Craniosynostosis associated with FGFR3 pro250arg mutation results in a range of clinical presentations including unsutural sporadic craniosynostosis

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Abstract

Several mutations involving the fibroblast growth factor receptor (FGFR) gene family have been identified in association with phenotypically distinct forms of craniosynostosis. One such point mutation, resulting in the substitution of proline by arginine in a critical region of the linker region between the first and second immunoglobulin-like domains, is associated with highly specific phenotypic consequences in that mutation at this point in FGFR1 results in Pfeiffer syndrome and analogous mutation in FGFR2 results in Apert syndrome. We now show that a much more variable clinical presentation accompanies analogous mutation in the FGFR3 gene. Specifically, mental retardation, apparently unrelated to the management of the craniosynostosis, appears to be a variable clinical consequence of this FGFR3 mutation.

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The craniosynostoses represent a diverse group of disorders, which, by definition, involve premature fusion of the cranial sutures. Many of these conditions are genetically determined and family studies have been an important element in the emergence of clinically distinct syndromes of craniosynostosis.¹⁻² For the most part these syndromes are well defined, although the precise classification of individual cases/families may be the subject of good natured dispute between friends.³⁻⁸ Apart from well established clinical differences setting it apart from several other syndromes of craniosynostosis,⁹ Saethre-Chotzen syndrome also seems to be molecularly distinct in that the linkage and cytogenetic data mapping the locus for this disorder to chromosome 7p exclude a causative association with mutations of the fibroblast growth factor receptors or their ligands.¹⁰⁻¹⁵ Indeed this has been confirmed with the recent identification of mutations in the TWIST gene in patients with Saethre-Chotzen syndrome.¹⁶⁻¹⁷

Following the description of fibroblast growth factor receptor 2 (FGFR2) mutations

in Crouzon syndrome,¹⁸ mutations in the FGFR1, 2, and 3 loci have now been extended to Pfeiffer syndrome,^{5,19} Apert syndrome,²⁰ Crouzon syndrome with acanthosis nigricans,²¹⁻²² Jackson-Weiss syndrome,²³ and Beare-Stevenson syndrome.²⁴ Craniosynostosis is common to all these conditions, the basis of clinical differentiation focusing on the associated features. Additionally, mutations of FGFR3 have been described in association with skeletal dysplasias, specifically achondroplasia, hypochondroplasia, and thanatophoric dysplasia both with and without cloverleaf skull malformation.²⁵

While in Pfeiffer syndrome the association of craniosynostosis with broad thumbs and halluces has been described in association with a range of mutations at the FGFR2 locus,^{5,26} the first mutation described was in the FGFR1 locus on chromosome 8.¹⁹ The same missense mutation was identified in five families with Pfeiffer syndrome, resulting in the substitution of proline 252 by arginine in the linker region between the second and third immunoglobulin-like domains of the extracellular component of the receptor. An analogous mutation in the FGFR2 gene, proline 253 substitution by arginine, was described as one of two mutations underlying Apert syndrome, in which the craniosynostosis is associated with syndactyly of the hands and feet.²⁰ More recently, Bellus *et al*²⁷ have described substitution of proline 250 by arginine in the FGFR3 gene in association with autosomal dominant craniosynostosis of non-specific phenotype.

Arising from the observation that two families thought clinically to represent Pfeiffer syndrome, but in whom linkage data were consistent with a 4p16.3 localisation, in contrast to the expected 8 centromere (FGFR1) or 10q25 (FGFR2), Bellus *et al*²⁷ sought mutations at the FGFR3 locus on 4p as the likely basis of the phenotype. The mutation identified, pro250arg, was then sought in a panel of 65 unrelated patients with craniosynostosis with and without limb involvement. A further eight positive cases were identified, reported as five cases of non-syndromic craniosynostosis and three with features consistent with Crouzon syndrome, Pfeiffer syndrome, and Saethre-Chotzen syndrome. The authors suggested that the phenotypic spectrum of this mutation

was broad, encompassing unilateral and bilateral coronal craniosynostosis, often associated with no clinical evidence of digital abnormalities but that radiological examination of the digits might show short, broad middle phalanges of the fingers and absent or hypoplastic middle phalanges of the toes.²⁷

An important consideration is that these observations derive from families with craniosynostosis segregating. The full range of phenotypic presentations of craniosynostosis in which it would be appropriate to look for this mutation is still unclear. Moreover, much of craniosynostosis is seen in apparently sporadic situations with clinically normal parents and the question of recurrence risk in future pregnancies is the major concern. It is uncertain as yet what contribution, if any, the identification of the pro250arg mutation in FGFR3 may make to this practical problem. Finally, since most patients with craniosynostosis do not have an identifiable syndromic form and the craniosynostosis often involves only a single suture, it would be valuable to know what proportion of unsutural cases might be the result of this mutation which appears to be associated with a variable phenotype. This communication addresses these issues in light of the results we have obtained from screening 165 patients with craniosynostosis for the FGFR3 pro250arg mutation.

