Case Report

Congenital lipoid adrenal hyperplasia: Immunohistochemical study of testosterone synthesis in Leydig cells

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Abbreviations & Acronyms
3β-HSD = 3β-hydroxysteroid dehydrogenase
17β-HSD = 17β-hydroxysteroid dehydrogenase
Ad4BP/SF-1 = adrenal 4-binding protein/steroidogenic factor 1
CAH = congenital lipoid adrenal hyperplasia
CT = computed tomography
CYP11A1 = cholesterol side chain cleavage P450
CYP17A1 = 17α-hydroxylase/17,20-lyase P450
LC = Leydig cell
MRI = magnetic resonance imaging
StAR = steroidogenic acute regulatory

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Introduction: Congenital lipoid adrenal hyperplasia is a rare disease that causes disorders of sex development. The 46,XY patient presents with female external genitalia and inguinal testes. We describe the case of a patient with congenital lipoid adrenal hyperplasia and investigated the testes of this patient in detail.

Case presentation: A 15-day-old 46,XY neonate presented with severe adrenal insufficiency. Congenital lipoid adrenal hyperplasia was diagnosed after detection of steroidogenic acute regulatory gene mutations. At 2 years and 5 months, she underwent bilateral gonadectomy. Leydig cells were observed both with and without lipid droplets in the testes of this patient. We also demonstrated immunohistochemically that some testosterone-synthesizing enzymes were maintained in this patient.

Conclusion: The results indicated transcription of testosterone-synthesizing enzymes remained despite lipid accumulation in this patient. The pattern of expression of testosterone-synthesizing enzymes suggested fetal Leydig cells may have remained after birth in the testes of this patient.

Key words: 46,XY, congenital lipoid adrenal hyperplasia, fetal Leydig cell, testosterone synthesis, undescended testes.

Keynote message

We reported the patient of 46,XY CAH with a focus on the steroid synthesis-related proteins. Our findings raised the possibility that the LCs partially retained the ability to produce testosterone, and fetal LCs might have remained after birth in the testes of 46,XY CAH.

Introduction

Lipoid CAH induces severe adrenal insufficiency and hypogonadism due to a lack of all the adrenocortical hormones, caused by mutations in the StAR gene. Normally, StAR protein plays a crucial role in the conversion of cholesterol to pregnenolone, helping to transport cholesterol from the outer to the inner mitochondrial membrane.[1] However, patients with 46,XY lipoid CAH are thought to be unable to synthesize testosterone and show female external genitalia and undescended testes in the inguinal canal. As lipoid CAH is extremely rare, few reports have described the biosynthesis of steroid hormone in LCs in detail. We present herein a case of lipoid CAH in which bilateral gonadectomy was performed. We performed sequence analysis of the StAR gene and examined various steroid synthesis-related proteins immunohistochemically in this patient. Finally, we discussed the results and the pathological conditions in lipoid CAH.

Case presentation

The patient was born at full term with completely normal female external genitalia. No abnormalities were identified in routine newborn screenings. At 15 days, she was brought to the hospital with poor feeding and poor weight gain. Hyperpigmentation suggestive of adrenal insufficiency was noted. Laboratory tests revealed severe hyponatremia (115 mEq/L) and hyperkalemia (8.6 mEq/L). She was immediately treated with hydrocortisone and examined

for all adrenocortical hormones and associated metabolites in serum and urine. Not only mineralocorticoids and glucocorticoids, but also adrenal androgens and their metabolic products were barely detectable. On the other hand, concentrations of adrenocorticotropic hormone were extremely high, at 387 pg/mL (normal 7.2–63.3 pg/mL). CT showed enlargement of bilateral adrenal glands (Fig. 1a). Bilateral gonads were not palpable but were detectable near the internal inguinal rings on ultrasonography and MRI (Fig. 1b). Since congenital adrenal hyperplasia was strongly suspected, chromosomal genetic testing was performed and revealed a 46,XY karyotype. Sequence analysis of the StAR gene revealed compound heterozygous mutations for p.Q258X and p.D246fs. From these results, lipoid CAH was diagnosed. Laparoscopic bilateral gonadectomy was performed at 2 years and 5 months. Both gonads were identified as normal testes accompanied by vas deferens and epididymis. The testes measured 16 × 10 × 6 mm on the right and 14 × 8 × 8 mm on the left (Fig. 2a,b). As of the time of writing, she is continuing adrenocortical hormone replacement therapy, and will receive combination estrogen replacement therapy at the time of puberty.

