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Mutation Screening in Patients With Syndromic Craniosynostoses Indicates That a Limited Number of Recurrent FGFR2 Mutations Accounts for Severe Forms of Pfeiffer Syndrome

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This study is dedicated to the memory of Elisabeth Lajeunie-Renier

Crouzon Syndrome (CS), Jackson-Weiss syndrome (JWS) and Pfeiffer syndrome (PS) are three craniosynostotic conditions caused by heterozygous mutations in Fibroblast Growth Factor Receptor (FGFR) genes. Screening a large cohort of 84 patients with CS, JWS or PS by direct sequencing of genomic DNA, enabled FGFR1, 2 or 3 mutation detection in 79 cases (94%). Mutations preferentially occurred in exons 8 and 10 of FGFR2 encoding the third Ig loop of the receptor. Among the 74 FGFR2 mutations that we identified, five were novel including four missense substitutions causing CS and a two bp deletion creating a premature stop codon and producing JWS. Five FGFR2 mutations were found in one of the two tyrosine kinase sub-domains and one in the Ig I loop. Interestingly, two FGFR2 mutations creating cysteine residues (W290C and Y340C) caused severe forms of PS while conversion of the same residues into another amino-acid resulted in Crouzon phenotype.

Our data provide conclusive evidence that the mutational spectrum of FGFR2 mutations in CS, JWS and PS is wider than originally thought. Genotype-phenotype analyses further indicate that FGFR2 mutations creating cysteine residues at positions 290 and 340 are associated with the most severe forms of PS with a poor prognosis.

KEY WORDS: FGFR1; FGFR2; FGFR3; Pfeiffer syndrome; Crouzon syndrome; Jackson-Weiss syndrome, mutations

INTRODUCTION

Crouzon (MIM# 123500), Jackson-Weiss (MIM# 123150) and Pfeiffer syndromes (MIM# 101600) are three clinically related but distinct craniosynostoses with common clinical features including craniosynostosis, ocular hypertelorism with proptosis and midface hypoplasia. They differ by the absence in CS and presence in PS and JWS of limb abnormalities. Clinical distinction between PS and JWS is based on the presence in JWS of broad great toes with medial deviation and tarsal-metatarsal coalescence in the absence of hand anomalies, but clinical overlap between the two entities does exist, making the diagnosis difficult [Cohen 2001]. In 1993, Pfeiffer syndrome has been subdivided into 3 types [Cohen 1993]. Type I, the most frequent form is usually associated with a benign course and satisfactory prognosis, whereas type II and III represent severe forms of the disease with a poor outcome and early demise in some cases. Subdivision was based on the presence (type II) or absence (type III) of cloverleaf skull.

In 1994, CS has been ascribed to *de novo* mutations in the *FGFR2* gene [Reardon et al., 1994]. *FGFR2* belongs to a family of four receptors comprising an extracellular ligand-binding domain, a transmembrane domain and an intracellular domain carrying the tyrosine kinase activity. Further studies have shown that *FGFR2* mutations also accounted for JWS and PS [Jabs et al., 1994; Lajeunie et al., 1995; Rutland et al., 1995], but genetic heterogeneity of this latter syndrome was demonstrated by the identification in several families of a recurrent *FGFR1* mutation causing mild forms of the disease [Muenke et al., 1994; Schell et al., 1995]. Sporadic cases of CS and PS have been associated with advanced paternal age and the origin of *FGFR2* mutations in these two conditions, like in Apert syndrome, was demonstrated to be exclusively paternal [Moloney et al., 1996, Glaser et al., 2000].

Surprisingly, the same *FGFR2* mutation can give rise to Crouzon, Jackson-Weiss or Pfeiffer syndrome [Rutland et al., 1995; Tartaglia et al., 1997]. Until 2002, *FGFR2*

mutations had been identified exclusively in the extracellular domain of the receptor mainly in exons 8 (IIIa) and 10 (IIIc) encoding the third immunoglobulin-like (Ig III) loop and appeared to account for only 50% of CS and PS cases [Passos-Bueno et al., 1999]. In 2002, novel mutations in other regions of the receptor including the IgII loop and the tyrosine kinase sub-domains TK1 and TK2 have been reported [Kan et al., 2002]. Likewise, this study demonstrated that in a clinically homogeneous group of CS and PS patients, FGFR2 mutations were detectable in more than 90% of all cases, thus making the existence of an additional locus [Passos-Bueno et al., 1999] very unlikely. A recurrent mutation, A391E, in a third gene, FGFR3, accounts for a peculiar form of CS associating craniosynostosis with acanthosis nigricans [Meyers et al., 95].

