Case Report

# CHARGE syndrome with both primary and secondary hypogonadism

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**Abbreviations & Acronyms**
17β-HSD = 17β-hydroxysteroid dehydrogenase
3β-HSD = 3β-hydroxysteroid dehydrogenase
ACTH = adrenocorticotropic hormone
Ad4BP/SF-1 = adrenal 4-binding protein/steroidogenic factor 1
CD = cluster of differentiation
CHD7 = chromodomain-helicase-deoxyribonucleic acid-binding protein 7
CT = computed tomography
FSH = follicle-stimulating hormone
GCNIS = germ cell neoplasm in situ
GH = growth hormone
GnRH = gonadotropin-releasing hormone
hCG = human chorionic gonadotropin
HE = hematoxylin and eosin
LH = luteinizing hormone
LH-RH = luteinizing hormone-releasing hormone
PRL = prolactin
SALL4 = Spalt-like transcription factor 4
TDS = testicular dysgenesis syndrome

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**Introduction:** CHARGE syndrome is a rare disorder that causes congenital abnormalities in multiple organs, including secondary hypogonadism. We report, herein, a unique case of CHARGE syndrome with both primary and secondary hypogonadism and discuss the possible causes and pathogenesis in this patient.

**Case presentation:** A 15-year-old boy with delayed secondary sexual characteristics and non-palpable testes was referred to our hospital. Physical examination and detection of a chromodomain-helicase-deoxyribonucleic acid-binding protein 7 gene mutation confirmed CHARGE syndrome. Hormone stimulation tests suggested both primary and secondary hypogonadism. Laparoscopic bilateral orchiectomy was performed because of decreased testosterone production and atrophy in both testes. Pathological examination of the testes revealed maturation arrest, germ cell neoplasm in situ, and decreased expression of steroid synthase.

**Conclusion:** This appears to be the first report of CHARGE syndrome with both primary and secondary hypogonadism demonstrated in endocrinological and histological examinations.

**Key words:** CHARGE syndrome, hypogonadism, testicular dysgenesis syndrome.

## Keynote message

This case appears to be the first report in which endocrinologal and histological studies demonstrated that the patient had not only secondary hypogonadism due to CHARGE syndrome but also primary hypogonadism. GCNIS and loss of testosterone production were also concomitant, suggesting that certain fetal triggers may have caused the TDS.

## Introduction

CHARGE syndrome is characterized by multiple congenital anomalies with an estimated incidence in newborns of approximately 1 in 20 000.[1] The name is an acronym for the classical symptoms of "ocular coloboma," "heart defects," "atresia of the choanae," "retarded growth and development," "genital hypoplasia," and "ear abnormalities." Hypogonadism in CHARGE syndrome is usually due to endocrine abnormalities in the hypothalamus and pituitary gland, resulting in decreased secretions of GnRH, LH, and FSH and in turn inducing secondary hypogonadism. We encountered a rare case of CHARGE syndrome with both primary and secondary hypogonadism. We discuss the possible causes of primary and secondary hypogonadism based on the results of molecular biological and immunohistological analyses.

## Case presentation

A 15-year-old boy was referred to our hospital with the main complaints of delayed secondary sexual characteristics and bilateral non-palpable testes. His medical history included ear anomalies (Fig. 1), hearing loss, mental retardation, short stature, posterior nasal atresia, and patent ductus arteriosus. The diagnosis of bilateral migrating testes was made at 3 and 7 years old, but the visits were then interrupted until he was 15 years old. Physical findings included short stature (105 cm; 2.5 standard deviations below the age-appropriate mean) at 7 years old

 

