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SHORT COMMUNICATION

FGF17, a gene involved in cerebellar development, is downregulated in a patient with Dandy–Walker malformation carrying a *de novo* 8p deletion

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Abstract Fibroblast growth factors (FGFs) are important signaling molecules which act during early vertebrate central nervous system development. *FGF17*, together with FGF8, is a key factor in the patterning of the mid-hindbrain region with a complex picture of spatiotemporal gene expression during the various stages of cerebellar development. Disruption or reduced expression of *fgf17* in mice has been associated with cerebellar vermis abnormalities. We have identified a *de novo* 2.3-Mb deletion of chromosome 8p21.2-p21.3 in a girl with severe growth retardation, seizures, and classical Dandy–Walker malformation. Analysis of gene expression in blood lymphocytes and skin fibroblasts revealed markedly reduced levels of *FGF17*, which is located 1 Mb from the proximal deletion breakpoint. This is the first report of a human cerebellar malformation associated with transcriptional downregulation of the *FGF17* gene.

Keywords *FGF17* · Cerebellum · Dandy–Walker malformation · Chromosome 8p

Introduction

Dandy–Walker malformation (DWM) (OMIM 220200) first described by Dandy and Blackfan in 1914 [1], is the most common human cerebellar malformation with an estimated incidence of 1/5,000 live births. It consists of a spectrum of developmental anomalies of the posterior fossa including: (1) a complete or partial agenesis of the cerebellar vermis, (2) cystic dilatation of the fourth ventricle, and (3) elevation of the roof of the tentorium cerebelli and the torcular. DWM is frequently associated with other brain abnormalities such as agenesis or dysgenesis of the corpus callosum or occipital encephalocele and neurological features includ-

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ing: developmental delay, ataxia, episodic tachypnea, seizures, nystagmus, dysarthria, hypotonia, and spasticity. DWM is etiologically heterogeneous; chromosomal abnormalities, single gene disorders as well as exposure to teratogens have been associated with this malformation. Heterozygous deletions of *ZIC1* and *ZIC4*, located in 3q, have been identified in patients with DWM [2]. Chromosome 13q deletions encompassing two other genes of the ZIC family have also been reported [3]. Deletions or duplications encompassing the transcription factor *FOXC1* on chromosome 6p25.3 have been found in patients with posterior fossa malformations ranging from mega cisterna magna or isolated vermis hypoplasia to classical DWM [4]. Moreover, DWM has been reported in patients with partial duplications of 8p or 8q and in a wide variety of chromosomal abnormalities [5]. Here, we report on a patient with severe growth retardation, developmental delay, and DWM, carrier of a 2.3-Mb *de novo* interstitial deletion of the short arm of chromosome 8. Gene expression studies in this patient revealed a markedly reduced level of *FGF17*, a gene involved in cerebellar vermis development, located at 1 Mb from the proximal deletion breakpoint in 8p21.3.

Case report

The girl was born by C-section at 40 weeks after a normal pregnancy, to nonconsanguineous healthy parents. Cerebellar vermis hypoplasia was detected by prenatal ultrasonography. Birth parameters were low: weight, 2,590 (3–10°); length, 46 cm (3°); and OFC, 32 cm (2°). The Apgar score was 9 and 10. Since 2 months of age, she suffered from gastroesophageal reflux and frequent gastrointestinal and respiratory infections. The child had neonatal hypotonia, motor development was delayed, she sat at 9 months, and started to walk with support after 3 years. A Griffith Scale performed at 24 months showed a global development of 22 months. EEG was normal until the age of 4.5 years, when she developed myoclonic seizures. Biochemical and metabolic workup only revealed a subclinical hypothyroidism and a mild hypochromic anemia. Cardiac, renal, and abdominal ultrasound examinations were normal. Ophthalmological and audiological investigations as well as a complete skeletal survey did not reveal any abnormality. She was examined at the age of 3 years and 10 months. The physical examination showed a dolicocephalic skull, high forehead, sparse hair, low-set and posteriorly rotated ears, bilateral epicanthic folds, a small nose with flat nasal bridge, and a thin upper lip. Generalized hypotonia and joint hyperlaxity, café-au-laits spots, and lentigines on the lower extremities were also present. Growth parameters were severely delayed: height, 68 cm (−7.5 SD); weight, 6.7 kg (−6 SD); OFC, 47.6 cm (−2 SD). At last evaluation, at the age of 5 years and 3 months, growth values were unchanged. Brain MRI studies performed at 1 week and 30 months of life confirmed the presence of a small upwardly rotated vermis associated with a retrocerebellar cyst, compatible with a diagnosis of Dandy–Walker malformation. The volume of the pituitary gland was slightly reduced (Fig. 1).

