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Molecular analysis of Pericentrin gene (PCNT) in a series of 24 Seckel/MOPD II families

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ABSTRACT

Microcephalic osteodysplastic primordial dwarfism type II (MOPD II, MIM 210720) and Seckel syndrome (SCKL, MIM 210600) belong to the primordial dwarfism group characterized by intrauterine growth retardation, severe proportionate short stature and marked microcephaly. MOPD II is distinct from SCKL by more severe growth retardation, radiological abnormalities and absent or mild mental retardation. Seckel syndrome is associated with defective ATR-dependent DNA damage signalling.

In 2008, loss-of-function mutations in the pericentrin gene (PCNT) have been identified in 28 patients, including 3 SCKL and 25 MOPDII cases [6, 7]. This gene encodes a centrosomal protein which plays a key role in the organization of mitotic spindles.

The aim of our study was to analyze PCNT in a large series of SCKL-MOPD II cases to further define the clinical spectrum associated with PCNT mutations. Among eighteen consanguineous families (13 SCKL and 5 MOPDII) and 6 isolated cases (3 SCKL and 3 MOPD II), we identified thirteen distinct mutations in 5/16 SCKL and 8/8 MOPDII including five stop mutations, five frameshift mutations, two splice site mutations and one apparent missense mutation affecting the last base of exon 19. Moreover, we demonstrated that this latter mutation leads to an abnormal splicing with a predicted premature termination of translation. The clinical analysis of the 5 SCKL cases with PCNT mutations showed that they all presented minor skeletal changes and clinical features compatible with MOPDII diagnosis. We therefore conclude that, despite variable severity, MOPDII is a genetically homogeneous condition due to loss-of function of pericentrin.

KEY WORDS

Seckel syndrome
MOPDII
Skeletal manifestations
PCNT
INTRODUCTION

Among the primordial dwarfisms, microcephalic osteodysplastic primordial dwarfism type II (MOPD II, MIM 210720) and Seckel syndrome (SCKL, MIM 210600) are both characterized by intrauterine growth retardation, severe proportionate short stature and microcephaly [1, 2]. MOPDII is distinct from SCKL by the severity of the growth retardation, the presence of skeleton abnormalities and the mild/absent mental retardation [3]. SCKL is a genetically heterogeneous condition associated with defective ATR-dependent DNA damage signalling [4]. The only reported genetic defect so far is a hypomorphic mutation in the ATR gene (Sckl1, 3q22.1-q24) [5]. In 2008, mutations in the pericentrin gene (PCNT) have been identified in 28 patients, including 3 patients with SCKL [6] and 25 with MOPDII [7]. This gene encodes a centrosomal protein, which acts both at structural and regulatory levels. First, pericentrin recruits several structural centrosomal proteins, particularly gamma tubulin ring complex which initiates microtubular nucleation and spindle organization [8, 9, 10, 11]. Second, it plays a role in cell cycle regulation through its interaction with the ATR pathway [6]. All mutations identified so far lead to premature translation termination and are responsible for pericentrin loss of function as demonstrated in PCNT-mutated cell lines issued from patients with SCKL or MOPDII.

To further define the clinical spectrum of patients with PCNT mutations, we analyzed PCNT in 24 families diagnosed either with SCKL or with MOPDII, including 18 consanguineous families and 6 cases from unrelated parents.

PATIENTS AND METHODS

Patients

Criteria for inclusion in the study were:

- Intrauterine and postnatal growth retardation with birth weight < -2 SD and postnatal height < -4 SD
- Microcephaly with an occipitofrontal circumference (OFC) < -4 SD
- Diagnosis of MOPDII or SCKL made by a clinical geneticist.

