LETTER TO THE EDITOR

Mutations of TSEN and CASK genes are prevalent in pontocerebellar hypoplasias type 2 and 4

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Sir, Pontocerebellar hypoplasias (PCH) represent a group of neurodegenerative autosomal recessive disorders with prenatal onset, atrophy or hypoplasia of the cerebellum, hypoplasia of the ventral pons, microcephaly, variable neocortical atrophy and severe mental and motor impairments (Barth, 2000).

Recently in two subtypes, PCH type 2 (associated with dyskinesia and/or dystonia and variable degrees of spasticity) and PCH type 4 (a more severe phenotype associated with perinatal symptoms, ventilator dependency and early death), mutations have been identified in three of the four different subunits of the transfer RNA-splicing endonuclease complex (TSEN54, TSEN34 and TSEN2) (Budde et al., 2008). Mutations in the calcium/calmodulin-dependent serine protein kinase (CASK) gene have also been associated with X-linked mental retardation (XLMR) with microcephaly, optic atrophy and brainstem and cerebellar hypoplasia (Najm et al., 2008).

Namavar et al. (2011) reported on a series of 169 patients affected with PCH and identified mutations in TSEN54 or RARS2 genes in 106 individuals. The authors display a strong correlation between TSEN54 mutations and a ‘dragonfly-like’ cerebellar pattern on magnetic resonance imaging, in which the cerebellar hemispheres are flat and severely reduced in size and the vermis is relatively spared. They also show that homozygosity for the common c.919G>T (p.Ala307Ser) missense mutation in TSEN54...
Table 1  Clinical and molecular characteristics in eight patients harbouring TSEN54 and CASK mutations

<table>
<thead>
<tr>
<th>Patients</th>
<th>Phenotype</th>
<th>Sex/age at diagnosis</th>
<th>Vital distress</th>
<th>Microcephaly</th>
<th>Chorea/dystonia</th>
<th>Ophthalmology</th>
<th>Spasticity</th>
<th>Swallowing difficulties</th>
<th>Cerebellar ataxia</th>
<th>Outcome</th>
<th>Molecular diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>PCH2</td>
<td>M/2 mo</td>
<td></td>
<td>+</td>
<td>+</td>
<td>OM paresis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Age 19 years,  unable to sit or stand, no speech, epilepsy</td>
<td>TSEN54 c.919 G&gt;T, p.Ala307Ser</td>
</tr>
<tr>
<td>P2</td>
<td>PCH2</td>
<td>F/4 mo</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Age 11 years, unable to sit or stand, no speech, epilepsy</td>
<td>TSEN54 c.919 G&gt;T, p.Ala307Ser</td>
</tr>
<tr>
<td>P3</td>
<td>PCH2</td>
<td>M/birth</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Deceased at age 24 mo</td>
<td>TSEN54 c.919 G&gt;T, p.Ala307Ser</td>
</tr>
<tr>
<td>P4</td>
<td>PCH4</td>
<td>F/birth</td>
<td>Neonatal respiratory distress</td>
<td>+</td>
<td>Unknown</td>
<td>Unknown</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Deceased at neonatal period</td>
<td>TSEN 54 c.919 G&gt;T, p.Ala307Ser</td>
</tr>
<tr>
<td>P5</td>
<td>PCH4</td>
<td>M/28 weeks of gestation</td>
<td>Unknown</td>
<td>+</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Un</td>
<td>Pregnancy medically terminated</td>
<td>TSEN 54 c.919 G&gt;T, p.Ala307Ser</td>
</tr>
<tr>
<td>P6</td>
<td>PCH2</td>
<td>F/9 mo</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Age 13 years, stands unsupported, walks with aid</td>
<td>CASK c.1970 G&gt;A p.Trp667X (de novo)</td>
</tr>
<tr>
<td>P7</td>
<td>PCH2</td>
<td>F/7 mo</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Normal</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>Age 8 years, stands unsupported, walks with aid, behaviour disturbance</td>
<td>CASK c.1577delG p.Arg526Serfs-X74 (de novo)</td>
</tr>
<tr>
<td>P8</td>
<td>PCH2</td>
<td>F/3 mo</td>
<td></td>
<td>+</td>
<td>+</td>
<td>Normal</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>Age 13 years, stands supported, no speech</td>
<td>CASK c.1968 G&gt;A p.Trp666X (de novo)</td>
</tr>
</tbody>
</table>

