

# Prevalence and Complications of Single-Gene and Chromosomal Disorders in Craniosynostosis

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## KEY WORDS

craniosynostosis, coronal synostosis, *FGFR2*, *FGFR3*, *TWIST1*, Muenke syndrome

## ABBREVIATIONS

CGH—comparative genomic hybridization

CI—confidence interval

[www.pediatrics.org/cgi/doi/10.1542/peds.2009-3491](http://www.pediatrics.org/cgi/doi/10.1542/peds.2009-3491)

doi:10.1542/peds.2009-3491

Accepted for publication Apr 19, 2010

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

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**FINANCIAL DISCLOSURE:** *The authors have indicated they have no financial relationships relevant to this article to disclose.*



**WHAT'S KNOWN ON THIS SUBJECT:** Several genes and chromosomal abnormalities are known to be associated with craniosynostosis, but their relative prevalence and how this knowledge can be used in clinical practice have not been documented in a large, prospective series.



**WHAT THIS STUDY ADDS:** The authors aimed to quantify the contributions of genetic lesions to craniosynostosis, to derive objective criteria to guide a cost-efficient strategy for genetic testing, to examine the implications of genetic diagnoses for management and prognoses, and to gain insights into pathogenesis.

## abstract



**OBJECTIVES:** We describe the first cohort-based analysis of the impact of genetic disorders in craniosynostosis. We aimed to refine the understanding of prognoses and pathogenesis and to provide rational criteria for clinical genetic testing.

**METHODS:** We undertook targeted molecular genetic and cytogenetic testing for 326 children who required surgery because of craniosynostosis, were born in 1993–2002, presented to a single craniofacial unit, and were monitored until the end of 2007.

**RESULTS:** Eighty-four children (and 64 relatives) had pathologic genetic alterations (86% single-gene mutations and 14% chromosomal abnormalities). The *FGFR3* P250R mutation was the single largest contributor (24%) to the genetic group. Genetic diagnoses accounted for 21% of all craniosynostosis cases and were associated with increased rates of many complications. Children with an initial clinical diagnosis of nonsyndromic craniosynostosis were more likely to have a causative mutation if the synostoses were unicoronal or bicoronal (10 of 48 cases) than if they were sagittal or metopic (0 of 55 cases;  $P = .0003$ ). Repeat craniofacial surgery was required for 58% of children with single-gene mutations but only 17% of those with chromosomal abnormalities ( $P = .01$ ).

**CONCLUSIONS:** Clinical genetic assessment is critical for the treatment of children with craniosynostosis. Genetic testing of nonsyndromic cases (at least for *FGFR3* P250R and *FGFR2* exons IIIa/c) should be targeted to patients with coronal or multisuture synostoses. Single-gene disorders that disrupt physiologic signaling in the cranial sutures often require reoperation, whereas chromosomal abnormalities follow a more-indolent course, which suggests a different, secondary origin of the associated craniosynostosis. *Pediatrics* 2010;126:e391–e400

The skull vault grows at narrow seams of unossified tissue, termed cranial sutures, which separate the individual bones. Craniosynostosis, the premature fusion of  $\geq 1$  cranial suture, affects 1 in 2100 to 2500 children<sup>1,2</sup> and has diverse presentations and causes. It may involve a single suture (most commonly the sagittal suture, followed in decreasing frequency by the coronal, metopic, and lambdoid sutures) or multiple sutures, may have a mechanical contribution (eg, external pressure on the fetal skull resulting from malposition or reduced internal expansive force resulting from suboptimal signaling of brain growth) or genetic predisposition, and may be isolated (nonsyndromic) or associated with other clinical signs as part of a syndrome.<sup>3–5</sup> Although a single corrective surgical procedure usually is sufficient for cases of nonsyndromic, single-suture synostosis, complex cases are challenging to manage. In the United Kingdom, a multidisciplinary team approach is used.<sup>6</sup> Here we explore the impact on clinical outcomes of genetic diagnoses associated with craniosynostosis, as evaluated at a single unit during a 15-year period starting in 1993.

During the same period, advances in molecular genetics have transformed diagnostic precision for 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 [Supplemental Table 5](#))<sup>4,5</sup> and, in the case of *FGFR2* and *TWIST1*, can occur occasionally in cases with apparently nonsyndromic clinical presentations.<sup>7–9</sup> Most important clinically

has been the discovery of a previously poorly delineated condition that is associated with a heterozygous P250R mutation encoded by *FGFR3* (fibroblast growth factor receptor 3) and is a common cause of coronal synostosis.<sup>10–12</sup> This entity (now termed Muenke syndrome) has rather nonspecific clinical features, craniosynostosis does not always occur, and the diagnosis can be confirmed only through molecular genetic testing.<sup>13</sup>

Although there have been previous surveys of genetic contributions to craniosynostosis,<sup>12,14–17</sup> none evaluated comprehensively a large, prospectively ascertained cohort of unelected patients. We had 4 major aims in undertaking this work, that is, (1) to quantify the contributions of identifiable genetic lesions to craniosynostosis, (2) to derive objective criteria to guide a cost-efficient strategy for genetic testing for this heterogeneous entity, (3) to examine the implications of genetic diagnoses for management and prognosis, and (4) to gain insights into pathogenesis.

