·Basic Research·

FGFR2 mutation in a Chinese family with unusual Crouzon syndrome

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Abstract

• AIM: To describe the clinical characteristics with genetic lesions in a Chinese family with Crouzon syndrome.

• METHODS: All five patients from this family were included and received comprehensive ophthalmic and systemic examinations. Direct sequencing of the *FGFR2* gene was employed for mutation identification. Crystal structure analysis was applied to analyze the structural changes associated with the substitution.

• RESULTS: All patients presented typical Crouzon features, including short stature, craniosynostosis, mandibular prognathism, shallow orbits with proptosis, and exotropia. Intrafamilial phenotypic diversities were observed. Atrophic optic nerves were exclusively detected in the proband and her son. Cranial magnetic resonance imaging implied a cystic lesion in her sellar and third ventricular regions. A missense mutation, *FGFR2* p.Cys342Trp, was found as disease causative. This substitution would generate conformational changes in the extracellular Ig-III domain of the FGFR-2 protein, thus altering its physical and biological properties.

• CONCLUSION: We describe the clinical presentations and genotypic lesions in a Chinese family with Crouzon syndrome. The intrafamilial phenotypic varieties in this family suggest that other genetic modifiers may also play a role in the pathogenesis of Crouzon syndrome.

• KEYWORDS: Crouzon syndrome; familial cases; phenotypic diversity; *FGFR2* mutation
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INTRODUCTION

Crouzon syndrome is one of the fibroblast growth factor receptor 2 (FGFR2)-related craniosynostosis syndromes with an incidence of 1.6 in 100 000 births [1]. Eight types of disorders comprise the *FGFR* -related craniosynostosis syndromes, including Pfeiffer syndrome, Apert syndrome, Crouzon syndrome, Beare-Stevenson syndrome, *FGFR2* -related isolated coronal synostosis, Jackson-Weiss syndrome, Crouzon syndrome with acanthosis nigricans (AN), and Muenke syndrome [1]. Clinically, patients with Crouzon syndrome are presented with craniofacial abnormalities, including significant proptosis, exotropia, and mandibular prognathism, whereas their intellect and extremities are often normal [2-5]. Reportedly, nearly 30% patients will develop progressive hydrocephalus, often with tonsillar herniation, and sacrococcygeal tail has also been described[6].

Families with Crouzon syndrome usually demonstrate an autosomal dominant inheritance pattern. By fay, only mutations in the *FGFR2* gene (MIM: 176943) have been found implicated in the etiology of Crouzon syndrome [7-9]. *FGFR2*, located on 10q26, encodes the FGFR-2 protein, a tyrosine-protein kinase acting as cell-surface receptor for fibroblast growth factors. FGFR-2 includes three extracellular immunoglobulin (Ig) like C2-type domains (Ig-I, Ig-II and Ig-III), a transmembrane domain, and a cytoplasmic tyrosine kinase domains. To date, nearly 60 *FGFR2* mutations have been reported in causing Crouzon syndrome, most of which

Table 1 Primers for mutation screening of the *FGFR2* gene

Exons	Product size	Forward primer	Reverse primer
Exon 2	384	CACTTGGGCTGGAGTGATTT	TTAACAATCTGCCCCCAGAC
Exon 3	397	CGTTCTCTCCTCTCCCTCCT	CCTTTTCACTTGGCCAAAAA
Exon 4	242	CCTGGGTTGTTGACTTTGCT	CAGAACTTCCCTCCATGCTC
Exon 5	321	TTTACTCATGGAGGGGAAGC	CGAGACTCCATCGCAAAAA
Exon 6	250	GAAAGCACAGTACTTGGTAT	AACGAGTCAAGCAAGAATGG
Exon 7	339	AGCCCTCTGGACAACACAAC	AAGAACCTGTGGCCAAACC
Exon 8	248	CCACAATCATTCCTGTGTCG	CAGTCAACCAAGAAAAGGGAAA
Exon 9	378	GCGTCAGTCTGGTGTGCTAA	GCACATGGAAGCTCACAGAA
Exon 10	295	GATACTCTGGCTGGGCTCTG	CCAATATCCCCATTTATAGCTGA
Exon 11	186	ACCCCATCACCAGATGCTAT	TTCACATGCCACAAAAGGAA
Exon 12	248	ACAGTAGCTGCCCATGAGTT	GGAAGCCCAGCCATTTCTA
Exon 13	340	GTTTTGCTGAATTGCCCAAG	AGCATGTCCAAATTGCCTGT
Exon 14	238	CTTTTGTTCTGGCGGTGTT	GGAACATTCTGAGCCTCACC
Exon 15	244	ACAGGGCATAGCCCTATTGA	GCAGCAGCCACTAAAGAAGG
Exon 16	294	AGCTGGGCGTGTTTAGGTTT	GGGCCTTCAAAAACGAGATA
Exon 17	243	CACGTCCCCATATTGCCTAT	GCATGTCTCACAAGACAACCA
Exon 18	404	TCCTGTCCCACGTCCAATAC	ATGGTCTCCCTGCTCAGTGT

