We report on two patients with an unusual combination of achondroplasia and surgically treated sagittal synostosis and scaphocephaly. The most common achondroplasia mutation, p.Gly380Arg in fibroblast growth factor receptor 3 (FGFR3), was detected in both patients. Molecular genetic testing of FGFR1, FGFR2, FGFR3 and TWIST1 genes failed to detect any additional mutations. There are several reports of achondroplasia with associated craniosynostosis, but no other cases of scaphocephaly in children with achondroplasia have been described. Recently it has been demonstrated that FGFR3 mutations affect not only endochondral ossification but also membranous ossification, providing new explanations for the craniofacial hallmarks in achondroplasia. Our report suggests that the association of isolated scaphocephaly and other craniosynostoses with achondroplasia may be under recognized.

How to Cite this Article:

Key words: achondroplasia; sagittal craniosynostosis; FGFR1 gene; FGFR2 gene; FGFR3 gene; TWIST1 gene
due to a p.Gly380Arg mutation in FGFR3, which is associated with scaphocephaly.

**CLINICAL REPORT**

**Patient 1**

The patient is the first male born to healthy nonconsanguineous Italian parents. The mother was a 24-year-old primigravida and the father was 36 years old at the time of conception. Decreased fetal length was detected by ultrasound in the 7th month of pregnancy and ACH was suspected. The pregnancy was complicated by gestational diabetes which was treated by diet therapy. He was born at 37 weeks of gestation by caesarean section after premature rupture of membranes. The birth weight was 3.75 kg (75th centile), length 46 cm (25th centile) and head circumference (OFC) 37 cm (10th centile) according to achondroplasia-specific growth charts [Aicardi et al., 1983; Hoover-Fong et al., 2007].

Molecular study of FGFR3 was performed and the second most common mutation for ACH (c.1138G>A, p.Gly380Arg) was detected. At the age of 3 years and 7 months he was admitted to our Dept. of Neurosurgery because of a suspicion of craniostenosis. On physical examination he had specific features of ACH including prominent forehead with frontal bossing, depressed nasal bridge, midface hypoplasia (Fig. 1A–B), short arms and legs, short fingers and toes, joint hypermobility, and flat feet. His weight was 10.5 kg (<5th centile), height 79 cm (10–25th centile) and OFC 56.5 cm (50th centile) according to achondroplasia-specific growth charts. In addition, he had a scaphocephalic skull with frontal bossing, bilateral agenesis of the superior crus of the ears (Fig. 2A–B), dyschromia on ankles, and a hypochromic spot on the back. Psychomotor delay with an IQ of 57 was determined using Griffiths scales. CT scan with 3D reformating revealed scaphocephaly due to isolated synostosis of the sagittal suture (Fig. 2E–F). Brain MRI identified Chiari I malformation, mild supratentorial ventriculomegaly and retrocerebellar arachnoid cyst (see Table I and Fig. 2C–D). There were no signs of intracranial hypertension on brain MRI as well as on ophthalmology examination. Surgical treatment for scaphocephaly (open cranial vault remodeling) was carried out with good outcome.

**Patient 2**

A 3-year-old male with ACH was referred to our Dept. of Neurosurgery for suspected scaphocephaly. He was the second child of nonconsanguineous and healthy parents. The father was 60 years old and the mother 29 years old at the time of conception. Family history was significant only for the presence of a unilateral hand anomaly (absent fingers 2–4; fusion of fingers 1 and 5) in the half-sister. Diagnosis of ACH in the proband was suspected by ultrasound during the 7th month of pregnancy. He was born at 36 + 2 weeks of gestation by caesarean section. His weight was 2.86 kg (3–25th centile), length 44.5 cm (10–25th centile) and OFC 34.5 cm (<10th centile) according to achondroplasia-specific growth charts. Molecular study of FGFR3 identified the most common mutation for ACH (c.1138G>A, p.Gly380Arg). Frequent episodes of sleep apnea and tonsilar hypertrophy occurred during the first years of life. Physical examination at 3 years of age showed short stature with rhizomelia, varus knees, joint hypermobility and flat feet. His weight was 10.5 kg (<5th centile), height 79 cm (10–25th centile) and OFC 56.5 cm (50th centile) according to achondroplasia-specific growth charts. In addition, he had a scaphocephalic skull with frontal bossing, bilateral agenesis of the superior crus of the ears (Fig. 2A–B), dyschromia on ankles, and a hypochromic spot on the back. Psychomotor delay with an IQ of 57 was determined using Griffiths scales. CT scan with 3D reformating revealed scaphocephaly due to isolated synostosis of the sagittal suture (Fig. 2E–F). Brain MRI identified Chiari I malformation, mild supratentorial ventriculomegaly and retrocerebellar arachnoid cyst (see Table I and Fig. 2C–D). There were no signs of intracranial hypertension on brain MRI as well as on ophthalmology examination. Surgical treatment for scaphocephaly (open cranial vault remodeling) was carried out with good outcome.

