Am. J. Hum. Genet. 60:555–564, 1997

A Unique Point Mutation in the Fibroblast Growth Factor Receptor 3 Gene (*FGFR3*) Defines a New Craniosynostosis Syndrome

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Summary

The underlying basis of many forms of syndromic craniosynostosis has been defined on a molecular level. However, many patients with familial or sporadic craniosynostosis do not have the classical findings of those craniosynostosis syndromes. Here we present 61 individuals from 20 unrelated families where coronal synostosis is due to an amino acid substitution (Pro250Arg) that results from a single point mutation in the fibroblast growth factor receptor 3 gene on chromosome 4p. In this instance, a new clinical syndrome is being defined on the basis of the molecular finding. In addition to the skull findings, some patients had abnormalities on radiographs of hands and feet, including thimble-like middle phalanges, coned epiphyses, and carpal and tarsal fusions. Brachydactyly was seen in some cases; none had clinically significant syndactyly or deviation of the great toe. Sensorineural hearing loss was present in some, and developmental delay was seen in a minority. While the radiological findings of hands and feet can be very helpful in diagnosing this syndrome, it is not in all cases clearly distinguishable on a clinical basis from other craniosynostosis syndromes. Therefore, this mutation should be tested for in patients with coronal synostosis.

Introduction

Craniosynostosis, the premature fusion of one or more of the cranial sutures, is a relatively common birth defect with an estimated incidence of 1/2,100–1/2,500 infants (Hunter and Rudd 1977; Lajeunie et al. 1995*a*). Craniosynostosis syndromes, including Pfeiffer, Crouzon, Jackson-Weiss, and Apert syndromes, previously classified on the basis of their clinical findings, have now been defined at a molecular level (Cohen 1995; Muenke and Schell 1995). Pfeiffer syndrome consists of craniosynostosis, which causes acrocephaly with broad and medially deviated great toes and broad thumbs (Pfeiffer 1964). Pfeiffer syndrome is heterogeneous because it is caused by a single recurring mutation (Pro252Arg) of the fibroblast growth factor receptor 1 gene (*FGFR1*) and by several different mutations affecting *FGFR2* (Muenke et al. 1994; Lajeunie et al. 1995*b;* Rutland et al. 1995; Schell et al. 1995; Meyers et al. 1996). Crouzon syndrome presents with craniosynostosis and ocular proptosis, with normal hands and feet. It is caused by multiple mutations in the *FGFR2* gene (Jabs et al. 1994; Reardon et al. 1994; Gorry et al. 1995; Ma et al. 1995; Oldridge et al. 1995; Park et al. 1995*a;* Steinberger et al. 1995), some of which are identical to those seen in Pfeiffer and Jackson-Weiss syndromes (Jabs et al. 1994; Rutland et al. 1995; Schell et al. 1995). Crouzon syndrome with acanthosis nigricans has been described with a specific Ala391Glu mutation in *FGFR3* (Meyers et al. 1995; Wilkes et al. 1996). Jackson-Weiss syndrome caused by an *FGFR2* mutation (Jabs et al. 1994) was originally described in a family with 138 affected individuals (Jackson et al. 1976) showing craniosynostosis with midface hypoplasia, broad great toes, and tarsal-metatarsal coalition or calcaneo-cuboidal fusion, usually without hand abnormalities. This syndrome presents with the widest intrafamilial variability among the FGFR-associated craniosynostosis syndromes, possibly because of the large size of the original kindred. In Apert syndrome, craniosynostosis occurs with symmetrical syndactyly of the hands and feet and is easily distinguishable from other craniosynostosis syndromes. One of two

Received September 20, 1996; accepted for publication December 3, 1996.

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0002-9297/97/6003-0011$02.00

FGFR2 mutations involving adjacent amino acids (Ser252Trp and Pro253Arg) has been found to cause Apert syndrome in nearly all patients studied (Park et al. 1995*b*; Wilkie et al. 1995*b*; Moloney et al. 1996). Recently, *FGFR2* mutations have been identified in Beare-Stevenson syndrome, a rare craniosynostosis syndrome characterized by furrowed skin (Przylepa et al. 1996).