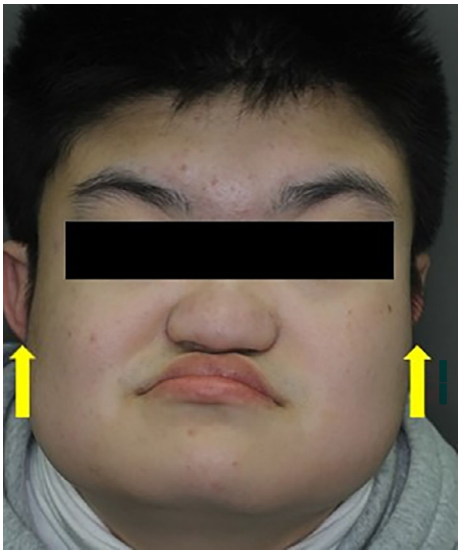

**Fig. 1** Bilateral auricular asymmetry (yellow arrows) in the patient.

and genitourinary abnormalities such as delayed development of pubic hair and micropenis (Tanner Stage 1 and 2, respectively). In addition, testes were not palpable bilaterally in the scrotum or inguinal canal. Neither testis was detectable on ultrasonography. CT of the head showed no abnormalities in the brain, including the hypothalamus and pituitary gland. Chromosomal G-banding analysis showed a 46, XY karyotype. CHARGE syndrome was suspected because the clinical characteristics fulfilled necessary symptoms (mental retardation and ear abnormalities), one major criterion (posterior nasal atresia), and two minor criteria (cryptorchidism and cardiac anomaly). Sequence analysis of the *CHD7* gene was performed, revealing an 8-nucleotide deletion in exon 4 (c.2099_2106delAATAAGAA) of *CHD7*. Finally, this patient was diagnosed with CHARGE syndrome.

Blood tests revealed low serum concentrations of FSH (0.33 mIU/mL), LH (0.10 mIU/mL), and total testosterone (<0.03 ng/mL). Serum ACTH, PRL, and GH levels were within the reference ranges. FSH and LH were hyporesponsive to GnRH stimulation. Moreover, the hCG stimulation test did not increase the serum testosterone concentration. These findings showed that the patient had both primary and secondary hypogonadism. Laparoscopic exploration was performed to identify the location of the testes. Testicular vessels leading into the inguinal canals were observed on both sides. Laparoscopic groin exploration was performed and atrophic testes were identified in the groin canals and pulled into the abdominal cavity. Since testosterone secretory function was absent, bilateral orchiectomy was performed. Both excised testes were atrophic (Fig. 2a,b). HE findings showed atrophic seminiferous tubules and only Sertoli cells were apparent (Fig. 3a). Germ cells were large and atypical with nuclear atypia and clear cytoplasm (Fig. 3b). Immunohistochemical staining of germ cells showed weak positivity for CD117 (Fig. 3c) and SALL4 (Fig. 3d). These pathological findings indicated maturation arrest of spermatogenesis and GCNIS in bilateral testes. The patient has been receiving testosterone replacement therapy since undergoing bilateral orchiectomy.

Immunohistochemical staining for testosterone-synthesizing enzymes in Leydig cells including P450scc, $P450_{17\alpha}$, 3β-HSD, and 17β-HSD was performed to clarify testicular function in this patient.[1,2] Testicular tissues from patients with obstructive azoospermia were used as a control. All these testosterone-synthesizing enzymes showed positivity in control tissues (Fig. 4a–d), but were negative in our patient (Fig. 4f–i). In addition, immunohistochemical staining for the transcription factor Ad4BP/SF-1, which regulates these enzymes,[3] showed positive results in both control tissues (Fig. 4e) and this case (Fig. 4j). These results suggested that testosterone synthesis was impaired not at the transcriptional level, but at the enzymatic level.

## Discussion

In CHARGE syndrome, the observed hypogonadism typically manifests as a secondary type, triggered by endocrine abnormalities in the hypothalamus and pituitary gland. The present case, however, involved a rare condition in which both primary and secondary hypogonadism were present simultaneously. Furthermore, pathological examination showed maturation arrest of spermatogenesis and GCNIS in bilateral testes.

