Adrenocortical carcinoma characterized by gynecomastia: A case report

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Abstract. We present a 4-yr-old boy with adrenocortical carcinoma (ACC), diagnosed due to the appearance of gynecomastia as the presenting symptom. Six months prior to admission, an acute growth spurt along with the development of bilateral breast swelling was observed. He did not present any features of virilization, including enlargement of the testes, increase in testis volume, and penis size. Laboratory investigations showed gonadotropin-independent hypergonadism, with low LH/FSH levels and elevated estradiol/testosterone levels. Abdominal computed tomography revealed a large heterogeneous mass adjacent to the right kidney and below the liver. Pathological investigations of the biopsy specimen demonstrated that the tumor was an ACC. Pre- and post-operative combination chemotherapy with mitotane was administered and surgical resection was carried out. Post-surgery, the elevated estradiol/testosterone concentrations reverted to within the reference range. Urinary steroid profile and tissue concentration analysis of estradiol and testosterone indicated the presence of estrogen in the ACC tissue. An investigation for TP53 gene aberrations revealed the presence of a germline point mutation in exon 4 (c.215C>G (p.Pro72Arg)). In ACC, the most common symptom is virilization, and feminization, characterized by gynecomastia, is very rare. However, a diagnostic possibility of ACC should be considered when we encounter patients who have developed gynecomastia without the influence of causative factors such as obesity or puberty, and do not present with the typical signs of virilization.

Key words: adrenocortical carcinoma, gynecomastia, TP53
Introduction

Adrenocortical carcinoma (ACC) is a very rare neoplasm, especially in children. The estimated annual incidence is approximately 0.2–0.3 cases per million people (1). Several reports indicate virilization as a major clinical symptom of ACC. Michalkiewicz et al. (2) investigated 254 patients below the age of 20 yr and diagnosed with ACC, in the USA and Brazil during 1990 to 2001. Although over half of these patients (55%) presented with virilization, the prevalence of feminization, characterized by gynecomastia, was not reported. Similarly, Kerkhofs et al. (1) investigated 12 cases of ACC in the Netherlands from 1993 to 2010, and even in these cases, feminization could not be detected. Thus, the presence of gynecomastia is considered very rare in ACC. We examined a 4-yr-old boy with bilateral gynecomastia that could be considered the dominating clinical feature leading to the diagnosis of ACC.

Case Report

Pre-admission findings and treatment

A 4-yr-8-mo-old boy visited our hospital due to gynecomastia and an acute growth spurt that persisted for six months. He had no past history of chronic diseases or medications. He was the second son of nonconsanguineous parents. There was no family history of malignant diseases among his first-degree relatives (parents and grandparents). His elder brother is in good health. His physical status on admission was as follows: height was 108.5 cm (+ 1.22 SD) and weight was 18.5 kg (+ 0.98 SD). His height had increased by 6.5 cm over the last 6 mo (Fig. 1A). A hard liver edge under the right hypochondrium could be identified on palpation. He had no skin pigmentation such as café-au-lait spots with “coast of Maine” border to consider the possibility of McCune-Albright syndrome. He had no anomalies such as macroglossia or hemihypertrrophy to indicate Beckwith-Wiedemann syndrome. He had symmetrical gynecomastia, and his breast glands were palpable with approximately 1.0 cm diameters under the nipple, without pigmentation (Fig. 1B). He had no genital anomalies, including hypospadias or bifid scrotum. His genital development indicated Tanner Stage I, with no pubic or axillary hair. The testicular volume was approximately 2 ml measured by Orchidometer (Sumitomo Pharmaceutical) and the stretched penile length approximately 4 cm. His voice remained high-pitched and he had no signs of hyperandrogenism, including acne and hirsutism.

