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CHD7 mutations and CHARGE syndrome in semicircular canal dysplasia

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Abstract

Objective—To determine whether patients with semicircular canal dysplasia have mutations in *CHD7*.

Background—CHARGE syndrome is a nonrandom clustering of congenital anomalies, including ocular *C*oloboma, *H*eart defects, choanal *A*tresia or stenosis, *R*etarded growth and development, *G*enital hypoplasia, and inner and outer *E*ar anomalies including deafness. Semicircular canal dysplasia has been included as a major diagnostic criterion for CHARGE syndrome. Mutations in the gene *CHD7* on chromosome 8q12.1 are a major cause of CHARGE syndrome, but the extent to which patients with semicircular canal dysplasia have *CHD7* mutations is not fully understood.

Study Design—Cross-sectional analysis of *CHD7* in 12 patients with semicircular canal dysplasia and variable clinical features of CHARGE syndrome.

Results—We identified six *CHD7* mutations, five of which occurred in patients who fulfilled Verloes' diagnostic criteria for typical CHARGE syndrome, and three which were previously unreported. Of the three remaining *CHD7* mutation positive patients, one had atypical CHARGE by diagnostic criteria. Four MRI records were available, which revealed two patients with cochlear nerve aplasia and one patient with Chiari 1 malformation.

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Conclusion—These data provide additional evidence that *CHD7* mutations are a significant cause of semicircular canal atresia in children with full or partial CHARGE syndrome.

Keywords

inner ear; chromodomain; multiple anomalies; development; deafness

Introduction

CHARGE syndrome was initially described as an association of congenital anomalies in 1979[1,2]. It is a relatively common, but often underappreciated cause of deafness, with a prevalence of 1:8,500 to 1:15,000[1–3]. CHARGE was originally defined by the presence of ocular *C*oloboma, *H*eart disease, *A*tresia choanae, *R*etarded growth, *G*enital hypoplasia, and *E*ar anomalies[4,5]. Affected individuals often have additional clinical features, including cranial nerve dysfunction, esophageal anomalies, facial clefts, feeding difficulties, arrhinencephaly, agenesis of the semicircular canals, hypothalamo-hypophyseal dysfunction, and characteristic square facies with a broad forehead and prominent nasal bridge[6–10]. Aplasia of the horizontal semicircular canal is the most specific abnormality of CHARGE syndrome[10,11]. Many other otologic abnormalities may be seen in CHARGE, including Mondini dysplasia of the cochlea, hypoplasia of cranial nerve VIII, and ossicular malformations[10,11].

Vissers and colleagues identified *CHD7* in 2004 as the major causative gene for CHARGE[12,13]. Recent studies suggest that the majority of patients with CHARGE syndrome have *CHD7* mutations[14–21]. *CHD7*, which encodes Chromodomain-helicase-DNA-binding-protein 7, is located on chromosome 8q12.1 and consists of 38 exons spanning a genomic size of 188 kb[22,23]. CHD7 is a member of a highly-conserved family of proteins that contain two N-terminal chromodomains (chromatin organization modifier domains), a SNF2-like ATPase/helicase domain, and a DNA-binding domain[22,23]. The triad of coloboma, choanal atresia, and abnormal semicircular canals (3C) is highly predictive of the presence of a CHD7 mutation[11,24].

Severe malformations of the posterior and lateral semicircular canals occur with haploinsufficiency for *CHD7*[15–17,24–26]. CHD7 is involved in the expression of patterning and pro-neural genes within the otic epithelium and ganglion, and deficiency results in decreased neurogenesis in the inner ear[27]. Mice with heterozygous loss of *Chd7* have delayed semicircular canal genesis and disrupted expression of genes required for semicircular canal formation, whereas mice with complete loss of *Chd7* have semicircular canal aplasia and vestibular organ agenesis[4]. In light of these recent findings, *CHD7* most likely has critical selector gene functions during inner ear morphogenesis[4].

