>A	Y155X	Saethre-Chotzen	1	d		44
	472T>C	F158L	Saethre-Chotzen	1	f	3	20
	485_488del	V162AfsX68	Saethre-Chotzen	1	f	1	44
	complete deletion	-	left coronal	1	d		26
Total				72	31d, 33f	62	

^aOmits heterozygous nonsynonymous mutations present in single individuals, thought to be coincidental or uninterpretable as follows: *FGFR1* 1408C>T (R470C) (similar to a reported mutation),⁴⁵ *TWIST1* 259_276del (A87_G92del) (family C04),⁴⁴ *EFNA4* 471_472delCCinsA (N157KfsX45),³⁸

^bNumber of different families.

^cMutation in mosaic state.

^dNot applicable - recessive mutation (parents 1st cousins).

^eFather heterozygous at 755C>T only.⁴²

^fThese children died of complications related to multislutture synostosis aged 3.9 years (T341P) and 2.1 years (C342R).

^gThis apparently synonymous substitution creates a cryptic splice donor site.⁴⁶

^hIncludes 1 pair of siblings. Not included in total are 5 additional individuals heterozygous for the P250R mutation and born in 1993-2002 who, on radiological assessment, did not have clinically significant craniosynostosis.

ⁱSiblings; an extensive family history is documented,⁴⁷ but only the father was additionally tested in this study.

^jSiblings.

Table 3

Utility of genetic testing in craniosynostosis where the initial diagnosis is uncertain^a

	Syndromic or familial				Non-syndromic				
Suture(s) involved	Total	Tested	Chromo somal	Single gene	Total	Tested	Chromo somal	Single gene	Gene and encoded mutation (number of cases) ^b
Sagittal, non-progressive	13	7	2	0	83	20	0	0	
Sagittal, progressive ^c	7	5	1	3	14	7	0	0	<i>FGFR2</i> : A344A (2) <i>FGFR3</i> : N540K (1)
Bicoronal	15	15	0	9	8	8	0	3	<i>FGFR3</i> : P250R (8+3N) <i>LMX1B</i> : L203F (1)
Unicoronal	8	8	1 ^d	3 ^d	42	40	0	7	<i>EFNB1</i> : -95T>G mosaic (1) <i>FGFR2</i> : A315S (1N), A337T (1N) <i>FGFR3</i> : P250R (1+4N) <i>TWIST1</i> : del (1), R39G (1N)
Metopic	16	14	4	0	36	28	0	0	
Lambdoid	0	0	0	0	10	7	0	0	
Multiple	18	17	4	2	11	9	0	1	<i>FGFR2</i> : C278Y (1N), C342Y (1) <i>FAM20C</i> : D437N (1)
Total	77	66	12 ^d	17 ^d	204	119	0	11	<i>EFNB1</i> (1); <i>FAM20C</i> (1); <i>FGFR2</i> (3+3N); <i>FGFR3</i> (10+7N); <i>LMX1B</i> (1); <i>TWIST1</i> (1+1N);

^aOmits 45 children with an obvious genetic diagnosis based on clinical features or known family history, who had mutations in *FGFR2* (21), *FGFR3* (4), *TWIST1* (14), *EFNB1* (5) and 47,XX,+21 (1).

^bNon-syndromic cases denoted N.

^cChildren requiring two or more corrective procedures.¹⁹

^dBoth figures include the child with a deletion of *TWIST1* associated with a t(7;8) translocation.

Table 4of follow-up, surgery and other complications in craniosynostosis.^a

	Total	Average length of follow-up (years±SEM)	Average number of procedures (±SEM)	>1 major procedure	2 stage initial procedure	Midface procedure	Hyper telorism correction	Craniofacial revisions (procedures)	Ventricular shunt	Secondary raised intra cranial pressure	Tracheo stomy	Ptosis correction	Hearing aid	Grommets	Upper limb surgery	DQ<70 /N ^b
	84	9.20±0.37	1.74±0.09	44 (52)	16 (19)	11 (13)	5 (6)	26	11 (13)	22 (26)	7 (8)	6 (7)	13 (15)	20 (24)	17 (20)	21/81 (26)
Non-syndromic	49	9.53±0.49	1.31±0.08	12 (24)	3 (6)	0 (0)	0 (0)	12	5 (10)	10 (20)	2 (4)	0 (0)	1 (2)	7 (14)	1 (2)	14/41 (34)
Autism risk genetic)	193	8.09±0.19	1.13±0.03	24 (12)	3 (2)	0 (0)	0 (0)	22	2 (1)	18 (9)	0 (0)	0 (0)	1 (1)	9 (5)	0 (0)	2/120 (2)
				3.5	5.5	∞	∞	2.4	4.5	2.7	10	∞	37	7.2	49	2.6

^a in parentheses are percentages of children. Differences between the proven genetic group and the other two groups combined, all significant at $P<0.05$ after correction for multiple testing (see SEM; standard error of the mean.

^b of children who had formal developmental testing.