

## Original

# Virilizing Adrenocortical Carcinoma Invading the Right Atrium with Histological High-Grade Malignancy and p53 Mutation in a 3-Year-Old Child: Indication of Post Operative Adjuvant Chemotherapy

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**Abstract.** We present a 3-yr-old girl with a virilizing adrenocortical carcinoma invading into the right atrium with histological high-grade malignancy and p53 mutation. Development of facial acne and pubic hair were noted at 3 yr and 2 mo. The levels of androgens were high. Diurnal variation in ACTH and cortisol were absent. Abdominal computed tomography revealed a large right suprarenal mass, with extension into the inferior vena cava and right atrium. Based on the diagnosis of a right virilizing adrenocortical tumor with Cushing syndrome, surgery was performed by a combined thoracoabdominal approach with the patient on cardiopulmonary bypass. The tumor was 7 × 5.5 × 3.5 cm in size, and weighed 95 g. The histological diagnosis was adrenocortical carcinoma with high-grade malignancy according to the category of Weiss. A heterozygous mutation of the p53 tumor-suppressor gene (codon 248 CGC→TGG) was found. We did not perform adjuvant chemotherapy because of radical resection on macroscopic observation and no metastasis in radiological findings. Five months after the surgery, her chest X ray and computed tomography revealed multiple lung metastases and a single liver metastasis. In this type of patient with histological high-grade malignancy and p53 mutations, postoperative adjuvant chemotherapy is indicated even if macroscopic total surgical removal had been performed.

**Key words:** virilizing adrenocortical carcinoma, right atrium, adjuvant chemotherapy, p53 mutation

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## Introduction

Adrenocortical tumors (ACT) are a rare malignancy, especially in children. The annual incidence has been calculated as 0.5 to 2 cases per million (1). They tend to extend rapidly by vascular and visceral invasion with distant metastases to lung, bone and brain. Only a few cases of extension into the right atrium have been

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reported in children and adults (2, 3).

ACT is often associated with the Li-Fraumeni and Beckwith-Wiedemann syndromes. The Li-Fraumeni syndrome is a rare autosomal dominant condition with incomplete penetration in which affected members develop many different types of tumors (1, 3). In addition to childhood sarcoma and pre-menopausal breast cancer, members of these families have an increased risk of the development of other malignancies, including leukemia, brain tumors, osteosarcomas and ACT (1, 3). Li-Fraumeni syndrome is associated with changes in the tumor suppressor gene p53 on chromosome 17p.

Surgery is the single most important procedure for successful treatment of ACT. The role of chemotherapy in the management of childhood ACT has not been established (3). Mitotane {1,1-dichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)-ethane, or o,p'-DDD}, an insecticide derivative that produces adrenocortical necrosis, has been used extensively in adults with ACT, but its efficacy in children is not known (3).

## Case Report

### Clinical course before admission

The patient was a 3-yr-old girl. Her mother's parents were of a consanguineous marriage. The mother and the grandmother both suffered from hereditary spherocytosis. No other member of the family suffered from tumors. A neonatal screening test for congenital adrenal hyperplasia (CAH), which measures 17 $\alpha$ -hydroxy progesterone (17OHP) levels on filter paper, was negative. The patient showed clitoromegaly, facial acne, lower voice, and hypertrichosis at the age of 3 yr and 2 mo. Four months later, she was noticed to have pubic hair. At a local hospital, she was diagnosed as having a simple virilizing type CAH on the ground of high 17OHP (5.6 ng/ml) and T (986 ng/dl). Neither an abdominal ultrasonogram nor computed tomography (CT) was performed. She was treated with glucocorticoid but it was not



**Fig. 1** External genitalia on admission.

effective against the virilization. At the age of 3 yr and 7 mo, she was admitted to our hospital for further examination.

### Physical status

Her height was 101 cm (+1.2 SD) and weight was 19 kg on admission. Percent of relative body weight was +20.2% and height velocity was 12 cm per year. Blood pressure was 126/70 mmHg. She had hirsutism, moon face, lower voice, facial acne, enlarged clitoris, and pubic hair (Tanner stage II) (Fig. 1). An elastic hard abdominal mass was felt at the right upper quadrant.

