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ORIGINAL ARTICLE

THREE NOVEL MUTATIONS OF *CHD7* GENE IN TWO TURKISH PATIENTS WITH CHARGE SYNDROME; A DOUBLE POINT MUTATION AND AN INSERTION

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ABSTRACT

The CHARGE (coloboma, heart defects, atresia, retardation, genital, ear) syndrome is a genetic disease characterized by ocular coloboma, choanal atresia or stenosis and semicircular canal abnormalities. Most of the patients clinically diagnosed with CHARGE syndrome have mutations in chromodomain helicase DNA-binding protein 7 (*CHD7*) gene. The *CHD7* gene is located on chromosome 8q12.1, and up to now, there are more than 500 pathogenic mutations identified in the literature. We report two patients diagnosed with CHARGE syndrome with two novel mutations in the *CHD7* gene: the first patient has double consecutive novel mutations in three adjacent codons, and the other has a novel insertion.

Keywords: CHARGE syndrome; Coloboma; *CHD7* gene; Choanal atresia; Double tandem base substitution.

INTRODUCTION

Coloboma, cardiac defects, choanal atresia/stenosis, retarded growth and development, genital hypoplasia and ear abnormalities/deafness are seen in CHARGE (Hall-Hittner syndrome) syndrome. In addition to these cardinal findings, dysmorphic facial features such as low set and dysmorphic ears, retrognathia, hypertelorism, microcephaly, facial asymmetry, cleft lip/palate, genital hypoplasia, limb abnormalities, hypogonadotropic hypogonadism and behavioral disorders can be seen [1-4]. Although this rare syndrome has an autosomal dominant pattern of inheritance, usually (97.0%) occurs as the result of a *de novo* mutation [5,6]. Mutations in the *CHD7* gene are responsible for 65.0-70.0% cases of CHARGE syndrome [7-9]. When typical cases who fully meet the formal diagnostic criteria, the incidence rises to 90.0-95.0% [6]. The *CHD7* gene is a member of chromodomain helicase DNA-binding (CHD) protein family that regulates transcription by chromatin remodeling [10]. It is suggested that CHD7 has a role in adenosine triphosphate (ATP)-dependent chromatin remodeling in embryonic stem cells [3]. We present two patients with novel mutations in the *CHD7* gene, who were clinically diagnosed as typical CHARGE syndrome according to Verloes criteria [11].

Case 1. A 2-month-old girl presented with choanal atresia, bilateral coloboma and hearing loss. She was the first child of non consanguineous parents. The pregnancy was medically followed and prenatal history was uneventful. She was born at term by cesarean delivery and her birth weight was 3190 g. During the first day of life, the patient presented to hospital with respiratory problems and left choana atresia was found.

On physical examination at 2 months of age, the patient weighted 4550 g (5 centile), height was 54 cm (29 centile) and head circumference was 37 cm (24 centile). She had characteristic facial findings: square face, unilateral microphthalmia, micrognathia

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and dysmorphic ears (Figure 1A). A careful ophtalmic examination assessment was performed. There were bilateral coloboma involved the optic disc, retina and choroid. Auditory brainstem response (ABR) audiometry showed bilateral decreased hearing threshold.

Abdominal and transfontanelle ultrasonographic examinations were unremarkable. Cranial magnetic resonance imaging (MRI) revealed bilateral frontal hypoplasia (interpreted as a normal variant) with hypoplastic right bulbus oculi. The patient also underwent echocardiography, which revealed an atrial septal defect (ASD) and moderate pulmonary hypertension. Cytogenetic analysis revealed a normal female karyotype. In sequence analysis for the *CHD7* gene, we identified two novel mutations in adjacent nucleotides in exon 2: c.1281T>G and c.1282C>G, affecting adjacent codons and resulting in two amino acid changes: tyrosine to stop codon (p.Y427*) and proline to alanine (p.P428A) (Figure 1B). None of these mutations has been described previously. Since neither the mother nor the father was a carrier of either mutation, we concluded that both mutations occurred *de novo* on one allele. Complex mutations of adjacent codons occur very rarely and to our knowledge, the adjacent CHD7 mutations occurring on the same allele have not been previously reported.

