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The Role of *CHD7* Mutations in Patients with Idiopathic Hypogonadotropic Hypogonadism and Kallmann Syndrome

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Abstract

Mutations in the chromodomain helicase DNA binding protein-7 (*CHD7*) cause CHARGE syndrome, which includes eye coloboma, heart malformations, atresia of the choanae, retardation of growth/development, genital anomalies, and ear abnormalities. CHARGE syndrome is usually sporadic, but is also autosomal dominant. *CHD7* encodes a large protein that participates in chromatin remodeling and transcription. Findings from studies of mouse models employing ENU-mutagenesis or gene-trap methods recapitulate human CHARGE syndrome. CHARGE patients may manifest anosmia and/or hypogonadism, features that overlap with idiopathic hypogonadotropic hypogonadism (IHH) and Kallmann syndrome (KS). Similarly, IHH/KS patients may also display partial CHARGE features. Therefore, it has been hypothesized that IHH/KS represents a milder allelic variant of CHARGE syndrome, which has been supported by the identification of heterozygous *CHD7* mutations in both normosmic IHH and KS. Developmental expression within the hypothalamus and the presence of human mutations indicate that *CHD7* has an important role in puberty and reproduction.

Keywords

CHARGE syndrome; *CHD7* gene; idiopathic hypogonadotropic hypogonadism; Kallmann syndrome; GnRH neuron; anosmia

Introduction

Integration of the hypothalamic-pituitary-gonadal axis is important for normal pubertal development and reproduction. The hypothalamus secretes pulsatile gonadotropin releasing hormone (GnRH), which then stimulates the pituitary to synthesize and secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH), which then stimulate gonadal sex steroids and gametes. Normal levels of sex steroids exert negative feedback upon the hypothalamus and pituitary.

However, patients with idiopathic hypogonadotropic hypogonadism (IHH) have low sex steroids. Despite the withdrawal of negative feedback, FSH and LH levels remain low or normal, both of which are inappropriate for hypogonadism. These findings indicate a defect in the hypothalamus or pituitary [Crowley et al. 1985]. IHH is estimated to occur in 1/10,000 males and 1/50,000 females. IHH/KS usually, but not always, results in irreversible

pubertal delay at age 17 in females with hypoenestrogenic amenorrhea and age 18 in males with low testosterone levels [Bhagavath et al. 2006].

Other than low gonadotropins, there is no other pituitary dysfunction as evidenced by normal imaging studies of the brain and serum pituitary hormones. IHH may be normosmic (nIHH) or be associated with olfactory dysfunction—Kallmann syndrome (KS). A variety of other anomalies have been observed in IHH/KS including midfacial defects, dental aplasia, neurologic abnormalities such as ataxia, synkinesia (mirror movements), deafness and visual abnormalities, renal agenesis, and heart defects [Bhagavath et al. 2006].

As outlined in Figure 1, a number of genes involved in IHH/KS have been identified since the first discovery of *KAL1* mutations in 1991 in X-linked KS [Franco et al. 1991, Legouis et al. 1991]. Two groups identified the *GNRHR* as the first gene involved in autosomal recessive normosmic IHH [de Roux et al. 1997, Layman et al. 1998]. A number of other genes have subsequently been identified. Most affect hypothalamic development and/or regulate GnRH. Some act on both the hypothalamus and pituitary, such as *NROB1* encoding DAX1 [Habiby et al. 1996]. Rarely, isolated deficiencies of either the beta-subunit of FSH [Layman et al. 1997, Matthews et al. 1993] or LH [Lofrano-Porto et al. 2007, Weiss et al. 1992] have been reported. Of particular interest is the observation that *GNRH1* mutations have only recently been found in nIHH patients [Bouligand et al. 2009, Chan et al. 2009] despite the report of the hypogonadal mouse with a *Gnrh1* deletion in 1986 [Mason et al. 1986]. Pituitary causes for IHH/KS should be considered, as many of these genes have been associated with combined pituitary hormone deficiency (Figure 1 and reviewed in [Layman 2010]. Nevertheless, the number of known genes only accounts for ~30% of patients as reviewed recently [Layman 2010] and in other articles of this edition. In addition, mutations in more than one gene are increasingly being reported [Pitteloud et al. 2007]. To date *KAL1* mutations cause Kallmann syndrome, while *GNRHR* mutations have only been found in normosmic IHH. *FGFR1* and *CHD7* are among the more commonly involved genes in both KS and nIHH [Layman 2010]. *CHD7* is the topic for this review.

CHARGE Syndrome

CHD7 was the gene found to be causative for CHARGE syndrome. The CHARGE syndrome acronym stands for Coloboma (hole in the eye), Hear malformations, Atresia of the choanae (the posterior nasopharynx), Retardation of growth and development, Genital anomalies, and Ear abnormalities—both auditory and vestibular [Blake and Prasad 2006, Blake et al. 1990]. Initially, the diagnosis was made if at least four of the six features were present.

