

A novel Fibroblast Growth Factor Receptor 2 (FGFR2) mutation associated with a mild Crouzon syndrome

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Introduction

Crouzon syndrome (CS) is an autosomal dominant disorder characterised by premature fusion of cranial sutures leading to the clinical condition of craniosynostosis, which is usually associated with skull distortion. Over the past years several mutations in fibroblast growth factor receptor (FGFR) genes 1, 2, 3 have been identified in both syndromic and non-syndromic craniosynostosis; the pathological phenotypes associated with these mutations are subject to a high degree of clinical variability. Most of these mutations have been described in the FGFR2 gene and result in Apert, Crouzon, Jackson-Weiss or Pfeiffer syndromes. Although the mutations associated with these syndromes and their clinical consequences are known, they result in a wide range of phenotypic expressions that make the genotype-phenotype correlation more difficult to understand. Here we describe a novel FGFR2 mutation associated with a particularly mild Crouzon phenotype.

Clinical summary

A 28-year-old, right-handed, male graduate student was referred to us because of a worsening headache characterized by constant band-like pain, tightness and pressure around the forehead and the back of the

head. He was the second child of non-consanguineous parents. Detailed questioning indicated that pregnancy and delivery had been unremarkable. He did not refer any disease and his history was negative for other symptoms. When asked specifically, he said he closely resembled his father, who died at the age of 43 in an accident and had been clinically diagnosed with Crouzon syndrome (CS). The physical inspection revealed mandibular prognathism, orbital hypertelorism and ocular proptosis, with no hand or foot abnormalities. The neurological examination and a complete neuropsychological evaluation did not reveal any abnormalities. The patient's Wechsler Adult Intelligence Scale (WAIS) score was 102; the Minnesota Multiphasic Personality Inventory (MMPI) revealed depressive features with anxious restlessness.

Laboratory testing results

After signing a written informed consent, the patient underwent several laboratory examinations. Magnetic resonance imaging (MRI) study of the brain (Fig. 1A-C), including cerebrospinal fluid dynamics, as well as EEG, ophthalmologic examination and visual electrophysiological tests (ERG, PERG, VEP), were all normal. The MRI exam revealed a thinning of the skull. A cranial-facial three-dimensional CT scan showed brachyturri-

cephaly and maxillary hypoplasia (Fig. 2A,B). No other abnormalities were observed at the radiographic skeletal survey. No auditory abnormalities were found at the hearing examination. The cardiac evaluation was unremarkable. Because of a suspect of hereditary craniofacial dysostosis, a molecular analysis was performed on genomic DNA isolated from the patient's peripheral blood lymphocytes. Sequencing of *FGFR2* exon 8 showed a sequence frameshift caused by a heterozygous 18 nucleotide duplication located between nucleotides 808 and 825 (Fig. 3), resolved only after cloning and re-

quencing the two alleles separately. This insertion does not change the open reading frame but leads to a protein that is 6 amino acids longer than normal. The proband's sister did not show either facial dysmorphism or neurological deficits, and her DNA screening for the specific mutation was negative.

FGFR2 mutation analysis

FGFR2 exons 8 and 10 were amplified with one unit of EurobioTaq DNA polymerase (Eurobio) using primers and PCR protocols developed in our laboratory. The cycling conditions were 30 s at 94°C, 30 s at