## METHODS

The study was approved by Oxfordshire Research Ethics Committee B (application C02.143), and written informed consent was obtained from each child's parent or guardian. We included all 326 children born in 1993–2002 who presented to the Oxford Craniofacial Unit from anywhere in the United Kingdom and underwent  $\geq 1$  major craniofacial surgical procedure (at Oxford hospitals or elsewhere) because of craniosynostosis. The diagnosis was confirmed through either standard or 3-dimensional computed tomography of the skull. After January 1995, all procedures were performed according to uniform surgical protocols by a single team; 22 children (6.7%) underwent their first surgical procedures elsewhere (more information on

surgical protocols<sup>18–20</sup> is provided in the [Supplemental Appendix](#)). 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  $< 5$  years in 306 cases (93.9%); therefore, our minimum of 5 years of follow-up monitoring after birth would have identified most children who eventually required surgery.

All children were assessed by a multispecialty team, including a clinical geneticist. Most children were monitored at least biennially, but 26 were lost to follow-up monitoring and 6 died; therefore, 294 (90.2%) continued to be monitored at the Oxford Craniofacial Unit when the study ended (December 31, 2007). Medical records were examined for significant events (see [Supplemental Appendix](#)). The average duration of follow-up monitoring from the first craniofacial procedure was  $7.04 \pm 3.30$  years (mean  $\pm$  SD).

Blood was obtained from 244 children for genetic analysis, including G-banded karyotyping and/or DNA extraction. Molecular genetic testing included analysis of previously documented mutation hotspots<sup>16</sup> in *FGFR1*, *FGFR2*, *FGFR3*, *TWIST1*, and *EFNB1* and several additional genes, with array comparative genomic hybridization (CGH) in selected cases (see [Supplemental Appendix](#)).

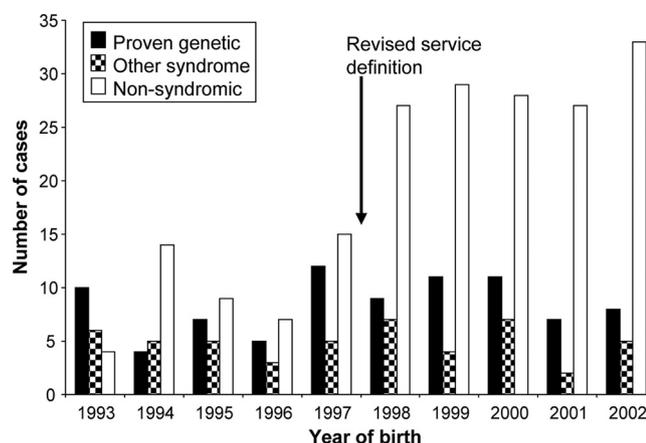
We developed 2 diagnostic classifications, one based on initial diagnoses (before genetic testing) and the other based on final diagnoses (after genetic testing). Children for whom a genetic diagnosis was considered to be readily apparent clinically (either on the basis of the referral diagnosis or after assessment of the family history and phenotype at the Oxford Craniofacial Unit) were excluded from analysis of the diagnostic utility of genetic testing;

in those cases, the proposed diagnosis was confirmed through appropriate molecular or cytogenetic testing. After all testing had been completed, cases were classified into 3 major categories of final diagnoses, that is, (1) proven genetic, when a causative chromosomal abnormality or pathogenic single-gene mutation was identified; (2) other syndrome, when additional malformations, a developmental quotient of  $<70$  (without obvious secondary cause), and/or a family history of craniosynostosis was present but no specific gene mutation, chromosomal abnormality, or other predisposition (such as prenatal exposure or extreme prematurity) was identified; and (3) nonsyndromic, when neither of the aforementioned conditions was applicable. The relationship between the initial and final diagnoses is summarized in Supplemental Figure 4. Statistical testing used  $\chi^2$  tests for data on annual case recruitment, heteroscedastic  $t$  tests (2-tailed) for comparisons of surgical outcomes, and Fisher's exact tests (2-tailed) for comparisons of  $2 \times 2$  numerical data.

