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Spectrum of Craniosynostosis Phenotypes Associated with Novel Mutations at the Fibroblast Growth Factor Receptor 2 Locus

Abstract

The causative relationship between several of the syndromic forms of craniosynostosis and mutations in the fibroblast growth factor receptor (FGFR) loci is now well established. However, within the group of patients with craniosynostosis, there are several families and sporadic cases whose clinical features differ in variable degrees from the classically described syndromes of craniosynostosis. In this communication we present novel FGFR2 mutations associated with a spectrum of craniosynostosis phenotypes in 4 sporadic cases and in one family in which craniosynostosis segregates. The mutation and phenotype data presented emphasise the clinical variability of mutations at this locus and underline the plasticity of the phenotype-genotype relationship in this important group of congenital malformation syndromes. Mutations found were tyrosine 105 to cysteine, glycine 338 to glutamic acid, serine 351 to cysteine and glycine 384 to arginine. These are the first reported mutations in the first immunoglobulin-like loop (tyrosine 105 to cysteine) and the transmembrane domain (glycine 384 to arginine) of FGFR2, providing further insights into the mechanism of abnormal receptor function in FGFR2 mutations.

Key Words

FGFR2
 Craniosynostosis
 Atypical phenotype

Introduction

Premature fusion of the cranial bone sutures, craniosynostosis, embraces an aetiologically heterogeneous group of disorders, many of which are genetically determined [1]. While over one hundred syndromes of craniosynostosis have been recognised [2], several 'common' phenotypes have emerged over the last hundred years. Most notable among these are the eponymous syndromes of Apert, Crouzon and Pfeiffer, all of which are inherited as autosomal dominant conditions. Crouzon, Apert and Pfeiffer syndromes are clinically distinct forms of cranio-

synostosis representing the phenotypic outcome of mutation of the fibroblast growth factor receptor (FGFR) 1, 2 and 3 genes [3, 4].

The FGFR gene family which encodes structurally related receptors of the tyrosine kinase, subclass IV, type has been extensively reviewed elsewhere [5]. To date 4 loci have been identified. The observation of FGFR2 mutations in Crouzon syndrome [6-10] has now been extended to include Apert [11, 12] and Pfeiffer syndromes [13-15]. Pfeiffer syndrome, however, shows locus heterogeneity, with some families having mutation at the FGFR1 locus on chromosome 8 [16]. While Apert syn-

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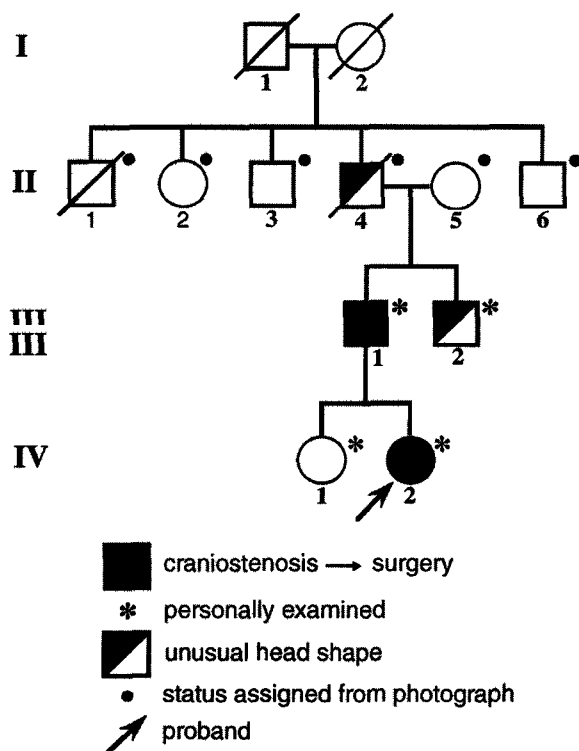


Fig. 1. Family tree of family 1.

