Pontine tegmental cap dysplasia: a novel brain malformation with a defect in axonal guidance

Peter G. Barth,1 Charles B. Majoie,2 Matthan W. A. Caan,2 Marian A. J. Weterman,3 Marten Kyllerman,4 Leo M. E. Smit,5 Richard A. Kaplan,6 Richard H. Haas,7 Frank Baas,3 Jan-Maarten Cobben8 and Bwee Tien Poll-The1

1Department of Pediatric Neurology, Emma Children’s Hospital/AMC, University of Amsterdam, 2Department of Radiology, Academic Medical Centre, University of Amsterdam, 3Laboratory of Neurogenetics, Academic Medical Centre, University of Amsterdam, 4Department of Paediatrics, The Queen Silvia Children’s Hospital, Sahlgrenska University Hospital, Göteborg, Sweden, 5Department of Pediatric Neurology, Free University Medical Centre, Amsterdam, Netherlands, 6Department of Pediatric Neurology, Kaiser Permanente Hospital, San Diego CA, 7Departments of Neurosciences and Pediatrics, University of California San Diego, La Jolla CA, USA and 8Department of Pediatrics, Section of Genetics, Emma Children’s Hospital/AMC, University of Amsterdam, Netherlands

Correspondence to: Bwee Tien Poll-The, Department of Pediatric Neurology, Emma Children’s Hospital/AMC, P.O. Box 22700 1100DE Amsterdam, Netherlands
E-mail: b.t.pollthe@amc.uva.nl

Four unrelated children are described with an identical brainstem and cerebellar malformation on MRI. The key findings are: vermal hypoplasia, subtotal absence of middle cerebellar peduncles, flattened ventral pons, vaulted pontine tegmentum, molar tooth aspect of the pontomesencephalic junction and absent inferior olivary prominence. Peripheral hearing impairment is present in all. Variable findings are: horizontal gaze palsy (1/4), impaired swallowing (2/4), facial palsy (3/4), bilateral sensory trigeminal nerve involvement (1/4), ataxia (2/4). Bony vertebral anomalies are found in 3/4. Additional MR studies in one patient using diffusion tensor imaging (DTI) with colour coding and fibre tracking revealed an ectopic transverse fibre bundle at the site of the pontine tegmentum and complete absence of transverse fibres in the ventral pons. The combined findings indicate an embryonic defect in axonal growth and guidance. Phenotypic analogy to mice with homozygous inactivation of Ntn1 encoding the secreted axonal guidance protein netrin1, or Dcc encoding its receptor Deleted in Colorectal Cancer led us to perform sequence analysis of NTN1 and DCC in all the patients. No pathogenic mutations were found. For the purpose of description the name ‘pontine tegmental cap dysplasia’ (PTCD) is proposed for the present malformation, referring to its most distinguishing feature on routine MRI.

Keywords: pontine hypoplasia; axonal guidance; molar tooth complex; netrin 1; deleted in colorectal cancer

Abbreviations: DTI = diffusion tensor imaging; FISH = fluorescent in situ hybridization; BAER = brainstem auditory evoked response; SSEP = somato sensory evoked potential; PTCD = pontine tegmental cap dysplasia.


Introduction

Hypoplasia of the ventral pons, usually combined with cerebellar hypoplasia, is seen in various conditions (Parisi and Dobyns, 2003) including disorders of N-glycosylation, especially CDG 1A (Ramaekers et al., 1997), disorders of O-glycosylation, causing deficient glycosylation of alpha-dystroglycan (Santavuori et al., 1998; Michele et al., 2002), pontocerebellar hypoplasias types 1 to 5 (Barth, 1993; Patel et al., 2006), chromosomal disorders (Arts et al., 1995) and extreme prematurity (Messerschmidt et al., 2005).