### Endocrine studies

The levels of androgens, especially serum dehydroepiandrosterone sulfate (DHEA-S) and T as well as urinary ketosteroids were high (Table 1-a). The diurnal variation in ACTH and cortisol were absent. The levels of ACTH were below measurement sensitivity, and serum cortisol levels were high at all times (Table 1-b). After a single bolus intravenous CRH (100  $\mu$ g/m<sup>2</sup>)

**Table 1-a** Steroid levels on admmission at 0900 h

|                  |       | control data  |                        |        |
|------------------|-------|---------------|------------------------|--------|
| testosterone     | 794.2 | <15           | ng/dl                  | (4), a |
| DHEA-S           | 9922  | <200          | ng/ml                  | (4), a |
| androstenedione  | 23    | <0.5          | ng/ml                  | (4), a |
| E <sub>2</sub>   | 21.3  | <10           | pg/ml                  | (4), a |
| 17OHP            | 3.7   | <0.9          | ng/ml                  | (4), a |
| DOC              | 0.81  | 0.083 ± 0.036 | ng/ml                  | (5), b |
| aldosterone      | 14.7  | 11.11 ± 3.8   | pg/ml                  | (6), c |
| PRA              | 1.7   | 6.18 ± 2.64   | ng/ml/h                | (6), c |
| urinary 17OHCS   | 6.5   | 1.0~5.6       | mg/day                 | (6), d |
| urinary 17KS     | 12.9  | <2.0          | mg/day                 | (6), d |
| urinary cortisol | 61.3  | 2~26.8        | μg/m <sup>2</sup> /day | (6), d |

DHEA-S: dehydroepiandrosterone sulfate, 17OHP: 17 $\alpha$ -hydroxy progesterone, DOC: 11-Deoxycorticosterone, PRA: plasma renin activity, 17OHCS: 17-hydroxycorticosteroid, 17KS: 17-ketosteroid. a: Normal values for girls aged 1–4 yr. b: Normal values for girls aged 1–5 yr. Data are given as the mean  $\pm$  SD. c: Normal values at age 3–5 yr. Data are given as the mean  $\pm$  SD. d: Normal values at age 2–6 yr.

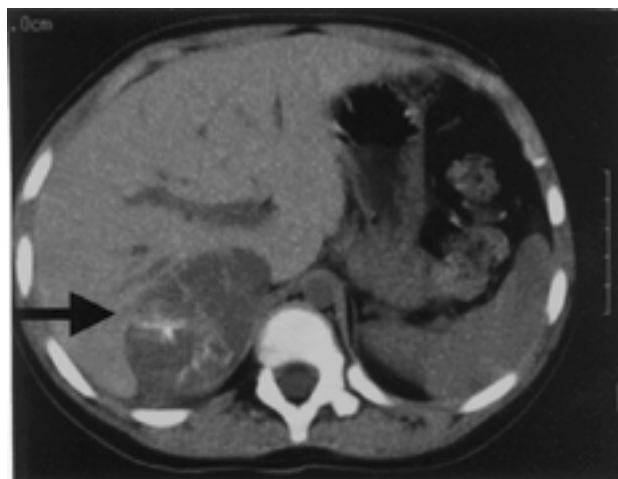
**Table 1-b** Diurnal variation in ACTH and cortisol levels

|                  | 6 A.M. | 12 A.M. | 6 P.M. | 12 P.M. |
|------------------|--------|---------|--------|---------|
| ACTH (pg/ml)     | <10    | <10     | <10    | <10     |
| cortisol (μg/dl) | 14.7   | 15.2    | 16.5   | 14.6    |

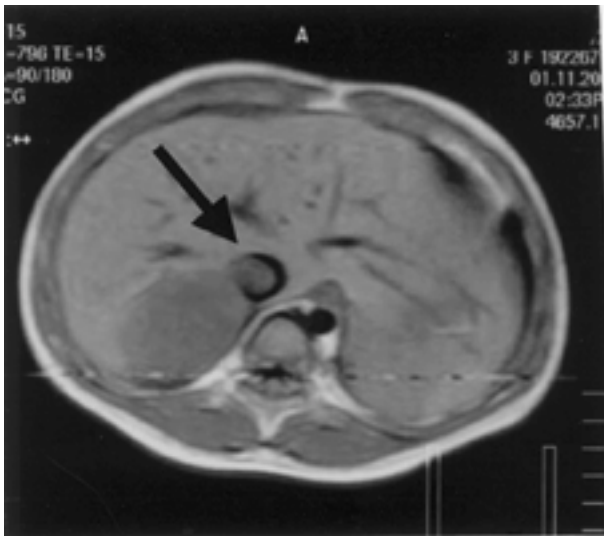
administration, serum cortisol and plasma ACTH levels changed from 15.9 to 17.7  $\mu$ g/dl, and from <10 to <10 pg/ml, respectively. She did not respond to an overnight dexamethasone suppression test (1 mg/1.73 m<sup>2</sup> oral administration; serum cortisol was 16.5  $\mu$ g/dl at 2300 h  $\rightarrow$  17.6  $\mu$ g/dl at 0900 h the next morning). These results showed an autonomous secretion of cortisol. Fasting blood glucose levels were 90–110 mg/dl.