Materials and methods

Whole genome array-CGH Array-CGH was performed using Agilent Human Genome CGH Microarray Kit 44BX4, with an estimated average resolution of 75 Kb. The procedures for reference and patient DNA digestion, labeling, and hybridization were performed according to the manufacturer's instructions. Images of the array were acquired with the Agilent scanner and analyzed by using the Agilent Feature Extraction software (v 9.5). A graphical overview of the results was obtained with CGH Analytics software (v 3.4.40).

CNVs validation The database of Genomic Variants (http://projects.tcag.ca/variation), DECIPHER (http://www.sanger.ac.uk/PostGenomics/decipher), and UCSC Genome browser (http://www.genome.ucsc.edu) were used to identify known benign genomic variants.

Gene expression analysis Total RNA from peripheral blood and skin fibroblasts of the patient and three healthy control subjects was isolated using TRIzol solution (Invitrogen), according to manufacturer's protocol. One microgram of each RNA sample was reverse transcribed with the SuperScript™ First-Strand Synthesis system and random hexamers as primers (Invitrogen Life Technologies). The expression of *FGF17*, *PDLIM2*, *hsa-miR-320* (upstream of the deletion) and *TNFRSF10D*, *NKX3-1*, *STC1*, and *ADAM7* (inside the deleted area) was measured by quantitative real-time PCR (qRT-PCR) in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using Power SYBR Green I dye chemistry. All experiments were performed in triplicate, and RNA samples of three healthy individuals were included as normal controls. Data analysis was performed using three different endogenous genes as internal controls (*GADPH*, *TBP*, *18S*, *and U6 for microRNA*). ΔΔCt values were indicated as fold reduction relative to control samples, which was set to onefold [6]. Only expression values for skin fibroblast are indicated. Each primer pair (sequences are available upon request) was designed by Primer Express 2.0 Software (Applied Biosystems).