We here report on hypoplasia of the ventral pons, combined with a dorsal vault projecting from the tegmentum into the fourth ventricle. Two previous single case reports on this condition mention associated deafness (Maeoka et al., 1997; Ouanounou et al., 2005) and absence of middle cerebellar peduncles (Ouanounou et al., 2005). Four new patients from non-related families are the object of the present investigation. The imaging and clinical features, neurological examination findings, were evaluated in each case. The composition of the pontine vault was analysed by multiplanar diffusion tensor imaging (DTI) in
one patient. DTI revealed an aberrant transverse fibre bundle, implying a key role for misdirected axonal guidance during the embryonic stage. Phenotypic analogies to induced netrin deficient (Netrin-1<sup>−/−</sup>) mice (Serafini et al., 1996) and to mice with induced deficiency in its receptor Deleted in Colorectal Cancer (Dcc<sup>−/−</sup>) (Fazeli et al., 1997) led us to analyse the human homologues of these genes, NTN1 and DCC, in all four patients.

**Patients and methods**

**Imaging studies**

Each patient was routinely referred to a centre for paediatric neurological examination and MR studies. MRI studies were done using field strengths of 1.5 T (patients 1–3), 1 T (patient 4) and 3 T (patient 1). Axial, sagittal and coronal T1 and/or T2 images, 3–5-mm slice thickness were obtained in all patients. Other images were obtained by transverse T2 (patient 3), axial IR (patients 2 and 3), axial 3DCISS (patient 1), axial FLAIR (patients 2 and 4), coronal IR (patients 2 and 4), sagittal 3DT1 (MPRAGE) slice thickness 1.3 mm (patient 3). DTI studies were done in patient 1 on a Philips Intera 3 Tesla MRI scanner, using 32 isocahedric diffusion directions. Other parameters were: TE 94 ms, TR 4831 ms, b 1000 s/mm², FOV 230–256 mm, scan matrix 70 × 112, image matrix 256 × 256, slice thickness 3 mm. Echoplanar distortions were corrected for (Mangin et al., 2002), using Brainvisa software. A full-brain fibre tractography was performed in an interactive environment DTII (Blas et al., 2005), containing around 16,000 fibres composed of 400,000 points. Clinical data are given in Table 1.

**Molecular genetics**

Genomic DNA was isolated from blood samples of patients using standard procedures. Primers were designed to amplify all exons (coding and for NTN1 also non-coding), exon–intron boundaries and at least 50 nt of flanking intron sequences by PCR. PCR primers and conditions are available upon request. After treatment with shrimp alkaline phosphatase (USB) and exonuclease I (New England Biolabs) PCR products were analysed by direct sequencing using the ABI Big Dye Terminator cycle sequencing kit and an ABI3730 sequencer (Applied Biosystems). The resulting sequence traces were compared with the NTN1 (NM_004822) and DCC (NM_005215) reference sequence using the Codon Code Aligner software. Identified changes were described according to international nomenclature (www.genomic.unimelb.edu.au/mdt/mutnomen) with numbers referring to the position in the cDNA relative to the start codon. Predictions for changes in splicing events were examined using the ESEfinder and NNSplice programs (http://rulai.cshl.edu/cgi-bin/tools/ESE/esefinder.cgi, www.fruitfly.org/seq_tools/splice.html).

**Results**

**Neuroimaging**

The following MRI findings were present in all patients, unless stated otherwise:

