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Clinical Report

Clinical Hypochondroplasia in a Family Caused by a Heterozygous Double Mutation in *FGFR3* Encoding GLY380LYS

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In classical achondroplasia (Ach), a glycine residue is replaced by an arginine at codon 380 in exon 10 of the fibroblast growth factor receptor 3 gene (*FGFR3*). Here we report on a mother and daughter with hypochondroplasia (Hch) caused by a new heterozygous double mutation (1138_1139GG > AA) at the same codon 380, but encoding a lysine instead of the usual arginine. Previous functional assays of these codon 380 amino acid substitutions demonstrated a lesser activation of receptor signaling by lysine compared to arginine [Webster and Donoghue, 1996; EMBO J 15:520–527]. This could explain the milder phenotype observed in our patients. Several other rare double mutations were previously described in both *FGFR2* and *FGFR3* and interpreted as resulting from positive selection of spermatogonial cells owing to gain-of-function in the encoded protein [Goriely et al., 2005; Proc Natl Acad Sci USA 102:6051–6056]. The present case contributes additional support for this hypothesis. © 2007 Wiley-Liss, Inc.

Key words: achondroplasia; hypochondroplasia; bone dysplasia; double mutation; *FGFR3*

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INTRODUCTION

Achondroplasia (Ach) and hypochondroplasia (Hch) are two common chondrodysplasias caused by heterozygous mutations in different regions of the fibroblast growth factor receptor 3 gene (*FGFR3*) [Francomano et al., 1994; Le Merrer et al., 1994; Rousseau et al., 1994; Shiang et al., 1994; Velinov et al., 1994; Bellus et al., 1995a,b; Prinos et al., 1995; reviewed by Vajo et al., 2000]. Biochemical analysis suggests that these mutations cause a gain-of-function leading to defects of differentiation of the cartilage growth plates of the long bones [Webster and Donoghue, 1996; Raffioni et al., 1998]. From a clinical and radiological point of view Ach and Hch present different characteristics including a well-known gestalt face in Ach and almost normal facial features in Hch [Prinster et al., 1998; Bellus et al., 2000].

In Ach virtually all patients have localized single nucleotide substitutions (either 1138G > A or 1138G > C) at codon 380 in exon 10, located within the transmembrane domain, causing the normal glycine residue to be replaced by an arginine (Gly380Arg) [Bellus et al., 1995a; Bonaventure et al., 1996]. In the milder condition of Hch, mutations are more widespread in *FGFR3* with a hotspot in the tyrosine kinase domain at codon 540 in exon 13 [Bellus et al., 1995b; Prinos et al., 1995; Prinster et al., 1998]. To our knowledge, mutations in the transmembrane domain have not previously been described in patients with Hch.

We observed a family with two female patients (mother and daughter) with radiological and clinical features of Hch, including an almost normal face and a heterozygous double mutation (1138_1139GG > AA) encoding Gly380Lys. This is the first substitution different from the usual arginine described at the

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classical Ach mutation codon. A similar phenomenon whereby rare double nucleotide mutations encode chemically similar amino acids to those arising from the usual single nucleotide mutations has been described for *FGFR2* [Goriely et al., 2003, 2005]. In addition, two different de novo *FGFR3* mutations in *cis* on the same allele were recently reported in a child with severe Ach [Rump et al., 2006]. Our report contributes to the further delineation of the genotype/phenotype correlation, and mechanisms of gain-of-function, associated with the *FGFR3* mutations.

CLINICAL REPORT

The propositus (case 1) was a 31-year-old female first referred to our Genetics Clinic at age 25 years, following lower limb bone lengthening. She was the second child of nonconsanguineous parents, and her mother also had short stature. Her elder sister's height was normal (160 cm). The pregnancy and delivery had been uneventful. On examination she was short (height 140 cm; -3.7 SD) with relative macrocephaly (head circumference 56 cm; >90 <98th centile). She had mild frontal bossing, a low nasal bridge, shortening of the upper limbs, broad hands with mild brachydactyly but no trident position, and moderate lumbar lordosis (Fig. 1A–C). Her intelligence was normal. Radiographs obtained during childhood (aged 2 and 4 years), showed a rather rectangular skull with a slightly prominent forehead, short long bones with metaphyseal flare, broad and short femoral necks, squared shortened iliac bones, unchanged lumbar vertebral interpedicular distances, and long distal fibulae (Fig. 1D–G). The clinical and radiological features suggested a diagnosis of Hch.