located in the Ig-III domain [10]. In addition, FGFR-2 is involved in intracellular signaling and plays an essential role in regulating cell proliferation, differentiation, migration, apoptosis, and embryonic development[11].

In the present study, we report the identification of an *FGFR2* mutation in a Chinese family with autosomal dominant Crouzon syndrome. Intrafamilial phenotypic diversities exist within this family. This mutation, located in a highly conserved spot of FGFR-2, would potentially induce a significant conformational change in the extracellular Ig-III domain.

SUBJECTS AND METHODS

Participants and Clinical Assessments This study, conformed to the tenets of the Declaration of Helsinki, was approved and prospectively reviewed by the Ethics Committee on Human Research of the People's Hospital of Ningxia Hui Autonomous Region. Written informed consents were obtained from all participants or their legal guardians. A large Chinese Hui family containing five patients and five unaffected family members was recruited from the People's Hospital of Ningxia Hui Autonomous Region. The detailed family pedigree and marriage status were presented in Figure 1A. All participants received detailed ophthalmic examinations, including best-corrected visual acuities (BCVAs) testing, slit-lamp biomicroscopy, intraocular press (IOP), anterior segment photography, and funduscopy, and the five patients (C1-II:1, III:3, III:4, IV:2 and IV:3) received additional physical examinations. Cerebral magnetic resonance imaging (MRI) of patients C1-III:3 and IV:2 was obtained using a General Electric 3D FIESTA (0.6 mm thick) MRI scanner (GE Healthcare) with head coils. Another 100 healthy controls free of Crouzon syndrome or other major eye problems were included. Peripheral blood samples were collected from all participants for genomic DNA extraction using a QIAmp DNA Blood kit (Qiagen, Valencia, CA, USA) per the manufacturer's protocols. Genomic DNA samples were preserved at -20℃ before use.

Mutational Screening All coding exons and flanking intronic regions of the *FGFR2* gene were amplified in all five patients (C1-II:1, III:3, III:4, IV:2 and IV:3) with polymerase chain reaction (PCR) using a previously described protocol[12]. Primer information was listed in Table 1. PCR products were sequenced in both directions using an ABI3730 Automated Sequencer (PE Biosystems, Foster City, CA, USA) and analyzed with Chromas (version 2.3; Technelysium Pty Ltd, Brisbane, QLD, Australia). Reference sequences for *FGFR2* were ENST00000358487 obtained from the ENSEMBL Human Genome Browser Map.

Pathogenic Analysis To confirm the evolutionary conservation of the mutated amino acid, we used the Vector NTI Advanced 11 software (Invitrogen, Grand Island, NY, USA) to align the orthologous sequences of FGFR-2 of the following species: *Homo sapiens* (ENSP00000351276), *Pan troglodytes* (ENSPTRP00000005308), *Canis lupus familiaris* (ENSCAFP00000009051), *Bos taurus* (ENSBTAP00000018708), *Sus scrofa* (ENSSSCP00000030305), *Mus musculus* (ENSMUSP00000112430), *Gallus gallus* (ENSGALP00000037940), *Danio rerio* (ENSDARP00000075360), *Drosophila melanogaster* (FBpp0075520), and *Caenorhabditis elegans* (F58A3.2c). SWISS-MODEL online server was applied to