Laboratory investigations and radiology

Blood cell counts, and liver and renal function test results were normal. An IGF-1 level of 191 ng/mL (reference range for 4-yr-old boys: 46–171) was slightly above the reference range. Total cholesterol levels were normal (186 mg/dL, reference range: 130–220). Suppressed gonadotropins levels (LH, < 0.1 mIU/mL; FSH, 0.13 mIU/mL), elevated estradiol (E2) levels (28.1 pg/mL, reference range: < 10), elevated testosterone (T) levels (0.82 ng/mL, reference range: < 0.1), and elevated dehydroepiandrosterone sulfate (DHEAS) levels (1,950 ng/mL, reference range: 130–830) were detected. The serum E2/T ratio was estimated to be 0.034. Tandem mass spectrometry analysis of filter paper samples of dried blood detected high androstenedione (AD4) levels (4.6 ng/mL, reference range: 0.14–0.34). Serum cortisol levels were in the normal range (16.5 μg/dL, reference range: 6.4–18.0) (Table 1). Bone age, estimated according to the 20-bone scoring of Tanner-Whitehouse 2 method, was nearly 8 yr-1 mo, approximately 4 yr higher than his chronological age. A large, heterogeneous tumor was detected below the liver on computed tomography (Fig. 2A).
Pre- and post-operative investigations and interventions

Suspecting neoplasms, including Wilm’s tumor and neuroblastoma, biopsy was carried out 2 d after admission, and pathological examination revealed that the mass was an ACC. The Weiss score was estimated to be 7/9. High nuclear grade, high mitotic rate (43/50 high-power fields), atypical mitosis, eosinophilic tumor cell cytoplasm, diffuse architecture, venous invasion, and capsular invasion were observed (Fig. 2B). Based on abdominal CT, invasion to the arteries or other adjacent organs was suspected because the mass was fixed to the right kidney.

Table 1  Blood concentrations of gonadotropins and steroid metabolites of the case on admission and after surgery

|                      | On admission | After operation | Reference range (4 yr old boy) |
|----------------------|--------------|----------------|--------------------------------|
| LH (mIU/mL)          | < 0.1        | < 0.1          | < 2.4                         |
| FSH (mIU/mL)         | 0.13         | 0.96           | 0.2–1.9                       |
| Estradiol (pg/mL)    | 28.1         | < 10           | < 10                          |
| Testosterone (ng/mL) | 0.82         | < 0.03         | 0.03–0.3                      |
| DHEAS (ng/mL)        | 1950         | < 20           | 130–830                       |
| Cortisol (μg/dL)     | 16.5         | < 0.05         | 6.4–18.0                      |
| Androstenedione (ng/mL) | 4.6       | < 0.3          | 0.14–0.34                     |
Stage III ACC was the final diagnosis, and pre- and post-operative chemotherapy was planned accordingly. Combined chemotherapy of ARAR0332 (etoposide-doxorubicin-cisplatin) with mitotane (120–150 mg/d; maximum dose, 2,500 mg/d) was administered following which the tumor contracted to an operable size. Surgical resection was carried out on day 106, followed by administration of GPOH-MET97 therapy along with mitotane. The resected tumor was approximately 8.0 cm in diameter and weighed 250 g.

During chemotherapy, his elevated sex hormones gradually decreased. After the operation, serum estradiol levels decreased to almost below the reference range. Cortisol levels decreased to an insufficient level, and glucocorticoid supplement had been initiated in advance (Fig. 3). Mineralocorticoid was added to the therapy on day 270. The patient was discharged on day 293, while still receiving mitotane and corticosteroids. Mitotane was discontinued 6 months after the surgery. Over a period of 1 yr after his operation, gynecomastia gradually disappeared. The growth rate also improved (Fig. 1A). Elevated T, DHEAS, AD4, and E2 levels were suppressed to below the reference ranges (Table 1). Relapse of ACC was not detected for 2 yr but the patient still needs hydrocortisone and fludrocortisone supplementation.

**Analysis of steroid metabolism in the urine and resected tumor tissue**

Spot urine samples were analyzed for steroid metabolites using gas chromatography/mass spectrometry (5). The value of the metabolites of AD4 and dehydroepiandrosterone (DHEA) were high. Metabolites of pregnenolone (P5), progesterone (P4), 17-OHP5, 17-OHP4, cortisol,
and estrogens were also elevated (Fig. 4A). These findings indicated an activated Δ5-steroids metabolic pathway (P5 converted to DHEAS), activated 3β-hydroxysteroid dehydrogenase (P5 converted to P4), and aromatase (DHEA and T converted to E2) (Fig. 5). After surgery, these metabolites decreased to almost under the 20-percentile levels, reported for 3 to 5-yr-old boys with normal hormone activity (Fig. 4B).

