Serum Makorin ring finger protein 3 values for predicting central precocious puberty in girls

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Abstract

Background/Aim: MKRN 3 is involved in regulating the initiation of puberty by inhibiting gonadotropin-releasing hormone (GnRH) secretion. This study evaluated the serum level of MKRN3 and investigated its diagnostic usefulness in girls with central precocious puberty (CPP).
Methods: In total, 41 girls with CPP and 35 age-matched normal control girls were enrolled. Serum values of MKRN3 and gonadotropin and estradiol concentrations were evaluated after 6 and 12 months of GnRH agonist (GnRHa) treatment in CPP patients. Serum levels of MKRN3 were measured in normal controls. A receiver operating characteristic curve (ROC) analysis was performed to assess the value of MKRN3 in diagnosing CPP.
Results: The MKRN3 concentrations were much lower in the patient group than in the control group ($p = 0.005$). Over one year of GnRHa treatment in patients, the gonadotropin concentrations were significantly decreased ($p < 0.05$), while the MKRN3 concentrations were unchanged ($p > 0.05$). MKRN3 levels were inversely correlated to standard deviation (SD) in height ($r = -0.46$, $p = 0.000$), SD in weight ($r = -0.32$, $p = 0.005$), Tanner stage ($r = -0.41$, $p = 0.000$) and bone age ($r = -0.46$, $p = 0.000$). There were no significant correlations between gonadotropin levels, estradiol and MKRN3 in CPP girls. Based on ROC analysis, the area under curve was 0.758 for MKRN3, with 82.9% sensitivity and 68.5% specificity.
Conclusions: Serum MKRN3 levels were lower in precocious puberty patients than in normal controls. The measurement of serum MKRN3 level may provide some help for CPP prediction, but relatively various values need further validation.

Introduction

MKRN3, the only known inhibitory component to date, encodes Makorin ring finger protein 3, a putative E3 ubiquitin ligase belonging to the Makorin family of zinc-finger proteins. *MKRN3* is located on chromosome 15 in the Prader-Willi syndrome-associated region (15q11–q13). A loss-of-function mutation in *MKRN3* results in central precocious puberty, and it is the most common genetic cause of CPP reported to date. Decreasing mRNA expression of hypothalamic *MKRN3* preceding pubertal onset has been observed in mice of both sexes. Furthermore, decreasing MKRN3 levels were observed in healthy girls and boys prior to the onset of puberty, supporting the inhibitory role of MKRN3 in hypothalamic GnRH secretion. Therefore, the serum level of MKRN3 need to be verified as a potential biomarker in predicting the onset of puberty, especially in patients with CPP.
In this study, we evaluated the relationships among serum MKRN3 level and endocrine parameters and assessed the diagnostic usefulness of the former. We also investigated changes in serum MKRN3 levels over 1 year of GnRH agonist treatment

Subjects and Methods

Subjects

Forty-one girls with idiopathic CPP aged 7.0-8.9 years and 35 age-matched normal controls who visited the Pediatric Endocrinology Department at the Hallym Medical Center for their growth checkup between March 1, 2013 and August 31, 2015 were enrolled. The patients all exhibited breast enlargement appearing before the age of 8 years. Bone ages (BA) were found to be at least 1 year ahead of the patients' chronological age (CA). A GnRH stimulation test was carried out in all patients to confirm that the HPG axis had been activated, as revealed by peak luteinizing hormone (LH) levels ≥ 5.0 IU/L on a chemiluminescent microparticle immunoassay
Inclusion criteria for the normal control group were follows: no evidence of breast budding and BA - CA < 1 year. All blood samples were centrifuged, and serum was separated and stored at -80'C before it was assayed.

Methods

Data on height, weight, BMI, pubertal status, and bone age were collected every 6months from clinical charts and electronic medical records. This study protocol was approved by the Institutional Review Board of the Hallym Medical Center (KANGDONG 2016-11-003).
Serum LH, FSH, and estradiol (E_2) were measured only in the patient group. Serum MKRN3 concentrations were determined using a commercially available Human MKRN3 ELISA Kit (MyBioSource, San Diego, CA, USA) with a detection limit of 7.8 pg/mL. The intra- and inter-assay coefficients of variation (CVs) listed by the manufacturer were < 8% and < 12%, respectively. The test worked as stated by the manufacturer. We measured the MKRN3 levels in baseline samples (before the initiation of GnRHa treatment in the patient group) in both groups. In the patient group, basal LH, follicle-stimulating hormone (FSH), and serum MKRN3 levels were measured before treatment and after 6 and 12 months of treatment.

