TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2018) 48: 911-915
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doi:10.3906/sag-1611-107

Phenotypic spectrum of CHARGE syndrome based on clinical characteristics

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Received: 25.11.2016 • **Accepted/Published Online:** 19.08.2018 • **Final Version:** 31.10.2018

Background/aim: CHARGE syndrome is a rare autosomal dominant disease with multiple congenital anomalies and cognitive impairment, which is caused by mutations in the *CHD7* gene. This study aimed to disclose the mild end of the phenotypic spectrum of CHARGE syndrome, which has a highly variable expressivity.

Materials and methods: Twenty-one patients who had at least one of the major symptoms of CHARGE syndrome (coloboma, choanal atresia, characteristic ear anomalies, semicircular canal hypoplasia, and cranial nerve anomalies) were included in the study. All patients were tested for karyotype analysis and *CHD7* gene mutation/deletion.

Results: In the study population, 6 different mutations were detected in 5 patients, and 2 different polymorphisms were detected in the CHD7 gene in 3 patients. MLPA analysis of all coding exons of the *CHD7* gene revealed no pathogenic deletion/duplication.

Conclusion: CHARGE syndrome should be considered as a differential diagnosis to detect the mild end of the spectrum, even if the patient does not fit the criteria.

Key words: Choanal atresia, coloboma, *CHD7* gene, semicircular canal hypoplasia

1. Introduction

CHARGE syndrome (Hall–Hittner syndrome; OMIM #214800) is a rare (incidence ranges between 1/8500 and 1/12,000) autosomal dominant disorder, which is characterized by ocular coloboma, heart defects, atresia of the choanae, retardation of growth, genital hypoplasia, and ear malformations. In addition, involvement of multiple systems is reported. Considering that these symptoms point to various other syndromes, the Blake and Verloes criteria were developed to facilitate differential diagnosis (1–4). Although a great number of patients are initially considered to have typical CHARGE syndrome according to these criteria, some of them receive a different diagnosis later during follow-up (2).

Chromodomain helicase DNA binding protein 7 (*CHD7*) gene mutations are found in at least 48% of patients with CHARGE syndrome. This rate rises to 90% in patients who are diagnosed with a typical presentation of CHARGE syndrome according to Blake and/or Verloes criteria (3). In addition, many patients with mutations of this gene exhibit a wide range of variable expressivity. For instance, *CHD7* gene mutations have been detected in patients who have only external ear and/or cardiac defects (5). The mild end of the phenotypic spectrum of *CHD7* mutations needs more clarification. In this case, if only patients complying with Blake and/or Verloes criteria are evaluated, a considerable portion of the patients (approximately 20%) will be underestimated (3).

In light of these findings, we evaluated all patients who had at least one major criterion and were suspected to have CHARGE syndrome during an initial examination. We discuss the clinical symptoms of all patients, with and without mutations, along with other syndromes considered in differential diagnosis.

2. Materials and methods

2.1. Patients

A total of 21 patients with preliminary diagnosis of CHARGE syndrome were included in the study, who were examined at the Department of Pediatric Genetics and Department of Medical Genetics between June 2014 and July 2015. They had at least one of the major symptoms (coloboma, choanal atresia, characteristic ear anomalies, semicircular canal hypoplasia, and cranial nerve anomalies). Medical

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history, physical examination findings and anthropometric measurements, and preliminary diagnoses that were considered during initial examination were also recorded.

All patients were screened for possible anomalies associated with CHARGE syndrome. All patients were tested for *CHD7* gene mutations/deletions. The parents of the patients were also screened for the *CHD7* gene in cases that had mutation/polymorphism of the gene.

The control group involved healthy age-matched children without any dysmorphic findings. Informed consent was obtained from all families in the study and control groups.

2.2. Methods

2.2.1. Karyotype analysis

Chromosome preparations were obtained from lymphocyte cultures and G banding was performed to identify individual chromosomes. Karyotyping was done according to guidelines of the International System for Human Cytogenetic Nomenclature.