Table 1. FGFR2 mutations identified in cases 1–5

Case	Nucleotide change	Amino acid change	Restriction site change
1, 2	G→A	Gly338Glu	<i>DdeI</i> +
3	A→G	Tyr105Cys	
4	C→G	Ser351Cys	
5	G→A	Gly384Arg	

drome is specifically related to one of two missense mutations resulting in the substitution of highly conserved amino acids in the linker region between the second and third immunoglobulin-like domain of the extracellular region of the receptor, Crouzon syndrome and FGFR2-related Pfeiffer syndrome are noteworthy for the range of mutations in the FGFR2 gene. More recently a form of Crouzon syndrome, specifically associated with acanthosis nigricans, has been shown to be caused by a missense

mutation within the transmembrane region of FGFR3 [4]. The families in whom these mutations have been identified do not usually show major intrafamilial clinical heterogeneity. The exception is Jackson-Weiss syndrome which is also caused by a mutation of the FGFR2 gene and was clinically very variable within the original reported family [7, 17]. Furthermore, an FGFR2 mutation has been detected in a small family in which 2 family members had only craniosynostosis, whereas another family member had broad thumbs and toes, in addition to craniosynostosis [18].

We now present 5 instances, 4 sporadic and 1 familial in which patients with unusual craniosynostosis phenotypes have been shown to have novel FGFR2 mutations. The mutations are summarised in table 1.

Methods

Molecular Analysis

DNA was extracted from lymphocytes by salting out [19]. Primers were designed to amplify various regions of FGFR2 from genomic DNA as detailed in table 2. Each PCR contained 200 ng genomic DNA, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM (cases 3 and 5) or 3 mM (cases 1, 2 and 4) MgCl₂, 200 μM dNTP, 25 pmol of each primer and 1 U *Taq* DNA polymerase in a final volume of 50 μl. The cycling conditions for all the PCRs were as follows: (1) 94°C × 30 s, (2) 57°C × 30 s, (3) 72°C × 30 s for 30 cycles.

SSCP Analysis

Radiolabelled PCR products were obtained by adding 0.1 μl α³²P-dCTP to each reaction. Denatured PCR products were electrophoresed, and the gels transferred to filter paper. After drying, the gels were autoradiographed.

DNA Sequencing

5'-Biotinylation of a single primer allowed the double-stranded PCR product to be captured on Dynabeads M-280 Streptavidin (Dyna, UK). The non-biotinylated strands were then removed using 0.15 M NaOH and sequenced.

DdeI Restriction Analysis

Digests were performed on diluted PCR product according to the manufacturer's specifications (Promega). The products were separated on a 3% Nusieve agarose gel.

Patients and Mutations

Pedigree 1

The family tree is shown in figure 1. The proband IV₂ is the second child of healthy non-consanguineous parents. She was born at term, weighing 3.85 kg. An unusual head shape was obvious at birth and the striking facial resemblance between the proband and her father was noted. A diagnosis of possible Crouzon syndrome was made. Surgery was required for raised intracranial pressure at the age



Fig. 2. Proband, IV₂, from birth to age 11 years. Note the change in facial appearance with age.

Table 2. FGFR2 primer sequences used for PCR in cases 1–5

Case	Forward primer	Reverse primer
1, 2, 4	5'ATCATTCTGTGTCGTCTAACC-3'	5'AAAAAACCCAGAGAGAAAGAACAGTATA-3'
3	5'CGCTGCCTGTTGAAAGATG-3'	5'GACATTCACCATGAAGTACCAAG-3'
5	5'GATGCGTCAGTCTGGTGTGCT-3'	5'GGATACGTTTGGTCAGCTTGTG-3'

of 3 months. Motor development was normal. At the age of 8 years recurrent headaches and raised intracranial pressure led to further surgery with remodelling of the forehead and supra-orbital ridge advancement. Clinical examination showed turribrachycephaly, mild proptosis, a broad nasal bridge, normal hands and minimal cutaneous syndactyly of the 2nd and 3rd toes. There was no facial asymmetry and no maxillary hypoplasia. Skull X-rays taken at that time show fusion of all sutures and copper-beating appearance, indicative of raised intracranial pressure. Clinical pictures (fig. 2) emphasise the changing phenotype with age, progressing from an initially non-specific form of craniosynostosis through a Saethre-Chotzen-like phase to a more Crouzon-like appearance.