Molecular screening in a large cohort of CS, JWS and PS patients diagnosed in our hospital was performed. The diagnosis was based on the clinical presentation of the proband and family members. Mutations in one of the three FGFR genes (FGFR 1, 2 or 3) were found in 94% of our cases. Five novel FGFR2 mutations were identified. FGFR2 mutations, although clustered mainly in exons 8 and 10, were also present with a lower frequency in exons 3, 14 and 16. The recurrent FGFR3 mutation A391E in exon 10 caused CS with acanthosis nigricans whereas the P252R FGFR1 mutation produced PS with slight facial anomalies and absence of neurological defects. Altogether our results confirm that CS and JWS are genetically homogeneous at the FGFR2 locus whereas PS is heterogeneous, being caused by FGFR1 or FGFR2 mutations. Clinical and radiological examination of PS patients further revealed that FGFR2 mutations creating cysteine residues at positions 290 and 340 are associated with the most severe phenotypes.

PATIENTS AND METHODS

Patients and samples

During the past 10 years, clinical diagnosis and surgical correction of the skull

deformation in patients with syndromic craniosynostoses was achieved by the same team of physicians and surgeons at the Craniofacial Surgery Department of the Hôpital Necker-Enfants Malades. Each patient showed characteristic clinical features including synostosis of one or several cranial sutures, ocular proptosis, maxillary hypoplasia and midface retrusion. Crouzon patients were distinguishable from PS and JWS by the absence of hand and foot anomalies. Among 2594 children with proven craniosynostosis, 116 children from 100 families were diagnosed as Crouzon patients and 35 children from 33 families as Pfeiffer patients. Diagnosis of JWS was established in two patients. In our series, the birth prevalence of CS was 1/50,000 and this entity appeared to account for 4.5 % of all craniosynostoses. PS was less frequent with an estimated birth prevalence of 1/150,000 and accounted for 1.3 % of all craniosynostoses. DNA samples were obtained from eighty four patients with Crouzon, Jackson-Weiss or Pfeiffer syndromes. Informed consents for molecular studies and photographs were obtained from all patients or their parents.

Mutation analysis

Molecular analysis was performed on a cohort of patients comprising 62 unrelated CS (24 familial cases and 38 sporadic cases) including two patients with acanthosis nigricans (one sporadic and one familial case), two familial cases of JWS and 20 unrelated PS (5 familial cases and 15 sporadic cases).

PCR amplification of genomic DNA was performed using previously described primers and conditions [Lajeunie et al., 1995, Kan et al., 2002]. PCR fragments were directly sequenced on an ABI 3100 capillary sequencer (Applied Biosystems) with BigDye terminator mix. Since FGFR2 mutations preferentially occur in exons 8 and 10, these two exons were tested first.

Novel mutations were validated by sequencing exons 8 and 10 in 65 control genomic

DNAs. When no mutation was found, screening of other exons was undertaken. Exon numbering for FGFR2 was based on a recently proposed nomenclature [Ingersoll et al 2002]. GenBank accession numbers for FGFR1: BC015035; FGFR2: AF410480; FGFR3: AY768549

RT-PCR amplifications

For RT-PCR studies, total RNA was extracted from cultured skin fibroblasts using the RNeasy Mini Kit (Qiagen). Complementary DNA was synthesized by priming with either random hexamers or oligo-dT in the presence of MuLV reverse transcriptase using the manufacturer's protocol (GeneAmp RNA PCR Core Kit, Roche). Twenty five to 40 PCR cycles were then performed to amplify fragments specific for either the IIIb isoform (exon 9) or the IIIc isoform (exon 10) of FGFR2 by using the following primers: 5'-AAGCACTCGGGGATAAATAG-3' (F) and 5'-GTTTTGGCAGGACAGTGAGC-3' (R) for exon 9 ; 5'-CACAGTGGTCGGAGGAGA-3' (F) and 5'-AGTTACATTCCGAATATAGAG-3' (R) for exon 10. Sense and antisense primers used for GAPDH amplification were as follows: 5'-CATGTGGGCCATGAGGTCCACCAC-3' and 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3'. Samples were analysed on 1% agarose gels. Specificity of all RT-PCR products was tested by direct sequencing