2.2.2. Sanger sequencing and MLPA analysis

Genomic DNA from the peripheral blood lymphocytes of all individuals was extracted with the QIAamp DNA Blood Mini Kit (QIAGEN GMBH, Hilden, Germany) using standard procedures. All coding exons and exon-intron boundaries of the *CHD7* gene were amplified by polymerase chain reaction (PCR) using HelixAmp Ready-2X-Multiplex version 2.0 PCR mix (NanoHelix). The sequences were evaluated using the CLC Genomics Workbench 3 sequencing program (QIAGEN). The "Ensembl.org" database (GRCh38.p3, GCA_000001405.18) with ENST00000423902 transcript ID of the *CHD7* gene was used for comparing the individual's sequence and the reference sequence. All variations were checked from mutation and SNP databases (Human Genome Mutation Database - HGMD, National Center for Biotechnology Information - NCBI/SNP, ensembl.org, chd7.org). Each variation was confirmed by bidirectional sequencing. Variation descriptions were done according to the nomenclature recommended by the Human Genomic Variation Society. Novel variations were screened in the control group. The control group consisted of 106 individuals who were admitted to our hospital for routine outpatient evaluation. None of them had a history of chronic systemic disease or genetic disorders. Furthermore, in silico programs, such as SIFT, PolyPhen 2, Mutation Taster, and KD4V, were used to describe the pathogenicity of novel variations in coding exons and exon-intron boundaries.

Multiplex ligation-dependent probe amplification (MLPA) analyses were performed to detect large deletions and duplications using the P201 SALSA MLPA Kit (MRC-Holland, the Netherlands). The PCR products were analyzed by ABI 3130 capillary electrophoresis system and Coffalyser software. Differences of the peak areas between the individual's sample and control samples were evaluated.

3. Results

Twenty-one patients were included in the study. Coloboma was the major presenting symptom in 11 patients, whereas choanal atresia was recorded in 13 patients, hypoplastic semicircular canals in 3 patients, and cranial nerve dysfunction in 8 patients.

In the study group, 6 different mutations were detected in 5 patients (Figures 1a–1e), and 2 different polymorphisms were detected in the *CHD7* gene in 3 patients. Three of 6 different mutations were novel. The patient who had coloboma, choanal atresia, and sensorineural deafness had two novel mutations in adjacent nucleotides in exon 2, c.1281 T>G and c.1282 C>G, which were affecting adjacent codons and resulting in two amino acid changes: (p.Y427X) and (p.P428A). The other novel frameshift mutation, c.4103_4104insGC (p.G3169Qfs*4), was detected in a patient who had choanal atresia, bilateral semicircular hypoplasia, and mixed type hearing loss. These novel mutations were not found in any parents or in 106 ethnically matched control individuals. MLPA analysis of all coding exons of the *CHD7* gene revealed no pathogenic deletion/duplication in the study group. Karyotype analysis of the patients was also normal.

One of the patients with a *CHD7* mutation was diagnosed with typical CHARGE syndrome according to the Blake criteria. On the other hand, 3 patients had symptoms consistent with typical CHARGE syndrome and 2 patients had symptoms consistent with atypical CHARGE syndrome according to the Verloes criteria. The clinical findings, mutation profiles, and potential differential diagnoses are summarized in the Table.

CHD7 mutations were not detected in 16 patients; 4 (25%) of these patients had findings consistent with typical CHARGE syndrome according to the Blake criteria. On the other hand, according to the Verloes criteria, only 3 (18%) of these patients had typical CHARGE findings. Accordingly, VACTERL (5/21) and VCFS (8/21) were the most likely differential diagnoses in cases with no mutation. Analysis of the 22q11 region did not show deletion in any of the patients. Two of the patients received the diagnosis of Ritscher–Schinzel syndrome and acromelic frontonasal dysostosis later during follow-up.

4. Discussion

Mutations in the *CHD7* gene, encoding chromodomain helicase DNA-binding protein, have been identified in 48%–90% of affected individuals (2). In the present study,

