American Journal of Medical Genetics 78:237–241 (1998)

Description of a New Mutation and Characterization of *FGFR1*, *FGFR2*, and *FGFR3* Mutations Among Brazilian Patients With Syndromic Craniosynostoses

Maria Rita Passos-Bueno,[1]* Andréa L. Sertié,[1] Antonio Richieri-Costa,[2] Luís G. Alonso,[3] Mayana Zatz,[1] Nivaldo Alonso,[4] Décio Brunoni,[3] and Sandra F.M. Ribeiro[4]

[1]*Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil*
[2]*Hospital de Pesquisa e Reabilitação de Lesões Lábio Palatais, Bauru, São Paulo, Brazil*
[3]*Departamento de Morfologia, Universidade Federal de São Paulo, São Paulo, Brazil*
[4]*Departamento de Cirurgia Plástica, Universidade de São Paulo, São Paulo, Brazil*

Dominant mutations in three fibroblast growth factor receptor genes (*FGFRs1-3*) cause Crouzon, Jackson-Weiss, Pfeiffer, and Apert syndromes. In the present study, 50 Brazilian patients with these four syndromes (27 Apert, 17 Crouzon, 5 Pfeiffer, and 1 Jackson-Weiss patients) were screened for mutations in the *FGFR1-3* genes. Except for one, all the Apert patients had either S252W (n = 16) or P253R (n = 10) mutations. The remaining Apert case is atypical with a mutation altering the splice site of *FGFR2* exon IIIc. The Pfeiffer patients had mutations in one of the FGFR genes: three in *FGFR2,* one in *FGFR1,* and one in *FGFR3.* In contrast, only 8 of the 17 Crouzon patients studied had a mutation in either *FGFR2* (n = 7) or *FGFR3* locus (n = 1). Mutations in the *FGFR2* locus account for most (93%) of our syndromic craniosynostotic cases, whereas 5% had mutations in the *FGFR3* locus and only 2% had mutations in the *FGFR1* gene. Except for one, all the other mutations were reported previously in craniosynostotic patients from other populations. Interestingly, the mutation C278F, previously described in Crouzon and Pfeiffer cases, was here identified in a familial case with Jackson-Weiss. Also, unexpectedly, a common mutation altering the splice site of the *FGFR2* exon IIIc was found in one Apert and two Pfeiffer patients. In addition, we identified a new mutation (A337P) in the *FGFR2* exon IIIc associated with Crouzon phenotype. Am. J. Med. Genet. 78:237–241, 1998.

© 1998 Wiley-Liss, Inc.

KEY WORDS: **craniosynostoses; *FGFR1; FGFR2; FGFR3;* new mutation**

INTRODUCTION

The craniosynostoses , a heterogeneous group of disorders affecting ~1 in 2,000 children, are generally defined as premature closure of one or more of the cranial sutures [Cohen, 1986; Gorlin et al., 1990]. Four conditions are best known: Crouzon syndrome, the mildest one, has only craniofacial manifestations; Jackson-Weiss syndrome with foot anomalies and Pfeiffer syndrome associated with foot and hand anomalies are the intermediate forms; and Apert, with bone and soft syndactyly of upper and lower limbs, is the most severe type [Gorlin et al., 1990].

It has been demonstrated that mutations in three of the four known fibroblast growth factor receptor *(FGFRs)* genes are responsible for these four conditions. A specific mutation (P252R) in the *FGFR1* locus was only identified among Pfeiffer patients, whereas the change P250R in *FGFR3* was found in Pfeiffer and Crouzon cases [Muenke et al., 1994; Schell et al., 1995; Bellus et al., 1996; Muenke et al., 1997]. However, several mutations in exons IIIa and IIIc of the *FGFR2* locus were associated with Crouzon, Pfeiffer, and Jackson-Weiss [Reardon et al., 1994; Jabs et al., 1994; Lajeunie et al., 1995; Oldridge et al., 1995; Park et al., 1995a; Rutland et al., 1995; Schell et al., 1995; Meyers et al., 1996].

Contract grant sponsor: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP); Contract grant sponsor: Conselho Nacional de Desenvovimento Científico e Tecnológico (CNPq); Contract grant sponsor: PRONEX; Contract grant sponsor: Howard Hughes Medical Institute.

*Correspondence to: Maria Rita Passos-Bueno, Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, São Paulo, Brazil 05508-900. E-mail: passos@usp.br

Received 9 September 1997; Accepted 24 April 1998

© 1998 Wiley-Liss, Inc.

