

## Original Article

# Endocrinological Characteristics of 25 Japanese Patients with CHARGE Syndrome

Yasuko Shoji<sup>1</sup>, Shinobu Ida<sup>1</sup>, Yuri Etani<sup>1</sup>, Hiroyuki Yamada<sup>1</sup>, Futoshi Kayatani<sup>2</sup>, Yasuhiro Suzuki<sup>3</sup>, Kenjiro Kosaki<sup>4</sup>, and Nobuhiko Okamoto<sup>5</sup>

<sup>1</sup>Department of Gastroenterology and Endocrinology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

<sup>2</sup>Department of Pediatric Cardiology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

<sup>3</sup>Department of Pediatric Neurology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

<sup>4</sup>Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan

<sup>5</sup>Department of Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

**Abstract.** CHARGE syndrome is a congenital disorder caused by mutation of the chromodomain helicase DNA binding protein 7 (*CHD7*) gene and is characterized by multiple anomalies including ocular coloboma, heart defects, choanal atresia, retarded growth and development, genital and/or urological abnormalities, ear anomalies, and hearing loss. In the present study, 76% of subjects had some type of endocrine disorder: short stature (72%), hypogonadotropic hypogonadism (60%), hypothyroidism (16%), and combined hypopituitarism (8%). A mutation in *CHD7* was found in 80% of subjects. Here, we report the phenotypic spectrum of 25 Japanese patients with CHARGE syndrome, including their endocrinological features.

**Key words:** CHARGE syndrome, *CHD7*, endocrinological features

---

## Introduction

CHARGE syndrome is a disorder with multiple anomalies. The occurrence of the

syndrome was estimated to be approximately 1:8,500 births by Issekutz *et al.* in 2005 (1). The syndrome was first reported in 1979 by Hall and Hinter, and Pagon *et al.* proposed its main features in 1981 (2). The syndrome was named for the acronym of coloboma of the eyes, heart defects, atresia choanae, retarded growth and development, genital and/or urological abnormalities, and ear anomalies and hearing loss. The criteria for CHARGE syndrome were defined by Blake *et al.* in 1998 (3) and updated by Verloes in 2005 (4). In 2004, chromodomain helicase DNA binding protein 7 (*CHD7*), located on chromosome 8q12.1, was identified as the

---

Received: May 27, 2013

Accepted: January 8, 2014

Correspondence author: Dr. Yasuko Shoji, Department of Gastroenterology and Endocrinology, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo, Izumi, Osaka 594-1101, Japan  
E-mail: yshoji@mch.pref.osaka.jp

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

main gene responsible for the syndrome (5). Patients with CHARGE syndrome have not only various clinical features but also various anomalies, and Japanese patients with the syndrome have been reported (6–8). The present study delineated the phenotypic spectrum of 25 Japanese patients with CHARGE syndrome including their endocrinological features.

### Subjects and Methods

The subjects were 25 Japanese patients (13 males and 12 females, aged from 1 to 25 yr) with CHARGE syndrome who were followed regularly at the authors' institution over the past 20 yr. A clinical geneticist diagnosed them by referring to Blake's and/or Verloes' criteria.

*CHD7* was analyzed in 21 subjects as described previously (6, 7). Briefly, all the coding exons 2–37 and their splice sites were PCR amplified from leukocyte genomic DNA of the patient, and the PCR products were screened for a mutation by denaturing high-performance liquid chromatography (DHPLC) using an automated instrument (WAVE, Transgenomic, Omaha, NE, USA). When abnormal heteroduplex patterns were detected, the corresponding PCR products were subjected to direct sequencing on an ABI PRISM 3100 autosequencer (Perkin-Elmer, Foster City, CA, USA).

Using the patients' medical records, we studied their phenotypes with a focus on endocrine disorders.

### Results

The characteristic clinical features of each patient are shown in Table 1. All patients had mental retardation and hearing loss. The patients also had congenital heart defects (76%), short stature (72%), genital disorders including micropenis and cryptorchidism (77% in males), ocular coloboma (56%), gastrointestinal disorders (52%), ear anomalies (44%), and cleft lip and/or palate (44%). Twenty-two patients (88%) had

some type of endocrine disorder, including short stature.