Case 2. A 4-month-old girl presented with right-sided choanal atresia, dysmorphic facial features and operated aberrant supraclavicular artery. She was the second child of non consanguineous parents; at birth the mother was 31 and the father was 32 years old. Family history was negative in regard to malformations and development. Right-sided choanal atresia and dysmorphic facial features were recognized after an uncomplicated 42-week pregnancy. Her birth weight was 3210 g. At birth she presented with respiratory problems due to right-sided choanal atresia. Cardiological examination demonstrated aberrant supraclavicular artery that was surgically corrected at the age of 2 months.

Physical examination at 4 months of age showed mild hypotonia, epicanthus, flat nasal bridge, small-curled low-set ears, pili asymmetry and bilateral clinodactyly of the second fingers of feet (Figure 2A). Her head circumference was 37 cm(<3 centile), height was 59 cm (90-95 centile), weight was 3900 g (3-25 centile). Ophtalmologic examination revealed bilateral posterior embryotoxon. A brain MRI was normal. Right-sided choanal atresia was confirmed by paranasal sinus computed tomography (CT), and bilateral semi-circular hypoplasia was noted. Audiological examination showed hearing impairment. Ultrasonographic examination was performed to rule out the suspected hip dislocation. After determining pili asymmetry hip dysplasia, ultrasongraphic examination revealed normal results. Her head circumference was 39.5 cm (<3 centile), height was 61 cm (3-10 centile), weight was 5310 g (<3 centile). Her

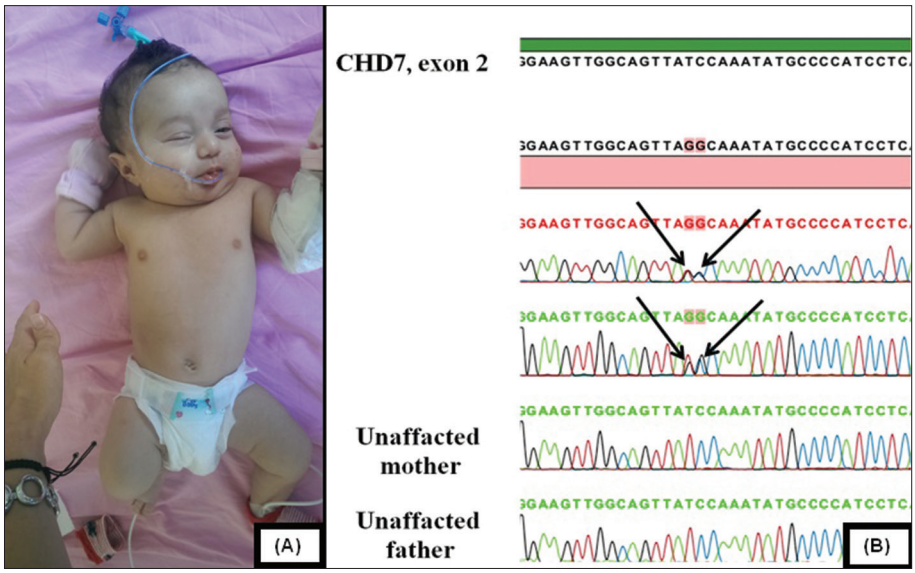

Figure 1. A) Photograph of the patient at 2 months of age. **B)** Two mutations in neighboring nucleotides in exon 2, c.1281T>G and c.1282C>G, affecting adjacent codons.

psychomotor development was delayed. She is now 3 years old and still unable to walk and talk.

A clinical suspicion of CHARGE syndrome was raised due to her dysmorphic features. In order to rule out Di George (DG) syndrome, fluorescent *in situ* hybridization (FISH) studies for 22q11 (for DG) and 8q12.1 (for CHARGE) were planned with routine high resolution cytogenetic analysis. All the results were normal. We discovered a novel *de novo* c.4103_4104insGC (p.G3169Qfs*4) mutation in the *CHD7* gene when we performed sequence analysis (Figure 2B). Molecular analysis of her parents showed normal results. Genetic counseling for future pregnancies was provided to the family.

DISCUSSION

The CHARGE syndrome occurs approximately 1 in 8,500 to 10,000 live births and shows characteristic clinical manifestations [12]. The diagnosis is primarily based on combinations of clinical findings that were grouped as major and minor characteristics. Three major or two major and two minor features refers to the diagnosis of typical CHARGE syndrome. Two major and one minor features refers to partial/incomplete CHARGE syndrome. The major characteristics of CHARGE syndrome are more specific to this disorder and relatively rare in other syndromes (Table 1) [11,13].