Therefore, the milder phenotypes can be caused by several mutations within and in different *FGFR* genes. In contrast, two canonical mutations in exon IIIa of the *FGFR2* gene (S252W: P253R) have been reported in most Apert patients [Wilkie et al., 1995; Park et al., 1995b; Oldridge et al., 1997]. Interestingly, common mutations may result in different phenotypes, although a plausible explanation for such findings has not been provided yet.

We are not aware of any comparative study of these four syndromic craniosynostoses within a single population and/or in Brazilian patients. Therefore, we performed a study of the *FGFR1-FGFR3* genes in 50 Brazilian patients with these four syndromes (27 with Apert, 17 with Crouzon, 5 with Pfeiffer, and 1 with Jackson-Weiss). The aims of this analysis were to verify (1) if these patients present a spectrum of mutations in the *FGFR1-3* genes similar to the one previously reported; (2) if the phenotype is the expected according to the type of the mutation identified; (3) if there is evidence for additional genetic heterogeneity of these phenotypes; and (4) the relative proportion of syndromic craniosynostotic patients caused by mutations in the *FGFR1, FGFR2,* or *FGFR3* genes.

PATIENTS

Fifty unrelated patients with Apert, Crouzon, Pfeiffer, or Jackson-Weiss syndrome were included. These patients were ascertained through three Brazilian craniofacial units (Hospital de Pesquisa e Reabilitação de Lesões Lábio Palatais and Hospital das Clínicas at the University of São Paulo and at the Federal University of São Paulo). All patients were examined by a clinical geneticist who documented the phenotype. Crouzon syndrome was distinguished from the other three conditions by the absence of hand and foot involvement. Patients with foot anomalies and the absence of broad thumbs were classified as Jackson-Weiss; whereas patients diagnosed with Pfeiffer syndrome had broad thumbs and great toes; Apert patients, besides a severe craniosysnotosis, had bone and cutaneous syndactyly of fingers and toes, which allowed the clinical distinction from Saethre-Chotzen syndrome.

METHODS

DNA was isolated from total blood according to standardized methods [Miller et al., 1988]. DNA analysis was performed in at least one affected individual per genealogy and whenever a new mutation was detected; 40 unrelated normal controls from the same ethnic background as the affected patients were screened for this mutation.

FGFR2 exons IIIa and IIIc were polymerase chain reaction (PCR) amplified using the condition reported previously [Oldridge et al., 1995; Slaney et al., 1996]. For the screening of Apert mutations S252W and P253R, the PCR products were digested with *Sfi*I and/or *Bst*UI, respectively [Slaney et al., 1996]. Patients without any of these two changes were screened for mutations in exons IIIa and IIIc through SSCP. Once one altered pattern was detected, then sequencing was performed. The primers used for sequencing were the same as those for PCR amplification. To confirm sequence changes, enzyme digestion was used whenever possible.

In the cases without a detected mutation in exons IIIa and IIIc of the *FGFR2* gene, the following mutations were analyzed through enzyme restriction digestion and/or SSCP analysis: P252R in the *FGFR1* [Muenke et al., 1994] and P250R in the *FGFR3* gene [Bellus et al., 1996]. In addition, the integrity of the Ser252 codon in *FGFR2* gene was tested by digestion of the amplified exon IIIa with the enzyme *Bsi*EI [Oldridge et al., 1997].

RESULTS

We first tested all of our patients for the S252W and P253R *FGFR2* mutations. Mutation P253R was found exclusively among Apert patients (n = 10 in 27). The S252W change was identified in 16 of the 27 Apert cases studied but also in one patient with Pfeiffer syndrome [Passos-Bueno et al., 1998].

One altered pattern in exon IIIa in two patients (one Crouzon and one Jackson-Weiss) and six distinct band shifts in exon IIIc in nine patients (six Crouzon, two Pfeiffer, and one Apert) were detected through SSCP analysis. Direct DNA sequencing of these PCR products showed that these abnormal bands were because of specific point mutations (Table I).

The two Pfeiffer cases in which we had not detected any mutation in the *FGFR2* exons were further tested for the P252R mutation within the *FGFR1* gene, and we found that one of them has this particular mutation (Table I).