Hearing loss in CHARGE syndrome may be due to middle ear, inner ear, and/or cranial nerve VIII abnormalities. Hearing loss in CHARGE is often mixed, but may be isolated conductive or sensorineural hearing loss. Improvement in hearing has been noted after cochlear bone-conduction implantation, cochlear implantation, or in the rare case in CHARGE patients, auditory brainstem implantation[6,8].

Absence or hypoplasia of the semicircular canals impairs balance, especially when combined with visual loss, and contributes to delays in motor development[10]. Vestibular anomalies in CHARGE syndrome result in a typical pattern of postural behavior. Abadie et al. reported a frequent inability to crawl on all fours without resting the head on the floor (5-point crawl), a prolonged duration of the developmental stage of standing with support, and an inability to ride a bike without stabilizers[28]. Following the first years of life, balance disturbances may be somewhat masked by visual compensation[29]. However, affected individuals often experience disequilibrium in the dark[29]. Agenesis of the semicircular canals can be readily visualized on computerized tomography or MRI[11].

The phenotypic spectrum of individuals with *CHD7* mutations and CHARGE has been examined in recent studies[12,14,15,22]. Certain isolated CHARGE features are more strongly associated with *CHD7* mutations than others. Felix et al. analyzed 184 patients with nonsyndromic cleft lifp and/or palate and found no *CHD7* mutations, suggesting that *CHD7* is not a major cause of isolated clefting[12]. Computed tomography scans of the temporal bone in CHARGE syndrome patients detect inner ear malformations 84% or more of the time[14]. In a retrospective review of 379 patients, *CHD7* mutation positive individuals had temporal bone anomalies (semicircular canal hypoplasia/aplasia, cochlear hypoplasia, and Mondini malformation) 98% (94/96) of the time vs *CHD7* mutation negative individuals having anomalies 75% (21/28) of the time (p-value 0.00004)[22]. Statistically significant differences were also demonstrated for facial nerve palsy (p-value 0.0005), retarded growth (p-value 0.007), developmental delay (p-value 0.008), and coloboma (p-value 0.044)[22]. We therefore ascertained 13 children with hearing loss and malformations of the semicircular canals for *CHD7* mutation analysis.

Materials and Methods

Subjects

13 patients seen at the University of Michigan Pediatric Otolaryngology outpatient clinic with hearing loss and semicircular canal malformations were selected for analysis. This constituted eight cases with a clinical diagnosis of CHARGE and five additional cases with a subset of CHARGE features. Parents of affected subjects were also invited to submit DNA for mutation analysis. Either a medical geneticist (DMM) or a pediatric otolaryngologist (GEG) examined most subjects, although a few subjects were evaluated at outside institutions, and a report of their exam was provided to our research team (Table 1). Our investigators noted several previously unrecognized features on careful clinical examination, including unilateral choanal atresia, temporal bone anomalies, submucous clefting, and partial facial nerve palsy. Certified audiologists assessed hearing loss using either air and bone conduction audiometry or auditory brainstem response testing. Middle and inner ear abnormalities were assessed by computed tomography of the temporal bones. Informed consent was obtained from participants and their parents. All protocols were approved by the University of Michigan Institutional Review Board.

CHD7 mutation analysis

Genomic DNA was extracted from whole blood. All 38 coding exons for *CHD7* were amplified from genomic DNA by PCR. Primer sequences are listed in table 2. Amplification conditions were 94°C for 3 min, followed by 31 cycles of annealing temperature of 54°to 58°C for 1 min, 72°C for 1 min 30°sec, 94°C for 45 sec, ending with an extension cycle of annealing temperature for 2 min, 72°C for 6 min, and 4°C for storage. Amplified exons were sequenced with dye terminator cycle sequencing. Forward and reverse sequences from exons were manually compared to published database sequences using Sequencher (Gene Codes Corporation, Ann Arbor, MI), and any variations were recorded. Full gene deletions were excluded through identification of single nucleotide polymorphisms, usually in the non-coding sequence (see Table 3). *De novo* mutations were identified by comparing the proband's DNA sequence to the parent's sequence.