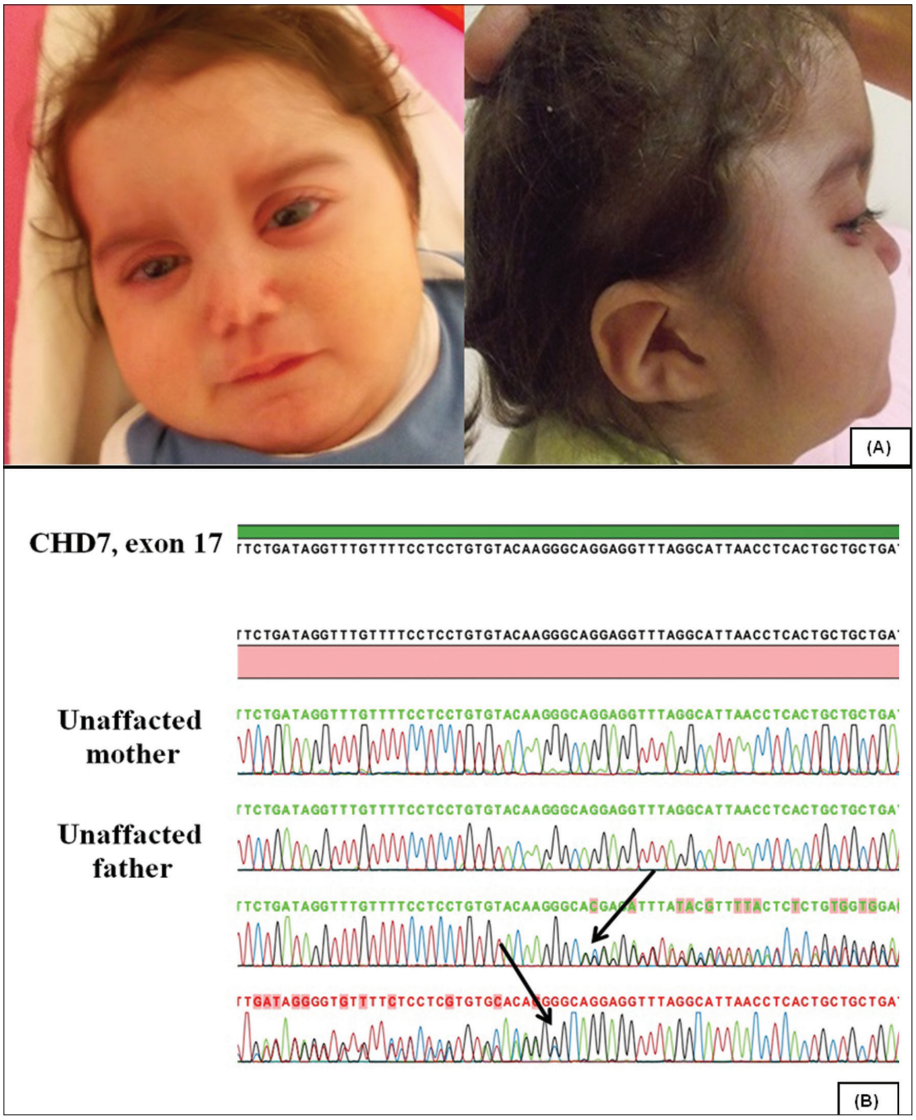

Figure 2. A) The patient at the age of 17 months and 2 years-9 months. **B)** The identification of a novel frameshift mutation. Chromotograms of *CHD7* exon 17 showing the c.4103_4104insGC mutation.

Table 1. Clinical characteristics of our patients as they scored to Verloes criteria. Both cases have two major and more than two minor malformations.