## RESULTS

### Referral Rates and Genetic Classification of Craniosynostosis in Cohort

The cohort comprised 326 children with radiologically proven craniosynostosis. The cases were classified (after genetic analysis) into proven genetic ( $n = 84$ , including 3 sibling pairs), other syndrome ( $n = 49$ , including 5 sibling pairs), and nonsyndromic ( $n = 193$ ) categories, as defined above. In the latter category, a predisposing factor was identified in 20 cases (see Supplemental Appendix). The numbers of children in each birth year according to final diagnosis are shown in Fig 1. Although there was no long-term change in the annual numbers of referrals in the proven genetic and



**FIGURE 1**

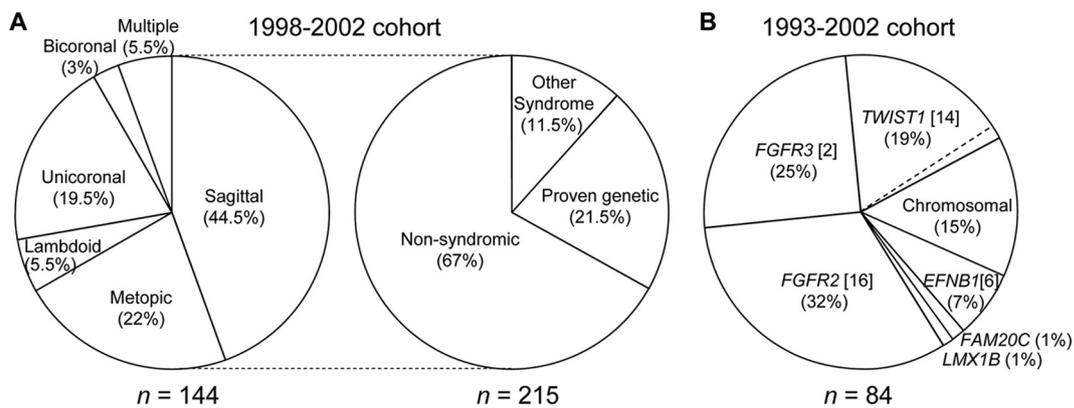
Craniosynostosis referrals to the Oxford Craniofacial Unit according to year of birth and final diagnostic category. The increased referral of nonsyndromic craniosynostosis cases after the revised service definition was issued by the National Specialist Commissioning Advisory Group in 1998 (arrow) should be noted.

other syndrome categories, nonsyndromic cases showed a marked stepwise increase ( $P < .001$ ) among children born in 1998 or later. This coincided with a revised definition for the craniofacial surgery service (operative from April 1, 1998), which stated that the designated craniofacial units were funded for the assessment of all patients with a suspected congenital craniofacial condition<sup>21</sup>; therefore, the lower numbers of nonsyndromic cases before 1998 likely reflect underreferral (confirmed by known changes in referral criteria used by specific surgical colleagues). To estimate the proportions of children in the 3 major diagnostic categories and those with different nonsyndromic synostoses, we used only data for children born in 1998–2002 ( $n = 215$ ) (Fig 2A and Supplemental Table 6). The relative prevalence of different single-suture synostoses, including marked gender biases in presentation, accords generally with previous findings.<sup>1,2,22,23</sup> To subclassify the proven genetic cases, we included children born in all years (Fig 2B), because there was no evidence for a change in these referrals over time (Fig 1 and Supplemental Table 6).

Twenty-one percent (95% confidence interval [CI]: 16%–27%) of children born in

1998–2002 (46 of 215 children) had a proven genetic cause for their craniosynostosis (Fig 2A). On the basis of all birth years, the major contributions to the proven genetic cases ( $n = 84$ ) were made by chromosomal 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.<sup>24,25</sup> There were no clearly pathogenic mutations in several other genes implicated previously in craniosynostosis (see Supplemental Appendix), including *FGFR1*, *MSX2*, and *EFNA4*, or in the Twist box encoded by *TWIST1*.

The 13 chromosomal abnormalities identified were very diverse (Table 1). The t(7p,8q) translocation was associated with deletion of *TWIST1* at 7p21.1,<sup>26</sup> and the deletions of 9p and 11q represent known associations with metopic synostoses.<sup>3,5</sup> Multiple-suture synostosis in the child with trisomy 6p21→pter might be attributable to excess dosage of the key osteogenic regulator *RUNX2*.<sup>16</sup> Two of the chromosomal abnormalities were submicroscopic, being detected through array CGH; both rearrangements (deletion of 17q21.31 and duplication of 22q11.21–q11.23) were described



**FIGURE 2**

Proportions of children with different types of craniosynostosis, according to final diagnosis. A, Nonsyndromic cases (left) and all cases (right) were analyzed for children born in 1998–2002, to minimize bias resulting from underascertainment of nonsyndromic cases. B, Proven genetic cases were analyzed for all birth years (1993–2002). The dashed line indicates categorical overlap in cases with combined *TWIST1* deletions and chromosomal translocations. Numbers in square brackets after gene names indicate the number of different mutations identified in the genes.