Table 2 Clinical features of the affected family members

Patient ID	Age (a)	Sex	OP	ES	BCVA (logMAR)		IOP (mm Hg)		Refractive status		MP	Height (cm)/ Weight (kg)	HC/CC (cm)
					OD	OS	OD	OS	OD	OS			
C1-II:1	71	M	Yes	Yes	1.0	0.8	8.9	7.4	+1.00DS/+0.25DC×80	+0.25DS	Yes	160/49	53/70
C1-III:3	41	F	Yes	Yes	LP	LP	13.8	15.1	+0.75DS	+1.00DS	Yes	145/52	56/75
C1-III:4	37	M	Yes	Yes	1.0	1.0	10.0	12.0	-1.75DS/-1.50DC×50	-1.75DS/-0.25DC×110	Yes	160/44	55/79
C1-IV:2	9	M	Yes	Yes	0.4	0.4	8.2	11.7	+0.25DS/+0.75DC×85	+1.25DS	Yes	125/21	49/60
C1-IV:3	14	M	Yes	Yes	0.6	1.0	17.1	13.3	-5.25DS/-2.25DC×15	+0.25DS/+0.75DC×175	Yes	147/30	51/65

M: Male; F: Female; OP: Ocular proptosis; ES: External strabismus; BCVA: Best corrected visual acuity; OD: Right eye; OS: Left eye; IOP: Intraocular pressure; MP: Mandibular prognathism; HC: Head circumference; CC: Chest circumference.