Saethre-Chotzen syndrome is variable, presenting coronal synostosis, ptosis, small ears with prominent crura, brachydactyly, mild cutaneous digital syndactyly, and broad, sometimes bifid, halluces in valgus position. While its underlying defect has not yet been defined, Saethre-Chotzen syndrome has been linked to the short arm of chromosome 7 (Brueton et al 1992; Lewanda et al. 1994; van Herwerden et al. 1994), and six associated chromosome translocations with breakpoints in 7p have been reported, further defining the locus (Reardon et al. 1993; Reid et al. 1993; Tsuji et al. 1994; Wilkie et al. 1995*c*).

A sizable group of patients with familial or sporadic craniosynostosis do not have one of the classical craniosynostosis syndromes. Here we report 20 unrelated families in which affected individuals have coronal synostosis and a unique point mutation in the *FGFR3* gene on chromosome 4p. In this instance, the clinical syndrome is being defined on the basis of the molecular finding.

Patients, Material, and Methods

Patients

Families 1–5 were evaluated through the genetics clinic at the Children's Hospital of Philadelphia, and families 6 and 13–18 were evaluated at the Radcliffe Infirmary, Oxford (fig. 1). The majority were referred as having sporadic or familial Pfeiffer syndrome, because of their present, though mild, radiological findings. Others had some findings consistent with Saethre-Chotzen or Crouzon syndrome or were thought to have nonsyndromic craniosynostosis.

Two families (4 and 9) were described previously (kindreds 2 and 12 in Robin et al. 1994) as Pfeiffer syndrome because their shortened middle phalanges on radiographs appeared similar to those reported by Martsolf et al. (1971) in a case of Pfeiffer syndrome. Another family (12) had been reported previously as having Jackson-Weiss syndrome (Adès et al. 1994) but was subsequently shown to have linkage to chromosome 4p, inconsistent with this diagnosis, and was called *Adelaide type craniosynostosis* (Hollway et al. 1995). Family 11 was described elsewhere as a distinct autosomal dominant craniosynostosis-brachydactyly syndrome (Glass et al. 1994).

All available radiographs were personally reviewed by one of the authors (R.I.M.): in families 1–5 (anterior-posterior [AP] radiographs of hands and AP and lateral radiographs of feet); in families 6, 13, 14, 16–18, and 20 (AP radiographs of hands and feet); and on patient III-1 in family 10 (AP radiographs of feet only). Reported radiographic findings were available as published previously on families 11 (Glass et al. 1994) and 12 (Adès et al. 1994). No radiographs were available for families 7–9, 15, and 19.

DNA Analysis

DNA analysis was performed as described by Bellus et al. (1996). In short, genomic DNA was PCR amplified using a primer pair homologous to intronic sequences flanking the 191-bp exon 7 of *FGFR3*. The PCR products were digested with *Nci*I (New England Biolabs), analyzed by gel electrophoresis on 2% Nusieve/1% agarose gels, and visualized by staining with ethidium bromide. The C→G transversion at position 749 of the coding cDNA sequence creates a new *Nci*I restriction site within the 341-bp PCR fragment, resulting in an additional 151-bp fragment in affected individuals (Bellus et al. 1996). In addition, exons 4, 6–10, and 12–19 of *FGFR3* were screened in families 4 and 9, and the findings were consistent with linkage to chromosome 4p (Bellus et al. 1996).

Results

An identical mutation, predicting an Pro250Arg amino acid substitution as described in detail by Bellus et al. (1996), was present in the *FGFR3* gene of all probands. This same mutation was found in all affected relatives studied. We were able to identify the mutation in 61 individuals from 20 kindreds, 8 (7 bicoronal; 1 unicoronal) of whom were sporadic and 53 of whom were familial cases (fig. 1). Of the 53 familial patients positive for the mutation, all but 6 had an abnormal skull shape (as evidenced by examination, history, or review of photographs, indicating craniosynostosis, e.g., brachycephaly or asymmetry) (fig. 1). Of the 46 individuals with abnormal skull shape, coronal synostosis was documented in 33, of whom 28 were bilateral and 5 were unilateral. (No further information about the skull shape was available on patient IV-18, who did not have craniosynostosis by age 20 mo [Adès et al. 1994], but he was subsequently lost to follow up.) Three of the six patients without craniosynostosis were normocephalic (individuals IV-3, IV-16, and IV-19 in family 12), and three were macrocephalic (individuals II-1, 6 III-2 in family 1 and III-13 in family 12) (fig. 1). However, all six individuals had abnormal findings on radiographs of the extremities.

