Case Report

Peripheral Precocious Puberty in a Male Caused by Leydig Cell Adenoma Harboring a Somatic Mutation of the *LHR* Gene: Report of a Case

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While a germline activating mutation of the luteinizing hormone receptor (LHR) gene is known to cause autonomous production of testosterone from testicular Leydig cells in male-limited precocious puberty, only a few studies have addressed the role of somatic LHR mutation in testicular pathology. The authors report a case of a 6-year-old boy who developed secondary sex characteristics including facial acne, enlarging genitalia, and aggressive behavior, for which serial biochemical evaluation confirmed the status of peripheral precocious puberty. Examination revealed asymmetrical testicular volume, following which a left testicular tumor was detected through ultrasonography. A left orchiectomy was performed, and histopathology revealed a well-circumscribed Leydig cell tumor. Molecular study of the exon 11 of the LHR gene revealed a missense mutation at the nucleotide position 1,732, leading to a substitution of histidine for aspartic acid at codon 578. Interestingly, the substitution was consistent with all previously reported LHR alteration in pediatric Leydig cell adenoma, but which had never before been reported in male-limited precocious puberty, suggesting that the mutation is a molecular signature of the adenoma.

Keywords: *Pediatric Leydig cell tumor, Luteinizing hormone receptor, LHR*

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Peripheral precocious puberty (PPP) in boys is a condition of early onset and rapid progression of puberty caused by increased sex steroids, independent of luteinizing hormone (LH) stimulation[1]. Endogenous sources of the sex hormones can be through extra-gonadal sources, such as adrenal tissue and hormone secreting tumors, or from gonadal causes including male-limited gonadotropin-independent precocious puberty and a Leydig cell tumor[1,2].

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Germline mutation of the *luteinizing hormone receptor* (*LHR*) gene (MIM 152790) is known to be an underlying cause of autonomous production of testosterone in a condition known as male-limited precocious puberty or testotoxicosis[3,4]. In this condition, a gain-of-function mutation of the receptor signals testicular cells to produce testosterone without LH stimulation[4]. Recently, there have been two case reports, reporting that somatic mutation of *LHR* had been detected in Leydig cell adenoma, the most prevalent hormone-producing tumor of the testis[5,6]. Interestingly, all reported *LHR* mutations in pediatric Leydig cell tumors have also found a substitution of aspartic acid by histidine at codon 578. In the present report, the authors describe the case of an Asp578His-associated Leydig cell tumor, which

was successfully treated by surgical removal of the affected testis.

Case Report

A 6-year-old boy was referred to us for an evaluation of precocious puberty. The patient had been in good health until one year prior to his referral when he began to show unusual acne on his forehead. The boy had been noted to have rapid body growth, presence of pubic hair, and an enlarging penis three months before being brought to our clinic. The boy's caretaker also reported that he sometimes showed aggressive behavior and preferred to play with older boys. On examination at the first visit, his height was 121.5 cm (90^{th} percentile) and weight was 28.5 cm (> 97^{th} percentile). He had widespread facial acne over his forehead (Fig. 1). No significant skin hyper-pigmentation was noted. The pubic hair was in Tanner stage 2. The resting penile length was 8.0 centimeters. The left and right testicular volume estimations were 6 and 2 ml, respectively.

His radiological bone age was 12 years. The serum electrolytes were normal. A hormone study reported testosterone level at 5.33 ng/ml, which had increased to 8.36 ng/ml three months after the initial visit. The LH was less than 0.100 mIU/ml, FSH was 0.179 mIU/ml and bata-HCG was less than 0.100 mIU/ml. The ACTH stimulation test showed normal physiologic response of 17-hydroxyprogesterone. Abdominal magnetic resonance imaging reported negative. Testicular ultrasonography revealed a hypervascular heterogenous echogenic mass, size about 2.3 x 1.0 cm in the left testis (Fig. 2). A unilateral left orchiectomy was performed. Cut surface of the specimen showed a well circumscribed mass, about 2 cm in diameter, of which histopathology was compatible with Leydig cell adenoma (Fig. 3). Tissue was collected from the surgical specimen and subsequently frozen for genetic study.