Results

In 12 subjects analyzed, six different heterozygous mutations in *CHD7* were identified (Table 1). One mutation (17%) was missense and five (83%) were nonsense. This is comparable to published frequencies of missense and nonsense mutations occurring 8% and 44% of the time, respectively; other reported mutations and their respective frequencies are frameshift deletions or insertions (34%), splice site (11%), larger deletions and duplication (2%) and translocations (<1%), and small in-frame deletions (<1%)[24]. The clinical characteristics and identified mutations for each subject are listed in Table 1. The open-access *CHD7* database was queried to reveal that four mutations were novel, while three others have been previously noted by other researchers (http://www.chd7.org/)[15–17,24]. Of the ninepatients with complete clinical information, five were given a diagnosis of CHARGE by both Blake's and Verloes' criteria. Of the six subjects with mutations, parental DNA was also analyzed for mutations in *CHD7*; mutations were found to be *de novo* in all six.

Brain MRI records were available for four of the six mutation-positive patients – patients 3, 4, 5, and 6 (Table 1). Left cochlear nerve aplasia was found in patients 3 and 6 on MRI (Figures 1 and 2). Patient 5 had Chiari I malformation. The remaining patient, patient 4, had an unremarkable MRI.

Discussion

Our mutation analysis of 12 patients is consistent with earlier studies suggesting that 60–80% of clinical CHARGE individuals have *CHD7* mutations[15,17,18]. We identified one subject (case #3) with a *CHD7* mutation who did not meet Blake's criteria but has atypical CHARGE based on Verloes' clinical criteria since she has no ocular coloboma, choanal atresia, or cardiovascular defects. This patient has the same *de novo* nonsense mutation (R947X) as a previously-described patient with CHARGE features including semicircular canal dysplasia[15].

Blake and colleagues delineated the most widely-accepted clinical diagnostic criteria for CHARGE based upon four major criteria or three major and one minor criterion[7]. Major

criteria included coloboma or microphthalmia, choanal atresia, typical ear anomalies (inner, middle, or outer), and cranial nerve dysfunction; minor criteria included genital hypoplasia, developmental delay, cardiovascular malformations, growth deficiency, orofacial clefting, tracheosophageal fistula, and distinctive[7]. Several studies have reported temporal bone anomalies in CHARGE individuals, particularly dysplasia of the semicircular canals, in the majority (84 to 100%) of patients who underwent temporal bone CT scans[9,30]. In 2005, Verloes proposed updated diagnostic criteria that include semicircular canal dysplasia as a major criterion, and eliminated sex-dependent evaluation[31]. Semicircular canal dysplasia occurs frequently and consistently in CHARGE, even in mildly affected individuals[11]. Our data provides additional supportive evidence that mutations in *CHD7* are a significant cause of semicircular canal dysplasia in children.

Semicircular canal dysplasia is one of the most common findings in the temporal bones of deaf individuals examined histologically or by computed tomography[32,33]. Semicircular canal dysplasia is more commonly seen in syndromic individuals, and in histologic samples presumably because of increased comorbidity.

Combined with data from recently developed mouse models, which exhibit completely penetrant defects in the lateral semicircular canal and variably penetrant defects in the posterior canal, our data indicate that *CHD7* deficiency is commonly associated with semicircular canal dysplasia[11,20–22,26]. Mice with *CHD7* deficiency exhibit defects in vestibular sensory epithelial innervation[35]. *CHD7* regulates inner ear neurogenesis, and *Chd7* conditional knockout and null mice have reduced vestibulo-cochlear ganglion size, neuron number, and expression of patterning and pro-neural genes[4]. Mutations in *CHD7* can result in a much milder phenotype than that of classical CHARGE syndrome[29]. Taken together, these findings raise the distinct possibility that subtle abnormalities of vestibular function may be an important additional clinical criterion for CHARGE syndrome, especially in less severely affected individuals.

These findings underscore the importance of temporal bone computed tomography in the evaluation of children with features of CHARGE syndrome, especially those with hearing loss. Similarly, these findings show the importance of careful examination for comorbidity in children with hearing loss and semicircular canal dysplasia on computed tomography of the temporal bones.

