Central nervous system anomalies in Pfeiffer syndrome (PS) due to mutations in the FGFR2 gene are poorly understood, even though PS is often associated with serious cognitive impairment. The aim of this study is to describe the neuropathological phenotype in PS. We present four severe fetal cases of sporadic PS with FGFR2 mutations who underwent termination followed by fetopathological and neuropathological examination. We studied the expression pattern of Fgfr2 in the mouse brain using radioactive fluorescence in situ hybridization. PS is associated with brain deformations due to the abnormal skull shape, but FGFR2 mutations also induce specific brain developmental anomalies: megalencephaly, midline disorders, amygdala, and hippocampus malformations, and ventricular wall alterations. The expression pattern of Fgfr2 in mice matches the distribution of malformations in humans. The brain anomalies in PS result from the combination of mechanical deformations and intrinsic developmental disorders due to FGFR2 hyperactivity. Several similarities are noted between these anomalies and the brain lesions observed in other syndromes due to mutations in FGFR receptor genes. The specific involvement of the hippocampus and the amygdala should encourage the precise cognitive screening of patients with mild forms of PS. © 2012 Wiley Periodicals, Inc.

Key words: Pfeiffer syndrome; craniosynostosis; intellectual disability; FGFR; central nervous system

INTRODUCTION

Pfeiffer syndrome (PS) comprises coronal craniosynostosis, broad thumbs and great toes and, in some cases, partial soft tissue syndactyly of the hands [Pfeiffer, 1964; Cohen, 1993]. Numerous germ line mutations for PS have been found in the FGFR1 and FGFR2 genes [Schell et al., 1995], but the most severe forms of the syndrome are due to four sporadic and recurrent FGFR2 mutations: p.W290C, p.Y340C, p.C342R, and p.S351C [Lajeunie et al., 2006]. Mice with those four specific activating mutations are not available, and the mechanisms leading from excessive Fgfr2 activity to the malformations found in PS are still not understood.

Severe sporadic forms of PS due to mutations in the FGFR2 gene are generally associated with intellectual disability, but the central nervous system anomalies in this disorder are not well known. Apert syndrome (AS), which is often associated with cognitive impairment and also due to FGFR2 mutations (p.S252W, p.P253R), may include some specific brain malformations [Cohen and Kreiborg, 1990]. In this study, we describe the neuropathology of four severe sporadic fetal cases of PS and analyze the expression pattern of Fgfr2 in the mouse brain.

How to Cite this Article:
provide a description of the neuropathological abnormalities of PS and (2) discuss the role of mechanical constraints versus intrinsic developmental disorders in their formation.

MATERIAL AND METHODS

Tissue fragments were obtained from four medically aborted fetuses following the informed consent of parents. In all cases, pregnancy was legally terminated after ultrasonographic and X-ray detection of craniostenosis. Full-body and skull radiographs were performed. Selected blocks were embedded in paraffin for neuropathological examination. Hemalun–phloxin and Hemalun–Luxol-fast-blue stains were used on 8 μm-thick sections of the brain stem, hindbrain, and spinal cord, and on 10 μm-thick sections of the whole brain. PCR amplification of genomic DNA and sequencing for the screening of FGFR2 mutations were performed using previously described primers and conditions [Lajeunie et al., 1995; Kan et al., 2002]. Radioactive in situ hybridization was carried out using 35S-labeled anti-sense probes on 12 μm-thick sections with the sections from case 2 was thus impossible (Figs. 2 and 3).

RESULTS

The extracranial abnormalities of the four fetuses are listed in Table I. The following focuses on neuropathology and craniofacial malformations.

Fetus 1

The brain weighed 552 g (>>90th centile), with abnormally round contours and an oblique sulcus lateralis in its superior part. Olfactory bulbs were normal. Marked gyration gave a hypermature aspect to the temporal lobes (Fig. 1).

There was an asymmetric enlargement of the lateral ventricles more so on the left than right side; the third ventricle and the midbrain aqueduct were moderately enlarged. Corpus callosum and fornix were normal. The layers of the septum pellucidum were very thin and fused anteriorly; the left layer was ruptured.

Histologically the cortex was abnormally folded; however, cortical cytoarchitecture was normal. Focal polymicrogyria was noted in the posterior part of the sulcus lateralis. Despite the bulging temporal lobes, the dentate gyrus, and Ammon’s horn were histologically unremarkable. Ependymal rosettes and gliosis were found in the walls of the lateral ventricles. Small focal spots of subependymal neuronal heterotopia were noted in the frontal horns and atrium, as well as periventricular white matter gliosis and spongiosis. Corticospinal fibers were fragmented in the pons but normal in the medulla oblongata. A moderate delay in myelination was noted in the brainstem.