No band shift was found in exon 7 of *FGFR1* and for exons IIIa and IIIc of *FGFR2* in the remaining 11 patients (10 Crouzon and 1 Pfeiffer). In addition, these patients have the ser252 codon in exon IIIa of *FGFR2* gene apparently intact because they have the expected restriction site for *Bsi*EI. These patients additionally were screened for the mutation P250R in *FGFR3* gene, and two of them (one Crouzon and one Pfeiffer) were found to have this specific change (Table I).

DISCUSSION

Wilkie et al. [1995] first demonstrated that Apert syndrome was caused by the mutations S252W or P253R within the exon IIIa of the *FGFR2* gene. In the present report we observed that except for one case, all the other Apert patients have either one of these mutations: 59% have the S252W and 37% have the P253R change, confirming that the range of mutations for this phenotype is very limited. Indeed, including the present patients, about 191 out of the reported 194 Apert patients have either one of these two mutations [Wilkie et al., 1995; Park et al., 1995b; Meyers et al., 1996; Moloney et al., 1996; Slaney et al., 1996; Oldridge et al., 1997; Passos-Bueno et al., 1997]. Except for one [Meyers et al., 1996], the other two exceptional Apert cases had their mutation identified. One of them had a phenylalanine instead of a serine at position 252, reinforc-

TABLE I. Mutations in *FGFR1, FGFR2,* and *FGFR3* Genes Among Patients With Syndromic Craniosynostosis

Locus	Exon	Location	Nucleotide change	Amino acid change	Type of mutation	Number of cases	Syndrome
2	IIIa	934	TGC–TGG	S252W	Missense	16	Apert
						1	Pfeiffer[b]
2	IIIa	937	CCT–CGT	P253R	Missense	10	Apert
2	IIIa	1012	TGC–TTC	C278F	Missense	1	Crouzon
						1	Jackson-Weiss
2	IIIc	1203	TGC–CGC	C342R	Missense	1	Crouzon
2	IIIc	1204	TGC–TTC	C324F	Missense	1	Crouzon
2	IIIc	1204	TGC–TAC	C342Y	Missense	2	Crouzon
2	IIIc	1188	GCT–CCT	A337P	Missense	1	Crouzon
2	IIIc	1191	GGG–CGG	G338R	Missense	1	Crouzon
2	IIIc	−2 acceptor site	A–G	Intronic	Splicing	2	Pfeiffer
						1	Apert[a]
1	5	755	CCT–CGT	P252R	Missense	1	Pfeiffer
3	7	749	CCG–CGG	P250R	Missense	1	Crouzon
						1	Pfeiffer

[a] This patient was previously reported [Passos-Bueno et al., 1997].

[b] Manuscript in press (Passos-Bueno et al., 1998).

ing that the specificity of this severe phenotype depends on the position of the mutations at the immunoglobulin-like (Ig)-II and Ig-III linker of the *FGFR2* gene [Oldridge et al., 1997]. However, the other exceptional case, which we previously reported, had an A to G transition at the 3′ acceptor splice site of the intron adjacent to exon IIIc [Passos-Bueno et al., 1997]. These results suggest that Apert-like patients with none of the canonical mutations should be investigated for other mutations within the *FGFR2* gene.

Among the 17 Crouzon patients studied, seven had causal mutation in the *FGFR2* gene. Six of these changes (C278F; G338R; C342Y; C342F; C342R) were reported previously among patients from other populations [Reardon et al., 1994; Oldridge et al., 1995; Meyers et al., 1996; Gorry et al., 1995; Steinberger et al., 1995]; but the mutation A337P is apparently being reported for the first time. This mutation creates a restriction site for Sau96I. The screening through enzyme restriction digestion of 40 control chromosomes showed that none of them have this change, and therefore, we considered it responsible for the phenotype in this patient. This mutation is just adjacent to the previously described mutation G338R. They both occur in very conserved amino acids, not only among *FGFR2* from different species but also within different *FGFRs*. The A337P is located five amino acids away from the cysteine at position 342, which forms the disulfide bond with the cysteine at position 278. These observations suggest that the A337P mutation possibly interferes in the formation of this bond.

The distribution of *FGFR2* mutations among Crouzon patients is clearly not random because 46% of the detected mutations were in the codons for the residues 278 and 342 [Reardon et al., 1994; Oldridge et al., 1995; Meyers et al., 1996; Gorry et al., 1995; Steinberger et al., 1995; and present study]. Because none of these mutations corresponds to a CpG to TpG change, additional studies will be necessary to understand why these sites are more prone to mutation.

