FGFR1 and *FGFR2* Mutations in Pfeiffer Syndrome

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Abstract: Pfeiffer syndrome (PS) (MIM 101600) is one of the most common syndromic forms of craniosynostosis. It is characterized by craniosysnostosis, midface hypoplasia, broad and medially deviated thumbs, and great toes with partial syndactyly of the digits. Here, we described clinical and genetic features of 12 unrelated Thai individuals with PS. All 12 patients were sporadic, and advanced paternal age was found in 50% of the cases. Polymerase chain reaction sequencing of *FGFR1* exon 5 and *FGFR2* exons 8, 10, 15, 16, and 17 was performed in all PS patients and revealed 9 recurrent mutations in all patients. Most of the mutations clustered in exons 8 and 10 (9/12) accounting for 75% of PS cases. The most frequently detected mutation, p.S351C, was associated with the severe form of PS in the Thai population. Less frequent mutations in exons 16 (p.K641R) and 17 (p.G663E) were also identified. In addition, the p.P252R mutation in *FGFR1* was detected in 1 PS patient with unilateral coronal craniosynostosis expanding the phenotypic spectrum of PS with this particular mutation. Knowing the mutation spectrum of the responsible genes could lead to the most effective strategy in identifying mutations causing Pfeiffer syndrome in the Thai population.

Key Words: Pfeiffer syndrome, *FGFR1*, *FGFR2*, mutations

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Pfeiffer syndrome (PS) (MIM 101600), one of the most common syndromic forms of craniosynostosis, is characterized by craniosysnostosis, midface hypoplasia, broad and medially deviated thumbs, and great toes with partial syndactyly of the digits. It is inherited in an autosomal dominant manner. Clinical severity ranges from mild craniofacial abnormalities with normal intellectual development to a cloverleaf skull with severe neurological involvement leading to early death in some cases.[1]

Gain of function mutations in the fibroblast growth factor receptor (*FGFR*) 1 or 2 genes have been found to be responsible for Pfeiffer syndrome.[2,3] At least 1 mutation in the *FGFR1* and 35 mutations in the *FGFR2* have been reported in Pfeiffer syndrome.[4–8] Mutations in the *FGFR1* are located in the linker region between the second and third immunoglobulin-like (Ig) domains 5, whereas mutations in the *FGFR2* are distributed in Ig III and both tyrosine kinase (TK) domains.[6,7]

Here, we reported clinical and molecular characterization of 12 Thai patients with Pfeiffer syndrome. Molecular analysis of the *FGFR1* and *FGFR2* genes by polymerase chain reaction (PCR) sequencing identified recurrent mutations in all cases with the majority in exons 8 and 10.

MATERIALS AND METHODS

All patients were recruited through Division of Medical Genetics and Metabolism, Department of Pediatrics and Craniofacial Center, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Each patient had characteristic features including craniosynostosis, ocular proptosis, midface hypoplasia, broad and medially deviated thumbs, and great toes with variable degree of cutaneous syndactyly. After informed consent was obtained, genomic DNA was extracted from peripheral leukocytes using Qiagen DNA extraction kits according to manufacturer's instructions (Qiagen, Valencia, CA). Polymerase chain reaction amplification of *FGFR2* exons 8 and 10 was performed as previously described.[9] The newly designed primers (*FGFR1* exon 5 and *FGFR2* exons 15, 16 and 17) and the PCR conditions were shown in Table 1. In brief, we used 100 ng of genomic DNA, 1XPCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of each primer, and 0.5 U Taq DNA polymerase (Fermentas) in a volume of 20 μl. The PCR products were then treated with ExoSAP-IT (USP Corporation, Cleveland, OH) according to the manufacturer's recommendations and sent for direct sequencing using forward and reverse primers at the Macrogen Inc., Seoul, Korea. The sequence was analyzed using Sequencher (version 4.2; Gene Codes Corporation, Ann Arbor, MI).

RESULTS

Twelve patients with clinical features consistent with Pfeiffer syndrome were included. There were 7 male and 5 female subjects. The clinical findings, which include clinical types, are summarized in Table 2. All 3 clinical types were seen in this study (Fig. 1). All

TABLE 1. Primers Used in PCR and Sequencing in *FGFR1* and *FGFR2*

Gene	Exon	Primer Sequence for PCR 5'-3'	Annealing Temperature (°C)	Product Size (bp)
FGFR1	5	CCCCAGCTCTGTTTTGAGAGGC	58	404
		CCCGAGACAGTGGTCTCCTTCC		
FGFR2	15	GGAGACCCTGGATTCTCTCTTTA	60	358
		AGGTTGTACAAGACATGCGAGG		
FGFR2	16	GTAGCTGGGACTACAGGTGCACAC	60	680
		ATCCTAGCGGTTGCTGATTATTC		
FGFR2	17	GGTCCTCATTGGGACTGATTTCTGC	58	432
		CCACCTTCTGTGCTCTTTGAAGCC		

cases were sporadic. Maternal and paternal age at birth of the proband ranged from 22 to 41 years (median = 29) and from 25 to 40 years (median = 35), respectively. Six of the patients' fathers were more than 35 years old when the children were born (Table 2).