### Radiological studies

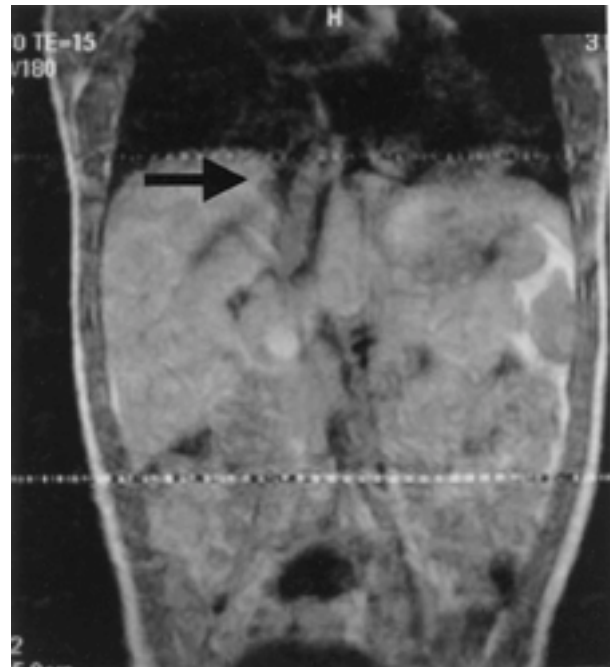
Abdominal magnetic resonance imaging (MRI), CT and ultrasonogram showed a large right suprarenal mass, with extension into the inferior vena cava and right atrium (Fig. 2, Fig. 3 a, b). She was diagnosed as having a right virilizing adrenal



**Fig. 2** Abdominal CT on admission: CT scan reveals a 5.3 cm  $\times$  4.5 cm right adrenal mass (arrow).



**Fig. 3a** Axial MRI on admission: MRI scan shows that the mass extended into the inferior vena cava (*arrow*).



**Fig. 3b** Coronal MRI on admission: MRI scan shows the mass extending through into right atrium (*arrow*).

tumor with Cushing syndrome. The brain, lung and liver were free from metastases in radiological findings.

### Operation and pathological findings

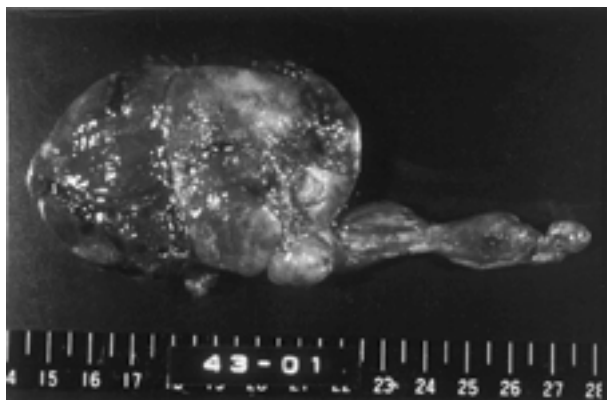
Surgery was performed by a combined thoracoabdominal approach with the patient on cardiopulmonary bypass. The right adrenal mass with intravascular invasion was totally removed. The surgeon confirmed that there was no metastasis to regional lymph nodes on macroscopic observation. Evaluation of the pathology of the circumference of the tumor was not performed. The tumor was  $7 \times 5.5 \times 3.5$  cm in size, and weighed 95 g, with areas of focal necrosis and hemorrhage (Fig. 4). Microscopic findings of a specimen showed marked dyskaryoses and abnormal mitoses (Fig. 5). The histological diagnosis was adrenocortical carcinoma with high grade malignancy according to the category of Weiss: high

nuclear grade (+), mitotic rate 150/50 HPF, atypical mitosis (+), eosinophilic cytoplasm in  $\geq 75\%$  of the tumor cells (+), necrosis (+), venous invasion (+), sinusoidal invasion (+), and Weiss criteria 7/9.