Diagnostic assessment was performed for all patients by their clinicians (Table 1). Seven of these families were previously clinically reported, by Faivre et al [12] (families 1,3,7, 8, 11 and 12) and Verloes et al [13] (family 19). Written informed consent was obtained from all subjects included in this study.
**Microsatellites marker analysis**

In all consanguineous families, microsatellites analysis of the PCNT locus was first performed and PCNT was sequenced only in compatible cases. The PCNT sequence analysis was performed in all cases from unrelated parents. Blood samples were obtained with informed consent from affected children, parents and unaffected siblings. Genomic DNA was extracted using Nucleospin® Blood XL kit (Macherey-Nagel). We established lymphoblastoid cell lines by EBV transformation and we performed a primary skin fibroblasts culture. Genotyping was performed using 4 flanking (D21S1903, D21S1897, D21SpolyATT, D21S1446) and one intragenic-PCNT (PCNT-IG) microsatellite markers in all consanguineous families and non-consanguineous families with at least two siblings.

**Mutation analysis**

PCNT exon and flanking intron sequences were amplified from patient DNA by PCR using 49 couples of primers designed with the Primer 3 software (Sequence of primers available on request). Sequencing reactions were performed on both strands using the BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Foster City, California) according to the manufacturer’s instructions.

**Functional consequences of c.3840G>C (p.Q1280H) mutation**

RNA was extracted from cultured fibroblasts and cDNA was synthetized using primers designed to overlap 2 consecutive exons (Sequence of primers: JUNCTION17/18-5': CCTGTCCCACAGCGAAAGAG; JUNCTION18/19-5': ACAGCTCCGCGCTGGAG; JUNCTION20/21-3': GGTTACCCTGAGTCTTGTGCAGC). RT-PCR products were expected to span exons 18 to 20 and exons 19 to 20. GAPDH expression was used as positive controls.

**RESULTS**

Genotyping analysis showed that 9/18 consanguineous families (4/13 SCKL and 5/5 MOPDII) were compatible with linkage to the PCNT locus.

The PCNT sequence analysis performed in these 9 consanguineous families and in 6 additional cases (from unrelated parents) allowed the identification of 13 distinct mutations in 13 patients (Table 2). All but one mutation were homozygous and cosegregate with the disease. In one patient (case 22), a single heterozygous mutation, inherited from the father, was detected. The 13 mutations were located throughout the gene and among them, five were nonsense mutations (exons 11, 15, 17, 28 and 34), five were frameshift mutations (exons 14, 16, 28A, 30), two were splice site mutations (intron 18 and intron 40) and one was an apparent missense mutation affecting the last base of exon 19 (c.3840G>C, p.Q1280H). None
of the mutations were identified in 400 control chromosomes. The c.3840G>C mutation was predicted to alter splicing. This was further confirmed by sequence analysis of RT-PCR products which demonstrated exon 19 skipping, predictive of a premature termination of translation (p.P1204GfsX11).

Among the 13 patients, 5 were clinically diagnosed as SCKL (patients 12-16) and 8 were diagnosed as MOPDII (patients 17-24, Table 1). The identification of PCNT mutations in 5 SCKL and 8 MOPDII patients prompted us to re-analyze the clinical features of all the patients with PCNT mutations and compare them to SCKL patients without mutation. We first observed that the five SCKL patients with PCNT mutations (families 12-16) presented a more severe growth retardation than the SCKL patients without PCNT mutation (-6 to -8 SD versus -4 to -5 SD), but less severe than the MOPDII patients with PCNT mutation (-7 to -13 SD). For two of them, the adult height is 120 cm and 140 cm respectively (Figure 1, from Hall JG). These patients presented also skeletal anomalies including gracile long bones, metaphyseal flaring, carpal condensation, and moderate hip dysplasia (Figures 2, 3, 4). These anomalies were not present during the first year of life, became more pronounced with time but were often less severe than those classically described in MOPDII patients. Importantly, we did not observe similar skeletal anomalies in 6 patients without PCNT mutation and with skeletal survey available. Finally, these patients have either normal intelligence or mild mental retardation. No difference was observed with respect to age of walking (normal to slightly delayed) and developmental course. Early developmental milestones were considered as normal with excellent social skills. Learning disability was noted after the age of 5 years. None of the 4 adult patients can live independently and they all perform “adapted work”.