Materials and methods

PATIENTS

Samples were analysed from 165 patients with craniosynostosis. Of these, 45 represented familial craniosynostosis with or without limb malformations for whom no mutation had been identified at the FGFR1 and FGFR2 loci, and 120 were clinically thought to represent sporadic cases. From available details of skull radiology, 47 patients with unsutural craniosynostosis were known in the series. This latter group included both familial and sporadically occurring patients.

LABORATORY ANALYSIS

DNA was extracted from lymphocytes by salting out.²⁸ Primers amplifying the intron between exons 6 and 7 of FGFR3 from genomic DNA were used. The primer sequences are 5'-CGGCAACTACACCTGCGTCGTG-3' forward from exon 6 of FGFR3 and 5'-CTTGAGCACGGTAACGTAGGG-3' reverse from the end of exon 7 of FGFR3. PCR conditions were 200 ng of genomic DNA, 10 mmol l⁻¹ Tris, pH 8.3, 1.5 mmol l⁻¹ MgCl₂, 16 mol l⁻¹ (NH₄)₂SO₄, 200 µmol l⁻¹ dNTP, 50 pmol of each primer, and 0.5 U *Taq* polymerase and DMSO was added to a final concentration of 10% in a total volume of 50 µl: 30 cycles of (1) 94°C × 30 seconds, (2) 58°C × 30 seconds, (3) 72°C × 40 seconds. The wild type, 351 bp product, which includes most of exon 7 of FGFR3, is cleaved by the enzyme *Nci*I giving fragments of 319 bp and 32 bp. In the presence of the C749G mutation which underlies the proline substitution, the 319 bp fragment is further cleaved by this enzyme into fragments of 151 and 168 bp (fig 1).

Figure 1 The undigested PCR product (Un) is 351 bp. *Nci*I restriction of the normal allele cleaves a 32 bp fragment, producing a 319 bp product. The C749G mutation in FGFR3 creates a *Nci*I site resulting in further digestion products of 168 bp and 151 bp. DNA size marker (M) is 100 bp ladder. Family 1 is shown, with affected father and children in lanes 1, 2, and 4 clearly being heterozygous for the mutation and the unaffected mother in lane 3 being homozygous wild type.

Results

Nine of 165 (5.5%) samples tested proved positive for the mutation. These were found to represent four familial (4/45=8.8%) and five sporadic cases (5/120=4.2%) in our series. Two cases presenting with unsutural craniosynostosis without limb malformation were found to have the mutation (3/47=6.4%).

CLINICAL FEATURES OF THE FGFR3 PRO250ARG MUTATION

Four families were identified with this mutation. Family 1 represents a two generational pedigree which presented with bicoronal craniosynostosis and broad thumbs and halluces in both affected offspring (fig 2). Despite surgical treatment for the craniosynostosis, both patients were assessed as having a mild to moderate degree of mental handicap, requiring special schooling. A likely diagnosis of Pfeiffer syndrome was suggested and examination of the parents showed that the father of the children himself had broad first and second toes with a normal skull both on clinical and radiological examination (fig 2). None of the three subjects carrying the mutation in this family showed cone shaped epiphyses on x ray.

Family 2 is a two generational pedigree, initially considered to represent Saethre-Chotzen syndrome, on the basis of the facial asymmetry, flat forehead, and prominent crus helcis in the affected patient as well as her affected son. The clinically affected mother had unicoronal synostosis radiologically and x ray in her son showed bilateral coronal suture craniosynostosis. This boy has developmental delay and attends special school. Grandparental examination was unremarkable except for brachydactyly in the grandfather. As the mutation was shown to arise de novo in his daughter, this finding is unrelated to the phenotype.

Similarly, family 3, represented by an affected mother and son, were considered to have Saethre-Chotzen syndrome, in view of the facial asymmetry and prominence of the ear crura in both affected subjects and the facial profile of the boy (fig 3). The craniosynostosis in this family involved the coronal sutures

Figure 2 Family 1 showing the craniosynostosis in both children associated with slightly wide halluces in the affected father (top) and children. Note also the somewhat broad second toes in the father.

Figure 3 Several clinical features in the patients reported are documented. Top left, case 2, family 3 to show the overlap with Saethre-Chotzen syndrome. Top middle and right, case 5, who has unicoronal synostosis and cone shaped epiphyses of the hand. Second line left and middle frames show case 7 who has multiple suture involvement confirmed on the x ray as well as profound developmental delay. Second line on the right is case 8 who had unsutural craniosynostosis involving the left coronal suture, as shown in her x ray at the bottom left. Case 8 also has cone shaped epiphyses, seen bottom middle. Case 9 is shown in the right hand frame, bottom line, with features which were thought clinically to be consistent with Saethre-Chotzen syndrome.

bilaterally. Family 4, comprising an affected mother and daughter, were thought to represent Saethre-Chotzen syndrome in view of the high, flat forehead, the prominent ear crura, and the absence of proptosis and limb malformations. Radiological examination again showed bilateral coronal suture involvement.