Hall [Hall 1979] first described the association of choanal atresia and multiple congenital anomalies. Hittner et al [Hittner et al. 1979] published a similar report of the association of coloboma, congenital heart disease, mental retardation, and abnormal appearing external ears associated with hearing loss. The disorder was initially known as the Hall-Hittner syndrome, but in 1981 the CHARGE acronym was coined by Pagan [Pagon et al. 1981]. CHARGE syndrome may be autosomal dominant and fully penetrant, but is usually sporadic with a prevalence of approximately 1/10,000 [Blake and Prasad 2006].

Blake [Blake et al. 1998] reported findings of an expert panel, who proposed major and minor criteria for CHARGE syndrome. The four major criteria consist of the four —“C” s”, which are eye coloboma, choanal atresia, cranial nerve abnormalities, and the characteristic CHARGE ear (Table 1). The eye coloboma are usually bilateral and involve the retino-choroidal region and optic nerve; and therefore can result in significant visual impairment unless just the iris is involved. Coloboma are found in 80–90% of CHARGE patients.

Choanal atresia occurring in 50–60% of patients can be unilateral or bilateral; and can result in breathing and/or feeding difficulties [Blake et al. 1998]. Cranial nerve abnormalities, identified in 90% of CHARGE patients, include cranial nerves I (resulting in anosmia), VII (facial palsy), VIII (deafness and vestibular dysfunction), and IX–XI (swallowing problems, reflux, and aspiration).

The characteristic CHARGE external ear is shown in Figure 2 [Blake et al. 1998]. Unlike the normal ear, which has a helix ending in the lobule, the antihelix ending in the antitragus inferiorly, and the opening (cavum conchae); the CHARGE ear is short and wide, protruding, and asymmetric with little or no lobe. There is decreased cartilage and the helix can end abruptly giving the appearance of being “snipped off.” The antihelix may be prominent and the conchae triangular. The CHARGE middle ear may show abnormal ossification or aplasia of the middle ear bones, which can result in hearing loss, while the inner ear may have decreased numbers of concentric turns in the cochlea (Mondini effect) and/or absent semicircular canals (which may be diagnosed by CT scan), leading to balance problems. The CHARGE ear was said to be present in 70–90% of CHARGE patients [Blake et al. 1998].

Blake’s minor criteria include features that are either less frequent or are not specific to CHARGE (Table 1). They include developmental delay of milestones, hypotonia, and mental retardation, which were universal, but also common in hundreds of other syndromes. The others include genital hypoplasia, cardiac anomalies such as conotruncal and aortic arch abnormalities, short stature, clefting, tracheoesophageal fistula, and dysmorphic facies. Blake suggested that the diagnosis of CHARGE Syndrome is highly likely if all four major or three major and three minor criteria are present. Probable/possible CHARGE includes 1–2 major and several minor criteria (Table 1).

Verloes also proposed diagnostic criteria for CHARGE syndrome, which included the three C’s—coloboma, choanal atresia, and canals (hypoplastic semicircular canals) as shown in Table 1 [Verloes 2005]. Minor criteria include cranial nerve VII–XII abnormalities, hypothalamic-pituitary dysfunction, ear abnormalities, malformations of the mediastinal viscera (heart and tracheoesophageal fistula), and mental retardation. Typical CHARGE is diagnosed by three major or two major and two minor criteria. Partial/incomplete CHARGE requires two major and at least of one five minor criteria, and atypical CHARGE is also defined as two major or one major and three minor criteria (Table 1). Other less common anomalies may also occur, such as renal agenesis, well-known to occur in Kallmann syndrome. Skeletal anomalies, thymic and parathyroid hypoplasia, and abdominal wall defects can occur. CHARGE syndrome can be a very debilitating disease with a life expectancy of five days in some patients to at least 46 years of age. Seventy percent of CHARGE patients survive to age 5, with most of the mortality within the first year of life and due to choanal atresia, heart defects, TE fistulas, and feeding and swallowing difficulties [Blake et al. 1990, Zentner et al. 2010].

The Molecular Basis of CHARGE Syndrome

The molecular basis of CHARGE syndrome was elucidated in 2004 by Vissers et al who were able to perform array comparative genomic hybridization (CGH) on two very informative patients [Vissers et al. 2004]. One had a 5 Mb deletion by CGH and the other patient had an apparently balanced chromosomal translocation involving chromosomes 6 and 8—t(6;8)(6p8p;6q8q) [Hurst et al. 1991] and no deletion by CGH. However, using a tiling resolution chromosome 8 array, the translocation patient was found to have two deletions: one that was 0.8 Mb and a second of 1.5 Mb. The shortest region of overlapping deletion of the two patients is shown in Figure 3, as well as the nine genes contained within

the common deletion region. These genes were subjected to DNA sequencing. Vissers et al [Vissers et al. 2004] identified that 10 of 17 CHARGE patients had heterozygous *CHD7* mutations including seven nonsense, two missense, and one potential splice mutant (Table 2). All but one patient had at least four of the original C-H-A-R-G-E acronym criteria (not Blake or Verloes) and had one or more of the following—vestibular, facial nerve abnormality, or clefting.

