Achondroplasia With Multiple-Suture Craniosynostosis: A Report of a New Case of This Rare Association

Beáta Bessenyei,1* Andrea Nagy,2 Erzsébet Balogh,1 László Novák,3 László Bognár,3 Alida C. Knegt,4 and Éva Oláh1

1Clinical Genetic Center, Department of Pediatrics, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary
2Department of Pediatrics, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary
3Department of Neurosurgery, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary
4Department of Clinical Genetics, Academic Medical Centrum, Amsterdam, The Netherlands

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We report on a female patient with an exceedingly rare combination of achondroplasia and multiple-suture craniosynostosis. Besides the specific features of achondroplasia, synostosis of the metopic, coronal, lambdoid, and squamosal sutures was found. Series of neurosurgical interventions were carried out, principally for acrocephaly and posterior plagiocephaly. The most common achondroplasia mutation, a p.Gly380Arg in the fibroblast growth factor receptor 3 (FGFR3) gene, was detected. Cytogenetic and array CGH analyses, as well as molecular genetic testing of FGFR1, 2, 3 and TWIST1 genes failed to identify any additional genetic alteration. It is suggested that this unusual phenotype is a result of variable expressivity of the common achondroplasia mutation. © 2013 Wiley Periodicals, Inc.

Key words: achondroplasia; multiple-suture; craniosynostosis

INTRODUCTION

Achondroplasia (ACH) is the most frequent form of non-lethal, short-limb dwarfism with an incidence of 5–15 in 100,000 live births. It is characterized by rhizomelic shortening of the limbs, macrocephaly with frontal bossing, hypoplasia of the midface and trident hands [Langer et al., 1967]. The phenotype is resulted from a decreased endochondral ossification caused by activating mutations in the fibroblast growth factor receptor 3 (FGFR3) [Shiang et al., 1994]. The inheritance of the disorder is autosomal dominant, the vast majority of patients have a p.Gly380Arg (c.1138G > A, or c.1138G > C) mutation in FGFR3 [Bonaventure et al., 1996].

Craniosynostosis, a cranial deformity due to the premature fusion of one or more cranial sutures resulted from an increased intramembranous ossification, may appear as an isolated disorder (non-syndromic form) or associated with additional clinical symptoms, principally dysmorphic features and in several diseases limb defects (syndromic form). While the isolated forms show the characteristics of multifactorial inheritance [Boyadjiev et al., 2007], the syndromic forms are often caused by mutations in the FGFR1, 2, 3 and TWIST1 genes, among others [Bellus et al., 1996; El Ghouzzi et al., 1997]. In about 5–15% of cases more than one suture is affected usually appearing as a part of a syndrome [Slater et al., 2008]. The overall incidence of craniosynostosis is 1:2,000 to 1:2,500.

Although skeletal dysplasia and craniosynostosis are etiologically and pathologically distinct groups of disorders, the two phenotypes may appear together in certain diseases such as thanatophoric and osteoglophonic dysplasias. Craniosynostosis, however, is not a common feature in ACH, only three cases have been published to date [Karadimas et al., 2006; Georgoulis et al., 2011; Hubbard et al., 2011].

Here, we report on a new case of this rare association due to a p. Gly380Arg mutation in FGFR3. Further genetic analyses failed to detect an additional genetic alteration.

Conflict of interest: none.

*Correspondence to:
Beáta Bessenyei, M.Sc., Clinical Genetic Center, Department of Pediatrics, Medical and Health Science Center, University of Debrecen, Debrecen, Nagyerdei krt. 98., Debrecen H-4032, Hungary.
E-mail: bessenyei.beata@med.unideb.hu

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The patient was a female born as the second child of healthy, non-consanguineous, Caucasian parents. The father was 31, the mother 36 years old at the time of conception. At 33 weeks of gestation, a cesarean was performed as shortening of the long tubular bones was detected by ultrasound examination. The newborn’s weight was 1,630 g (10–25th centile), length 36 cm (−3 SD) and head circumference (HC) 33 cm (97th centile). On physical examination, she had the specific features of ACH: macrocephaly, midface hypoplasia, depressed nasal bridge, short arms and legs. X-ray pictures showed the classical trident hands, and the shortening of the femur and humerus. In addition, she had abnormal skull configuration with acrocephaly, and left, posterior plagiocephaly (Fig. 1).

Imaging studies (CT with 3D reformatting) at 2 months revealed multiple-suture synostosis involving complete closures of the metopic, the left lambdoid and squamosal sutures and partial, bilateral closure of the coronal suture (Fig. 2). Series of reconstructive operative procedures were carried out at 3, 8, 16, 26 months and 4 and 5 years of age. At 5 months, ventriculoperitoneal shunt was inserted for treatment of hydrocephalus. At the age of 2 years, occipital craniotomy was performed due to foramen magnum stenosis. On examination at 3 years of age, her weight was 8,000 g (−3.5 SD), height was 69 cm (−6.5 SD), and HC was 46 cm (3rd centile). She had genu valgum, scoliosis, and strabism. Her mental development was normal. As her growth delay was more severe than expected, phosphate metabolism (calcium, phosphate, alkaline phosphatase, vitamin D, parathormone, osteocalcin, beta-CTx, P1NP) was checked, but it gave normal result.

