Genotypic and phenotypic spectrum of *CCDC141* variants in a Chinese cohort with congenital hypogonadotropic hypogonadism

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Abstract

Objective: To identify *CCDC141* variants in a large Chinese cohort with congenital hypogonadotropic hypogonadism (CHH) and to assess the contribution of *CCDC141* to CHH.

Design: Detailed phenotyping was conducted in CHH patients with *CCDC141* variants and co-segregation analysis was performed, when possible.

Methods: Whole-exome sequencing was performed in 177 CHH patients and 450 unrelated, ethnically matched controls from China.

Results: Seven novel *CCDC141* rare sequencing variants (RSVs) were identified in 12 CHH pedigrees. Four of the variants were private mutations; however, p.Q409X, p.Q871X and p.G1488S were identified in more than one patient. Up to 75% (9/12) of patients had mutations in other CHH-associated genes, which is significantly higher than CHH patients without *CCDC141* RSVs. The co-segregation analysis for eight CHH families showed that 75% (6/8) *CCDC141* RSVs were inherited from their fertile parents. Over half (58.3%, 8/18) of the patients exhibited other clinical deformities in addition to hypogonadism. One patient harbouring a *CCDC141* RSV showed a reversal of CHH after sex-steroid replacement.

Conclusions: Our results broaden the genotypic spectrum of *CCDC141* in CHH, as *CCDC141* RSVs alone do not appear sufficient to cause CHH. The phenotypic spectrum in patients with *CCDC141* RSVs is much wider than originally believed.

European Journal of
Endocrinology
(2020) **183**, 245–254

Introduction

Congenital hypogonadotropic hypogonadism (CHH), due to a deficiency in the secretion of gonadotropin-releasing hormone, is a rare genetic disorder characterised by abnormal puberty and infertility and is frequently associated with non-reproductive phenotypes. Anosmia/hyposmia (i.e. lack of or a defective sense of smell),

termed Kallmann syndrome (KS) in CHH patients, has been observed to co-occur in ~50% of them, with the remaining being normosmic (nCHH) (1). Other phenotypes associated with CHH include synkinesia, cerebellar ataxia, hearing loss, eye movement disorders, renal aplasia, high arched palate, labial or palatine cleft,

skeletal anomalies, dental agenesis and cardiovascular defects (2, 3, 4, 5).

During embryonic development, GnRH neurons originating in the nasal placode will migrate along the olfactory axons towards the CNS. Upon arriving at the CNS, olfactory axons will sneak into the olfactory bulb, whereas GnRH neurons will migrate caudally on a bundle of non-sensory olfactory axons towards the hypothalamus (6). Pathological changes in any of these processes can lead to CHH. To date, over 30 genes have been found to be associated with CHH and many encode receptor-ligand pairs, such as *FGFR1* (OMIM 136350) and *FGF8* (OMIM 600483), *GNRHR* (OMIM 138850) and *GNRH1* (OMIM 152760), *TACR3* (OMIM 162332) and *TAC3* (OMIM 162330), *KISS1R* (OMIM 604161) and *KISS1* (OMIM 603286), as well as *PROKR2* (OMIM 607123) and *PROK2* (OMIM 607002) (7, 8, 9, 10, 11, 12, 13). Furthermore, disruption in the dynamic remodelling of the cell's cytoskeleton during migration of GnRH neurons may also result in CHH (14, 15, 16). Recently, Topaloglu *et al.* reported that the cytoskeletal protein, *CCDC141*, is expressed in GnRH neurons as well as olfactory fibres and that its knockdown reduced migration of GnRH neurons (17). They also identified inactivating *CCDC141* variants in 3.3% of CHH individuals (18); however, the contribution of *CCDC141* to CHH, in particular, has not been reported in other cohorts.

Our objective in this study was to identify *CCDC141* variants in a Chinese CHH cohort of 177 patients and to assess the contribution of *CCDC141* to CHH.

