Molecular analysis of *FGFR 2* and associated clinical observations in two Chinese families with Crouzon syndrome

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Abstract. Crouzon syndrome, a dominantly inherited disorder and the most common type of craniosynostosis syndrome, is caused by mutations in the fibroblast growth factor receptor 2 (*FGFR 2*) gene, and characterized by craniosynostosis, shallow orbits, ocular proptosis, midface hypoplasia and a curved, beak-like nose. The purpose of the present study was to investigate the fibroblast growth factor receptor 2 *(FGFR 2)* gene in two Chinese families with Crouzon syndrome and to characterize the associated clinical features. Two families underwent complete ophthalmic examination, and three patients in two families were diagnosed with Crouzon syndrome. Genomic DNA was extracted from leukocytes of peripheral blood samples, which were collected from the family members and 200 unrelated control subjects from the same population. Exons 8 and 10 of the *FGFR 2* gene were amplified using polymerase chain reaction analysis and were directly sequenced. Ophthalmic examinations, including best-corrected visual acuity, slit-lamp examination, fundus examination and Computerized Tomography scans, and physical examinations were performed to exclude systemic diseases. These patients were affected with shallow orbits and ocular proptosis, accompanied by midface hypoplasia, craniosynostosis, strabismus or papilloedema, with clinically normal hands and feet. A heterozygous *FGFR 2* missense mutation, c.811-812insGAG (p.273insGlu) in exon 8 was identified in the affected individual, but not in the unaffected family members or the normal control individuals in family 1. In family 2, another heterozygous *FGFR 2* missense mutation, c.842A>G (P.Tyr281Cys or Y281C), in exon 8 was identified in the affected boy and his mother, but not in the unaffected family members or the normal control individuals. Although *FGFR 2* gene mutations and polymorphisms have been reported in various ethnic groups, particularly in the area of osteology, the present study reported for the first time, to the best of our knowledge, the identification of two novel *FGFR 2* gene mutations in Chinese patients with Crouzon syndrome.

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Introduction

Crouzon syndrome, a type of craniosynostosis, is a dominantly inherited disorder, which is predominantly caused by mutations in the fibroblast growth factor receptor 2 (*FGFR 2*) gene and is characterized by craniosynostosis, shallow orbits, ocular proptosis, midface hypoplasia and a curved, beak-like nose (1-4).

The most common genetic mutations of *FGFR 2*, located at chromosome 10q26, are localized at exons IIIa (exon 8) and IIIc (exon 10) that encode the extracellular immunoglobulin-like III (IgIII) domain of the protein (5-7).

Although Crouzon syndrome is inherited as an autosomal dominant trait, several cases present as *de novo* mutations arising from unaffected parents (8-11). The present study reported the mutational analyses of three patients with Crouzon syndrome from separate families at the gene level and reported its associated clinical features, resulting in the identification of two heterozygous mutations.

Patients and methods

Crouzon syndrome families. Two probands in two Chinese families were diagnosed as having Crouzon syndrome at the Zhongshan Ophthalmic Center (Guangzhou, China). The proband of family 1 (Fig. 1) was a one-year-old girl and was the second child of healthy unrelated parents, vaginally delivered maturely at 39 weeks. The second proband, in family 2, (Fig. 2) was a three-year-old boy and was also the second child of his family. For the present study, ophthalmic examinations of these two families were performed, as follows: Visual acuity was examined using the Early Treatment Diabetic Retinopathy Study chart (Precision Vision, LaSalle, IL, USA). Anterior segment images were captured using a BX 900 Slit Lamp (Haag-StreitAG, Köniz, Switzerland). Anterior segment measurements were obtained using Pentacam® HR version 70700 (OCULUS Optikgeräte GmbH, Wetzlar, Germany). Fundus imaging was performed using a Heidelberg Retina Angiograph (Heidelberg Engineering GmbH, Heidelberg, Germany). In addition, physical examinations, including blood examination, a urine test, electrocardiogram, chest X-ray, blood biochemistry test, blood lipid and blood coagulation tests, were performed to exclude systemic diseases. The study was approved by the ethics committee of Zhongshan Ophthalmic Center, Sun Yat-Sen University.

Sample collection. The affected families were identified at Zhongshan Ophthalmic Center. In addition, 200 subjects from the same population, but without diagnostic features of Crouzon syndrome, were recruited to serve as normal controls. Informed consent was obtained from all participating individuals and, in accordance with the principles of the Declaration of Helsinki, venous blood samples were collected from the two families and 200 controls for genomic DNA extraction from peripheral blood leukocytes, using the a DNA extraction kit (Qiagen, Inc., Valencia, CA, USA) with standard protocols.

