Scientific Foundations

FGFR2 Mutations among Thai Children with Crouzon and Apert Syndromes

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Crouzon and Apert syndromes have been reported to be associated with mutations in *Fibroblast Growth Factor Receptor 2 (FGFR2)* gene in various ethnic groups, but never in Southeast Asian subjects. Therefore, the authors conducted a study to characterize 11 Thai patients: four with Crouzon syndrome and seven with Apert syndrome. All cases are sporadic. Mean paternal and maternal ages were 38.7 and 28.6 years, respectively. Molecularly, all patients were found to have mutations in the *FGFR2* gene. Three mutations (C278F, S347C, S351C) were detected in all Crouzon patients with two having S351C. The seven patients with Apert syndrome have either S252W or P253R mutation. The authors' findings that sporadic cases were associated with advanced paternal age and that they all had mutations in *FGFR2* are consistent with previous reports. This is another observation supporting the causative role of *FGFR2* mutations in Crouzon and Apert syndromes.

Key Words: Crouzon syndrome, Apert syndrome, fibroblast growth factor receptor, mutation analysis.

Crouzon syndrome (MIM 123500), one of the most common syndromic craniosynostoses, is characterized by abnormal head shape due to premature closure of the cranial sutures, prominent eyes secondary to shallow orbits, and maxillary hypoplasia.[1] Apert syndrome (MIM 101200), one of the most serious syndromic craniosynostoses, is characterized by symmetrical syndactyly of the hands and feet and abnormal craniofacial features resembling those of Crouzon syndrome.[2] Intelligence varies from normalcy to mental retardation.[3]

Both syndromes are known to be inherited in an autosomal dominant manner. Recently, an exclusive paternal origin of *de novo* mutations associated with advanced paternal age has been described.[4,5] In 1994, Crouzon syndrome was found to be associated with mutations in *Fibroblast growth factor receptor 2 (FGFR2)*.[6,7] One year later, a similar association between Apert syndrome and *FGFR2* was evidenced.[8] Fibroblast growth factors (FGF) constitute a family of related mitogens. Four FGFRs (FGFR1–4) make up a family of structurally related receptors encoded by four different genes.[9] These receptors are composed of three extracellular immunoglobulin (Ig)-like domains, a transmembrane domain, and a tyrosine kinase domain.[10] The *FGFR2* gene on chromosome 10q25.3–26 consists of 20 exons spanning a region of greater than 120 kb.[11] Besides Crouzon and Apert syndromes, specific point mutations in *FGFR2* have been associated with several other craniosynostoses including Pfeiffer syndrome (MIM 101600),[12] Beare-Stevenson cutis gyrata syndrome (MIM 123790),[13] Jackson-Weiss syndrome (MIM 123150),[6] and some cases of Antley-Bixler syndrome (MIM 207410).[14]

To our knowledge, mutations in the *FGFR2* gene have been associated with both Crouzon and Apert syndromes in various ethnic groups including Japanese[15] and Taiwanese subjects,[16] but not in Southeast Asians subjects. We therefore conducted a study to clinically and molecularly characterize 11 Thai patients with craniofacial dysostosis.

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Materials and Methods

Patients were ascertained through the Genetics Clinic and Craniofacial Clinic of the King Chulalongkorn Memorial Hospital, Bangkok, Thailand during a 2-year period, January 2000 to December 2001.

The study was approved by the local ethics committee. After informed consent was obtained, 6 milliliters of peripheral blood were obtained for DNA isolation by a standard method. For patients with Crouzon syndrome, *FGFR2* exon 8 and *FGFR2* exon 10 were polymerase-chain-reaction (PCR) amplified and subsequently sequenced using previously described methods.[17] For patients with Apert syndrome, only the *FGFR2* exon 8 was amplified. Subsequently, the PCR-amplified 322-bp fragment was divided into two tubes and digested with *Mbo*I or *Bgl*I (New England Biolabs, Beverly, MA) for identification of the presence of S252W or P253R, respectively. After overnight incubation at 37°C, digested products were electrophoresed through a 12% polyacrylamide gel or 3% agarose gel before being stained with ethidium bromide. To confirm the presence of the mutations, PCR products from two patients, each with S252W or P253R, were also directly sequenced using an automated DNA sequencer (ABI Prism 310 Genetic Analyzer, Perkin Elmer, Wellesley, MA).