Proband III₁ gave a history of previous neurosurgery in childhood although details of this are unavailable. His craniosynostosis only

came to light after his daughter's birth. Although the proptosis in adulthood was more marked than in his daughter, a review of photographs at different ages also shows an evolving phenotype (fig. 3). Intelligence was normal.

The proband's uncle, III₂, was similarly affected but declined formal examination. The proband's sister, mother and paternal grandmother were all clinically normal, but it was evident from family photographs that the paternal grandfather had also been affected.

A mutation was demonstrated in exon B of the FGFR2 gene, specifically a G to A change resulting in glycine 338 being replaced by glutamic acid. The mutation created a *DdeI* restriction site, and the sequencing result was confirmed in this way in all affected individuals.



Fig. 3. Proband's father, III, from infancy to adulthood. Note the emergence of proptosis as a feature of the phenotype in adult life.

Case 2

This case was born to non-consanguineous parents at 36 weeks' gestation. The parents were each aged 30 years at the time of birth. Initial concern stemmed from the observation, at the age of 5 months, that the boy's anterior fontanelle was bulging. CT scan of the brain showed ventricular dilation of moderate degree compatible with Arnold-Chiari malformation, and a shunt was inserted. At this time obstructive sleep apnoea was documented and attributed to laryngomalacia. He was re-presented aged 18 months with persistent headache. Herniation of the cerebellar tonsils into the posterior cervical canal was demonstrated radiologically. A posterior fossa decompression and a cerebellar tonsillopexy were successfully undertaken subsequently. Since the obstructive sleep apnoea continued to cause concern, a tonsillectomy and a pharyngoplasty were performed.

On evaluation at the age of 9 years, the clinical features noted were proptosis, hypertelorism, maxillary hypoplasia and strabismus involving the left superior oblique muscle (fig. 4). The cranial contours were normal with no palpably raised sutures. The hands and feet were normal. A possible diagnosis of Crouzon syndrome was considered, but the absence of craniosynostosis on skull X-ray and CT scan was felt to be inconsistent with this. His developmental history was normal and his school performance at age 9 was good.

Molecular analysis of exon B of the FGFR2 gene has shown a G to A mutation at glycine 338 resulting in substitution by glutamic acid, identical with that recorded in pedigree 1 above. This mutation creates a new restriction site *DdeI*, and the mutation has been confirmed as being *de novo* in this way (table 1).

Case 3

This case was born in 1980 to non-consanguineous parents after a normal pregnancy and delivery. Skull asymmetry was present from birth, but initial concern during the first few months of life focused on choanal stenosis and his mouth breathing, which resolved without intervention. Craniosynostosis of the sagittal and, possibly, coronal sutures was diagnosed radiologically. Initial surgery at the age of 1 year has been followed by further remodelling procedures at ages 5 and 8 with a frontal advance at age 13. Clinical examination at the age of 5 showed a developmentally normal boy with asymmetrical dolichocephaly and a moderate degree of proptosis (fig. 5). Notably, his limbs and digits were normal. He was tentatively diagnosed as 'atypical Crouzon syndrome'. Radiological evaluation of the skull at this time showed a very asymmetrical skull vault with left sided flattening and digital marking of the parietal bones suggestive of raised intracranial pressure (fig. 6). Skeletal radiology was normal.

Molecular analysis of his FGFR2 gene has shown an A to G mutation in the codon encoding tyrosine 105 in the first immunoglobulin-