The frequency of mutations among our Crouzon patients (47%) is comparable with that in previous studies, including those that have sequenced the exons IIIa and IIIc as a method for screening mutations in the *FGFR2* gene among patients with this phenotype [Meyers et al., 1996]. Our data thus reinforce that there may be locus or allelic heterogeneity for this phenotype.

The Jackson-Weiss propositus included in the present report inherited the gene from his mother who also has craniosynostosis and foot anomalies. A broad hallux was observed in the propositus and his mother; the only hand alteration was bilateral clinodactyly of the 5th finger in the propositus (Fig.1). These patients have the C278F change, which has been described in Crouzon and Pfeiffer patients [Oldridge et al., 1995; Meyers et al., 1996]. Therefore, the present findings increase the spectrum of clinical variability caused by this mutation and additionally reinforces the existence of other genes or environmental factors playing an important role in the expression of the mutated allele. It is possible that polymorphic changes in other genes, which can also cause craniosynostosis, such as MSX2 for the Boston form [Jabs et al., 1993] and TWIST for Saethre-Choetzen [Howard et al., 1997; El Ghouzzi et al., 1997], might interfere in the expression of the *FGFR2* mutated alleles.

It is interesting to note that our five Pfeiffer cases had their mutation identified: three in *FGFR2* (60%), one in *FGFR1* (20%), and one in *FGFR3* (20%). Two (one isolated and one familial) had the A to G transition at the 3′ acceptor splice site of the intron adjacent to exon IIIc. This mutation was reported previously in two Pfeiffer cases from other populations [Schell et al., 1995; Lajeunie et al., 1995] and in one Brazilian Apert patient [Passos-Bueno et al., 1997]. The fact that we observed that 40% of our Pfeiffer cases (2 in 5) or 6% of our total cases (3 in 50) have this A to G transition and that this change was also detected in patients from other populations, additionally suggest that mutations along the *FGFR2* gene might not be at random.

The clinical distinction of these four craniosynostoses and the association with their respective mutations is undoubtedly important for a better understanding of the function of *FGFR2* gene. However, if we consider these four clinical syndromes as a unique entity but

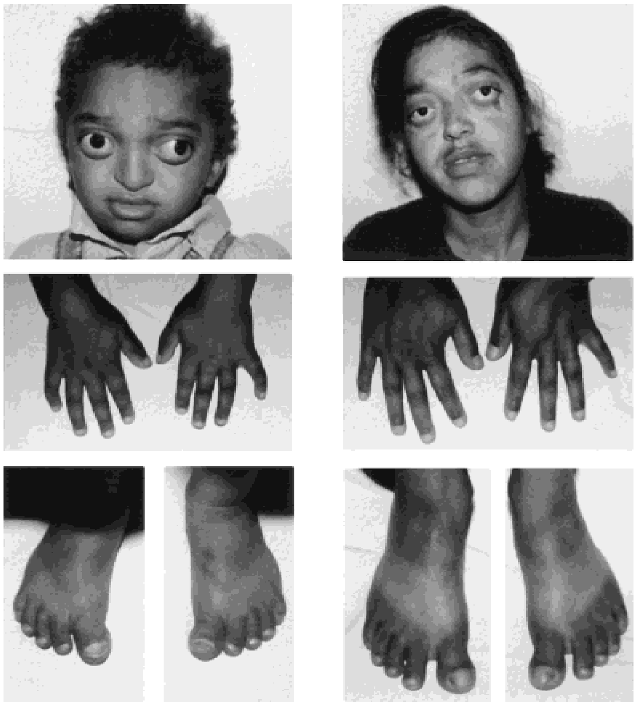

Fig. 1. Photos of the propositus **(left)** with Jackson-Weiss syndrome and his affected mother **(right),** both heterozygous for the C278F mutation. Enlargement of toes was observed in both cases; clinodactyly of the 5th finger was observed only in the propositus.

with different phenotypic effects of the mutated alleles, we could suggest that most cases are caused by mutations in the *FGFR2* gene (93%), followed by cases caused by mutations in the *FGFR3* gene (5%) while mutations in *FGFR1* (2%) accounts for four cases. This proportion might be biased because Apert patients are more likely to be ascertained because of their more severe phenotype. If we exclude these patients, we still observe a higher proportion of cases caused by mutations in the *FGFR2* gene (79% in *FGFR2,* 11 in 14; 14% in *FGFR3,* 2 in 14; and 7% in *FGFR1,* 1 in 14). These findings are in accordance to the recent review of Cohen [1997].