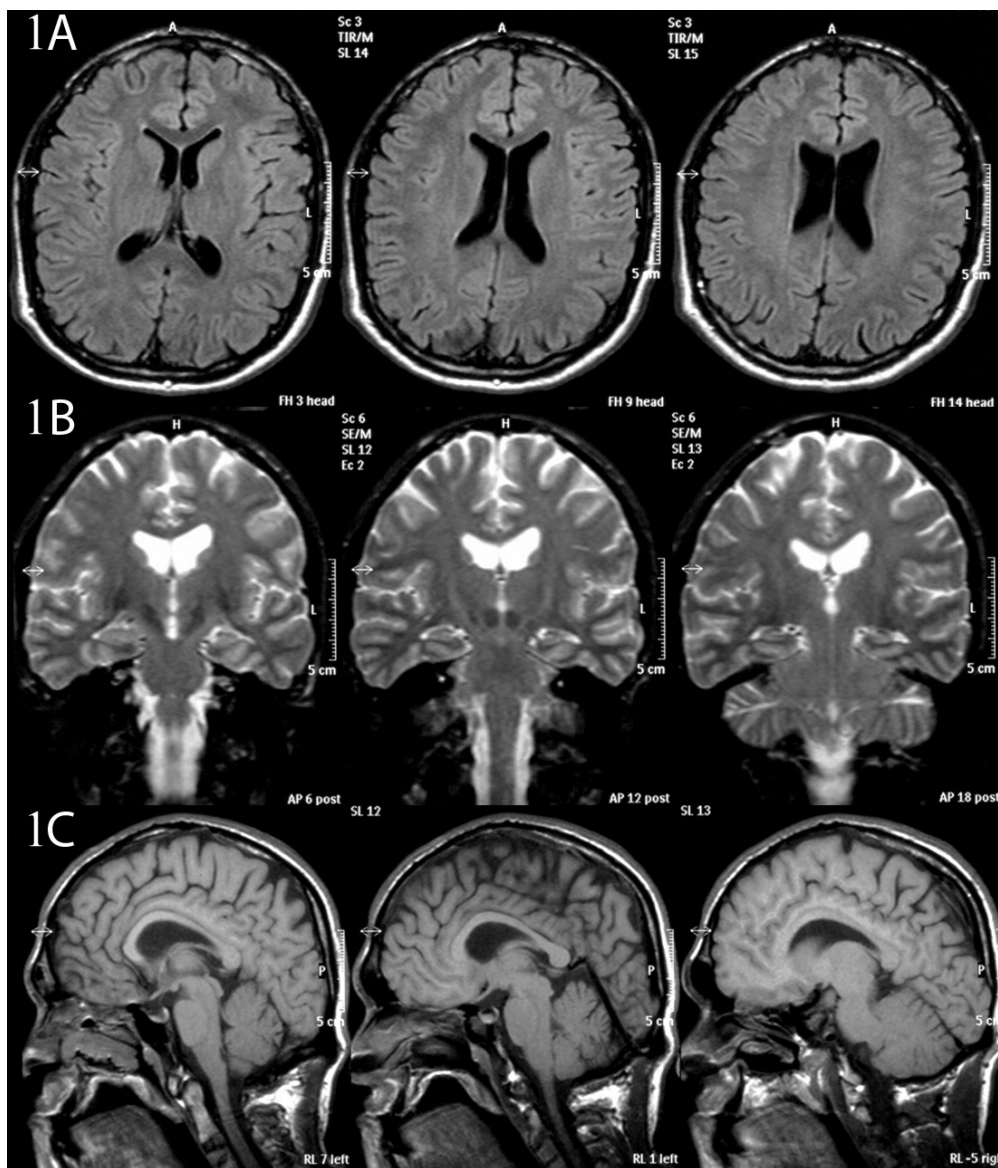


Fig. 1. - MR imaging brain study. Axial T2 FLAIR (A), Coronal T2 (B), Sagittal T1-weighted (C) images: thinning of the skull, normal brain anatomy, symmetrical ventricles, no parenchymal pathological changes.

60°C, 30 s at 72°C per 35 cycles. The PCR products were directly sequenced in both directions using a Big-Die-Terminator Cycle Sequencing kit (Applied Biosystems). Sequencing reactions were purified with Multiscreen Filter Plates (Millipore Corporation, Bedford, USA) and run on a 3130xl Genetic Analyzer (Applied Biosystems). The exon 8 amplicon containing the sequence frameshift, was cloned in the pCR 2.1-TOPO vector using the TOPO TA Cloning kit (Invitrogen BV, Groningen, NL). The two separated alleles were sequenced as described above.