### Clinical features

#### *Endocrinological findings*

Short stature was the most frequent problem (72%), and the mean height was  $-3.04$  standard deviation (SD) in 12 males (from  $-5.4$  to  $-0.9$  SD) and  $-3.64$  SD in 10 females (from  $-6.4$  to  $-0.2$  SD), excluding 3 patients with growth hormone replacement therapy for GH deficiency (GHD) at the time of this study. In the 3 subjects (cases 16, 21 and 24) with GHD, the mean acquired degree of change in height ( $\Delta$ Ht SDS) was  $+1.33$  SD during growth hormone therapy ( $0.175$  mg/kg/wk), and the mean duration of growth hormone replacement therapy was 2.2 yr (Table 2). They had normal findings for the pituitary on head magnetic resonance imaging.

Meanwhile, there were 15 patients (52%) with hypogonadotropic hypogonadism (HH), including cases with suspected delayed puberty and 10 boys with genital hypoplasia. Three patients (12%) had received gonadal hormone therapy: hCG 1500 units once a week i.m. in case 8 and Kaufmann treatment in cases 12 and 21. Among the subjects with HH, 6 patients with relatively mild mental retardation (cases 4, 8, 9, 14, 15 and 21) were suspected of anosmia by self-evaluation for strong odors, such as spicy food. Four patients (16%) had hypothyroidism: cases 4 and 22 had central hypothyroidism with a low response in the thyrotropin-releasing hormone loading test, and cases 8 and 13 had primary hypothyroidism and received thyroxine replacement therapy. The growth rate improved in case 4, from  $-4.2$  to  $-2.8$  SD, for 4 yr before puberty after thyroxine replacement. Two cases (8%) with combined pituitary hormone deficiency with GHD and gonadotropin deficiency received growth hormone and gonadal hormone replacement therapy. One patient had hypertension (case 21). Electrolyte abnormalities were not observed in any of the patients.

### Others

Ocular findings: Sixteen patients (64%) had ocular defects: coloboma with a various combination of iris, retina and disc defects in 14 patients, microphthalmus in 4 patients, nystagmus in 2 patients, and ptosis of the eyelid and amblyopia in 1 patient each. Although coloboma seems to be found in 75% of patients (7), representing one of the major features of CHARGE syndrome, its frequency was 56% in our subjects.

Otorhinolaryngological findings: All patients had hearing loss, and inner ear anomalies on temporal head computed tomography were found in 12 subjects. External ear anomalies were found in 13 subjects, with a suspicion of anosmia in 6 subjects, atresia choanae in 3 subjects, and laryngomalacia and vocal cord palsy in 1 subject each. All subjects with atresia choanae had a cleft lip and palate.

Oral and maxillofacial findings: Thirteen (52%) patients had oral and maxillofacial complications: cleft lip and/or palate in 11 patients, jaw deformity in 1 patient and deficit of the depressor muscle at the angle of the mouth in 1 patient.

Cardiovascular findings: Nineteen patients (76%) had cardiovascular anomalies: ventricular septal defect in 7 patients, double-outlet right ventricle in 3 patients, endocardial cushion defect in 1 patient, coarctation of the aorta in 2 patients, atrial septal defect in 6 patients, patent ductus arteriosus in 12 patients, pulmonary artery stenosis in 2 patients, vascular ring in 2 patients, aortic arch interruption in 1 patient, and transposition of the great arteries in 1 patient.

Neurological findings: All patients had various degrees of mental retardation. Nevertheless, we should note the unreliability of the results for the development assessment test used because of the hearing loss and dystopia of the patients. We found VII cranial nerve palsy in 6 patients, epilepsy in 4 patients, hypoplasia of the cerebellar vermis in 2 patients, and flaccid palsy and hydrocephalus in 1 patient each.

Gastrointestinal findings: Gastrointestinal disorders were found in 13 patients (52%): gastroesophageal reflux in 8 patients including 4 patients received fundoplication), esophageal closure in 4 patients, esophageal hiatal hernia in 3 patients, and duodenal closure, tracheoesophageal fistula and intestinal malrotation in 1 patient each.

Genital and urological findings: Genital and urological features were found in 12 patients (48%): genital hypoplasia including micropenis and cryptorchidism in 8 patients and unilateral renal aplasia, hydronephrosis and vesicoureteral reflux in 1 patient each.

In addition, there were 2 patients with tracheostenosis, 1 patient with congenital scoliosis, 1 patient with leukoderma and 1 patient with polysyndactyly.