Based on the urine steroid profile, sex steroid metabolites in frozen ACC tissues were measured by using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Trace amounts of E2 (70 pg/g) and T (440 pg/g) were detected by this method. The E2/T ratio was 0.154, greater than that observed in serum (0.034).
Immunochemistry staining, to demonstrate the existence of steroid metabolic enzymes, was not performed. We were also unable to estimate the expression of CYP19A1 mRNA, or perform western blot analysis for the detection of CYP19A1 protein in the ACC tissues because of insufficient tumor specimens.

**Analysis of TP53 gene mutation**

After obtaining informed consent from the patient’s family, according to the Declaration of Helsinki, and approval from the ethics committee of the Hokkaido Medical Center for Child Health and Rehabilitation, DNA analysis for TP53 gene was performed using the patient’s peripheral mononuclear leucocytes. A heterozygous point mutation was detected in exon 4 of the TP53 gene (c.215C>G (p.Pro72Arg)) (Fig. 6). His parents refused further gene analysis in other family members.

**Discussion**

We present a rare case of gynecomastia without any signs of virilization in a boy who was diagnosed with ACC. Gynecomastia is supposed to occur commonly in male adolescents as a normal part of puberty due to transient physiologic imbalance in steroid hormones or hypersensitivity of estrogen receptors with a reported prevalence of 4–69%. Obesity also might influence the pathogenesis of gynecomastia by increasing peripheral aromatase enzyme activity leading to an imbalance in E2 and T levels (6).

The characteristic features of our ACC patient were as follows: gynecomastia at prepubertal age without obesity, acute growth spurt with significant bone maturation, elevated levels of serum E2, high percentage of urinary estrogen metabolites, followed by remission of gynecomastia and return of the normal growth rate after tumor resection. These factors indicated that his gynecomastia was not physiological and could be one of the most important clinical symptoms of ACC. In breast or prostate cancer, the E2/T ratio is known to act as an indicator of tissue aromatase activity (7, 8). In our case, the E2/T ratio in ACC tissue was higher than that in

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**Fig. 5.** A schematic representation of the steroid metabolic pathway in the patient’s ACC tissue. According to laboratory data and the urine steroid profile generated on admission, activity of 17α-hydroxylase (converted P5 to DHEA through 17α-OHP5), 3β-hydroxysteroid dehydrogenase (converted P5 to P4), and aromatase (converted DHEA and testosterone to estradiol) is mainly accelerated. StAR: Steroidogenic acute regulatory protein, SULT2A1: Sulfotransferase family 2A member 1.
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Fig. 6. The scheme for TP53 gene analysis. A heterogeneous germline point mutation was detected in exon 4 (c.215C>G (p.Pro72Arg)).

Table 2 Patients with adrenocortical carcinoma (ACC) presenting with gynecomastia (below the age of 20 yr)

| Age and sex | Time for diagnosis | Other symptoms | E2 (pg/mL) | T (ng/mL) | E2/T |
|-------------|--------------------|----------------|------------|-----------|-------|
| Watanabe (9)  | 1.5 yr Boy | 1 yr | Penile size enlargement Pubic hair | 349 | 2.6 | 0.134 |
| Kawamura (10) | 10 yr Boy | 2 yr | Pubic hair | 166 | 1.23 | 0.135 |
| Sato (11) | 9 yr Boy | 2 yr | Growth spurt Pubic hair | < 25 | 0.42 | < 0.06 |
| Sindgikar (12) | 6 yr Boy | 6 mo | Growth spurt | 28.9 | -- | -- |
| Whohltman (13) | 1 yr Girl | 1 yr | Clitoromegaly Pubic hair | 17 | 0.43 | 0.039 |
| Itami (14) | 6 yr Boy | 1 yr | Penile size enlargement Pubic hair | 40 | 0.26 | 0.154 |
| Our case | 4 yr Boy | 6 mo | Growth spurt | 28.1 | 0.82 | 0.034 |
the serum, indicating abundant tissue estrogen production due to increased aromatase activity in the tumor.