Histopathological findings

Light microscopy showed seminiferous tubules mainly comprising spermatogonia and Sertoli cells, with no spermatocytes or spermatids (Fig. 2c). Two types of LCs were identified, filled with and without lipid droplets in the testicular interstitium. The nuclei of LCs depressed by excessive lipid droplets were more clearly observable under electron microscopy (Fig. 2d). Testosterone-synthesizing enzymes in the testis of this patient were investigated by immunostaining and Western blotting methods using antibodies reacting with StAR protein, Ad4BP/SF-1, CYP11A1, CYP17A1, 3β-HSD, and 17β-HSD. As control samples, we used biopsy tissues from three individuals with cryptorchidism (n = 3; mean age, 5). All these steroid synthesis-related proteins were observed in LCs of control samples using immunohistochemistry (Fig. 3). Western blotting analysis showed negative results for StAR protein in the testis of this patient (data not shown). LCs both with and without lipid droplets in this patient expressed all the steroid synthesis-related proteins except StAR, 3β-HSD, and 17β-HSD (Fig. 3). This was part of a study approved by the ethics committee of Fukushima Medical University School of Medicine (2245). Informed consent was obtained after explaining the purpose and methods.

Discussion

Lipoid CAH is a rare disorder caused by StAR gene mutations. Since StAR gene was identified as the gene responsible for lipoid CAH, various mutations of the have been reported. The p.Q258X mutation has been reported mainly in Asian patients.[2] To the best of our knowledge, this report presents the first description of heterozygous mutations in p.Q258X and p.D246fs of StAR gene. In lipoid CAH, as cholesterol is not being converted into pregnenolone, the excess cholesterol esters are stored in the steroidogenic cells.[3] The testes of newborn StAR-knockout mice have been shown to contain the lipid droplets in the interstitial space, and lipid deposition increases with age.[4] In fact, lipid

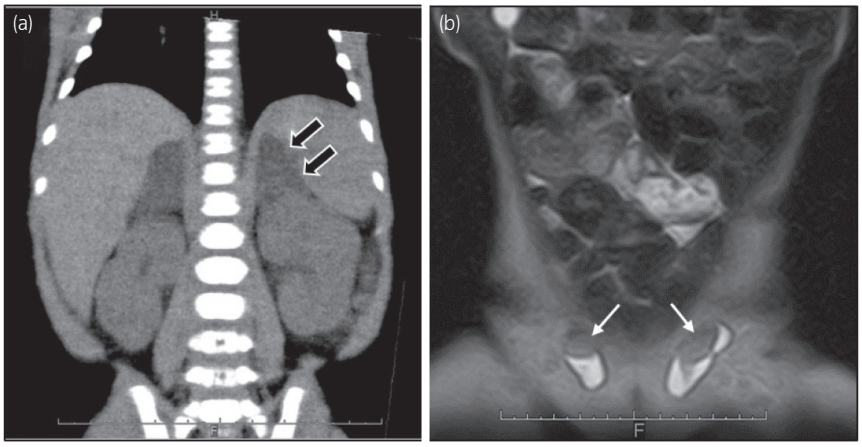

Fig. 1 Finding from CT and MRI. (a) Abdominal CT shows enlarged adrenal glands (black arrows) at 28 days old. (b) Abdominal MRI (T2-weighted imaging) shows gonads in the inguinal canals bilaterally (white arrows) at 2 years and 5 months old.

