

Phenotypic spectrum of neonatal CHARGE syndrome

Espectro fenotípico de Síndrome de CHARGE neonatal

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Abstract

CHARGE syndrome is a genetic disorder of wide phenotypic variability, of autosomal dominant inheritance, caused by pathogenic variants in the *CHD7* gene. **Objective:** To describe the broad phenotypic spectrum of neonatal CHARGE syndrome, heterozygous for the *CHD7* gene, and the usefulness of genome sequencing in diagnostic confirmation, considering differential diagnoses. **Clinical Case:** 34-week preterm newborn, with severe prenatal history of polyhydramnios, increased nuchal translucency, and hyperechogenic cardiac focus, with a TORCH study that ruled out congenital infection. Peripheral facial paralysis, choanal atresia, multiple dysmorphisms, congenital heart disease, and bilateral retinochoroidal coloboma were observed at birth. The neuroimaging study showed hypoplasia of the cochlea and bilateral semicircular canals, and pontocerebellar hypoplasia. The auditory evoked potentials showed deep right-sided sensorineural hearing loss and left anacusis. The patient developed hypocalcemia and immunological alterations, confirming hypoparathyroidism and thymus hypoplasia. The karyogram was normal and 22q11.2 microdeletion was excluded through multiplex ligation-dependent probe amplification (MPLA). A pathogenic variant in the *CHD7* gene was detected that confirmed the clinical suspicion of CHARGE syndrome. **Conclusions:** The overlap of clinical characteristics of CHARGE syndrome requires molecular genetic confirmation, considering differences in evolution, therapies, and recurrence risks with other genetic syndromes.

Keywords:

CHARGE syndrome;
facial palsy;
newborn;
CHD7 gen;
hypoacusia

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Introduction

CHARGE Syndrome is a complex syndrome of autosomal dominant inheritance, and most of the cases are isolated. It has a wide phenotypic spectrum and may compromise almost all body organs and systems. Its prevalence is 1/8,500-15,000 live births with a high and variable comorbidity, and also the presence of lethal cases underdiagnosed in the neonatal period¹.

In 1981, Pagon² created the term 'CHARGE association' as an acronym to describe the presence of Coloboma of the eye, Heart defects, Atresia of the nasal choanae, Retardation of growth and/or development, Genitourinary anomalies, and Ear anomalies and deafness. Other anomalies have been added over the years such as brain and cranial nerves alterations, cochlear dysplasia, scoliosis, hemivertebrae, kidney abnormalities, omphalocele/umbilical hernia, cleft lip and palate, thymus/parathyroid abnormalities, and autism spectrum disorder with a phenotype constantly expanding. Some anomalies have been incorporated to the major and/or minor clinical criteria (Table 1) described by Blake, Verloes and Hale in 1998, 2005 and 2015³⁻⁵.

In 2004, when Vissers et al⁶ described alterations in the *CHD7* gene, this 'CHARGE association', defined as the non-random occurrence of a several anomalies combination observed in more than one individual without identified etiology, it became a syndrome⁷.

The *CHD7* gene (chromodomain helicase DNA-binding) located on region q12 of the chromosome 8 is crucial for the mitigation of neural crest cells that are divided into five subpopulations (cranial, cardiac, vagal, sacral, and trunk), affecting a wide variety of tissues including thymus/parathyroid structures⁸. 90% of typical cases that met diagnostic criteria and between 65 to 70% of those typical and with suspicion of CHARGE syndrome occur due to *CHD7* gene alterations⁹. Most of the cases are *de novo*, so if neither parent is a carrier, the recurrence risk is lower than 3%.

Considering the described prevalence (in Chile, we should have 20 cases per year), we believe that there is a high rate of underdiagnosis due to the overlap of symptoms with other syndromes and the high morbidity and mortality in the neonatal period. Among the differential diagnosis are the 22q11.2 microdeletion syndrome, Kallmann syndrome, Kabuki syndrome, Treacher Collins syndrome, Mowat Wilson syndrome, and the 3M syndrome^{10,11}.

The objective of this article is to describe the wide phenotypic spectrum of the CHARGE syndrome in a female newborn, and the usefulness of the sequencing in diagnostic confirmation and warning about the suspicion of this condition and its differential diagnoses.

This article was approved by the Institutional Ethics Committee and the parents signed informed consent.