The fetus carried a single de novo heterozygous base substitution in the FGFR2 gene (p.S351C). The fetus was diagnosed with PS (type 2).

Fetus 3

The brain weighed 232 g (90th centile). The right occipito-frontal diameter (OFD) was 95 mm and the left OFD was 88 mm. The bitemporal diameter was 80 mm. The falx cerebri was shortened. The frontal, parietal, and occipital lobes were narrow and compressed, as opposed to the bulging temporal lobes. Operculization of the insula was absent. Gyration was unusual with numerous aberrant large and deep sulci. The olfactory bulbs were present. Coronal sectioning disclosed an asymmetric ventriculomegaly, normal corpus callosum and unremarkable septum pellucidum and fornices. The white matter presented with diffuse edema affecting mainly the periventricular regions. The midbrain aqueduct was moderately enlarged (Fig. 4).

Histological examination showed periventricular ependymal rosettes with subependymal gliosis. The white matter was subjected to microglial activation and showed diffuse micro-calcifications. The basal ganglia were normal. The neocortex lamination and
<table>
<thead>
<tr>
<th>Age at delivery (WG)</th>
<th>Gender</th>
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<th>Siblings</th>
<th>3rd Trimester sonography</th>
<th>Amniotic fluid/caryotype</th>
<th>Weight (g)</th>
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<th>Clinical type</th>
<th>FGFR2 mutation</th>
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<th>Main neuropathological findings</th>
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<tr>
<td>Fetus #1</td>
<td>Female</td>
<td>39 years</td>
<td>11-year old brother and 6-year old sister, both healthy, from previous partner, 1 spontaneous abortion with current partner</td>
<td>Severe hydrocephalus, hydroamnios</td>
<td>46,XX</td>
<td>2,450</td>
<td>552</td>
<td>3</td>
<td>p.S35 1C</td>
<td>Conical ribs</td>
<td>Broad first metatarsals and big toes with various deviation of all toes and prominent heels</td>
<td>Triangular first phalanx</td>
<td>Tarsal and cuboid points</td>
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<td>Meckel's anomaly</td>
<td>Ventricle dilatation</td>
<td>Aberrant gyration</td>
<td>[Hypermature aspect of the temporal lobes]</td>
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<tr>
<td>Fetus #2</td>
<td>Female</td>
<td>NA</td>
<td>NA</td>
<td>Severe craniofacial malformation</td>
<td>t[13;14] 46,XX</td>
<td>650</td>
<td>111</td>
<td>2</td>
<td>p.Y340C</td>
<td>Short neck</td>
<td>Vertebral fusions [C6-T5]</td>
<td>Dense iliac wings with irregular borders</td>
<td>Elbow and knee synostosis</td>
</tr>
</tbody>
</table>

(Continued)
| Fetus #3 | 28 + 1/2 days | Female | 41 years | 2-year old girl, healthy | Severe craniodorsal malformations and spinal anomalies | 46,XX | 1,460 | 232 | 2 | p.Y340C | Vertebral fusions [body and processae spinosae], C4-T1 and T3-T8 | Fetal kyphosis | Short sacrum | Missing acromions | Bilateral humero-radial fusion | Broad first metacarpal and metatarsal bones with shortened first phalanges | Pilonidal cyst | Membranous ventricular septum defect [1.5 mm] | Megalencephaly | Ventricular dilatation | No insular operculization | Abnormal gyration | Large temporal lobes | White matter gliosis | Ependymal abrasion, subependymal rosettes, subependymal gliosis |
| Fetus #4 | 35 | 29 years | NA | Lateral and third ventricle dilatations, multiple spinal malformations, single umbilical artery, oligohydramnios and foetal hypokinesia | 46,XX | 2,550 | 462 | 2 | p.Y340C | Fusion of the posterior arches of the cervical vertebrae | Elbow and knee arthrogryposis | Broad and short thumbs and big toes; second phalanges of the thumb missing | Enlargement of the head of the first metatarsals | Cuboid point [advanced maturation] | Left talipes | Single umbilical artery [left artery missing] | Common mesenteric artery | Lung hypoplasia | Megalencephaly | Ventricular dilatation | Abnormal gyration | White matter gliosis | Chiari type I malformation | Large temporal lobes, hippocampus and amygdala disorganization |
differentiation, including temporal neocortex were unremarkable. Ammon’s horn did not show any significant anomaly. The corticospinal fibers were normal and symmetrical. The brain stem and the spinal cord were normal.

The fetus had a de novo heterozygous mutation in the extracellular domain of \( \text{FGFR2} \) (p.Y340C). The fetus was diagnosed with PS (type 2).