Other facial findings included midface hypoplasia (16/27), downslanting palpebral fissures (17/34), ptosis (12/44), and highly arched palate (21/40) (fig. 2; table 1). Mild-to-moderate sensorineural hearing loss was reported in 17/46 patients, 7 of whom are part of the

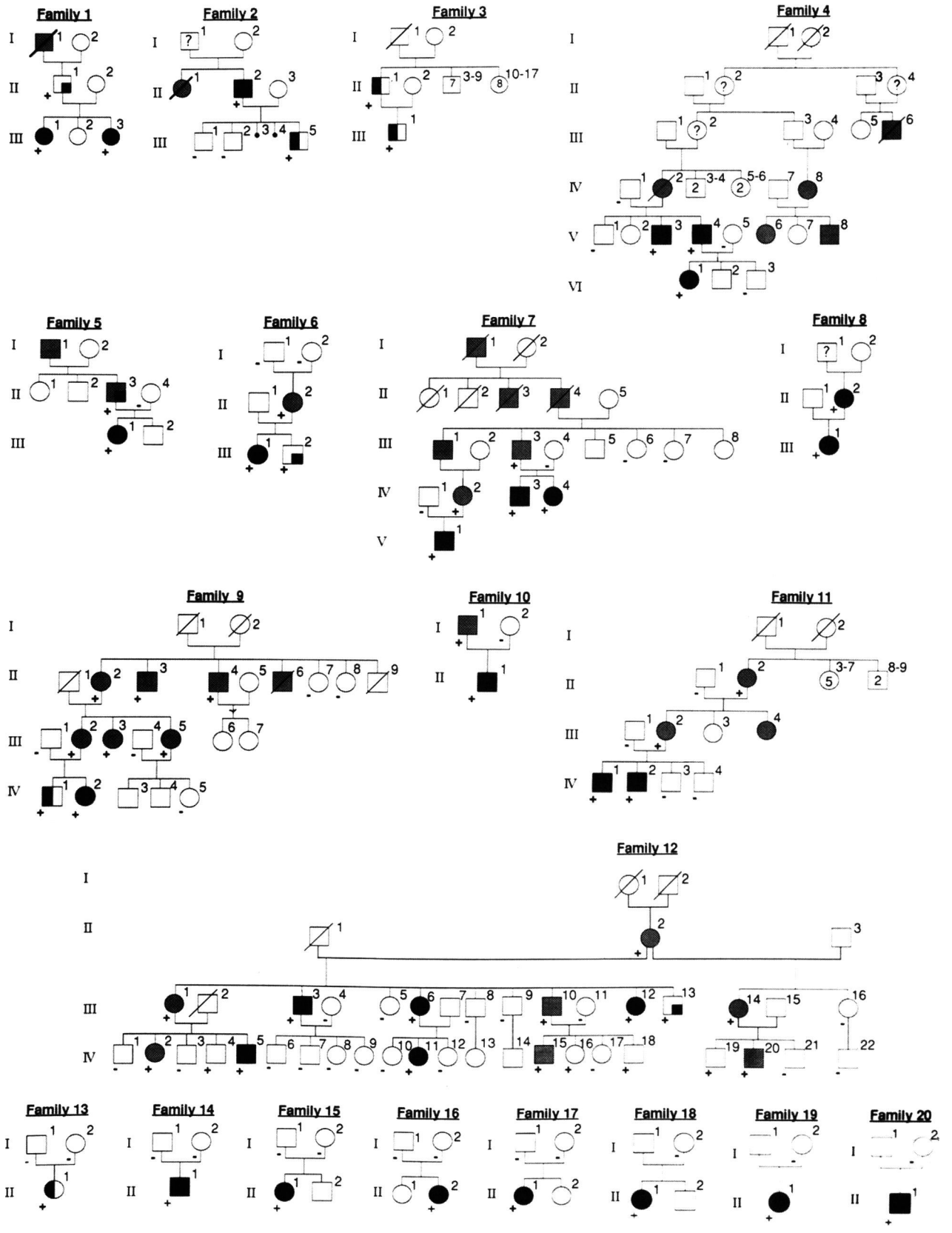

Figure 1 Pedigrees of 20 craniosynostosis families. Clinical findings consist of bicorononal synostosis (■), unicoronal synostosis (◧), macrocephaly (◻), and abnormal skull shape (■) consistent with craniosynostosis by examination, history, or photographic review. The Pro250Arg mutation in *FGFR3* was present (+) or was not present (−) as denoted.

