C278F mutation in *FGFR2* gene causes two different types of syndromic craniosynostosis in two Chinese patients

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Abstract. The current study was performed with aim to investigate the fibroblast growth factor receptor 2 *(FGFR2)* gene in two Chinese families with two different forms of syndromic craniosynostosis, and to characterize their associated clinical features. Two families underwent complete ophthalmic examinations, and two patients from each family were diagnosed with craniosynostosis. Genomic DNA was extracted from leukocytes of peripheral blood collected from these two families and from 200 unrelated subjects within the same population as controls. Exons 8 and 10 of the *FGFR2* gene were amplified by polymerase chain reaction and directly sequenced. Ophthalmic examinations of the two patients revealed shallow orbits and ocular proptosis, accompanied by midface hypoplasia and craniosynostosis. Case 1 had retinal detachment, abnormal limbs and hands, while case 2 exhibited normal hands and feet upon clinical examination. A heterozygous *FGFR2* missense mutation c.833G>T (C278F) in exon 8 was identified in these two patients, but not in unaffected family members or the normal controls. Although *FGFR2* gene mutations and polymorphisms have been studied in various ethnic groups, we report a mutation of *FGFR2* in two different Chinese patients with two different types of syndromic craniosynostosis.

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Introduction

Craniosynostosis is a group of connective disorders that primarily presents with premature fusion of cranial sutures, and has a birth prevalence of 1 in 2,000-3,000 live births (1,2). Many syndromes associated with craniosynostosis have been studied, the majority of which exhibit dominant inheritance (3). These syndromes are clinically classified according to the presence of specific craniofacial abnormalities with or without specific limb involvement (2,4). Among these autosomal dominant forms of the craniofacial complex, Crouzon syndrome is the most distinctive and common disorder characterized by the triad of premature craniosynostosis, orbital proptosis and midfacial hypoplasia (5,6). Historically, Crouzon syndrome was distinguished clinically by the absence of limb defects. Sometimes, it is a difficult diagnostic for clinicians (7,8). Since clinical classification of craniosynostosis syndromes is challenging, investigations of the underlying gene mutations have great value for differentiation of these disorders (9), and may help to determine the molecular basis of the syndrome.

The molecular analysis of fibroblast growth factor receptor (*FGFR*) genes is of great value in the clinical by providing confirmatory diagnosis, and also allows prenatal diagnosis (10). FGFRs are transmembrane signal-transduction molecules. Upon binding of fibroblast growth factors (FGFs) to their extracellular region, FGFRs dimerize to induce trans-autophosphorylation of the intracellular tyrosine kinase region (11,12). The sequence structure of FGFRs share great similarity, which is characterized by three extracellular immunoglobulin-like domains (IgI, IgII and IgIII), a single transmembrane domain and a split tyrosine kinase (TK1/TK2) domain. *FGFR2* gene is located at 10q26, and

~95% of cases have mutations in exons IIIa (exon 8) and IIIc (exon 10) (13).

The present study performed mutational analysis of two Chinese families with craniosynostosis syndrome at the gene level, and identified one recurrent heterozygous mutation and characterized its associated clinical features.

Patients and methods

Ethics. All experimental protocols and the methods were performed in accordance with the standard guidelines and approved by the Ethics Committee of Zhongshan Ophthalmic Center (Guangzhou, China). Written informed consent, for participation in the present study and the publication of images, was obtained from all participating subjects in accordance with the Declaration of Helsinki.

A Crouzon syndrome family. Two probands in two Chinese families were diagnosed with craniosynostosis at Zhongshan Ophthalmic Center. The proband of family 1 (Fig. 1) was a 53-year old man, whose sister did not have craniosynostosis. The proband of family 2 (Fig. 2) was a 7-year old girl who was the only child of her family. Ophthalmic examinations were performed in these two families, as follows: Visual acuity was tested using the Early Treatment Diabetic Retinopathy Study chart (Precision Vision, Woodstock, IL, USA); anterior segment photographs were captured using the BX 900 Slit Lamp (Haag-Streit AG, Köniz, Switzerland); fundus photography was performed using a Heidelberg Retina Angiograph (Heidelberg Engineering GmbH, Heidelberg, Germany); computed tomography (CT) and physical examinations, including blood and urine tests, electrocardiogram, X-ray, blood biochemistry test, blood lipid and blood coagulation tests were conducted to exclude systemic diseases.

Sample collection. The affected families were identified at Zhongshan Ophthalmic Center. As a normal control, two hundred subjects without diagnostic features of craniosynostosis syndrome from the same population were recruited. Venous blood samples were collected, and genomic DNA was extracted from peripheral blood leucocytes using standard protocols and a DNA extraction kit (Qiagen GmbH, Hilden, Germany). Patients with primary microcephaly, postural plagiocephaly, incomplete data, no visual perception or lost from follow-up were excluded from the study.