Subjects and methods

Subjects and clinical evaluation

This study consisted of 177 unrelated patients (146 males, mean age at diagnosis: 21.9 years), who were previously diagnosed with CHH based on (1) failure to enter spontaneous puberty by age 18 or through medically induced puberty before this age, (2) low sex-steroid levels in association with inappropriately low/normal gonadotropin levels and an abnormal/normal response to a GnRH stimulation test, (3) exclusion from other anterior pituitary dysfunctions, (4) normal radiological imaging of the hypothalamic and pituitary areas, and (5) for KS, anosmia/hyposmia according to formal testing (UPSIT score <5th percentile by age; University of Pennsylvania Smell Identification Test). All patients (112 with KS and 65 with nCHH) were recruited from the People's Hospital

of Henan Province (Zhengzhou, China) and Xiangya Hospital (Changsha, China).

The brain and olfactory bulbs were examined using MRI, when possible. Bone densitometry, body weight, skin texture, height, limb development, tooth development, testis size, sperm count and medication history were collected and analysed retrospectively. The patients or their adult parents signed an informed consent form. A group of 450 unrelated, ethnically matched individuals served as the control. The study was approved by the ethics committee of the School of Life Sciences, Central South University (No. 2017030801).

Whole-exome sequencing and co-segregation analysis

Genetic testing was performed by whole-exome sequencing (WES) according to previously outlined methods (19). Briefly, genomic DNA was fragmented and 180–300 bp products were chosen. Exons and flanking intronic regions were captured by Agilent SureSelect Human All Exon V6 enrichment platform (Agilent Technologies) and subsequently sequenced on Illumina HiSeq 4000 platform (Illumina, San Diego, CA). The average sequencing depth was of 50×. Copy number variations analysis was performed using ExomeDepth algorithm as described previously (20). We screened for rare sequencing variants (RSVs, <1% in the dbSNP, ExAC, ESP6500, Genome Aggregation Database (gnomAD), Chinese Millionome Database (CMDB) and 1000 Genomes) in *CCDC141* and a panel of genes involved in CHH, including *AXL*, *DUSP6*, *FEZF1*, *FGF8*, *FGF17*, *FGFR1*, *FLRT3*, *GNRH1*, *GNRHR*, *HESX1*, *HS6ST1*, *IL17RD*, *ANOS1*, *KISS1*, *KISS1R*, *NSMF*, *PROK2*, *PROKR2*, *SEMA3A*, *SEMA3E*, *PLXNA1*, *SEMA7A*, *SPRY4*, *TAC3*, *TACR3*, *WDR11*, *LEP*, *LEPR*, *NROB1*, *PCSK1*, *STUB1*, *CHD7*, *DMXL2*, *SOX2*, *PNPLA6*, *RNF216*, *SOX10* and *OTUD4* (21, 22, 23). Variants were named according to the Human Genome Variation Society's nomenclature (24). The detected variants were verified by PCR-Sanger sequencing with special primers (Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article). Co-segregation analysis was carried out on all available family members.

Interpretation of RSVs

Mutations were interpreted according to the guidelines by the American College of Medical Genetics and Genomics (ACMG) (25). Missense variants were automated in InterVar (<http://wintervar.wglab.org/>) and supplemented

manually, including inputting information on phenotypes, inheritance and segregation. Variants were divided into five categories: benign (B), likely benign, uncertain significance (U), likely pathogenic (LP) and pathogenic (P). Furthermore, four commonly used *in silico* tools were utilised to predict the function of the detected variants, including Polyphen2, MutationTaster, SIFT and Combined Annotation Dependent Depletion. A variant was considered deleterious if it was predicted to be damaging by at least two of the *in silico* tools.