CHD7

The gene for CHARGE syndrome is chromodomain helicase DNA binding protein-7 (*CHD7*), and is one of 9 CHD proteins that have in common the ability to hydrolyze ATP to alter nucleosome structure (DNA wrapped in histones) [Marfella and Imbalzano 2007]. The gene at chromosome 8q12 consists of 38 exons spanning 188kb [Vissers et al. 2004]. The large 10kb transcript codes for a 2997 amino acid protein with five consensus nuclear localization signals, which was experimentally demonstrated to be nuclear [Schnetz et al. 2009]. The protein structure reveals two N-terminal chromodomains that function to bind histones, while SNF2/helicase domains are important in chromatin remodeling (Figure 4). There are three conserved regions CR1-3, SANT, and BRK domains, the functions of which are unknown. The leucine zipper region at the C-terminus is likely important in transcription.

Schnetz et al [Schnetz et al. 2009] used ChIP-chip analysis and demonstrated that CHD7 binds histone-3 methylated at Lys 4 (H3K4). In addition to chromatin remodeling, CHD7 may function in DNA binding, transcription regulation, cell cycle regulation, apoptosis, and embryonic stem cell pluripotency [Zentner et al. 2010]. Utilizing yeast-2 hybrid studies, CHD7 is also known to bind related protein CHD8 [Batsukh et al. 2010]. More recent data indicate that CHD7 does affect enhancer elements. Using chromatin immunoprecipitation and massively parallel DNA sequencing (ChIP-Seq), CHD7 bound more than 10,000 sites on chromatin in mouse ES cells, most of which demonstrated features of gene enhancer elements [Schnetz et al. 2010]. These binding sites were predominantly located distal to transcription start sites and appeared to modulate genes either positively or negatively. In addition, CHD7 co-localized with the enhancer protein P300, as well as with ES cell master regulators (OCT4, SOX2, and NANOG). Therefore, these investigators hypothesized that enhancer-mediated gene dysregulation may be involved in the disease pathogenesis and that the critical CHD7 target genes could be up or down-regulated by *CHD7* mutations [Schnetz et al. 2010].

Human *CHD7* Mutations in CHARGE Syndrome

Many investigators have subsequently contribute to understanding the molecular basis of CHARGE syndrome [Delahaye et al. 2007, Jongmans et al. 2006, Jongmans et al. 2008, Lalani et al. 2006, Ogata et al. 2006, Sanlaville et al. 2006, Sanlaville and Verloes 2007, Vissers et al. 2004, Vuorela et al. 2007, Zentner et al. 2010]. Sanlaville [Sanlaville et al. 2006, Sanlaville and Verloes 2007] and Jongmans [Jongmans et al. 2006, Jongmans et al. 2008] have contributed greatly to the literature of *CHD7* mutations in CHARGE; however, Zentner [Zentner et al. 2010] recently reviewed the literature. They reported that 67% of 379 CHARGE patients had *CHD7* mutations. Seventy percent were nonsense or frameshift, and most of the remainder were missense or splice site mutations. Most *CHD7* mutations occur sporadically, but can be autosomal dominantly inherited [Zentner et al. 2010]. Germline mosaicism has also been reported. Mutations are distributed across the large, 38-exon gene; and to date, there is no correlation of genotype with phenotype. The mutations are heterozygous, loss of function, and are likely due to haploinsufficiency.

A *CHD7* Single Nucleotide Polymorphism (SNP) in Idiopathic Scoliosis

In a cohort of 53 multiplex families of European descent with idiopathic scoliosis (IS), Gao et al [Gao et al. 2007] performed haplotype analysis, which revealed linkage and association of 8q12 loci with a LOD score 2.77. Within the 4-cM region between markers D8S1113 and D8S1136, located is a candidate gene *CHD7*. Since >60% of CHARGE syndrome patients may develop late-onset scoliosis, they hypothesized that milder variants of *CHD7* may be involved in IS susceptibility. By a fine-mapping study within *CHD7*, a known SNP rs4738824 in intron 2 has been identified in a nucleotide which appears perfectly conserved across nine vertebrate species [Gao et al. 2007]. This SNP is located in a possible binding site for caudal-type (cdx) homeodomain-containing transcription factors and speculated to confer functional effects upon *CHD7*. Consistent with this finding, high *Chd7* expression was shown in mouse spinal cord at E11 [Kim et al. 2008].

