The Results of *CHD7* Analysis in Clinically Well-Characterized Patients with Kallmann Syndrome

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Context: Kallmann syndrome (KS) and CHARGE syndrome are rare heritable disorders in which anosmia and hypogonadotropic hypogonadism co-occur. KS is genetically heterogeneous, and there are at least eight genes involved in its pathogenesis, whereas CHARGE syndrome is caused by autosomal dominant mutations in only one gene, the *CHD7* gene. Two independent studies showed that *CHD7* mutations can also be found in a minority of KS patients.

Objective: We aimed to investigate whether *CHD7* mutations can give rise to isolated KS or whether additional features of CHARGE syndrome always occur.

Design: We performed *CHD7* analysis in a cohort of 36 clinically well-characterized Dutch patients with KS but without mutations in *KAL1* and with known status for the KS genes with incomplete penetrance, *FGFR1*, *PROK2*, *PROKR2*, and *FGF8*.

Results: We identified three heterozygous *CHD7* mutations. The *CHD7*-positive patients were carefully reexamined and were all found to have additional features of CHARGE syndrome.

Conclusion: The yield of *CHD7* analysis in patients with isolated KS seems very low but increases when additional CHARGE features are present. Therefore, we recommend performing *CHD7* analysis in KS patients who have at least two additional CHARGE features or semicircular canal anomalies. Identifying a *CHD7* mutation has important clinical implications for the surveillance and genetic counseling of patients. **(*J Clin Endocrinol Metab* 97: E858–E862, 2012)**

Kallmann syndrome (KS) is characterized by the combination of anosmia and hypogonadotropic hypogonadism (HH) (1). Anosmia, or the inability to smell, is the result of olfactory bulb defects (2), whereas HH presents as absent or impaired pubertal maturation and is caused by GnRH deficiency (3). KS is clinically and genetically very heterogeneous (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) (3–10). At present, mutations in eight genes explain approximately 25–35% of KS cases. Heterozygous loss-of-function mutations in the *CHD7* gene were identified in patients with normosmic idiopathic HH (nIHH), KS, and CHARGE syndrome (11, 12).

CHARGE syndrome is a highly variable disorder in which congenital anomalies, multisensory impairment, and variable mental retardation can occur (OMIM 214800). CHARGE is an acronym for ocular coloboma, heart defects, choanal atresia, retardation of growth and/or development, genital hypoplasia and ear anomalies combined with deafness (13). HH and anosmia are present

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Abbreviations: CT, Computed tomography; HH, hypogonadotropic hypogonadism; HRT, hormone replacement therapy; KS, Kallmann syndrome; nIHH, normosmic idiopathic HH; UPSIT, University of Pennsylvania Smell Identification Test.

in the majority of patients with CHARGE syndrome (14). Recently, we showed that HH and anosmia co-occur in CHARGE syndrome (4), which means that KS is part of the phenotypic spectrum of CHARGE syndrome. *CHD7* mutations are found in more than 90% of patients with typical CHARGE syndrome (5).

Conversely, *CHD7* mutations are not a major cause of KS, because only 3–5% of patients with nIHH/KS were found to have a *CHD7* mutation in two independent studies (11, 12). The first study identified seven *CHD7* mutations in a cohort of 197 patients with nIHH/KS (seven of 197 = 3.6%) (12). Four of the *CHD7*-positive patients had nIHH, whereas three patients had KS. In the *CHD7*-positive patients, no other features of CHARGE syndrome were present, except for cleft lip/palate and hearing loss, which can occur in both CHARGE syndrome and KS. The authors concluded that *CHD7* mutations can give rise to isolated nIHH and KS. The second study, performed by our group, found three *CHD7* mutations in a cohort of 56 Japanese/North American nIHH/KS patients in whom mutations in *KAL1*, *FGFR1*, *PROK2*, and *PROKR2* had been excluded (three of 56 = 5.4%) (11). The three *CHD7*-positive patients were all diagnosed with KS but on extensive clinical reevaluation were found to have several other features of CHARGE syndrome.

Because of the conflicting data from these two studies, we decided to investigate whether *CHD7* mutations can give rise to isolated KS in an independent cohort. We therefore analyzed the *CHD7* gene in 36 clinically well-characterized Dutch KS patients.

Patients and Methods

Patients

A cohort of 36 Dutch KS patients (seven women, 29 men), without a hemizygous mutation in *KAL1* in the male patients, was informed about this study via their pediatric endocrinologist, endocrinologist, gynecologist, or clinical geneticist. The patients gave their informed consent for sequence analysis of *FGFR1*, *PROK2*, *PROKR2*, *FGF8*, and *CHD7* and for collection of their medical data via a questionnaire and/or retrospective chart review. The KS diagnosis was based on the presence of HH, defined as no pubertal maturation in combination with normal or low serum gonadotropins and low sex steroids, in combination with a smell deficit identified from the patient's history and/or formal smell testing. The *CHD7*-positive patients who were identified in this study, were carefully reevaluated for features of CHARGE syndrome and underwent formal smell testing [University of Pennsylvania Smell Identification Test (UPSIT); Sensonics Inc., Haddon Heights, NJ; www.sensonics.com) (15)]. This study was approved by the ethical review board of the University Medical Center Groningen (UMCG).