Results

There were 11 patients included in the study: four with Crouzon syndrome and seven with Apert syndrome. Male-to-female ratio was 3:8. Ages ranged from 2 months to 10 years. The clinical findings are summarized in Table 1. All cases were sporadic. Mean paternal and maternal ages were 38.7 and 28.6 years, respectively. Three of the patients' fathers were more than 35 years old (57, 59 and 62 for patients 5, 8 and 7, respectively) when the patients were born.

With the methods used, mutations in *FGFR2* were identified in all cases. There were three different mutations in four patients with Crouzon syndrome: one (C278F) in exon 8, and the other two (S347C and S351C) in exon 10. All seven patients with Apert syndrome had either S252W or P253R mutation (Table 1 and Figure 1).

Discussion

We identified 11 Thai children with syndromic craniosynostoses. All were sporadic. About one quarter of the reported cases of Crouzon syndrome had no family history while the majority of Apert cases were sporadic.[18] Sporadic cases presumably represented fresh mutations. The advanced paternal age of our patients supports the findings from previous reports that *de novo* mutations in both syndromes were exclusively paternal in origin and associated with advanced paternal age.[4,5]

All of the mutations identified in our patients were missense and previously described in other ethnic background studies.[19] Two patients with Crouzon syndrome (patients 3 and 4) with the same S351C mutation had severe clinical manifestations and died in their infancy. The phenotypes of previously reported cases with this S351C mutation varied from Crouzon, Pfeiffer, Antley-Bixler, or unclassified

Table 1. Clinical Features of the 11 Thai Children with Crouzon or Apert Syndrome

Patient ID	*Clinical diagnosis*	*Sex*	*Age**	*Paternal/Maternal age‡ (y)*	*Development and clinical course*	*Mutation*
1	Crouzon	F	10 y	27/22	IQ = 53, moderate mixed hearing loss	C278F (833G > T)
2	Crouzon	F	18 mo	32/24	Normal development. Sudden unexplained death at age 2 years.	S347C (1040C > G)
3	Crouzon	M	2 mo	34/33	Died of pneumonia at age 2 months	S351C (1052C > G)
4	Crouzon	F	1 y	33/34	Hydrocephalus, compressive optic neuropathy, hearing loss, died of aspiration pneumonia at age 1 year.	S351C (1052C >G)
5	Apert	M	18 mo	57/34	DQ = 67, hearing loss, cleft soft palate	P253R (758C > G)
6	Apert	F	30 mo	25/23	DQ = 46, cleft palate	S252W (755C > G)
7	Apert	F	3 y	62/37	IQ = 60	S252W (755C > G)
8	Apert	F	2 y	59/24	Incomplete cleft palate	P253R (758C > G)
9	Apert	F	3 mo	30/25	—	S252W (755C > G)
10	Apert	F	9 y	33/30	IQ = 55, cleft soft palate	S252W (755C > G)
11	Apert	M	20 mo	34/29	DQ = 89	P253R (758C > G)

*Age at last visit.
‡Parental ages when child was born.
DQ, developmental quotient; IQ, intelligence quotient.