Mutation detection. Exons 8 and 10 of the *FGFR 2* gene were amplified using polymerase chain reaction (PCR) analysis with the following primers: *FGFR2-8* (IIIa), forward 5'-GGT CTCTCATTCTCCCATCCC-3', reverse 5'-CCAACAGGA AATCAAAGAACC-3' (product size, 325 bp); *FGFR2-10* (IIIc), forward 5'-CCTCCACAATCATTCCTGTGTC-3', reverse 5'-ATAGCAGTCAACCAAGAAAAGGG-3' (product size, 257 bp) (9-10) (Beijing Genomics Institute, Guangzhou, China). Briefly, PCR was performed with a 50 μl reaction volume with 2 μl each primer, 2 μl DNA, 25 μl buffer mix and 19 μl H_2O. All reagents used for PCR were purchased from (Takara Bio, Inc., Tokyo, Japan).

The cycling profile included one cycle at 94˚C for 5 min, followed by 40 cycles at 94˚C for 45 sec, 61˚C for 45 sec, and 72˚C for 45 sec, with one cycle at 72˚C for 10 min. The PCR products were sequenced from both directions using an ABI3730 automated sequencer (PE Biosystems, Foster City, CA, USA). The sequencing results were analyzed using Chromas (version 2.3; Technelysium Pty., Ltd., Brisbane, QLD, Australia), and they were compared with the reference sequences in the database at the National Center for Biotechnology Information (NCBI; NC_000010).

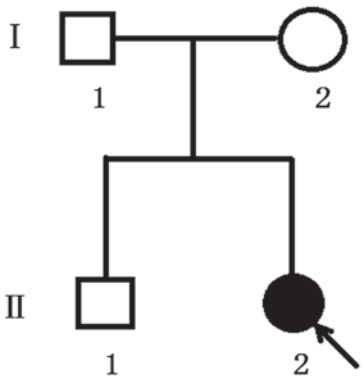

Figure 1. Pedigree of a Chinese family (family 1) with Crouzon syndrome. Squares denote males, circles denote females. Shaded symbol indicates ophthalmologist-confirmed Crouzon syndrome. Arrow indicates the proband.

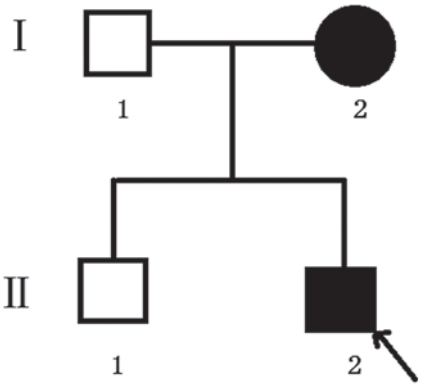

Figure 2. Pedigree of a Chinese family (family 2) with Crouzon syndrome. Squares denote males, circles denote females. The shaded symbols indicate ophthalmologist-confirmed Crouzon syndrome. The arrow indicates the proband.

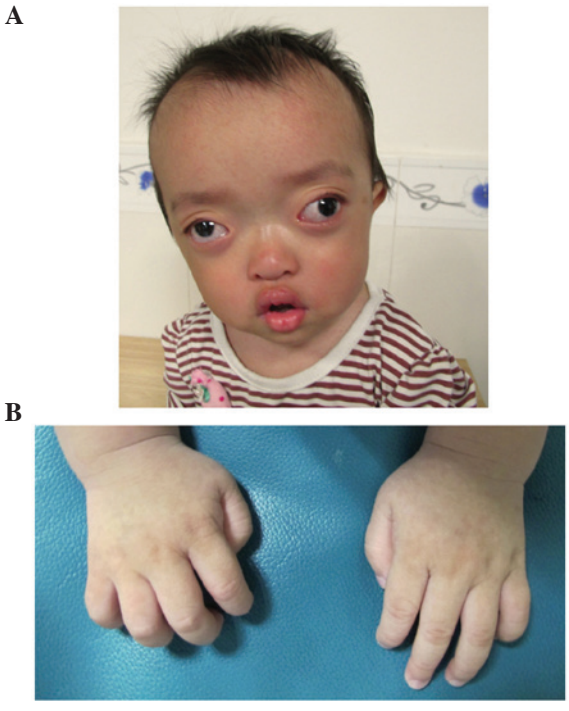

Figure 3. Images of the proband of family 1. (A) Proband of family 1 exhibited ocular proptosis, strabismus and midface hypoplasia. (B) Clinical image of the hands of the proband of family 1.