Subsequently, her mother (case 2) was examined at the age of 71 years. She was born to parents (now both deceased) of normal stature; her father was 47 years old at the time of her birth. She had three siblings, all female and now deceased, also of normal stature. She presented with more severe dwarfism than her daughter (height 120 cm; -7 SD) but her face was only mildly dysmorphic (slight frontal bossing and low nasal bridge similar to the daughter; Fig. 2A,B). She had broad hands with moderate brachydactyly, but fingers were not in the trident position (Fig. 2C); *genu varum* with bowing of the legs; and moderate hyperlordosis. Radiological studies revealed narrow interpediculate distances of the lumbar vertebrae and anteroposterior shortening of the lumbar pedicles (Fig. 2D–F). A computed tomography scan demonstrated spinal stenosis between L2 and L5 but she refused surgical intervention. These findings were considered consistent with severe Hch.

MOLECULAR ANALYSIS

A molecular study of the *FGFR3* gene was performed. Genomic DNA was extracted from 2–5 ml whole blood from the two affected individuals. Subsequently, exons 9, 10, 13, and 15 of *FGFR3* were

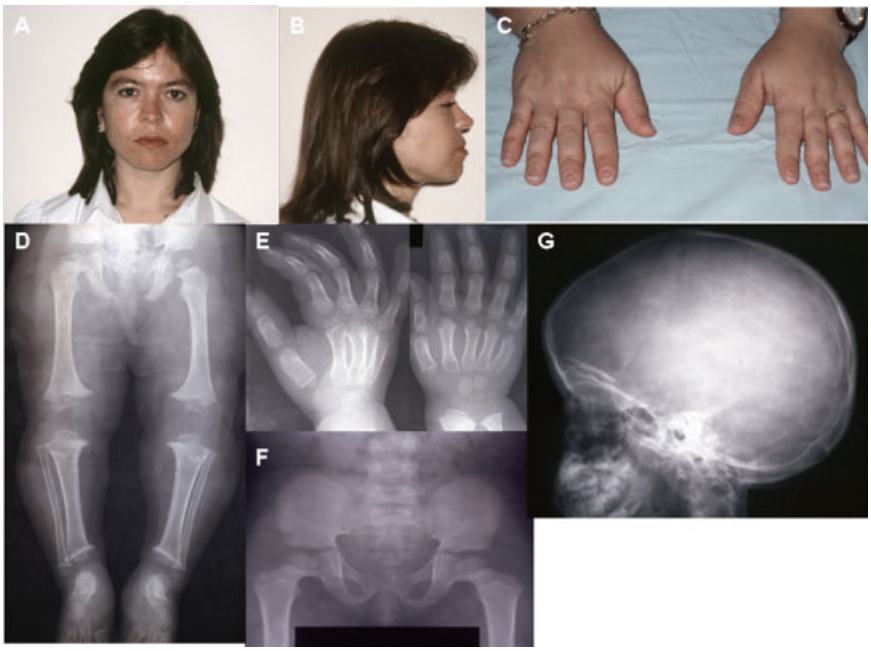

FIG. 1. The proposita, at age 31. **A** and **B**: Normal face presenting only a mild frontal bossing and a low nasal bridge. **C** and **E**: Broad hands with mild brachydactyly but no trident position. **D** and **F**: Radiological features at 2 and 4 years showed short long bones with metaphyseal flare, broad and short femoral neck, squared shortened iliac bones, unchanged interpedicular distance. **G**: Rectangular skull with a slightly prominent forehead. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