Mutation detection. Exons 8 and 10 of the *FGFR2* gene were amplified by polymerase chain reaction (PCR) with previously established primers (11,14,15). Briefly, PCR was performed in 50 μl reactions using the PCR Amplification kit (Takara Bio, Inc., Otsu, Japan). The cycling profile was initiated by one cycle at 94˚C for 5 min, followed by 40 cycles at 94˚C for 45 sec, 52-66˚C for 45 sec and 72˚C for 45 sec, and ended with one cycle at 72˚C for 10 min. The PCR products were sequenced from both directions with an ABI3730 Automated Sequencer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The sequencing results were analyzed using Chromas (version 2.3; Technelysium Pty Ltd, Brisbane, Australia) by comparing with the reference sequences in the database at the National Center for Biotechnology Information (NCBI; NC_000010).

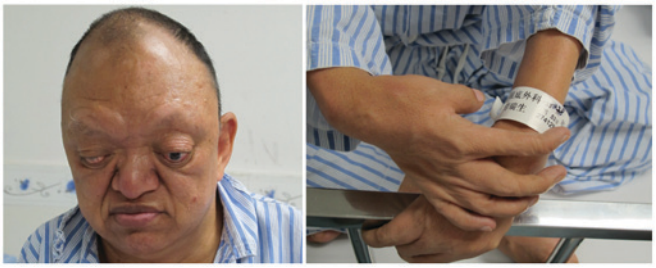

Figure 1. The proband of family 1 was a 53-year-old man with craniosynostosis. The patient had shallow orbits and ocular proptosis, accompanied by midface hypoplasia, craniosynostosis with clinically abnormal hands and feet, without broad thumbs.

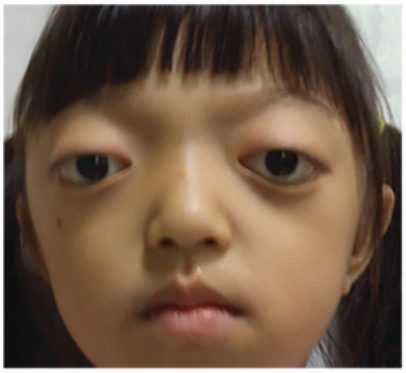

Figure 2. The second proband of family 2 was a 7-year-old girl with craniosynostosis, and she had shallow orbits and ocular proptosis, accompanied by midface hypoplasia, craniosynostosis with clinically normal hands and feet.

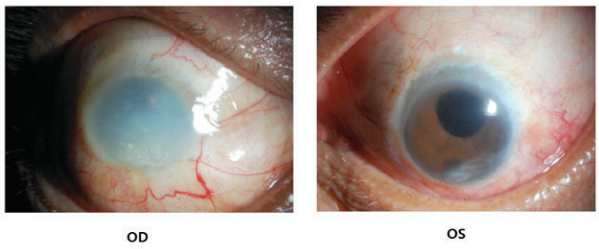

Figure 3. Eye abnormalities in case 1. The cornea was small with a diameter of 9 mm and fully white in the right eye. The left eye experienced retinal detachment 2 years ago, and had been operated on with vitrectomy, cataract surgery and silicone oil implantation. Microcornea and absent lens are observed. OS, oculus sinister; OD, oculus dextrus.

Results

Clinical data. The Chinese families studied in this project were from the southern area of China. Two individuals, in two separate families, were identified as having similar disease and other systemic diseases were excluded (data not shown). These patients exhibited from shallow orbits and ocular proptosis, accompanied by midface hypoplasia, craniosynostosis and a curved, beaklike nose. Besides these manifestations, case 1 in family 1 had retinal detachment and clinically abnormal hands and feet, without broad thumbs (Fig. 1).

The best-corrected visual acuity of case 1 as measured using a LogMAR chart. The visual acuity was no light

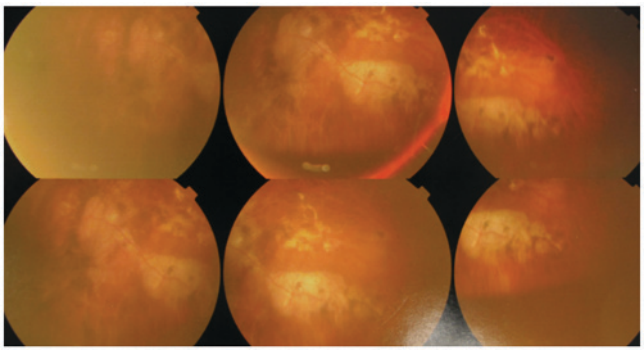

Figure 4. Retinal atrophy in fundus photographs of case 1.