Mouse Models of CHARGE Syndrome

Mouse models corroborate the human CHARGE phenotype. Two approaches have been used to study the function of *CHD7* in mouse—ENU mutagenesis [Bergman et al. 2010b, Bosman et al. 2005] and gene-trap [Hurd et al. 2007, Layman et al. 2009]. Bosman et al used ENU mutagenesis and identified 9 mice with heterozygous *Chd7* mutations, most of which were nonsense [Bosman et al. 2005]. Hurd et al used a gene-trap method inserting β -galactosidase sequence in between exons 1 and 2 of *Chd7* [Hurd et al. 2007]. Homozygous mutations were lethal, while heterozygous mutations recapitulated CHARGE. As shown in Table 3, the features of the mice resemble human CHARGE syndrome. The most obvious phenotype was circling behavior indicating vestibular dysfunction due to hypoplastic semicircular canals (lateral ones most commonly). Interestingly, coloboma were not present in either type of mouse.

The Relationship of CHARGE Syndrome to Idiopathic Hypogonadotropic Hypogonadism (IHH)/Kallmann Syndrome (KS)

What is the evidence that CHARGE patients have hypogonadotropic hypogonadism? Wheeler et al studied nine CHARGE patients prior to the identification of *CHD7* as the causative gene [Wheeler et al. 2000]. The patients included two males with micropenis and seven with pubertal failure. All nine had low serum gonadotropins and the authors concluded that they may have IHH. Unfortunately, there are no details of pubertal staging, sex steroid levels, and they could not exclude the reversible constitutional delay of puberty. However, four patients were old enough to be classified as IHH, while the other five were hypogonadotropic, but prepubertal. Chalouhi et al studied 14 children with CHARGE and found that all were anosmic [Chalouhi et al. 2005]. Pinto reported that 19 of 20 CHARGE boys had micropenis and/or cryptorchidism, which could be consistent with IHH, though all were prepubertal [Pinto et al. 2005]. In addition, seven of nine neonatal boys under five months of age had low testosterone, suggesting hypogonadism. None of seven girls over 12 years of age started puberty spontaneously, and five had subnormal responses to GnRH. MRI and smell tests were abnormal. Ogata demonstrated that a 14 7/12 year old CHARGE patient with a *CHD7* mutation had a KS phenotype [Ogata et al. 2006]. She had coloboma, external ear defects, feeding abnormalities, cardiac defects (atrial and ventricular septal defects, patent ductus arteriosus, and right aortic arch) and bilateral hearing impairment.

CHD7 Mutations in IHH/KS

Recognizing overlapping phenotypic features of both CHARGE and IHH/KS, Kim et al [Kim et al. 2008] hypothesized that IHH/KS might be a mild allelic variant of CHARGE. Using a

candidate gene approach, the 37 protein coding exons and splice junctions of *CHD7* were sequenced in 101 IHH/KS patients not known to have CHARGE syndrome. In 96 additional IHH/KS patients, DNA sequencing of exons 6–10 encoding the two highly conserved chromodomains was performed. Putative mutations were sequenced in 180 controls (and in an additional 96 Turkish controls for the 2 Turkish patients who had mutations). Three of 51 (6%) of IHH and 3/50 (6%) of KS patients had sporadic heterozygous *CHD7* mutations [Kim et al. 2008]. Of the 96 screened only for exons encoding the chromodomains, one additional mutation was identified in a female KS patient with cleft lip/palate, and hearing loss. These seven mutations were not seen in the SNP database of in 180–276 controls.

Three mutations occurred in chromodomains (two splice and one missense); four additional missense mutations were predicted to affect protein structure and binding (Figure 4). One of the missense mutations was located in the N-terminus, while the other three were in the C-terminus around the leucine zipper region. The Ser834Phe missense [Delahaye et al. 2007] and the IVS6+5G>C splice [Jongmans et al. 2008] mutations have been previously identified in CHARGE patients. Of interest, *CHD7* mutations in IHH/KS clustered around exons 2,6,8, and 38 unlike in CHARGE where they are distributed across the gene (Figure 4).

Potential Mechanisms of *CHD7* Mutations in IHH/KS

A putative splicing mutation IVS8+5G>A identified in a nIHH male without other anomalies was studied by RT-PCR of lymphoblast RNA, cloning, and sequencing. These studies revealed exon 8 skipping in the transcript [Kim et al. 2008]. This mutant introduces 16 aberrant out-of-frame amino acid residues causing a frameshift and subsequent termination codon at residue 849. This predicts that 70% of the protein will be truncated. Importantly, this mutation removes half of the first and all of the second chromodomains, likely rendering a nonfunctional *CHD7* protein (Figure 4). Another intronic IVS6+5G>C mutation was identified in a female with KS with cleft lip/palate and mild hearing loss without other anomalies. Using similar techniques, exon 6 skipping was identified for this splice mutation (Figure 4). This mutation results in an inframe deletion of 22 of the 66 amino acids of chromodomain 1. Interesting, this mutation reported previously by Jongmans [Jongmans et al. 2008] was predicted to dramatically impair splicing by in silico methods, which Kim et al [Kim et al. 2008] demonstrated in vitro.