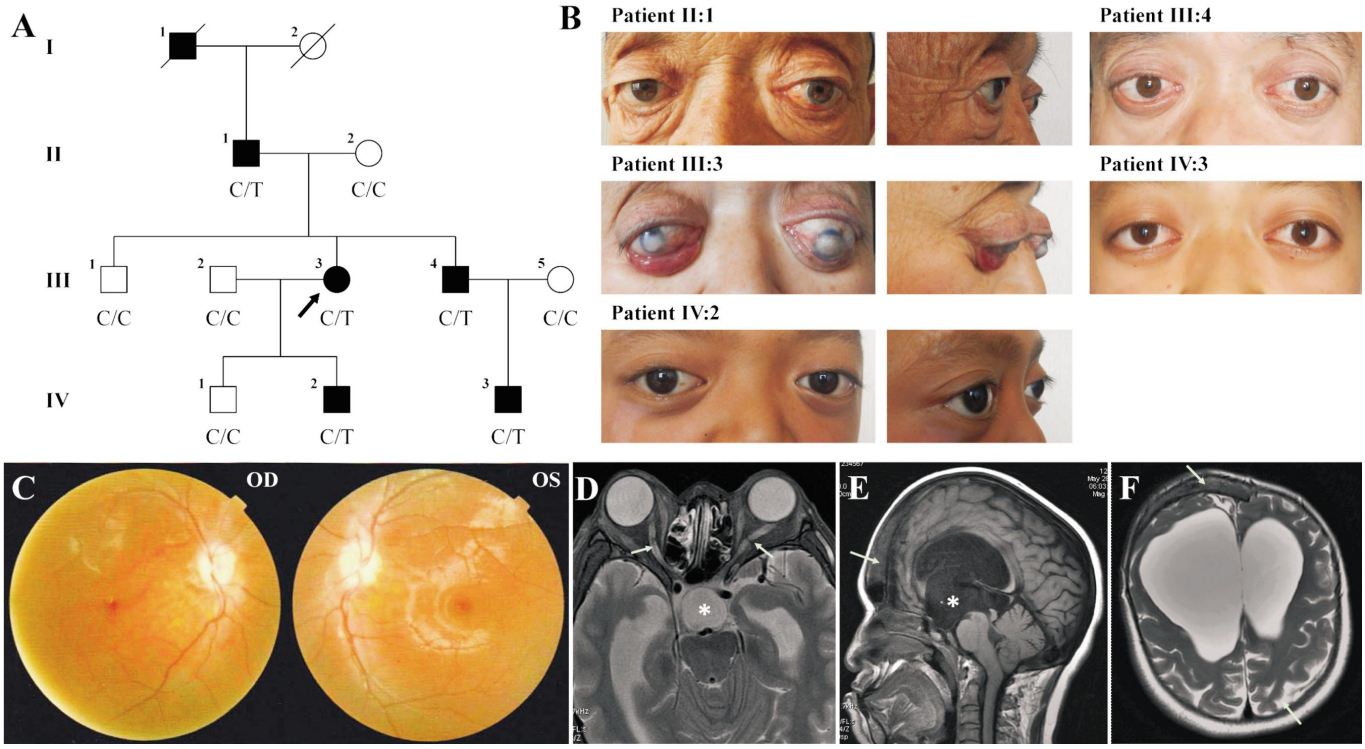

Figure 1 Pedigree and clinical assessments of family C1 A: The pedigree of family C1 indicates an autosomal dominant inheritance pattern. *FGFR2* genotypes are annotated below the pedigree symbols. Filled and empty symbols represent affected and unaffected family members, respectively. Proband (C1-III:3) is indicated by arrow. B: Ocular proptosis in all five patients from family C1. C: Fundus photos of patient C1-IV:2 indicate optic disc pallor in both eyes. D: Cerebral axial T2 weighted imaging (WI) of the proband C1-III:3 implies bilateral shallow orbits with proptosis, thickened and distorted optic nerves (arrows), and dilated paracele. A cystic lesion in the sellar and the third ventricular regions was marked by an asterisk. E: Cerebral sagittal T1 WI of the proband indicates hyperostosis of the frontal bone (arrow), the cystic lesion (asterisk), and dilated paracele. Chiari malformation was not found in this patient. F: Cerebral axial T2 WI of the proband suggests hyperostosis of the frontal bone (superior arrow), dilated paracele and subarachnoid space (inferior arrow), and cerebral atrophy.

construct the crystal structures of the wild type and mutant FGFR-2 as previously described [13-14]. Predicted structures were displayed with PyMol software (version 1.5).

RESULTS

Clinical Findings Personal and family histories were carefully reviewed for this family. Systemic evaluations revealed a clinical diagnosis of Crouzon syndrome for all patients in this family with their clinical details summarized in Table 2. Phenotypic varieties existed among the five patients. Upon physical examination, all five patients in family C1 were presented with short stature, craniosynostosis, and mandibular prognathism. Consistent with previous findings, radiographic metacarpal-phalangeal profile revealed shortening in patients C1-IV:2 (age 9) and IV:3 (age 14)[15], and no other anomalies in extremities were revealed. The head circumference of patient C1-III:3 was within the normal range, which was probably induced by the long term intracranial hypertension. MRI examination indicated severe hydrocephalus in patient C1-III:3 (Figure 1F) and very slight changes in patient IV:2 (data not shown). In patient C1-III:3, a series of changes accompanied by hydrocephalus, including hyperostosis of the frontal bone, cerebral atrophy, dilated paracele and subarachnoid space, were also observed (Figures 1D-1F). Noteworthy, an additional cystic lesion in the sellar and the third ventricular regions were also found in this patient (Figures 1D-1E).

Shallow orbits, proptosis, and exotropia since early childhood were also found in this family (Figure 1B and 1D). Two

