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Mutation Analysis of Crouzon Syndrome in Taiwanese Patients

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Crouzon syndrome is an autosomal-dominant disorder that causes premature fusion of the cranial suture. Crouzon, Pfeiffer, and Apert syndromes are caused by mutations in the extracellular, third immunoglobulin-like domain, and adjacent linker regions (exons IIIa and IIIc) of the fibroblast growth factor receptor 2 (*FGFR2*) gene. We screened 12 Crouzon syndrome patients for mutations in exons IIIa and IIIc of the *FGFR2* gene by polymerase chain reaction (PCR) and direct sequencing. Mutations were detected in nine of 12 patients at amino acid positions 278 , 281, 289, 342, and 354. More than half of the studied Crouzon patients carried a mutation resulting in either the loss or gain of a cysteine residue. A novel missense Ser354Phe substitution at exon IIIc of the human *FGFR2* gene was found. According to our results, sequencing analysis of IgIII domain of the *FGFR2* gene can lead to a genetic diagnosis of Crouzon syndrome. J. Clin. Lab. Anal. 20:23–26, 2006.

Key words: Crouzon syndrome; *FGFR2* gene; mutation; IgIII

INTRODUCTION

Craniosynostosis is a group of diseases that are generally defined as causing premature closure of one or more of the cranial sutures (1). More than 100 syndromes in which craniosynostosis is a feature have been described (2). Crouzon syndrome is the most distinctive and common disorder of the autosomal-dominant form of the craniofacial complex, and is characterized by the triad of premature craniosynostosis, orbital proptosis, and midfacial hypoplasia (1). Molecular analysis has identified mutations in the gene coding for fibroblast growth factor receptor 2 (FGFR2) on chromosome 10q25-26 (3). Recent studies have demonstrated that mutations in three of the four known *FGFR* genes are responsible for a variety of craniosynostosis syndromes, including Crouzon (4–6), Apert (7,8), Pfeiffer (9,10), and Jackson-Weiss (3,9,11) syndromes. Crouzon syndrome shows a broader spectrum of mutations in the *FGFR2* gene, and occasionally patients with Crouzon syndrome share identical mutations (Cys278Phe, Cys342Arg, and Cys342Tyr) with patients with Pfeiffer and Jackson-Weiss syndromes, respectively (3,8,10,11). The majority of mutations in *FGFR2* are missense substitutions clustered around the third extracellular immunoglobulin-like domain, encoded by exons IIIa and IIIc. Considering that craniosynostosis is essentially diagnosed based on phenotype, these findings suggest a possible pheno-

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type–genotype correlation between specific *FGFR* mutations and phenotypic variations in Crouzon syndrome.

To define the genotype of Taiwan patients with Crouzon syndrome, we performed mutation analyses on 12 unrelated patients with the syndrome.

MATERIALS AND METHODS

The study group consisted of 12 patients with craniosynostosis syndrome. All had the characteristic features of craniosynostosis (i.e., moderate to severe ocular proptosis, shallow orbits, hypertelorism, and midface hypoplasia). Genomic DNA was extracted from EDTA-anticoagulated peripheral blood taken from each patient. The coding exon IIIa and exon IIIc of the *FGFR2* gene were amplified by polymerase chain reaction (PCR). The primers specific to exon IIIa of the *FGFR2* gene (forward primer 5′-TGACAGCCTCT GACAACACAAC-3′ and reverse primer 5′-GGAAAT CAAAGAACCTGTGGC-3′) produced a 349 bp PCR product. For exon IIIc of *FGFR2*, forward primer 5′-ATCATTCCTGTGTCGTCTAAC-3′ and reverse primer 5′-AAAAAACCCA GAGAGA AAGAACAGTA TA-3′ produced a 225 bp PCR product. The PCR was performed for 35 cycles of initial denaturation at 94°C for 15 sec, 60°C (exon IIIa) or 58°C (exon IIIc) for 20 sec, 72°C for 30 sec, and a final extension at 72°C for 10 min using a Perkin Elmer 2400 thermal cycler (Applied Biosystems, Foster City, CA). The PCR fragments were purified and sequenced by an automatic 377 DNA sequencer (Applied Biosystems). The control subjects were selected randomly and confirmed by a medical doctor to be free of craniosynostosis. Our study was reviewed by the local ethics committee, and informed consent was obtained from all patients and controls before they were included in the study.

RESULTS

Mutations were detected in nine of the 12 Crouzon patients and represented amino acid changes at positions 278 , 281, 289, 342, and 354 (Table 1). Three of the 12 patients carried the Cys342Arg mutation, which is the most frequent mutation found in Taiwanese patients. The Tyr281Cys illustrates a gain of a cysteine residue located just three amino acids away from Cys278. Another mutation, A→C transversion at nucleotide 866, was found to cause a Gln289Pro substitution, which was previously detected by Oldridge et al. (12).

A novel C→T transition at nucleotide 1073 (Fig. 1) of the *FGFR2* gene was detected (10,13) in exon IIIc of the gene. This mutation will lead to phenylalanine to serine at amino acid 354 of the *FGFR2* gene. To clarify those with nucleotide sequence changes are mutations or polymorphisms, we analyzed 50 normal individuals by PCR-based restriction analysis. The Phe354Ser mutation was not found in the 50 normal individuals.