For the missense mutations, amino acid conservation was analyzed. The His55 involved in the His55Arg was completely conserved, while Ala2789 involved in the Ala2789Thr was conserved in seven of eight species [Kim et al. 2008]. The Ser834 (Ser834Phe) in chromodomain 1 was completely conserved, and the Pro2880 (Pro2880Leu) was conserved in seven of eight orthologs. Lastly the Lys2948 (involved in the Lys2948Glu), though predicted to be intolerant by SIFT, was conserved in six of eight orthologs (Table 4).

Protein modeling was performed using 3D Jury, EMBOSS, and PHYRE [Kim et al. 2008]. Shown are the 300 amino acid C-terminal residues of *CHD7* in Figure 5. Pro2880Leu and Lys2948Glu, both of which are highly conserved and predicted intolerant by SIFT (sorting intolerant from tolerant), are located in highly flexible loop regions suggesting that the mutations would be deleterious (Figure 5A). The same model was rotated 180° to show Ala2789Thr, which is located in a highly flexible loop region. Although predicted to be tolerated by SIFT, Ala2789 is highly conserved and predicted to be deleterious by protein modeling (Figure 5B). In Figure 5C, the 155 amino acids around Ser834 (mutated to Phe) are shown. There is a strong stacking interaction of mutant Phe834 (shown in blue) with the ring of Tyr881 (shown in green). Therefore, the Phe834 mutant is strongly predicted to affect protein stability.

Three of the seven mutations affect the highly conserved chromodomains—two are splicing mutants and one is a missense mutation [Kim et al. 2008]. It is known that chromodomains interact with histone tails and that chromodomain deletion impairs nucleosome binding [Bouazoune et al. 2002, Marfella and Imbalzano 2007]. Therefore, these three mutations, two occurring in nIHH and one in KS, are highly likely to be deleterious. Two have been observed previously in CHARGE syndrome [Delahaye et al. 2007, Jongmans et al. 2008]. These three mutations and the four other missense mutations—two in nIHH and two with KS (complete or incomplete)—are summarized in Table 4. Using SIFT, three of four missense mutations were predicted intolerant. The three missense mutations in exon 38 were predicted to be deleterious by protein modeling. His55Arg is completely conserved and predicted intolerant by SIFT, but it is not within a known protein domain.

CHD7 Mutations in Both IHH/KS and CHARGE

The IVS6+5G>C mutation has been previously reported in CHARGE *de novo* [Jongmans et al. 2008]. Two brothers, ages 4 and 7, had multiple features including mild mental retardation, micropenis and cryptorchidism, anosmia, CHARGE external ear, vestibular (balance) problems, and hearing loss. One brother had bilateral cleft lip/palate. No coloboma, choanal atresia, heart defects, or short stature were present. The patient reported by Kim et al also had a *de novo* mutation and displayed mild hearing impairment, cleft lip/cleft palate, short stature, and KS [Kim et al. 2008]. By Blake's criteria [Blake et al. 1998], both patients have possible CHARGE based upon the presence of 1–2 major and several minor criteria. By Verloes' criteria [Verloes 2005], the patient of Kim et al [Kim et al. 2008] does not have CHARGE, while the patient from Jongmans [Jongmans et al. 2008] has atypical CHARGE based upon one major and three minor criteria (Table 1, Table 5). Clearly Jongman's patient has a more severe phenotype. It should be noted that the patient of Kim et al [Kim et al. 2008] did not have mutations in 11 other IHH/KS genes including *KALI*, *FGFR1*, *FGF8*, *PROKR2*, *PROK2*, *TAC3*, *TACR3*, *GNRHR*, *KISS1R*, *GNRH1*, or *NELF* (unpublished).

The Ser834Phe mutation was found in a CHARGE patient by Delahaye et al [Delahaye et al. 2007]. This patient had multiple congenital anomalies, as did his brother (Table 6). The patient reported by Kim et al [Kim et al. 2008] was a normosmic male with cleft lip and no other anomalies; and did not have CHARGE syndrome by Blake's criteria (or Verloes), whereas Delahaye's has possible CHARGE by Blake's criteria and atypical CHARGE by Verloes' criteria (Table 6). Similarly, this nIHH patient reported by Kim et al [Kim et al. 2008] did not possess mutations in the same 11 other IHH/KS genes studied.