**MATERIALS AND METHODS**

Written informed consent was obtained from the patient’s parents. Amplification and sequencing analysis of exon 10 of FGFR3 was done in both patients.

Sequence analysis of the following hotspot regions were performed using: FGFR1 (NM_023110.2 – NCBI36) exon 7; FGFR2 (NM_000141.4 – NCBI36) exon 7 and 8; FGFR3 (NM_000174.2 – GRCh37) exon7; TWIST1 (NM_000474.3 – GRCh37) exon1. PCR amplification (primer sequences and PCR conditions are available on request), followed by direct sequencing on an AB 3730 sequencer with BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems).

Array-CGH was performed using Human Genome CGH Microarray Kit G3 180 (Agilent Technologies, Palo Alto, USA) with ~13 Kbp overall median probe spacing. Labelling and hybridization were performed following the protocols provided by the manufacturers. A graphical overview was obtained using the Agilent Genomic Workbench Lite Edition Software 6.5.0.18. Genomic positions refer to the Human Genome February 2009 assembly (GRCh37/hg19).

**RESULTS**

Sequence analysis of exon 10 of FGFR3 performed at birth identified the two most common mutations, c.1138G >A in Patient 1 and c.1138G>C in Patient 2 both causing a p.Gly380Arg change, confirming the diagnosis of ACH in both patients. To study whether an additional genetic alteration causing craniosynostosis was present, cytogenetic and further molecular analyses were performed. Mutational analysis of the hotspot regions of FGFR1, FGFR2, FGFR3 and TWIST1 failed to detect any further genetic alteration. Screening by Array-CGH analysis of both patients identified a 601,5 Kb deletion at 3q26.31 band (175,097,493–175,699,047) in
FIG. 1. Clinical and neuroradiological findings in Patient 1 (A-B) with prominent forehead and frontal bossing, depressed nasal bridge, deeply set eyes, hypertelorism, midface hypoplasia, scaphocephalic skull and large and low-set ears. (C) Sagittal MRI T1 sequence demonstrates narrow foramen magnum, stenosis of the spinal canal and small corpus callosum. (D) Sagittal MPR (multi planar reconstruction) reformatted CT scan reveals increase of the anteroposterior diameter of the skull with marked frontal bossing, a small odontoid process (white arrowhead) and foramen magnum stenosis. Note the typical occipital bony spur and prominent posterior lip of foramen magnum. (E) Vertex and (F) lateral 3D reformatted views of the skull demonstrate fusion of the sagittal, squamosal and lambdoid sutures resulting in bathmocephaly, a variant of scaphocephaly.
DISCUSSION

FGFR3 gain-of-function mutation leads to both chondrodysplasias and craniosynostosis. Among FGFR3-related chondrodysplasias, craniosynostosis is a main feature in MS, CAN and in most TD2 cases. Uni or bilateral coronal synostosis is the major craniofacial deformation in MS and CAN, whereas cloverleaf skull is prevalent in TD2 [Cohen, 2009]. However, the involvement of multiple sutures is also reported in MS and CAN providing evidence for the phenotypic variability in FGFR3-related craniosynostosis [Doherty et al., 2007; Di Rocco et al., 2011; Ridgway et al., 2011; Doumit et al., 2014]. Moreover, several isolated cases of non-syndromic craniosynostosis due to FGFR3 mutations have been reported so far [Tsai et al., 2000; Schindler et al., 2002; Barroso et al., 2011; Roscioli et al., 2013].