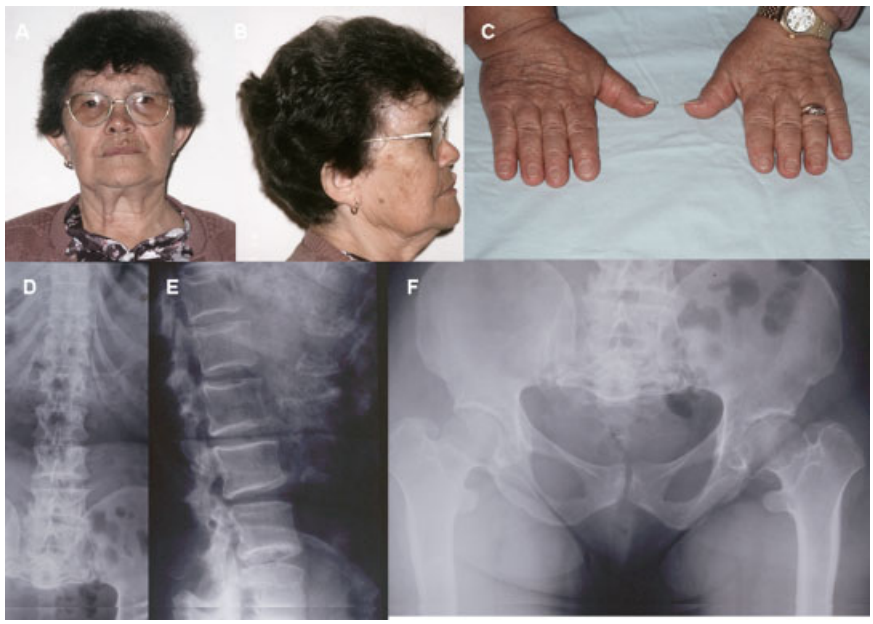

FIG. 2. The proposita's mother, at age 71. **A** and **B**: Her face was not dysmorphic (slight frontal bossing and low nasal bridge). **C**: Broad hands with moderate brachydactyly but not in trident position. **D**, **E**, and **F**: Radiologic narrow interpediculate distance in lumbar vertebrae and anteroposterior shortening of the lumbar pedicles. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

amplified by PCR. The primers pairs used to amplify exon 10 (including the intron-exon boundaries) were 10F (5′-AGGCCAGGCCTCAACGCC-3′) and 10R (5′-GGCAGCTCAGAACCTGGTATC-3′). The sequences of primer pairs used to amplify exons 9, 13, and 15 are available on request. PCR reactions were performed in a 50 µl reaction volume, using 10 µM of each primer, 5 µl of 10× reaction buffer [160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl (pH 8.8), 0.1% Tween-20] (Bioline Ltd, London, UK), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 1 U of BioTaq polymerase (Bioline). A 100 ng aliquot of genomic DNA was denatured for 5 min at 94°C followed by 35 cycles of amplification (45 sec at 95°C; 45 sec at 65°C; 45 sec at 72°C) followed by a 10 min extension of 72°C. PCR products of 220, 256, 453, and 378 bp covering exons 9, 10, 13, and 15, respectively, were purified using Exonuclease I and shrimp alkaline phosphatase and sequenced on an automated sequencer (ABI PRISM^R 3100-Avant), using a BigDye v3,1 sequencing kit (Applied Biosystems, Foster City, CA) with fluorescent dye terminators. Sequence analysis was performed on both strands of the PCR-amplified exons of *FGFR3*.

DNA sequencing of exons 9, 13, and 15 of *FGFR3* was normal, but sequencing of exon 10 identified the same heterozygous double mutation, 1138_1139GG > AA, in both mother and daughter (Fig. 3), indicating that the two changes must be present in *cis* on the same *FGFR3* allele. Hence the substitutions, which involve the canonical Gly380 codon mutated in Ach, encode a heterozygous Gly380Lys change instead of the usual Gly380Arg described in classical Ach.

DISCUSSION

The fibroblast growth factor receptor genes are all characterized by three immunoglobulin-like domains, a single transmembrane segment, and a split tyrosine kinase domain. Dominantly acting mutations in *FGFR1*, *FGFR2*, and *FGFR3* cause a variety of short-limbed dwarfism and craniosynostosis syndromes [Passos-Bueno et al., 1999; Wilkie, 2005]. The complex pattern of genotype/phenotype correlations is related both to the role of the mutated genes in many aspects of development, and to the multiple different mechanisms of gain-of-function in the mutant receptors [Robertson et al., 2000].