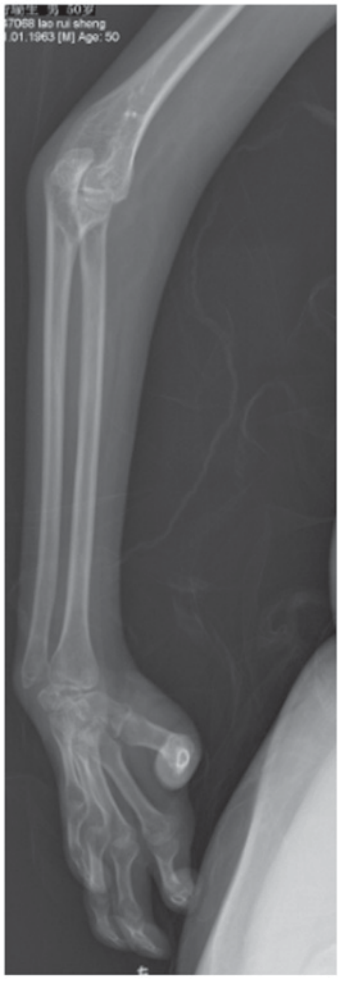

Figure 5. X-ray demonstrated abnormal limbs of case 1.

perception of the right eye (oculus dextrus; OD) and counting figure/50CM of the left eye (oculus sinister; OS). The patient could see nothing when he was born due to congenital ocular abnormalities. The diameter of the cornea was ~9 mm and the cornea was completely white, obscuring the lens and the fundus (Fig. 3A). Retinal detachment occurred 2 years ago, and had been treated with vitrectomy, cataract surgery and silicone oil implantation, so the lens could not be observed (Fig. 3B). Some retinal atrophy was observed in the fundus photographs (Fig. 4) and the intraocular pressure was 34 mmHg. X-ray showed abnormal limbs (Fig. 5).

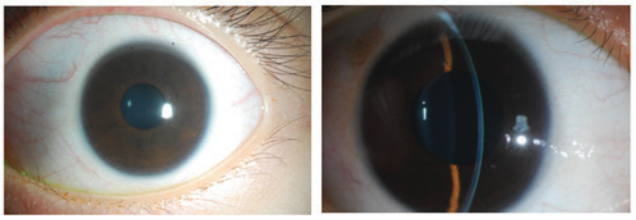

Figure 6. Images of the eyes of case 2. The anterior segment photograph demonstrated that the corneas were normal in size and transparency, and the lenses were positioned normally and remained clear.

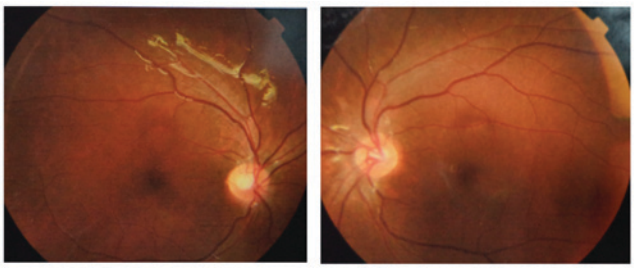

Figure 7. No abnormalities were detected in the retinas, choroids, or optic nerves of the fundus photographs of case 2.

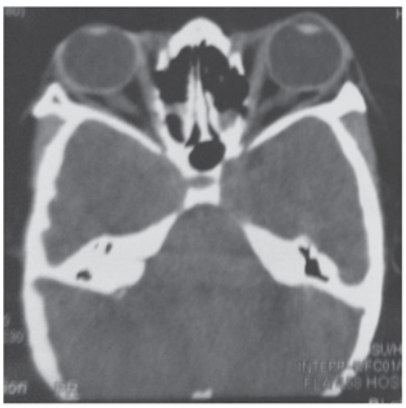

Figure 8. Computed tomography of the skull of the patient was performed and revealed shallow orbits in case 2.

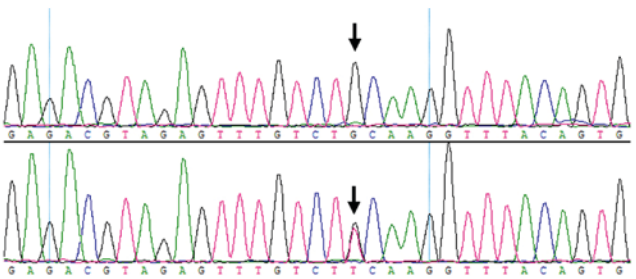

Figure 9. A heterozygous fibroblast growth factor receptor 2 missense mutation c.833G>T (C278F) in exon 8 was identified in the affected individuals (cases 1 and 2), but not in any of the unaffected family members and the normal controls. The mutation causes the cysteine 278 codon (TGC) to change to a phenylalanine codon (TTC).