The variable spectrum of phenotypic severity in FGFR3 disorders may be due to the different effects of FGFR3 mutations on chondrocyte and osteoblast related signaling pathways such as the MAPK, P38, PLCg, STAT and PKC pathways [Matsushita et al., 2009; Su et al., 2010; Marie et al., 2012]. There is evidence that FGFR3 and MAPK signaling in chondrocytes promotes synchondrosis closure resulting in premature fusion of ossification centers in the spine and cranial base in humans and in chondrodysplasia murine models [Matsushita et al., 2009; Laurita et al., 2011]. It was recently observed in two ACH patients and the Fgfr3 mouse that the disruption of endochondral ossification causes skull base anomalies with premature fusion of the basal synchondroses and reduction of the size of the foramen magnum [Di Rocco et al., 2014]. Moreover the same study revealed for the first time the effect of FGFR3 mutations on membranous ossification in ACH. In fact, non-ossified gaps in frontal bones were found in patients with ACH and in the Fgfr3Y367C/+ mouse. This observation may be due to the high levels of FGFR3 expression detected in frontal bones (compared with that in parietal bones) that could be damaged by the activating FGFR3 mutations [Quarto et al., 2009]. Therefore, CT scan showed premature fusion of sagittal and squamous sphenoidal sutures in some patients with ACH, while a premature fusion of the coronal sutures was observed in dwarf mice. These findings are supported by several clinical reports of suture synostosis in patients with ACH or hypochondroplasia [Angle et al., 1998]. However, the association with craniosynostosis has been reported in only five patients with ACH to date. The first case was described by Karadimas et al. [2006] who reported a 32-gestational week fetus with typical sonographic findings of ACH associated with multiple craniosynostoses resembling cloverleaf skull deformity. Afterwards, Hubbard et al. [2011] reported on a 2-year-old ACH patient with associated isolated frontosphenoidal craniosynostosis. Recently, two other cases of multiple-suture synostosis associated with molecularly proven ACH were identified by Georgoulis et al. [2011] and Bessenyei et al. [2013]. Finally, a case of ACH associated with unspecified craniosynostosis is reported in a recent study of more than two hundred cases of craniosynostosis [Seruya et al., 2013] (see Table I).
FIG. 2. Clinical and neuroradiological findings in Patient 2 (A-B) with scaphocephalic skull and frontal bossing, ears with agenesis of the superior crus bilaterally. (C) MRI T1 sequences demonstrate narrow spinal canal, a retrocerebellar arachnoid cyst and Chiari I malformation (white arrow) with mild supratentorial ventricular dilatation. (D) Sagittal MPR reformatted CT scan shows disproportionate sagittal elongation of the skull, a J-shaped sella and short clivus and small foramen magnum. (E) Vertex and (F) lateral 3D reformatted views of the skull demonstrate scaphocephaly due to fusion of the sagittal suture.
We report on two patients with ACH and CT scan proven sagittal synostosis and scaphocephaly which was surgically treated. ACH was molecularly proven for both patients, and we did not identify any additional craniosynostosis-causing pathogenic mutations in the hotspot regions of FGFR1, FGFR 2, FGFR 3 and TWIST1. Array-CGH analysis showed a deletion of 601,05Kb at band q26.32 on chromosome 3 in Patient 2 and his healthy mother. This deletion encompasses only one gene, NAALADL2 (N-acetylated alpha-linked acidic dipeptidaselike 2), previously reported deleted in a patient with microcephaly, seizure and severe intellectual disability [Millson et al., 2012] and involved in a complex chromosomes rearrangement in another patient with intellectual disability [Borg et al., 2005]. Based on genome-wide association studies, NAALADL2 may also be involved in kidney function, Kawasaki disease, systemic lupus erythematosus and other conditions [Hwang et al., 2007; Burgner et al., 2009; Chung et al., 2011]. However, NAALADL2 has not been related to chondrodysplasias nor to craniosynostosis to date. Interestingly, both patients had mild psychomotor delay. In this regard, it may be possible that the deletion of NAALADL2 in Patient 2 could be contributing to his intellectual disability although his mother seems to have normal intelligence. In addition, Patient 1 also had mild psychomotor delay. It is known that most individuals with ACH have delayed developmental milestones [Ireland et al., 2011, 2012], but normal cognitive functioning [Thompson et al., 1999; Trotter et al., 2005], In our patients, the cognitive impairment could be related the presence of craniosynostosis [Da Costa et al., 2013].

Thus our report is consistent with the recent findings that FGFR3 mutations can adversely affect membranous bone ossification and provides further evidence for the involvement of FGFR3 in craniofacial development. In view of these observations it is plausible to suppose that scaphocephaly and other craniosynostosis conditions are underdiagnosed in ACH, and should be investigated by CT with 3D reformatting when abnormal head shape or a palpable ridge along the suture are present. However, it remains to be elucidated why the same mutation could cause only ACH phenotype in most individuals, while others have an associated craniosynostosis. It is plausible that additional factors such modifier gene(s), epigenetic or environmental factors may also play a pivotal role in phenotype determination.

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REFERENCES