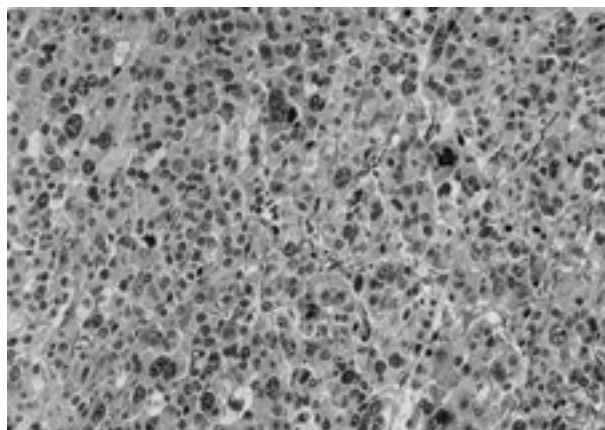
### Clinical course after operation

Due to hypercortisolism and atrophy of the left adrenal gland, she was treated with steroid replacement. After the operation, the manifestations of virilization disappeared gradually except for the lower voice. Serum androgens rapidly decreased to the normal level (Table 2). We decided not to perform chemotherapy because of radical resection on macroscopic observation and no metastasis in radiological findings before the operation.

Five months postoperation, the mother again noticed the child's acne and the pubic hair and we confirmed acne, pubic hair, clitoromegaly and lower voice. Neither hypertrichosis, moonface, nor



**Fig. 4** Right adrenal tumor was  $7 \times 5.5 \times 3.5$  cm in size, and weighed 95 g, with areas of focal necrosis and hemorrhage.



**Fig. 5** Histology of the right adrenal mass.

**Table 2** The changes in steroid levels after surgery

| Postoperative period        | DHEA-S<br>(ng/ml)  | Testosterone<br>(ng/dl) | E <sub>2</sub><br>(pg/ml) | U-17KS<br>(mg/day) |
|-----------------------------|--------------------|-------------------------|---------------------------|--------------------|
| 2 mo                        | 13                 | <5                      | 6.7                       | 1.0                |
| 3 mo                        | 64                 | 7.5                     | 17.5                      |                    |
| 4 mo                        | 357                | 66.6                    | 29.4                      |                    |
| 5 mo                        | 483                | 114.8                   | 29.8                      | 2.5                |
|                             | chemotherapy start |                         |                           |                    |
| 3 mo after chemotherapy     | 37                 | <5                      | 31.2                      | 1.3                |
| <i>control data; (4, 6)</i> | <200               | <15                     | <10                       | <2.0               |

DHEA-S: dehydroepiandrosterone sulfate, U-17KS: urinary 17-ketosteroid.

hypertension was found. Her serum DHEA-S and T levels were re-increased (Table 2). Chest X ray and CT revealed multiple lung metastases and a single liver metastasis (Fig. 6). We performed combined chemotherapy including mitotane, following the protocol by Arico *et al.* (7): vincristine  $1.5 \text{ mg/m}^2$  i.v., methyl-prednisolone  $600 \text{ mg/m}^2$  i.v., carmustine (MCNU)  $75 \text{ mg/m}^2$  i.v., procarbazine  $75 \text{ mg/m}^2$  orally, hydroxyurea  $1500 \text{ mg/m}^2$  orally, cisplatin  $60 \text{ mg/m}^2$  i.v., cytosine-arabioside  $300 \text{ mg/m}^2$  i.v., and cyclophosphamide  $300 \text{ mg/m}^2$  i.v. Shrinkage of the tumors was observed.

### Analysis of the P53 gene

DNA analysis of her peripheral mononuclear leukocytes for the p53 gene was performed after obtaining informed consent and was also certified by the ethnics committee of our hospital. A heterozygous mutation of the p53 tumor-suppressor gene (codon 248 CGC→TGG) was found. Analysis of the p53 gene of her family members has not been performed.

Chemotherapy with multiple agents including mitotane has been continuously performed.



**Fig. 6** Chest CT at five months after surgery: CT scan shows multiple lung metastases.

## Discussion

Adrenocortical tumors (ACT) are rare in the general population. Almost all child cases previously reported were presented with clinical signs of endocrine dysfunction, i.e., hormonal secretion resulted in clinical consequences: virilization syndrome, Cushing syndrome, feminization syndrome, or a mixed Cushing-virilization syndrome. In children, virilization and mixed Cushing-virilization are common symptoms (8). Our case showed signs of a mixed Cushing-virilization syndrome. She had pubic hair, lowed voice, acne, hirsutism, clitoromegaly as signs of virilization, and mild obesity, moon face, and hypertension as a sign of Cushing syndrome. She did not show retarded growth or impaired glucose metabolism. She predominantly showed virilization compared with Cushing syndrome.