Chd7 Expression During Development

Kim et al [Kim et al. 2008] have shown expression of *Chd7* mRNA in a variety of rat tissues and in GnRH neuronal cells (not shown). To determine *Chd7* expression during development, in situ hybridization was performed [Kim et al. 2008]. By E10.5–E11, high *Chd7* expression was seen in the olfactory placode (Figure 6A), spinal cord (Figure 6B), and in the nasopharynx and eye (Figure 6C). At E10.5–E11, high levels of *Chd7* expression were particularly noteworthy in the olfactory placode, which gives rise to GnRH neurons. As development progressed, label intensity declined and became more restricted to the central and peripheral nervous system. At E14 (Figure 6D,E), high signal intensity was observed in the olfactory epithelium, cochlea, anterior pituitary gland, and spinal cord. In the adult (Figure 6F–I), intense *Chd7* expression was restricted to the preoptic area of the hypothalamus, cerebellum, and hippocampus. Expression of *Chd7*, therefore, was consistent with its involvement in GnRH and olfactory neuron development, which begins about day E10.5–E11 and is essentially completed by E18.5 in the mouse [Wierman et al. 2004].

However, it is not known if *CHD7* directly affects GnRH neuron development—this will require future study.

Additional *CHD7* Mutations in IHH/KS

Following the publication from Kim et al [Kim et al. 2008], Jongmans et al [Jongmans et al. 2009] studied 56 IHH/KS patients and identified three heterozygous *CHD7* mutations (two nonsense and one missense) not seen in 300 controls (Figure 4). The phenotypic features are summarized in Table 7. By Blake's criteria [Blake et al. 1998], all three had possible CHARGE, although patients 2 and 3 had atypical CHARGE by Verloes' criteria [Verloes 2005], while patient 1 did not have CHARGE. Of interest, patient #3 in Table 7 (#8 in their publication) had an R2024X mutation, which was previously reported by Vissers et al and displayed a phenotype of coloboma, a heart defect, retardation of growth and development, an ear abnormality including deafness, vestibulocochlear dysfunction, and facial nerve palsy [Vissers et al. 2004]. Several additional studies have been published regarding *CHD7* mutations and IHH/KS, however, the patients clearly have CHARGE syndrome [Bergman et al. 2010a, Dauber et al. 2010, Foppiani et al. 2010], and in one the patient is not old enough to be classified as IHH [Dauber et al. 2010].

These findings support that IHH/KS may be a mild allelic form of CHARGE syndrome and *CHD7* has been designated as *KAL5*. CHARGE syndrome is a more severe, commonly debilitating disorder, in which two-thirds of the mutations are frameshift mutations spanning the entire gene [Zentner et al. 2010]. Unfortunately, to date, there are no consistent genotype/phenotype correlations. In contrast, IHH/KS is a much less severe disease in most patients, which is consistent with the identification of less deleterious missense mutations. Interestingly, the seven *CHD7* mutations reported by Kim et al were confined to the regions of exons 2,6,8, and 38, and they occurred in both nIHH and KS, some of whom had some CHARGE features, but also some who did not [Kim et al. 2008].

CHD7 was the second most common gene, behind *FGFR1* [Pitteloud et al. 2006] to be involved in either nIHH or KS [Kim et al. 2008]. Although commonly sporadic, because of its potential autosomal dominant inheritance and reports of germline mosaicism, genetic counseling is important. These findings suggest that a careful clinical history and physical exam of IHH/KS patients for CHARGE features should be performed. What is not clear for the clinician is how far to go in the evaluation. An ear exam can be done (and compared to photos of the CHARGE ear), but should hearing or vestibular problems be tested if the patient is completely asymptomatic? Similarly, it is reasonable to have an ophthalmologic exam, but on the other hand, most coloboma will present with visual loss. Should a cardiac echo be done if there is no heart disease or murmur?

It is likely that pediatric geneticists and pediatric endocrinologists will ascertain the most severe cases of CHARGE, while those presenting to medical endocrinologists, medical geneticists, or reproductive endocrinologists for pubertal delay will likely have a less severe phenotype. A search for associated anomalies must be undertaken by proper clinical studies with predefined measures, appropriate controls, and individuals measuring outcomes should be blinded to the diagnosis to prevent anticipation bias.

In conclusion, compelling evidence that IHH/KS is a mild allelic variant of CHARGE has been reported. DNA sequencing should be considered in IHH/KS patients with any features of CHARGE, although it must be appreciated that *CHD7* mutations have been found in patients without these phenotypic features. *CHD7* expression during development also suggests a role for *CHD7* in the GnRH migratory pathway, which needs to be studied further.

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HYPOTHALAMUS

Figure 1.
Known IHH/KS genes are shown.

Figure 2.

The normal ear is shown on the left; and the CHARGE ear is shown on the right.

Figure 3.