### Mutation analysis of *CHD7*

In the 21 patients analyzed, 17 patients (80%) had a mutation in *CHD7*: a nonsense mutation in 7 patients (41%), frameshift mutation in 5 patients (29%), splice site mutation in 3 patients (17%) and gene deletion in 2 patients (10%). No mutation in *CHD7* was found in 4 patients (19%). No novel mutation was found in this study.

## Discussion

The endocrinological findings in CHARGE syndrome have been reported in some studies (8–11). Although HH was commonly reported, the frequency of HH was less than that of the previous study for girls before puberty. Additionally, HH in association with anosmia is a symptom that overlaps with Kallmann syndrome, which classically consists of an impaired sense of smell and gonadotropin deficiency. Bergman *et al.* also suggested that anosmia seems to be a predictor for the occurrence of HH in CHARGE syndrome because genital hypoplasia is a predictor only in boys and there is no way to make a diagnosis until puberty in girls (12). In our study, 6 patients with HH including suspected cases had smell

**Table 1** Characteristics of the 25 patients with CHARGE syndrome

No.	Age (yr)	Sex	CHD7 analysis	Height SDS	Body weight SDS	Endocrine findings	Ocular findings	Otorhinolaryngological findings
1	1	m	c.490C>T E164X	-4.2	-3.6	short stature		hearing loss (B) inner ear anomalies (B)
2	4	m	c.6157C>T R2053X	-3	-2.1	short stature s/o HH	coloboma (B) microphthalmos (R)	hearing loss (L) inner ear anomalies (B) atresia choanae
3	5	m	c.4947C>TA Y1649X	-3.3	-2.1	short stature s/o HH	coloboma*	hearing loss (B) inner ear anomalies (B)
4	12	m	c.6991A>T K2331X	-3.2	-1.8	short stature central hypothyroidism s/o HH	coloboma (B)	hearing loss (B) inner ear anomalies (B) anosmia
5	2	f	c.3907A>T K1303X	-3.9	-4.2	short stature	coloboma (B) microphthalmos (L)	hearing loss (B) inner and outer ear anomalies (B)
6	5	f	c.4015C>T R1339X	-5	-3.3	short stature		hearing loss (B) inner ear anomalies (B) laryngomalacia
7	4	m	c.4723-4724insG E1575G fsX8	-0.9	-1	s/o HH		hearing loss (B) inner ear anomalies (B)
8	20	m	c.6326delT V2109A	-1.3	-1.8	HH (HCG) hypothyroidism (ectopic)		hearing loss (B) inner ear anomalies (B) anosmia
9	6	f	c.6745-6746insG D2249GfsX2	-1.1	-0.8		coloboma (L) ptosis (L)	hearing loss (B) inner ear anomalies (B) anosmia
10	7	f	1295delA fs462X	-4.4	-2.8	short stature	coloboma (L) nystagmus	hearing loss (B) inner ear anomaly (B) atresia choanae
11	13	f	c.2500-2504delTCCTTA	-2.9	-2.1	short stature HH	coloboma*	hearing loss (B) outer ear anomaly (B)
12	24	f	7486-7487insGC	-1.4	0.1	HH (HRT)		
13	9	m	IVS21-2A>G	-5.4	-2.7	short stature hypothyroidism s/o HH	nystagmus	hearing loss (B) outer ear anomaly (B)
14	12	m	IVS25-7G>A	-4.8	-2.8	short stature s/o HH	coloboma* microphthalmos*	hearing loss* outer ear anomaly* anosmia
15	12	m	IVS26+1G>T	-3.4	-2.1	short stature s/o HH	coloboma (B) microphthalmos (L)	hearing loss (B) outer ear anomaly anosmia
16	11	m	deletion	-2.2	-2.1	short stature GHD		hearing loss (B) outer ear anomaly (B)
17	20	f	deletion	-6.4	-4.4	short stature HH	coloboma*	hearing loss (B) outer ear anomaly (B)
18	3	m	no mutation	-2.3	-1.3	short stature s/o HH		hearing loss (B) outer ear anomaly (B)
19	6	m	no mutation	-1.5	-1.1		coloboma*	hearing loss (B) outer ear anomaly (B)
20	6	f	no mutation	-0.2	0			hearing loss (L)
21	25	f	no mutation	-1	0	GHD HH (HRT) hypertension		hearing loss (B) inner ear anomalies (B)
22	2	m	NT	-3.2	-2.3	short stature s/o HH central hypothyroidism	coloboma*	hearing loss (B) outer ear anomaly (B) vocal palsy
23	4	f	NT	-2.4	-1.3	short stature	coloboma*	hearing loss (B) atresia choanae outer ear anomaly (B)
24	12	f	NT	-2.5	-0.2	short stature GHD s/o HH	amblyopia*	hearing loss (B) inner ear anomalies (B)
25	18	f	NT	-8.7	-5	short stature	coloboma*	hearing loss*