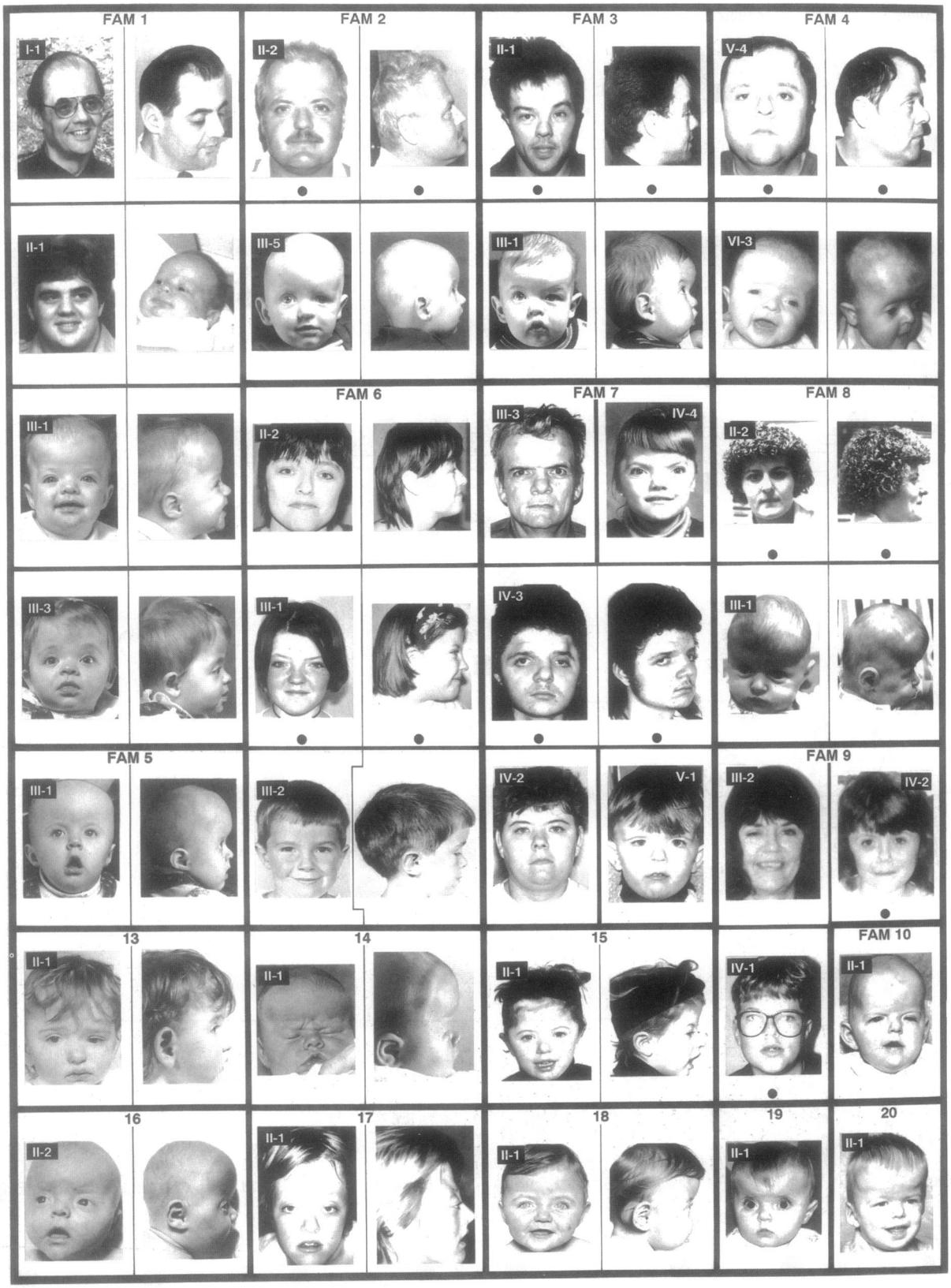

Figure 2 Facial findings of affected individuals from 18 families. Pedigree positions are shown in figure 1. Black circles (●) denote postoperative photographs.

family previously reported as Adelaide type craniosynostosis (Adès et al. 1994; Hollway et al. 1995). The majority appeared to be of normal intelligence, although formal IQ testing was not available. Mental retardation was found in three patients, and developmental delay or "borderline intelligence" in 14/46 patients.