**TABLE 1** Chromosomal Abnormalities Among Children With Craniosynostosis

Karyotype From Peripheral Blood	Sutures Involved
47,XX,+mar ish der(2)(wcp2+)[4]/46,XX[30] <sup>a</sup>	Metopic
46,XY,der(12)t(3;12)(p26.3;p13.33)mat	Metopic
46,XY,der(10)t(3;10)(q2?7;q2?6.1)	R and L squamosotemporal
46,XY,del(4)(q25q28.2)	Sagittal to multiple
46,XY,add(4)(q32.3q33)	Sagittal
46,XX,der(7),t(6;7)(p21;q36) <sup>b</sup>	Multiple
46,XY,t(7;8)(p21;q13) <sup>c</sup>	L coronal
46,XX,del(9)(p22.1)	Metopic
46,XY,del(11)(q23.3)	Metopic
46,XX,del(17)(q21.31) <sup>d</sup>	Sagittal
47,XX,+21	Sagittal, R coronal
46,XX,dup(22)(q11.21q11.23) <sup>e</sup>	Sagittal, R coronal
46,XY,inv(22)(p1?1.2q13?1) <sup>f</sup>	Metopic, bicoronal

Abnormalities arose de novo unless otherwise indicated. R indicates right; L, left.

<sup>a</sup> Twenty-four of 30 cells examined from skin contained the der(2) marker.

<sup>b</sup> The patient died perioperatively at 0.5 years of age.

<sup>c</sup> The translocation included a deletion of the *TWIST1* gene at the 7p21 breakpoint<sup>26</sup> and was classified primarily as a single-gene mutation.

<sup>d</sup> The submicroscopic deletion was detected through array CGH.<sup>27</sup>

<sup>e</sup> The submicroscopic duplication was detected through array CGH; the mother, with an apparently identical duplication, did not have craniosynostosis. The duplication was similar to LCR22D-22G rearrangements.<sup>28</sup>

<sup>f</sup> A sibling with an apparently identical inversion had autistic spectrum disorder but not craniosynostosis; both parents demonstrated normal cytogenetic findings.

previously in multiple cases, but no previously reported case involved craniosynostosis.<sup>27,28</sup> Molecular analysis of inv(22), which was noted as a de novo change for 2 brothers, revealed a more-complex rearrangement with  $\geq 4$  breaks (C. Babbs, written personal communication, 2010). A causal relationship to the craniosynostosis (as a low-frequency complication) was judged to be likely in the latter 3 cases, because of the relatively large extent of the rearrangements and the associated clinical problems, in-

cluding learning disabilities. Overall, synostosis of the midline (metopic/sagittal) sutures predominated in association with chromosomal abnormalities (11 of 13 cases) and was present exclusively in 7 cases. None of the aneuploid chromosomal regions overlapped between cases, which indicates marked causal heterogeneity. The frequent association of metopic synostosis with chromosomal abnormalities was demonstrated in a separate study from our unit.<sup>29</sup>

In contrast to the rarity of individual chromosomal abnormalities, some single-gene mutations were present in multiple patients (Table 2); of these, the *FGFR3* P250R substitution was the most common (24% [95% CI: 15%–34%] of the genetic cases and 5.6% [95% CI: 2.9%–9.6%] of all cases among children born in 1998–2002). In addition to the 20 children with this mutation who had craniosynostosis, 5 children (counting only children born in 1993–2002) with *FGFR3* P250R were first-degree relatives of affected individuals but did not themselves have clinically significant craniosynostosis. With the assumption of an overall prevalence of craniosynostosis in childhood<sup>1</sup> of 1 case per 2100 population and 80% penetrance, we estimate from these data that the population prevalence of the *FGFR3* P250R mutation is  $\sim 1$  case per 30 000.

To evaluate critically the added diagnostic value provided by genetic testing, we assessed in each case whether clinical features and/or a family history of a known mutation suggested an obvious diagnosis. This was so for 45 of 84 children with a proven genetic cause (Table 3 and Supplemental Figure 4). We then analyzed how the remaining 39 cases with positive genetic