m, male; f, female; s/o, suspected of; AS, aortic stenosis; ASD, atrial septal defect; CoA, coarctation of the aorta; DORV, double-outlet right ventricle; ECD, endocardial cushion defect; GER, gastroesophageal reflux; GHD, GH deficiency; HH, hypogonadotropic hypogonadism; PDA, patent ductus arteriosus; PS, pulmonary artery stenosis; TGA, transposition of the great arteries; VSD, ventricular septal defect; VUR, vesicoureteral reflux; (B), bilateral; (R), right; (L), left; \*, unknown side.

**Table 1** Continued.

No.	Oral and maxillofacial findings	Cardiovascular findings	Neurological findings	Gastrointestinal findings	Genito-urinary findings	Others
1	cleft lip and palate	VSD	MR	esophageal hiatal hernia	micropenis cryptorchidism	
2			MR			
3			MR		micropenis	
4	cleft lip and palate		MR	GER	cryptorchidism	
5	cleft lip and palate	VSD ASD PDA hypoplastic LV aortic arch interruption	MR facial nerve palsy	GER		
6		PDA	MR facial nerve palsy	GER		
7	deficit of the depressor muscle of the mouth angle	PDA	MR	esophageal hiatal hernia	micropenis	scoliosis
8		VSD AS PS PDA aortic arch interruption	MR		cryptorchidism	
9	cleft lip and palate	PDA ASD	MR	GER		
10		VSD PDA CoA	MR flaccid palsy hypoplasia of cerebellar vermis	esophageal atresia GER	hydronephrosis	tracheal stenosis
11	cleft lip and palate		MR facial nerve palsy			
12	cleft lip and palate	VSD PDA	MR			
13		ECD PDA	MR facial nerve palsy	esophageal atresia	VUR micropenis	
14	cleft lip and palate		MR epilepsy	esophageal atresia	micropenis cryptorchidism	polysyndactyly
15		VSD PDA	MR facial nerve palsy		micropenis	leukoderma
16	cleft lip and palate jaw deformity	vascular ring	MR			
17			MR epilepsy	GER duodenal atresia esophageal hiatal hernia intestinal malrotation		
18		vascular ring PDA ASD	MR epilepsy facial nerve palsy		micropenis	
19		AP window	MR			
20	cleft lip	ASD PDA	MR			scoliosis
21		VSD CoA	MR	tracheoesophageal fistula		anosmia
22	cleft palate	DORV PS	MR epilepsy	GER	micropenis	
23	cleft lip and palate	DORV PS	MR	GER	unilateral renal aplasia	tracheostenosis
24		TGA PDA	MR			
25		DORV PDA	MR			

**Table 2** Summary of patients with GHD

Case	Provocation test (pre, peak)	GH duration (yr)	$\Delta$ Ht SDS during GH
16	Arginine (1.6 mg/dl, 3.7 mg /dl) L-dopa (0.4 mg /dl, 4.2 mg /dl)	3	0.7
21	Arginine (0.1 mg /dl, 3.8 mg /dl) Insulin (0.1 mg /dl, 3.0 mg /dl)	1.6	0.9
24	Arginine (0.1 mg /dl, 1.0 mg /dl) L-dopa (0.1 mg /dl, 1.3 mg /dl)	2	2.4

$\Delta$ Ht SDS, degree of change in height standard deviation score.

disorders based on their self-evaluations. However, the frequency was unclear because it is possible that the patients with severe mental retardation did not answer correctly.

As for the other symptoms, we observed GHD, hypothyroidism and combined hypopituitarism with GHD and HH. However, growth hormone secretion was not assessed in the majority of these patients unless they or their family desired for growth therapy, and we could not determine the precise frequency of GHD in our patients.