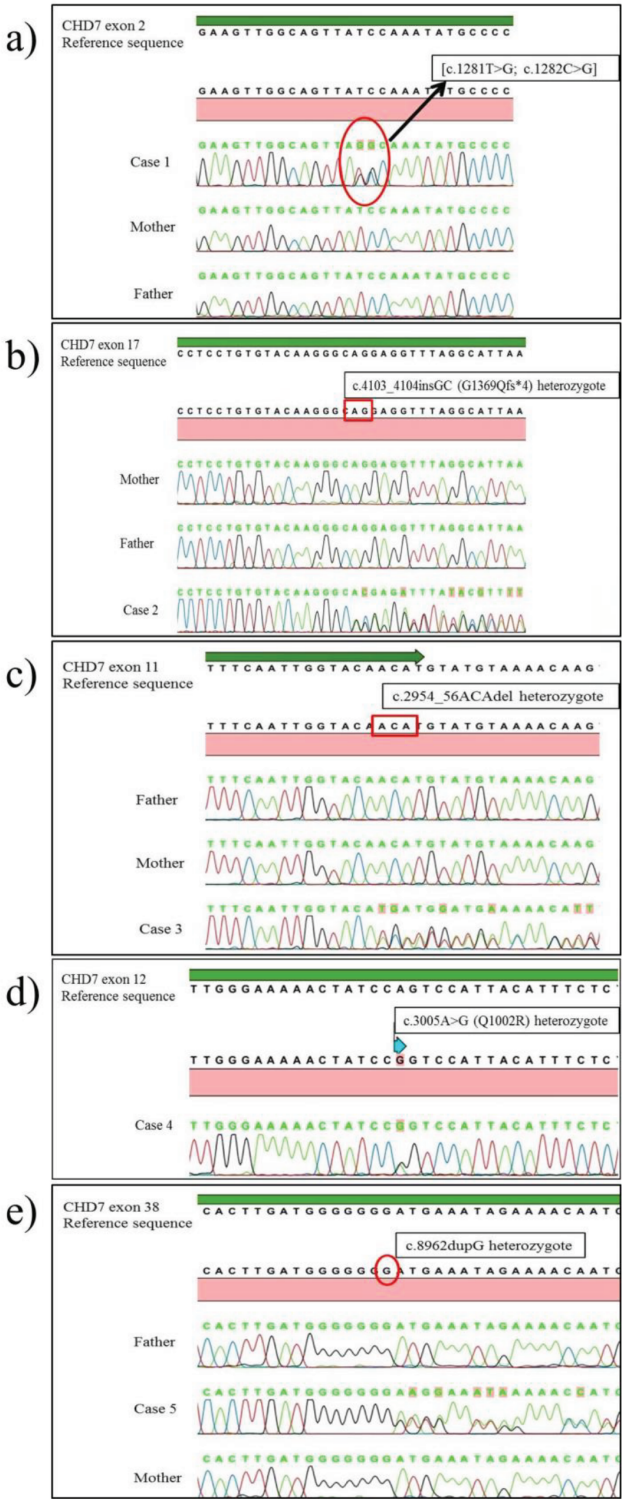

Figure 1. Sequence analysis of mutation-positive cases: patient 1 (a), patient 2 (b), patient 3 (c), patient 4 (d), patient 5 (e).

this rate was approximately 24%. It is difficult to generalize the findings due to the small sample size, which consisted of patients with a single major symptom. In addition, our results show that performing a mutation screening only in patients who comply with criteria would result in underestimation of approximately 20% of the patients if evaluated by the Blake criteria. However, only 3 of 5 patients with mutations had typical CHARGE syndrome features according to the Verloes criteria, whereas only 1 of 5 patients had typical CHARGE syndrome characteristics according to the Blake criteria.

Coloboma is a major symptom of CHARGE syndrome. Unilateral or bilateral coloboma with/without microphthalmia have been reported in 65%–75% of individuals with CHARGE syndrome (4). In our cohort the incidence was 58% (11/19) among all patients and 40% (2/5) in cases with mutation. Another finding, choanal atresia or stenosis, has been reported in 50%–60% of CHARGE syndrome cases (2). As a major abnormality choanal atresia has been noted in 72% (13/18) of our patients. When cases with mutations are taken into consideration, this abnormality has been noted in 80% (4/5) of our patients.

Ear anomalies have been reported in 80%–100% of patients with CHARGE syndrome. Most include external ear anomalies (lop or cup-shaped, low set with decreased vertical height) (2). In the current study, 10 of the mutation-negative cases and 4 of 5 mutation-positive cases had external ear malformations. Furthermore, mixed, conductive, or sensory causes of hearing loss have been reported in 86%–89% of patients with CHARGE syndrome. Audiogram/ABR should be included in routine examinations (1,4,6). Among our patients who had deafness, sensorineural and mixed type hearing deficiencies were detected in 5 and 6 patients, respectively. Of cases with mutations, the 1st and 3rd cases had sensorineural and mixed type hearing deficiency, respectively.