**TABLE 2** Pathogenic Single-Gene Mutations Among Children With Craniosynostosis

Gene	Nucleotide Change	Amino Acid Change	Diagnosis	No. of Cases	De Novo, Familial, or Not Tested <sup>a</sup>	No. of Additional Family Members With Mutation	Reference on Mutation Related to Case	
<i>EFNB1</i>	−95T>G <sup>b</sup>		Craniofrontonasal syndrome (male)	1	De novo			
	1A>G	M1V	Craniofrontonasal syndrome	1	De novo		40	
	406 + 1G>A		Craniofrontonasal syndrome	1	Not tested		40	
	463A>C	T155P	Craniofrontonasal syndrome	1	De novo		40	
	472A>G	M158V	Craniofrontonasal syndrome	1	Familial	1	40	
<i>FAM20C</i>	474G>T	M158I	Craniofrontonasal syndrome	1	Familial	1	40	
	1309G>A	D437N	Raine syndrome	1	Not applicable <sup>c</sup>		24	
<i>FGFR2</i>	755C>G	S252W	Apert syndrome	4	4 de novo		41	
	755_756CG>TC	S252F	Apert syndrome	1	De novo <sup>d</sup>		42	
	758C>G	P253R	Apert syndrome	5	5 de novo		41	
	833G>A	C278Y	Multisuture	1	Familial	3	16	
	940-2A>G		Pfeiffer syndrome	1	De novo		43	
	940-2A>T		Pfeiffer syndrome	1	De novo		43	
	943G>T	A315S	R coronal	1	Familial	2	7	
	1009G>A	A337T	L coronal	1	Familial	6	16	
	1012G>C	G338R	Crouzon syndrome	1	Familial	1		
	1021A>C	T341P	Pfeiffer syndrome <sup>e</sup>	1	Not tested		41	
	1024T>C	C342R	Pfeiffer syndrome <sup>e</sup>	1	De novo		16	
	1025G>A	C342Y	Crouzon syndrome	3	3 de novo		41	
	1025G>A	C342Y	Multisuture	1	De novo		41	
	1025G>C	C342S	Pfeiffer syndrome	1	De novo		41	
	1032G>A	A344A <sup>f</sup>	Sagittal	2	2 familial	5	16, 41	
	1040C>G	S347C	Crouzon syndrome	1	De novo		41	
	1988G>A	G663E	Pfeiffer syndrome	1	Not tested			
	<i>FGFR3</i>	749C>G	P250R	Muenke syndrome	20 <sup>g</sup>	4 de novo, 14 familial, 1 not tested	27	18
		1620C>A	N540K	Hypochondroplasia	1	De novo		
<i>LMX1B</i>	607C>T	L203F	Nail-patella syndrome	1	Familial	1		
<i>TWIST1</i>	115C>G	R39G	R coronal	1	Familial	2	8	
	230delA, 232T>C	K77SfsX48	Saethre-Chotzen syndrome	2 <sup>h</sup>	Familial	1	26	
	310G>T	E104X	Saethre-Chotzen syndrome	1	De novo		26	
	331delG	V111SfsX14	Saethre-Chotzen syndrome	1	Familial	2	20	
	355delC	Q119fsX6	Saethre-Chotzen syndrome	1	Familial	1	44	
	368C>G	S123W	Saethre-Chotzen syndrome	1	Familial	1	26	
	376G>T	E126X	Saethre-Chotzen syndrome	1	Familial	1	20	
	379_381dup	A127dup	Saethre-Chotzen syndrome	1	De novo		44	
	396_416dup	K133_P139dup	Saethre-Chotzen syndrome	2 <sup>i</sup>	Familial	1	16	
	406C>T	P136S	Saethre-Chotzen syndrome	1	Familial	2	20	
	465C>A	Y155X	Saethre-Chotzen syndrome	1	De novo		44	
	472T>C	F158L	Saethre-Chotzen syndrome	1	Familial	3	20	
	485_488del	V162AfsX68	Saethre-Chotzen syndrome	1	Familial	1	44	
		Complete deletion		L coronal	1	De novo		26
	Total				72	31 de novo, 33 familial	62	

Results omit heterozygous, nonsynonymous mutations that were present in single cases and were thought to be coincidental or uninterpretable, as follows: *FGFR1*, 1408C>T (R470C) (similar to a reported mutation)<sup>45</sup>; *TWIST1*, 259\_276del (A87\_G92del)<sup>44</sup>; *EFNA4*, 471\_472delCCinsA (N157KfsX45)<sup>38</sup>. R indicates right; L, left.

<sup>a</sup> The numbers of different families are indicated.

<sup>b</sup> Mutation in mosaic state.

<sup>c</sup> Recessive mutation (parents were first cousins).

<sup>d</sup> The father was heterozygous at 755C>T only.<sup>42</sup>

<sup>e</sup> These children died as a result of complications related to multisuture synostoses, at 3.9 years (T341P) and 2.1 years (C342R) of age.

<sup>f</sup> This apparently synonymous substitution creates a cryptic splice donor site.<sup>46</sup>

<sup>g</sup> Includes 1 pair of siblings. Not included in the total were 5 additional individuals who were heterozygous for the P250R mutation and were born in 1993–2002 but, in radiologic assessments, did not have clinically significant craniosynostosis.

<sup>h</sup> Siblings; an extensive family history is documented,<sup>47</sup> but only the father was additionally tested in this study.