Patients 5 to 9 were all sporadic cases, two of whom presented clinically with plagiocephaly in the newborn period and were shown radiologically to have unicoronal synostosis. Although two of these five cases had cone shaped epiphyses radiologically (fig 3), only one patient showed unequivocal clinical evidence of digital abnormality, a broad thumb in case 6 (table 1), who has already been reported elsewhere as he also had DiGeorge syndrome with confirmed 22q deletion.²⁹ It is worth noting the severe developmental delay in case 7, whose mutation had arisen de novo (fig 4) and whose craniosynostosis involved all sutures except the lambdoid (fig 3). There was no evidence of raised intracranial pressure in this patient and, despite extensive investigation, no other basis for the developmental delay was found.

Discussion

The clinical profile of the patients in whom we have identified the FGFR3 pro250arg mutation shows wide variability in presentation. In this respect the FGFR3 mutation differs from the clinical observations in patients with substitution of the analogous proline in FGFR1 and FGFR2. The proline 252 to arginine substitution in FGFR1 consistently results in a Pfeiffer syndrome phenotype, whereas the proline 253 to arginine substitution in FGFR2 is one of only two adjacent amino acid substitutions underlying Apert syndrome. The specificity of the Apert syndrome association with the proline 253 to arginine substitution in FGFR2 has been further underlined by the recent report of Oldridge *et al*³⁰ in which a rare amino acid substitution known to cause Apert syndrome (serine 252 to phenylalanine) is modified if the adjacent amino acid (proline 253 in wild type) is also altered to serine. These authors showed a range of phenotypes associated with different combinations of amino acid substitutions at the serine 252 proline 253 sequence in wild type FGFR2. In view of these findings, the specificity of the the proline 253 arginine substitution with the Apert syndrome phenotype is all the more remarkable, leading the authors to suggest that the conformation of the mutant FGFR2 giving rise to syndactyly is a function not just of the particular amino acid but also of specific amino acids at neighbouring sites. All four human FGFRs show identical amino acid sequence in this region,³¹ the only mutation in the patients we describe being the proline to arginine substitution. Given the absence of adjacent amino acid changes as phenotype modifiers, as seen in the cases of Oldridge *et al*,³⁰ the phenotypic spectrum in the cases we report is surprising.

The group of patients we describe highlight a number of important practical points with

Table 1 Clinical details of families and patients identified in this report

	Suture	Broad thumbs	Hallux	Cone shaped epiphyses	Mental retardation	Other features	Presumptive clinical diagnosis
Family 1							
Case 1	None	No	Broad	No	No	Broad 2nd toes	?Pfeiffer syndrome
Case 2	Bicoronal	No	Broad	No	Yes	No	
Case 3	Bicoronal	No	Broad	No	Yes	No	
Family 2							
Case 1	Left coronal	No	No	?	No	No	Saethre-Chotzen syndrome
Case 2	Bicoronal	No	No	?	Yes	Cleft palate	
Family 3							
Case 2	Bicoronal	No	Broad	?	No	No	Saethre-Chotzen syndrome
Family 4							
Case 2	Bicoronal	No	No	?	No	Flat forehead	Saethre-Chotzen syndrome
Sporadic							
Case 5	Left coronal	No	Broad	Yes	No	No	Non-specific
Case 6	Multiple	Yes	No	No	?	Del(22q11)	Non-specific
Case 7	Multiple	No	No	No	Yes	2/3 toe syndactyly	?deletion 7p21
Case 8	Left coronal	No	No	Yes	No	No	Non-specific
Case 9	Bicoronal	No	No	No	No	No	Saethre-Chotzen syndrome

Figure 4 The de novo nature of the mutation in case 8. The affected patient is shown to be heterozygous for the mutation in lane 3, his unaffected parents in lanes 1 and 2.

respect to diagnosis and counselling in patients with craniosynostosis. Firstly, there is significant overlap between the Saethre-Chotzen syndrome phenotype and that of FGFR3 proline 250 arginine substitution. Secondly, mental retardation not related to the craniosynostosis is part of the clinical spectrum in both familial and sporadic cases. Thirdly, the presence or absence of limb abnormalities are not a guide to identification of patients with this underlying mutation. Fourthly, five of our nine cases represent new mutations, suggesting that the C to G transversion which underlies the proline 250 arginine substitution may be a relatively common mutation. Fifthly, the identification of this mutation in association with unisutural craniosynostosis in three cases emphasises the caution with which recurrence risks should be approached in craniosynostosis. Finally, the presence of cone shaped epiphyses in two of the eight cases for whom hand radio-

logy was available may assist clinicians in guiding laboratory colleagues as to which mutation to seek in individual cases.

The systematic classification of craniosynostosis syndromes based on associated clinical features has led, in recent years, to the addition of a number of subtypes described in individual families,^{32,33} whose clinical features did not fit easily with the known syndromic forms. While one of these has been found to be associated with a point mutation in the MSX2 gene on chromosome 5,³⁴ another has been mapped to chromosome 4p, in the region of FGFR3.³¹ Perhaps mutations at the FGFR3 locus, encompassing a range of phenotypic presentations such as we describe, may account for several patients with craniosynostosis for whom mutations have not been identified at the other loci known to underlie craniosynostosis.

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