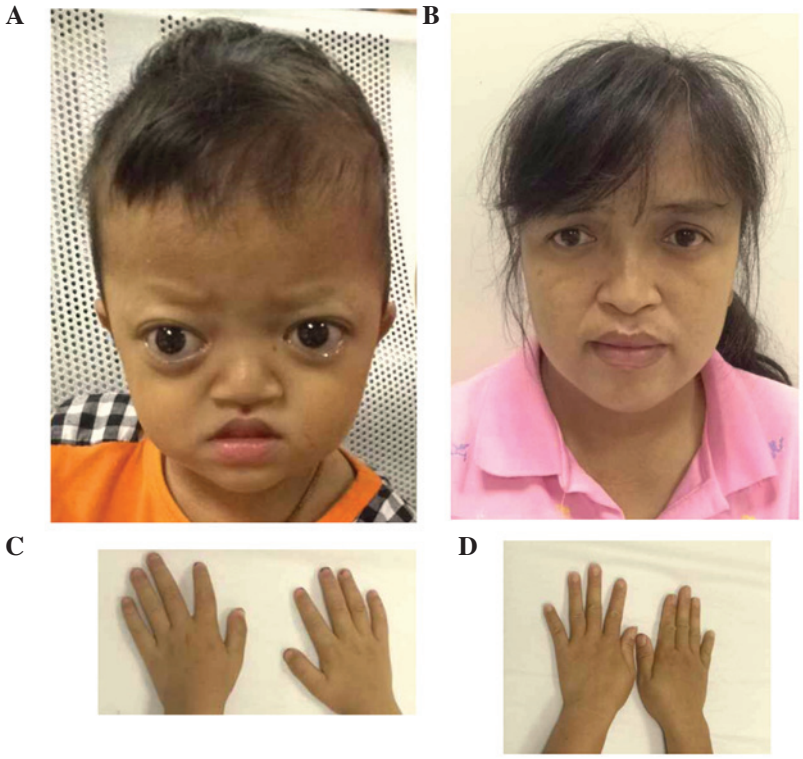

Figure 4. Images of the proband of family 2 and his mother. (A) Proband of family 2 exhibited ocular proptosis and midface hypoplasia. (B) Mother of the proband of family 2, who presented with a similar appearance to her son. (C) Clinically normal hands of the proband of family 2. (D) Clinically normal hands of the mother of the proband of family 2.

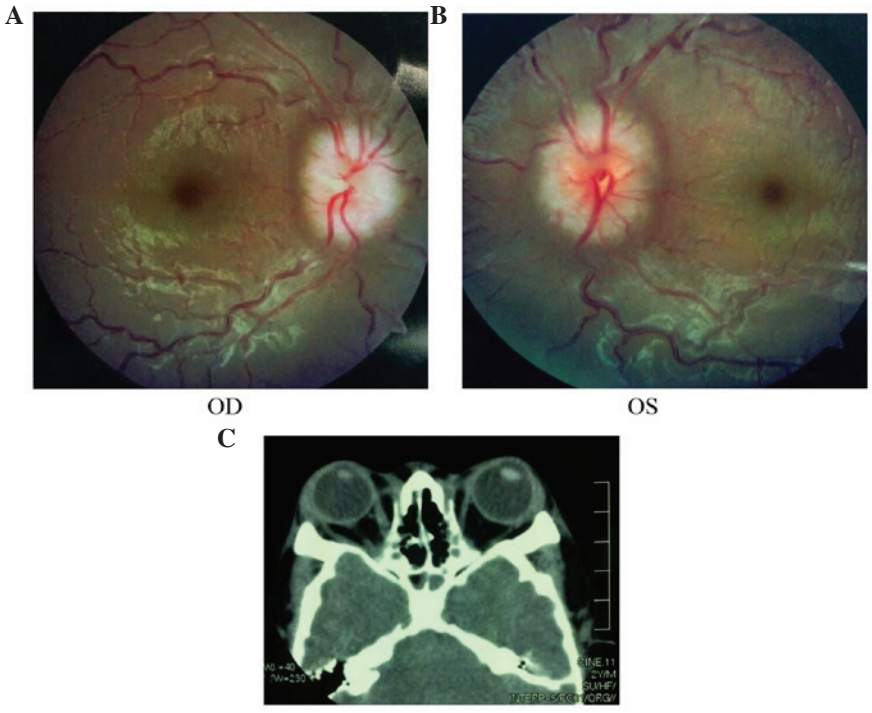

Figure 5. Images of the examination of the proband of family 2. (A and B) Papilloedema of both eyes were noted. (C) Shallow orbits were found on Computed Tomography examination. OD, right eye; OS, left eye.