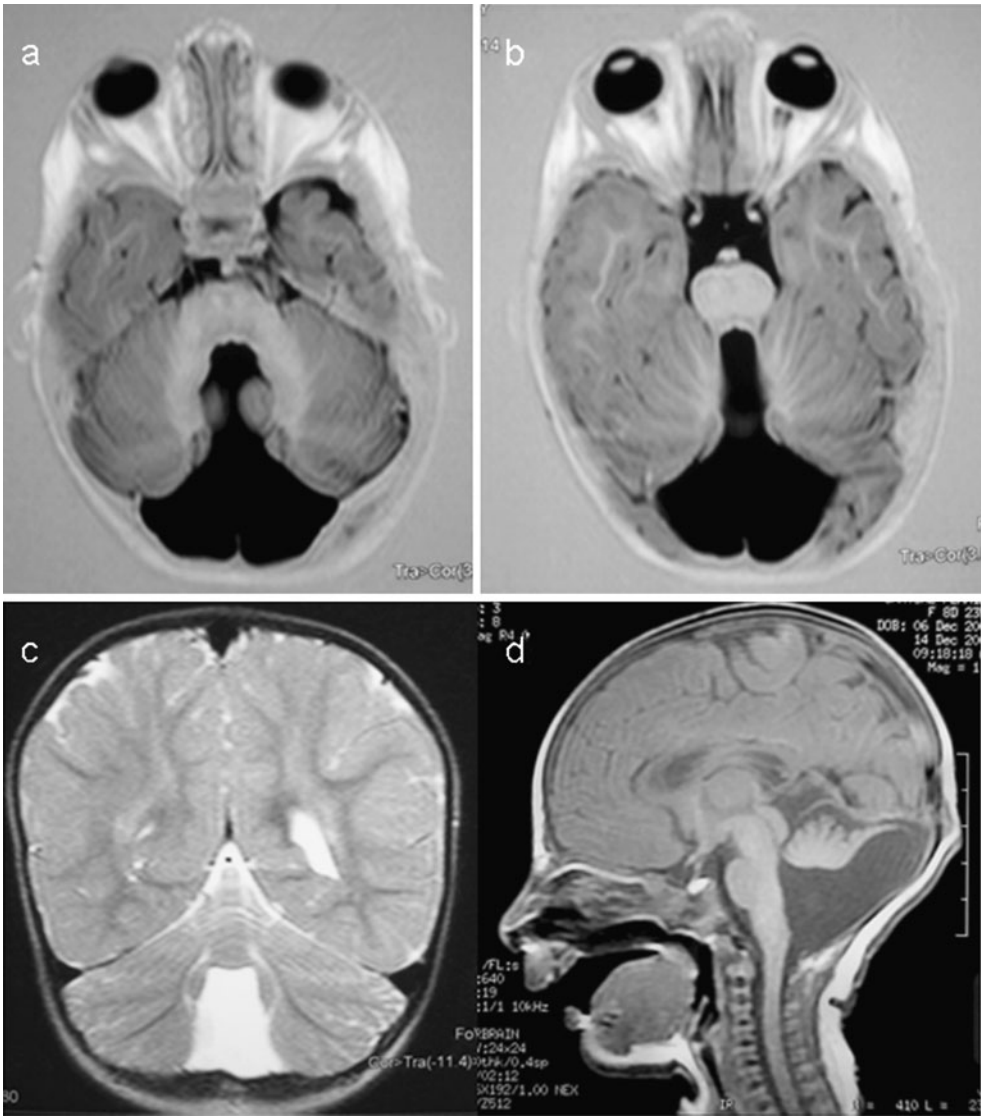

Fig. 1 Neuroimaging studies of the patient showing classical DWM. **a** and **b** Serial axial inversion recovery images (patient aged 2 years) showing severe cerebellar vermis hypoplasia; **c** coronal T2-weighted image confirming severe cerebellar vermis hypoplasia; **d** sagittal T1-weighted image (patient aged 1 month) showing severe cerebellar vermis hypoplasia with upwardly rotated vermis and a retrocerebellar cyst

Results

Cytogenetic and molecular data The standard karyotype was normal. Array-CGH analysis showed a heterozygous interstitial deletion of the short arm of chromosome 8 spanning about 2.3 Mb of genomic DNA, with the proximal [chr8:23,117,561 bp] and distal [chr8:25,349,074 bp] breakpoints mapping to chromosome bands 8p21.2 and 8p21.3, respectively (Fig. 2). This rearrangement was confirmed by FISH analysis using a locus-specific probe. The parental chromosomes were negative, arguing for a *de novo* origin of the deletion.

FGF17 expression analysis The *FGF17* expression in blood lymphocytes and skin fibroblasts (0.44) was reduced to more than 50% compared to the same tissues in control individuals (Fig. 3). The expression of the neighboring gene *PDLIM2* (0.98) as well as the expression values of two other transcripts located in the vicinity of *FGF17*: *hsa-miR-320* (1.16) and *U6* (0.69) were normal compared to control RNAs whose value was set to 1. In the deleted area, the haploinsufficient genes *TNFRSF10D* (1.2) and *STC1* (1.1) were normally expressed while *NKX3-1* (0.45) and *ADAM7* (0.45) showed reduced expression values compared to controls.