Other features suggestive of MOPDII diagnosis were present in the clinically diagnosed SCKL patients with PCNT mutations including truncal obesity and death at 20 years of age of rupture of CNS vessels (patient 15), initial feeding difficulties and development of typical pigmentation anomalies with time (patients 13 and 14), polycystic ovaries (patient 14), subglottic stenosis (patient 13), microdontia (patient 12), high-pitched voice, stridor and upper respiratory tract infections (patient 16).

Finally, facial features were highly suggestive of MOPD diagnosis for 11 patients with PCNT mutations including a broad nose with hypoplastic tip, thin alae nasi, with columella lying below the alae nasi, long midface, prominent cheeks, small jaw and large eyes in the youngest children. Facial features were also changing with time, variable with the ethnic origin (patient 18) and less characteristic in the eldest patient of our series (patient 15). By contrast facial features of the patients with no PCNT mutation (1-11) were quite variable.
DISCUSSION

We report here the identification of 13 distinct mutations in 8 MOPDII and 5 SCKL patients. As previously reported by Griffith [6] and Rauch [7], mutations are distributed throughout the gene. We did not find any recurrent mutations in our series. However, the c.3109G>T mutation (exon 15) was previously reported by Rauch et al in a patient also originating from Turkey (patient 1). In one patient (patient 22), one mutation only was detected by direct sequencing but unfortunately RNA was not available. This might be due to the limit of our screening and a partial deletion of PCNT gene cannot be excluded. Our study provides also the first example of a “missense” mutation (c.3840G>C) but we demonstrated that this mutation impairs exon 19 splicing, leading to premature termination of translation. We conclude, as previously suggested, that all identified mutations are loss of function mutations.

We identified PCNT mutations in all MOPDII cases, confirming the genetic homogeneity of this disorder. Moreover, the retrospective analysis of the 5 SCKL patients with PCNT mutation also suggests that they all belong to the MOPDII spectrum. However, they were diagnosed as SCKL, based on the absence of severe skeletal manifestations and on their final stature >110 cm, which usually excludes the diagnosis of MOPDII [3]. Our study also supports that SCKL spectrum is heterogeneous and suffers from variable definition in the literature and from clinicians in practice. Indeed, Seckel syndrome has often been used as a generic term used for primordial dwarfism, without more specific diagnosis. Recently, D’Angelo and Di Bartolomeo reported two cases of SCKL with intracranial anomalies, suggestive of MOPDII diagnosis [16, 17]. Similarly, SCKL patients with bone dysplasia suggestive of MOPDII have been reported [18, 19]. From our study, we suggest that MOPDII spectrum is wider than previously defined. However, in all patients with PCNT mutations we have consistently observed 1) distinct facial features 2) growth retardation <-5SD and microcephaly <-4SD, 3) mild to absent mental retardation 4) skeletal manifestations including hip dysplasia ranging from short femoral neck to severe coxa vara; carpal condensation, and gracile long bones with metaphyseal flaring. Other suggestive features occasionally observed included 1) vascular anomalies and cutis marmorata 2) high pitched voice 3) microdontia, 4) hyperinsulinism, 5) subglottic stenosis, 6) pigmentation anomalies with areas of hypo- and hyperpigmentation.
We also observed in two patients with \textit{PCNT} mutation a liver involvement varying in severity from cytolysis to cirrhosis, with the same histological features than those described in the literature for patients with MOPDI diagnosis, consisting in ductular cholestasis, inflammatory infiltrate, and giant multinucleate hepatocytes. Although these findings are not specific, they may suggest the existence of biliar epithelium anomalies in MOPDII spectrum [20].