Discussion

Crouzon syndrome is an autosomal dominant cranio-facial dysostosis, with a high penetrance transmission, caused by mutations in the gene encoding the FGFR2, at 10q26. The mutations have been located mainly within two exons, 8 and 10, which encode the extracellular IgIIIa/c domain of the protein (Reardon et al., 1994; Passos-Bueno et al., 1999). The characteristic facial features of CS, i.e. brachycephaly, maxillary hypoplasia and prominent mandible, are associated with extraskeletal manifestations, including ocular signs (exophthalmos and strabismus, ocular anterior chamber abnormalities, opaque cornea, thickened irides and ciliary bodies). Moreover, conductive hearing loss, usually resulting from ear canal malformations, particularly impaired Eustachian tube function, associated with an abnormal configuration of the nasopharynx and cleft palate, has been found in patients with CS. Sensorineural hearing loss has also been reported (Orvidas et al., 1999). Moreover, several bone disease-related neurological complications, such as hydrocephalus, papilledema, optic nerve atrophy, mental deficiency, chronic tonsillar herniation (Chiari I) and syringomyelia, have been described. Recent neurobiological findings regarding selective white matter involvement (hypoplasia/agenesis of the corpus callosum, absent septum pellucidum, ventriculomegaly) even suggest that these brain malformations in syndromic craniosynostoses are much more likely to be a primary neurological disorder, thus shifting the pathophysiological model from a mechanical to molecular signalling disturbance (Raybaud et al., 2007). However, a different subset of mutations seems to be associated with non-syndromic craniosynostosis, without facial features

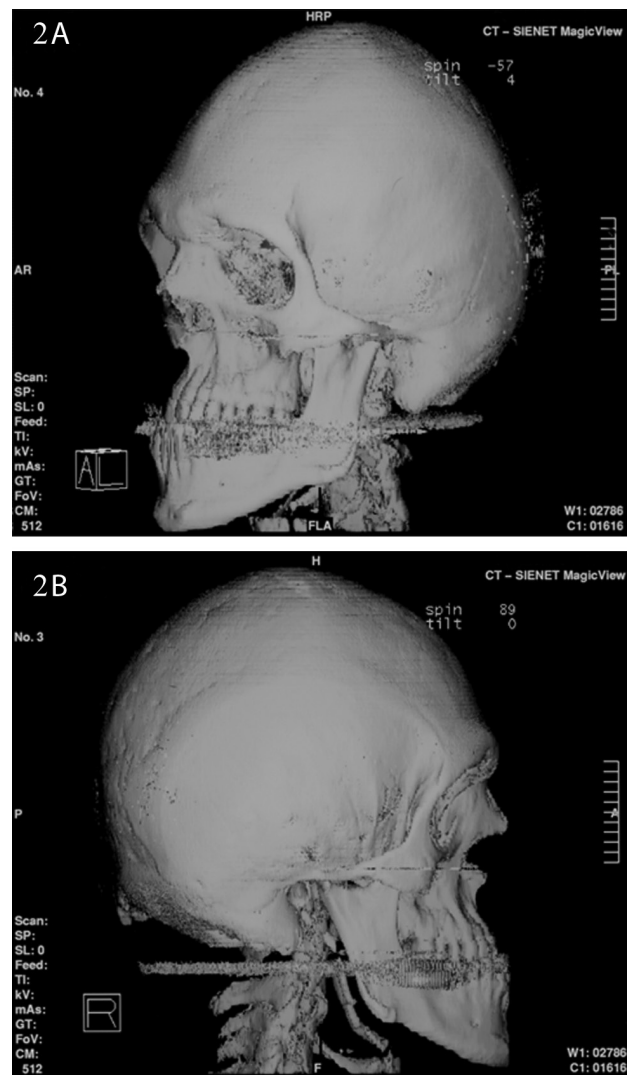


Fig. 2. - Cranial-facial three-dimensional CT. Anterolateral (a) and lateral (b) projections: turricephaly and maxillary hypoplasia.