Results

Phenotypic and genotypic summary of the CHH patients

A total of 177 unrelated patients (146 men) were included in this study. Among them, 63.3% (112/177) were diagnosed with KS and 36.7% (65/177) with nCHH, which is comparable to other cohorts (2, 3, 4, 5) (Table 1). It should be noted that up to 24% of the males in this cohort presented with unilateral or bilateral cryptorchidism, whereas only 5.9% males presented with cryptorchidism in a CHH cohort studied by Nair *et al.* (4). Additional pathologies were also identified in these patients, including congenital hearing loss (9.7%), dental agenesis (11.8%), skeletal anomalies (10.1%), ocular defects (10.1%), ataxia (2.8%), cleft lip/palate (1.7%), renal agenesis (4%), mirror movement (2.8%) and external ear defects (4%). Furthermore, family histories of CHH, delayed puberty or isolated congenital olfactory defects were identified in 21.9% (43/177) of patients. The occurrence rates of some of these pathologies within other CHH/KS cohorts are listed in Table 1.

Exome sequencing was performed on 177 CHH probands and 66.1% (117/177) of patients harboured deleterious RSVs in 34 CHH-associated genes (Fig. 1). The most frequently mutated gene was *PROKR2* (14.7% of probands), followed by *CHD7* (10.2%), *ANOS1* (8.5%) and *FGFR1* (8.5%). No mutation was identified in *TAC3*, *HESX1*, *FLRT3*, *LEP* and *NROB1*.

CCDC141 RSVs were significantly enriched in CHH patients vs controls

Seven heterozygous *CCDC141* RSVs, including two protein truncating variants and five missense variants, were identified in 12 out of the 177 CHH probands (Fig. 2). Only two missense RSVs (*p.G1488S CCDC141* and

p.P7L CCDC141) were found in the controls. The allele frequency of *CCDC141* RSVs was significantly higher in CHH patients compared to the controls (6.8%, 12/177 vs 0.2%, 2/450; $P=0.000$, Fisher's exact test).

Four out of seven *CCDC141* variants identified in the patients were private mutations (*p.R123G*, *p.Q256K*, *p.R1384S* and *p.Q1407K*); however, *p.Q409X*, *p.Q871X* and *p.G1488S* were identified in multiple unrelated patients. These variants were not found in the controls except *p.G1488S CCDC141*. The occurrence frequencies of *p.R123G*, *p.Q409X*, *p.Q871X* and *p.G1488S* variants were 0.00008, 0.0002, 0.000026 and 0.0015, respectively, in the gnomAD database, whereas the other RSVs were not detected in this database (Table 2). All RSVs were predicted to cause disease according to MutationTaster, and all variants except *p.R1384S* were predicted to be probably damaging or damaging according to PolyPhen-2 (Table 2). Per the ACMG guidelines, two variants (*p.Q409X* and *p.Q871X*) were pathogenic and the remaining were of uncertain significance (Table 2).

Nine out of 12 probands also carried variants in other CHH-associated genes, including *PCSK1*, *ANOS1*, *PROKR2*, *AXL*, *SOX10*, *HS6ST1*, *PNPLA6* and *FGFR1* (Table 2). The frequency of one patient carrying variants in at least two genes was significantly higher in the *CCDC141* RSVs' positive group (75%, 9/12) relative to the *CCDC141* RSVs' negative group (31.4%, 33/105; $P=0.008$, Fisher's exact test).

Clinical characteristics of CHH patients harbouring CCDC141 RSVs

Clinical data from the 12 probands with *CCDC141* RSVs are summarised in Table 3. The mean age at diagnosis was 22.6 years. Seven males were affected with KS, while five patients (three males and two females) had nCHH. Cryptorchidism occurred in four KS patients and one nCHH patient. Eight patients exhibited non-reproductive clinical deformities in addition to hypogonadism. Dental agenesis was detected in three patients (probands 2, 4 and 11), mainly manifesting as small and missing teeth. Hypopigmentation was seen in proband 8, carrying *p.Q871X CCDC141* and *p.L80V SOX10* mutations. Neurological defects were also common in these patients. Colour weakness was found in probands 3 and II:4 in Family 2. Congenital high myopia was found in probands 4 and 10. Congenital anhidrosis was seen in proband 4. Delayed mental development was seen in proband 11.