While this study further demonstrates that MOPDII is caused by \textit{PCNT} mutations, the pathogenic mechanisms underlying the clinical features observed in these patients remain unclear [21]. First, microcephaly could be related to structural centrosomal abnormalities similar to those observed in primary microcephaly [22]. Second, defect in ATR-dependent DNA damage signalling has been demonstrated in other conditions characterized by short stature and microcephaly and may thereby also account for short stature observed in MOPDII cases [23, 24]. Other specific clinical features like vessels anomalies, generalized bone dysplasia and hepatitis remain unexplained so far, since they have not described in patients with ATR mutations or other centrosomal genes such as ASPM.

Finally, O’Driscoll and collaborators suggested that the ATR signalling pathway was unusually sensitive to haploinsufficiency and established a correlation between ATR-pathway dysfunction and growth retardation [23]. Similarly, Rauch et al reported a significant reduction of the mean height of heterozygous MOPDII parents [7]. We did not observe such a reduction in the mean of parental heights (ranging from -1 to +2 SD) but ethnical variability of growth charts may interfere with parental height analysis.

In conclusion, we identified 13 \textit{PCNT} loss of function mutations in 13 patients who all presented diagnostic criteria for MOPDII. However, we observed a wider variability in the severity of the short stature and skeletal manifestations than previously admitted, modifying the MOPDII clinical spectrum. The distinction between SCKL and MOPDII appears to be crucial for the appropriate management, keeping in mind the risk of vascular anomalies in MOPDII.

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Legends to figures

Figure 1: Comparison of growth curves of patients from our cohort with those of patients from MOPD II cohort reported by Hall JG et al (from Hall JG, 2004)

Figure 2: Comparison of Hip X-rays in patients with PCNT mutations. Note the hip dislocation and coxa vara more pronounced in MOPD II patients

Figure 3: Comparison of lower limb X-rays in patients with PCNT mutations. Note the gracile long bones in both groups.

Figure 4: Comparison of hand X-rays in patients with PCNT mutations. Note the carpal fusion, the clinodactyly and the delayed bone age.