Discussion

The deleted region encompasses 2.3 Mb of genomic DNA and contains 19 genes: seven transcripts (*TNFRSF10A*, *R3HCC1*, *LOXL2*, *CHMP7*, *ENTPD4*, *SLC25A37*, and *AC051642.5*) can be safely excluded as they were also lacking in a previously reported patient carrier of parentally inherited 8p deletion (DECIPHER 2046); similarly,

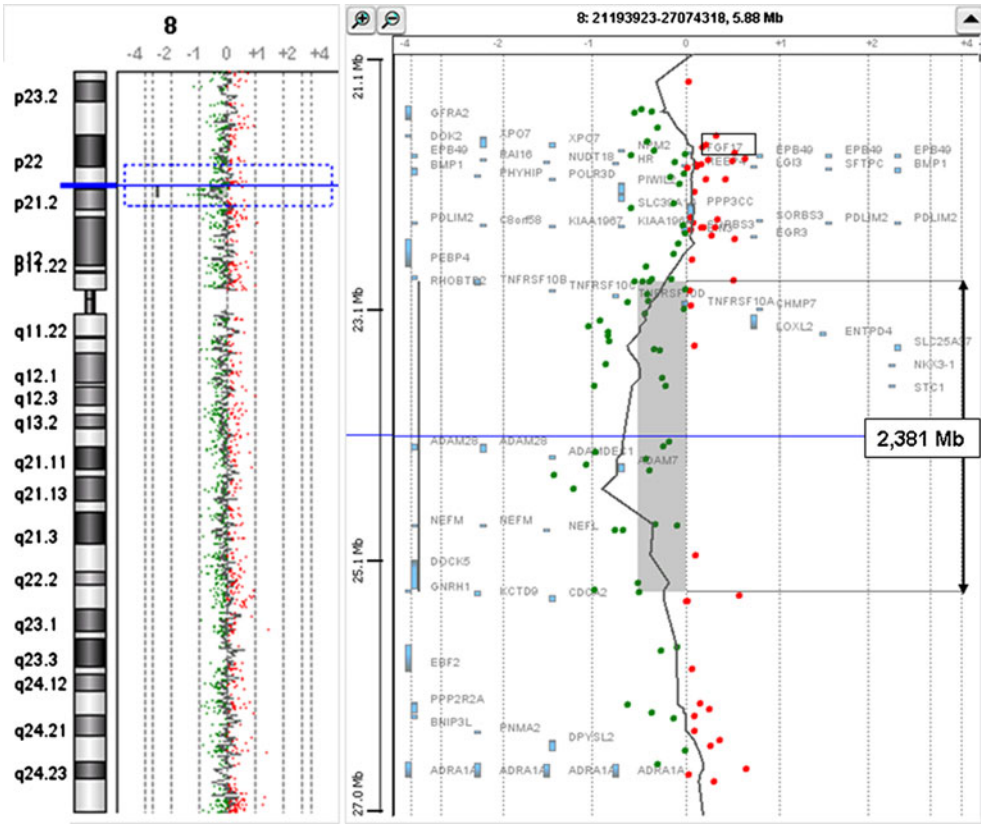

Fig. 2 Graphical overview of array-CGH analysis showing the deletion of about 2.3 Mb in the 8p21.2-p21.3 genomic region from A_14_P124616 (23,117,561 bp) to A_14_P110189 (25,349,074 bp) probes, based on the March 2006 release (UCSC Genome Browser). *FGF17* is indicated by a *square box*

CHMP7 or *DOCK5* are deleted in two small benign CNVs [7]. Thus, 12 potentially relevant genes are left: *TNFRSF10D*, *NKX3-1*, *NKX2-6*, *STC1*, *ADAM28*, *ADAMEC1*, *ADAM7*, *NEFM*, *NEFL*, *GNRH1*, *KCTD9*, and *CDCA2*.

Haploinsuffiency for *FGF17* and *NKX3-1* could account for the severe growth retardation observed in the patient: FGF signaling is essential for the regulation of endochondral bone differentiation. *FGF17*, together with FGF1, 2, and 19, has been identified as the predominant ligand expressed in the human fetal growth plate cartilage [8].