1. Flat profile of the ventral pons (Fig. 1).
2. An abnormal curved structure covering the middle third of the pontine tegmentum and projecting into the fourth ventricle (Figs. 1 and 2).
3. Subtotal absence of the middle cerebellar peduncles (MCP) (Fig. 3).
4. Shortening of the isthmus of the mesencephalon, lateralized course of the superior cerebellar peduncles due to broadening of the anterior end of the fourth ventricle. Shape and orientation of the superior cerebellar peduncles in axial and transverse images imparts the impression of a molar tooth (Fig. 4).
5. Hypoplasia and malformation of the vermis (Fig. 2), normal shape and size of the cerebellar hemispheres in three patients, mild hypoplasia of the cerebellar hemispheres in one patient (4).
6. The inferior cerebellar peduncles are present though smaller than normal.
7. The shape of the medulla oblongata on transverse sections is altered due to absence or alteration of the inferior olivary nucleus (Fig. 5).
8. Both cochleae can be identified in all patients. The inner auditory meati, identified in patients 1 and 3, have reduced diameters. The 7th and 8th nerves, visualized only in patient 1, are severely reduced in width.
9. In one patient (patient 1) on transverse section of the pons close to the exit of trigeminal nerves a band of high fractional anisotropy, suggestive of a fibre tract, borders on the fourth ventricle. A narrow strip connects the structure with the cerebellum (not shown).
10. Fractional anisotropy with colour coding (Figs. 6 and 7) and fibre tractography images (Fig. 7), performed in patient 1 show a transverse directed fibre bundle in the pons directly beneath the fourth ventricle. Extensions from this structure project towards the cerebellum. This suggests that the projection of the ectopic bundle parallels the normal transverse pontine fibres that project to the cerebellar hemispheres. Transverse sections of the mesencephalon show a transverse band across the midline indicating presence of the commissure of the superior cerebellar peduncles (not shown).
11. Supratentorial findings are mild lateral ventricular dilatation in 3/4 patients (1, 2 and 4) and hippocampal dysplasia (lack of inrolling) in 1 patient (1) (Fig. 3).

**Mutation analysis of NTN-1 and DCC genes**

DNA from all patients was screened for the presence of mutations. In addition to several known polymorphisms (see supplementary data), 10 as yet unidentified alterations were found (Table 2). However, all detected changes were located outside of the coding regions except for
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
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<td>Dutch</td>
<td>Swedish</td>
<td>African (Burundi)</td>
<td>American—Irish</td>
</tr>
<tr>
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<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Unaffected sibs</td>
<td>1</td>
<td>4 pat. half sibs</td>
<td>1</td>
<td>1 + (1 spont ab.)</td>
</tr>
<tr>
<td>Family history</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Latest visit y; m</td>
<td>2; 3</td>
<td>7; 1</td>
<td>5; 6</td>
<td>5; 9</td>
</tr>
<tr>
<td>FOC SD</td>
<td>0</td>
<td>1.4a</td>
<td>1.3b</td>
<td>—</td>
</tr>
<tr>
<td>Length SD</td>
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<td>—2.2a</td>
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<tr>
<td>Weight SD</td>
<td>—2</td>
<td>—1.8a</td>
<td>—1.3b</td>
<td>—1.3</td>
</tr>
</tbody>
</table>

**Brainstem sensory nerves involved**
- V, VIII-acoustic: Corneal anestheasia l>r, bilateral sensory deafness
- VIII-acoustic: Bilateral vestibular, Dysequilibrium

**Brainstem motor nerves involved**
- VII r>l, IX

**Supranuclear eye movements**
- Not affected

**Swallowing**
- Impaired; Temporary gastrostomy

**Speech**
- Deaf-mute, Sign language

**Gait/Posture**
- Sitting with own hand support, unable to walk

**Cerebellar motor symptoms**
- No test possible

**Pyramidal tract symptoms**
- No

**Other neurological findings**
- No

**Learning disorder**
- Non-verbal contact, learning sign language

**External dysmophria**
- No

**Extracranial malformations**
- Bilateral ureteral reflux, Th4 thoracic hemivertebra

**Karyotype**
- 46XY; 22q11 Normal on FISH
- 46XX; 22q11 Normal on FISH
- 46XX
- 46XY

**BAER**
- Absent
- Absent
- No recognizable pattern

**EEG**
- Normal
- Normal
- Normal

**SSEP**
- Crossed cortical response
- ND
- ND

**Transcranial magnetic stimulation of cortex**
- Right stimulation: crossed response in left m. abd. pollic. brev.; left stimulation: no response
- ND
- ND
- ND

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Abbreviations: FOC = fronto-occipital circumference; EEG = electroencephalogram; BAER = brainstem auditory evoked response; SSEP = somatosensory cortical evoked response; ND = not done.