In Ach, virtually all patients have a single point mutation at *FGFR3* codon 380, within the transmembrane domain [Bellus et al., 1995a; Bonaventure et al., 1996]. We believe that our family is both, the first described, with a double mutation (1138_1139GG > AA) at the same codon as Ach, and the first case of a transmembrane mutation associated with Hch. Notably, the two affected members showed less severe clinical features than those normally described in Ach and more similar to molecularly confirmed cases of Hch.

The double mutation identified in this family encoded Gly380Lys, therefore a normal glycine residue was replaced by lysine instead of the arginine found in the classical heterozygous Ach. To elucidate the molecular basis of the Ach mutation, transformation assays were performed by Webster and Donoghue [1996] on chimeric Neu/FGFR3 receptor constructs, demonstrating constitutive activation of receptor signaling by the Gly380Arg mutation. These results

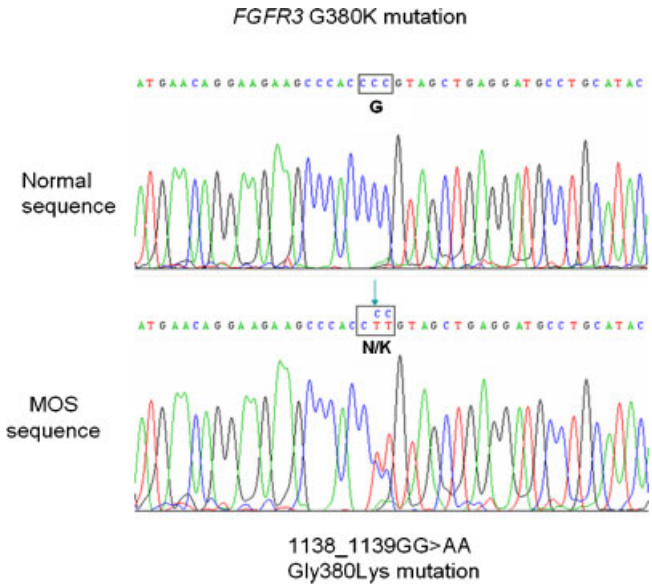

FIG. 3. DNA sequencing of *FGFR3* using the 10R primer, showing heterozygosity for the same double substitution (1138_1139 GG > AA) in both patients. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

suggested that this activation is mediated by hydrogen bonding of the large basic arginine side chain to a neighboring receptor. Importantly the same authors demonstrated that substitution to lysine (also basic but less bulky than arginine) was also activating in this transformation assay, although more weakly than arginine [Webster and Donoghue, 1996]. This last conclusion is consistent with the milder phenotypes observed in our patients.

The occurrence of double mutations on the same (*cis*) allele is expected to be extremely rare as a random occurrence [Kondrashov, 2002], yet such mutations have been described in several Mendelian disorders with important consequences for the patient's phenotype and genetic counseling [Savov et al., 1995]. Notably, there is a close analogy between the double mutation found in this family, mutations arising in *FGFR2* causing Apert Syndrome [Goriely et al., 2003, 2005] and with a recently described double mutation of *FGFR3* (Leu337Arg in *cis* with Gly380Arg) in a severe case of Ach [Rump et al., 2006]. In both *FGFR2* and *FGFR3*, new germline gain-of-function mutations have been shown to originate exclusively from the father and to show a paternal age effect (reviewed by Rannan-Eliya et al. [2004]). Moreover, at the serine 252 codon in *FGFR2*, double mutations encoding chemically similar amino acids (phenylalanine and tyrosine) to those arising from the usual single nucleotide change (encoding tryptophan) causing Apert syndrome have been described either in patients or in sperm [Goriely et al., 2003, 2005]. These rare double mutations in both *FGFR2* and *FGFR3* may occur as a result of positive selection for gain-of-function mutations in spermatogonial cells [Goriely et al., 2005]. In the present case, we can speculate that the double mutation originated from the healthy maternal grandfather, age 47 years, at the time of the affected mother's birth.

For a better understanding of the complex pattern of genotype/phenotype correlations of the FGFR members of the receptor tyrosine kinase family, further clinical and molecular studies of patients and families with these clinical pathologies are recommended. These studies are also crucial to the development and monitoring of the new therapeutic approaches recently emerged towards the overactivation of these receptors [Robertson et al., 2000; Horton, 2006].

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