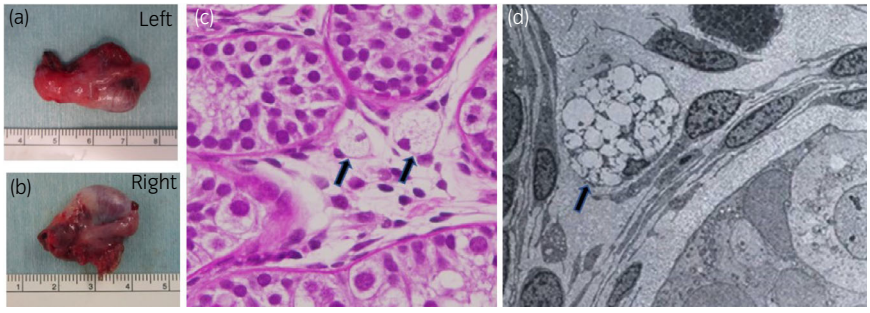

Fig. 2 Extracted gonad and histopathological findings. (a) The left testis with epididymis is 16 × 10 × 6 mm in size. (b) The right testis with epididymis is 14 × 8 × 8 mm in size. (c) LCs are enlarged and filled with lipid droplets (black arrows) in the interstitium (hematoxylin-eosin stain, ×400). (d) LCs filled with lipoid droplets (black arrow) are clearly observed under electron microscopy.