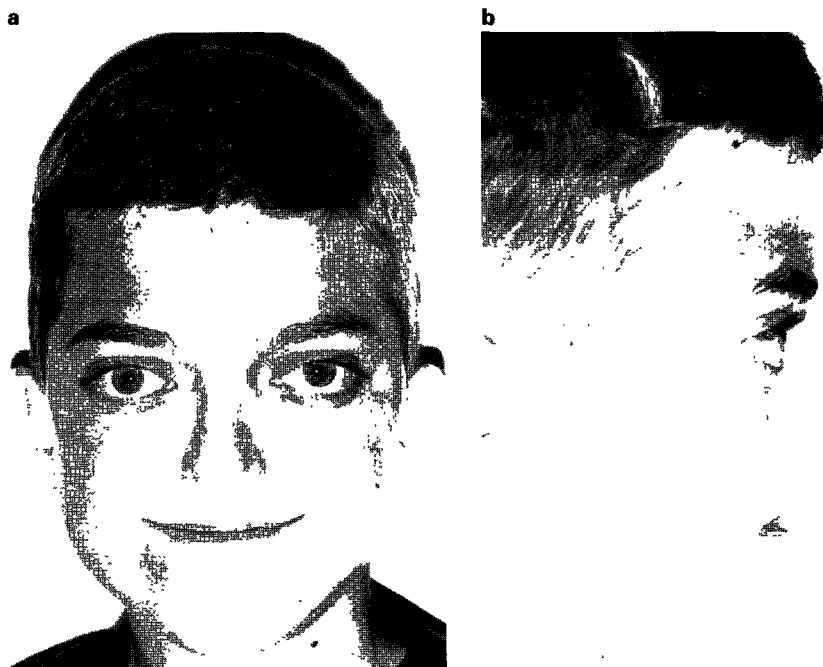


Fig. 4. Anteroposterior (a) and lateral (b) views of case 2.

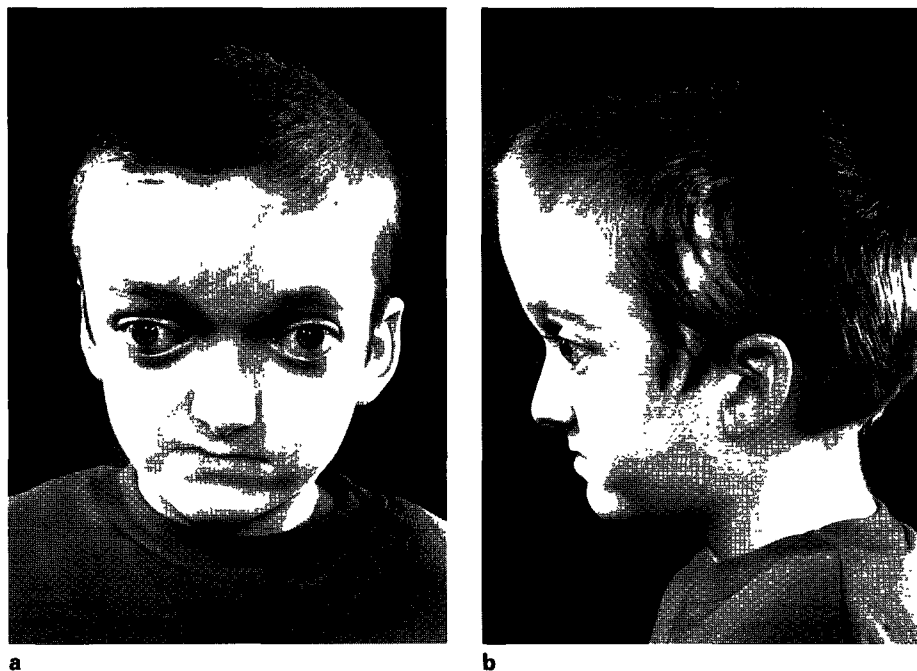


Fig. 5. Anteroposterior (a) and lateral (b) views of case 3. Note the proptosis and dolichocephaly.

like domain of the extracellular region of FGFR2, resulting in replacement by cysteine. No SSCP changes were observed in the two exons encoding the IgII to IgIII linker and the IgIII domain. Parental DNA was not available for analysis, but the same SSCP change was not seen in 75 samples.

Case 4

This girl presented with choanal atresia within 24 h of birth, which required surgical intervention in the first few days of life. The dysmorphic features noted were proptosis, enlarged and tense fonta-

nelles as well as midface hypoplasia, similar to Crouzon syndrome. The skull was cloverleaf. Unlike Crouzon syndrome, however, the elbow joints were fixed and humero-ulnar synostosis was demonstrated radiologically (fig. 7). The fingers and toes were normal with no features of Pfeiffer syndrome. The subsequent course was dominated by respiratory complications with sleep apnoea, and neurological development was significantly delayed when assessed at the age of 2 years.