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*Measurements at 4.5 y; bMeasurement at 2.8 y.*
two silent mutations; one in exon 3 of NTN1 in patient 1 that was also present in the mother of the patient and one in exon 2 of DCC in patient 4. Identified alterations that were predicted to have no effects on the splicing mechanisms of the corresponding mRNA or located at a distance larger than 50 bp from the exon were not analysed further. Changes that were located closer to the exons or predicted to result in effects on splicing as compared to the reference sequences were screened for their presence in at least 160 normal chromosomes. All appeared to be polymorphisms except for the changes in exon 3 of NTN1 and near exons 7 and 17 of DCC that were each present in one allele in one patient only.

Discussion

A distinct pattern of hindbrain malformation affecting the pons, medulla oblongata and cerebellum was found in four unrelated children, two males and two females. The pontine abnormality involves flattening of its ventral and vaulting of its dorsal border, coupled with near absence...
of the middle cerebellar peduncles. Further analysis by DTI revealed the virtual absence of transverse pontine fibres and the presence of an ectopic bundle of transverse fibres occupying the place of the pontine tegmentum in one patient. The latter finding has not been reported previously. It should be mentioned that long fibre tracking is a relatively new technique and neuropathological and neurophysiological evaluation are presently unavailable to ascertain the neuroradiological findings. Clinical findings include involvement of cranial nerves, with the acoustic nerves involved in all, followed by facial motor and sensory trigeminal nerves. A swallowing disorder was present in two, caused by involvement of the glosopharyngeal

Fig. 2 Patient 1. 8 months. Abnormal pontine structure is seen on corresponding midsagittal (upper) and axial T2w (lower) images (arrows). The vermis is hypoplastic and dysplastic with anterior shift of the fastigium.

Fig. 3 Patient 4.10 months. Coronal T2w image shows the absence of both middle cerebellar peduncles, indicated by asterisks. The lateral ventricles are slightly enlarged and the hippocampi are dysplastic.

Fig. 4 Patient 3. 4y5m. Axial T1w image shows dorsoventral narrowing of the mesencephalic isthmus, with abnormal shape and orientation of the superior cerebellar peduncles imparting a 'molar tooth' appearance.

Fig. 5 Patient 1. 5 months. Axial T1w image shows the medulla oblongata. Arrows indicate the absent contours of the inferior olivary nuclei.
Fig. 6 Axial 3 T, 3 mm slices, fractional anisotropy (fa). Colour coding: blue coloured fibres perpendicular to plane of section, red coloured fibres tangential to plane of section, direction LR/RL, green fibres tangential to plane of section, direction AP/PA. A and B from pons level at exit of trigeminal nerves. A Control (female 27 years); B Patient 1. 3y 1 m. Numbers indicate: 1 = corticospinal tract (blue); 2 = transverse pontine (pontocerebellar) fibres (red) 3 = medial lemniscus (blue); 4 = ectopic transverse fibre bundle (red).

Fig. 7 Three-dimensional image of fibre tracts reconstructed from axial 3 T fa slices. Colour coding as in Fig. 6. A Control (female 27 years); B Patient 1. 3 yl. Numbers indicate: 1 = corticospinal tract (blue); 2 = transverse pontine (pontocerebellar) fibres (red); 3 = ectopic transverse fibre tract (red); 4 = middle cerebellar peduncle (green); 5 = fibres passing from the ectopic transverse bundle to the cerebellum (green).