DNA analysis

DNA was extracted from peripheral blood lymphocytes using standard procedures. All individual exons of the *CHD7* gene were amplified by PCR, and direct sequencing was performed on an ABI 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA) as described previously (14). The GenBank accession number NM_017780.2 was used as reference sequence for the *CHD7* gene. The A of ATG was designated number 1. The intron sequences of the *CHD7* gene can be found in NG_007009.1.

Results

The clinical features of the 36 Dutch KS patients and the results of DNA analysis are summarized in Table 1 and Supplemental Table 2. Heterozygous *CHD7* mutations were identified in three patients, who were carefully reexamined for additional features of CHARGE syndrome.

Patient 1 was diagnosed with KS at age 16 and received hormone replacement therapy (HRT). She had a bilateral cleft lip/palate and mixed hearing loss. At age 20, she was

TABLE 1. Dutch patients with KS who were found to have a mutation in the *CHD7* gene: *KAL1*, *FGFR1*, *PROK2*, *PROKR2*, and *FGF8* status, results of *CHD7* analysis, and an overview of the clinical characteristics and family history

Patient no. (n = 3)	Sex	*KAL1, FGFR1, PROK2, PROKR2,* and *FGF8* status[a]	*CHD7* results	Clinical characteristics besides hypogonadotropic hypogonadism and anosmia	Family history[b]
1	F	—	c.4015C>T; p.Arg1339X	Cleft lip and palate, bilateral mixed hearing loss, retinal coloboma, balance disturbance, mild scoliosis, olfactory bulb aplasia	—
2	M	—	c.5316G>A; p.Trp1772X	Bilateral hearing loss, cleft palate, short stature, bicuspid aortic valve, abnormal external ears, synkinesia	—
3	M	—	c.6322G>A; p.Gly2108Arg *de novo*	Bilateral sensorineural hearing loss, hypoplasia of cochlea and semicircular canals	—

F, Female; M, male.

[a] —, Normal results of *KAL1*, *FGFR1, PROK2*, *PROKR2*, and *FGF8* analysis.

[b] —, Negative family history.

reevaluated at the UMCG's multidisciplinary CHARGE outpatient clinic and was found to have a *CHD7* nonsense mutation (c.4015C>T; p.Arg1339X). Her history revealed delayed motor development and feeding difficulties. She also had balance disturbance [but a computed tomography (CT) scan of the temporal bone had not been performed], mild scoliosis, and impaired vision. Ophthalmological reexamination revealed bilateral retinal colobomas. Her external ears were normal, and ultrasound of the heart and kidneys showed no abnormalities. Anosmia was confirmed by formal smell testing (UPSIT score 12 of 40), and reevaluation of an earlier magnetic resonance imaging brain scan showed olfactory bulb aplasia. In retrospect, she has typical CHARGE syndrome.

Patient 2 was diagnosed with KS at age 16 and was started on HRT. He had severe bilateral hearing loss. He was reexamined at age 31 at the Department of Human Genetics, Radboud University Nijmegen Medical Center, and was shown to harbor a *CHD7* nonsense mutation (c.5316G>A; p.Trp1772X). His history revealed mildly delayed motor development and a cleft palate. He had short stature, synkinesia, and small earlobes. An ultrasound of heart and kidneys revealed a bicuspid aortic valve and normal kidneys. He has not undergone imaging of the inner ear. Formal smell testing showed that he had anosmia (UPSIT score 7 of 40). After reevaluation, he was found to have typical CHARGE syndrome.

Patient 3 was diagnosed with KS at age 14 and started with HRT. He had severe bilateral sensorineural hearing loss. He was reevaluated at age 17 at the UMCG's CHARGE outpatient clinic. *CHD7* analysis revealed a *de novo CHD7* missense mutation (c.6322G>A; p.Gly2108Arg). The glycine at position 2108 is highly conserved, and the amino acid substitution is considered pathogenic by three prediction programs (SIFT, PolyPhen, and Align GVGD) (16–18). In addition, this variant was previously identified in three other index patients with CHARGE syndrome and is therefore highly likely to be pathogenic (www.CHD7.org; Janssen, N., J. E. H. Bergman, M. A. Swertz, L. Tranebjaerg, M. Lodahl, J. Schoots, R. M. W. Hofstra, C. M. A. van Ravenswaaij-Arts, and L. H. Hoefsloot, submitted manuscript). Patient 3 had delayed motor development and balance disturbance. He has a normal intelligence and normal external ears, eyes, kidneys, and heart. Anosmia was confirmed with the UPSIT (score 9 of 40). A temporal bone CT scan showed hypoplasia of the cochlea and semicircular canals, a very specific and frequent feature in CHARGE syndrome, but otherwise, the patient did not fulfill the clinical criteria for CHARGE syndrome (5).