MATERIALS AND METHODS

Written informed consent was obtained from the patient’s parents. Peripheral blood sample was collected from the patient, and genomic DNA was extracted using QIAamp DNA Blood Mini kit (Qiagen, Germantown, MD). Amplification of exon 10 of \( \text{FGFR3} \) was performed by polymerase chain reaction (PCR) according to Shiang et al. [1994]. PCR product was directly sequenced on
an ABI 3100 sequencer (Applied Biosystems, Foster City, CA) with BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems).

Karyotyping based on Giemsa–Trypsin–Giemsa banding was performed on metaphase peripheral blood lymphocytes using standard methods. Hot-spot regions of FGFR1 (exon IgIIIa), FGFR2 (exon IgIIIa and IgIIIc), FGFR3 (p.Pro250Arg site), and TWIST1 (entire coding region) were amplified by PCR (primer sequences and PCR conditions are available on request), followed by direct sequencing on an ABI 3100 sequencer with BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Array CGH was performed using Agilent 180K oligo-array, Amadid 023363 (Agilent Technologies, Inc., Santa Clara, CA).

**RESULTS**

Sequence analysis of exon 10 of FGFR3 identified the common c.1138G > A (p.Gly380Arg) mutation confirming the diagnosis of ACH. To study whether an additional genetic alteration causing craniosynostosis was present along with the common ACH mutation, cytogenetic and further molecular analyses were performed.

Conventional karyotyping after GTG-banding showed a normal female karyotype: 46, XX. Mutation analysis of hot-spot regions of FGFR1, 2, 3 and TWIST1 failed to detect any further genetic alteration. Array CGH analysis showed a normal, female array profile.

**DISCUSSION**

Craniosynostosis is exceedingly rare in the FGFR3-related chondrodysplasias except thanatophoric dysplasia. Mutations in FGFR3, however, can be identified in two craniosynostosis syndromes, namely Muenke and crouzonodermoskeletal syndromes, reflecting that this gene has an effect on intramembranous ossification as well. In FGFR3-related chondrodysplasias gain of function mutations can be detected causing an enhanced inhibition of chondrocyte proliferation and differentiation. It was shown, that the level of mutated receptor activities and the severity of the associated disorders are related [Naski et al., 1996]. The p.Gly380Arg mutation, accounting for about 98% of the ACH cases, increases the efficiency of phosphorylation within the unliganded FGFR3 dimers, while cross-linking and dimerization propensities are not affected [He et al., 2010]. In the severe, occasionally craniosynostosis-associated thanatophoric dysplasia, mutated FGFR3 receptors are more strongly activated than in ACH. Muenke syndrome is a FGFR3-related disorder, characterized by the disturbance of intramembranous ossification leading to abnormal suture formation. However, the growth of the long bones are not affected. This can be explained by the molecular behavior of the mutated receptor. The p.Pro250Arg Muenke mutation located in the ligand binding domain, enhances the affinity of the receptor for unnatural ligands, FGF2 and FGF9, and one of its cognate ligands, FGF1 [Ibrahimi et al., 2004]. Increased FGF9 signaling was shown to cause craniosynostosis in mice and human, so the altered ligand specificity, in the presence of the Muenke mutation, is the molecular reason for the syndrome [Harada et al., 2009; Wu et al., 2009.] All together, the type and the degree of the effect of a FGFR mutation on ligand binding, receptor dimerization, phosphorylation and kinase activity determine a specific pathological phenotype. However, it remains a question, why a certain mutation can cause different phenotypes.

Craniosynostosis associated with ACH has been reported only in three cases to date. Karadimas et al. [2006] reported a 32-gestational week fetus with severe shortening of all long bones, a narrow thorax, and multiple craniosynostosis resembling cloverleaf skull deformity. Molecular analysis of fetal DNA isolated from the amniotic fluid sample revealed the most frequent mutation of ACH (p.Gly380Arg). The pregnancy was terminated based on the parents’ decision. Further analysis of FGFR2 and FGFR3 was not performed, because of the limited amount of fetal DNA. Hubbard et al. [2011] reported a boy with ACH and left frontal plagiocephaly. At the age of 2, CT scan of the head with 3D reformatting demonstrated the fusion of the left frontosphenoidal suture as well as the metopic one. Recently, multiple-suture synostosis was identified in a molecularly proven ACH patient by Georgoulis et al. [2011]. This case resembles our patient as the same sutures were involved. However, in our case additional genetic tests were also performed. Cloverleaf skull deformity was reported in a hypochondroplasia case, a disorder...
allelic with ACH [Angle et al., 1998]. The most common hypochondroplasia mutation p.Asn540Lys was identified in that patient, while sequence analysis of FGFR2 exon IIIa and IIIc yielded normal result.

In the patient presented here multiple-suture craniosynostosis leading to acrocephaly and posterior plagiocephaly has been demonstrated. Achondroplasia was molecularly proven, but second pathogenetic mutation causing craniosynostosis could not be identified in the hot-spot regions of FGFR1, 2, 3 and TWIST1. Cytogenetic and array CGH analysis also gave normal result. Although growth retardation of the patient was more severe than expected in the presence of the classical ACH mutation, disturbance of phosphocalcic metabolism could be ruled out.

Our case and the reported ones suggest that this combined phenotype may be related to a variable expressivity of the common mutation of ACH. Similarly, identical mutations in FGFR2 can lead to variable phenotypes of distinct conditions, such as Crouzon, Pfeiffer, and Jackson–Weiss syndromes, indicating that additional factors must also contribute to the phenotype. It is suspected, that modifier gene(s) may exist in the genome altering the phenotypic outcome of the ACH mutation. In addition, epigenetic or environmental influences may also modify the manifestation of the disease.

REFERENCES