Table 1. Clinical criteria for CHARGE syndrome

Verloes's Criteria	Blake's Criteria	Hale's Criteria
Major Criteria		
1. Ocular Coloboma	1. Coloboma, Microphthalmia	1. Coloboma
2. Choanal Atresia	2. Choanal Atresia or cleft palate	2. Choanal Atresia or cleft palate
3. Hypoplastic semicircular canals	3. Characteristic external ear anomaly, middle/inner ear malformations, mixed deafness	3. Abnormal external, middle or inner ears, including hypoplastic semicircular canals
	4. Cranial nerve dysfunction	4. Pathogenic <i>CHD7</i> variant
Minor Criteria		
1. Heart or esophagus malformation	1. Cardiovascular malformations	1. Cranial nerve dysfunction including hearing loss
2. Malformation of the middle or external ear	2. Trachea-esophageal defects	2. Dysphagia/feeding difficulties
3. Rhombencephalic dysfunction including sensorial deafness	3. Genital hypoplasia or delayed pubertal development	3. Structural brain anomalies
4. Hypothalamo-hypophyseal dysfunction (gonadotrophin or growth hormone deficiency)	4. Orofacial cleft	4. Developmental delay/Intellectual disability/autism
5. Mental retardation	5. Developmental delay	5. Hypothalamo-hypophyseal dysfunction (gonadotrophin or growth hormone deficiency) and genital anomalies
	6. Growth deficiency	6. Heart or esophagus malformation
	7. Characteristic facial features	7. Renal anomalies skeletal/limb anomalies
Typical CHARGE: 3 major or 2 major + 2 minor	Typical CHARGE: 4 major or 3 major + 3 minor	CHARGE: 2 major + any number of minor
Partial CHARGE: 2 major + 1 minor		
Atypical CHARGE: 2 major or 1 major + 3 minor		

From Busa T. Prenatal findings in children with early postnatal diagnosis of CHARGE syndrome. *Prenat Diagn.* 2016 Jun;36(6):561-7.

Clinical case

Second child of young non-consanguineous parents, without relevant family history. During pregnancy, the mother presents severe symptomatic polyhydramnios managed with amnioreduction since the 31 weeks (negative cytochemical and bacterial culture). Prenatal ultrasounds showed a large-for-gestational-age fetus, nasal fold, nuchal edema, and a cardiac hyperechogenic focus. The amniocentesis test was negative for congenital infection. The child born by cesarean section at 34 weeks of gestational age after lung maturation with corticosteroids due to imminent preterm birth. The birth weight was 2,660 g (p75), height 45 cm (p50), and head circumference 31 cm (p10) (Alarcón-Pittaluga curves). APGAR score of the child was 2-7 (1' and 5') requiring intubation for mechanical ventilation where choanal atresia was observed.

The neurogenetic evaluation showed craniofacial dysmorphisms such as square face, facial asymmetry, low and narrow forehead, dysmorphic, low-set and posteriorly rotated ears, short neck, widely spaced nipples, short sternum, and brachydactyly (figure 1). In the neurological examination was possible to observe cranial nerves dysfunctions (peripheral facial palsy, bulbar dysfunctions with swallowing disorders), and central hypotonia. A CHARGE syndrome diagnosis was suggested based on these findings. The neuro-ophthalmological examination showed bilateral chorioretinal coloboma with no posterior embryotoxon



Figure 1. Square Face, right peripheral facial paralysis.

or iris coloboma. The echocardiography showed severe coarctation of the aorta with right-to-left shunt, ventricular septal defects, and patent ductus arteriosus (PDA) which required prostaglandins and subsequently surgery.

CT scan and, subsequently, brain MRI confirmed right choanal atresia and showed cochlear and bilateral semicircular canal dysplasia and hypoplasia, and pontocerebellar hypoplasia (Figure 2). The brainstem auditory evoked potentials showed profound right-sided