The symptoms of CHARGE syndrome are similar to those of various other congenital diseases, making the syndrome difficult to distinguish from those diseases based on symptoms alone. Genetic diagnosis is the most precise and definitive method and is recommended for the diagnosis of CHARGE syndrome. We, therefore, investigated mutations in *CHD7*, as these gene mutations have been reported in about 90–95% of CHARGE syndrome.[4] Zailong *et al*. reported that the most common type of mutation is frameshift (41%), followed by nonsense mutation (32%). In this case, next-generation sequencing revealed a frameshift mutation, c.2099_2106del, in *CHD7*. This variant has not previously been reported in the literature. *CHD7* is normally related to

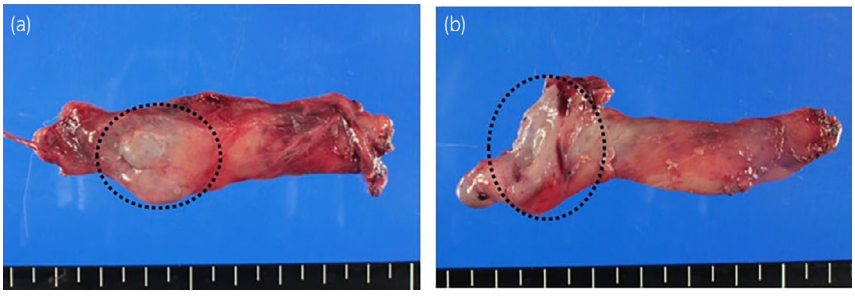


**Fig. 2** Gross appearance of the specimen showing atrophy of the left (a) and right (b) testes (black circles).

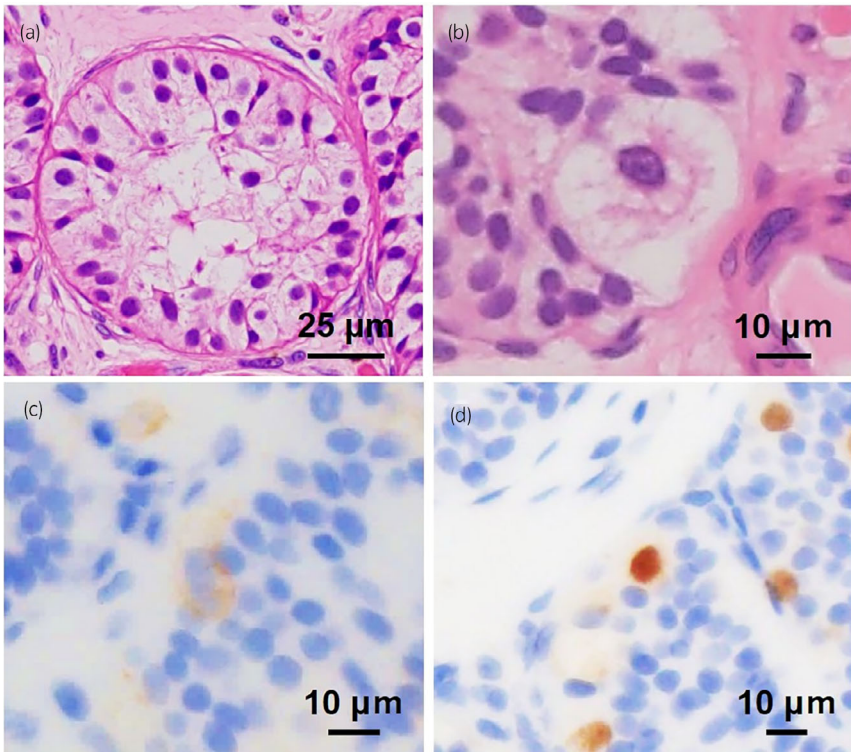


**Fig. 3** Microscopic findings of the testes. The seminiferous tubules appear atrophic and only Sertoli cells are evident (HE staining; scale bar = 25 μm) (a). Large atypical cells with nuclear atypia and clear cytoplasm are observed in the seminiferous tubules (HE staining; scale bar = 10 μm) (b). Immunohistochemical staining showed CD117 (c) and SALL4 (d) positivity (scale bars = 25 μm).

