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MUTATIONAL ANALYSIS OF *TAC* AND *TACR3* IN IDIOPATHIC CENTRAL PRECOCIOUS PUBERTY

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Abstract

Background: The genetic background of idiopathic central precocious puberty (ICPP) is not well understood, and is thought to arise from the effect of multiple genes. Familial ICPP have been reported suggesting the existence of monogenic causes of ICPP. The neurokinin B (NKB) system has recently been implicated in the regulation of the human reproductive axis. In humans, NKB and its receptor are encoded by the TAC3 and TACR3 genes, respectively. Mutations in these genes have been suggested to be causative for ICPP.

Methods: ICPP was defined by pubertal onset before 8 yrs of age in girls, and a pubertal LH response to GnRH testing. Twenty eight girls with ICPP were included in the study (age at diagnosis was 5.72 ± 2.59; bone age, 6.12 ± 2.81, height at the start of treatment, 0.90 ± 1.48 SD). LHRH test was performed and was pubertal in all subjects (LH 20.35 ± 32.37 mIU/ml; FSH 23.32 ± 15.72 mIU/ml). The coding regions of *TAC* and *TACR3* were sequenced.

Results: No rare variants were detected in *TAC* and *TACR3* in the 28 subjects with ICPP.

Conclusions: We confirmed that mutations in *TAC* and *TACR3* are not a common cause for ICPP.

Key words: *TAC, TACR3*, idiopathic central precocious puberty, timing of puberty.

Introduction

Besides great advances in medicine and endocrinology the complex events that trigger the onset of puberty remain enigmatic. Nowadays puberty begins earlier than a few decades ago, and it is believed that multiple factors are involved such as environmental, genetic and racial/ethnic [1]. Central precocious puberty (CPP) results from premature activation of hypothalamic GnRH secreting neurons, leading to precocious development of secondary sexual characteristics, acceleration in linear growth and progressive bone age advancement [2]. The term "idiopathic" for CPP was given due to unknown factors influencing the premature activation of the hypothalamic-pituitary-gonadal (HPG) axis. Monogenic causes and familial occurrence of ICPP have been reported [3]. In a study by de Vries et al. [4], a 27.5% prevalence of familial cases of CPP has been reported, which strongly suggest a genetic origin. Over the last decade, the neuropeptide kisspeptin emerged as an important excitatory neuroregulator for the release of GnRH. At the present kisspeptin is recognized as the most potent known stimulator of GnRH-dependent LH secretion and is considered as a crucial factor for acquisition of normal reproductive function and the onset of puberty [5]. Two- gain -of -function mutations in *KISS1* and *KISS1R* have been identified recently as genetic causes of CPP [6, 7]. Other candidate genes for CPP include *GNRH1*,

GNRHR, LIN28B, TAC and *TACR3* [8–10]. The aim of this study was to evaluate the role of *TAC* and *TACR3* in the pathogenesis of ICPP.

Methods

Subjects

Twenty eight girls with ICPP attending the outpatient clinic at the Department of Endocrinology and Genetics, University Hospital for Sick Children, Medical Faculty, Skopje, Macedonia were recruited for this study. Written informed consent was obtained from all patients and/or their parents. All patients presented with breast budding as first sign of puberty, and all were pre-menarcheal at diagnosis. The girls were diagnosed with ICPP if the following criteria were met: age at onset of breast development < 8 yrs, peak LH-level > 5IU/l in response to rapid-acting GnRH (0.1 mg of Relefact LH-RH), and a non-pathological brain MRI. In addition, bone age and sex steroid hormones were evaluated. One hundred thirty-two healthy controls were recruited among patients with normal pubertal development with available DNA.

Mutation analysis

DNA was extracted from peripheral blood using standard procedures. The entire coding regions and the intron-exon junctions of TAC3 and TACR3 genes were amplified by polymerase chain reaction using specific primers and automatically sequenced.

Amplification reactions were performed in a final volume of 25 μl containing 200 ng genomic DNA, 0.2 mM dNTPs, 1.5 mM PCRx Enhancer Solution (Invitrogen), 0.6 pmol each primer, 1X PCR buffer, and 1U Go Taq DNA polymerase (Promega, Madison, WI) and carried out for 35 cycles: denaturation at 95°C for 30 sec, annealing at 55–56°C for 30 sec, extension at 72°C for 1 min, followed by a final extension for 10 min at 72°C. The PCR products were checked on 1% agarose gel electrophoresis, purified and automatically sequenced in an ABI Prism Genetic Analyzer 3100 automatic DNA sequencer (Applied Biosystems, Foster City, CA).