FGFR2 analysis showed a point mutation in the codon specifying serine 351, within the juxtamembrane region. A TCC to TCG muta-

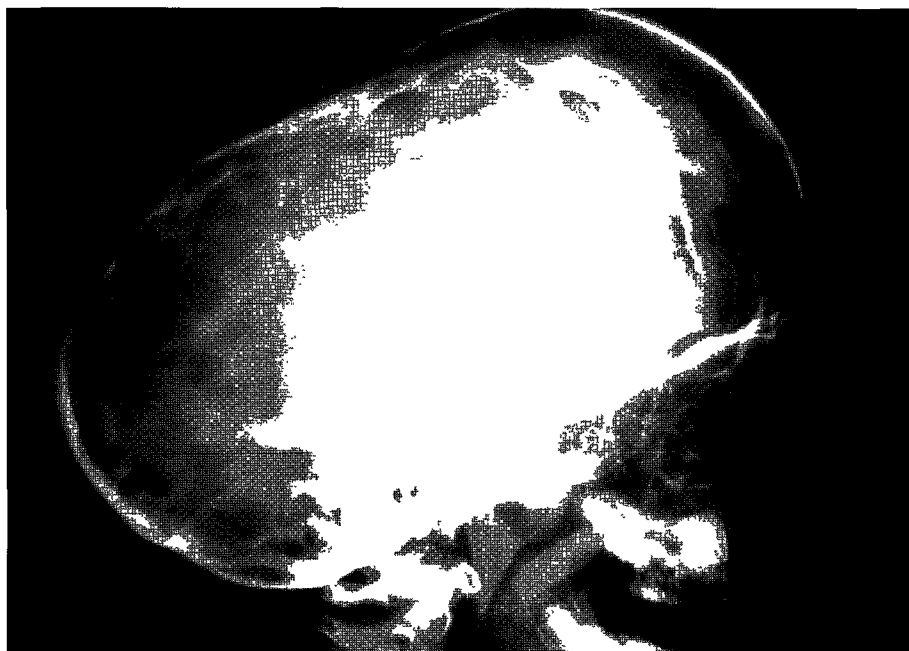


Fig. 6. Lateral skull X-ray of case 3. Note particularly the absence of sutures and digital marking.

tion was identified resulting in substitution of a cysteine for the serine. The family history was reported to be negative and consent to publish clinical photographs was not given. Parental DNA was not available for analysis.

Case 5

This case was born in 1960 and presented with respiratory difficulties and skull asymmetry at the age of 2 months. Investigation revealed choanal stenosis and a high arched palate. Skull X-ray confirmed premature synostosis of the right coronal suture and the anterior aspect of the sagittal suture, accounting for a mild degree of facial asymmetry. Clinical examination of both parents was normal. Surgery to the fused sutures was undertaken aged 2 years in view of the increasing degree of skull asymmetry (fig. 8).

Mutation analysis demonstrated a *de novo* point mutation of glycine 384 to arginine (resulting from a GGG to AGG) within the transmembrane domain.

Discussion

To date, over two dozen individual mutations of FGFR2 have been reported in patients with Crouzon syndrome and 11 in FGFR2 related Pfeiffer syndrome. It is clear that the phenotypic effects are not solely governed by the individual mutation identified. For instance, Crouzon and Pfeiffer syndromes have been observed for each of 3 individual missense mutations in the codon encoding cysteine 342 [13–15], and a case with Crouzon syndrome has also been found with the same mutation as in the original Jackson-Weiss family [7, 10]. The scientific explana-



Fig. 7. Radiological demonstration of the humero-ulnar synostosis of the elbow joint in case 4.

tion of these clinical and molecular observations remains unknown. Several aspects of the cases we now present further emphasise the plasticity of the phenotype-genotype relationship in FGFR2-related craniosynostosis.