Height was normal in 34 of 39 patients. Five patients had heights below the third centile. Because all of these are members of family 12, this may be due to shared genetic background rather than the *FGFR3* mutation.

Clinical abnormalities of hands and feet, such as clino- and brachydactyly, were noted in some patients (fig. 3; table 1). Broad great toes were noted in 6/23 patients. Medial or lateral hallucal deviation did not occur in any patient (fig. 3). Approximately half of our patients (11/19) had no limb abnormalities, on clinical examination. None had clinically significant syndactyly.

Radiographs of the hands showed broad, thimble-like middle phalanges (12/20), coned epiphyses (6/8), and carpal fusion (2/16) (fig. 4; tables 1 and 2). In 7/20 patients, no radiographic abnormalities were noted in the hands. Radiographs of the feet demonstrated broad, short middle phalanges (2/16), calcaneo-cuboidal fusion (6/17), and coned epiphyses (6/7). No patient had tarso-metatarsal coalition. In 9/20 patients, no abnormalities were observed on foot radiographs. Normal radiographs of both hands and feet were seen in 5/21 patients who had significant craniofacial anomalies.

Table 1

Summary of Clinical Findings in Affected Individuals

Finding	No. of Affected Individuals/No. of Patients Tested
Surgery for bicoronal synostosis	28/49
Surgery for unicoronal synostosis	5/49
Macrocephaly only	3/49
Midface hypoplasia	16/27
Downslanting palpebral fissures	17/34
Ptosis	12/44
High, arched palate	21/40
Sensorineural hearing loss	17/46
Developmental delay/MR	17/46
Hands:	
Brachydactyly	13/44
Clinodactyly	14/33
Feet:	
Broad big toe	6/23
Brachydactyly	11/42
Radiographs of hands:	
Coned epiphyses	6/8
Carpal fusion	2/16
Thimble-like middle phalanx	12/20
Absent/fused middle phalanx V	2/19
Normal	7/20
Radiographs of feet:	
Coned epiphyses	6/7
Calcaneo/cuboidal fusion	6/17
Short, broad middle phalanx	2/16
Normal	9/20

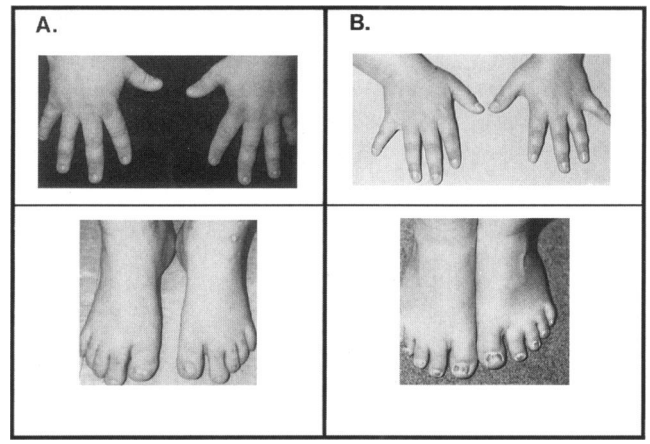

Figure 3 Brachydactyly of hands and feet. *A*, Family 4, V-4. *B*, Family 18, II-1.

Of 20 kindreds, the Pro250Arg mutation was shown to be familial in 12 and a new mutation in 5 cases; in 3 apparently sporadic cases, full parental studies were not available (fig. 1). The high number of familial mutations is likely due to ascertainment bias, because most cases were collected retrospectively. A prospective study is in progress. Of note, there was unilateral synostosis in all affected members in one family (3; fig. 1), and both uni- and bicoronal synostosis in two families (2 and 9; fig. 1).

