A novel mutation (a886g) in exon 5 of FGFR2 in members of a family with Crouzon phenotype and plagiocephaly

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Abstract

We identified a novel mutation in members of a family with signs of Crouzon syndrome and plagiocephaly. In affected members of the family an A→G transition was found at position 886 in exon 5 of the fibroblast growth factor receptor 2 (FGFR2) gene. The base change results in the replacement of a lysine by glutamic acid in Ig-like loop III of FGFR2. The unusual finding of plagiocephaly in these Crouzon patients may either be the result of the type of mutation or because of genetic and environmental factors that affect the phenotype in addition to the mutated FGF receptor.

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Crouzon syndrome is a clinically defined craniofacial dysostosis characterised by ocular proptosis owing to shallow orbits, hypertelorism, and craniosynostosis. Craniosynostosis is commonly caused by premature closure of the coronal, lambdoid, and sagittal sutures. Neurological symptoms observed in Crouzon syndrome include mental retardation, seizures, and optic atrophy.1

Crouzon syndrome is transmitted as an autosomal dominant trait with variable expression. Molecular genetic analyses have identified mutations in the gene coding for fibroblast growth factor receptor 2 (FGFR2) on chromosome 10 (10q26) as the underlying cause of the disorder.2 All mutations observed in Crouzon syndrome to date have been detected in the two exons (exons 5 and 7) of the gene that encodes the Ig-like chain III (IIIa and IIIc) of the receptor.3-7 The mutations probably interfere with normal FGF binding.

Mutations in the FGFR2 gene have also been observed in the clinically defined craniosynostosis syndromes of Apert, Pfeiffer, and Jackson-Weiss.8-13 With the possible exception of Apert syndrome,4 14 it is not possible to correlate the type of FGFR2 mutation with the clinical condition. In fact, identical mutations have been described in Crouzon and Pfeiffer syndromes and in Crouzon and Jackson-Weiss syndromes.8 10 Thus the clinically distinct syndromes are extremes of a spectrum of cranial malformations with the eponymous syndromes at opposite ends of this spectrum.

Here we describe a novel mutation in exon 5 of FGFR2 in a family with Crouzon syndrome and plagiocephaly. Since plagiocephaly is not normally a finding in clinically defined Crouzon syndrome, the present study supports the idea that eponymous craniosynostosis syndromes are phenotypic extremes of FGFR2 mutations rather than nosological entities.

EDTA blood was obtained from all subjects shown in the pedigree (fig 1) except III.1 and DNA was extracted according to standard procedures. III.1 was diagnosed prenatally on DNA extracted from amniotic fluid cells. Despite the detection of a mutation, the parents decided against termination of pregnancy. Primers used for the amplification of exon 5 of FGFR2 were those described by Slaney et al15 (primer A) and Park et al16 (primer B). Single strand conformation polymorphism (SSCP) analysis17 of the exon 5 amplification products was performed as described previously.18 Amplification products from those members of the family that had shown a band shift on SSCP analysis were sequenced directly using sequenase (Amersham) according to standard procedures.19 Both strands were sequenced in all cases.

A pedigree of the three generation family described is given in fig 1. There were three affected males and one affected female.

I.1 was a 47 year old man with mild manifestations of Crouzon syndrome, including hypertelorism, divergent strabismus, and midface hypoplasia. At the age of 37 years no sutureal remnants were seen on radiographs, nor were there signs of raised intracranial pressure. He did not have any neurological or ophthalm-
logical abnormalities. Both hands and feet were normal.

II.1 was the 21 year old daughter of II.1 with severe manifestations of Crouzon syndrome. She developed headaches and seizures at 4 years of age. Bilateral optic atrophy and right amaurosis were first observed at 7 years. Cranial findings at the age of 7 included hypertelorism, mild proptosis, brachycephaly, and discrete anterior right plagiocephaly. She had midface hypoplasia and a long philtrum. Radiography showed pansynostosis, increased digital markings, and fusion of cervical vertebrae 2 and 3. Surgery was performed at the age of 7 to alleviate increased intracranial pressure. Seizures recurred at 13 years. Mental performance was normal. There were no abnormalities of either hands or feet.

II.2 was the 9 year old brother of II.1 with severe manifestations of Crouzon syndrome. He was first seen at 1 year and presented with mild anterior plagiocephaly. Radiography showed right unilateral synostosis of the coronal suture. At this stage, the remaining sutures were normal. At 5 years bilateral optic atrophy was diagnosed. Pansynostosis was detected on radiography. Owing to severely increased intracranial pressure, surgery was performed. He did not develop seizures and his psychomotor development was normal. At present, hypertelorism, mild proptosis, and midfacial hypoplasia are striking. There were no abnormalities of either hands or feet. His big toes were somewhat broadened (fig 2).

II.3 was the unaffected sister of II.1 and II.2. III.1 was the 6 month old son of II.1. At 6 weeks of age he presented with severe dolichocephaly, anterior plagiocephaly, proptosis, and hypertelorism (fig 3).

Radiography showed premature closure of the sagittal and the right coronal suture resulting in right anterior plagiocephaly. At surgery at 5 months of age, synostosis was found of both coronal sutures in addition to the sagittal suture. There were early signs of premature closure of the lambdoid sutures. Intracranial pressure was slightly increased. There were no neurological abnormalities. Hands and feet were normal.

SSCP analysis of exon 5 showed band shifts in all affected but not in unaffected members of the pedigree in fig 1 (not shown). An A→G transition at position 886 (codon 292) was found by sequencing of exon 5 in the affected subjects (fig 4). Both the wild type and the mutated nucleotide were equally pronounced on cycle sequencing in all affected subjects. There is no evidence of mosaicism in any of the patients. The base change results in the replacement of a lysine by glutamic acid in the first third of Ig-like chain III of FGFR2. The exchange of the basic amino acid lysine for the acidic glutamic acid probably alters structural integrity and thus the function of the receptor.

Although the mutation at codon 292 described here has not previously been recognised in autosomal dominant craniosynostosis, many point mutations at different positions of exons 5 and 7 of FGFR2 are known. These two exons code for Ig-like chain III of FGFR2 that is required for ligand binding. A muta-
tion in exon 7 at amino acid position 342 has been most frequently observed in FGFR associated craniosynostosis.\textsuperscript{4} It results in the replacement of a cysteine by another amino acid, thus interfering with the formation of a disulfide bridge within the receptor molecule. This in turn affects the structure of the receptor as was shown by molecular modelling.\textsuperscript{22} Alteration of the receptor might either interfere with ligand binding or result in constitutive activation of the receptor. Support for the latter notion comes from experiments examining the common FGFR2 mutation at amino acid position 342 in a \textit{Xenopus} oocyte system. Here constitutive activation of the mutated receptor was found that resulted in the induction of mesoderm even in the absence of FGF.\textsuperscript{23}

Plagiocephaly is not a finding in clinically defined Crouzon syndrome, which is characterised by symmetrical premature fusion of sutures. The asymmetrical synostosis of the coronal suture observed in the present patients may be the result of the phenotypic effect of the novel mutation in FGFR2 described here. According to this notion the mutation would give rise to a milder phenotype. This, however, applies to patient I.1 only. The remaining patients were quite severely affected and required surgery to alleviate increased intracranial pressure. Therefore, we suggest that in addition to the type of mutation, additional genetic (for example, determinants of side of premature closure of coronal suture) and environmental factors cause the unusual findings in the present family. Support for this notion comes from the observation of great phenotypic variation in the manifestations of FGFR associated craniosynostosis even within affected members of the same family.\textsuperscript{24}

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