None of the patients with a *CHD7* mutation had an additional mutation in *FGFR1*, *PROK2*, *PROKR2*, or *FGF8*, whereas six other patients of our cohort harbored a heterozygous missense variant in the *PROKR2* gene [c.254G>A; p.Arg85His (4x), c.254G>T; p.Arg85Leu, and c.791G>A; p.Arg264His], and one patient had a heterozygous variant in *FGF8* (c.86_103dup; p.Gly29_Arg34dup) (see Supplemental Table 2 and Information).

Discussion

Our research question was whether *CHD7* mutations could be identified in KS patients without additional CHARGE features. We restricted our study to KS patients, because we have previously shown that HH is associated with anosmia in patients with a *CHD7* mutation (4), and therefore we assumed that the chance to find a *CHD7* mutation in normosmic IHH patients without CHARGE features would be even lower.

We identified three *CHD7* mutations in 36 Dutch KS patients (three of 36 = 8.3%). All three patients had additional features of CHARGE syndrome on careful reexamination, which is in agreement with our previous study (11). Hearing loss was the most frequent feature seen in the *CHD7*-positive KS patients in our study (Table 1) and in other published studies (11, 12); it was found in seven of 13 *CHD7*-positive KS patients. However, hearing loss can also occur in patients with a mutation in the *KAL1*, *FGFR1*, or *FGF8* gene (Supplemental Table 1) (7, 9). Other features that were repeatedly found in *CHD7*-positive KS patients were a cleft lip/palate (five of 13), short stature (three of 13), and balance disturbance (three of 13) (Table 1) (11, 12).

We did not identify a *CHD7* mutation in 30 KS patients without additional CHARGE features (Supplemental Table 2), which suggests that *CHD7* mutations are not a frequent cause of isolated KS. Recently, *CHD7* analysis was also performed in a cohort of 30 Finnish KS patients (19). Although three KS patients displayed additional CHARGE features, no *CHD7* mutations were identified in this cohort. Additional studies in large cohorts of clinically well-characterized KS patients are needed to estimate the frequency of *CHD7* mutations in KS patients more reliably. In addition, it would be useful to know whether the *CHD7*-positive patients in the study by Kim *et al.* (12) underwent formal smell testing and were carefully reevaluated after the *CHD7* mutation was identified, because otherwise subtle features of CHARGE syndrome could have been missed. Another limitation of the study by Kim *et al.* (12) is that it is unclear whether *CHD7* analysis was performed in the parents of the patients with a *CHD7* missense variant to give further proof that the five identified missense variants are indeed pathogenic.

Based on the results of this study and the literature, our advice is to evaluate KS patients carefully for features of CHARGE syndrome by taking a detailed case history and physical examination. If the case history reveals that walking without support was delayed, that the patient was unable to crawl without resting the head on the floor (5-point crawling), and that the patient was unable to ride a bicycle without side-stabilizers, the patient probably suffered from balance disturbance (20). Imaging of the semicircular canals (preferably a temporal bone CT scan) is indicated in all KS patients who are suspected of balance disturbance. Our advice is to perform *CHD7* analysis in KS patients who have at least two of the following features of CHARGE syndrome: ocular coloboma, choanal atresia/stenosis, characteristic external ear anomaly, cranial nerve dysfunction (facial palsy, sensorineural hearing loss, or hypoplastic cranial nerves on imaging), or balance disturbance. In addition, *CHD7* analysis is recommended in all KS patients with semicircular canal anomalies, irrespective of other CHARGE features. These recommendations are in line with our 2011 recommendation for *CHD7* analysis in patients suspected of CHARGE syndrome (5).

Identifying a *CHD7* mutation has important clinical implications. First, the *CHD7*-positive patient should be screened for additional CHARGE features, because subtle features can remain undetected but can have therapeutic consequences, *e.g.* unilateral renal agenesis. For recommendations on screening, we refer to the clinical surveillance schedule in Bergman *et al.* (5). Second, genetic counseling is indicated, because the patient has a 50% chance of transmitting the *CHD7* mutation to his or her offspring. The offspring may develop a more severe manifestation of CHARGE syndrome, because the syndrome is highly variable, even within families (5). The possibility of prenatal diagnosis and preimplantation genetic diagnosis should therefore be discussed.

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