Hormonal findings in our case revealed an increase in androgens, especially serum DHEA-S, T and urinary 17-ketosteroids. Although 17OHP was slightly high, it was not enough of an increase to diagnose her as having a 21-hydroxylase deficiency. A cortisol excess was confirmed by

assays for serum cortisol, urinary 17-hydroxycorticosteroids, and urinary free cortisol. Autonomous secretion of cortisol was demonstrated. Radiological findings showed a large right adrenal mass. She was diagnosed as having a right virilizing adrenal tumor with Cushing syndrome.

Distant metastases most frequently involves the lung and liver and less commonly the regional lymph nodes, inferior vena cava, brain, diaphragm, or bone (1). Initial metastases have been found in 5% to 64% of patients in separate published studies (1). In our case, CT and MRI showed tumor extension to the inferior vena cava and right atrium. The friable necrotic nature of these tumors, especially in the caval and atrial thrombus, is most important. Only a few cases of extension into the right atrium have been reported in children and adults (2, 3). In one past report, a patient died in the operating room during mobilization of the tumor thrombus (2). We therefore performed a combined thoracoabdominal approach with the patient on cardiopulmonary bypass.

Because of heterogeneity and the rarity of ACT, prognostic factors have been difficult to establish. Tumor size (9), histology (10), DNA ploidy (11) and other clinical, surgical, and laboratory parameters (3, 9) have been reportedly associated with the outcome, but findings have been inconsistent. Pediatric series published in the 1990s have revealed overall survival rates ranging from 43% to 91% (1). Four stages, I-IV, have been proposed for childhood ACT, depending on tumor size and clinical as well as surgical parameters (3). Patients with stages I and II have the best chance of a cure, whereas patients with stages III and IV have a poor prognoses.

Our case would be classified as stage I (Tumor totally excised with negative margins. The tumor weight was < 200g, there was no evidence of metastasis, and abnormal hormone levels returned to normal after surgery). While based on the Weiss category (10), it would be classified as

adrenocortical carcinoma with high-grade malignancy. In our case, the tumor invaded the vena cava and was categorized as a malignant tumor on the basis of histological findings, but the tumor was totally excised on macroscopic observation and abnormal hormone levels returned to normal after surgery. The role of adjuvant chemotherapy in the management of childhood ACT has not been established, so we did not perform adjuvant chemotherapy, and observed her carefully.

Unfortunately, she developed multiple lung metastases and single liver metastasis five months postoperation. We decided to treat her with aggressive chemotherapy. Arico *et al.* (7) reported one girl with relapsed disseminated adrenocortical carcinoma who showed good control of the disease after combined chemotherapy according to the eight-drug-in-one-day protocol. Mitotane blocks 11- $\beta$  hydroxylation and decreases cortisol production. It is also capable of producing selective adrenocortical necrosis, both in the ACT and in metastases (12). We performed eight-drug-in-one-day protocol and mitotane treatment. In our case, the tumor showed signs of shrinkage after chemotherapy, and the abnormal hormone levels returned to normal again. Thus, the intensified chemotherapy seemed to be effective for suppression of recurrent ACT. From now on, careful observation and continuous medical treatment will be necessary.

The etiology of ACT is not well understood. Li-Fraumeni syndrome is associated with changes in the tumor suppressor gene p53 on chromosome 17p (3). The Li-Fraumeni syndrome is a rare autosomal dominant condition with incomplete penetration in which affected members develop many different types of tumors (3). In families with the Li-Fraumeni syndrome the frequency of ACT is 100 times more than that in the general population (13). On the other hand, ACT in childhood cancer is associated with the highest frequency of germline p53 mutations (13). We found a mutation of the p53 tumor-suppressor

gene (codon 248 CGC→TGG). No other member suffered from the tumors in her family, and analysis of the p53 gene was not performed. P53 mutations in children with sporadic ACT suggest that children may represent probands with which to ascertain Li-Fraumeni syndrome families. In children, p53 positivity has been associated with clinically malignant ACT and p53 negativity has been associated with clinically benign ACT (14). She has a predisposition to suffer from ACT and a poor prognosis regardless of treatment. Immediate post-operative adjuvant chemotherapy might be indicated in childhood ACT with p53 gene mutations.

In summary, we present a 3-year-old girl with adrenocortical carcinoma, a rare tumor condition in children, with invasion of the inferior vena cava and right atrium. Surgery was performed by a combined thoracoabdominal approach with the patient on cardiopulmonary bypass. The adrenal tumor with intravascular invasion was totally removed. Although macroscopic total surgical removal was successfully performed, the nature of the tumor may be at high potential risk for recurrence and metastases due to histological high-grade malignancy and a p53 mutation. Immediate post-operative adjuvant chemotherapy might be indicated in these cases.

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