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Original Article

Clinicogenetic Study of Turkish Patients With Syndromic Craniosynostosis and Literature Review

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ABSTRACT

BACKGROUND: Fibroblast growth factor receptor 2 mutations have been associated with the craniosynostotic conditions of Apert, Crouzon, Pfeiffer, Saethre-Chotzen, Jackson-Weiss, Beare-Stevenson cutis gyrata, and Antley-Bixler syndromes in various ethnic groups. **METHODS:** Thirty-three unrelated Turkish patients (12 with Apert syndrome, 14 with Crouzon syndrome, six with Pfeiffer syndrome, and one with Saethre-Chotzen syndrome) and 67 nonsyndromic craniosynostosis patients were screened for mutations in exons IIIa and IIIc of the *FGFR2* gene by denaturing high-performance liquid chromatography and confirmed by direct sequencing. **RESULTS:** We detected several pathogenic mutations in 11/33 (33%) patients with Apert syndrome (four with p.Pro253Arg; seven with p.Ser252Trp) and 8/33 (24%) patients with Crouzon syndrome (three with p.Trp290Arg, one with p.Cys342Tyr, p.Cys278Phe, p.Gln289Pro, and a novel p.Tyr340Asn mutation) and five (15%) with Pfeiffer syndrome (p.Cys342Arg, p.Pro253Arg, p.Trp290Arg, and p.Ser351Cys). No *FGFR2* gene mutation was detected in any of the patients with Saethre-Chotzen syndrome and nonsyndromic craniosynostosis. **CONCLUSIONS:** Our results indicate that the majority of Turkish patients with syndromic craniosynostosis have detectable genetic changes with an overall frequency of 72.7%. Because this is the first molecular genetic report from a Turkish cohort, the identified spectrum profile of *FGFR2* mutations of the syndromic craniosynostotic patients would be very helpful for understanding the genotype–phenotype relationship and has a great value for diagnosis, prognosis, and genetic counseling.

Keywords: Apert syndrome, Crouzon syndrome, Pfeiffer syndrome, craniosynostosis, DHPLC

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Introduction

Craniosynostosis is the pathologic condition that results from premature fusion of one or more cranial sutures. These syndromes feature distinctive facial features and variable hand and foot anomalies. Involvement ranges from severe with neonatal death to mild with no clinical manifestations, with a prevalence of 1 in 2100-3000.[1,2] The classical craniosynostosis syndromes are autosomal dominant disorders that include Apert (MIM 101200), Crouzon (MIM 123500), Pfeiffer (MIM 101600), Saethre-Chotzen (MIM 101400), Jackson-Weiss (MIM 123150), Beare-Stevenson cutis gyrata (MIM 123790), and Antley-Bixler syndrome (MIM 207410).[1-5]

It is well known that craniosynostosis is genetically heterogeneous, and several mutations in fibroblast growth factor receptors (*FGFR*) 1, 2, 3, and *TWIST* genes have been identified. Upon ligand binding of fibroblast growth factors (FGFs) to the extracellular domain, the receptors dimerize and, by the activation of the intracellular tyrosine kinase domain, initiates a cascade of signals that regulate multiple cellular activities, cell growth, differentiation, and embryonic development.[6-10]

B.G.N. and S.P. contributed equally to the work described in this paper.

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The molecular defects most frequently occur in *FGFR2* (NM_001144916.1), a tyrosine kinase receptor gene, which has been mapped to 10q25.3-26. Mutation analysis of *FGFR2* gene detected >98% for Apert syndrome, around 50% for Crouzon syndrome, and 67% for Pfeiffer syndrome.[11]

From a clinical view, two mutations, p.Ser252Trp and p.Pro253Arg, in the immunoglobulin-like extracellular subdomain exon IIIa of the *FGFR2* gene account for 98% of Apert syndrome patients, making diagnosis easy and allowing genetic counseling in difficult cases. However, a broad range of mutations throughout the extracellular domain of *FGFR2* causes the overlapping of cranial phenotypes of Crouzon and Pfeiffer syndromes and related craniofacial dysostoses.[12,13]

Mutations in the *FGFR2* gene have been identified in both Apert and Crouzon syndromes in various ethnic groups, including Japanese, Chinese, Taiwanese, and Southeast Asian patients.[6-10] In the current study, we performed denaturing high-performance liquid chromatography (DHPLC) analysis, followed by sequencing analysis in 100 Anatolian-origin Caucasian Turkish patients with craniosynostosis. We demonstrated the mutations associated with syndromic craniosynostosis patients and discussed the genotype–phenotype correlations of such mutations in comparison with the literature.