Family 1 and case 2 share the same mutation. This mutation is in that region of FGFR2 – the second half of

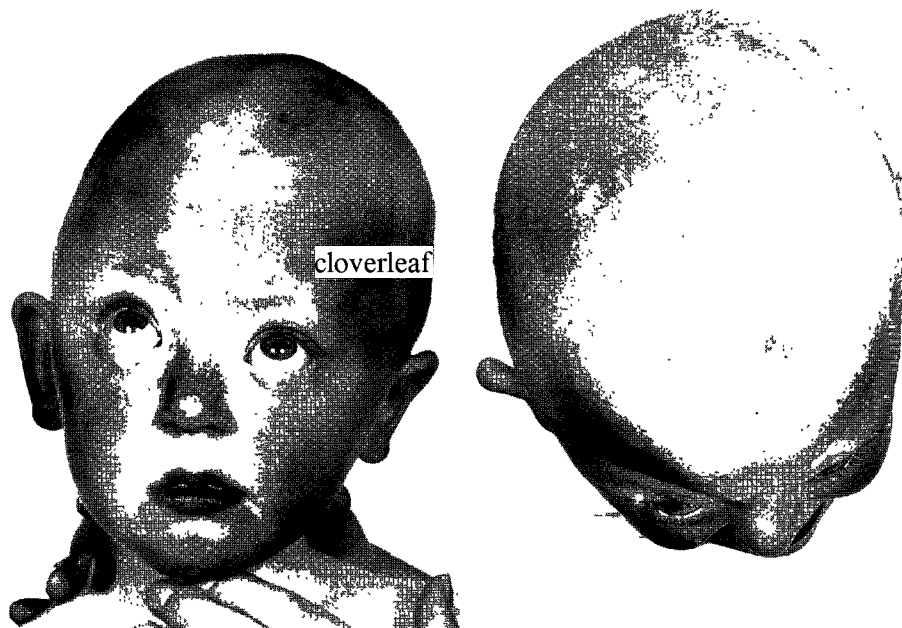


Fig. 8. Case 5 at the age of 2 years. Note the skull asymmetry and the absence of proptosis or maxillary hypoplasia.

the third immunoglobulin-like domain – where most of the Crouzon syndrome mutations have been concentrated. In the 3 cases detailed, the diagnosis of Crouzon syndrome had been considered but doubted in all instances on the basis of atypical features. In family 1, the mild degree of proptosis and the relative absence of mid-face hypoplasia in childhood caused an experienced clinician to question the Crouzon syndrome diagnosis. From a phenotypic perspective, the sequence of changes seen in figures 2 and 3 indicates the progressive nature of the phenotype. Similar families with ‘insidious onset’ of craniosynostosis have been reported in the literature [20]. Difficulties in precise clinical classification have been noted in these families also. It is worth noting that neurological complications beset all 3 cases in whom this mutation is described and neurosurgery was required in all 3. The causal relationship of the mutation and phenotype is established by its *de novo* appearance in case 2. Although this is the first published report of this particular change, mutation of glycine 338 to arginine, as opposed to glutamic acid, has been found in two instances of typical Crouzon syndrome [10 and our unpubl. obs.]. Case 2 is unusual in having no demonstrable craniosynostosis on X-ray; however, a mother and son with an unusual form of Crouzon syndrome but without craniosynostosis, associated with a tryptophan to glycine substitution at position 290 of FGFR2, have been previously reported [9].

Case 3 would probably be accepted as a case of Crouzon syndrome, but he has a novel mutation discussed below.

Case 4 represents an interesting phenotype. Whereas her facial features would be consistent with Crouzon syndrome, as, indeed, would the mutation observed (within the region of highest concentration of Crouzon-related mutations and the nature of the mutation, creating a cysteine, would also be typical of Crouzon syndrome), the cloverleaf skull and the bilateral humero-ulnar synostosis are atypical. Kreiborg [21], in a survey of 61 Crouzon syndrome cases, noted elbow stiffness in 16% and radial head subluxation in 2 of these cases, but did not record actual bony synostosis of the elbow as part of the Crouzon syndrome spectrum. Serine 351 to cysteine is a newly reported mutation, however, serine 347 and serine 354 to cysteine have both been repeatedly reported in classical Crouzon syndrome including cases from our own series. Comparable mutations have been found in FGFR3 in individuals with thanatophoric dysplasia type I. Both serine 371 and tyrosine 373, which lie in the juxtamembrane region, have been found mutated to cysteine [22].