Discussion

Here we present detailed clinical findings in 20 kindreds with a newly recognized craniosynostosis syndrome in which affected individuals have a common mutation, Pro250Arg, located between the second and third immunoglobulin-like domains of the FGFR3 protein. The mutation was identified first in two families that were linked to markers on chromosome 4p (Bellus et al. 1996). It is of note that this mutation occurs at the analogous position within *FGFR3* as the mutations in *FGFR1* (Pro252Arg) and *FGFR2* (Pro253Arg) previously described in Pfeiffer (Muenke et al. 1994) and Apert syndromes (Wilkie et al. 1995*b*), respectively.

On the basis of this *FGFR3* (Pro250Arg) mutation, we were able to define a new craniosynostosis syndrome with inter- and intrafamilial variability whose main characteristics include bilateral or unilateral coronal synostosis and specific bone anomalies of the hands and feet in some affected individuals. It is of note that some mutation carriers did not show any signs of craniosynostosis, having only macrocephaly or normal head size. Other facial findings such as midface hypoplasia,

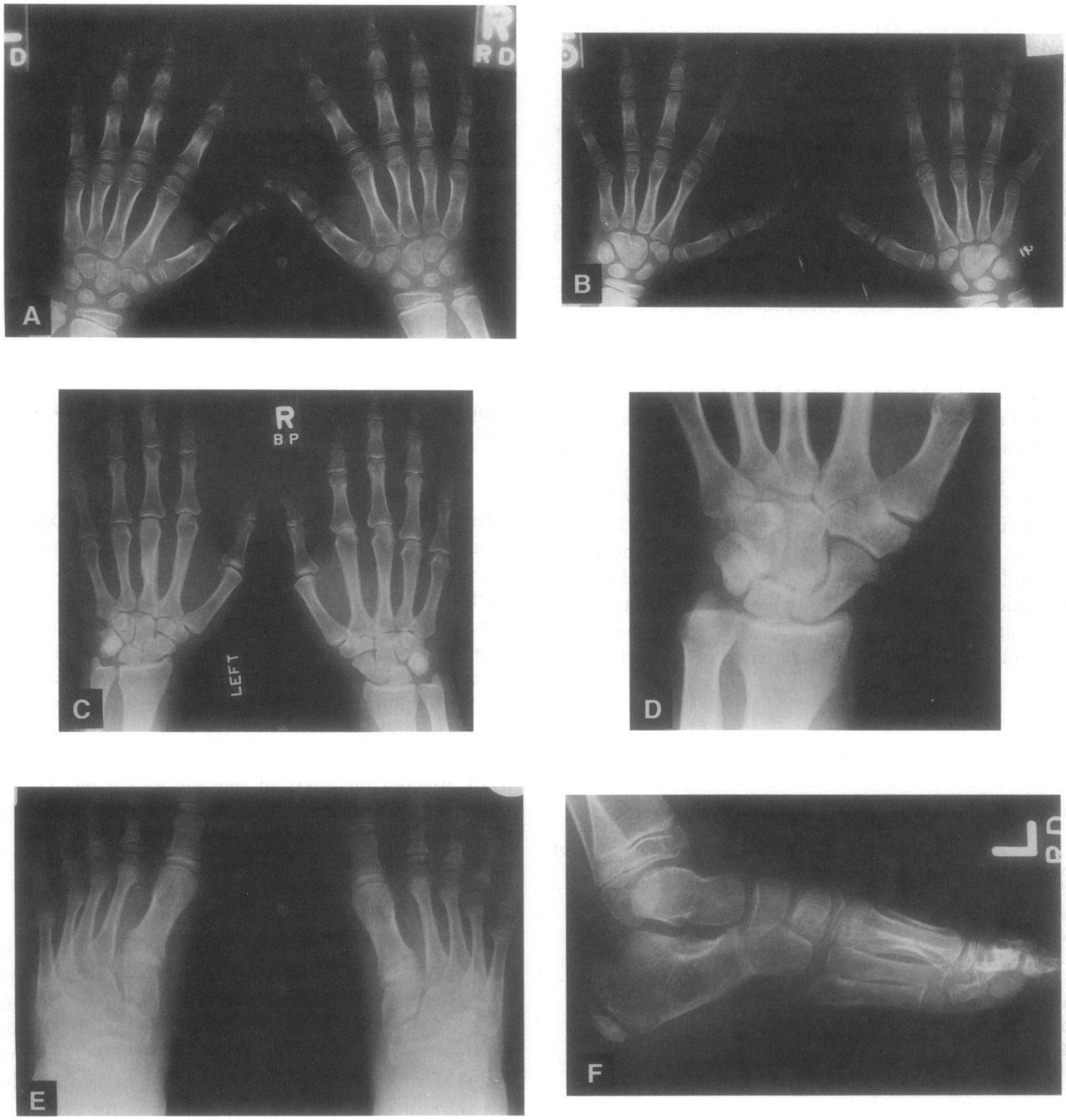

Figure 4 Radiographs of hands and feet in affected individuals. *A*, Family 4, VI-1. The middle phalanges of the second through fifth fingers are short and thimble-like in shape. *B*, Family 5, III-1. The epiphyses of the middle phalanges of the second and fifth fingers are cone-shaped. The capitate and hamate bones of the wrists are fused. *C*, Family 1, II-1. The middle phalanges of the second and fifth fingers are short. *D*, Family 2, II-2. The trapezium and trapezoid bones are fused. *E*, Family 18, II-1. The epiphyses of the proximal phalanges of the second, third, and fourth toes are coned. *F*, Family 4, VI-3. Calcaneo-cuboidal fusion.

ptosis, and downslanting palpebral fissures occurred but were not present in all cases and were usually not striking. Sensorineural hearing loss was noted in some and should be tested for in all patients. We anticipate that the same mutation will be identified in other previously described families that do not present with classical syndromes, e.g., the family described as being "suggestive of Saethre-Chotzen syndrome" by von Gernet et al. (1996) and subsequently linked to 4p by Golla et al. (1996).