Patients and Methods

Patients

One hundred Turkish patients (43 girls and 57 boys) with craniosynostosis were ascertained through the School of Medicine, Department of Clinical Genetics at Akdeniz University, Antalya, and Department of Medical Genetics in Istanbul University, Istanbul, Turkey. Clinical and genetic studies were performed in concordance with the institutional review board protocols approved by the ethics committees of the University of Akdeniz and the University of Istanbul and the most recent version of the Helsinki Declaration. Signed informed consent forms were obtained from all the participating individuals and parental guardians.

Clinical studies

Thirty-three unrelated Turkish patients with a clinical diagnosis of syndromic craniosynostosis (Apert syndrome [n = 12], Crouzon syndrome [n = 14], Pfeiffer syndrome [n = 6], Saethre-Chotzen syndrome [n = 1]), and nonsyndromic craniosynostosis patients (n = 67) were screened for *FGFR2* gene mutations. The mean age of the syndromic patients was 5.8 years (1 month-33 years) and 2.8 years (1-12 years) in nonsyndromic patients. Maternal and paternal mean ages were 29.2 and 35.8 years, respectively. All patients were examined by clinical geneticists from both the university hospitals and were diagnosed as being affected by one of the common craniosynostotic syndromes or nonsyndromic craniosynostosis. All patients had skull radiography and some received a three-dimensional computed tomography scan review to verify diagnosis. None of the patients had chromosomal abnormalities.

DHPLC and sequence analysis

Genomic DNA of each patient was extracted from whole blood by a nonenzymatic DNA isolation protocol.[14] Amplification of genomic DNA was performed using previously described primers and conditions in a standard polymerase chain reaction.[15] The amplicons were heated to 95°C for 5 minutes, and then allowed to cool slowly to 25°C to form heteroduplexes. We established the optimal DHPLC parameters of each of the hot-spot exons (IIIa, IIIc) for the *FGFR2* gene using WAVE Maker Software version 1.6.2 (Transgenomic™ Limited, Glasgow, UK). The optimum temperature was determined by running a melt profile between 3°C and 5°C, based on the software predicted temperature, as 58-61°C for *FGFR2* exon IIIa, and 57-60°C for exon IIIc. Each anomalous elution peak was then subjected to direct sequencing in both directions using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analyzed on an ABI Prism 377 capillary Sequencer system (Applied Biosystems). The sequencing data were compared with GenBank (NM_022976) sequences by using MacVector™ (version 7.0). Mutation nomenclature was done based on Human Genome Variations Guidelines.