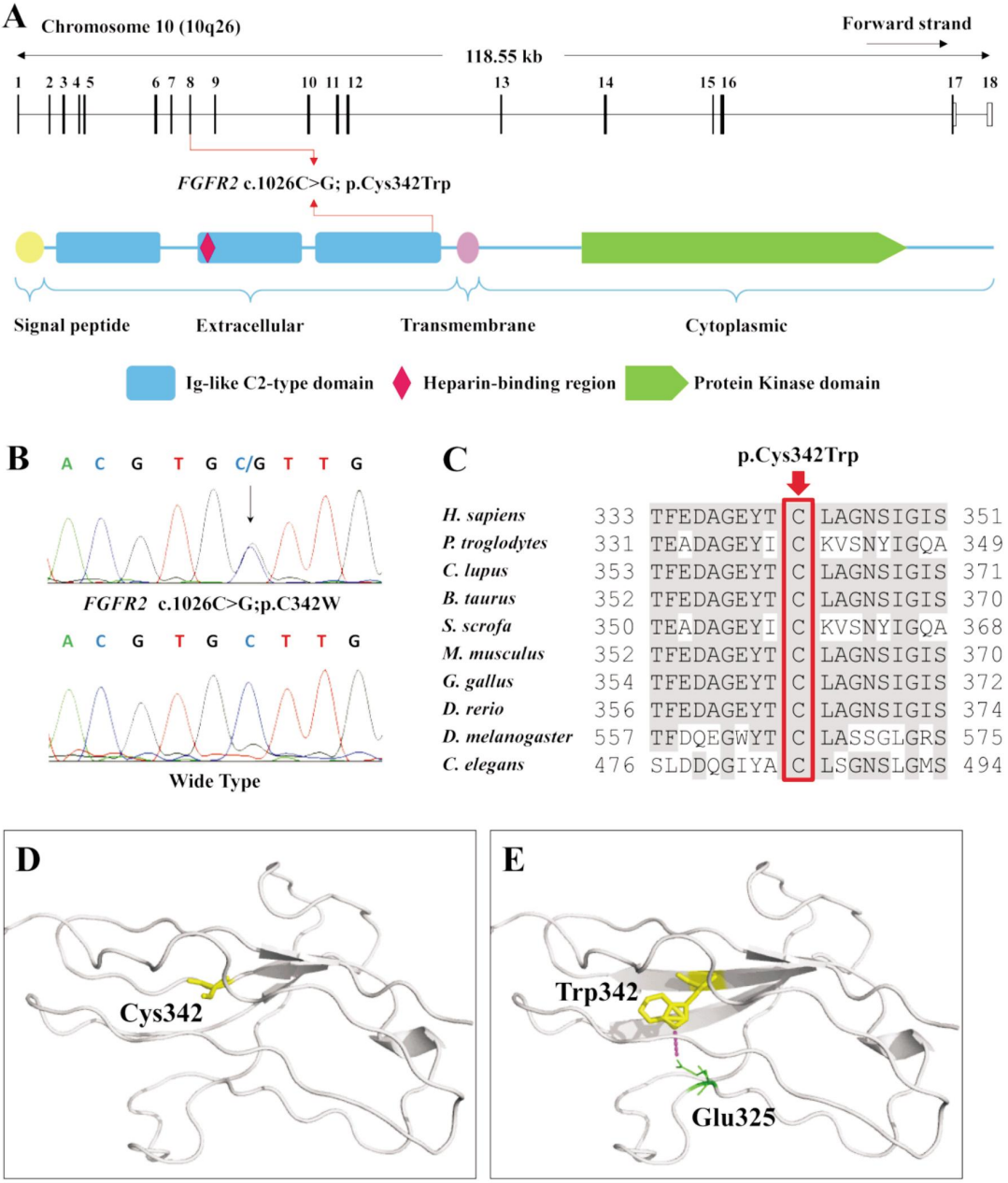

Figure 2 The *FGFR2* mutation identified in family C1 A: Schematic representation of the relative linear location of the identified *FGFR2* mutations in context of genome structure (upper) and protein structure (below). B: Sequencing chromatograms of the identified *FGFR2* c.1026C>G mutation (upper) and the wild type sequence (below). C: Residue Cys342, indicated by the arrow, is evolutionary conserved among all tested species. D-E: Predicted crystal structures of the wild type (D) and mutant (E) FGFR-2. A hydrogen bond between Trp342 and Glu325 is generated by the substitution. The elongation of two β sheets is also revealed in the mutant protein.

patients, C1-III:3 and IV:2, showed remarkably reduced BCVAs. Bilateral thickened and distorted optic nerves were found in the proband C1-III:3 (Figure 1D), while her fundus appearance was not attainable due to severe exposure keratitis and corneal ulceration caused by subluxation of her eyeballs (Figure 1B). Optic atrophy was also noticed in patient C1-IV:2 (Figure 1C), resulting in his poor vision. Funduscopy and MRI tests indicated no obvious anomalies in the optic nerves and fundus of the other three patients.