**Fetus 4**

The brain weighed 462 g (>90th centile) and was cloverleaf shaped. The frontal lobes were curved forward. The Sylvian fissures and the temporal sulci were in a vertical position, the Sylvian fissures were large without operculization, the gyral pattern was abnormal and the depth of sulci was very irregular. A Chiari type I malformation was found (Fig. 5).

There was enlargement of the lateral and third ventricles. The anterior part of the septum pellucidum was ruptured. The corpus callosum was present. The brain parenchyma was diffusely congested.

However, despite the gyral abnormalities, the cortical cytoarchitecture and differentiation were unremarkable. The white matter was microvacuolated and showed signs of venous congestion. Ventricular wall abnormalities consisted of superficial periventricular

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**FIG. 1.** Fetus 1—PS due to p.S351C FGFR2 mutation (type 3) 34 WG + 5 days. a: Gross view of the fetus: severe macrocephaly and skull dysmorphia. b: Lateral and lower views of the brain: megalencephaly and hypermature gyration. c: Coronal sections: lateral and third ventricles and midbrain aqueduct enlargement; focal polymicrogyria (box). d: Details of the focal polymicrogyria. e: Subependymal rosettes. f: Subependymal nodules of heterotopia. g: White matter gliosis and spongiosis. h: Corticospinal tract fragmentation in the pons.

**FIG. 2.** Fetus 2—PS due to p.Y340C FGFR2 mutation (type 2) 24 WG + 4 days. a: Gross view of the fetus: cloverleaf skull. b: Lateral, upper and lower views of the brain: large temporal lobes. c,d: Posterior coronal sections: disproportion between temporal and frontoparietal sizes. e: Age-match controls: balanced frontoparietal and temporal sizes. f: Anterior coronal section: amygdala and anterior white commissure. g: Splenium agenesis with Probst bundles (black arrows); abnormal position of the lateral geniculate body (red arrow).
abrasion and ependymal rosettes surrounded by numerous abnormal reactive astrocytes (gliosis).

The corpus callosum was normal. The corticospinal tract was normal at the level of internal capsule. The anterior white commissure was normal. The amygdalae had a disorganized structure. Due to the large size of the temporal lobes, the usual landmarks on coronal sections could not be localized at their usual position and precise matching with the control sections was impossible (see Fetus 2). In the region of the hippocampus, the dentate gyrus could be identified as well as the pyramidal cell layer and the zonal differentiation of Ammon’s horn but the curling of the whole structure was defective, leading to very unusual aspects on the sections. The basal ganglia were unremarkable.

The upper part of the midbrain aqueduct was dilated. The corticospinal tract was fragmented in the midbrain and the pons, but the pyramis medullae oblongatae was normal, as was cerebellum.

The fetus had a de novo heterozygous mutation in the extracellular domain of FGFR2 (p.Y340C). The fetus was diagnosed with PS (type 2).

Radioactive in situ hybridization
In wild-type mice, Fgfr2 was expressed at E12.5 along the ventricles in the ventricular and subventricular zones of the pallium, in the posterior medial pallium (cortical hem), in the ganglionic eminences of the subpallium, and in the choroid plexus. This expression pattern persisted at E13.5 and E15.5. The main structures affected in the brain of fetuses with PS thus expressed Fgfr2 at early stages in mouse brain development: the cortical hem, which contributes to the formation of the dentate gyrus and the hippocampus, the ependyma and the choroid plexus, the neurogenic periventricular layers, and the ganglionic eminences, which take part in the formation of the amygdala (Fig. 6).

DISCUSSION
These four fetuses with PS shared neuropathological characteristics such as brain overgrowth (megalencephaly) and ventricular dilatation. Furthermore, we observed that two cases bearing the activating p.Y340C mutation had striking abnormalities of hippocampus and amygdala. We then showed that the areas where Fgfr2 was expressed in the mouse brain matched the areas presenting with abnormalities in the four fetuses.

Phenotype–Genotype Correlations
Most severe sporadic PS cases have been linked to four recurrent activating mutations in the FGFR2 gene: p.W290C, p.Y340C, p.C342R, and p.S351C [Cornejo-Roldan et al., 1999; Lajeunie et al., 2006] and specific phenotype–genotype correlations at the organ level have been proposed. The p.S351C FGFR2 mutation may be associated with a cartilaginous tracheal sleeve, vertebral fusion, and sacrococcygeal inversion [Gonzales et al., 2005; Oliveira et al., 2006]. One fetus in our series carried the p.S351C mutation and presented with a cartilaginous tracheal sleeve; sacrococcygeal inversion was not found but minor caudal anomalies such as sacral
dimple and pilonidal cyst were present. The p.Y340C mutation phenotype is less well characterized but is known to be associated with multiple pterygia [Baynam et al., 2008]. Three fetuses in our series carried the p.Y340C mutation and Fetus 3 presented with small pterygia, which could not nevertheless be formally differentiated from secondary pterygia due to fetal immobility. Two of three cases with the p.Y340C mutation had distinctive hippocampus and amygdala malformations (Figs. 2 and 5), and these anomalies could be part of the malformations associated with this specific mutation.