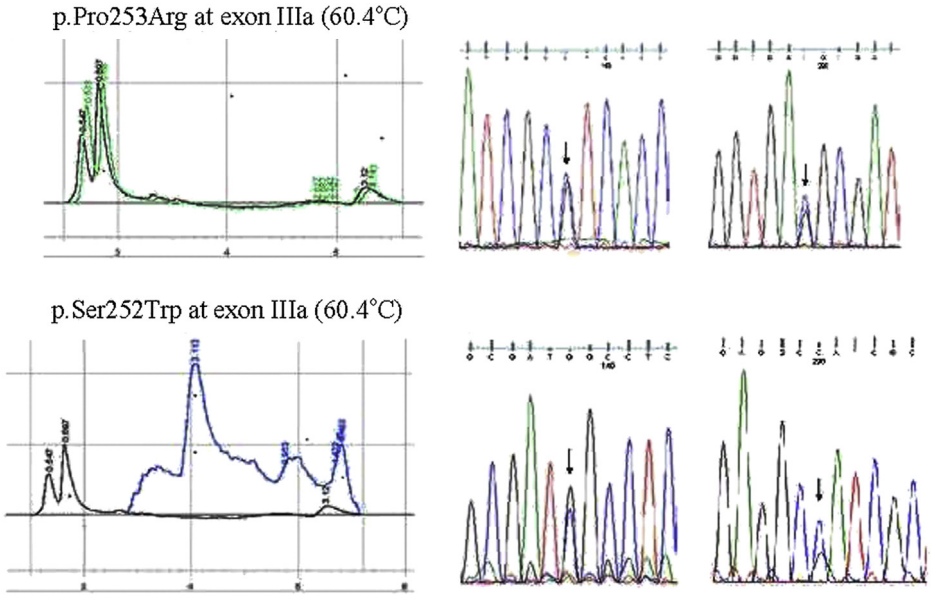

FIGURE 1.
Denaturing high-performance liquid chromatography (DHPLC) elution chromatograms and sequencing analysis of Apert syndrome patients.

TABLE 1.
Clinical features of Turkish syndromic craniosynostosis patients

Patient	Sex	Age mo/yr	Maternal/Paternal Age (year)	Clinical Diagnosis	*FGFR-2* Mutation	Nucleotide Change
1	M	1 mo	22/26	AS	p.Pro253Arg	c.758c > G
2	F	6 mo	30/30	AS	p.Pro253Arg	c.758c > G
3	F	12 yr	26/27	AS	p.Pro253Arg	c.758c > G
4	F	3 yr	34/38	AS	p.Pro253Arg	c.758c > G
5	F	6 mo	36/46	AS	p.Ser252Trp	c.755 C > G
6	M	3 mo	29/38	AS	p.Ser252Trp	c.755 C > G
7	F	5 mo	28/36	AS	p.Ser252Trp	c.755 C > G
8	M	3 mo	24/26	AS	p.Ser252Trp	c.755 C > G
9	F	12 yr	23/28	AS	p.Ser252Trp	c.755 C > G
10	F	1 mo	29/38	AS	p.Ser252Trp	c.755 C > G
11	M	12 yr	30/33	AS	p.Ser252Trp	c.755 C > G
12	M	2 yr	24/24	AS	-	
13	M	2 yr	18/40	CS	p.Trp290Arg	c.868 G > C
14	F	10 yr	33/30	CS	p.Trp290Arg	c.868 G > C
15	F	33 yr	22/28	CS	p.Trp290Arg	c.868 G > C
16	M	4.5 mo	25/26	CS	p.Cys342Tyr	c.1025 G > A
17	F	12 yr	40/41	CS	p.Cys278Phe	c.833 G > T
18	M	8 yr	39/48	CS	p.Gln289Pro	c.866 A > C
19	M	2 yr	39/32	CS	p.Tyr340Asn*	c.1018 T > A
20	F	48 yr	24/28	CS	p.Tyr340Asn*	c.1018 T > A
21	M	4 yr	32/35	CS	-	
22	M	7 yr	40/43	CS	-	
23	M	16 yr	42/47	CS	-	
24	F	7 yr	33/35	CS	-	
25	M	14 yr	25/35	CS	-	
26	M	10 yr	27/30	CS	-	
27	F	1 mo	35/40	PS	p.Cys342Arg	c.1024 T > C
28	F	1 mo	20/64	PS	p.Pro253Arg	c.758 C > G
29	F	12 yr	24/28	PS	p.Trp290Cys	c.870 G > C
30	M	4 yr	28/32	PS	p.Ser351Cys	c.1052 C > G
31	M	3 mo	26/30	PS	p.Ser351Cys	c.1052 C > G
32	F	6 yr	30/35	PS	-	

Abbreviations:
(−) = Presence
(+) = Absence
* = Novel mutation
AS = Apert syndrome
CH = Cerebellar herniation
CNSSA = Central nervous system structural anomaly
COS = Choanal stenosis
CS = Crouzon syndrome
CTO = Cryptorchidism
E = Epilepsia
F = Female
FL = Familial
GH = Gingival hypertrophy
HC = Hydrocephaly
HD = Heart defect
HN = Hydronephrosis
Ig = Immunoglobulin-like loop
IH = Inguinal hernia
M = Male
N = Nephrolithiasis
PS = Pfeiffer syndrome
S = Sporadic
SA = Sacrococcygeal appendage