TABLE 1. FGFR2 mutations in Crouzon syndrome

Exon	Location[a]	Nucleotide change[a]	Amino acid change[a]	Patients
Novel mutation				
IIIc	1073	C>T	Ser354Phe	1
Mutation previously detected				
IIIa	833	G>T	Cys278Phe	2
IIIa	842	A>G	Tyr281Cys	1
IIIa	866	A>C	Gln289Pro	1
IIIc	1025	G>A	Cys342Tyr	1
IIIc	1024	T>G	Cys342Arg	3

[a]cDNA numbering considering the initiator Met codon as nucleotide +1; FGFR2 GenBank accession number = X52832.

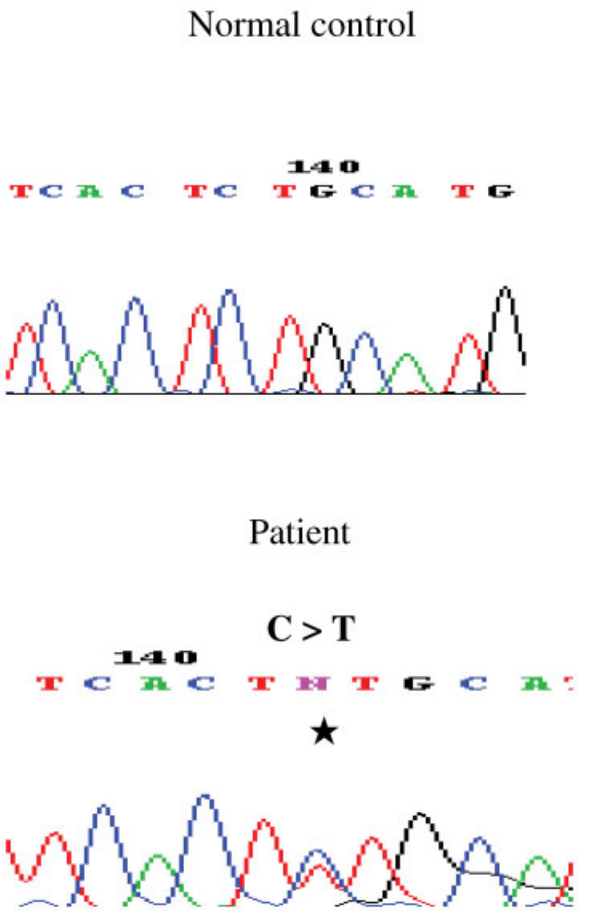

Fig. 1. Electropherograms of the sequence analyses of the *FGFR2* gene. A novel mutation at 1073 C→T in exon IIIc of the *FGFR2* gene in the patients is shown in comparison with the normal control sequence (10,13).

DISCUSSION

Dominant mutations in the *FGFR2* gene were recently identified as the cause of four phenotypically distinct craniosynostosis syndromes (Crouzon, Jackson-Weiss, Pfeiffer, and Apert syndromes). Most *FGFR2* mutations affect the structure of the extracellular domain of the FGFR2 protein, are de novo when found in sporadic cases, segregate with the syndrome in familial cases, and are not found in the normal population. The diagnosis of Crouzon syndrome was made according to the clinical signs of the patients, including acrocephaly, exophthalmos, and maxillary hypoplasia with "parrot-beak" nose, short upper lip, high narrow palate, narrowly spaced teeth, and prognathism (14). A total of 12 patients diagnosed as having Crouzon syndrome were subjected to DNA sequencing analysis on the IgIII domain of the *FGFR2* gene. Six different mutations were detected in nine patients, and one of them was a novel mutation on the *FGFR2* gene IgIII domain.

To date, most of the patients diagnosed with Crouzon syndrome have been shown to carry mutations of the *FGFR2* gene (10,15). However, mutations remain to be identified in ~50–73% of patients. These may be due to genetic heterogeneity, which has been observed in other craniosynostosis syndromes, such as Apert and Pfeiffer syndromes. Three patients with no detected mutations may have a mutation (or mutations) located on different exons of the *FGFR2* gene (15–17).

The mutations that affect conserved residues in the IgIII loop or on its margins (Phe276Val, lle288Ser, Gln289Pro, and Thr341Pro) may reduce the stability of the core loop domain, as suggested by three-dimensional modeling of the IgIII domain of *FGFR2* (16). Mutations were detected at Ser 354; however, the amino acid changes were very different. The exchange of Ser354 by Cys caused a severe form of Crouzon syndrome. The unpaired new cysteine residue may induce a ligand-independent dimerization of receptor molecules. In the present study, a serine to phenylalanine change at position 354 was detected, which is a novel mutation. According to the predicted structure of the *FGFR2* IgIII domain (18), Ser354 may be involved in the receptor–ligand interactions. A mutation in Ser354 will potentially interfere with the binding function of this *FGFR2* IgIII domain.

The novel mutation Ser354 lies close to the disulfide-bonded cysteines, Cys342. These craniosynostosis mutations are in a position to disrupt the formation of a disulfide bond. In our previous study (summarized in Table 1) (6), most of the mutations resulted in a gain or loss of cysteine residues. The cysteines of both sites form the S-S bond in the third immunoglobulin-like domain, which controls receptor activity to the ligand. Point mutations at positions 289, 301, 314, 341, 343, 351, and 354 will lead to a structure change and loss of ligand binding activity of the IgIII domain (18). It is clear that the same mutation may lead to phenotypic heterogeneity, and a mutation such as Cys342Arg in *FGFR2* can result in Crouzon and Pfeiffer syndromes (5,10). This molecular analysis of the *FGFR* gene not only provides useful information to help clinicians confirm a diagnosis and perform prenatal diagnoses, it will also have a great impact on the clinical classification of craniosynostosis syndrome.

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