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Investigation of imprinting alterations in *MKRN3* and *DLK1* in a cohort of girls with central precocious puberty through specific DNA methylation analysis

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Background

Loss of imprinting has been implicated in the pathogenesis of several human diseases[1].
In the last years, monogenic causes of central precocious puberty (CPP) were identified in families with loss-of-function mutations in two paternally expressed imprinting genes: *MKRN3* and *DLK1*[2,3].
However, the role of imprinting defects in CPP has not been described so far.

Objective

To investigate the methylation status at primary differentially methylated regions (DMR) of *MKRN3* and *DLK1* in a cohort of patients with central precocious puberty.

Patients and methods

One hundred and fifteen girls with CPP (107 sporadic, 8 familial) were selected for analysis. All had normal brain MRI. Leukocyte DNA was obtained from all patients. *MKRN3* and *DLK1* pathogenic variants were initially excluded by DNA sequencing analysis. Bisulfite treatment followed by by Allele-Specific Methylated Multiplex Real-Time Quantitative PCR was performed with leukocyte DNA, analyzing separately the methylation index (MI) of *MKRN3*:TSS-DMR and *DLK1/MEG3*:IG-DMR for each patient. The MI results were compared with the following controls: 50 adults, 15 pre-pubertal girls and 18 pubertal girls.

Results

- Mean age at puberty onset was 6.1 ±1.9 years for all girls with CPP.
- Hypomethylation at DLK1/MEG3:IG-DMR was identified in two patients with sporadic CPP (patients 1 and 2).
- Both girls had been firstly referred to pediatric endocrinology for presenting precocious menarche. Age at menarche was 8.7 years in patient 1, and 7.9 years in patient 2.
- During follow-up, other clinical findings were noticed in both: being born small for gestational age, prominent forehead, small hands/feet, short stature, speech and motor delay, overweight/obesity. Patient 2 presented early onset type 2 diabetes.
- Subsequent studies were performed for delineation of molecular mechanisms. SNP array identified a maternal uniparental disomy at chromosome 14 in patient 1. While patient 2 presented normal genomic microarray and normal microsatellites analysis, indicating a mechanism of epimutation. Both mechanisms are associated with Temple syndrome.
- In the remaining patients, methylation index for *DLK1/MEG3*:IG-DMR resulted normal (Table 1).
- For all patients, methylation index at *MKRN3*:TSS-DMR resulted normal (Table 2).
- Besides that, there were no significant correlations between age at puberty onset and: 1) methylation index at *DLK1/MEG3*:IG-DMR (p=0.69) and 2) methylation index at *MKRN3*:TSS-DMR (p=0.45).

Patient 1
DLK1/MEG3:IG-DMR methylation index 16%

Patient 2
DLK1/MEG3:IG-DMR methylation index 11%

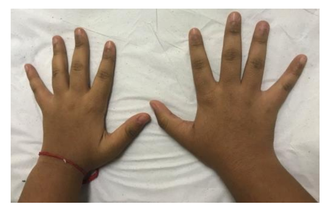

Table 1. Methylation index at *DLK1/MEG3*:IG-DMR

Patients	***Mean ±SD (%)***
Girls with CPP (n=113)	49 ±2
✓Sporadic CPP (n=105)	49 ±5
✓Familial CPP (n=8)	51 ±1
Controls	***Mean ±SD (%) (Min-Max)***
Adult (n=50)	49 ±1.5 (46-52)
Pre-pubertal girls (n=15)	50 ±2 (46-54)
Pubertal girls (n=18)	48 ±1.5 (45-51)

Table 2. Methylation index at *MKRN3*:TSS-DMR

Patients	***Mean ±SD (%)***
Girls with CPP (n=115)	49 ±6
✓Sporadic CPP (n=107)	49 ±6
✓Familial CPP (n=8)	45 ±7
Controls	***Mean ±SD (%) (Min-Max)***
Adult (n=50)	47 ±6 (35-59)
Pre-pubertal girls (n=15)	49 ±5 (39-59)
Pubertal girls (n=18)	49 ±6 (37-61)

Conclusions

- There was no leukocyte DNA methylation defect at *MKRN3* imprinting control region in girls with central precocious puberty.
- *DLK1/MEG3*:IG-DMR hypomethylation was identified in two patients with central precocious puberty and additional findings of Temple syndrome.

References:
1. Geoffron *et al.* J Clin Endocrinol Metab, 2018
2. Abreu *et al.* N Engl J Med, 2013
3. Dauber *et al.* J Clin Endocrinol Metab, 2017

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