Conclusion

Our data lends further support to the inclusion of vestibular dysfunction and/or semicircular canal dysplasia as a major criterion for CHARGE syndrome. History of balance disturbances and delayed gross motor development, in conjunction with close clinical examination, can uncover additional information suggestive of *CHD7* mutations. We provide additional evidence that mutations in *CHD7* are a significant cause of semicircular canal dysplasia. It seems reasonable to consider screening children with nonsyndromic hearing loss and semicircular canal dysplasia for mutations in *CHD7*.

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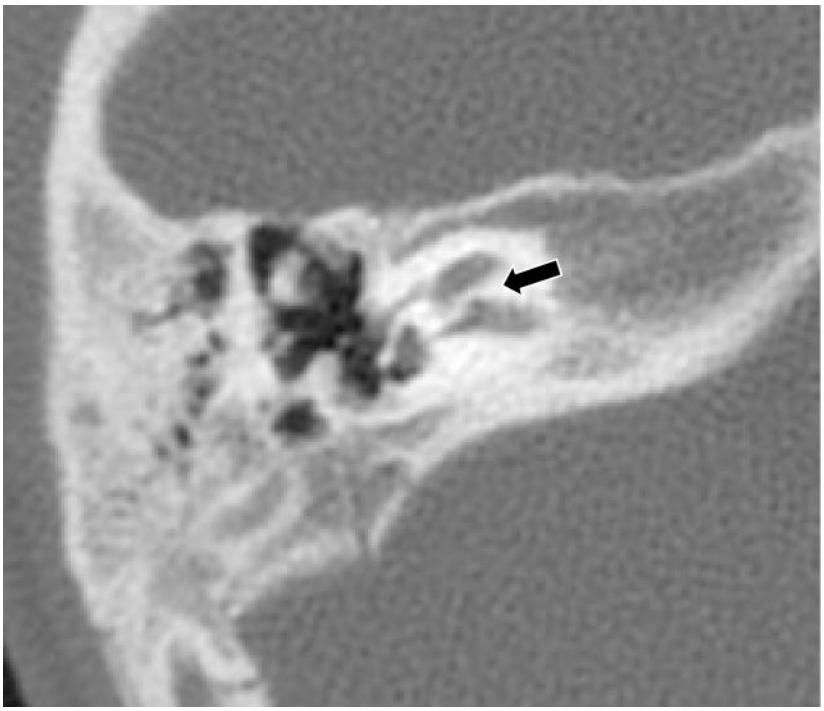

Figure 1.

Right Axial CT from patient 3 at the level of the cochlear canal shows lack of resorption of the bone that usually forms the cochlear canal (denoted by black arrow). Absence of the cochlear canal is usually indicative of aplasia of the cochlear nerve.