PCR-sequencing revealed mutations in the *FGFR1* or *FGFR2* in all patients. Of these 12, there were nine different recurrent mutations (Table 2). Most of the mutations clustered in exons 8 and 10 (9/12) accounting for 75% of PS cases. Less frequent mutations in exons 16 (p.K641R) and 17 (p.G663E) were also detected.

DISCUSSION

We previously reported *FGFR* mutations in Thai patients with syndromic craniosynostosis and skeletal dysplasia.[10–12] This study is the first to describe the clinical manifestation of PS and evaluate the mutation spectrum of the responsible genes in the Thai population. Polymerase chain reaction sequencing of *FGFR1* exon 5 and *FGFR2* exons 8, 10, 15, 16, and 17 successfully identified disease-causing mutations in all 12 patients with clinical features consistent with PS. None of the parents were affected. Mutation analysis revealed recurrent *de novo* mutations in every case. Advanced paternal age was found in 50% of the patients. Of 12 unrelated PS patients, 9 had mutations occurring in *FGFR2* exons 8 and 10, accounting for 75%. Less frequent mutations in the TK2 domain were also detected in the 2 PS patients.

Mutations in both *FGFR1* and *FGFR2* result in PS. The c.755C > G (p.P252R) is the only mutation identified in *FGFR1*, which causes PS with mild phenotypic abnormalities.[2,5] Our study identified the p.P252R in 1 patient with right coronal craniosynostosis and broad and medially deviated thumbs and great toes (Fig. 2). Pfeiffer syndrome patients with the p.P252R mutation in *FGFR1* has been reported to have mild craniofacial involvement.[2] To our knowledge, this is the first PS patient with this particular mutation

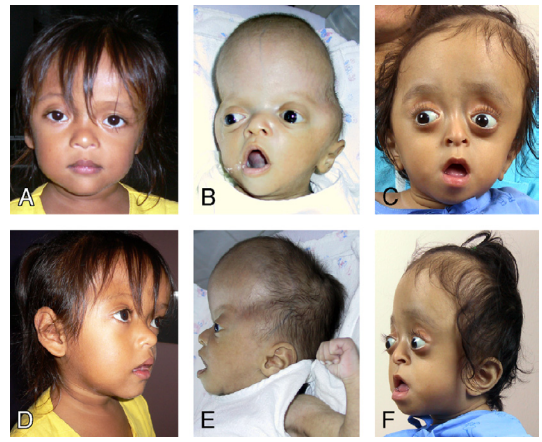

FIGURE 1. Craniofacial features of PS patients with different severity. (A, D) Patient 1 with PS type 1. (B, E) Patient 10 with PS type 2 and (C, F) Patient 7 with PS type 3. (A, B, C) showing anterior view. (D, E, F) showing lateral view.

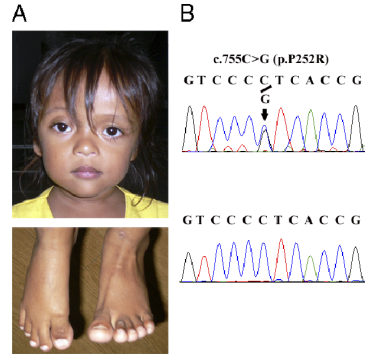

FIGURE 2. p.P252R in *FGFR1* in a PS patient with unilateral coronal craniosynostosis. (A) Unilateral coronal craniosynostosis (upper) and broad medially deviated great toes (lower). (B) Chromatograms showing a heterozygous c.755C > G (p.P252R) mutation (an arrow) and normal sequences.