On a follow-up visit at the fourth orchiectomy month, the testosterone level had decreased to 1.02 ng/ml and the LH had risen to 3.86 mIU/ml. The child continued to have facial acne and the caretaker

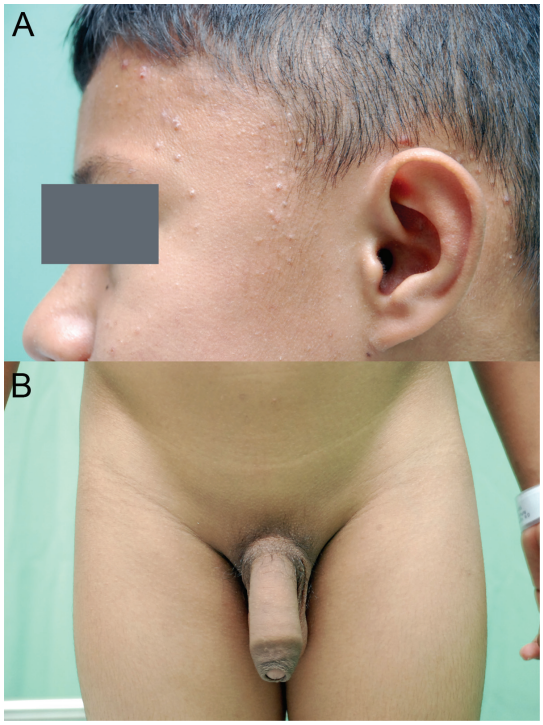

Fig. 1 Clinical photographs showing: A) facial acne at the forehead, and B) enlarging penis size and growing pubic hair at Tanner stage 2

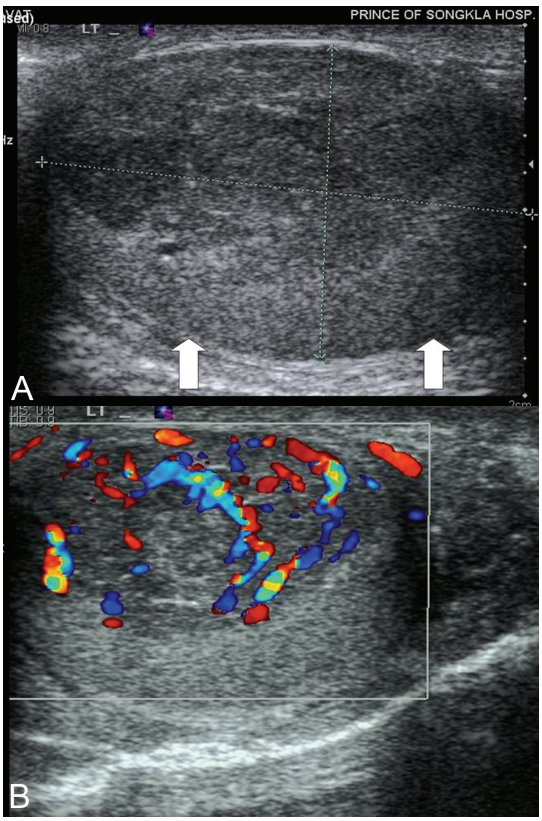

Fig. 2 A) Longitudinal US of the left testis revealed rather well-defined heterogeneous intratesticular mass. A rim of normal testicular parenchyma surrounds the mass (arrow). B) Color Doppler flow reveals increased tumor vascularity