<sup>i</sup> Siblings.

test results (11 with chromosomal abnormalities, 27 with single-gene mutations, and 1 with both) presented (Table 3). All cases with chromosomal

abnormalities involved a syndrome, representing 12 (16%) of 77 cases initially placed in this diagnostic group. An additional 16 (21%) of 77 children

with a syndrome had a single-gene mutation, including 9 *FGFR3* P250R mutations (5 cases had been misdiagnosed originally as Crouzon or Saethre-

**TABLE 3** Utility of Genetic Testing in Craniosynostosis When Initial Diagnosis Is Uncertain

Sutures Involved	<i>n</i>								Gene and Encoded Mutation (No. of Cases)
	Syndromic or Familial				Nonsyndromic				
	Total	Tested	Chromosomal	Single-Gene	Total	Tested	Chromosomal	Single-Gene	
Sagittal, nonprogressive	13	7	2	0	83	20	0	0	
Sagittal, progressive <sup>a</sup>	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 + 3 nonsyndromic); <i>LMX1B</i> , L203F (1)
Unicoronal	8	8	1 <sup>b</sup>	3 <sup>b</sup>	42	40	0	7	<i>EFNB1</i> , -95T>G mosaic (1); <i>FGFR2</i> , A315S (1 nonsyndromic), A337T (1 nonsyndromic); <i>FGFR3</i> , P250R (1 + 4 nonsyndromic); <i>TWIST1</i> , del (1), R39G (1 nonsyndromic)
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 (1 nonsyndromic), C342Y (1); <i>FAM20C</i> , D437N (1)
Total	77	66	12 <sup>b</sup>	17 <sup>b</sup>	204	119	0	11	<i>EFNB1</i> (1); <i>FAM20C</i> (1); <i>FGFR2</i> (3 + 3 nonsyndromic); <i>FGFR3</i> (10 + 7 nonsyndromic); <i>LMX1B</i> (1); <i>TWIST1</i> (1 + 1 nonsyndromic)

Results omit 45 children with obvious genetic diagnoses, on the basis of clinical features or known family history, who had mutations in *FGFR2* ( $n = 21$ ), *FGFR3* ( $n = 4$ ), *TWIST1* ( $n = 14$ ), *EFNB1* ( $n = 5$ ), and 47,XX,+21 ( $n = 1$ ).

<sup>a</sup> Cases that required  $\geq 2$  corrective procedures.<sup>19</sup>

<sup>b</sup> Both values include the child with a deletion of *TWIST1* associated with a t(7;8) translocation.

Chotzen syndrome and the rest of the patients had a family history of unusual head shapes). Single-gene mutations also were present in 11 (5.4%) of 204 cases classified originally as nonsyndromic synostosis (Table 3); *FGFR3* P250R again was the most common individual mutation (7 cases). In contrast to the predominance of midline suture synostoses in cases with chromosomal abnormalities, 10 of 11 of the apparently nonsyndromic cases with single-gene mutations had unicoronal or bicoronal synostoses (Table 3). Children with nonsyndromic unicoronal synostoses and single-gene mutations were more likely than children without mutations to require squint correction (4 of 7 children vs 5 of 35 children;  $P = .005$ ) and hearing aids (2 of 7 children vs 0 of 35 children;  $P = .025$ ). The high prevalence of strabismus associated with *FGFR2* and *FGFR3* mutations was noted previously.<sup>30</sup>

### Indications for Cytogenetic and Molecular Genetic Testing

The data presented in Table 3 may be used to formulate rational criteria for

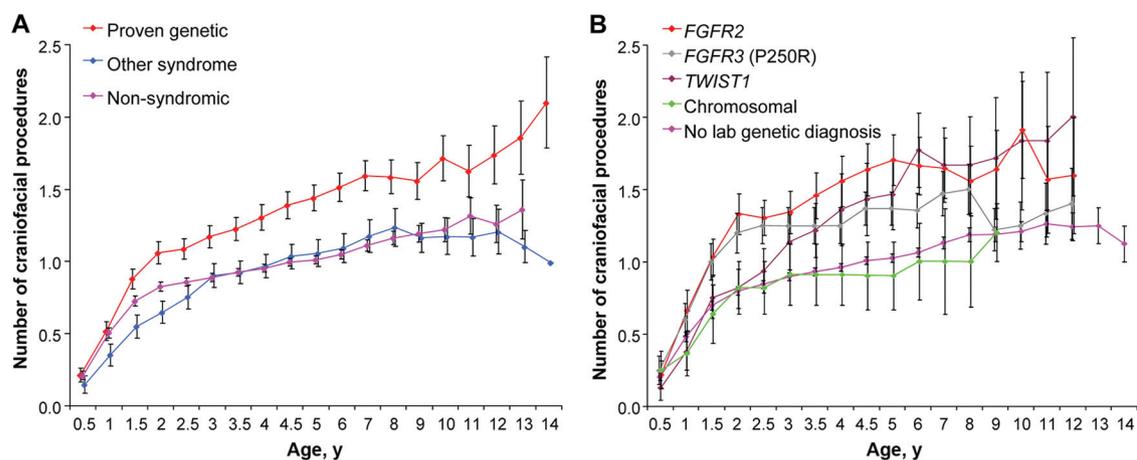
cytogenetic and molecular genetic testing in cases of craniosynostosis. Cytogenetic analysis clearly should be considered for all syndromic cases (although it may be deferred when a single-gene mutation is suspected and subsequently confirmed), but no clinically significant chromosomal abnormalities were identified in 92 mutation-negative, nonsyndromic cases known to have been tested; these values include negative array CGH results for 14 individuals with sagittal synostoses and 13 with metopic synostoses. Array CGH was performed for 30 syndromic cases without a known genetic basis and identified microdeletions judged to be clinically contributory in 2 cases (6.7% [95% CI: 1%–22%]), a rate significantly lower ( $P = .05$ ) than a recently reported value of 28% for the prevalence of submicroscopic abnormalities in cases of syndromic craniosynostosis of unknown cause.<sup>31</sup>