Table 2 Unknown sequence alterations in NTN1 and DCC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exon</th>
<th>Position and mutation</th>
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<th>Pat 1</th>
<th>Pat 2</th>
<th>Pat 3</th>
<th>Pat 4</th>
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<tbody>
<tr>
<td><strong>NTN1</strong></td>
<td>1</td>
<td>c.1-91G&gt;T</td>
<td>35/170</td>
<td>±</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>c.1128C&gt;T p. Ala376Ala</td>
<td>0/170</td>
<td>±</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>4</td>
<td>c.1208-102C&gt;G</td>
<td>4/160</td>
<td></td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>c.1358+58G&gt;A</td>
<td>ND</td>
<td></td>
<td>±</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>c.1412-21.T&gt;12insC</td>
<td>ND</td>
<td></td>
<td>+/+</td>
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<td>±</td>
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<td></td>
<td>8</td>
<td>c.1487+123C&gt;G</td>
<td>ND</td>
<td></td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td><strong>DCC</strong></td>
<td>2</td>
<td>c.231T&gt;C p.Asp77Asp</td>
<td>31/170</td>
<td></td>
<td></td>
<td></td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>c.1141+17C&gt;T</td>
<td>0/170</td>
<td>±</td>
<td></td>
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<tr>
<td></td>
<td>17</td>
<td>c.2456-28c.2456-25delGTTT</td>
<td>0/174</td>
<td></td>
<td></td>
<td></td>
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Summary of detected nucleotide changes of NTN-1 and DCC in patients with pontine dysplasia. ± and +/+ indicate heterozygous or homozygous changes respectively. ND = not determined.
and nerves. Horizontal gaze, affected in two, may represent pontine tegmental or cerebellar vermal lesions or both. Gross deficits in motor and cognitive achievements in patients 1 and 4 cannot be readily explained on the basis of a brainstem disorder alone and may have a supratentorial origin. This consideration also applies to the seizure disorder in patient 4 and episodes of loss of conscience in patient 3. MRI findings of ventricular dilatation in three cases and hippocampal dysplasia in one also point to supratentorial involvement. A hindbrain anomaly similar to the one presented in this study has been described before in two single case reports that bear morphological and clinical similarity to the present series (Maeoka et al., 1997; Ouanounou et al., 2005). Maeoka et al. (1997) reported a 2-year-old girl with sensory deafness and truncal ataxia and mild developmental delay. The MRI findings are essentially similar to the present series, but do not mention the middle cerebellar peduncles. Ouanounou et al. (2005) reported a 3-month-old boy with bilateral 6th and 7th nerve deficits, classified as Moebius syndrome. The MRI findings are similar to the present series, and include the absence of the middle cerebellar peduncles. This patient also had moderate dilatation of the lateral ventricles. In addition the last author mentioned small internal auditory canals without comment on hearing.

The present disorder shares some of its imaging features with Joubert syndrome (molar tooth complex). Shared features are vermal hypoplasia, anterior displacement of the fastigium of the fourth ventricle and molar tooth sign. Clinical findings in typical Joubert syndrome include severe to moderate mental retardation, ataxia, intermittent hyperpnea–apnea, nystagmus and severe ocular motor apraxia (Steinlin et al., 1997). Findings in the present series that overlap with Joubert syndrome are mental retardation (patients 3 and 4), ataxia (patients 2 and 3) and mild oculomotor apraxia (patient 3).

Key findings in PTCD not present in Joubert syndrome are: severe pontine hypoplasia, dorsal pontine vaulting, near absence of the middle cerebellar peduncles, ectopic commissural fibres and cranial nerve involvement (Steinlin et al., 1997; Yachnis and Rorke, 1999; Parisi and Dobyns, 2003; Gleeson et al., 2004; Valente et al., 2006; Alorainy et al., 2006). Although the differences with Joubert syndrome are obvious, axonal guidance pathology is present in both disorders leaving the possibility of related molecular pathways. Three findings in PTCD: (1) ventral pontine hypoplasia, (2) the near absence of the middle cerebellar peduncles and (3) the absence of transverse pontine fibres (DTI) point to early loss of ventral pontine neurons. Known mechanisms that can account for early loss of ventral pontine neurons are:

1. Impairment of fetal migration of pontine neurons. Mice deficient for the Large gene, with resultant deficiency of alpha-dystroglycan develop pontine hypoplasia by impaired neuronal migration towards the ventral pons (Qu et al., 2006), offering a model for understanding the pontine hypoplasia in O-glycosylation disorders such as Walker–Warburg syndrome and Muscle–Eye–Brain disease. No abnormal commissures are reported in this model.

2. Degenerative disease. Pontine hypoplasia in pontocerebellar hypoplasias 1 and 2 is due to a degenerative process which causes progressive loss of pontine and other neurons without signs of axonal misrouting (Barth, 1993).