Mapping of overlapping deleted regions of chromosome 8q12 are shown along with the relative positions of the nine genes with the region. Mutations were identified in *CHD7* shown in bold [Visser et al. 2004].

Figure 4.

The CHD7 protein, along with its recognized domains, is shown. Mutation location is shown for the seven mutations reported by Kim et al [Kim et al. 2008] in black (n=5) or blue (the two that have been previously reported in CHARGE—see text). Three mutations reported by Jongmans et al [Jongmans et al. 2009] are shown in brown.

Figure 5.

Protein modeling of four different CHD7 mutations (shown in red) reproduced with permission from Kim et al [Kim et al. 2008] is shown. (A) 300 amino acids of CHD7 revealing Pro2880Leu and Lys2948Glu are shown. (B) When the model in A is rotated 180° the Ala2789Thr location also becomes visible. (C) The Phe834 residue, mutated in the Ser834Phe, demonstrates stacking with Tyr881.

Figure 6.

Chd7 expression during development is shown in the mouse (see text for details).

Reproduced with permission from Kim et al [Kim et al. 2008]. Abbreviations: OP = olfactory placode; GCL = granule cell layer of the cerebellum; OE = olfactory epithelium; aPG = anterior pituitary gland; pPG = posterior pituitary gland; SC = spinal cord; CA1 = CA1-region of the hippocampus; CA3 = CA3-region of the hippocampus; CO = cochlea; E = eye; NP = nasal pit.

Table 1

Blake's Diagnostic Criteria [Blake et al. 1998] for CHARGE syndrome are compared with Verloes' Criteria [Verloes 2005].

	Blake	Verloes
Major		
	Coloboma	Coloboma
	Choanal atresia	Choanal atresia
	Cranial nerve abnormality	Canals-hypoplastic semicircular
	CHARGE ear	
Minor		
		Rhombencephalic dysfunction (cranial nerve VII-XII palsies and neurosensory deafness)
		Abnormal external or middle ear
	Developmental delay (delayed motor milestones, mental retardation, hypotonia)	Mental retardation
	Genital hypoplasia (micropenis, cryptorchidism, hypoplastic labia, delayed puberty)	Hypothalamic-pituitary dysfunction (including growth hormone & gonadotropin deficiency)
	Growth deficiency	
	Cardiovascular malformations (conotruncal defects such as TOF; aortic arch and AV abnormalities)	Malformation of mediastinal organs (heart and esophagus)
	Tracheoesophageal-fistula	
	Cleft lip/palate	
	Characteristic facies	
Occasional		
	Thymic/parathyroid hypoplasia	
	Renal anomalies (hydronephrosis, dysgenesis, horseshow/ectopic kidney)	
	Hand anomalies (polydactyly, ectrodactyly, altered palmar flexion creases, thumb hypoplasia)	
	General appearance (webbed neck, sloped shoulders, accessory/hypoplastic nipples)	
	Abdominal wall defects (omphalocele or umbilical hernia)	
	Scoliosis, hemivertebrae	
Diagnosis		
Highly likely	All 4 major; or 3 major & 3 minor	
Probable/Possible	1 or 2 major & several minor	
Typical		3 major or 2 major & 2 minor
Partial/Incomplete		2 major & 1 minor
Atypical		2 major; or 1 major & 3 minor

Table 2

The Description of *CHD7* Mutations in CHARGE Patients

Mutation	Sex	Phenotype				
		Coloboma	Heart	Atresia Choanae	Retardation	Genital Hypoplasia
Del 8q12	F	+	+	-	+	-
Del 8q12	F	+	+	-	+	-
p.R157X	F	+	+	-	+	-
p.G360X	F	-	+	+	+	-
p.Q572X	M	-	-	-	+	+
p.I1028V	F	+	-	-	+	-
p.L1257R	F	+	-	-	+	+
p.Y1806X	F	-	+	+	+	+
p.C2017X	M	+	-	-	+	+
p.R2024X	F	+	+	-	+	-
p.Y2608X	F	-	+	+	+	-
IVS26-7G>A	M	+	-	-	+	+

Presence (+) or absence (-) of CHARGE acronym findings in those with mutations. F = female; M = male. All mutations were either de novo or unknown. Data from *Visser et al. [Visser et al. 2004]*. All with mutations had 4 criteria and 1 of the following: vestibulocochlear abnormalities, facial nerve palsy, or cleft lip/palate.

Table 3

Organs affected in mouse models of CHARGE syndrome

Organ Affected	Method of Mouse Creation	
	ENU mutagenesis	Gene-trap
Vestibular	+	+
Genital	+	ND
Heart	+	ND
Cleft lip/palate	+	ND
Choanae	+	ND
Coloboma	–	–
Reduced body weight	+	+
Olfactory bulb/sense of smell	+	+

ENU mutagenesis from Bosman et al [Bosman et al. 2005] and gene-trap data from Hurd et al [Hurd et al. 2007]. ND = not determined.