Molecular genetic analysis also is indicated in syndromic cases; in nonsyndromic cases, all children with sagittal ( $n = 27$ ), metopic ( $n = 28$ ), or lamb-

doid ( $n = 7$ ) synostoses had negative test results, in contrast to 3 (37.5%) of 8 bicoronal cases and 7 (17.5%) of 40 unicoronal cases in which a single-gene mutation was identified. The majority of the nonsyndromic cases with mutations had *FGFR3* P250R ( $n = 7$ ), but mutations in *FGFR2* ( $n = 3$ ) and *TWIST1* ( $n = 1$ ) also were identified. Therefore, the positive diagnostic yield from molecular genetic testing in nonsyndromic synostosis cases was substantially higher among children with fusion of the coronal sutures only, compared with those with fusion of the midline sutures ( $P = .0003$ ).

### Surgical Prognosis and Genetic Causes

The numbers of children who underwent 1, 2, 3, or 4 major craniofacial procedures (as defined above) were 246, 62, 13, and 5, respectively, and the timing of these procedures is shown in Supplemental Figure 5. There are several reasons why a child with craniosynostosis might require  $>1$  corrective procedure. For severe turri-brachycephaly, our operative protocol



**FIGURE 3**

Numbers of major craniofacial procedures for children monitored for completed half years (up to 5 years of age) or whole years (>5 years of age), for ages with  $\geq 5$  children in a given category. A, All cases were analyzed according to final diagnosis. B, Selected cases in the proven genetic category were subdivided according to whether they had mutations in *FGFR2*, *FGFR3* (P250R), or *TWIST1* or a chromosomal abnormality and were compared with all cases without a laboratory genetic diagnosis (other syndrome and nonsyndromic categories combined). Error bars indicate SEMs. The change in the age scale at 5 years should be noted.

involves an initial 2-stage procedure with posterior release followed by definitive frontoorbital advancement and remodeling.<sup>18,20</sup> Additional unplanned revisions may be necessary because of secondarily increased intracranial pressure or progressive calvarial deformity.<sup>18–20</sup> Later on, children with significant midface hypoplasia and exorbitism may require midface advancement,<sup>32</sup> and those with craniofrontonasal syndrome may require correction of hypertelorism.<sup>33</sup>

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. With the average number of procedures as an index of craniofacial severity, Fig 3A shows that the proven genetic cases consistently had more-severe surgical courses than did the other 2 groups (significant at  $P < .05$  for ages 2–7 years). We were interested in determining 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 did those without a

genetic diagnosis ( $P < .05$  for each gene for ages 4.5–7 years). In contrast, children with chromosomal abnormalities had surgical courses similar to those of children without a genetic diagnosis, with repeat surgery being required for only 17%, compared with 58% with single-gene mutations ( $P = .01$ ).

In addition to major craniofacial procedures, children may experience 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 did those in the 2 other diagnostic categories combined (summarized in Table 4 for measures that reached statistical significance; detailed data are presented in Supplemental Table 7). Requirements for midface procedures, ventricular shunting, and tracheostomy were associated predominantly with *FGFR2* mutations, whereas the need for ptosis correction, fitting of hearing aids, and upper-limb surgery largely represented associations with particular syndromes (Saethre-

Chotzen syndrome, Muenke syndrome, and Apert syndrome, respectively). Significant learning disabilities (developmental quotient of  $<70$ ), which were present in 21 (26%) of 81 proven genetic cases, were most commonly associated with chromosomal abnormalities (8 of 11 cases) and Apert syndrome (5 of 9 cases).

The 6 children (1.8%) in the cohort who died all had syndromes, namely, either Pfeiffer syndrome with an identified *FGFR2* mutation ( $n = 2$ ), or chromosomal abnormality ( $n = 1$ ) (Tables 1 and 2), or unknown genetic basis ( $n = 3$ ). Four deaths (at 0.5, 1.2, 2.1, and 3.9 years of age) were directly attributable to complications of craniosynostosis, which involved multiple sutures in all cases. The cases included 1 perioperative death, which occurred at 0.5 years of age in a child with an unbalanced t(6;7) chromosomal translocation and uncorrected tetralogy of Fallot, who required emergency intervention because of severely increased intracranial pressure.