ACKNOWLEDGMENTS

We thank Constância Urbani, Antonia M.P. Cerqueira, Marta Canovas, Eloísa S. Moreira, Dr. Mariz Vainzof, Prof. M. Ferreira , Dr. F. Kok, and Dr. C.A.E. Kim for referring patients; Dr. Andrew Wilkie for valuable suggestions; and Alexander Kneppers and Dr. E. Bakker for helping in sequencing. This work was supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP); Conselho Nacional de Desenvovimento Científico e Tecnológico (CNPq); PRONEX. In addition, the research of M.R.P.B. was supported in part by an International Research Scholars grant from the Howard Hughes Medical Institute.

REFERENCES

Bellus GA, Gaudenz K, Zackai EH, Clarke LA, Szabo J, Francomano CA, Muenke M (1996): Identical mutations in three different fibroblast growth factor receptor gene in autosomal dominant craniosynostosis syndromes. Nature Genet 14:174–176.

Cohen MM Jr (1986): "Craniosynostosis: Diagnosis, Evaluation and Management." New York: Raven Press.

Cohen MM (1997): Immunolocalization of transforming growth factor B1, B2 and B3 and insulin-like growth factor I in premature cranial suture fusion. Plast Reconstr Surg 99(Suppl 2):310–316.

El Ghouzzi V, Le Merrer M, Perrin-Schimitt F, Lajeunie E, Benit P, Renier D, Bourgeois P, Bolcato-Bellemin A, Munnich A, Bonaventure J (1997): Mutations of the TWIST gene in the Saethre-Chotzen syndrome. Nature Genet 15:42–46.

Gorlin RJ, Cohen MM, Levin LS (1990): "Syndrome of the Head and Neck." 3rd ed. Oxford University Press, pp 519–539.

Gorry MC, Preston RA, White GJ, Zhang Y, Singhal V, Losken HW, Parker MG, Nwokoro NA, Post JC, Erlich GD (1995): Crouzon syndrome: Mutations in two spliceoforms of *FGFR2* and a common point mutation shared with Jackson-Weiss syndrome. Hum Mol Genet 4:1387–1390.

Howard TD, Paznekas W, Green ED, Chiang LC, Ma N, Ortiz de Luna RI, Delgado CG, Gonzalez-Ramos M, Kline AD, Jabs EW (1997): Mutations in TWIST, a basic helix-loop-helix trasncription factor, in Saethre-Chotzen syndrome. Nature Genet 15:36–41.

Jabs EW, Li X, Scott AF, Meyers G, Chen W, Eccles M, Mao J-I, Charnas LR, Jackson CE, Jaye M (1994): Jackson-Weiss and Crouzon syndromes are allelic with mutations in fibroblast growth factor receptor 2. Nature Genet 8:175–179.

Jabs EW, Müller U, Li X, Ma L, Luo W, Haworth IS, Klisak I, Sparkes R, Warman ML, Mulliken JB, Snead ML, Maxson R (1993): A mutation in the homeodomain of the human MSX2 gene in a family affected with autosomal dominant cranioysnostosis. Cell 75:443–450.

Lajeunie E, Ma HW, Bonaventure J, Munnich A, Merrer ML (1995): *FGFR2* mutations in Pfeiffer syndrome. Nature Genet 9:108.

Meyers GA, Day D, Goldberg R, Daentl DL, Przylepa KA, Abrams LJ, Graham JM Jr, Feingold M, Moeschler JB, Rawnsley E, Scott AF, Jabs EW (1996): *FGFR2* exon IIIa and IIIc mutations in Crouzon, Jackson-Weiss and Pfeiffer syndromes: Evidence for missense changes, insertions and a deletion due to alternative RNA splicing. Am J Hum Genet 58:491–498.

Miller S, Dykes DD, Plesky HF (1988): A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215.

Moloney DM, Slaney SF, Oldridge M, Wall AS, Sahlin P, Stenman G, Wilkie AOM (1996): Exclusive paternal origin of new mutations in Apert syndrome. Nature Genet 13:48–53.

