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Genetics of congenital hypogonadotropic hypogonadism in Denmark

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ABSTRACT

Congenital hypogonadotropic hypogonadism (CHH) is a rare disorder characterized by incomplete/absent puberty caused by deficiency or defective action of gonadotropin-releasing hormone (GnRH). The phenotypic features of patients with CHH vary from genital hypoplasia and absent puberty to reversal of HH later in life. We examined the genetics and clinical features of CHH in Denmark. Forty-one male patients were screened for mutations in *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *GNRHR*, *TAC3*, *TACR3*, and *KISS1R*. *CHD7* was screened in two patients with hearing loss. In 12 patients, a molecular genetic cause for CHH was found. Four patients had mutations in *KAL1* (C105VfsX13, C53X, ex5-8del, R257X), and five in *FGFR1* (G97S, R209C, A512V, R646W, and c.1614C>T, (p.I538I), predicted to affect splicing). All 9 had severe HH (cryptorchidism and/or micropenis), and 2 had cleft lip/palate. One patient with a previously reported homozygous R262Q mutation in *GNRHR* displayed fascinating temporal variation in his phenotype. Two patients with hearing loss had *CHD7* mutations (c.7832_7841del (p.K2611MfsX25) and c.2443-2A>C), confirming that CHH patients with CHARGE syndrome-associated features should be screened for mutations in *CHD7*.

1. Introduction

Congenital hypogonadotropic hypogonadism (CHH) is a rare disorder characterized by incomplete or absent puberty caused by the lack or deficient number of hypothalamic gonadotropin-releasing hormone (GnRH) neurons, disturbed secretion or action of GnRH, or both [Seminara et al., 1998]. When HH presents with deficient sense of smell (anosmia), the condition is termed Kallmann syndrome (KS). X-linked, autosomal dominant, and autosomal recessive, as well as di- and oligogenic inheritance of CHH have all been described [Falardeau et al., 2008; Pitteloud et al., 2007a; Sykiotis et al., 2010], and several genes have been connected with the disorder, including *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *CHD7*, *GNRHR*, *GNRH1*, *KISS1R*, *KISS1*, *TAC3*, and *TACR3* [Bianco and Kaiser, 2009; Brioude et al., 2010]. In the majority of cases, however, the molecular genetic cause remains unresolved, implying the existence of additional genes underlying the condition [Martin et al., 2011]. We have previously described the phenotypic and genotypic features of Finnish patients with CHH [Laitinen et al., 2011a; Laitinen et al., 2012a]. They had mutations in *FGFR1*, *KAL1*, *CHD7*, or *GNRHR*, whereas biallelic mutations in *KISS1*, *KISS1R*, *TAC3*, *TACR3*, *PROK2*, *PROKR2* or *GNRH1* genes were not found [Laitinen et al., 2011a; Laitinen et al., 2012a]. Herein, we describe genotypic and phenotypic features of CHH in another Nordic country, as a relatively large series of Danish patients was examined.

2. Patient data

All patients with hypogonadotropic hypogonadism in our tertiary referral center (pediatric endocrinology and andrology) were identified through our register. Thus, patients registered with an ICD10 code corresponding to IHH (DE23.0D) were included. The diagnosis was validated by careful evaluation of the patient record files. All clinical (medical history, phenotypic description, auxological data) and biochemical data were reordered, and results from DEXA and brain MRI included when available, as well as the sense of smell, if reported. The data on sense of smell are based on patient records; tested or self-reported. DNA was collected from our

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biobank and available in 41 male patients with CHH (our unit primarily diagnoses and treats male patients).

3. Methods

DNA from peripheral blood lymphocytes was purified using the QuickGene-810 Nucleic Acid Isolation System (Fujifilm, Life Science-Products, Tokyo, Japan), by means of the QIAmp 96 DNA Blood kit (Qiagen, Inc., Chatsworth, CA, USA), or Nucleo Spin 96 Blood kit (Macherey–Nagel, Select Science Ltd, Corston, UK). Forty-one patients with congenital HH were screened for mutations in *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *GNRHR*, *TAC3*, *TACR3*, and *KISS1R*. In addition, *CHD7* was screened for mutations in two patients with hearing loss. The *GNRHR* mutations Q106R and R262Q as well as the *TAC3* M1L mutation were also screened in 95 healthy prepubertal girls from The COPENHAGEN Puberty Study [Aksglaede et al., 2009; Tommiska et al., 2011]. The coding exons and exon-intron boundaries of the genes were PCR-amplified from the genomic DNA of the patients. PCR products were purified with ExoSAP-IT treatment (Amersham Biosciences, Piscataway, NJ, USA), and bi-directionally sequenced using the ABI BigDyeTerminator Cycle Sequencing Kit (v3.1) and ABI Prism 3730xl DNA Analyzer automated sequencer (Applied Biosystems, Foster City, CA, USA). The sequences were aligned and read with Sequencher® 4.9 software (Gene Codes Corporation, AnnArbor, MI, USA). All primer sequences and PCR conditions are available upon request.