TABLE 2. Clinical and Molecular Characteristics of Thai Patients with Pfeiffer Syndrome

Patient ID	Sex	Age*	Paternal/Maternal Age†	Type, Cranial and Brain Abnormality	Gene	Mutation
1	F	30 mo	31/29	Type 1, right coronal synostosis	*FGFR1*	p.P252R (c.755C>G)
2	M	3 mo	25/22	Type 3, aqueductal stenosis, hydrocephalus, multiple synostosis	*FGFR2*	p.S267P (c.799C > T)
3	M	8 yr	25/23	Type 3, IQ = 64 (WISC –III) at 8 years old	*FGFR2*	p.C278F (c.833G > T)
4	M	7 mo	39/34	Type 1, multiple synostosis	*FGFR2*	p.C278F (c.833G > T)
5	F	3 yr 7 mo	28/28	Type 1, multiple synostosis	*FGFR2*	p.T341P (c.1021A > C)
6‡	M	6 mo	40/41	Type 1, bilateral coronal synostosis	*FGFR2*	p.A344P (c.1030G > C)
7	F	7 mo	39/34	Type 3, multiple synostosis	*FGFR2*	p.S347C (c.1040C > G)
8	F	1 mo	NA/NA	Type 2, cloverleaf skull (dead)	*FGFR2*	p.S351C (c.1052C > G)
9	F	1 mo	31/29	Type 2, cloverleaf skull, hydrocephalus	*FGFR2*	p.S351C (c.1052C > G)
10	M	1 mo	35/36	Type 2, cloverleaf skull, hydrocephalus (dead)	*FGFR2*	p.S351C (c.1052C > G)
11	M	4 mo	39/22	Type 1, bilateral coronal synostosis	*FGFR2*	p.K641R (c.1922A > G)
12	M	5 mo	38/28	Type 1, hydrocephalus	*FGFR2*	p.G663E (c.1988G > A)

*Age at first visit.
†Parental age when the child was born.
‡Previously reported in Shotelersuk et al., 2001b.
NA, nonavailable.

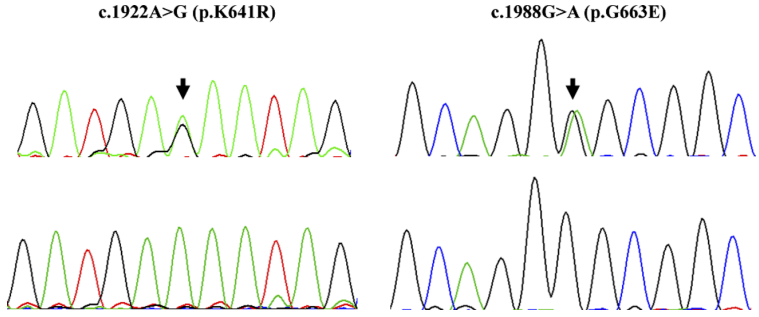

FIGURE 3. Mutations in the tyrosine kinase domain of FGFR2. Chromatograms show (left upper panel) heterozygous c.1922A > G (p.K641R) and (right upper panel) c.1988G > A (p.G663E) mutations in PS patients. The normal sequences are shown in the lower panel.

having unilateral coronal craniosynostosis. Interestingly, the unilateral coronal craniosynostosis can also be found in Muenke et al[13] and other FGFR-related craniosynostosis syndromes. Definite diagnosis and appropriate genetic counseling could therefore be facilitated by molecular genetic testing of the responsible genes. The p.P252R in *FGFR1* was also identified in patients with characteristic abnormalities of the digits but mild or absent skull involvement.[14] It has been suggested that a search for the p.P252R in *FGFR1* should be performed in individuals with characteristic appearance of the digits even in the absence of craniosynostosis.[14] Our findings in the PS case with unilateral craniosynostosis support this recommendation.

Eight different recurrent mutations with three involving the cysteine residue were identified in *FGFR2*. The c.799C > T (p.S267C), c.833G > T (p.C278F), and c.1040C > G (p.S347C) mutations identified in our patients have been previously reported in patients with either Pfeiffer or Crouzon syndrome. It has been demonstrated that similar mutations can lead to different craniosynostosis syndromes because of modifier genes and different environmental influences.[5] The c.1052C>G (p.S351C) was the most frequent mutation identified in our study, accounting for 25% (3/12 patients with PS). All had severe phenotype with 2 of them passed away at approximately 2 years of age because of respiratory compromise. Our findings are consistent with the previous reports suggesting an association between the p.S351C and the severe form of PS.[15,16] The less frequent mutations in the TK2 domain, p.K641R, and p.G663E were also detected (Fig. 3). Both mutations were previously reported in other population.[6] The identification in the Thai population therefore supports their pathogenicity.

In conclusion, we successfully identified pathogenic mutations in all 12 Thai PS cases by PCR sequencing of *FGFR1* exon 5 and *FGFR2* exons 8, 10, 15, 16, and 17. All were recurrent and de novo. The majority of the mutations were located at exons 8 and 10, accounting for 75% of cases. The p.S351C was the most frequently detected and associated with the severe form of PS in this study. Revealing the common causative mutations will help facilitate genetic testing, leading to rapid and reliable diagnosis and proper genetic counseling of PS in the Thai population.

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