RESULTS

Mutation screening was performed by direct sequencing of FGFR1, 2 and 3 genes. In FGFR1 and FGFR3 genes, sequence analyses were restricted to exons 7 and 10 respectively, whereas 16 exons of the FGFR2 gene were studied. FGFR mutations were detected in 79/84 (94%) unrelated patients (Table 1). The recurrent FGFR1 mutation P252R was recorded in 3 Pfeiffer cases (one familial and two sporadic). In two Crouzon patients with *acanthosis nigricans*, the recurrent A391E FGFR3 mutation was

identified. All other mutations causing CS, JWS and PS were found in the FGFR2 gene. As expected, mutations in exons 8 and 10 of the FGFR2 gene were largely predominant in our cohort, representing 92% (68/74) of the overall FGFR2 mutations. Among these 68 mutations, 36 (53%) either created or eliminated cysteine residues, thus generating unpaired cysteine able to induce disulfide bond formation between two mutant receptors. Mutations outside exons 8 and 10 (6/74) were located in either exon 3 encoding the first Ig-like loop (Y105C) or exons 14 and 16 encoding the tyrosine kinase sub-domains. Mutations N549H in the TK1 and K659N in the TK2 domains are homologous, respectively, to the N540K and K650N FGFR3 mutations causing hypochondroplasia. In 5 patients (4 CS and 1 PS) no FGFR2 mutation was found. Analysis of exon 7 of FGFR1, exons 7 and 10 of FGFR3 and exon 1 of TWIST also failed to reveal an abnormal sequence in those cases.

CROUZON PATIENTS

Missense substitutions were detected in 57/60 unrelated cases including 21 familial forms and 36 sporadic cases. In familial forms, presence of the single base change was confirmed by DNA sequencing of at least one additional affected member. The Y105C mutation in exon 3 was found in one familial case. Three other family members carried the same substitution. Sixteen missense mutations corresponding to 10 distinct heterozygous amino-acid changes were identified in exon 8. Two of these mutations (I288N and Y308C) are novel (Table 2). They were found neither in the DNA of unaffected parents, nor in 65 control DNA samples. Thirty three mutations corresponding to 12 distinct amino-acid changes occurred in exon 10. The most frequent mutation C342Y was detected in 12 unrelated cases (22%). The L357S mutation is reported for the first time.

Clinical re-examination of Crouzon patients carrying FGFR2 mutations showed that proptosis although variable was systematically present in all patients. Coronal

sutures were the most frequently affected as 86% of patients presented bicoronal fusions (brachycephaly), 18% exhibited both coronal and sagittal fusions (oxycephaly) and 5% showed pansynostosis (fusion of all sutures). In the remaining 9%, fusion was restricted to the sagittal suture (scaphocephaly) or the sagittal and lambdoids.

A close examination of patients carrying novel FGFR2 mutations revealed that the Y308C mutation occurred in a girl with a typical form of CS. The L357S mutation was detected in a familial case and segregated with the disease. The proband was a 12 year-old girl with pansynostosis and chronic tonsillar herniation. Her older brother who had sagittal and bicoronal synostosis had never required surgery because facial anomalies were mild.

JACKSON-WEISS PATIENTS

An heterozygous dinucleotide deletion (AC) at position 958-959 was detected in exon 10 of FGFR2 in a familial form of JWS with typical tarsal/metatarsal coalescence in the absence of hand anomalies (Fig. 1a-d). This frameshift mutation that occurred in a mother and her son, was predicted to induce translation of four illegitimate amino-acids, immediately followed by a premature termination codon (TGA) at position 324 of the receptor (Fig. 2b). RT-PCR analysis of mRNA extracted from fibroblasts of the affected mother, revealed an absence of the mutant transcripts, indicating that the 2bp deletion induced RNA instability of the IIIc transcripts (Fig. 2a). Interestingly, ectopic expression of the FGFR2 IIIb transcripts was observed in the mother's fibroblasts (Fig. 2c) suggesting that illegitimate expression of the IIIb isoform in cells of mesenchymal origin might account for the phenotype.