Results

Clinical data. The Chinese families included in the present study were from the southern area of China. In two successive generations, one individual was found to have a congenital disease. The proband of family 1 was referred by her local pediatrician at the age of 2 months, owing to concerns about her elongated head shape and a possible diagnosis of sagittal

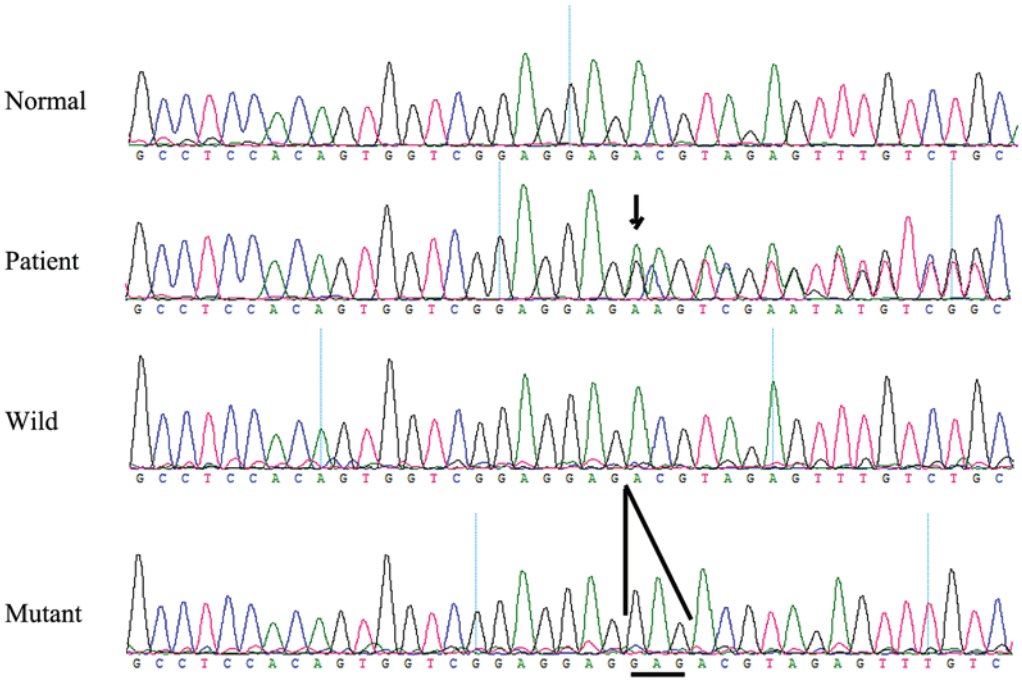

Figure 6. DNA sequence of a region of the *FGFR 2* gene in the affected and unaffected individuals of family 1. A heterozygous *FGFR 2* missense mutation, c.811-812insGAG (p.273insGlu), in exon 8 was identified in the affected individual, but not in any of the unaffected family members or the normal controls. The mutation caused the insertion of glutamic acid in the position of 273 of *FGFR 2*. Patient; c.811-812insGAG (p.273insGlu) mutation in exon 8 in the affected individuals; wild, sequence of the normal allele of exon 8 subcloned into the pGEM-T vector (used as a control); mutant, heterozygous missense mutation, c.811-812insGAG (p.273insGlu), in exon 8 in the affected individuals. This mutation caused the insertion of glutamic acid in position of 273 of the *FGFR 2* gene. *FGFR 2*, fibroblast growth factor receptor 2.