Major Signs (the three Cs)	**Case 1**	**Case 2**
Coloboma (iris or choroid, with or without microphthalmia)	Bilateral retinal/choroid coloboma	–
Atresia of choanae	Unilateral microphthalmia; left sided chonanal atresia	Right sided choanal atresia
Hypoplastic semicircular canals	Not tested	Bilateral semicircular canal hypoplasia
Minor Signs	**Case 1**	**Case 2**
Rhombencephalic dysfunction (brainstem dysfunctions, cranial nerve VII to XII palsies and neursensory deafness)	Deafness	Deafness
Hypothalmo-hypophyseal dysfunction (including growth hormone and gonadotrophin deficiencies)	Not tested; dysmorphic ears	Not tested; dysmorphic ears
Abnormal middle or external ear	Atrial septal defect	Aberrant suplaclavicular artery
Malformations of mediastinal organs (heart, esophagus)	Not tested (age related)	–
Mental retardation	Developmental retardation (hypotonia)	+
Mutations	**c.1281T>G; p.Y427* and c.1282C>G; p.428A**	**c.4103_4104insGC; p.G3169Qfs*4**

Coloboma and ophthalmic findings are found in 75.0-90.0% of CHARGE patients [6]. Coloboma may be present in bilateral choroid, retina and the optic nerve. Retinal coloboma is present more often in CHARGE patients. In some cases, retinal coloboma can occur with microphtalmia and this occurrence predicts poor prognosis [14,15]. Typical colobama usually causes field defects in CHARGE syndrome [16]. Choanal atresia or stenosis is found in approximately half of the patients and noticed due to respiratory difficulty during the neonatal period [12,17]. Laryngomalacia, tracheomalacia and subglottic stenosis can be seen [6]. Cleft palate/lip can cooccur occur with coanal atresia [18]. Cardiac malformations are usually present in patients with CHARGE syndrome, and common features are tetralogy of Fallot, ASD and patent ductus arteriosus [16]. Hypoplastic left/right heart and double outlet right ventricle are rarely present in CHARGE syndrome. Almost all patients with CHARGE syndrome have ear malformations, especially semi-circular canal abnormalities and sensorineural hearing loss [15]. Due to the pituitary hormone deficiencies, growth retardation appears during childhood [16]. In addition to growth failure, hypothalamo-hypophyseal dysfunction leads to hypogonadotropic hypogonadism, genital hypoplasia and delayed puberty in patients with CHARGE syndrome [6].

The *CHD7* gene consists of 38 exons and only the first exon is non coding [8]. This gene is a member of ATP-dependent chromatin remodeling protein family [19]. The function of the CHD family is not yet clear, but it is known that ATP-dependent chromatin remodeling has a key role in embryonic development [19,20]. In the embryonic period, the CHD7 protein activates several gene expressions that required embryonic stem cell differentiation into the neural progenitors. In CHARGE patients, disruption of CHD7 function during development inhibits gene expression and impairs differentiation of embryonic stem cells. These functional distinctions might partly explain the symptoms of the syndrome [21].

More than 500 pathogenic alterations have been described in the *CHD7* gene. Mutational spectrum is reported as follows: nonsense mutations 46.0%, frameshift mutations 24.0%, missense mutations 15.0%, splice site mutations 10.0%, intronic mutations approximately 3.0%, chromosomal rearrangements and deletions, including *CHD7* gene locus, approximately 1.0% [8]. The CHD7 protein contains an ATPase domain [helicase C-terminal (HELICc)] and DEXDc domains, a pair of breast tumor kinase (BRK) domains and a SANT (SWI3, ADA2, N-CoR and TFIIIB) domain [21]. Previously, five different pathogenic mutations in the HELICc domain were published [22]. In our article, patient

2 has a novel insertion mutation in exon 17 that seems to disrupt the HELICc domain of the CHD7 protein. The phenotype-genotype correlation is not yet clearly known.

Mechanisms of the double nucleotide substitution are not completely understood. One of these is tandem base substitution (TBS) that is described as the presence of at least two successive nucleotide substitutions without any insertion or deletion of bases [23]. The other mechanism may occur as concurrent mutations are considered simultaneously. The ratio of these mutations are seen in 93.9% of human inherited diseases [24]. In the first case, we detected a novel double base mutation that is described for the first time in the literature. The first mutation (c.1281 T>G; p.Y427*) leads to a truncated protein and this may cause typical CHARGE syndrome findings in this case.

The majority of patients with CHARGE syndrome have a normal karyotype; rarely can chromosomal abnormalities be seen [8]. Therefore, cytogenetic analysis must be performed to exclude chromosomal abnormalities and other syndromes overlapping CHARGE syndrome.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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