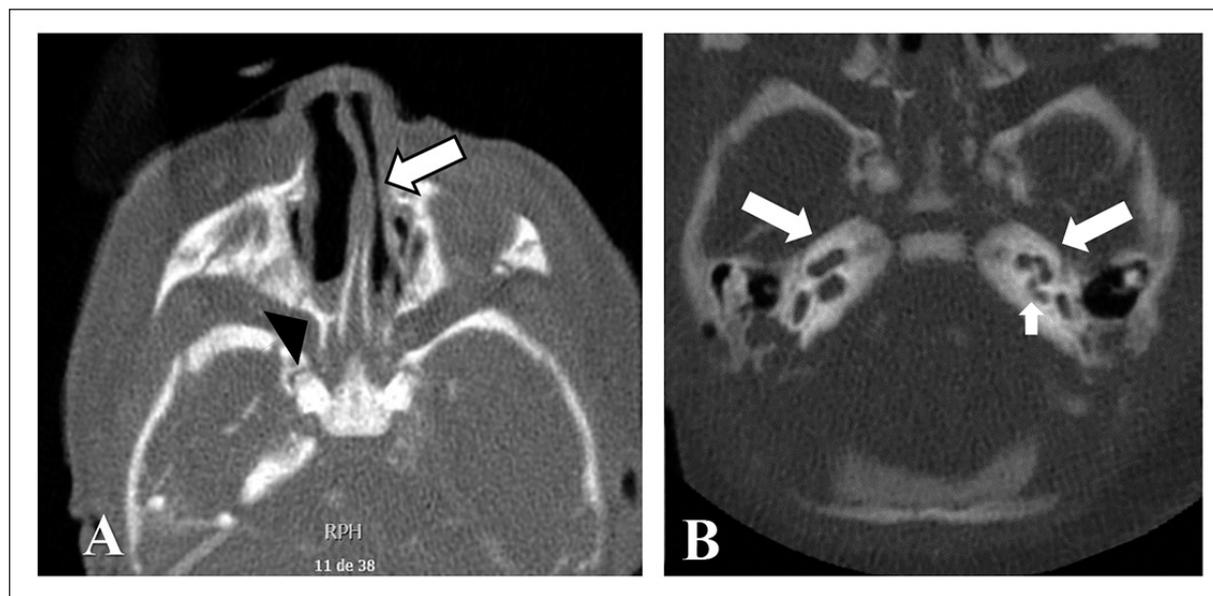


Figure 2. **A)** Axial paranasal cavities computed tomography (CT) image, deviation of left convex nasal septum (White Arrow), with thickening of the vomer and right choanal atresia (black arrow head). **B)** Axial ears CT image, bilateral cochlear dysplasia, more prominent at right with decreased size of apical and middle spire (white arrows). At left ear, a decrease in caliber of cochlear nerve canal (short arrow) which indicates hypoplasia/absence of the cochlear branch of VIII nerve.

sensorineural hearing loss and total left-sided hearing loss.

The patient required calcium treatment due to persistent hypocalcemia since the second day of life, with ionized calcium levels lower than 2.9 (adjusted to pH 7, normal level 4.4-6 mg/dL), and hypoparathyroidism was subsequently confirmed. Subsequent studies due to severe recurrent infections diagnosed immunodeficiency with lymphocyte subpopulations, total T lymphocyte, low T helper and T suppressor cells, and hypogammaglobulinemia. She was treated with gamma globulin and immunization schedule modifications. Chest CT scan showed thymic hypoplasia.

With the karyogram and the multiplex ligation-dependent probe amplification (MLPA), it was possible to rule out 22q11.2 microdeletion, *CHD7* gene molecular study detected the pathogenic variant c.1926_1927delGA (p.lys644Glufs*31) confirming CHARGE syndrome.

Also during the course of the condition, the patient presented multiple episodes of apnea and periodic breathing, and the fiberoptic bronchoscopy showed severe laryngomalacia with omega-shaped epiglottis and collapsible arytenoid cartilage that required tracheostomy, and a Nissen fundoplication and gastrostomy were performed due to severe swallowing dysfunction. She was discharged on the fifth month of life with outpatient follow-up.

Discussion

The CHARGE syndrome presents high variability in its clinical expression with a combination of multiple inconstant and nonspecific associated abnormalities. Nonetheless, the suspected diagnosis is clinical and the scores of the different diagnostic criteria of Blake, Verloes, and Hale^{4,5,12} have demonstrated their strength when contrasted with patients with pathogenic variants in *CHD7* (see Table 1).

The prenatal diagnosis is infrequent y probably these cases represent the most severe form of the clinical spectrum. Among the prenatal findings, 25% of cases present polyhydramnios, therefore, Legendre¹³ in these cases suggests looking for major criteria signs of CHARGE syndrome, both in directed ultrasound and in fetal MRI. In our case, we believe that the clinical spectrum of our patient was serious. Due to the nuchal edema, polyhydramnios, and hyperechogenic heart tissues, a prenatal chromosome study was suggested but not performed.