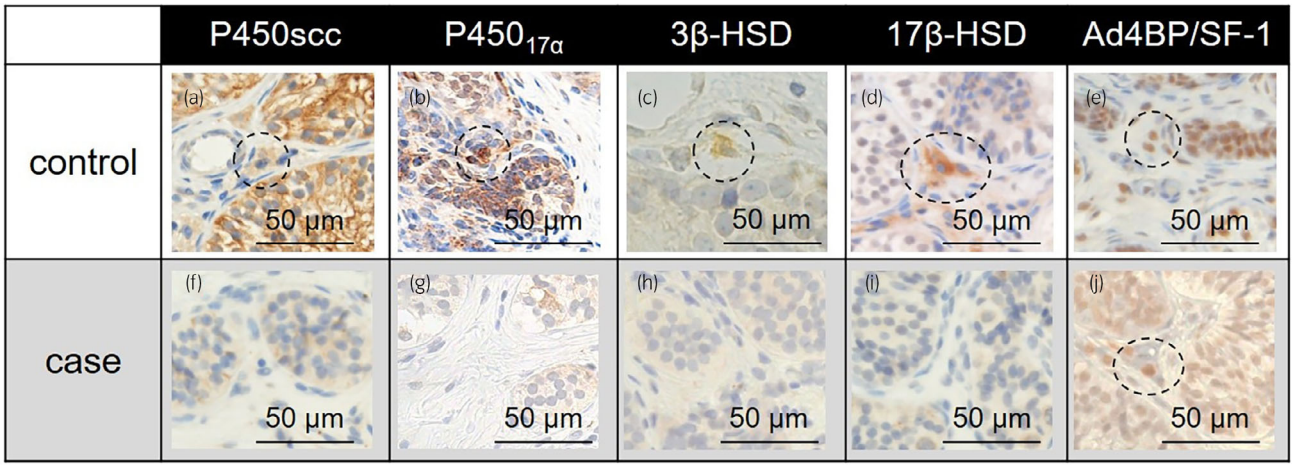


**Fig. 4** Immunohistochemical findings of steroidogenic enzymes and transcription factor Ad4BP/SF-1. The upper row (a–e) shows the testes in a patient with obstructive azoospermia, with Leydig cells positive for P450scc (a), P450$_{17\alpha}$ (b), 3β-HSD (c), 17β-HSD (d), and Ad4BP/SF-1 (e). The lower row (f–j) shows the testes from the present case, demonstrating negative staining for P450scc (f), P450$_{17\alpha}$ (g), 3β-HSD (h), and 17β-HSD (i), but positive staining for Ad4BP/SF-1 (j) (scale bars = 50 μm).

the formation and migration of GnRH neurons in the hypothalamus. Defects in *CHD7* cause improper migration of GnRH neurons, leading to impaired secretion of FSH, LH, and consequently testosterone.[5] Judging from the endocrine pathogenesis of typical CHARGE syndrome, LH-RH loading should theoretically show testosterone secretion from the testes. However, sufficient testosterone secretion from the testes was not observed in this case.

Leydig cells are responsible for synthesizing testosterone to induce androgenization and sustain male reproductive functions. Immunohistochemical staining of Ad4BP/SF-1 was positive in this case, while P450scc, P450$_{17\alpha}$, 3β-HSD, and 17β-HSD were negative. Thus, histopathological examination suggested that the pathogenesis of Leydig cell dysfunction was at the level of the steroid-synthesizing enzymes, rather than at the transcriptional level. To the best of our knowledge, a reduction in steroid synthase expression has not previously been reported in patients with cryptorchidism or male infertility.