Identified Mutation and Pathogenic Assessments Genetic assessments revealed a heterozygous missense mutation, *FGFR2* c.1026C>G, cosegregated with the disease phenotype in this family, and absent in 100 healthy controls (Figures 1A, 2A-2B). This mutation resulted in the replacement of hydrophilic cysteine with hydrophobic tryptophan at codon 342 (p.Cys342Trp) in the Ig-III domain (Figure 2A), and the mutational spot was found highly conserved among all tested species (Figure 2C). Crystal structures of the wild type and mutant FGFR-2 (residues 153-360) was constructed based on the human FGFR-2 [protein data bank (PDB) ID: 4J23] with the sequence identity of 100% and similarity of 63%[16]. The constructed crystal structure revealed that the substitution would cause significant conformational changes, including the generation

of a hydrogen bond between Trp342 and Glu325, and the elongation of two β sheets (Figures 2D, 2E), which might change the physical and biological properties of FGFR-2.

DISCUSSION

Familial Crouzon syndrome is usually caused by *FGFR2* mutations. In the present study, we report the identification of a missense *FGFR2* mutation, p.Cys342Trp, in a Chinese family with autosomal dominant Crouzon syndrome. This mutation has previously been found in a Japanese sporadic case and four Caucasian cases [17-19], but never in the Chinese population. Crystal structural modeling indicates that this mutation would lead to a significant conformational change in the extracellular Ig-III loop by generating a hydrogen bond between Trp342 and Glu325, and elongating two β sheets.

FGFR-2 is an essential protein involves in multiple signaling pathways. Ligand binding will phosphorylate a few proteins, including 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase gamma-1 (PLCG1), fibroblast growth factor receptor substrate 2 (FRS2), and serine/threonine-protein kinase PAK 4 (PAK4), thus resulting in the activation of corresponding signaling cascades [20-23]. Most Crouzon syndrome causative *FGFR2* mutation would lead to a gain or loss of cysteine residues, which form the disulfide bonds in the Ig-III loop, and regulate the binding of FGFR-2 to the ligand [1]. The mutation identified in this study is a missense substitution from cysteine to tryptophan at residue 342 of FGFR-2, which also eliminates a cysteine. Thus, we highly hypothesize that this mutation would cause constitutive kinase activation or impair regular FGFR-2 maturation, internalization, or degradation, thereby resulting in aberrant signaling[24].

Clinically, intrafamilial phenotypic diversity exists within this family. Changes in optic nerves have never been described in patients with Crouzon syndrome. Atrophic optic nerves were found in two patients from this family with hydrocephalus, but not in the other three patients. We therefore, for the first time, characterized the unusual changes in optic nerves in patients with Crouzon syndrome. An additional cystic lesion also found in the sellar and third ventricular regions in one patient with Crouzon syndrome. Recent developments in molecular genetics diagnosis help to provide better insights into the genotype-phenotype correlations. Some forms of inherited ocular diseases show significant genetic and clinical heterogeneities. Inherited ocular diseases present all three Mendelian inheritance patterns and involve numerous disease causative genes and mutations. Different mutation in the same gene, or even the same mutation, can clinically be correlated with a wide phenotypic spectrum. It is therefore presumed that other genetics modifiers, like the epigenetic modifying factors, or ethnic background may play an important role in the pathogenesis or progression of these diseases.

In summary, in the present study, we report the genetic and clinical findings in a Chinese family with autosomal dominant Crouzon syndrome. The unusual changes of optic nerves are described in this family carrying *FGFR2* p.Cys342Trp, which has never been found in the Chinese population. The phenotypic varieties in this family further suggest the potential role of other factors in the pathogenesis of Crouzon syndrome. Our study also indicates that clinicians should pay attention to the optic nerve changes in patients with Crouzon syndrome. MRI and funduscopy should be recommended to these patients by clinicians.

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