Muenke M, Gripp KW, McDonald-McGinn M, Gaudenz K, Whitaker LA, Bartlett SP, Markowitz RI, Robin NH, Nwokoro N, Mulvihill JJ, Losken HW, Mulliken JB, Guttmacher AE, Wilroy RS, Clarke LA, Hollway G, Adés LC, Hann EA, Mulley JC, Cohen MM Jr, Bellus GA, Francomano CA, Moloney DM, Wall AS, Wilkie AOM, Zackai EH (1997): A unique point mutation in the fibroblast growth factor receptor 3 gene (*FGFR3*) defines a new craniosynostosis syndrome. Am J Hum Genet 60:555–564.

Muenke M, Schell U, Hehr A, Robin NH, Losken HW, Schinzel A, Pulleyn LJ, Rutland P, Reardon W, Malcolm S, Winter RM (1994): A common mutation in the fibroblast growth factor receptor 1 gene in Pfeiffer syndrome. Nature Genet 8:269–274.

Oldridge M, Lunt PW, Zackai EH, McDonald-McGinn DM, Muenke M, Moloney DM, Twigg SRF, Heath JK, Howard TD, Hoganson G, Gagnon DM, Jabs EW, Willkie AOM (1997): Genotype-phenotype correlation for nucleotide substitutions in the IgII-IgIII linked of *FGFR2.* Hum Mol Genet 6:137–143.

Oldridge M, Wilkie AOM, Slaney SF, Poole MD, Pulleyn LJ, Rutland P, Hockley AD, Wake MJC, Goldin JH, Winter RM, Reardon W, Malcolm S (1995): Mutations in the third immunoglobulin domain of the fibroblast growth factor receptor-2 gene in Crouzon syndrome. Hum Mol Genet 4:1077–1082.

Park W-J, Meyers GA, Li X, Theda C, Day D, Orlow SJ, Jones MC, Jabs EW (1995a): Novel *FGFR2* mutations in Crouzon and Jackson-Weiss syndromes show allelic heterogeneity and phenotypic variability. Hum Mol Genet 4:1229–1233.

Park W-J, Theda C, Maestri NE, Meyers GA, Fryburg JS, Dufresne C, Cohen MM Jr, Jabs EW (1995b): Analysis of phenotypic features and *FGFR2* mutations in Apert syndrome. Am J Hum Genet 57:321–328.

Passos-Bueno MR, Richieri-Costa A, Sertié AL (1998): The canonical Apert mutation (S252W) in a Pfeiffer patient. J Med Genet (in press).

Passos-Bueno MR, Sertié AL, Zatz M, Richieri-Costa A (1997): A Pfeiffer mutation in an Apert patient: How wide is the spectrum of variability due to mutations in the *FGFR2* gene? Am J Med Genet 71:243–245.

Reardon W, Winter RM, Rutland P, Pulleyn LJ, Jones BM, Malcolm S (1994): Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. Nature Genet 8:98–103.

Rutland P, Pulleyen LJ, Reardon W, Baraitser M, Hayward R, Jones B, Malcolm S, Winter RM, Oldridge M, Slaney SF, Poole MD, Wilkie AOM (1995): Identical mutations in the *FGFR2* gene cause both Pfeiffer and Crouzon syndrome phenotypes. Nature Genet 9:173–176.

Schell U, Heher A, Feldman GJ, Robin NH, Zackai EH, Die-Smulders C, Viskochil DH, Stewart JM, Wolff G, Ohashi H, Price RA, Cohen MM Jr, Muenke M (1995): Mutations in *FGFR1* and *FGFR2* cause familial and sporadic Pfeiffer syndrome. Hum Mol Genet 4:323–328.

Slaney SF, Oldridge M, Hurst JA, Morriss-Kay GM, Hall CM, Poole MD, Wilkie AOM (1996): Differential effects of *FGFR2* mutations on syndactyly and cleft palate in Apert syndrome. Am J Hum Genet 58:923–932.

Steinberger D, Mulliken JB, Müller U (1995): Predisposition for cysteine substitutions in the immunoglobulin-like chain of *FGFR2* in Crouzon syndrome. Hum Genet 96:113–115.

Wilkie AOM, Slaney SF, Oldridge M, Poole MD, Ashworth GJ, Hockley AD, Hayward RD, David DJ, Pulleyn LJ, Rutland P, Malcolm S, Winter RM, Reardon W (1995): Apert syndrome results from localized mutations of *FGFR2* and is allelic with Crouzon syndrome. Nature Genet 9:165–172.