Dysfunction of the Leydig cells also has a negative influence on Sertoli cell function. The dysfunction of Sertoli cells impairs germ cell differentiation, leading to maturation arrest

of spermatogenesis, GCNIS, or testicular germ cell cancer.[6] Actually, the present case was also complicated by maturation arrest of spermatogenesis and GCNIS. Skakkebaek *et al*. proposed the concept of TDS,[7] referring to a series of syndromes in which Leydig cell dysfunction caused by environmental factors during the fetal period leads to decreased testosterone secretion and abnormalities in sex differentiation such as hypogonadism and cryptorchidism. The causes of TDS might be environmental and/or genetic factors during the fetal period.[7] Although risk factors related to testicular dysfunction were not investigated in this case, we speculated that some factors during the fetal period might have caused maturation arrest of spermatogenesis, GCNIS, decreased steroid synthesis, and cryptorchidism. It is possible that GCNIS could have been prevented if surgery for non-palpable testes had been performed a little earlier.

In conclusion, we have provided here the first report of CHARGE syndrome with both primary and secondary hypogonadism which was endocrinologically and histologically demonstrated. Immunohistochemistry also suggested GCNIS and loss of testosterone production. TDS caused by the action of environmental and/or genetic factors during the fetal period might exist in the background of this case.

## Registry and Registration No. of the study/trial

None.

## Author contributions

Yuki Yoshida: Writing – original draft. Soichiro Ogawa: Writing – review and editing. Satoru Meguro: Writing – review and editing. Akifumi Onagi: Writing – review and editing. Ryo Tanji: Writing – review and editing. Kanako Matsuoka: Writing – review and editing. Seiji Hoshi: Writing – review and editing. Junya Hata: Writing – review and editing. Yuichi Sato: Writing – review and editing. Hidenori Akaihata: Writing – review and editing. Masao Kataoka: Writing – review and editing. Motohide Uemura: Writing – review and editing. Yoshiyuki Kojima: Supervision; writing – review and editing.

## Conflict of interest

The authors declare no conflict of interest.

## Approval of the research protocol by an Institutional Reviewer Board

Not applicable.

## Informed consent

Not applicable.

## References

1 Miller WL. Molecular biology of steroid hormone synthesis. *Endocr. Rev.* 1988; **9**: 295–318.
2 Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr. Rev.* 2011; **32**: 81–151.
3 Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. *Endocr. Rev.* 1997; **18**: 361–77.
4 Hsu P, Ma A, Wilson M *et al*. CHARGE syndrome: a review. *J. Paediatr. Child Health* 2014; **50**: 504–11.
5 Kim JH, Choi Y, Hwang S, Kim G-H, Yoo H-W, Choi J-H. Phenotypic spectrum of patients with mutations in CHD7: clinical implications of endocrinological findings. *Endocr Connect* 2022; **11**: e210522. https://doi.org/10.1530/EC-21-0522.
6 Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM *et al*. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. *Physiol. Rev.* 2016; **96**: 55–97.
7 Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum. Reprod.* 2001; **16**: 972–8.

## Editorial Comment

### Editorial Comment to CHARGE syndrome with both primary and secondary hypogonadism

CHARGE syndrome, which is a disorder with a combination of malformations, rarely occurs and, however, is an important cause of congenital hypogonadism. Most often dysfunction of chromodomain-helicase-DNA-binding protein (CHD) 7 gene results in this disorder, causing multiple malformations and dysfunctions. CHD7 gene dysfunction impairs the function of the hypothalamus and pituitary gland which results in secondary hypogonadism.

Yoshida *et al*.[1] reported a case of CHARGE syndrome with secondary but also primary hypogonadism that occurred in a 15-year-old boy. After stimulation with human chorionic gonadotropin (hCG), the patient's serum testosterone level did not elevate, which suggested the presence of mixed hypogonadism. The author decided to perform bilateral orchidectomy, and pathological examination revealed germ cell neoplasia in situ (GCNIS). Thus, the author hypothesized that[1] dysfunction of the Leydig cell function was involved by decreased expression of steroid-synthesizing enzymes and[2] pathogenesis of GCNIS was involved in Sertoli cell degeneration associated with Leydig cell dysfunction.