Table 1 Prevalence of main additional phenotypes in CHH among different CHH cohorts.

Phenotypes	CHH				KS				
	This report (n = 177)	Quinton <i>et al.</i> (2) (n = 215)	Nair <i>et al.</i> (4) (n = 135)	Bonomi <i>et al.</i> (5) (n = 459)	This report (n = 112)	Costa-Barbosa <i>et al.</i> (3) (n = 219)	Quinton <i>et al.</i> (2) (n = 112)	Nair <i>et al.</i> (4) (n = 79)	Bonomi <i>et al.</i> (5) (n = 192)
Anosmia/hyposmia	63.3%	52%	68.4%	41.8%	100%	100%	100%	100%	100%
Cryptorchidism	24%	NA	5.9%	NA	26.8%	NA	NA	7.6	NA
Mirror movement	2.8%	20%	11.5%	6.1%	3.5%	19%	31%	13.3%	11%
Renal agenesis	4.0%	10%	1.40%	5.1%	5.4%	8%	15%	1.4%	12%
Ocular defects	10.1%	20%	NA	NA	12.9%	NA	27%	NA	NA
Hearing loss	9.7%	5%	16%	7%	11.7%	15%	8%	18.7%	6%
Cleft lip/palate	1.7%	5%	0.7%	NA	2.7%	6%	4%	0	NA
Dental agenesis	11.8%	NA	0.7%	12%	16%	14%	NA	0	15%
Limb deformities	4.0%	NA	31.6%	NA	5.4%	5%	NA	27.4%	NA
Skeletal deformities	10.1%	NA	NA	NA	12.5%	13%	NA	NA	NA
External ear defect	4.0%	NA	NA	NA	4.5%	NA	NA	NA	NA
Ataxia	2.8%	NA	NA	NA	1.8%	NA	NA	NA	NA

NA, not available.

Pedigree investigation

We also analysed the variants in the available family members of eight probands (Fig. 3 and Table 3). Interestingly, six probands (1, 2, 3, 4, 5 and 11) inherited their *CCDC141* RSVs from the unaffected parents, whereas probands 7 and 8 carried *de novo* *CCDC141* RSVs.

In Family 1, the proband (III:7) was diagnosed as KS, carrying *p.N355I DUSP6* and *p.Q1407K CCDC141* variants. His brother (III:8), carrying *p.N355I DUSP6*, showed anosmia and his father (II:5), harbouring *p.Q1407K CCDC141*, was normal. In Family 2, the proband (III:2) inherited a *p.R123G CCDC141* mutation from his father and a *p.V162I PCSK1* mutation from his mother. The proband showed dental agenesis and ataxia in infancy, in addition to nCHH. Both parents showed normal puberty and were fertile. However, his father (II:4), carrying the *p.R123G CCDC141* mutation, showed colour weakness. His mother (II:5), carrying the *p.V162I PCSK1* mutation, showed sparse armpit hair. The maternal uncle (II:8), harbouring the *p.V162I PCSK1* mutation, showed hypospadias and non-liquefaction of sperm, but displayed normal sexual characteristics and had a baby after one cycle of treatment. Interestingly, the maternal grandma (I:4), who also carried *p.R123G CCDC141* and *p.V162I PCSK1*, manifested delayed puberty and no armpit or pubic hair, but showed normal fertility (Fig. 3 and Table 3).

In Family 3, the proband (II:2), carrying trigenic mutations of *p.Q256K CCDC141*, *p.R270H PROKR2* and *p.R617Q SEMA3A*, showed left cryptorchidism and colour weakness in addition to nCHH. His mother (I:2), carrying two mutations of *p.R270H PROKR2* and *p.R617Q SEMA3A*, and his father (I:1), carrying only *p.Q256K CCDC141*, were unaffected. And his sister (II:3), with the *p.Q256K CCDC141* and *p.R270H PROKR2* mutations, showed reduced armpit and pubic hair, but normal puberty and fertility (Fig. 3 and Table 3).