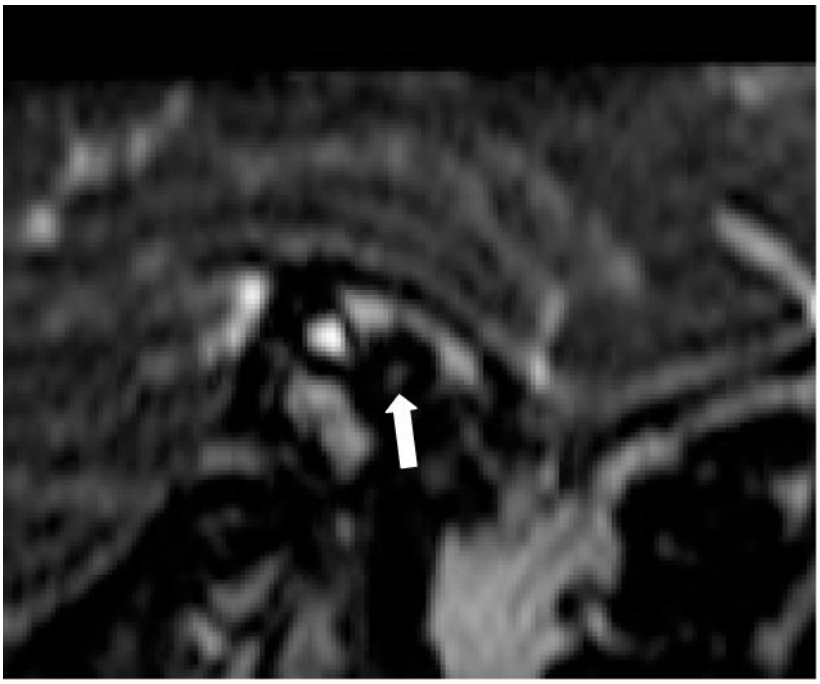

Figure 2.

Left Sagittal MRI from patient 3 through the internal auditory canal. A well-demarcated 4-nerve bundle of facial, cochlear, and vestibular nerves should be visible but cannot be discerned in the image above (denoted by white arrow). Cochlear nerve aplasia was confirmed clinically by stimulation.

Table 1

Clinical findings of subjects enrolled in the present study. Positive clinical findings are marked with a + sign. In some cases, detailed otolaryngologic or genetics examination was required to identify findings that had previously not been identified. Subject 8 had notable choanal stenosis (*) without complete choanal atresia. Bolded mutations have not previously been reported in the literature.

	Major							Minor										
Patient	**Coloboma**	**Choanal Atresia**	**Inner Ear Malformations**	**External Ear Malformations**	**Hearing Loss**	**Cranial Nerve Dysfunction**	**Genital Hypoplasia**	**Developmental Delay**	**Cardiovascular Defects**	**Growth Deficiency**	**Cleft Lip/Palate**	**TE Fistula**	**Dysphagia**	**DNA**	**Exon**	**Protein**	**Blake Criteria CHARGE status**	**Verloes Criteria CHARGE status**
1			+		+	+		+										
2	+	+	+	+	+	+					+		+	**2254A>T**	5	R752X	+	typ
3			+	+	+	+		+			+	+	+	2839C>T	11	R947X		atyp
4	+		+	+	+	+		+	+					5458C>T	26	R1820X		typ
5	+	+	+	+	+			+	+					3881T>C	16	L1294P	+	typ
6	+		+	+	+	+		+	+				+	**2764C>T**	10	Q922X		typ
7	+		+	+	+			+										typ
8	+	*	+	+	+			+		+	+	+		**1774C>T**	3	Q592X	+	typ
9	+	+	+	+	+			+	+	+							+	typ
10			+	+	+													atyp
11			+		+			+		+		+	+					atyp
12	+		+	+	+		+			+	+		+				+	typ