Results

Spectrum of FGFR2 mutations

In this study, all Turkish Anatolian craniosynostosis patients (n = 100) were sporadic, except in two patients with Crouzon syndrome in which both the mother and child were affected. Sequence analysis identified heterozygous mutations in all 11 Apert syndrome patients, with either the p.Pro253Arg (c.758 C > G) or p.Ser252Trp (c.755 C > G) mutation (Fig 1, Table 1). Among the patients with Crouzon syndrome, mutations were identified in eight; three with

TABLE 1.
Clinical features of Turkish syndromic craniosynostosis patients *(Continued)*

Protein Domain	Familial/Sporadic	Typical Craniofacial Features	Palatal Anomaly	Extremity Anomaly/ Radioulnar Synostosis	Other	Early Death
IgII-III	S	+		+		
IgII-III	S	+	+	+/+	CNSSA, HD	
IgII-III	S	+	+	+		
IgII-III	S	+	+	+		
IgII-III	S	+		+		+
IgII-III	S	+	+		HD	
IgII-III	S	+	+	+		
IgII-III	S	+		+	CNSSA	
IgII-III	S	+		+		
IgII-III	S	+	+	+	HD	
IgII-III	S	+	+	+		
	S	+	+	+		
IgIIIa	S	+	+			
IgIIIa	FL	+	+		E	
IgIIIa	FL	+	+		E	
IgIIIc	S	+	+	+	IH	
IgIIIa	S	+	+		HD	
IgIIIa	S	+	+	+	CNNSA, IH	
IgIIIc	FL	+	+			
IgIIIc	FL	+	+			
	S	+	+		CNNSA, IH	
	S	+	+		CNNSA	
	S	+	+			
	S	+	+			
	S	+	+		CH, HC	
	S	+				
IgIIIc	S	+		+/+	HD	
IgII-III	S	+	+	+/+	CNSSA, HC, HD, IH	+
IgIIIa	S	+		+	HN, SA	+
IgIIIa	S	+	+	+/−	HC, HD, N, GH	+
IgIIIa	S	+	+	+/−	COS, CTO, HC,	
	S	+	+	+/−		

heterozygous p.Trp290Arg (c.868 G > C) mutation; one each with heterozygous p.Cys342Tyr (c.1025 G > A), p.Cys278Phe (c.833 G > T), p.Gln289Pro (c.866 A > C); and a novel mutation p.Tyr340Asn (c.1018 T > A) (Fig 2).

The p.Trp290Arg mutation was detected in one familial case of Crouzon syndrome in which the parents were first-degree cousins (patients 14 and 15). The other sporadic patients with p.Trp290Arg, p.Cys278Phe, and p.Gln289Pro mutations had a father of advanced age (>40 years old).

In addition to reported mutations, a novel T to A transversion at nucleotide 1018 of the *FGFR2* gene was detected in exon IIIc. This novel missense mutation leads to transversion of tyrosine to asparagine at amino acid 340 of the gene. To clarify whether it is a mutation or polymorphism, we analyzed 50 chromosomes of healthy subjects by sequencing (unpublished observation). The p.Tyr340Asn mutation was not found in any of the subjects. Regarding Pfeiffer syndrome patients, we identified four different