3. A defect in axonal growth and guidance resulting in neuronal loss and ectopic commissures. There are no known human equivalents. Loss of embryonic axonal guidance with ectopic commissures however was found in mice with induced homozygous defects of Ntn1 (Serafini et al., 1996) or Dcc (Fazeli et al., 1997). The induced defects affect the fate of pontine and olivary neurons which originate from the ventral rhombic lip, a proliferative zone that borders on the roof plate of the fourth ventricle (Alcántara et al., 2000; Wingate, 2001). The migrating neurons normally remain ipsilateral to their site of origin while their outgrowing axons cross the midline to reach the opposite cerebellar hemispheres as mossy- and climbing fibres (Sotelo, 2004; Marillat et al., 2004). Netrin-1, the gene product of Ntn1, is widely expressed in the CNS as a factor inducing axonal growth (reviewed by Barallobre et al., 2005). As a secretory product of the floor plate it interacts with the extracellular domains of growth cone receptors DCC (Deleted in Colorectal Cancer) and UNC5. Homozygous inactivation of the Ntn1 or Dcc genes in mice cause multiple commissural defects, including the corpus callosum, hippocampal commissures, pons and commissural crossing at the ventral spinal cord. Upon failure of axons to connect to their synaptic targets redundant DCC receptors bind to caspase, thereby inducing apoptosis and loss of neurons (Barallobre et al., 2005). In both conditions loss of pontine neurons is accompanied by the formation of an ectopic cerebellar commissure (Serafini et al., 1996; Fazeli et al., 1997). While Ntn1 and Dcc are widely expressed throughout the nervous system and also in non-neural tissues, their distribution pattern is not identical. E.g. Ntn1 is expressed in post-natal rat cochlea while Dcc is not (Gillespie et al., 2005). Phenotypic analogies between the Ntn1 deficient mouse (Serafini et al., 1996), the DCC deficient mouse and (Fazeli et al., 1997) and PTCD led us to evaluate the human homologues NNTN1, and DCC by sequence analysis in all four patients. No mutations were detected within the coding regions.
of NTN1 that would lead to amino acid changes thereby possibly affecting protein function. In addition to several known polymorphisms, 10 hitherto unknown nucleotide changes were found. Based on their relative distance to the exons, lack of predicted effects on splicing events, or occurrence in a control population, all except three were clearly polymorphisms. These three were all present in a heterozygous state in one patient only. Two were intronic changes, without or with very weak predicted effects on splicing. The third one, a silent mutation in exon 3, which was also found in the mother, does not affect the composition of the protein although it can not be excluded that this mutation would exert effects on splicing. Assuming similar dose dependency of netrin-1 and Dcc in mouse and man, both alleles should show pathogenic mutations. Therefore, it seems unlikely that the observed changes represent pathogenic mutations, practically excluding NTN1 and DCC as candidate genes for this disorder. A related defect, ROBO3 mutation causes the rare human disorder horizontal gaze palsy with progressive scoliosis (HGPPS) (Jen et al., 2004). The mouse homologue of ROBO3, the growth cone factor Rig-1/Robo3 allows one-way ventral midline passage of axons by interfering with the axon repellent action of the secreted ground floor factor slat (Sabatier et al., 2005). Typical structural abnormalities in human ROBO3 mutation identified by DTI consist of absent midline crossing at the pontine and mesencephalic levels but ectopic fibre bundles have not been identified in either ROBO3 mutation (Sicotte et al., 2006) or its mouse equivalent (Sabatier et al., 2004). As additional tests we performed SSEP and transcranial magnetic stimulation in one PTCD patient. Only contralateral responses were obtained (Table 1, patient 1), which is unlike the ipsilateral response expected in HGPPS (Jen et al., 2004). Although we did not test for ROBO3 mutations in this study phenotypic differences between HGPPS and PTCD make ROBO3 an unlikely candidate gene for the latter. Other genes involved in the interplay of axonal growth cones and growth factors, including the downstream of netrin-1/DCC operating signal molecules offer rational targets for further research.

Supplementary material
Supplementary material is available at Brain online.

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