In Family 4, the proband (II:5) inherited the *p.Q409X CCDC141* variant from his normal mother. Family analysis found that his sister (II:2) manifested delayed puberty and two of his nieces (III:2 and III:3) showed dental agenesis. In Family 5, the proband (II:1) inherited two maternal variants of *p.Q409X CCDC141* and *p.Q174R GNRHR*, as well as a paternal variant of *p.Q65X GNRHR*. Both his father and mother showed normal puberty.

In Family 8, the proband (III:9) carried a *de novo* *p.Q871X CCDC141* variant and inherited a *p.L80V SOX10* mutation from his mother. He showed bilateral cryptorchidism, hypopigmentation, a white forelock, and

Figure 1

Prevalence of deleterious rare mutations in 34 CHH-associated genes in this cohort.

mild hearing loss in addition to KS. His mother (II:11) and maternal grandma (I:2), both carrying the *p.L80V SOX10* mutation, showed hypopigmentation, a white forelock and anosmia, but had normal reproductive function and hearing. The maternal uncle (II:1) showed hypogonadism, but with self-reported normal smell. It is a pity we could not determine his genotype. Additionally, two maternal cousins (III:4 and III:6) showed normal puberty and smell, but displayed repeated stillbirth. It seemed that *p.L80V SOX10* may lead to various reproductive and non-reproductive pathologies and that *CCDC141* might play an additive role in reproductive pathologies.

In Family 11, the proband (II:1) carried a *de novo p.E670K FGFR1* variant as well as two paternal variants of *p.G1488S CCDC141* and *p.G24S HS6ST1*, manifested KS with delayed mental development. However, his father, carrying two variants of *p.G1488S CCDC141* and *p.G24S HS6ST1*, appeared normal.

Phenotypes in patients with the same *CCDC141* mutations

p.Q409X CCDC141 was seen in three male probands (4, 5 and 6) and two of them (probands 4 and 5) were diagnosed as nCHH, while proband 6 was diagnosed as KS.

These patients showed some similar phenotypes, such as Tanner II pubic hair, small penis (<3 cm in length), small testicular volume (<5 mL), a nearly identical BMI (23–23.9 kg/m²) and low bone mass. Proband 5 had no additional phenotypes, whereas probands 4 and 6 did. Proband 4 had congenital anhidrosis (displayed as rarely sweating and feeling a needle-like sting when hot), congenital high myopia and dental agenesis with loss of some teeth. Proband 6 suffered from dental agenesis and right cryptorchidism.

Probands 7 and 8, carrying the *de novo p.Q871X CCDC141* mutation, had a micropenis, cryptorchidism, no pubic hair and a normal BMI. However, proband 8, who carried a *p.L80V SOX10* mutation, displayed hypopigmentation, a white forelock and hearing loss.

p.G1488S CCDC141 was seen in two male probands (10 and 11) and one female proband (12). Probands 10 and 11 were diagnosed as KS and were overweight, with a BMI of 31 kg/m² and 34 kg/m², respectively. They also had a micropenis and a small testicular volume (<5 mL). However, proband 10 suffered from congenital high myopia, whereas proband 11 showed right cryptorchidism, delayed mental development and a delay in major motion during their early years. Proband 12 was 20 years of age with a BMI of 24 kg/m². She experienced menarche at the age of 17, followed by irregular and rare menstrual cycles before suffering from hypogonadotropic hypogonadism (HH) at the age of 18. Fortunately, after a cycle of treatment, she displayed a reversal of HH.