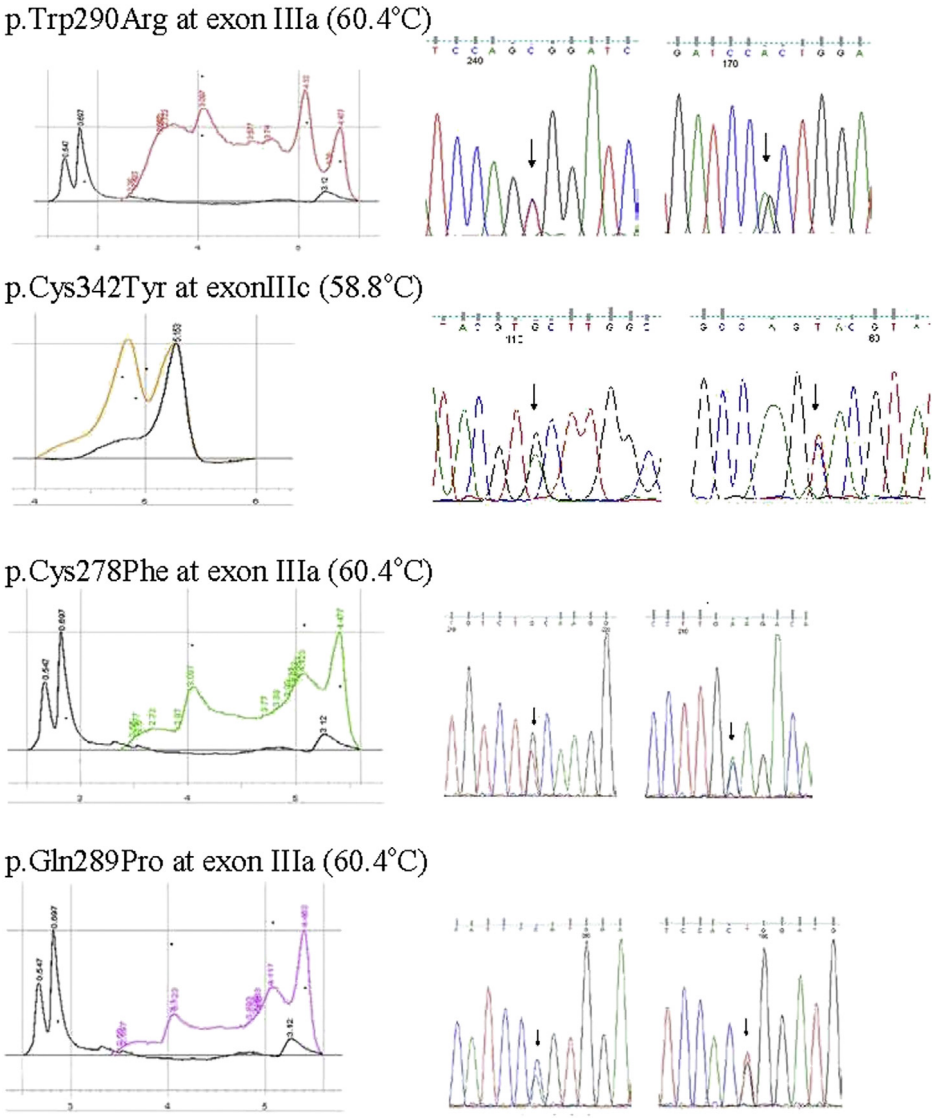

FIGURE 2.
Denaturing high-performance liquid chromatography (DHPLC) elution chromatograms and sequencing analysis of Crouzon syndrome patients.

mutations—p.Cys342Arg (c.1024 T > C), p.Pro253Arg (c.758 C > G), p.Trp290Cys (c.870 G > C), and p.Ser351Cys (c.1052 C > G)—in five patients (Fig 3).

No *FGFR2* gene mutation was detected in any of the patients with Saethre-Chotzen syndrome or with non-syndromic craniosynostosis. The clinical characteristics and *FGFR2* mutations of syndromic craniosynostosis patients are presented in Table 1.

Discussion

Identical mutations in the *FGFR2* gene have been reported to cause different phenotypes of craniosynostosis, and even different syndromes, whereas a wide range of mutations in the *FGFR2* gene produce a variety of overlapping phenotypes that are usually difficult to classify.[16] Until now, 101 *FGFR2* gene mutations have been reported to be related to craniosynostosis phenotypes in the Human Gene Mutation Database. The mutations in patients from various ethnic backgrounds demonstrated similar spectrum, with the majority (>90%) of patients with Apert syndrome heterozygous for either of the two mutations (p.Ser252Trp and p.Pro253Arg) and a broader spectrum for patients with Crouzon, Pfeiffer, and Saethre-Chotzen syndromes (Table 2). In this study, we found the mutation detection rate to be 73% of the patients. A recent study of *FGFRs 1, 2, 3* and *TWIST* genes in 99 Canadian patients with craniosynostosis revealed a mutation detection rate of 51%.[17] Analysis of exons IIIa and IIIc only of the *FGFR2* gene revealed that the mutation detection rate in patients with Apert and Crouzon syndromes from different populations varied from 31% to 100%, as shown in Table 2. We detected mutations in 11 of 12 patients with Apert syndrome (91.6%), eight of 14 patients with Crouzon syndrome (57%), and five of six patients with Pfeiffer syndrome (83%), with an overall detection rate of 24/33 (72.7%) in the Turkish syndromic craniosynostosis population. Additionally, we did not detect any mutation in patients with Saethre-Chotzen syndrome, which is consistent with the reports that the