(de Ravel et al., 2005). Identical mutations in the FGFR2 gene have been reported to cause different phenotypes, even different syndromes (Rutland et al., 1995; Gorry et al., 1995), while a wide range of mutations in FGFR genes produce a variety of overlapping phenotypes that are often difficult to classify (Kan et al., 2002).

Fibroblast growth factors (FGFs) and their receptors (FGFRs) constitute an elaborate signalling system that is involved in the developmental and repair processes of virtually all mammalian tissues. Four FGFR tyrosine kinase receptors are known to date. They are transmembrane proteins consisting of an extracellular ligand-binding domain, composed of three immunoglobulin (Ig)-like domains containing characteris-

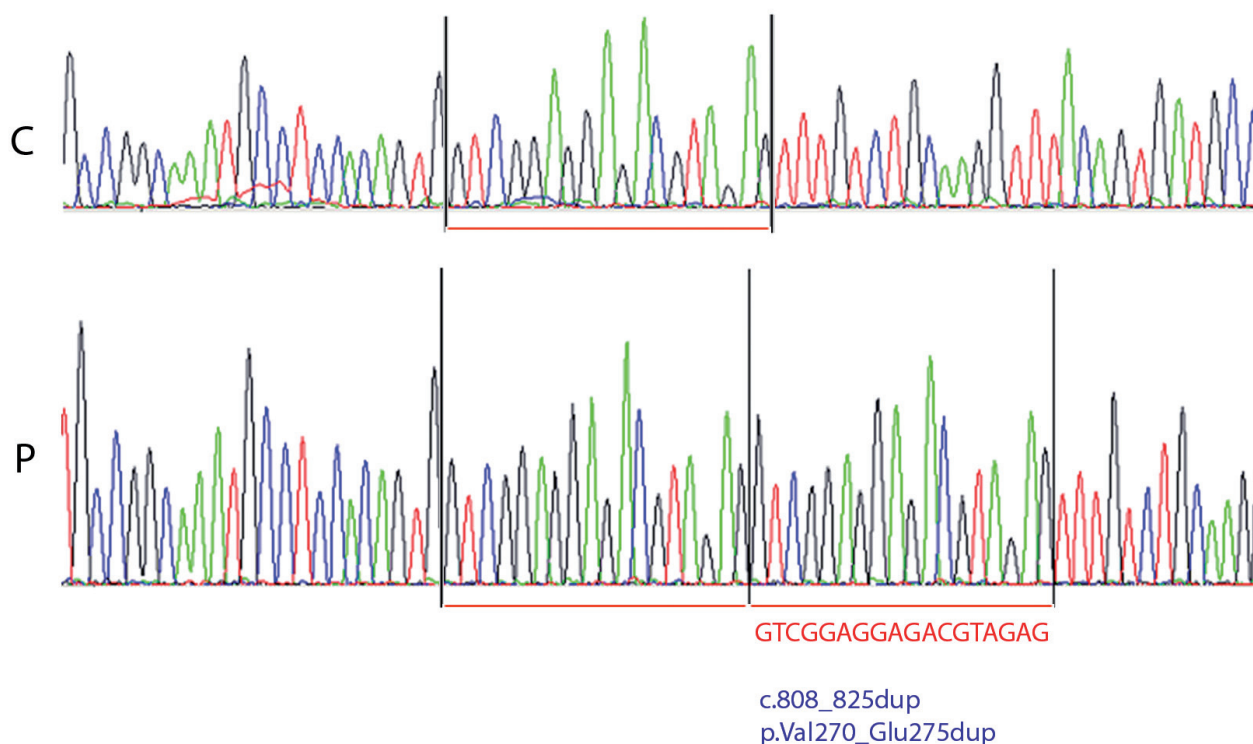


Fig. 3. - Results of molecular analysis. DNA sequencing of FGFR2 exon 8 showed a frameshift caused by a heterozygous insertion defined only by cloning. The sequence of the cloned mutant allele showed an 18 nucleotide duplication in exon 8 (IIIa domain), located between nucleotides 808 and 825. The mutation does not change the open reading frame but leads to a protein that is 6 amino acids longer than normal. P: patient; C: control.