Figure S1. Facial features in two sisters with no PCNT mutation (A and B, family 7) and two patients with PCNT mutation (C1 and C2, Patient 14; D1 and D2, Patient 17). Note in patient with PCNT mutations the characteristic nose with hypoplastic alae nasi, prominent cheeks and small jaw while in the two patients with no PCNT mutation the facial features are marked by the severe microcephaly with large nose and large ears.
<table>
<thead>
<tr>
<th>Family /SCKL*</th>
<th>Ethnic origin/ Gender</th>
<th>CS /Coefficient f</th>
<th>Birth : WOG/ Weight(g)/Height(cm)/HP(cm)</th>
<th>Postnatal growth : Weight/Height/HC(SDS)</th>
<th>Mental retardation</th>
<th>Other clinical features</th>
<th>Radiological features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 /SCKL* (case 4[12])</td>
<td>Algeria F</td>
<td>Y f=1/64</td>
<td>41/2160/45/32</td>
<td>-4/-4/-4</td>
<td>Mild</td>
<td>Café au lait spots. VSD. Pyelic bifidity. Osteosarcoma</td>
<td>Mild bowing of the femora</td>
</tr>
<tr>
<td>2/SCKL</td>
<td>NA M</td>
<td>Y</td>
<td>NA NA NA NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>3 /SCKL* (case 3[12])</td>
<td>Mali M</td>
<td>Y f=1/8</td>
<td>FT/2000/40/24</td>
<td>-4/-5.5/-10</td>
<td>Severe</td>
<td>Hypertonia. Ichtyosis. Abnormal gyration pattern</td>
<td>Slight platyspondyly</td>
</tr>
<tr>
<td>4/SCKL</td>
<td>France M</td>
<td>Y f=1/16</td>
<td>NA NA NA NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5/SCKL</td>
<td>Algeria F/F</td>
<td>Y</td>
<td>NA NA NA NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6/SCKL</td>
<td>Algeria M</td>
<td>Y f=1/32</td>
<td>FT/3300/47/30</td>
<td>-4/-4/6</td>
<td>Mild</td>
<td>Hip dislocation. Chromosomal breakage</td>
<td>Normal</td>
</tr>
<tr>
<td>7/SCKL* (case 2[12])</td>
<td>Morocco F/F</td>
<td>Y f=1/16</td>
<td>FT/1400/40/28 FT/NA/40/NA</td>
<td>Mild/ N</td>
<td>Delayed puberty. Cataract Cataract</td>
<td>Thoracolumbar scoliosis</td>
<td></td>
</tr>
<tr>
<td>9/SCKL</td>
<td>Lebanon F</td>
<td>Y</td>
<td>NA</td>
<td>NA NA NA NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>10/SCKL</td>
<td>France F/F</td>
<td>N</td>
<td>FT/2030/41/NA NA/NA/NA/NA</td>
<td>-3.5/-7/-7</td>
<td>N/ Mild</td>
<td>Pyelic duplication Scoliosis</td>
<td>NA Severe scoliosis</td>
</tr>
<tr>
<td>11/SCKL* (case 6[12])</td>
<td>France M/F</td>
<td>N</td>
<td>NA NA NA NA</td>
<td>NA/-4/-8 NA/-4/-6</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>13/SCKL</td>
<td>Morocco F</td>
<td>Y f=1/16</td>
<td>32/950/34/25,5</td>
<td>-5/-8/-6.6</td>
<td>N</td>
<td>Café au lait spots, areas of depigmentation Hepatocytic cytosis. Subglottic stenosis Recurrent upper respiratory tract infections</td>
<td>Coxa vara</td>
</tr>
<tr>
<td>15/SCKL</td>
<td>France M</td>
<td>N</td>
<td>FT/1720/40/27,3</td>
<td>-2.7/-6/-8</td>
<td>Mild</td>
<td>Horseshoe kidney. Clinodactyly of fifth finger. Rupture of CNS vessels leading to death (20 years)</td>
<td>High vertebral bodies. Thick diaphyseal cortex. Short femoral neck</td>
</tr>
</tbody>
</table>
### Table 1: Clinical and radiological features of the 24 families.

Patients 1-11: Patients with Seckel diagnosis - *PCNT* excluded  
Patients 12-16: Patients with Seckel diagnosis - *PCNT* mutation identified  
Patients 17-24: Patients with MOPDII diagnosis - *PCNT* mutation identified  

<table>
<thead>
<tr>
<th>No.</th>
<th>Family Details</th>
<th>Sex</th>
<th>Age</th>
<th>Weight</th>
<th>Length</th>
<th>Head Circumference</th>
<th>PCNT Status</th>
<th>Other Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>17/</td>
<td>MOPDII Turkey</td>
<td>M</td>
<td>Y</td>
<td>1/16</td>
<td>36/1290/35/27</td>
<td>-6/-9/-7</td>
<td>Mild</td>
<td>Poor sucking, Vomiting, Hyperlaxity, Horseshoe kidney, Hypertonia, Subglottic stenosis, Recurrent upper respiratory tract infections, Micropenis, High squeaky, nasal voice</td>
</tr>
<tr>
<td>18/</td>
<td>MOPDII Sri Lanka/M</td>
<td>Y</td>
<td>f=1/16</td>
<td>NA</td>
<td>NA/-12/-12</td>
<td>NA</td>
<td>Polikiddermia, Anal septal defect</td>
<td>Hypoplastic distal phalanges, Carpal fusion, Hip dislocation, Overtubulated and thick diaphyseal cortex</td>
</tr>
<tr>
<td>19/</td>
<td>MOPDII Morocco/F/M</td>
<td>Y</td>
<td>f=1/16</td>
<td>37/770/30/24</td>
<td>39/1190/33/27,6</td>
<td>-7/-11/-12</td>
<td>Severe/severe</td>
<td>Hypertonia, Micropenis</td>
</tr>
<tr>
<td>20/</td>
<td>MOPDII Algeria/M</td>
<td>Y</td>
<td>f=1/16</td>
<td>FT/NA/30/NA</td>
<td>-10/-13/-10</td>
<td>Mild</td>
<td>Café au lait spots, Moya-moya disease complicated with rupture of CNS vessels</td>
<td>Coxa vara, Carpal fusion</td>
</tr>
<tr>
<td>21/</td>
<td>MOPDII Italy</td>
<td>M</td>
<td>N</td>
<td>31/585/31/23,5</td>
<td>-8/-12/-12</td>
<td>N</td>
<td>Micropenis, Café au lait spots, Livedo reticularis, Cirrhosis, High squeaky, nasal voice</td>
<td>Coxa vara</td>
</tr>
<tr>
<td>22/</td>
<td>MOPDII France/M</td>
<td>N</td>
<td>37/1400/40/29,5</td>
<td>-9/-7/-6</td>
<td>N</td>
<td>Anemia, Body asymmetry, Radial head dislocation</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>23/</td>
<td>MOPDII Morocco/F</td>
<td>N</td>
<td>37/870/33,5/24</td>
<td>-6/-7/-9</td>
<td>N</td>
<td>High squeaky voice, Café-au-lait spots</td>
<td>Coxa vara, Short femoral neck, Delayed bone age</td>
<td></td>
</tr>
<tr>
<td>24/</td>
<td>MOPDII Morocco/F</td>
<td>Y</td>
<td>37/1300/36/26</td>
<td>-5,5/-7/-6,5</td>
<td>N</td>
<td>Cranial multiple osteolysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 2: Mutations identified in our series.