Table 4

Phenotype and genotype of seven CHD7 mutations in IHH/KS as reported by Kim et al [Kim et al. 2008]

Pt.	Phenotype	Sex	Origin	Exon/Intron	Mutation	Effect
1	nIHH; no anomalies	M	Turkey	Intron 8	IVS8+5G>A	Splicing; Premature Stop
2	KS; cleft lip & palate; hearing impairment	F	USA	Intron 6	IVS6+5G>C	Splicing; 22 AA deletion
3	nIHH; cleft lip; cryptorchidism	M	USA	Exon 8	Ser834Phe	SIFT: intolerant; Conserved 8/8; Protein modeling
4	KS; no anomalies	M	USA	Exon 2	His55Arg	SIFT: intolerant; Conserved: 8/8
5	nIHH; no anomalies	M	Turkey	Exon 38	Ala2789Thr	SIFT: tolerant; Conserved: 7/8; Protein modeling
6	nIHH; cryptorchidism	M	USA	Exon 38	Pro2880Leu	SIFT: intolerant; Conserved 7/8; Protein modeling
7	KS; no anomalies	M	USA	Exon 38	Lys2948Glu	SIFT: intolerant; Conserved: 6/8; Protein modeling

M = male; F = female; SIFT = sorting intolerant from tolerant. Conservation of amino acid residues affected is compared with known orthologs and is also shown.

IVS6+5G>C *CHD7* mutation as reported by Kim et al [Kim et al. 2008] and Jongmans et al [Jongmans et al. 2008]

Table 5

	Blake Criteria	Kim	Jongmans	Verloes Criteria	Kim	Jongmans
Major	Coloboma	-	-	Coloboma	-	-
	Choanae	-	-	Choanae	-	-
	Cranial nerve	+	+			
	CHARGE ear	-	+	Canals	-	+
Minor				Rhombencephalic dysfunction	+	+
				Ear (middle & external)	-	+
	Developmental delay	-	+	Mental retardation	-	+
	Genital hypoplasia	+	+	Hypothalamic- pituitary dysfunction	+	+
	Growth deficiency	+	-			
	Heart	-	-	Malformation mediastinal organs (heart, esophagus)	-	-
	TE fistula	-	-			
	Cleft lip/palate	+	+			
	Characteristic facies	-	+			

By Blake's criteria, both patients have possible CHARGE; and by Verloes' Criteria, the patient of Kim et al does not have CHARGE, while the patient from Jongmans has atypical CHARGE.

Table 6

Ser834Phe *CHD7* mutation as reported by Kim et al [Kim et al. 2008] and Delahaye et al [Delahaye et al. 2007]

	Blake Criteria	Kim	Delahaye	Verloes Criteria	Kim	Delahaye
Major	Coloboma	-	-	Coloboma	-	-
	Choanae	-	-	Choanae	-	-
	Cranial nerve	-	+			
	CHARGE ear	-	+	Canals	-	+
Minor				Rhombencephalic dysfunction	-	+
				Ear (middle & external)	-	+
	Developmental delay	-	+	Mental retardation	-	+
	Genital hypoplasia	+	+	Hypothalamic- pituitary dysfunction	+	+
	Growth deficiency	-	+			
	Heart	-	+	Malformation mediastinal organs (heart, esophagus)	-	+
	TE fistula	-	+			
	Cleft lip/palate	+	+			
	Characteristic facies	-	+			

By Blake's criteria, the patient of Kim does not have CHARGE, but Delahaye's has possible CHARGE. By Verloes' Criteria, the patient of Kim et al does not have CHARGE, while the patient from Delahaye has atypical CHARGE.

Table 7

Three *CHD7* mutations in IHH/KS patients as reported by Jongmans et al [Jongmans et al. 2009]

	Blake Criteria	1	2	3	Verloes Criteria	1	2	3
Major	Coloboma	-	-	+	Coloboma	-	-	+
	Choanae	-	-	-	Choanae	-	-	-
	Cranial nerve	+	+	+				
	CHARGE ear	-	+	-	Canals	-	+	-
Minor					Rhombencephalic dysfunction	-	+	+
					Ear (middle & external)	-	+	-
	Developmental delay	-	-	+	Mental retardation	-	+	+
	Genital hypoplasia	+	+	+	Hypothalamic-pituitary dysfunction	+	+	+
	Growth deficiency	+	-	+				
	Heart	-	-	-	Malformation mediastinal organs (heart, esophagus)	-	-	-
	TE fistula	-	-	-				
	Cleft lip/palate	-	+	+				
	Characteristic facies	-	-	-				

Mutation 1 is E2935X; mutation 2 is I2117N; the correct notation for mutation 3 (patient 8 in their report) is R2024X (corrected from R2935X).