Results

The mean age at onset of puberty was 7.5 yrs (6.5–7.9; 5.72 ± 2.59 yrs), median 0.75 years. Height at onset of therapy in SD score was 0.90 ± 1.48. There was a mean bone age advancement of 1.4 yrs (-0.1 to 2.8) (median + 0.66 years). Maximum peak levels of LH were well above the upper normal levels.

Concerning the genetic analysis, no rare variants were detected in *TAC* or *TACR3* in the 28 subjects with ICPP.

Discussion

Kisspeptin plays a critical role in the development of puberty. Eventually, sex steroids appear to play a major role in kisspeptin expression [11, 12]. In 2003, the presence of deletions and inactivating mutations of *KISS1R* in patients with idiopathic hypogonadotropic hypogonadism was reported [13, 14]. Recently, the kisspeptin system has also been implicated in the pathogenesis of ICPP [6, 7, 15]. In our previous study we tested the same cohort of 28 girls with ICPP but we did not find any pathogenic mutation in *KISS1* and *KISS1R* [16].

Other genes including *GNRH1*, *GNRHR, LIN28B, TAC* and *TACR3* were considered candidate genes for ICPP. There is evidence that NKB is highly expressed in hypothalamic neurons that also express kisspeptin and that NKB/NK3R are involved in the regulation of pubertal development [12]. Therefore one might hypothesize that activating mutations in NKB or in NK3R, could be identified in children with ICPP. In addition loss-of-function mutations in the *TAC3* and *TACR3* genes were found in patients with normosmic IHH, which is characterized by an absence of pubertal development and low circulating levels of LH and gonadal steroids [17–22].

A group from Brazil described new rare variants in the NKB (p. A63P) and in the NK3R (p. A449S) in two girls with central precocious puberty and constitutional delay of growth and puberty, respectively [23]. Their preliminary study suggested that these two new variants were unlikely to have a direct causative role in the precocious puberty and constitutional delay of growth and puberty phenotype.

The results of our study did not reveal any pathogenic mutation in *TAC* and *TACR3* genes in girls with ICPP and confirm the results of the Brazilian study. It seems that *TAC* and *TACR3* are not major genes involved in pathogenesis of ICPP.

Considering the low incidence of mutations in these genes in ICPP, it is possible that other factors involved in the GNRH regulation, yet to be discovered, will show to have a role in the pathogenesis of ICPP. New DNA techniques such as whole exome sequencing and molecular karyotypisation might be powerful tools in uncovering these still unknown genes.

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Резиме

МУТАЦИСКА АНАЛИЗА НА *TAC* И *TACR3* КАЈ ИДИОПАТСКИ ЦЕНТРАЛЕН ПРЕДВРЕМЕН ПУБЕРТЕТ

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Вовед: Генетската основа на идиопатски централен предвремен пубертет (ИЦПП) сè уште не е добро разјаснета. Генетската активација на почетокот на пубертетот се мисли дека произлегува од ефектот на мултипли гени. Фамилијарен ИЦПП сугерира постоење на моногенетски форми на ИЦПП. Неурокининот Б (НКБ) систем е инволвиран во регулацијата на хумана репродуктивна оска. Но, како НКБ-системот ги манифестира своите ефекти на централната невроендокрина контрола на хуманата репродукција сè уште останува енигма. Кај луѓето, НКБ и неговиот рецептор ги кодираат *TAC3* и *TACR3* гените. Сугерирано е дека мутации во овие гени се одговорни за ИЦПП.

Методи: ИЦПП е дефиниран со пубертетски почеток пред осум години кај девојчиња, и пубертетска вредност на LH одговор на GnRH тестирање. Дваесет и осум девојчиња со ИЦПП беа вклучени во студијата (возраст на дијагноза 5,72 ± 2,59; коскена возраст 6,12 ± 2,81, висина на почеток на терапија 0,90 ± 1,48 SD). LHRH тестот беше направен и беше со пубертетски вредности кај сите пациенти (LH 20,35 ± 32,37 mIU/ml; FSH 23,32 ± 15,72 mIU/ml). Кодирачките региони на *TAC* и *TACR3* беа секвенционирани.

Резултати: Не е откриена ниту една ретка варијанта во *TAC* и *TACR3* кај ниеден од 28 пациенти со ИЦПП.

Заклучок: Мутациите во *TAC* и *TACR3* генот не се причина за ИЦПП кај испитуваните пациенти.

Клучни зборови: *TAC, TACR3*, идиопатски централен предвремен пубертет, појава на пубертет.