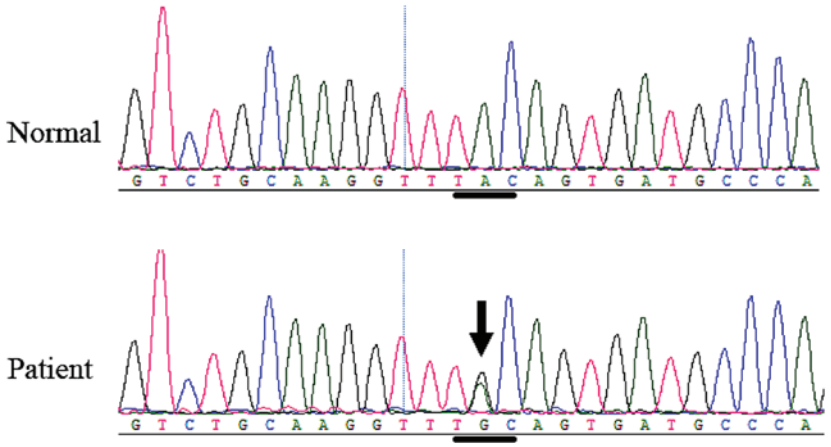

Figure 7. DNA sequence of the reigon of the *FGFR 2* gene in the affected and unaffected individuals of family 2. In family 2, the heterozygous *FGFR 2* missense mutation, c.842A>G (P.Tyr281Cys or Y281C), in exon 8 was identified in the affected boy and his mother, but not in any of the unaffected family members or the normal control individuals. The mutation caused the tyrosine 281 codon to change to a cysteine codon (arrow). *FGFR 2*, fibroblast growth factor receptor 2.

synostosis. The proband had otherwise been developing well with normal feeding and steady weight gain following birth. There was no history of learning difficulties or genetic problems in the family. The parents considered her development to be equivalent to her age-matched peers.

On examination, the proband exhibited shallow orbits and ocular proptosis, accompanied by midface hypoplasia, craniosynostosis and a curved, beak-like nose (Fig. 3A), with clinically normal hands and feet (Fig. 3B). The proband also showed exotropia of the left eye, although the corneas were normal in size and transparency, and the lenses were positioned normally and remained clear. As the child was just 1 year old, it was not possible to assess visual acuity, however, visual tracking was present. The parents and the old brother of the proband showed normal visual acuity and eye examinations.

The proband of family 2 had midface hypoplasia and craniosynostosis (Fig. 4A). His mother's condition was less severe, with normal visual acuity (Fig. 4B). The proband and his mother had clinically normal hands and feet (Fig. 4C and D). The proband had papilloedema of both eyes (Fig. 5A and B) and had a history of intracranial hypertension (1 year previously). The Computed Tomography examination revealed

shallow orbits (Fig. 5C). No abnormalities were detected in the corneas or lens.

Mutation screening. A heterozygous *FGFR 2* missense mutation, c.811-812insGAG (p.273insGlu), in exon 8 (Fig. 6) was identified in the affected individual, but was not identified in any of the unaffected family members or the normal control individuals. This mutation causes the insertion of glutamic acid in position of 273 of the *FGFR 2 gene*. In family 2, another heterozygous *FGFR 2* missense mutation, c.842A>G (P.Tyr281Cys or Y281C), in exon 8 (Fig. 7) was identified in the affected boy and his mother, but was not identified in any of the unaffected family members or the normal control individuals. The mutation causes the tyrosine 281 codon to change to a cysteine codon.

Discussion

In the present study, two mutations in exon 8 of the *FGFR 2* gene were identified, which were associated with Crouzon syndrome: A *de novo* mutation, c.811-812insGAG (p.273insGlu), in family 1 and a familial mutation, c.842A>G (p.Tyr281Cys or Y281C), in family 2. These mutations, rather than representing a rare polymorphism in the normal population, were the causative mutations in these two families.

The c.811-812insGAG (p.273insGlu) mutation was identified for the first time in the *FGFR 2* gene in Chinese patients, and, to the best of our knowledge, has not previously been reported either in China or elsewhere. However, the c.842A>G (P.Tyr281Cys or Y281C) mutation, identified in family 2, has been previously reported outside of China (12). Although Crouzon syndrome is inherited as an autosomal dominant trait, several cases are sporadic and present as *de novo* mutations arising from unaffected parents, as observed in family 1 in the present study.

The most common genetic mutation of *FGFR 2* has been localized in the third Ig-like domain and in the flanking linker regions, coded by exons IIIa (exon 8) and IIIc (exon 10) (13,14). The two mutations found in the two families in the present study were in exon IIIa. The mutations may disrupt the intra-Ig domain disulfide bond, thus leading to changes in FGF signaling, and may induce the activation or downregulation of FGFR 2 (15-20).

Okajima *et al* (21) reported that certain patients with an *FGFR 2* mutation may have Peters anomaly, optic nerve hypoplasia, scleralization of the cornea and corectopia in craniosynostosis syndromes. In the present study, the proband of family 2 had papilloedema, which expands the clinical manifestations of Crouzon syndrome.

In conclusion, the present study identified two mutations of *FGFR 2* in two Chinese families with Crouzon syndrome. This finding expands the mutation spectrum of *FGFR 2*, and is useful and valuable for genetic counseling and prenatal diagnosis in families with Crouzon syndrome with ocular disorders.

Acknowledgements

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