In this newly described entity, stature is normal, in contrast to other *FGFR3* mutations in which dysplasia of the long bones is invariable, as in achondroplasia (Rousseau et al. 1994; Shiang et al. 1994; Bellus et al.

Table 2

Summary of Radiographic Findings in Individuals from 13 Families Whose Radiographs Were Available for Review

	Family 1			Family 2		Family 3		Family 4		Family 5		Family 6									
Finding	II-1	III-1	III-3	II-2	III-3	II-1	III-1	V-4	VI-3	II-3	III-1	II-2	III-1	III-2	Family 10, II-1	Family 13, II-1	Family 14, II-1	Family 16, II-1	Family 17, II-1	Family 18, II-2	Family 20, II-1	Total
Hands:																						
Coned epiphyses	ND	+	ND	ND	ND	ND	ND	ND	–	ND	+	ND	–	+	ND	+	ND	+	ND	+	ND	6/8
Capitate/hamate fusion	–	–	ND	+	ND	–	ND	–	–	–	+	–	–	–	ND	–	ND	–	–	–	–	2/16
Thimble-like middle phalanx	+	+	+	–	–	–	+	–	+	–	+	–	–	+	ND	+	+	+	+	+	–	12/20
Absent/fused middle phalanx V	–	–	–	+	–	–	–	–	–	–	–	–	–	–	ND	+	ND	–	–	–	–	2/19
Feet:																						
Coned epiphyses	ND	–	ND	ND	ND	ND	ND	ND	+	ND	+	ND	ND	ND	+	+	ND	+	ND	+	ND	6/7
Calcaneo/cuboidal fusion	+	–	ND	–	ND	–	ND	–	+	–	–	+	+	+	–	–	ND	–	–	+	–	6/17
Short, broad middle phalanx	–	–	+	–	–	–	–	–	–	–	–	+	ND	ND	ND	–	ND	–	ND	–	–	2/16

Note.—ND = not determined; a minus sign (–) = abnormality not found; a plus sign (+) = abnormality found.

1995*a*), hypochondroplasia (Bellus et al. 1995*b*), and thanatophoric dysplasia (Tavormina et al. 1995*b*). However, the hands and feet in this newly defined syndrome were often reported to be short by the patients themselves, and brachydactyly was found in some, although it was not clinically significant. Although the hands and feet were phenotypically suggestive of the syndrome, radiographic investigation proved to be even more valuable, because, in some instances, the radiographs were abnormal in the hands and feet when they were normal clinically. Radiological abnormalities were noted in a large number of patients, the most characteristic being the thimble-like middle phalanges of the hands, but they were not functionally significant. This finding, together with carpal and tarsal fusion and coned epiphyses, differentiates this syndrome from other previously defined craniosynostosis syndromes. Although the radiological abnormalities cannot be considered cardinal manifestations of this syndrome, their presence in patients with coronal synostosis or in first-degree relatives is suggestive of this specific mutation.