The effects of identified missense mutations were predicted with the web version of PolyPhen-2 software (v.2.2.2). The effect on splicing of the identified synonymous change in *FGFR1* was predicted with the online bioinformatics tool Human Splicing Finder (v. 2.4.1).

4. Ethical issues

Patients gave their informed consent, and the study was approved by the regional ethical committee (KF01328087) and the Danish Data Protection Agency (2006-41-7251).

5. Results and discussion

The patients enrolled to this study were from a single tertiary referral center, in which the patients with CHH are followed up from birth to senescence. This system is probably beneficial for the patients, as the potentially vulnerable transition period from the pediatric to adult health care system can be avoided [Laitinen et al., 2012b]. Similar to our earlier results in Finnish patients [Laitinen et al., 2011a], a relatively high proportion of Danish males with CHH had a history of microphallus (23/41, 56%), a phenotypic finding consistent with profound neonatal congenital gonadotropin deficiency. Thirteen patients were reported to have a normal sense of smell (patient's own statement or tested), and 16 were hyposmic/anosmic; for 12 patients this was not reported at all. Because of the incomplete data on olfaction, *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *GNRHR*, *TAC3*, *TACR3*, and *KISS1R* were analyzed in all patients regardless of the reported sense of smell. *CHD7* was screened in two patients with hearing loss. Twelve (29%) of the 41 Danish patients with CHH were detected as having a conclusive mutation in *FGFR1*, *KAL1*, *GNRHR*, or *CHD7*. The phenotypes of these patients are summarized in Table 1.

Five patients (12%) had a mutation in *FGFR1* (c.289G>A (p.G97S), c.625C>T (p.R209C), c.1535C>T (p.A512V), c.1936C>T (p.R646W), and a synonymous change c.1614C>T, (p.I538I), predicted to affect splicing). They all had cryptorchidism and/or

Table 1
Clinical features of the Danish HH patients with *FGFR1*, *KAL1*, *GNRHR*, and *CHD7* mutations.

Proband	Family history	Gene	Mutation	History of			Olfaction		Associated phenotypes
				Micro-penis	Cryptor-chidism	Puberty	Self-reported	Clinical examination	
1	Anosmic father	*FGFR1*	c.625C>T (p.R209C)	Yes	Yes	No[a]		A	
2	No	*FGFR1*	c.1936C>T (p.R646W)	Yes	Yes	No		H	Cleft lip or palate, tooth agenesis
3	Brother with cleft lip and palate, tooth agenesis	*FGFR1*	c.1535C>T (p.A512V)	Yes	Yes	No	H		Cleft lip or palate; normal MRI
4	No	*FGFR1*	c.1614C>T (p.I538I, affects splicing[b])	Yes		No	N		
5	Two anosmic sons	*FGFR1 TAC3*	c.289G>A (p.G97S)[d] c.1A>T (p.M1L)	Yes	No	Partial[c]	A		
6	No	*KAL1*	c.309C>A + c.310delT (p.S103R + p.C105VfsX13)			No		A	Normal MRI
7	Brother with KS	*KAL1*	c.154_157duplAGGT (p.C53X)		Yes	No		A	Hypoplastic olfactory groove and sulci in MRI; olfactory bulbs not visible, renal US normal
8	No	*KAL1*	del ex5-8	Yes	Yes	No			Hearing loss, rheumatoid arthritis, normal MRI
9	No	*KAL1*	c.769C>T (p.R257X)[e]		Yes	No	A		
10	No	*GNRHR*	c.785G>A/c.785G>A (p.R262Q/p.R262Q)[f]			Delayed; reversal; LOH	N		
11	No	*CHD7*	c.7832_7841del (p.K2611MfsX25)	Yes	Yes	No		A	Cleft lip and palate, esophagotracheal fistula, hearing loss
12	No	*CHD7*	c.2443-2A>C	Yes	Yes	No	N		Syndactyly, hearing loss

Forty-one Danish patients with congenital hypogonadotropic hypogonadism (CHH) were screened for mutations in *KAL1*, *FGFR1*, *FGF8*, *PROK2*, *PROKR2*, *GNRHR*, *TAC3*, *TACR3*, and *KISS1R*. In addition, *CHD7* was screened for mutations in two patients with hearing loss. In 12 patients, a molecular genetic cause for CHH was found. Mutations were found in *KAL1*, *FGFR1*, *GNRHR*, and *CHD7*.

KS, Kallmann syndrome; LOH, late-onset hypogonadism; A, anosmia; H, hyposmia; N, normosmia; MRI, magnetic resonance imaging; US, ultrasound.

[a] Testicular volume less than 4 mL.
[b] Predicted by Human Splicing Finder.
[c] Testicular volume 6 mL at the age of 32.
[d] Mutation first reported in Jarzabek et al. [2012].
[e] Mutation previously reported in Hardelin et al. [1993] and Bhagavath et al. [2007].
[f] Case previously reported in Tommiska et al. [2013].