**Ventricular Dilatation and Chiari Malformation in PS: Mechanical and Developmental Origins**

Our four cases exhibited various degrees of ventricular dilatation, without obvious signs of obstruction. In fact, ventricular dilatation in syndromic craniosynostosis may not be strictly mechanical [Cinalli et al., 1995; Collmann et al., 2005]. In mice, our results and published data confirm that Fgfr2 is expressed in the ependyma and the choroid plexus during embryonic development (Fig. 6 and [Wilke et al., 1997; Reid and Ferretti, 2003; Yaylaoglu et al., 2005]),
as well as diffusely in the white matter [Wilke et al., 1997]. Ventricular dilatation in PS could thus partly result from FGFR2 hyperactivation.

Only one case in our series (Fetus 4) had Chiari malformation (Fig. 5), although this anomaly is found in 50% of the pediatric PS cases [Cinalli et al., 1995]. Interestingly, Fetus 4 is the oldest fetus we describe (35 WG). In megalencephaly—capillary malformation syndrome [Conway et al., 2007] and in Costello syndrome [Gripp et al., 2010], Chiari malformation develops in the last prenatal or early post-natal stages. Both conditions present with megalencephaly and ventricular dilatation, and result from activating mutations in signaling pathways downstream of the FGFRs [Conway et al., 2007; Gripp et al., 2010]. Similarly, in PS, it appears that Chiari malformation has a late onset.

**Analogy With Other FGFR Mutations**

The best-known brain phenotype due to a FGFR2 mutation is the Apert syndrome (AS) [Maksem and Roessmann, 1979; de León et al., 1987; Cohen and Kreiborg, 1990] but apart from an over-convolution of the temporal cortex [Raybaud and Di, 2007], specific temporal malformations have not been described in this syndrome. Other CNS malformations in AS include megalencephaly, hydrocephalus and ventricular wall abnormalities. The pyramidal tract abnormalities described in AS by Cohen and Kreiborg [1990] and Maksem and Roessmann [1979] may relate to the cortico-spinal tract fragmentation we report on in one case and can be interpreted as a consequence of abnormal axonal guidance (Fig. 1).

Megalencephaly (defined by a brain weight ≥2 SD) was found in three cases. Morphologically, the overgrowth affected predominantly the temporal lobes and resembled the anomalies found in FGFR3 mutations causing thanatophoric dysplasia (TD), a lethal form of chondrodysplasia characterized by temporal lobe enlargement, deep transverse sulci across the inferomedial temporal surface and hippocampal dysplasia [Hevner, 2005]. Nevertheless, we did not observe abnormal transverse sulci across the temporal lobe in PS and the gyrus dentatus dysplasia

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we report on is very different from the descriptions provided by [Hevner, 2005] in TD.

In TD, the temporal overgrowth is supposedly caused by hyperactivity of FGFR3 in the cortical hem, a signaling center adjacent to the dorsal midline and the choroid plexus [Hevner, 2005]. According to this author, hippocampal dysplasia, polymicrogyria, and subependymal neuronal heterotopia would then be in part secondary to the primitive temporal overgrowth. The expression pattern of Fgfr2 we found in mice brains supports this hypothesis: our results show an early expression of this gene in the posterior part of the medial pallium, corresponding to the cortical hem mentioned by [Hevner, 2005], as well as in the neurogenic periventricular regions of the pallium (Fig. 6). These results confirm and precise previous expression pattern studies [Bansal et al., 2003].

In summary, in the light of the data on brain malformations in other FGFR mutations, the specific brain abnormalities in PS are the distinctive temporal and amygdala dysplasia. TD and AS share some neuropathological features with PS, such as megalencephaly and ventricular dilatation. Based on the expression pattern of Fgfr2 in the mouse brain and on previous experimental and neuropathological reports, these abnormalities could be related to a hyperactivity of the FGF pathway predominantly occurring in the medial pallium, the medial ganglionic eminence, the choroid plexus, and the ependyma. A specific disturbance of the differentiation of the temporal lobe and the amygdala in PS, due to a hyperactivity of FGFR2, can thus be suspected. These results should encourage focused imaging and neuropsychological studies in mild forms of PS in order to screen for cognitive disorders related to a dysfunction of the temporal lobe and adapt the rehabilitation programs accordingly. Similar abnormalities may also be found in other FGFR related syndromes after closer neuropathological investigations.

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REFERENCES