The best-corrected visual acuity of case 2 in family 2 was measured by LogMAR, and was 0.00 (OD) and 0.10 (OS). The results of the optometry were -0.50DS (OD) and +3.00DS

(OS). Applanation tonometry revealed normal bilateral intraocular pressure. The corneas were transparent and normal in size, and the lenses were clear and were positioned normally (Fig. 6). No abnormalities were found in the retinas, choroids or optic nerves (Fig. 7). CT of the skull of this patient (Fig. 8) revealed shallow orbits.

Mutation screening. A heterozygous *FGFR2* missense mutation c.833G>T (C278F) in exon 8 was identified in both affected individuals, but not in any of the unaffected family members or the normal controls. The mutation causes a change from the cysteine 278 codon (TGC) to a phenylalanine codon (TTC; Fig. 8). As the parents of case 1 had been dead for several years, it cannot be determined whether the mutation was inherited. The parents of Case 2 had no mutations with normal appearance, therefore, case 2 was caused by a *de novo* mutation.

Discussion

The current study identified a mutation of the *FGFR2* gene that is associated with craniosynostosis syndrome: c.833G>T (C278F) in exon 8. This mutation is the causative mutation in two patients with craniosynostosis syndrome from two different families, rather than a rare polymorphism in the normal population.

Craniosynostosis are a group of connective disorders characterized by premature fusion of cranial sutures. Three clinically associated craniosynostosis, Crouzon, Jackson-Weiss (JW) and Pfeiffer syndromes (PS), share common clinical features including craniosynostosis, ocular hypertelorism with proptosis and midface hypoplasia (10,16). The craniosynostosis syndromes differ by the absence of limb abnormalities in Crouzon syndrome and presence of limb abnormalities in PS and JW. Previously, clinical distinction between PS and JW was determined by the presence of broad great toes with medial deviation and tarsal-metatarsal coalescence without hand anomalies in JW (9,17). Craniosynostosis is associated with a variety of ophthalmic abnormalities. For example, patients with craniosynostosis may have anterior segment dysgenesis and present Peters anomaly, which is characterized by microphthalmia, microcornea, sclerocornea, glaucoma, chorioretinal coloboma, corneal perforation, and retinal detachment (9). Case 1 in the present study presented with retinal detachment and microcornea. Some cases of Peters anomaly without systemic complications have an autosomal dominant or recessive inheritance pattern. Cases with short limb dwarfism are designated as Peters-plus syndrome, which is currently believed to be autosomal recessive. Additionally, Peters anomaly has been reported in Pfeiffer syndrome (9,17). Therefore, case 1 was diagnosed as Pfeiffer syndrome with Peters-plus syndrome and case 2 was diagnosed as Crouzon syndrome. Surprisingly, the same *FGFR2* mutation can lead to CS, PS or JW phenotype (8,18,19).

To date, ~16 genes associated with craniosynostosis have been identified. Although craniosynostosis can be classified into several different syndromes based on clinical phenotypes, correlation between genotype and phenotype has not yet been established. In the majority of cases, pathogenic *FGFR* mutations are missense, which all confer gain-of-function to the mutated protein; some mutations are highly recurrent (10,20). The c.833G>T (C278F) was identified in the *FGFR2* gene in two different Chinese patients with different kinds of syndromic craniosynostosis in the current study; however, it occurred at a hotspot for mutation, which has been reported in other ethnic groups (17,18).

It is clear that the same mutation may result in heterogeneous phenotypes, as the C278F mutation in *FGFR2* can lead to Crouzon syndrome and Pfeiffer syndrome. The mechanism by which the same genotype causes different phenotypes remains unclear. One potential explanation may be due to other unidentified polymorphisms within *FGFR2*. Cys 278 is part of the disulfide bridge that stabilizes the IgIII loop in all FGFR proteins and is the most conserved amino acid of the extracellular domains in the Ig superfamily. The loss of Cys 278 destroys the disulfide bond in the Ig-III domain, leaving the other cysteine at position 342 potentially unpaired to participate in intermolecular disulfide bonding, which potentially results in a ligand-independent dimerization of receptor molecules and, thus, constitutive activation (21,22). Similar hypotheses apply to mutations affecting Cys 342 in other Crouzon syndrome patients (23,24). Mutations of either Cys 342 or Cys 278 can cause a severe craniosynostosis phenotype. In addition, although the same C278F mutation in *FGFR2* can lead to different types of craniosynostosis, the treatments are similar and surgery is usually required in severe cases (25-27).

In summary, this study identified an *FGFR2* in two Chinese patients with syndromic craniosynostosis. The finding expands the reported mutation spectrum of *FGFR2*, and is of great value for genetic counseling and prenatal diagnosis in families with syndromic craniosynostosis.

Acknowledgements

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