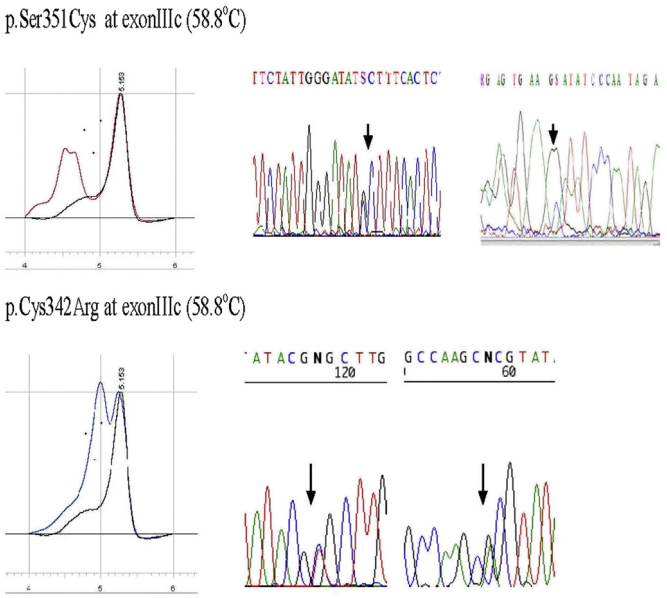

FIGURE 3.
Denaturing high-performance liquid chromatography (DHPLC) elution chromatograms and sequencing analysis of Pfeiffer syndrome patients.

majority of the patients have mutations in the *FGFR3* or *TWIST* genes, which are not analyzed in this study.[2,18] In addition, no *FGFR2* gene mutation was detected in Turkish patients with nonsyndromic craniosynostosis. A different subset of mutations seems to be associated with craniosynostosis, without facial features and extremity abnormalities.

Apert syndrome has been found to account for about 4.5% of all cases of craniosynostosis, which results almost exclusively from one of two point mutations (p.Ser252Trp or p.Pro253Arg) in *FGFR2*. The majority of cases are sporadic, resulting from new mutations.[19] The frequency of the p.Ser252Trp mutation has been noted to be much higher than that of the p.Pro253Arg mutation in French,[20] Canadian,[17] Taiwanese,[10] and Brazilian patients.[21] Consistent with the literature, we found more p.Ser252Trp (n = 7) than the p.Pro253Arg (n = 4) mutations. Crouzon syndrome is the most common form of craniosynostosis syndrome, for which more than 50 different mutations have been described. We identified five *FGFR2* mutations, including p.Trp290Arg, p.Cys342Tyr, p.Cys278Phe, p.Gln289Pro, and a novel p.Tyr340Asn in 8/14 (57%) of the Crouzon patients. This proportion is much higher than that reported in two previous studies in which *FGFR2* mutations were detected in 7/15 and 6/28 Crouzon patients, respectively.[15,22] Pfeiffer syndrome is another severe form of the disease, and approximately 95% of these cases have been attributed to *FGFR2* mutations.[23] We identified mutations in 4/5 (80%) of the Pfeiffer patients. This proportion is higher than that reported in two previous studies in which *FGFR2* mutations were detected in 9/17 and 3/8 Pfeiffer patients, respectively.[15] The higher percentage of our results can be attributed to the stringency of the clinical diagnosis.

Clinical findings in patients with FGFR2 *mutations*

Apert syndrome

From the clinical perspective, further analysis indicated that patients with a p.Ser252Trp mutation more commonly presented with a cleft palate, marker of more severe craniofacial abnormalities, syndactyly, and radiohumeral synostosis.[6,24] In the current study, all patients had syndactyly, except one with p.Ser252Trp mutation (patient 6). Five of the patients with the p.Ser252Trp mutation had high palate, three of the patients had cleft palate, one patient had renal anomaly (patient 7), and one had an early death (patient 5). Although congenital heart defects are noted in about 10% of the patients with Apert syndrome, we detected them in three patients—one with p.Pro253Arg and the other two with p.Ser252Trp. Central nervous system structure anomalies were detected in two patients—one with p.Pro253Arg and one with p.Ser252Trp. Based on these findings, severity of craniofacial malformations, congenital heart defects, and central nervous system structure anomalies seen in Apert syndrome are not distinguishable between these two mutations; however, early death was noted in one patient with p.Ser252Trp (patient 5).