Discussion

In this study, we systematically analysed the *CCDC141* RSVs in a large cohort of Chinese CHH patients via WES. Overall, *CCDC141* RSVs were significantly enriched in the CHH cohort vs our ethnically matched controls (6.8% vs 0.2%, *P*=0.000), supporting the involvement of *CCDC141* in CHH. However, up to 75% of *CCDC141* RSVs carriers had at least one mutation in other CHH-associated genes, and the frequency of multiple rare

Figure 2

Distribution of *CCDC141* RSVs identified in our study. Arabic numerals in the superscript represent the frequency of the mutation in this study. A full colour version of this figure is available at <https://doi.org/10.1530/EJE-19-1018>.

Table 2 The molecular genetic characteristics of the CHH probands with identified *CCDC141* variants.

ID	Dx	c.HGVS	p.HGVS	Segregation	MAF (gnomAD)		CMDDB	ACMG	In silico prediction			
					Matched	All			CADD	SIFT	Poly2	Mut T
1	KS	CCDC141: c.4219C>A	p.Q1407K	Paternal	0.001	0.0000828	0	U(PM1)	24.7	T	D	D
		DUSP6: c.1064A>T	p.N355I	Not paternal	0	0	0	LP	19.2	T	D	D
2	nCHH	CCDC141 c.367A>G	p.R123G	Maternal	0.0012	0.00008	0	U(PP1)	24.1	D	P	D
		PCSK1 c.484G>A	p.V162I	Paternal	0	0	0	U	32	D	D	D
3	nCHH	CCDC141c.766C>A	p.Q256K	Paternal	0	0	0	U(PM2)	24.5	D	P	D
		SEMA3A c.1850G>A	p.R617Q	Maternal	0.000058	0.000004	0	LB	21.4	T	B	D
		PROKR2 c.809G>A	p.R270H	Maternal	0.000058	0.0001	0	U	34	D	D	D
4	nCHH	CCDC141 c.1225C>T	p.Q409X	NA	0.0031	0.0002	0.0028	P(PVS1 PP1 PP3)	37	.	.	.
5	nCHH	CCDC141 c.1225C>T	p.Q409X	NA	0.0031	0.0002	0.0028	P(PVS1 PP1 PP3)	37	.	.	.
		GNRHR c.521A>G	p.Q174R	NA	0.0001	0.000008	0	U	24.2	D	P	D
		GNRHR c.193C>T	p.Q65X	NA	0	0	0	LP	37	.	.	.
6	KS	CCDC141 c.1225C>T	p.Q409X	NA	0.0031	0.0002	0.0028	P(PVS1 PP1 PP3)	37	.	.	.
		AXL c.449T>C	p.V150A	NA	0.0022	0.0002	0	LB	24	T	D	N
7	KS	CCDC141 c.2611C>T	p.Q871X	De novo	0.0003	0.000026	0	P(PVS1 PS2 PP3)	37	.	.	.
		ANOS1 c.1678G>A	p.V560I	De novo	0.011	0.0008	0.00834	U	2.6	T	B	N
8	KS	CCDC141 c.2611C>T	p.Q871X	De novo	0.0003	0.000026	0	P(PVS1 PS2 PP3)	37	.	.	.
		SOX10 c.238C>G	p.L80V	Maternal	0	0	0	LP	24.5	D	D	D
9	KS	CCDC141 c.4152G>C	p.R1384S	NA	0	0	0	U(PM2)	22.4	T	B	D
		PNPLA6 c.3371G>A	p.C1124Y	NA	0	0.000004	0	U	25	D	B	D
10	KS	CCDC141 c.4462G>A	p.G1488S	NA	0.0066	0.0015	0.0067	U(PS1 BS1)	34	D	D	D
11	KS	CCDC141 c.4462G>A	p.G1488S	Paternal	0.0066	0.0015	0.0067	U(PS1 BS1)	34	D	D	D
		HS6ST1 c.70 G>A	p.G24S	Paternal	0.0001	0.0005	0.0001	U	21.4	T	B	D
		FGFR1 c.2008G>A	p.E670K	De novo	0	0	0	LP	35	D	D	D
12	nCHH	CCDC141 c.4462G>A	p.G1488S	NA	0.0066	0.0015	0.0067	U(PS1 BS1)	34	D	D	D