According to a previous report, there is no significant correlation between phenotype and genotype in CHARGE syndrome. However, Jogmans *et al.* reported that 85% of boys and 70% of girls with gonadotropin deficiency had a *CHD7* mutation (13). In our subjects with a *CHD7* mutation, 94.1% had some type of endocrinological problem, and 64.7% had HH. In subjects without *CHD7* mutation, 75% had some endocrinological problems, and 50% had HH. In addition, 76.4% with a *CHD7* mutation and *CHD7* deletion and 62.8% without a *CHD7* mutation had short statures. This suggests that the syndrome has a relatively higher frequency of endocrinological disorders with *CHD7* mutation and deletion, but all patients must be assessed periodically for endocrinological status on routine follow-up.

### Conclusions

We reported the phenotype of CHARGE syndrome, including endocrinological findings.

Endocrine disorders were also one of the core symptoms in CHARGE syndrome, and it is thus necessary to assess the endocrine status of patients with this syndrome. *CHD7* mutations were detected in 80% of the patients who underwent gene analysis.

The phenotypic spectrum of CHARGE syndrome is heterogeneous, and it is sometimes difficult to make a diagnosis because of unfulfilled criteria. It is helpful to make a diagnosis according to *CHD7* mutation analysis. The authors believe that accumulation of data for delineation of the phenotypic spectrum of CHARGE syndrome will lead to better clinical management of patients.

### References

1. Issekutz KA, Graham JM Jr, Prasad C, Smith IM, Blake KD. An epidemiological analysis of CHARGE syndrome: preliminary results from a Canadian study. *Am J Med Genet A* 2005;133A:309–17. [[Medline](#)] [[CrossRef](#)]
2. Pagon RA, Graham JM Jr, Zonana J, Yong SL. Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association. *J Pediatr* 1981;99:223–7. [[Medline](#)] [[CrossRef](#)]
3. Blake KD, Davenport SLH, Hall BD, Hefner MA, Pagon RA, Williams MS, *et al.* CHARGE association: an update and review for the primary pediatrician. *Clin Pediatr (Phila)* 1998;37:159–73. [[Medline](#)] [[CrossRef](#)]
4. Verloes A. Updated diagnostic criteria for CHARGE syndrome: a proposal. *Am J Med Genet A* 2005;133A:306–8. [[Medline](#)] [[CrossRef](#)]

5. Vissers LE, van Ravenswaaij CM, Admiraal R, Hurst JA, de Vries BB, Janssen IM, *et al.* Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet* 2004;36:955–7. [[Medline](#)] [[CrossRef](#)]
6. Aramaki M, Udaka T, Kosaki R, Makita Y, Okamoto N, Yoshihashi H, *et al.* Phenotypic spectrum of CHARGE syndrome with CHD7 mutations. *J Pediatr* 2006;148:410–4. [[Medline](#)] [[CrossRef](#)]
7. Ogata T, Fujiwara I, Ogawa E, Sato N, Udaka T, Kosaki K. Kallmann syndrome phenotype in a female patient with CHARGE syndrome and CHD7 mutation. *Endocr J* 2006;53:741–3. [[Medline](#)] [[CrossRef](#)]
8. Zenter GE, Layman WS, Scacheri PC. Molecular and phenotypic aspects of CHD7 mutations in CHARGE syndrome. *Am J Med Genet PART A* 152A:674–86.
9. Asakura Y, Toyota Y, Muroya K, Kurosawa K, Fujita K, Aida N, *et al.* Endocrine and radiological studies in patients with molecularly confirmed CHARGE syndrome. *J Clin Endocrinol Metab* 2008;93:920–4. [[Medline](#)] [[CrossRef](#)]
10. Ishikawa A, Enomoto K, Furuya N, Muroya K, Asakura Y, Adachi M, *et al.* Clinical features in 26 patients with CHARGE syndrome. *The Journal of the Japan Pediatric Society* 2012;116:1357–64.
11. Wheeler PG, Quigley CA, Sadeghi-Nejad A, Weaver DD. Hypogonadism and CHARGE association. *Am J Med Genet* 2000;94:228–31. [[Medline](#)] [[CrossRef](#)]
12. Bergman JE, Bocca G, Hoefsloot LH, Meiners LC, van Ravenswaaij-Arts CM. Anosmia predicts hypogonadotropic hypogonadism in CHARGE syndrome. *J Pediatr* 2011;158:474–9. [[Medline](#)] [[CrossRef](#)]
13. Jongmans MC, Admiraal RJ, van der Donk KP, Vissers LE, Baas AF, Kapusta L, *et al.* CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene. *J Med Genet* 2006;43:306–14. [[Medline](#)] [[CrossRef](#)]