Results

Table 1. Baseline characteristics of the subjects.

	Patients (n = 41)	Controls (n = 35)	*p value*
Age at diagnosis (years)	8.42 ± 0.48	8.32 ± 0.46	0.341
Height SDS	1.37 ± 0.86	-0.18 ± 0.65	<0.001*
BMI SDS	0.90 ± 0.77	-0.11 ± 1.02	<0.001*
Tanner stage	2.53 ± 0.50	1.00 ± 0.00	<0.001*
BA-CA (years)	2.05 ± 0.74	-0.22 ± 0.76	<0.001*
Basal LH (IU/L) [a]	1.36 ± 1.11	ns	ns
Basal FSH (IU/L) [a]	3.41 ± 1.81	ns	ns
Peak LH (IU/L) [a]	10.90 ± 4.98	ns	ns
Peak FSH (IU/L) [a]	17.03 ± 7.35	ns	ns
MKRN3 (pg/mL)	521.00 ± 608.50	1282.24 ± 791.26	<0.001*

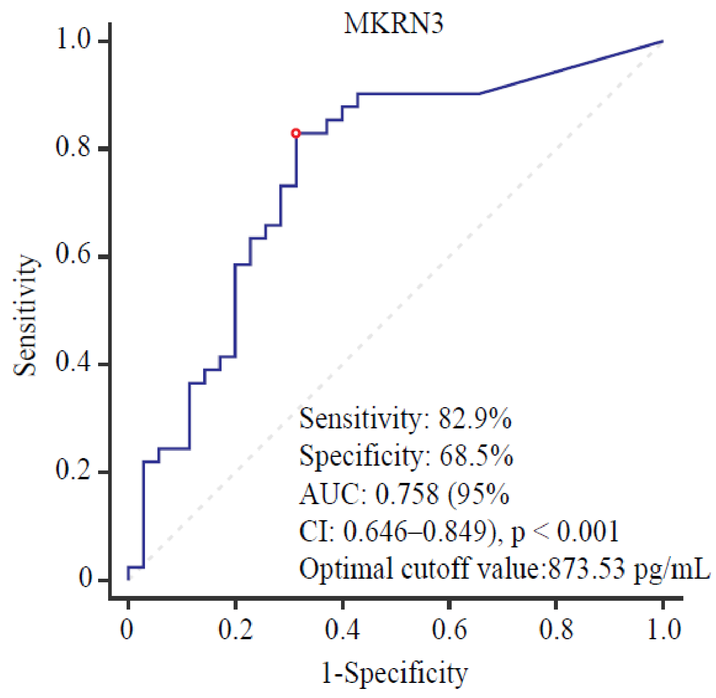
Table 2. Changes in serum MKRN3 and gonadotropin levels before, during, and after GnRHa treatment (mean ± SE).

	CPP (0)	CPP (6)	CPP (12)	*p value*
LH (IU/L)	1.36 ± 0.17	0.77 ± 0.07	0.79 ± 0.12	0.017*
FSH (IU/L)	3.41 ± 0.28	0.97 ± 0.11	1.09 ± 0.09	<0.001*
MKRN3 (pg/mL)	521.00 ± 95.03	455.67 ± 99.01	481.39 ± 100.51	0.649

Table 3. Pearson's correlations between serum MKRN3 and other variables.

	r	*p value*
BA (years)	–0.461	0.000*
Tanner stage	–0.405	0.000*
Height SDS	–0.46	0.000*
Weight SDS	–0.32	0.005*

Figure 1. ROC curve for MKRN3 level in the diagnosis of CPP



Conclusions

In conclusion, MKRN3 acts as a inhibitory factor of hypothalamic GnRH secretion and decrease as puberty begins. Additionally, GnRHa treatment reduced gonadotropin but did not seem to affect MKRN3 in CPP girls. A low MKRN3 level is a useful predictor of CPP but the value are relatively variable. Therefore, serum MKRN3 level might be used as a potential biomarker for CPP prediction after more validation study. A larger prospective study is warranted to confirm these initial exploratory results.