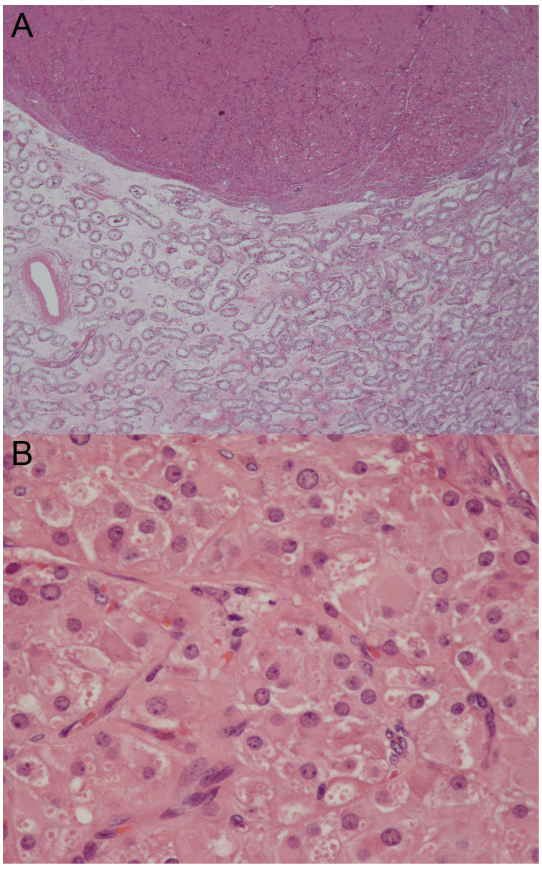

Fig. 3 Histopathology of the tumor showing A) well circumscribed mass composed of large polygonal cells with abundant eosinophilic granular cytoplasm. B) The nuclei are round with a small nucleolus. Reinke crystals (not shown) are rarely observed. (H&E, 4X and 20X)

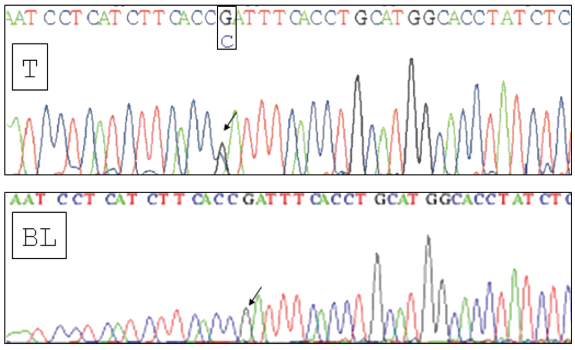

Fig. 4 Molecular studies revealed nucleotide substitution at the position 1732 of the *LHR* gene, found only in the tumor tissue (T) and negative in blood (BL). The alteration caused a missense mutation at codon 578 (GAT→CAT), leading to a substitution of aspartic acid by histidine

reported his school teachers complaining about aggressive behavior. The pubic hair and the penis had not changed remarkably from the pre-operative examination.

On molecular study of *LHR* exon 11, using polymerase chain reaction (PCR) and direct nucleotide sequencing, a heterozygous mutation at nucleotide position 1732 of the *LHR* gene was detected in the tumor tissue (Fig. 4). This mutation theoretically leads to amino acid substitution at codon 578 from aspartic acid to histidine. The study of adjacent normal testicular tissue and peripheral blood showed a wildtype sequence. (The sequences of the primers used in the present study can be provided on request.)

Discussion

The approach to a boy with isosexual precocity usually begins with excluding adrenal causes, congenital adrenal hyperplasia and sex-steroid producing adrenal tumors[2]. In the presented patient, response to the ACTH stimulation was not compatible for congenital adrenal hyperplasia and an abdominal magnetic resonance study showed no adrenal mass. The source of sex hormones was then localized to autonomous testosterone production from testicular tissue. Taken together with the asymmetry of testicular volume and an ultrasound result showing a testicular mass, functioning Leydig cell adenoma was the most likely diagnosis.

Leydig cell tumors are sex cord stromal tumors that arise from Leydig cells, which are interstitial cells that produce testosterone[7,8]. The tumor has a bimodal age distribution, early during the prepubertal period and later between 30 and 60 years of age. In the pediatric age group, the mean age at presentation of Leydig cell adenoma is 7 years[9], which is generally higher than the common age of presentation of male-limited precocious puberty (MPP). In the latter condition, excessive testosterone is produced from Leydig cells throughout the testicular tissue, secondary to a germline mutation of the *LHR* gene.

