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

Jorieke E. H. Bergman, Willem de Ronde, Marjolijn C. J. Jongmans, Bruce H. R. Wolffenbuttel, Sten L. S. Drop, Ad Hermus, Gianni Bocca, Lies H. Hoefsloot, and Conny M. A. van Ravenswaaij-Arts

Departments of Genetics (J.E.H.B., C.M.A.v.R.-A.), Endocrinology (B.H.R.W.), and Pediatric Endocrinology (G.B.), University of Groningen, University Medical Center Groningen, 9700 RB Groningen, The Netherlands; Department of Internal Medicine (W.d.R.), Kennemer Gasthuis, 2000 AK Haarlem, The Netherlands; Departments of Human Genetics (M.C.J.J., L.H.H.) and Endocrinology (A.H.), Radboud University Nijmegen Medical Center, 6525 GA Nijmegen, The Netherlands; and Department of Endocrinology (S.L.S.D.), Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands

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

F, Female; M, male.

—, Normal results of KAL1, FGFR1, PROK2, PROKR2, and FGF8 analysis.

—, 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 on 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.CH7D.org; Janssen, N., J. E. H. Bergman, M. A. Swertz, L. Tranebaerg, 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 Supplementary 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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Address all correspondence and requests for reprints to: Conny M. A. van Ravenswaaij-Arts, Department of Genetics, University Medical Center Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands. E-mail: c.m.a.van.ravenswaaij@umcg.nl.

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