tic cysteine residues, a transmembrane domain and an intracellular domain, which carries the tyrosine kinase activity. Ligand-binding specificity of FGFRs depends on the third extracellular Ig-like domain, which is subject to alternative splicing that generates a variety of receptor isoforms. Three different splice variants termed IgIIIa, IgIIIb and IgIIIc have been identified (Givol et al., 1992; Reuss et al., 2003). The FGFR2c isoform is required for the normal functioning of the osteoblast lineage and cooperates with FGFR3, a regulator of the chondrocyte series, during endochondral osteogenesis. Unregulated FGF signaling during intramembranous ossification, which is usually related to gain-of-function mutations in the FGFR2 gene, is associated with syndromic craniosynostoses, characterised by an association of limb abnormalities and facial dysmorphism with cranial distortion due to premature fusion of calvarial and skull base sutures (Eswarakumar et al., 2004). Moreover, significant brain abnormalities have been reported in all syndromes.

The FGFR2 mutation in the IIIa domain, which we describe and that, to our knowledge, has not been reported before, gives rise to a longer protein (6 amino acids) without changing the open reading frame. This mutation may be considered a possible cause of CS as it is located in a functional domain of the protein, i.e., in the region with the highest impact on FGF receptor binding specificity, in which other mutations related to this syndrome have been reported. Previously described mutations causing craniosynostosis are widely distributed across the FGFR2 protein, yet the majority localize to some amino acids that form the S-S bond in the IgIIIa/IIIc domain, involving the three-dimensional configuration of key structural points of the receptor (Kan et al., 2002). Severe phenotypes have been associated with mutations causing loss or addition of cysteine residues, which form S-S bonds, thereby resulting in the disruption of the protein's structure, dimerization and continuous ligand-independent activation of the receptor (Schaefer et al., 1998; Eswarakumar et al., 2005). The nucleotide

duplication found in our case, in which unpaired cysteine residues are not involved and the relative integrity of the loop in the functional protein binding domain is preserved, might explain the observed milder phenotype. The mechanism underlying receptor activation in non-cysteine mutations, however, remains unclear (Robertson et al., 1998). The genetics of the craniosynostosis syndrome is probably more complex than what its simple autosomal-dominant inheritance pattern suggests. Indeed, other disease-modifying genetic factors controlling the abnormal gain-of-function that accompanies FGFR signalling might play a role (Ito et al., 2005). In addition, hypotheses concerning the role of changes in extracellular matrix composition in the altered osteogenic process have recently been formulated (Carinci et al., 2007). In conclusion, the genotype-phenotype correlation in all these syndromes remains to be clarified.