Crouzon syndrome

FGFR mutations have not been found to be associated with genital anomalies, but recent studies have shown that rare mutations in the *FGFR2* gene may influence the risk of hypospadias.[25,26]

In the present study, hypospadias was detected only in the patient with a p.Cys342Tyr mutation (patient 16). Mutations of p.Trp290Arg and p.Gln289Pro are known to produce similar phenotypic features; in addition, these mutations also result in high palate, pear-shaped nose, and midfacial hypoplasia, which are the characteristics of Crouzon syndrome.[27] It is known that seizures occur in 12% of Crouzon symptom patients, and we detected epilepsia only in familial cases with p.Trp290Arg (patients 14 and 15).[28] The p.Cys278Phe mutation is also known to be associated with various clinical syndromes, including Crouzon, Pfeiffer, and Jackson-Weiss syndromes[8]; in the current study, a congenital heart defect was noted only in the patient with this mutation (patient 17). Although inguinal hernia is not a classic finding of Crouzon syndrome, this condition was detected in three patients, one with p.Trp290Arg and the other one with p.Gln289Pro. Furthermore, we found a novel p.Tyr340Asn mutation in one of our familial Crouzon patients (patients 19 and 20). Both of the patients exhibited typical craniofacial features; however, other findings, including mental retardation, hydrocephaly, central nervous system structure anomaly, seizures, and hearing defects were not detected. This clinical profile suggests that mutation may be associated with mild clinical form of Crouzon syndrome (unpublished observation).

Pfeiffer syndrome

The p.Pro253Arg mutation has been described very rarely in patients and is observed in both Apert and Pfeiffer syndromes.[29] Our patient with a p.Pro253Arg mutation exhibited all craniofacial features, central nervous system anomaly and hydrocephaly, multiple joint contractures, radiohumeral synostosis, inguinal hernia,

TABLE 2.
FGFR2 gene mutations in Caucasian and Asian populations

Origin	Diagnosis	Number of Patients Studied	Mutation	% Detection Rate	Number of Patients With Mutations
Brazil (Passos-Bueno et al., 1998[21])	Apert syndrome	27	p.Ser252Trp	59.25	16
			p.Pro253Arg	37	10
			Splice site	3.70	1
	Crouzon syndrome	17	p.Ala337Pro	5.88	1
			p.Cys278Arg	5.88	1
			p.Cys342Arg	5.88	1
			p.Cys324Phe	5.88	1
			p.Cys342Tyr	5.88	1
			p.Gly338Arg	5.88	1
			p.Pro250Arg	5.88	1
	Pfeiffer syndrome	5	p.Pro252Arg	20	1
			p.Pro250Arg	20	1
			p.Ser252Trp	20	1
			Splice site	40	2
Canada (Chun et al., 2003[17])	Apert syndrome	17	p.Ser252Trp	76	13
			p.Pro253Arg	18	3
	Crouzon syndrome	15	p.Cys342Tyr	13	2
			p.Cys342Ser	7	1
			p.Gln289Pro	7	1
			p.His254Tyr	7	1
			p.Ser354Phe	7	1
			p.Trp290Arg	7	1
	Pfeiffer syndrome	17	p.Cys342Arg	12	2
			p.Ser347Cys	12	2
			p.Cys278Phe	6	1
			p.Cys342Ser	6	1
			p.Cys342Trp	6	1
			c.940-1G > A	6	1
			c.940-3_946del10insACC	6	1
	Saethre-Chotzen syndrome	11	p.Pro250Arg	37	4
Germany (Kress et al., 2000[22])	Crouzon syndrome	28	p.Cys278Phe	10.71	3
			p.Cys278Thr	3.50	1
			p.Pro263Leu	3.50	1
			p.Phe276Val	3.50	1
	Pfeiffer syndrome	8	p.Gly289Pro	25	2
			p.Ile288Ser	13	1
France (Lajeunie et al., 1999[20])	Apert syndrome	36	p.Pro253Arg	97	35
			p.Ser252Phe	3	1
Japan (Matsumoto et al., 1998[6])	Apert syndrome	3	p.Cys934Gly	100	3
Japan (Nagase et al., 1998[7])	Apert syndrome	1	p.Ser242Trp	100	1
	Crouzon syndrome	1	p.Cys342Trp	100	1
	Pfeiffer syndrome	3	p.Asp321Ala	33	1
			p.Cys342Ser	33	1
			p.Thr341Pro	33	1
Japan (Sakai et al., 2001[8])	Apert syndrome	6	p.Ser252Trp	83	5
			p.Pro253Arg	16.66	1
	Crouzon syndrome	9	pCys342Ser	22	2
			p.Ser354Cys	22	2
			p.Cys342Arg	11	1
			p.Cys342Thr	11	1
			p.Ser252Leu	11	1
			p.Trp290Gly	11	1
Taiwan (Tsai et al., 1999[10])	Apert syndrome	15	p.Ser252Trp	87	13
			p.Pro253Arg	13.33	2
Taiwan (Tsai et al., 2001[31])	Crouzon syndrome	9	p.Trp281Cys	67	6