Dx, diagnosis; HGVS, Human Genome Variation Society; KS, Kallmann syndrome; MAF, minor allele frequency in gnomAD database; matched, ethnically- matched population in gnomAD; NA, not available; all: all populations in gnomAD; CMDDB, Chinese millionome database. ACMG criteria: B, benign; LB, likely benign; LP, likely pathogenic; P, pathogenic; U, uncertain significance. CADD: Combined Annotation Dependent Deletion; CADD, SIFT, Poly2 (PolyPhen-2) and Mut T (Mutation Taster) were used for *in silico* prediction for the effect of the variants. SIFT: D, deleterious; T, tolerated. PolyPhen-2: B, benign; D, probably damaging; P, possibly damaging. Mutation Taster: A, disease_causing_automatic; D, disease_causing; N, polymorphism. PM1: Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation. PP1: Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease. PM2: Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium. PV51: null variant (nonsense, frameshift, canonical +/- 2 splice sites, initiation codon, single or multixon deletion) in a gene where LOF is a known mechanism of disease. PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.). BS1: Allele frequency is greater than expected for disorder. PS2: *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history. PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).

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Table 3 Clinical and laboratory findings of the CHH probands carrying *CCDC141* RSVs.

No.	Sex	Dx	Age	LH	FSH	TS	E	OB	Bone density	Other phenotypes
1	M	KS	23	0.6	0.57	0.1	9.7	Agenesis	low	Right cryptorchidism
2	F	nCHH	19	0.1	0.15	1.2	20	Normal	low	Dental agenesis, ataxia
3	M	nCHH	29	1.6	0.3	0.2	10	Normal	low	Left cryptorchidism, colour weakness
4	M	nCHH	26	0.14	0.61	0.3	22	NA	low	Dental agenesis, congenital anhidrosis, high myopia
5	M	nCHH	17	0.09	0.31	0.5	12.6	Normal	low	
6	M	KS	33	0.09	0.1	0.2	12.5	Hypogenesis	low	Right cryptorchidism
7	M	KS	23	0.1	0.1	0.5	13	Agenesis	low	
8	M	KS	19	0.14	0.1	0.1	12	Agenesis	osteoporosis	Bilateral cryptorchidism, HL hypopigmentation
9	M	KS	26	0.83	0.12	0.2	11	NA	osteoporosis	Right cryptorchidism
10	M	KS	19	0.6	0.56	0.8	15	Agenesis	low	Congenital high myopia
11	M	KS	19	0.34	0.12	0.3	12	NA	low	Dental agenesis, delayed mental development, cryptorchidism
12	F	nCHH	18	0.6	0.45	1.1	21	NA	low	

Age means the age of diagnosis. Normal adult men reference range for testosterone (TS): 1.75–7.81 ng/mL, for Estradiol (E2): <53 pg/mL, for luteinizing hormone (LH): 1.2–8.6 mIU/mL, and for follicle-stimulating hormone (FSH): 1.3–19.3 mIU/mL. Normal adult female reference range for TS: 0.1–1.5 ng/mL, for E2: 31–136 pg/mL, for LH: 1.9–12.5 mIU/L, and FSH 1.3–11 mIU/L.

Dx, diagnosis; F, Female; HL, hearing loss; KS, Kallmann syndrome; M, Male; NA, not available; nCHH, normosmic Idiopathic hypogonadotropic hypogonadism; OB, olfactory bulbs.

variants in one patient was significantly higher in the *CCDC141* RSVs' positive group relative to the *CCDC141* RSVs' negative group. Moreover, the co-segregation analysis for the eight available CHH families showed that 75% (6/8) of *CCDC141* RSVs were inherited from unaffected parents. In sum, our results confirm the findings from the pioneering investigation by Hutchins *et al.* (18) reporting that inactivating *CCDC141* variants at least partially contributes to CHH.