PFEIFFER PATIENTS

FGFR mutations were detected in 19/20 (95%) Pfeiffer samples. Three patients carried the recurrent P252R FGFR1 substitution that gave rise to a relatively mild

phenotype (Table 3). Sixteen patients including 4 familial forms and 12 sporadic cases harbored a FGFR2 mutation. Although variable, the phenotype was more severe (Fig. 1j) than in patients carrying FGFR1 mutations as attested by marked facial deformities, common hydrocephaly (in 10/16 patients), mental retardation and premature death (Table 3). In exon 8, the W290C FGFR2 mutation was identified in three cases, two of them died prematurely and the third was prenatally diagnosed by ultrasound examination at 29 weeks of gestation. Radiological and clinical examination after termination of pregnancy confirmed the diagnosis of Pfeiffer type II with very severe proptosis, cloverleaf skull and humero-radio-ulnar synostosis (Fig. 1k). In exon 10, we found 11 mutations of 6 different types including the common splice mutation (940-2A→G) that creates a cryptic donor site. Both patients harboring the Y340C mutation had a severe phenotype consistent with the diagnosis of Pfeiffer type II. One case died in the early childhood of respiratory distress while the second case was prenatally diagnosed and pregnancy was terminated at 25 weeks of gestation. Radiological examination showed cloverleaf skull, elbow ankylosis with bilateral humero-radio-ulnar synostosis, broad and deviated big toes and vertebral anomalies including sacrococcygeal eversion (Fig. 1e-g)

Mutations in exons 14 and 16 encoding the tyrosine kinase domains TK1 and TK2 were identified in two sporadic cases. Mutation in the TK2 domain was associated with a more severe phenotype (Fig. 1h, i) than in the TK1.

DISCUSSION

We have carried out a molecular study of three FGFR genes in a large series of 84 cases with CS, JWS and PS. Based on previous studies [Kan et al., 2002; Cornejo-Roldan et al., 1999] and our own experience, the mutation detection rate appears to depend largely on the accuracy of the original diagnosis and the sensitivity of the detection method. In our study, all patients were examined by the same physicians so

that the clinical criteria for phenotypic classification were highly homogeneous allowing recognition of the Crouzonoid facies.

Based on our clinical diagnosis and using direct sequencing, we identified FGFR2 mutations in 95% (57/60) of our Crouzon patients. This situation is similar to two previous studies [Kress et al., 2000; Kan et al., 2002] in which mutations were found in 25/28 and 18/20 Crouzon patients respectively. In PS, we found 95% of mutations in our cohort of 20 patients. A similar percentage was obtained in the Oxford series [Kan et al 2002]. In contrast, Cornejo-Roldan et al. [1999] studying a total of 78 unrelated Pfeiffer patients identified FGFR mutations in only 40 cases (51%). The difference between the three studies could rely on the stringency of clinical diagnosis. In the Cornejo-Roldan's group, the criteria for inclusion in the Pfeiffer group might be too broad. Hence, the presence of mild radiological findings like shortened middle phalanges could be insufficient to make a diagnosis of PS.

Although previous studies have failed to disclose genotype-phenotype relationships in craniosynostoses caused by FGFR2 mutations, analysis of amino-acid substitutions eliminating cysteine 342 in our cohort of patients suggest that the phenotype relies on the nature of the newly created residue. Tyrosine would preferentially cause CS (12/12) while serine, arginine or tryptophane would mainly account for PS (5/6). A similar situation exists in FGFR3-related skeletal dysplasias. Substitutions of lysine 650 by methionine or glutamic residues give rise to thanatophoric dysplasia or SADDAN whereas replacement of lysine by glutamine, asparagine or threonine generates hypochondroplasia, a much milder condition [Bellus et al., 1996; Bellus et al., 2000]. Differences in the receptor activation levels due to conformational changes of the receptor three-dimensional structure induced by amino-acids of variable charge and size could explain this result.