The association of cardiac, craniofacial, and airway abnormalities and cranial nerves alterations has high morbidity and mortality in the neonatal period. Our

patient required prostaglandins for stabilization and later correction of her congenital heart disease and a tracheostomy/gastrostomy to prevent reflux and aspiration and optimize nutrition.

The most frequent cranial nerve abnormalities are in the facial nerve (peripheral facial palsy), cochlear nerve (deafness), olfactory tracts, and bulbar dysfunctions (IX-XII) which are rarely diagnosed. These findings during clinical examination should be complemented with swallowing studies, laryngoscopy, esophageal pH tests and polysomnography.

Our patient met major criteria for the CHARGE syndrome diagnosis, described by the three authors mentioned above but along with these criteria a neonatal phenotype of the 22q11.2 deletion (Di George) was overlapped with congenital heart disease, hypocalcemia, and immunodeficiency which is infrequently described in CHARGE syndrome and is not part of major or minor criteria (table 1). The congenital heart disease of our patient corresponded to coarctation of the aorta which is described in 22q11.2 deletion but is infrequent in the CHARGE syndrome. Cardiac malformations in this syndrome represent 75-85% of cases but they are not a major criterion, and the Tetralogy of Fallot is the most frequently described.

The prevalence of immunological abnormalities in patients with CHARGE syndrome is little described in the literature, and of these, most have no molecular confirmation^{14,15}. This alteration was considered as a risk factor for serious and recurrent infections in our patient. Due to the severity of this associated anomaly, early immunological evaluation is suggested in patients with CHARGE syndrome to optimize therapeutic and preventive management.

The clinical characteristics overlapping between the 22q11.2 deletion and the CHARGE syndrome could be explained by the interaction between the *TBX1* gene (one of the candidate genes for anomalies associated with 22q11.2 deletion) and the *CHD7* gene, and that they are required in the pharyngeal ectoderm for the pharyngeal arches, thymus, and semicircular canals development¹⁶.

Another differential diagnoses of CHARGE syndrome are the Kabuki syndrome, renal-coloboma syndrome, Cat-eye syndrome, Joubert syndrome, Branchiootorenal (BOR) syndrome, retinoic acid embryopathy, and VACTERL association^{10,11,17,18}.

Given the multiple systems potentially affected in the CHARGE syndrome and not described in the clinical diagnostic criteria of Verloes, Blake, and Hale, it is necessary to specifically search for them to prevent serious complications. Consider the support of multiple specialists because major criteria such as coloboma, usually retrochoroidal, middle and inner ear disorders,

and choanal atresia require ophthalmologists and radiologists for detection. Multidisciplinary teams are also required that bring together geneticists, otolaryngologists, maxillofacial surgeons, neurologists, neuro-rehabilitation (developmental delay, anomalies of I, II, VII, VIII, IX and X cranial nerves), cardiologists (conotruncal septal anomalies), endocrinologists (growth retardation, hypocalcemia, hypoparathyroidism), immunologists, and urologists¹⁹⁻²².

Conclusion

Despite the complexity of the diagnosis and pathogenesis of CHARGE syndrome since the CHD7 gene alterations affect a large number of development pathways, a clinical diagnosis is possible considering the criteria of Blake, Verloes, or Hales. However, when there is a wide phenotypic spectrum and it shares several symptoms with other syndromes as our case, confirmation with molecular diagnosis is necessary. With the accurate diagnosis, it is possible to know the natural history of the disease, to follow-up according to international recommendations, and perform genetic counseling to the patient and his/her family.

Ethical Responsibilities

Human Beings and animals protection: Disclosure the authors state that the procedures were followed according to the Declaration of Helsinki and the World Medical Association regarding human experimentation developed for the medical community.

Data confidentiality: The authors state that they have followed the protocols of their Center and Local regulations on the publication of patient data.

Rights to privacy and informed consent: The authors have obtained the informed consent of the parents (tutors) of the patients and/or subjects referred to in the article. This document is in the possession of the correspondence author.

Conflicts of Interest

Authors declare no conflict of interest regarding the present study.

Financial Disclosure

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