(#) This mutation was previously identified by Rauch in a MOPDII patient with the same Turkish ethnic background

<table>
<thead>
<tr>
<th>Family</th>
<th>Diagnosis</th>
<th>Identified mutation</th>
<th>Position</th>
<th>Status</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>SCKL</td>
<td>c.1753C&gt;T</td>
<td>Exon 11</td>
<td>homozygous</td>
<td>p.Arg585X</td>
</tr>
</tbody>
</table>
| 13     | SCKL      | c.3840G>C           | Exon 19  | homozygous | p.Gln1280His  
|        |           |                     |          |        | Splicesite: Pro1204Glyfs*11 |
| 14     | SCKL      | c.6176_6189delGTCA  | Exon 30  | homozygous | p.Gln2060Argfs*48 |
|        |           | CTGCCGAAG            |          |        |         |
| 15     | SCKL      | c.3271_3272delTT    | Exon 16  | homozygous | p.Leu1091Valfs*101 |
| 16     | SCKL      | c.5266dupA          | Exon 28  | homozygous | p.Met1756Asnfs*53 |
| 17     | MOPDII    | c.3109G>T           | Exon 15  | homozygous | p.Glu1037X (#) |
| 18     | MOPDII    | c.9099+2T>C         | Intron 40 | homozygous | Splicesite |
| 19     | MOPDII    | c.6316_6325delGTTC  | Exon 30  | homozygous | p.Leu2106Alafs*18 |
|        |           | GAGAGCA              |          |        |         |
| 20     | MOPDII    | c.2326_2327delGA    | Exon 14  | homozygous | p.Glu776Lysfs*3 |
| 21     | MOPDII    | c.3608-2A>G         | Intron 18 | homozygous | Splicesite |
| 22     | MOPDII    | c.5578G>T           | Exon 28  | heterozygous | p.Glu1860X |
| 23     | MOPDII    | c.7338C>A           | Exon 34  | homozygous | p.Cys2446X |
| 24     | MOPDII    | c.3382C>G           | Exon 17  | homozygous | p.Gln1128X |
Diagnosis of SCKL and PCNT mutation

Diagnosis of MOPDII and PCNT mutation

Diagnosis of SCKL and PCNT excluded

Patients clinically diagnosed as SCKL

Patients clinically diagnosed as MOPDII

19 years

5 ½ years

2 years

5 years

20 years