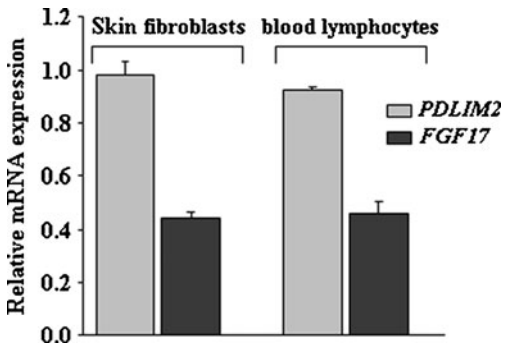

Fig. 3 qRT-PCR expression analysis of *FGF17* and *PDLIM2* transcripts. Histogram showing the expression of *FGF17* and PDLIM2 mRNAs in blood lymphocytes and skin fibroblasts in the patient compared to three normal controls whose value was set to one. Expression values were normalized with those obtained from the housekeeping gene *GADPH*. Similar results were obtained with *TBP* and *18S*

NKX3-1 belongs to the NK homeobox gene family and encodes for a transcriptional repressor which contributes to maintain chondrogenic cell fate and promote early skeletal tissue differentiation [9]. *NKX3-1*, *ADAM7* are expressed in Rathke's pouch [10, 11], and members of the FGF8 subfamily are required for the proliferation and patterning of progenitor cells in the developing anterior pituitary [12]. Interestingly, the adenohypophysis was found to be slightly reduced in the patient.

Genomic imbalances have been shown to contribute to disease phenotypes by modifying not only the expression of genes within the aneuploid segments but also of normal copy number genes. The "position effect" of structural rearrangements on the expression of genes that lie outside their boundaries has been reported as far as 2–7 Mb away from the breakpoints [13]. Human *FGF17* is located at 1 Mb to the proximal deletion breakpoint in 8p21.3. Its expression in the peripheral lymphocytes and skin fibroblasts of the patient was found to be reduced to less than 50% compared to control tissues. The finding of a normal expression of other transcripts (*PDLIM2*, *hsa-miR-320*, and *U6*) located in the vicinity of *FGF17* argues against a general "silencing" mechanism imposed on the entire region and most likely suggests the implication of downstream long-range regulatory enhancer or repressor elements present in the deleted

segment. The possibility that genes or regulatory elements located inside the deletion may directly or indirectly affect the expression of *FGF17* cannot be excluded. Interestingly, evolutionary conserved noncoding elements which were found upstream of *fgf17* in teleost genomes, following chromosomal rearrangements that occurred during the teleost/tetrapod split, could be located downstream of the gene, as it has been demonstrated for the *fgf8* locus [14].

In six out of eight patients with an inversion duplication involving various segments of chromosome 8p including 8p21.3, neuroimaging studies showed hypoplasia of the (inferior) cerebellar vermis, a thin or absent corpus callosum, enlargement of the ventricular system, and hippocampal maldevelopment [15]. DWM has also been reported in a patient with a 8p21.3-p23.3 duplication [16]. Development of the vertebrate cerebellum and midbrain is coordinated by ligands of the FGF8 family (Fgf8, *Fgf17*, and Fgf18) produced by the isthmic organizer at the mid-hindbrain (MHB) junction. Disruption of *fgf17* in mice decreases precursor cell proliferation in the vermis anlage and in the most caudal part of the midbrain [17, 18]. Interestingly, downregulation of *fgf17* expression has been shown in *Mid1* mutant, the mouse ortholog of the Opitz GBBB syndrome characterized by midline developmental defects, in which mispecification of the MHB boundary results in an abnormal development of the most anterior lobes of the cerebellar vermis [19]. Mice mutant for *Gyplican 1* (*Gpc1*), a neuronal cell surface heparan sulfate proteoglycan, have a significant reduction in brain size with abnormalities in cerebellar foliation, and in particular, absence or partial agenesis of the most anterior lobe of the cerebellar vermis; indeed, *fgf17* expression was found to be downregulated in these mutants [20]. All these data strongly suggest that *FGF17* is critical for cerebellar vermis development and that misexpression of human *FGF17* may represent one of the key factors in the pathogenesis of 8p-related cerebellar vermis hypoplasia.

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