It is clear from the presenting diagnoses that this molecularly defined syndrome has previously been confused with a number of different clinical entities, including the Saethre-Chotzen, Pfeiffer, Crouzon, and Jackson-Weiss syndromes. Our analysis indicates that certain features of these latter syndromes are rare or absent in patients with the Pro250Arg *FGFR3* mutation. Hence, classical cases of Saethre-Chotzen syndrome may be diagnosed when a combination of tear-duct abnormalities, cleft palate, parietal foramina, brachydactyly, cutaneous 2/3 syndactyly of the hands, and bifid or laterally deviated halluces are present. Similarly, Crouzon and Pfeiffer syndromes are characterized by a different facial morphology with more severe ocular proptosis, involvement of multiple cranial sutures, and, in the case of Pfeiffer syndrome, broad, medially deviated halluces. Tarsal-metatarsal coalition is seen in Jackson-Weiss syndrome. Except in such classical cases, however, it may be impossible to exclude the Pro250Arg mutation by clinical criteria, and we would recommend that this mutation be tested for in all other patients with unilateral or bilateral coronal synostosis. Given the variable and sometimes mild manifestations, it will also be important to test all at risk relatives of confirmed cases.

The pathogenetic mechanism of the Pro250Arg substitution in patients with craniosynostosis is currently uncertain. Loss of function appears unlikely, since hemizygous deletion of *FGFR3*, which frequently occurs in the 4p− (Wolf-Hirschhorn) syndrome, is not associated with craniosynostosis (Cohen 1986), and mice heterozygous for null mutations in *FGFR3* are phenotypically normal (Colvin et al. 1996; Deng et al. 1996). In humans, substitutions of two neighboring amino acids in FGFR3, Arg248Cys and Ser249Cys, cause thanatophoric dysplasia type 1 (Tavormina et al. 1995*a*, 1995*b*; Rousseau et al. 1996): the former substitution has been shown in vitro to activate FGFR3 signaling constitutively by ligand-independent, covalent cross-linking of receptor dimers (Naski et al. 1996). The striking contrast in phenotype between thanatophoric dysplasia and FGFR3 craniosynostosis suggests that the Pro250Arg substitution acts by a qualitatively different gain-of-function mechanism. This is likely to reflect a conserved feature of FGFR function, given that equivalent Pro→Arg substitutions have been identified in FGFR1 (Muenke et al. 1994) and FGFR2 (Wilkie et al. 1995*b*). Several explanations have been suggested for the mechanism and specificity of these Pro→Arg substitutions (Wilkie et al. 1995*a*; Bellus et al. 1996), but in the only experimental study reported no consistent differences between the properties of wild-type and mutant Pro→Arg FGFR1 proteins were demonstrated in *Xenopus* embryos (Neilson and Friesel 1996).

In the present study, we identified both familial and sporadic *FGFR3* mutations. In their study of nonsyndromic coronal synostosis, Lajeunie et al. (1995*a*) found that 26/65 bicoronal cases and 12/127 unilateral cases were familial. It remains to be determined how many of the previously considered nonsyndromic cases of uni- and bicoronal synostosis are due to this mutation. To determine the frequency of this newly identified *FGFR3* mutation, a prospective study in patients who do not have any of the classical craniosynostosis syndromes is now underway.

Note added in proof.—Recently, mutations of the *TWIST* gene, a basic helix-loop-helix transcription factor, have been identified in Saethre-Chotzen syndrome (El-Ghoussi et al. 1977; Howard et al. 1997).

Acknowledgments

We are grateful to all family members for their participation and help in this study. We thank Ian Glass and Tony Hockley for referring family 11 and Edward C. Maynard for referring family 19; Geraldine Ashworth, Maureen E. Smith, and Wendy C. McKinnon for assistance with the clinical studies; and Laurie Lunney for technical assistance in preparing the manuscript. K.W.G. is sponsored by the Howard Hughes Medical Institute. This work was supported by the Wellcome Trust (D.M.M. and A.O.M.W.) and by NIH grants R29HD28732 and RO1HD29862 (to M.M.).

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