Table 2

TABLE OF PRIMERS FOR CHD7

CHD7x2.1FGGGACCAAATTCAGATGTACAAA; AGCTGCTGTCCACAAAGGAT

CHD7x2.2FCGGTCAGATGGGTGTCTACC; ACAGCATTGGGGTATCTTGG

CHD7x2.3FTCCAATGAATCAGTCCGTACC; CATGTTGAATTTCACACTGCAA

CHD7X2-1078F TTCCATCACCACCCCTCTAC;CTGGATTGCTTGTGGGTCTC

CHD7X3 FTTTCAGAAACATCAGCCACTAA; TCCCACCCCTCATTTCATAG

CHD7x4F GCCAATATGTATGGATTTATCAGTTG; CATCGCTATGGAGATAATGTTCC

CHD7x5bF TCACTGCAAGCTCCACCTC;TGCAGTGAACAGGGCTTTTT

CHD7x6F GTGGTAGCAAAGGGGAATGA; TGGGGTCAAATATCCCAAAG

CHD7x7F GTGAAGGTCCTTTGCTGCTC; CCCAGGCCATGATGACTAAA

CHD7x8F TGTTGCTCAGCAGCCTTAAT; ATGCAAGTTGACAGCACCAA

CHD7X9F TTTTATATTGCTGTGACCCAAAA; CACAGTCCAAGGCTCTGACC

CHD7x10FTATGTATGTATGTGGTCAAATGAATC; AGGAGGTCGCTCCTGTTTC

CHD7x11FGGAAACAGAGCGTGTGGTAAG; TTTTCAATAACTAAAGGAAAGGAACT

CHD7X12 FGCCTTTGGGTATGCATTTGT; CAGTGATCATCCAAGGCAAA

CHD7x13FTGCCAAAATAACTTGAAAACAGAA; GCATCAAATTCTGAGCAACG

CHD7x14FTCTGTTTTCATGCCTGATTCC; TTGCCATTTCATGGGCTAAT

CHD7x15FCACTGGGCTTTGAAAAATGAA; CACCATGAAATCCCCAGTCT

CHD7x16FCAGTTTGCAATGGGTTTTGA; GATGCACTTCCCCCATTCTA

CHD7x17FCCCTATTTGCTCTGAGATTAGTTC; CTGAGTGACGACGCAACATT

CHD7x18FTTTGGTGGGGAGACAGAAAC; TGCTGCATTTTTCTCAAAGAG

CHD7x19FGCAGCATTTGTTTAGTCTGCAA; CCAAAGCATAAAGAAAGCTTCA

CHD7x20FTGTCTGGCATAAGTGGAGGA; AGCCTGGTCTGCTTTCTCAC

CHD7x21FCTGGCAAAAGTGGGCTAAGA; GGGGTGTCACACAAATTCAA

CHD7x22FTCTTGCACCCTGGATTTCTT; AAGTTCCTGGTGGCTTTGTG

CHD7x23FAGCCACCAGGAACTTTTGTG; GCTCAGGTCCCTCAGTTGAC

CHD7x24FCACCATCTGTCACGCTTCAA; CCATGATGTTTTCCGGCTAC

CHD7x25FCCCACCATGCTCAGATGTTT; TGGTAGACGCCAAGAGTCCT

CHD7x26 FAGAAATCCTCCCAGGCATCT; GAGATTGCGGGAAATGACA

CHD7x27FTGCTTTTGATGTCAAACCCATA; TCCTTGAAAGCAAAGCAAGAA

CHD7x28FTGCCCACATAAGACTTGTTAAA; CCACGTGAACAATGACTGCT

CHD7x29FCCCTTTCCCACACTGTCATT; GAGCCTTTCTTTGGTGGTCA

CHD7x30 FGAAGCGGAAAGGGAAGCTATGCGGTACAGAGTTCGAGAGG

CHD7x31.1FCCCTTGAATTCTCCCCAAGT; TGACTGGAGGAGTCTGGACA

CHD7x31.2FCCCTGAGTTATCCTTCTTGGA; CTCGTGAAAAAGAGCAGGTG

CHD7x32FTGGAGCTGATTAGTATTCACA; CCCTAATCCTTTTTGGTCAGC

CHD7x33FTCTTTTGCATCTTGATGGATG; TTCTAAGCAAGGCCAGTGAAA

CHD7x34FCAGCTCTGTGCACCAGTCAT; AGCTGTCAACACGTGCAATC

CHD7x35 FCTCCTGACCTCAGGTGATCC; CGCGCATCTTCAAATAACTG

CHD7x36FTCTGACAGTTCTCTTTGGCATT; ATTCGGGCAGACAGGATTC

CHD7x37FGAAGGGGGAGGGAGTAGATT; TGATGTATTATGTCAATTCTTTTAAGC

TABLE OF PRIMERS FOR CHD7
CHD7x38.1FGTTCACCACAGAGGCTCACA; CAGATCCATTCGCAGAGTCA
CHD7x38.2FGGAGAGGAGAAAGGAAATGAGA; TGACCAAGATACCTTTTGACACA
CHD7x38.3FTTTTGACAAGTGGTAGTCCTACTGTT; CAAGACCACGAGACAATGGA
CHD7x38.4FGAGGCGCAGCAATAATAAGG; GCCAGAATGCTGTATTTGCAT