DYS = dysmetria; F = female; M = male; mo = months; NYS = nystagmus; OM = oculomotor; TA = trunk ataxia; TRE = tremor; UN = unknown; y = years; + = present; + + = severe.
Figure 1 Brain MRI scans from patients affected with TSEN54 and CASK mutations. (A) Sagittal and coronal brain magnetic resonance images of a foetus (Patient 1, 28 weeks of gestation) and two children (9 months and 14 years old) affected with TSEN54 mutations. In all cases, a PCH is found with a characteristic ‘dragonfly-like’ cerebellar pattern (white arrow). The cerebellar hemispheres are flat and severely reduced in size on the coronal plane with no atrophy. The corpus callosum appears thin but no atrophy of the cortex is observed. (B) Sagittal and coronal brain magnetic resonance images of three children (22 months, 13 months and 9 years old) affected with CASK mutations. In one patient (Patient 8), we found PCH with the ‘dragonfly-like’ cerebellar pattern i.e. flattening and severely reduced size of the cerebellar hemispheres (white arrow). In Patients 6 and 7, we found an attenuated pons and mild hypoplasia of the cerebellar hemispheres with a ‘butterfly-like’ pattern but no atrophy. In all cases, the corpus callosum is normal and there is no atrophy at the supratentorial level.
is clinically associated with a PCH2 phenotype whereas the presence of a nonsense or splice site mutation is associated with a PCH4 phenotype.

We studied a population of 11 patients (seven female, four male) and a male foetus from 13 families presenting with a PCH2 or PCH4 phenotype identified upon clinical and brain MRI criteria (Table 1). Eight patients displayed a PCH2 phenotype while four patients were compatible with a more severe PCH4 phenotype leading to death in the neonatal period in two of them, or to a medical termination of the pregnancy in the 3rd trimester for the foetus. We studied the mutation prevalence of TSEN (TSEN 54, 34, 15, 2) and the X-linked CASK genes in this series of patients.

We identified five patients from four families displaying mutations in the TSEN54 gene and three unrelated patients harbouring mutations in the CASK gene. Their clinical presentation and molecular findings are summarized in Table 1 and their MRI findings in Fig. 1. Similarly to the study by Navamar et al. (2011), we found nonsense or splice site mutations in TSEN54 to be associated with a severe PCH4 phenotype (two siblings, one deceased at birth and his foetus brother) whereas homozygosity for the common mutation (Ala307Ser) was associated with a milder, PCH2 phenotype (three unrelated patients) even though one of our patients (Patient 3, Table 1) died at age 24 months. Interestingly, CASK mutations were all de novo and were identified in female patients only, presenting a PCH2 phenotype although more attenuated in comparison to the PCH2 phenotype of patients bearing TSEN54 mutations in our series. We identified a novel TSEN54 variant (c.370-2 A > G) found to affect splicing
and three novel CASK mutations (c.1968 G > A, c.1970 G > A and c.1577delG) leading to a truncated protein (data not shown). These variations have not been found in 250 control chromosomes. No mutations were found in the TSEN2, TSEN34 and TSEN15 genes.

Brain MRI scan analyses were performed in all patients and we identified the characteristic ‘dragonfly-like’ cerebellar pattern in all patients affected with TSEN54 mutations: cerebellar hemispheres appearing flat and severely reduced in size, with no atrophy, as described by Namavar et al. (2011) (Fig. 1). Additionally, the corpus callosum appeared thin in patients with both PCH2 and PCH4 but with no cortical atrophy. Interestingly in one of the patients carrying a CASK mutation, we also identified a ‘dragonfly-like’ cerebellar pattern (Patient 8, Fig. 1) showing that the latter is not specific to TSEN54 mutations as suggested by Namavar et al. (2011). In the two other patients harbouring CASK mutations, we found an attenuated pons and mild hypoplasia of the cerebellar hemispheres with a ‘butterfly-like’ pattern but no atrophy. In all cases, the corpus callosum was normal and there was no atrophy at the supratentorial level. Based on these clinical, molecular and MRI findings, we propose a new diagnostic algorithm (Fig. 2).

In conclusion, our findings in a small series of patients affected with PCH show a strong correlation between the PCH2 and PCH4 phenotypes and TSEN54 mutations with a high prevalence of a common mutation as reported by Namavar et al. (2011) and others. Additionally we report patients presenting with a PCH2 phenotype, harbouring novel CASK mutations. In brain MRI we found the characteristic ‘dragonfly-like’ image in patients affected with both TSEN54 and CASK mutations showing that this pattern cannot discriminate the two genetic conditions. Finally, we propose a diagnostic algorithm for PCH type 2 and 4 involving TSEN54 and CASK analysis and based upon clinical and MRI criteria.

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**References**