Mutations affecting tryptophan 290 were also of particular interest. Three different amino-acid substitutions have been recorded W290C, W290G and W290R. The four

patients carrying W290G and W290R substitutions had Crouzon syndrome. Similarly, four previously reported patients with the same mutations have been diagnosed as Crouzon [Passos-Bueno et al., 1999]. By contrast, the three patients carrying the W290C mutation in our series had a severe sub-lethal form of Pfeiffer syndrome. The same mutation has been described in at least seven additional patients. Six of them had a severe form of PS with cloverleaf skull and severe proptosis and two of them died prematurely [Tartaglia et al., 1997, Schaefer et al., 1998; Kan et al., 2002; Zackai et al., 2003; Nazarro et al., 2004]. The seventh patient presented a severe non classifiable craniosynostosis syndrome with limb and joint anomalies and mental retardation [Shotelersuk et al., 2002]. Taken together, these observations strongly suggest that conversion of tryptophane 290 into cysteine is likely to induce formation of disulfide bonded dimer receptors resulting in a severe phenotype, mostly Pfeiffer syndrome type II [Cohen, 1993], characterized by marked disabling and early death.

Of note was the relatively uncommon Y340C substitution. This mutation has been previously described in three patients with PS [Cornejo-Roldan et al., 1999; Kan et al., 2002, Blaumeiser et al., 2004] and in our series, it was identified in two PS cases. Both exhibited a very severe form of the disease with cloverleaf skull, severe ocular proptosis, hydrocephalus, abnormal great toes and thumbs consistent with the diagnosis of PS type II. Premature death occurred in one case. It is tempting to conclude that this mutation, like the W290C substitution, is preferentially associated with severe forms of PS with a poor prognosis regarding neurological functions and survival. Interestingly, another amino-acid substitution creating an unpaired cysteine residue, namely S351C, also results in severe forms of Pfeiffer syndrome (type III) in most reported cases [Gripp et al., 1998, Sweeney et al., 2002]. Although confusion might exist between PS type III and the so-called 'Antley-Bixler-like' phenotype, we consider that the heterozygous S351C FGFR2 mutation is a hallmark of severe forms of PS, as typical forms of Antley-Bixler syndrome are caused by recessive mutations in the P450

oxido-reductase (POR) gene [Fluck et al., 2004].

Unlike FGFR3, mutations in the tyrosine kinase subdomains of FGFR2 have been described only recently [Kan et al., 2002] and since, only one additional case has been reported [Zankl et al., 2004]. In our series, five additional cases were identified including three CS and two PS. In keeping with previous data, the K641R mutation caused PS. The N549H mutation caused both CS and a mild form of PS suggesting that mutations in the TK domains are phenotypically less deleterious than mutations creating cysteine residues in the extracellular domain.

Although no definitive genotype-phenotype relationships can be drawn from the literature on craniosynostoses and FGFR genes, our results suggest that some specific FGFR2 residues namely tryptophan 290 and tyrosine 340 account for the most severe forms of Pfeiffer syndrome when converted into cysteine residues.

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Legends

Figure 1. Phenotype of a mother and her son with Jackson-Weiss syndrome. Both patients have brachycephaly, midface hypoplasia, facial dysmorphism and proptosis (a, b). Note partial tarsal fusion and extra delta-shaped small bone between the first and the second phalanx of the left hallux in the boy (at 15 years) and complete tarsal fusion in the mother

(c,d).

Radiographs of a 25-week-old foetus with Pfeiffer syndrome carrying the Y340C FGFR2 mutation. Lateral and frontal views showing humero-radio-ulnar synostosis and sacral anomalies (e), cloverleaf skull (f) and abnormal shape of the first phalanxes of big toes (g).

Severe proptosis, marked brachycephaly with underossified cranial vault (h), mild syndactyly and flat thumbs (i) in a patient with Pfeiffer syndrome carrying a K641R FGFR2 mutation.

Facial and lateral radiographs of a Pfeiffer patient carrying a C342R FGFR2 mutation, showing multisynostoses of sagittal and coronal sutures responsible for severe scaphocephaly (j).