The *LHR* gene, located on human chromosome 2p21, encodes for a transmembrane receptor expressed on the cell membranes of testicular Leydig cells[4]. Physiologically, Leydig cells produce testosterone when the receptor is stimulated by luteinizing hormone or its analog, human chorionic gonadotropin. The mutation hotspots in the *LHR* gene associated with LH independent precocious puberty are on exon 11, which encodes the transmembrane domain of an LHR receptor[3,4]. Over half of the MPP mutations reported

have been reported at residue 578 on the sixth transmembrane protein. In germline mutation causing MPP, aspartic acid of residue 578 is substituted by tyrosine, glycine or glutamic acid, but never histidine.

On the other hand, Asp578His is detected in Leydig cell adenoma[5,6], especially in the pediatric age group. On a systemic screening of 29 adult Leydig cell tumors in the UK[10], only one case of a 65-year-old man was found to have this identical mutation in the *LHR* gene. Another screening in adult patients in Brazil gave negative results[11]. The genotype-phenotype specificity might be explained by the in-vitro evidence that showed the most potent activation occurring with this type of genetic variation[5].

In the presented patient, the characteristic well-circumscribed adenoma and clearance of testosterone after the left orchiectomy suggested that the pathology was a neoplastic process that had arisen within the boy's normal testicular tissue.

The secondary sex characteristics in the presented patient seemed not to disappear after the orchiectomy, although the testosterone level had decreased. This phenomenon may indicate that the effects of testosterone on the end organs or the behavioral changes may be quite persistent or even irreversible even after the abnormal production has been controlled.

In summary, the authors have added another case of pediatric Leydig cell adenoma harboring a somatic Asp578His substitution, with the suggestion that the alteration may be a specific molecular signature of this childhood tumor.

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พัฒนาการทางเพศก่อนวัยในเด็กชายอันเกิดจากเนื้องอกของเซลล์ Leydig ซึ่งสัมพันธ์กับการกลายพันธุ์ของยีน LHR: รายงานผู้ป่วย

สุรศักดิ์ สังขทัต ณ อยุธยา, สมรมาศ กันเงิน, สมจิตร จารุรัตน์ศิริกุล, ธีราวุฒิ ทับทวี, เวลาวี ไชยพันธุ์, ศักดา ภัทรภิญโญกุล, ปิยวรรณ เชียงไกรเวช

ได้เสนอรายงานผู้ป่วยเด็กชายอายุ 6 ปี รายหนึ่งซึ่งมีพัฒนาการทางเพศก่อนวัยกล่าวคือ มีสิวขึ้นบริเวณใบหน้า อวัยวะเพศขยายขนาด มีขนบริเวณหัวหน่าว และมีพฤติกรรมก้าวร้าว การสืบค้นทางต่อมไร้ท่อพบว่าเข้าได้กับภาวะ peripheral precocious puberty การตรวจร่างกายพบมีอัณฑะด้านซ้ายโต ซึ่งได้รับการตรวจยืนยันด้วยคลื่นเสียงความถี่สูงว่ามีก้อนทูมภายใน ผู้ป่วยได้รับการตัดอัณฑะด้านนั้น ผลการตรวจทางพยาธิวิทยาพบเป็นเนื้องอกของเซลล์ Leydig การศึกษาทางอณูชีววิทยาพบมีการกลายพันธุ์แบบ somatic ของจีน LHR ที่ตำแหน่ง exon ที่ 11 ตำแหน่ง nucleotide ที่ 1732 ซึ่งสามารถทำนายได้ว่าทำให้เกิดการทดแทนกรดอะมิโนที่ 578 จาก aspartic acid เป็น histidine

การกลายพันธุ์ตำแหน่งนี้ตรงกับที่เคยมีรายงานก่อนหน้าในรายผู้ป่วยป่วยซึ่งเป็นเนื้องอกของเซลล์ Leydig และไม่เคยพบในกลุ่มที่เป็น male-limited precocious puberty และชี้ว่าน่าจะเป็นการกลายพันธุ์ที่จำเพาะต่อเนื้องอกนี้