Case 5 does not have features of Crouzon syndrome or any other syndromic form of craniosynostosis. The unique mutation in this case is discussed below.

Several variants of FGFR2 are described. One of the most common varies in the extracellular domain of the receptor, where the first immunoglobulin-like loop is fre-

quently absent and has been suggested to be unnecessary for ligand binding [5]. Mutation in the first immunoglobulin-like domain has not been reported previously in craniosynostosis. Case 3 of this report represents the first example of craniosynostosis-related mutation in this area of the FGFR2 gene. The identification of this mutation emphasises that immunoglobulin-like domain 1 must also be important and needs to be considered as a possible site of mutation in patients with craniosynostosis. Parental DNA was not available for analysis but the change was not observed (by SSCP) in another 150 chromosomes. Not only is a cysteine created, but the tyrosine which is removed two residues away from the conserved cysteine of the loop is very strongly conserved in immunoglobulin-like loops [23].

The mutation of a residue in the transmembrane domain of FGFR2 in case 5 has not been observed hitherto. Although craniosynostosis as a manifestation of mutation of the transmembranous region has been recorded in FGFR3, specifically with respect to Crouzon and acanthosis nigricans, case 5 of the current report confirms that mutation at this region of the FGFR2 gene may also be associated with skull malformation. This case was quite atypical and the clinical features could not be confused with classical Crouzon syndrome, nor indeed with any of

the known syndromes of craniosynostosis. Specifically, this woman did not show any evidence of acanthosis nigricans. A glycine 380 to arginine mutation of FGFR3 is found in the majority of cases of achondroplasia. The mutation, glycine 384 to arginine in our patient, is in a similar position within FGFR2 and involves the same amino acid substitution [24, 25]. However, as the glycine 384 to arginine mutation has only been described once, it is unlikely to share the same exceptionally high mutation rate as glycine 380 to arginine in FGFR3. This is presumably because it does not involve a CpG doublet, in contrast to the achondroplasia mutation. None of the mutations described here involve a CpG dinucleotide.

Our observation of such a wide range of phenotypes suggests that clinicians should consider the possibility of an FGFR2 mutation in patients with non-classical craniosynostosis. The importance of screening the whole molecule is underlined.

Acknowledgements

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Erratum

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 Due to a technical failure a portion of the text under 'Results' was omitted in the paper 'Tetrasomy 18p de novo: Parental Origin and Different Mechanisms of Formation' by Bugge et al., *Eur J Hum Genet* 1996;4:160-167. Printed below is the complete text for 'Results'. We apologize for any inconvenience this may have caused.

Tetrasomy 18p de novo: Parental Origin and Different Mechanisms of Formation

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Results

Parental Age Distribution

Range for mothers 24-39 years, mean maternal age 29 years; range for fathers 25-45 years, mean paternal age 32.1 years (table 1).

Chromosome analysis revealed 47 chromosomes in all 9 cases; the size and banding pattern of the extra small metacentric chromosome were compatible with an isochromosome of the short arm of chromosome 18. All cases were nonmosaic. All parental karyotypes were normal.

In situ hybridization with a chromosome 18 centromere-specific probe showed a monocentromeric signal on the marker chromosome in all cases. FISH using an 18p-

specific library showed labeling of the short arms of chromosomes 18 and the whole of i(18p). No labeling of the marker chromosome was observed after FISH with an 18q-specific library (fig. 1).

DNA analysis indicated that all nine i(18p) chromosomes were of maternal origin (table 2, fig. 2). A uniparental origin of the normal chromosomes 18 could be excluded. In 6 cases (cases 1-6), the 18p markers showed that the maternal heterozygous alleles were reduced to homozygosity in the proband in the pericentromeric region, while they remained heterozygous (nonreduced) in the telomeric region. In cases 7 and 8, all informative markers in the proband were nonreduced and in case 9, 6 informative markers showed reduction to homozygosity for maternal alleles (table 2).