Semicircular canal defects, which are accepted as major signs, may be considered as inner ear abnormalities, and they are seen in as many as 95% of patients with CHARGE syndrome (7). Computed tomography (CT) examination is required to confirm semicircular canal hypoplasia/aplasia, cochlear hypoplasia, and Mondini defect (decreased number turns of cochlea). On the other hand, most centers, including ours, do not use CT as part of the initial screening, unless other indications are present. Some patients require cochlear implants. In such cases, MRI examination is recommended to rule out aberrant facial nerve (8–11). Only one of our cases with *CHD7* mutation had semicircular canal hypoplasia. This malformation was not detected in mutation-negative cases, but abnormalities of semicircular canals were detected in two patients.

Involvements of cranial nerves (I, II, V, especially VII and VIII, and IX, X, and XI) are commonly observed in CHARGE syndrome, which are usually asymmetric (1,2). In our patients with *CHD7* mutations, one had facial

Table. Clinical findings and differential diagnosis options of mutation-positive cases.

Patient no.	Major signs*	Minor signs**	Differential diagnosis of the patients	Mutations
1	Coloboma (optic disc, retina, and choroid) Choanal atresia Posteriorly rotated, cup-shaped ears Sensorineural deafness	Square face Microphthalmia Micrognathia Cardiovascular malformation	CHARGE syndrome Fryns–microphthalmia syndrome Cat-eye syndrome	c.1281 T>G (p.Y427X) c.1282 C>G (p.P428A)
2	Choanal atresia Small-curled low-set ears Bilateral semicircular hypoplasia Hearing loss (mixed type)	Delayed psychomotor development Mild hypotonia Epicanthus Bilateral posterior embryotoxon Flat nasal bridge Cardiovascular malformation (aberrant supraclavicular artery)	CHARGE syndrome Apert syndrome Velocardiofacial syndrome (VCFS)	c.4103_4104insGC (p.G3169Qfs*4)
3	Choanal atresia Cup-shaped malformed ears Facial paralysis Hearing loss (mixed type)	Triangular face Low hairline and bilateral simian crease Cardiovascular malformation	CHARGE syndrome Velocardiofacial syndrome (VCFS) Floating harbor S Moebius syndrome Teratogen exposure (methimazole)	c.2954_56del (ACAdel)
4	Coloboma Choanal atresia	Retrognathia Depressed nasal bridge Cleft palate Cardiovascular malformation	CHARGE syndrome Velocardiofacial syndrome (VCFS)	c.3005A>G (Q1002R)
5	Low-set, cup-shaped ears Optic disc hypoplasia Hearing loss (mixed type)	Mild mental retardation Microphthalmia Microcornea Retinal detachment Cardiovascular malformation Renal hypoplasia Hypogonadotropic hypogonadism	Warburg Micro syndrome CHARGE syndrome	c.8962dupG (D2988fs)

paralysis and 4 had sensorineural or mixed type hearing loss. Absence or anomalies of cranial nerve I causes anosmia. Pathologies of the eighth nerve appear as neural or combined deafness; involvement of nerves IX, X, and XI appear as swallowing/feeding difficulties (80%) (2).

Besides CHARGE syndrome, 18 different clinical cases associated with *CHD7* mutations are described in the Human Gene Mutation Database. These include isolated temporal bone malformation, autism, hypogonadotropic hypogonadism, Kallmann syndrome, and isolated mental retardation (http://www.hgmd.cf.ac.uk/ac/index.php). Furthermore, the identification of novel *CHD7* mutations with various phenotypes in CHARGE patients will improve the clinical practice and genotype/phenotype correlations.

In this study *CHD7* gene analysis was performed for 21 patients, which led to the identification of six *CHD7* alterations. These included 1 deletion, 3 point mutations, 1 duplication, 1 insertion, and a splice site mutation in 5 patients. Double mutation was present in one patient. The mild end of the phenotypic spectrum of *CHD7* mutations needed to be clearly defined. Therefore, all patients with suspicion of CHARGE syndrome, even if only one major symptom was present, were evaluated.

In conclusion, the analysis of *CHD7* gene mutation/deletion in our patients with at least one major criterion is not sufficient to disclose the mild end of the spectrum of CHARGE syndrome. On the other hand, CHARGE syndrome should be considered as a differential diagnosis in the case of clinical suspicion even if the patient does not fit the Blake criteria. This is necessary in order to not miss the mild end of the spectrum.

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