Table 2 (*continued*)

Origin	Diagnosis	Number of Patients Studied	Mutation	% Detection Rate	Number of Patients With Mutations
Thailand (Shotelersuk et al., 2003[9])	Apert syndrome	7	p.Ser252Trp	57	4
			p.Pro253Arg	42.85	3
	Crouzon syndrome	4	p.Ser351Cys	50	2
			p.Cys278Phe	25	1
			p.Ser347Cys	25	1
United (States Mulliken et al., 1999[32])	Apert syndrome	13	p.Ser252Trp p.Pro253Arg	100	
	Crouzon syndrome	5	p.Pro250Arg	40	2
	Pfeiffer syndrome	3		100	3
Turkey (this study)	Apert syndrome	12	p.Pro253Arg	33	4
			p.Ser252Trp	58	7
	Crouzon syndrome	14	p.Cys278Phe	7.14	1
			p.Gln289Pro	7.14	1
			p.Trp290Arg	21	3 (one familial)
			p.Tyr340Asn*	14	2 (familial)
			p.Cys342Tyr	7.14	1
	Pfeiffer syndrome	6	p.Pro253Arg	17	1
			p.Trp290Cys	17	1
			p.Cys342Arg	17	1
			p.Ser351Cys	33	2
	Saethre-Chotzen syndrome	1	Negative		Negative
	Unclassified craniosynostosis	67	Negative		Negative

* Novel mutation.

and congenital heart defect, which is a rare condition in Pfeiffer syndrome (patient 28). Furthermore, another patient with heterozygous p.Trp290Arg mutation had infrequent findings of syndromes, such as hydronephrosis and sacrococcygeal appendage, and also died early. Interestingly, until now, only a few patients with syndromic craniosynostosis have been known to show sacrococcygeal defects. Angeline et al. presented a case of Pfeiffer syndrome with sacrococcygeal defect and detected the p.Trp290Arg mutation in *FGFR2*.[30] As stated previously, p.Trp290Arg, p.Tyr340Cys, p.Cys342Arg, and p.Ser351Cys mutations are associated with severe forms of Pfeiffer syndrome characterized by multiple malformations, including defective neurological functions and early death.[12] Severe Pfeiffer phenotypes and early death have been observed in our patients with p.Pro253Arg, p.Trp290Cys, and p.Ser351Cys mutations. Patient 31, with the p.Ser351Cys mutation, might have had a poor prognosis with multiple joint contractures and severe hydrocephaly that was operated on at 1 month of age. In our series, Pfeiffer patients detected with p.Cys342Arg mutation had a better prognosis (patient 27).

In summary, craniosynostosis is a heterogeneous disorder. Our data indicate that all the nucleotide substitutions found in these patients have led to severe phenotypes. The spectrum and frequency of *FGFR2* mutations in Turkish patients with syndromic craniosynostosis are still largely unknown. These patients underscore the importance of comprehensive mutational analysis of Turkish-Caucasian patients. Because we found a novel mutation in Pfeiffer cases, it is clear that the elucidation of the *FGFR2* mutations in patients with clinical features of syndromic craniosynostosis offers a significant benefit to those families in terms of prognosis, genetic counseling, and prenatal diagnosis.

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