Co-existing virilization was found to be more frequent when we reviewed previously reported cases of ACC with gynecomastia (Table 2) (9–14). According to a former statistical report on ACC, the major steroid metabolites obtained from ACC tissues were androgens (1, 2). Androgens produced in ACC tissues are converted to estrogens by activated aromatase activity in tumor tissues (9). This process can induce elevation of both androgens and estrogens levels in most feminizing ACC cases resulting in expression of co-existing virilization.

Two possibilities could explain the lack of virilization in our case. First, the aromatase activity of the tumor tissue was more active, compared to previously reported cases, although the serum estradiol levels and the E2/T ratio of our case was lower compared to former reported values (Table 2). Second, the diagnosis of ACC might have been made at an earlier stage of the clinical course. Sindgikar et al. (12) have reported a 6-yr-old boy with ACC diagnosed due to the appearance of gynecomastia and acute growth spurt, which is similar to our case. In both studies, the patients were diagnosed with ACC approximately 6 mo after the first appearance of gynecomastia. Some cases of ACC may be obscured by the presence of virilization. Although the initial clinical symptom in these patients was gynecomastia, the diagnosis as ACC was made approximately 1 to 2 yr after the appearance of gynecomastia (10, 14), indicating that virilization might become apparent only in the later stages of the ACC disease course.

Our ACC case has another specific feature, a germline mutation in the TP53 gene, the tumor suppressor gene that encodes the p53 protein. In a study of 88 children with ACC, Wasserman et al. (15) have reported the presence of germline TP53 mutations in nearly 50% of the patients, although they did not present with clinical features, such as virilization and/or feminization. Similarly, a 52-yr-old man with ACC with the same mutation has been reported; however, his tumor was a non-functioning ACC, and clinical features including the existence of gynecomastia have not been described (16). Therefore, our case might be the first reported case with feminizing ACC accompanied by a TP53 mutation. Recently, Fujisawa et al. (17) investigated the steroid metabolic pathway in ACC tissue obtained from a Brazilian boy with virilization and detected a TP53 gene mutation: p.Arg337His, common in South Brazil. They showed the presence of combined steroidogenic properties of fetal adrenal and Leydig cells, and several postzygotic carcinogenic events. This report confirms that androgen synthesis in ACC with TP53 mutation is regulated genetically to some extent.

TP53 gene mutation in our case could be discussed in association with aromatase overexpression. A previous report confirmed aromatase overexpression in ACC tissue from a feminizing 18-mo-old boy (9). In addition, the transcripts associated with aromatase promoter II have been prominently characterized in ACC tissue of a 54-yr-old man (18). However, TP53 gene mutation status was not investigated in these two cases. On the other hand, in breast tissue, mutations in the TP53 response elements increase aromatase activity, although normal p53 protein regulates the aromatase activity negatively via the connecting aromatase promoter II (19). Our case, together with these studies, could indicate that the TP53 gene mutation is associated with aromatase hyperactivity in ACC tissue and contributes to increase estrogen synthesis.

Li-Fraumeni syndrome (LFS) is a rare autosomal dominant disease associated with changes in the tumor suppressor gene, TP53, located on chromosome 17p. Approximately 50% of LFS patients are known to develop at least one LFS-associated cancer (breast cancer, sarcoma, brain tumor, or ACC) by the age of 30 yr, and 90% develop malignant disease by the age of 60 yr (20). According to the Chompret’s
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criteria of LFS (21), our patient is ‘a proband with ACC at any age of onset, regardless of the family history’, indicating presence of LFS. In addition, pathological examination of his ACC tumor presented with a relative high score of the Weiss criteria, indicating likely cancer recurrence and poor prognosis (22).

LFS in children without any clinical symptoms of cancer, regular blood tests and ultrasonography (every 4 mo) are recommended for early detection of malignant tumors (23). Although our patient was already diagnosed with ACC, there is a need for careful and regular follow-up, in accordance with the course of childhood LFS, for earlier detection of ACC relapse and other malignant diseases because of the presence of a higher Weiss’s score and a TP53 gene mutation.

**Conclusion**

We report a boy with ACC diagnosed due to the presence of gynecomastia without virilization, carrying a TP53 germline mutation. Chemotherapy and surgical treatment induced remission for 2 years. Gynecomastia without virilization could represent a feature of ACC and we should consider it when we encounter a case of breast swelling.

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