References

- Carinci P., Becchetti E., Baroni T., Carinci F., Pezzetti F., Stabellini G., Locci P., Scapoli L., Tognon M., Volinia S., Bodo M. Extracellular matrix and the growth factors in the pathogenesis of some craniofacial malformations. *Eur. J. Histochem.*, **51** (Suppl 1): 105-115, 2007.
- de Ravel T.J.L., Taylor I.B., Van Oostveldt A.J.T., Fryns J.P., Wilkie A.O.M. A further mutation of the FGFR2 tyrosine kinase domain in mild Crouzon syndrome. *Eur. J. Hum. Genet.*, **13**: 503-505, 2005.
- Eswarakumar V.P., Horowitz M.C., Locklin R., Morriss-Kay G.M., Lonai P. A gain-of-function mutation of *Fgfr2c* demonstrates the roles of this receptor variant in osteogenesis. *PNAS*, **101**: 12555-12560, 2004.
- Eswarakumar V.P., Lax I., Schlessinger J. Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev.*, **16**: 139-149, 2005.
- Givol D. and Yayon A. Complexity of FGF receptors: genetic basis for structural diversity and functional specificity. *FASEB J.*, **6**: 3362-3369, 1992.
- Gorry M.C., Preston R.A., White G.J., Zhang Y., Shinghal V.K., Losken H.W., Parker M.G., Nwokoro N.A., Post J.C., Ehrlich G.D. Crouzon syndrome: mutations in two spliceforms of FGFR2 and a common point mutation shared with Jackson-Weiss syndrome. *Hum. Mol. Genet.*, **4**: 1387-1390, 1995.
- Ito S., Sekido K., Kanno H., Tanaka M., Yamaguchi K., Yamamoto I. Phenotypic diversity in patients with craniosynostoses unrelated to Apert syndrome: the role of fibroblast growth factor receptor gene mutations. *J. Neurosurg.*, **102** (Suppl 1): 23-30, 2005.
- Kan S., Elanko N., Johnson D., Cornejo-Roldan L., Cook J., Reich E.W., Tomkins S., Verloes A., Twigg S.R.F., Rannan-Eliya S., McDonald-McGinn D.M., Zackai E.H., Wall S.A., Muenke M., Wilkie A.O.M. Genomic screening of fibroblast growth-factor receptor 2 reveals a wide spectrum of mutations in patients with syndromic craniosynostosis. *Am. J. Hum. Genet.*, **70**: 472-486, 2002.
- Orvidas L.J., Fabry L.B., Diacova S., McDonald T.J. Hearing and otopathology in Crouzon syndrome. *Laryngoscope*, **109**: 1372-1375, 1999.
- Passos-Bueno M.R., Wilcox W.R., Jabs E.W., Sertié A.L., Alonso L.G., Kitoh H. Clinical spectrum of fibroblast growth factor receptor mutations. *Hum. Mutat.*, **14**: 115-125, 1999.
- Raybaud C. and Di Rocco C. Brain malformation in syndromic craniosynostoses, a primary disorder of white matter: a review. *Childs Nerv. Syst.*, **23**: 1379-1388, 2007.
- Reardon W., Winter R.M., Rutland P., Pulleyn L.J., Jones B.M., Malcolm S. Mutations in the fibroblast growth factor receptor 2 gene cause Crouzon syndrome. *Nat. Genet.*, **8**: 98-103, 1994.
- Reuss B., von Bohlen und Halbach O. Fibroblast growth factors and their receptors in the central nervous system. *Cell Tissue Res.*, **313**: 139-157, 2003.
- Robertson S.C., Meyer A. N., Hart K.C., Galvin B.D., Webster M.K., Donoghue D.J. Activating mutations in the extracellular domain of the fibroblast growth factor receptor 2 function by disruption of the disulfide bond in the third immunoglobulin-like domain. *Proc. Natl. Acad. Sci. USA*, **95**: 4567-4572, 1998.
- Rutland P., Pulleyn L.J., Reardon W., Baraitser M., Hayward R., Jones B., Malcom S., Winter R.M., Oldridge M., Slaney S.F., Poole M.D., Wilkie A.O.M. Identical mutations in the *FGFR2* gene cause both Pfeiffer and Crouzon syndrome phenotypes. *Nat. Genet.*, **9**: 173-176, 1995.
- Schaefer F., Anderson C., Can B., Say B. Novel mutation in the FGFR2 gene at the same codon as the Crouzon syndrome mutations in a severe Pfeiffer syndrome type 2 case. *Am. J. Med. Genet.*, **75**: 252-255, 1998.