Radiographs of a 29-week-old foetus with Pfeiffer syndrome carrying the W290C FGFR2 mutation. Brachycephaly with cloverleaf skull, bilateral humero-radio-ulnar synostosis and large and deviated thumbs are visible (k).

Figure 2. Direct sequencing of genomic DNA from the JWS mother showing a heterozygous frameshift deletion (delAC) in exon 10 of FGFR2 (b). The normal sequence of the RT-PCR product derived from the patient fibroblasts indicates that the mutant allele is not detectable at the cDNA level (a). RT-PCR amplification of the IIIc isoform of FGFR2 encoded by exon 10 gives a signal both in the patient and control cells whereas amplification of the IIIb isoform encoded by exon 9 gives a signal in the patient's fibroblasts only (c). M : affected mother; C : control

TABLE 1. Patients analyzed for FGFR mutations

Clinical diagnosis	Number of patients	Patients with FGFR2 mutation	Patients with FGFR1 mutation	Patients with FGFR3 mutation	Patients with no FGFR mutation
Crouzon syndrome	60	57 (95%)	0	0	3 (5%)
Pfeiffer syndrome	20	16 (80%)	3 (15%)	0	1 (5%)
Crouzon syndrome with A.N.	2	0	0	2	0
Jackson-Weiss syndrome	2	1	0	0	1
Total	84	74 (88%)	3 (3.5%)	2 (2.5%)	5 (6%)

·AN = acanthosis nigricans

TABLE 2. FGFR mutations* identified in the study

Gene	Nucleotide change	Mutation	Protein domain	Familial/ Sporadic	Crouzon	Pfeiffer	Crouzon + A.N. ^a	Jackson-Weiss
FGFR2	314A>G	Y105C	Ig I ^b	F	1	-	-	-
FGFR2	799T>C	S267P	Ig IIIa	S	2	-	-	-
FGFR2	800C>T	S267F	Ig IIIa	F	1	-	-	-
FGFR2	826T>G	F276V	Ig IIIa	S	1	-	-	-
FGFR2	833G>T	C278F	Ig IIIa	S	5	-	-	-
FGFR2	833G>A	C278Y	Ig IIIa	S	1	-	-	-
FGFR2			Ig IIIa	S	1	-	-	-
	863T>A	I288N						
FGFR2	866A>C	Q289P	Ig IIIa	2F + 1S	3	-	-	-
FGFR2	868T>G	W290G	Ig IIIa	F	1	-	-	-
FGFR2	870G>T	W290C	Ig IIIa	S	-	3	-	-
FGFR2		Y308C	Ig IIIa	S	1	-	-	-
	923A>G							
FGFR2	940 -2	Splice	Ig IIIc	S	2	-	-	-
	A>G	acceptor						
FGFR2	958delA	Premature stop codon	Ig IIIc	F	-	-	-	1
FGFR2	962A>C	D321A	Ig IIIc	S	1	-	-	-
FGFR2	1009G>C	A337P	Ig IIIc	S	1	-	-	-
FGFR2	1012G>C	G338R	Ig IIIc	3S + 1F	4	-	-	-
FGFR2	1018T>C	Y340H	Ig IIIc	2S + 1F	3	-	-	-
FGFR2	1019A>C	Y340S	Ig IIIc	S	1	-	-	-
FGFR2	1019A>G	Y340C	Ig IIIc	S	-	2	-	-
FGFR2	1021A>C	T341P	Ig IIIc	S	1	-	-	-
FGFR2	1024T>C	C342R	Ig IIIc	S	-	1	-	-
FGFR2	1025G>A	C342Y	Ig IIIc	9S + 3F	12	-	-	-
FGFR2	1025G>C	C342S	Ig IIIc	4S + 1F	1(F)	4(S)	-	-
FGFR2	1026C>G	C342W	Ig IIIc	2F + 1S	2(F)	1(S)	-	-
FGFR2	1032G>A	A344A	Ig IIIc	2F + 2S	4	-	-	-
		Cryptic site						
FGFR2	1040C>G	S347C	Ig IIIc	S	2	-	-	-
FGFR2	1061C>T	S354F	Ig IIIc	S	1	-	-	-
FGFR2	1070T>C	L357S	Ig IIIc	F	1	-	-	-
FGFR2	1645A>C	N549H	TK1 ^c	S	2	1	-	-
FGFR2	1922A>G	K641R	TK2	S	-	1	-	-
FGFR2	1977G>T	K659N	TK2	S	1	-	-	-
FGFR1	755C>G	P252R	IgII-IgIII linker	2S + 1F	-	3	-	-
FGFR3	1172C>A	A391E	TM ^d	1S + 1F	-	-	2	-