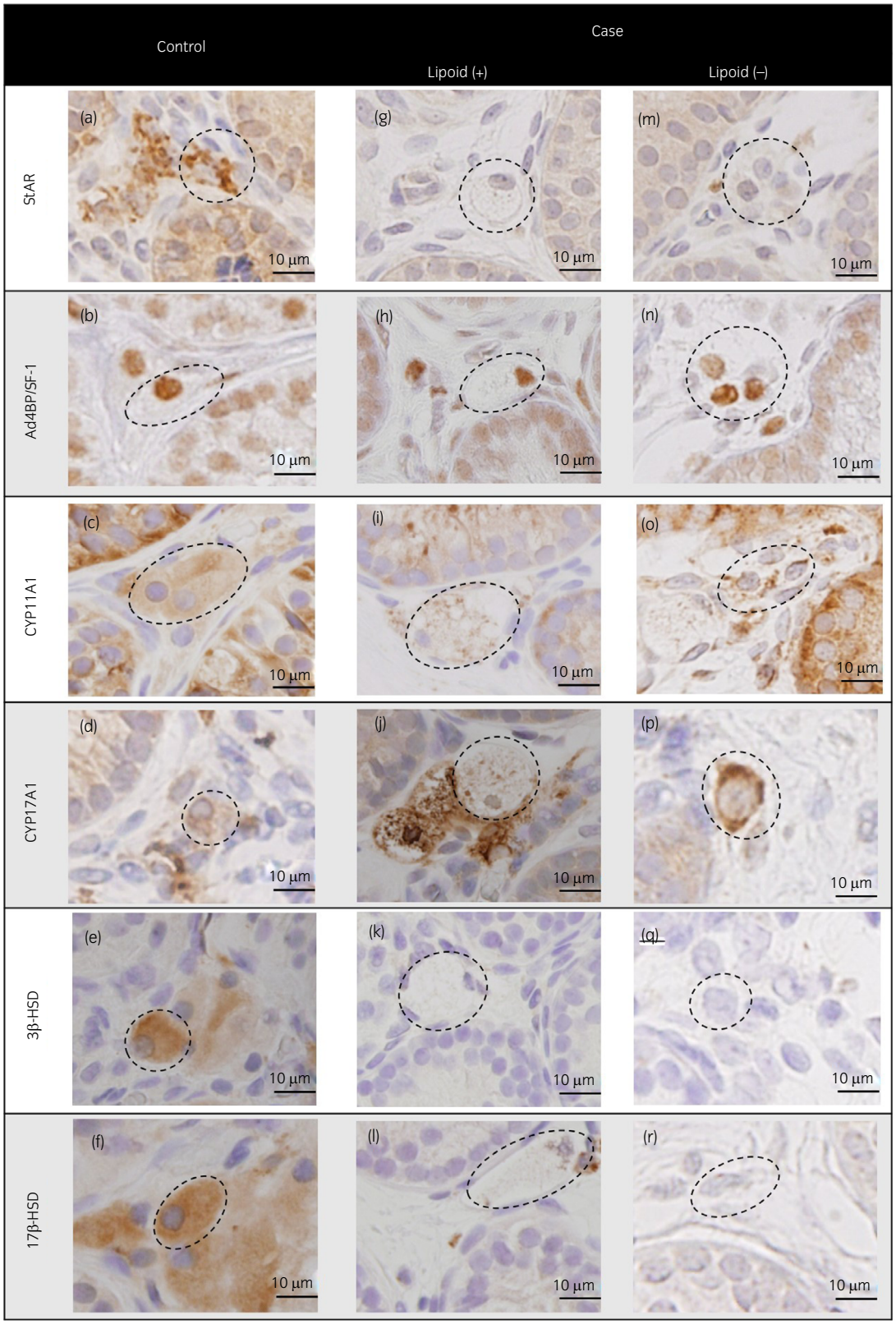

Fig. 3 Immunopathological findings. Immunohistochemistry of StAR protein, Ad4BP/SF-1, CYP11A1, CYP17A1, 3β-HSD, and 17β-HSD. Left column shows testes from boys with cryptorchidism (controls). LCs of controls appear positive for StAR (a), Ad4BP/SF1 (b), CYP11A1 (c), CYP17A1 (d), 3β-HSD (e) and 17β-HSD (f). On the other hand, the middle and right columns show the testes from our case. LCs with lipid droplets are positive for Ad4BP/SF-1 (h), CYP11A1 (i) and CYP17A1 (j), and negative for StAR (g), 3β-HSD (k) and 17β-HSD (l). LCs without lipid droplets are positive for Ad4BP/SF-1 (n), CYP11A1 (o) and CYP17A1 (p), and negative for StAR (m), 3β-HSD (q) and 17β-HSD (r). Original magnification ×1000.

accumulation of LCs was shown in the gonad after later childhood.[5,6] However, excessive lipid accumulation of LCs has not been shown in the gonads before early childhood.[7,8] LCs both with and without lipid droplets were identified in the present case. From these insights, we considered that lipid accumulation also increases with age in humans. We performed immunohistochemistry to clarify the condition of steroidogenic enzymes in LCs of this patient with lipoid CAH.

LCs are known to synthesize testosterone to induce androgenization and maintain male reproductive functions.[9] Ad4BP/SF-1 is a nuclear receptor transcription factor for androgenesis and plays an important role in steroidogenesis and gonadal development.[10] Ad4BP/SF-1 is known to be expressed in the gonads and adrenal cortex. In this patient, Ad4BP/SF-1 was expressed in LCs both with and without lipid droplets, suggesting that transcription of steroid-synthesizing enzymes potentially remained, irrespective of lipid accumulation. Some groups have hypothesized that cholesterol accumulation in steroidogenic cells destroys the residual steroidogenic capacity.[5,6] If this hypothesis is correct, 3β-HSD and 17β-HSD should be expressed in LCs lacking lipid accumulation. However, the expressions of steroidogenic enzymes were detected regardless of the degree of lipid accumulation. We thus considered another hypothesis. The mammalian testis contains two types of LCs: fetal LCs; and adult LCs.[11] After birth, fetal LCs disappear and adult LCs appear sequentially. Fetal LCs in mice express steroidogenic enzymes such as StAR protein, CYP11A1, and CYP17A1, but do not express 17β-HSD or 3β-HSD.[11] In this patient, LCs both with and without lipoid droplets appeared negative for 3β-HSD and 17β-HSD. Given these findings, human testes may contain these two types of LCs, and immature LCs may remain after birth in the testes of patients with lipoid CAH. It may be effective for this patient to maintain the testicular tissue inside the body, when innovative therapy for lipoid CAH is developed. However, we chose bilateral gonadectomy in this patient because of malignant potency.[12] As this represents the first case report to examine this issue, further studies are thus needed to confirm our hypothesis.

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Conflict of interest

The authors declare no conflict of interest.

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