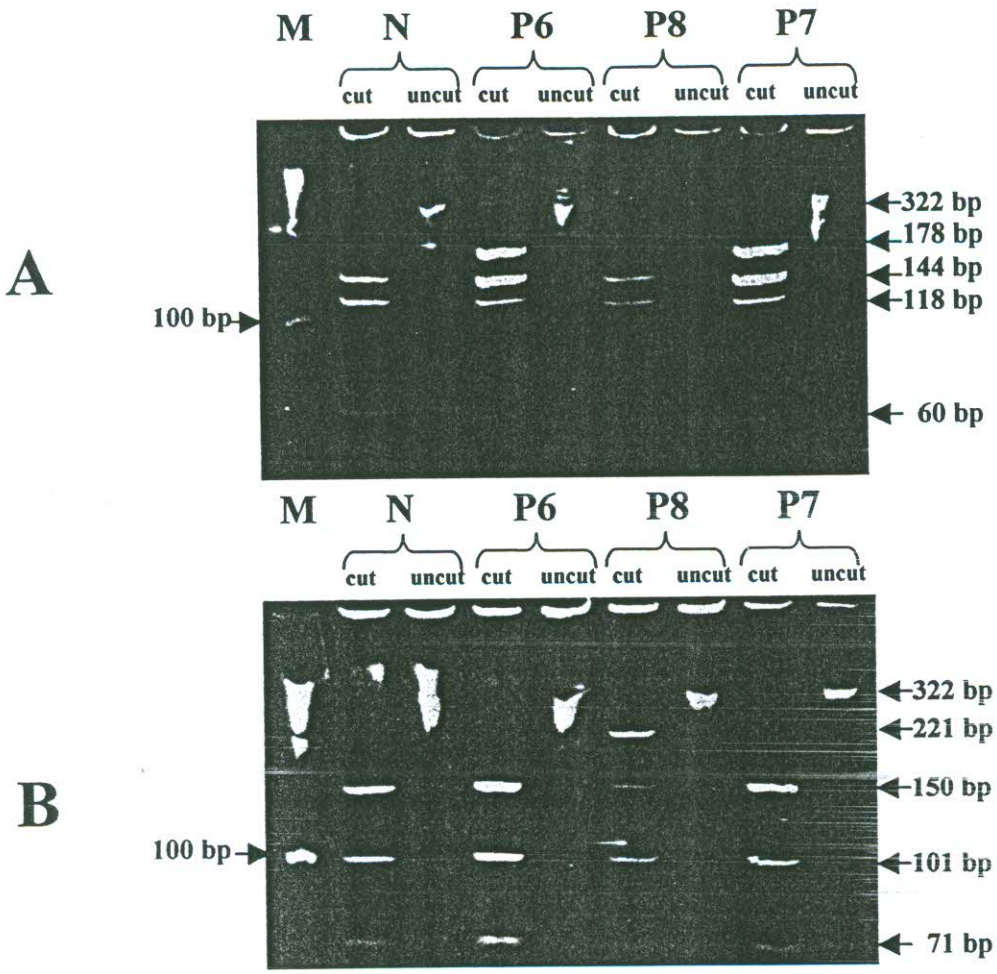

Fig 1 Two mutations causing Apert syndrome arising in *FGFR2* exon 8 are detected by restriction enzyme analysis and electrophoresed on 12% polyacrylamide gel stained with ethidium bromide. (**A**) The detection of S252W by *Mbo*I, in which individuals with this mutation (P6 and P7) show the 178 bp fragment in addition to the 144, 118, and 60 bp fragments seen in normal individuals. (**B**) The detection of P253L by *Bgl*I. The presence of the new band of 221 bp in addition to the 150, 101, and 70 bp fragments indicates that P8 is heterozygous for the P253L. In both (**A**) and (**B**), M in lane 1 represents a 100 bp marker with the band 100 bp indicated with an arrow; lanes 2 and 3 are controls (N); lanes 4 and 5 are from patient 6 (P6); lanes 6 and 7 are from patient 8 (P8); lanes 8 and 9 are from patient 7 (P7). Cut, PCR products digested by restriction enzymes; uncut, PCR product without adding restriction enzyme and showing only the undigested 322 bp fragment.

craniosynostosis syndromes.[14,20–22] The C278F mutation found in patient 1 was also associated with various clinical syndromes including Crouzon, Pfeiffer, and Jackson-Weiss syndromes.[23–25] Nonetheless, the S347C mutation found in patient 2 has been associated only with Crouzon syndrome.[6,24,26]

While the spectrum of *FGFR2* mutations causing Crouzon syndrome is wide, those causing Apert syndrome are much more restricted.[27] Four of our patients with Apert syndrome had S252W, while the other three had P253R. These two mutations account for approximately 99% of Apert syndrome mutations in a ratio of about 2:1.[8,28] Four of our patients with Apert syndrome had cleft palates: two with S252W and the other two with P253R. Severity of craniofacial malformations and syndactyly was not distinguishable between the two mutations. Negative correlations between phenotypic features and *FGFR2* mutations was consistent with a published study,[29] although some others did report significant correlations.[30,31]

Conclusion

We identified 11 unrelated Thai patients with Crouzon and Apert syndromes. They all had mutations in the *FGFR2* gene supporting the obser-

vation that mutations in the *FGFR2* are responsible for the phenotypes across different ethnic groups. Moreover, the identified molecular abnormalities could be used in developing diagnostic tools to identify prenatal cases in families at risk.

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