*Nucleotide and amino acid numbers refer to the following GenBank accession numbers:

FGFR1: BC 015035; FGFR2: AF 410480; FGFR3: AY 768549. Novel mutations are in bold.

^aA.N. : acanthosis nigricans; ^bIg : immunoglobulin-like loop; ^cTK: tyrosine kinase;

^dTM : transmembrane

TABLE 3. Clinical features of Pfeiffer patients carrying FGFR2 or FGFR1 mutations

Patient	Mutation	Fascio stenosis	Proptosis	Suture fusions	Limb anomalies	Synd actyly	Arnold- Chiari	Elbow ankylosis	Hydro cephaly	Mental retardation
PS 1 (dead)	W290C (FGFR2)	severe	mild	Sagittal+ metopic	Thumbs + halluces	+	?	-	?	?
PS2 (dead)	W290C (FGFR2)	severe	moderate	Bicor. + sagittal	Thumbs + halluces	+	+	-	+	?
PS 3 (foetus)	W290C (FGFR2)	severe			Thumbs + halluces			+		?

PS 4	D321A (FGFR2)	moderate	moderate	Bicor.	Thumbs + halluces	+	-	-	-	+
PS 5 (foetus)	Y340C (FGFR2)	severe	Severe	Pansyn.	Thumbs + halluces	+	?	-	+	?
PS 6 (dead)	Y340C (FGFR2)	severe	Severe	Bicor.	Thumbs + halluces	+	+	-	+	?
PS 7	C342R (FGFR2)	severe	severe	Pansyn.	Thumbs + halluces	+	-	-	+	-
PS 8 (dead)	C342S (FGFR2)	severe	very severe	Pansyn.	Thumbs + halluces	+	?	-	+	?
PS 9	C342S (FGFR2)	severe	very severe	Bicor.	Thumbs + halluces	+	-	-	-	+
PS 10	C342S (FGFR2)	severe	very severe	Sagittal	Thumbs	-	-	-	-	-
PS 11	C342S (FGFR2)	severe	very severe	lambdoids	Thumbs + halluces	+	-	+	+	+
PS 12 (dead)	C342W (FGFR2)	severe	moderate	Bicor. + sagittal	Thumbs + halluces	+	+	-	+	?
PS 13	N549H (FGFR2)	mild	moderate	Bicor.	Thumbs + halluces	+	+	-	+	?
PS 14	K641R (FGFR2)	severe	moderate	Bicor. + sagittal	Thumbs + halluces	+	+	-	+	-
PS 15	Sp940-2 A>G(R2)	moderate	moderate	Bicor. + metopic	Thumbs + halluces	+	-	-	-	+
PS 16	Sp940-2 A>G(R2)	severe	moderate	Bicor. +	Thumbs + halluces	+	-	-	+	+
PS 17	P252R (FGFR1)	moderate	moderate	Bicor.	Thumbs + halluces	+	-	-	-	-
PS 18	P252R (FGFR1)	moderate	moderate	Unicor.	Thumbs + halluces	+	-	-	-	-
PS 19	P252R (FGFR1)	moderate	moderate	Bicor.	Thumbs + halluces	+	-	-	-	-

*Bicor. = bicoronal, fusion of two coronal sutures (brachycephaly); Unicor.= unicoronal, fusion of one coronal suture (plagiocephaly); Pansyn.= pansynostosis, fusion of all sutures.

[†]Premature death due to respiratory distress.

[‡]Pregnancy was interrupted at 25 weeks after ultrasound examination and detection of multiple malformations.

[§]Cloverleaf skull