## DISCUSSION

This series provides the most accurate demographic data available for esti-

**TABLE 4** Follow-up Periods, Types of Surgery, and Complications According to Diagnostic Category

Diagnostic Category	No. of Cases	Follow-up Period, Mean ± SEM, y	No. of Procedures, Mean ± SEM	>1 Major Procedure	n (%)										DQ of <70, n/N (%) <sup>a</sup>	
					2-Stage Initial Procedure	Midface Procedure	Hypertelorism Correction	Craniofacial Revisions (Procedures)	Ventricular Shunt	Secondary Increased Intracranial Pressure	Tracheostomy	Ptosis Correction	Hearing Aid	Grommets		Upper-Limb Surgery
Proven genetic	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)
Other syndrome	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)
Nonsyndromic	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)
Relative risk for proven genetic				3.5	5.5	∞	∞	2.4	4.5	2.7	10	∞	37	7.2	49	2.6

Differences between the proven genetic group and the other 2 groups combined all were significant at  $P < .05$ , after correction for multiple testing (see Supplemental Table 7). DQ indicates developmental quotient.

<sup>a</sup> N indicates the number of children who underwent formal developmental testing.

mation of 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 and Table 4). In addition to the 84 proven genetic cases, mutation-positive cases were identified in 36 families, involving 64 affected relatives (Tables 1 and 2), which indicates the need for extended genetic counseling in many families.

For clinical management, a particularly important category includes cases in which the initial clinical diagnosis was uncertain. Through combined cytogenetic and molecular genetic analysis, we achieved a new, specific diagnosis in 39 (12%) of 326 cases (Table 3). Approximately two-fifths of those children ( $n = 16$ ) had only 1 mutation (*FGFR3*P250R), and 7 of those cases presented with apparently nonsyndromic coronal craniosynostosis. An additional 6 children with uncertain initial diagnoses harbored mutations in *FGFR2* (Table 3). The identification of causative mutations is important for clinical management, because it indicates a higher risk for repeat craniofacial procedures (Fig 3),<sup>18–20,34,35</sup> as well as having implications for genetic counseling of the extended family and future strategies for targeted pharmacologic inhibition of genetically determined craniosynostosis.<sup>36</sup> In contrast, the evidence does not support routine testing for children with nonsyndromic sagittal, metopic, or (with less confidence) lambdoid synostoses (Table 3), which together represent 48% of cases (Fig 2A). The striking difference in the molecular epidemiological features of coronal synostoses, compared with synostoses of other cranial sutures, probably reflects the distinctive origin of coronal sutures at the boundary between 2 different embryonic tissues (the neural crest and the cephalic mesoderm).<sup>37,38</sup>

Our data provide trajectories for the surgical outcomes of different classes of craniosynostosis. Although variations in the surgical treatment of children with *FGFR2*, *FGFR3*, and *TWIST1* mutations were noted (Supplemental Table 7), all mutations were associated with a greater number of repeat craniofacial procedures at most ages, compared with 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.<sup>37</sup> Surprisingly, children with chromosomal abnormalities tended to present later and with a different pattern of suture involvement (metopic or sagittal), compared with those with single-gene mutations. They rarely required repeat craniofacial surgery (Fig 3B and Supplemental Table 7), which suggests that intrinsic signaling abnormalities in the cranial sutures were less likely to be present. Because the associated abnormal karyotypes were very diverse (Table 1)<sup>3,31</sup> and impaired cognitive development was frequent, an alternative possibility is that, in these chromosomal cases, the craniosynostosis had a biomechanical origin (reduced transduction of stretch forces exerted by the growing brain). We conclude that, although a few recurrent chromosomal abnormalities (such as deletions in 7p21 [*TWIST1*], 9p22–p24, and 11q23–q24 and duplication of 5q35 [*MSX2*]) clearly predispose subjects genetically to craniosynostosis, attempts to identify specific craniosynostosis genes at many other putative loci will fail.

Our data also provide insight into the cause of craniosynostosis among children with other syndromes. This group is highly heterogeneous; among proposed diagnoses, only Kabuki syndrome<sup>39</sup> was present recurrently (2 cases). The cumulative numbers of craniofacial procedures for the group

with other syndromes were lower than those for the group with identified single-gene disorders and were similar overall to those for the nonsyndromic group (Fig 3). This relatively benign surgical course suggests that only a minority of subjects had intrinsic abnormalities of biogenesis or signaling in the cranial sutures.

## CONCLUSIONS

This study highlights the added value that clinical genetic assessment and, where indicated, cytogenetic and molecular genetic testing can bring to the treatment of children with craniosynostosis, informing both the surgical prognosis and the estimation of recurrence risks, and provides a benchmark for future surgical series. We

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*Pediatrics* 2010;126:e391

DOI: 10.1542/peds.2009-3491 originally published online July 19, 2010;

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