For some probands, mutations in other CHH-associated genes seemed sufficient to induce CHH, so the simultaneous *CCDC141* RSVs might only be 'transient mutations' that have no role in the phenotypes. For instance, proband 7 carried a *de novo* *p.V560I ANOS1* mutation, which alone may be sufficient to cause CHH in an X-chromosome-linked manner (26). Proband 8 carried a *p.L80V SOX10* mutation, which may lead to KS with autosomal dominant inheritance (27, 28). Indeed, a panel of *SOX10*-associated phenotypes, such as hypopigmentation, a white forelock and anosmia were segregated along with the *SOX10* mutation in this family. In addition, proband 5 carried a *p.Q409X CCDC141* mutation and compound heterozygous *GNRHR* mutations, which may have been sufficient to cause CHH (9). Interestingly, his mother, carrying two variants of *p.Q409X CCDC141* and *p.Q174R GNRHR*, appeared normal. Proband 11 inherited *p.G1488S CCDC141* and *p.G24S HS6ST1* from his unaffected father and also carried a *de novo* *p.E670K FGFR1* mutation, which may have been enough to result in CHH (29, 30).

In some circumstances, *CCDC141* seems to act synergistically with other CHH-associated genes to cause CHH. For instance, the KS phenotype of proband 1 (III:7 in Family 1) may be the synergistic effect of *p.N355I DUSP6* and *p.Q1407K CCDC141*, as his father (II:5), with *p.Q1407K CCDC141*, and brother (III:8), with *p.N355I DUSP6*, showed normal puberty. Interestingly, seven individuals in this pedigree showed variations in anosmia; however, we were not able to elucidate the genotypes for most due to their refusal. Proband 2 (III:2 in Family 2), carrying *p.R123G CCDC141* and *p.V162I PCSK1*, manifested dental agenesis, ataxia in infancy and nCHH. Curiously, individuals who only carried one of these variants (II:4, II:7, II:8 with *p.R123G CCDC141*; II:5, II:10 with *p.V162I PCSK1*) had normal puberty (Fig. 2 and Table 3). Of note, the proband's maternal grandma (I:4), who also carried these two variants, manifested delayed puberty and no armpit or pubic hair, but showed normal fertility. It thus seems that delayed/absent puberty are consequences from mutations in both *CCDC141* and *PCSK1*. Proband 3 (II:2 in Family 3), with three mutations (*p.Q256K CCDC141*, *p.R270H PROKR2* and *p.R617Q SEMA3A*), had KS with cryptorchidism and colour weakness. However, individuals who only carried one or two mutations (I:2, II:3, I:1) had normal puberty and fertility. In this circumstance, it seems that these three mutations are necessary for the pathogenicity of CHH, though we cannot rule out the possible contribution of other unidentified genes or environmental factors to the CHH phenotypes in these pedigrees.

Patients who carried monogenic *CCDC141* RSVs showed some phenotypes that were unrelated to those known to pertain to the *CCDC141* mutant, such as dental agenesis for proband 4 and anosmia for proband 10, implying that they may carry mutations in other yet unidentified CHH-related gene(s). Actually, in the previous study, oligogenic inheritance/locus heterogeneity and spontaneous clinical reversals were commonly seen in patients with *CCDC141* RSVs (18). Therefore, our data reinforce the notion that *CCDC141* RSVs may be implicated in the 'second hits' to CHH and account for the variable expressivity and incomplete penetrance of CHH in some patients.

CCDC141, a coiled-coil domain-containing protein, functions as a cross-linker between Myosin II and the disrupted-in-schizophrenia 1 (DISC1)-centrosome complex. A malfunctioning *Ccdc141* may lead to neurological and psychiatric disorders (15, 31). Recently, Yanagi *et al.* reported that *CCDC141*-deficient mice exhibited delayed radial migration with aberrant neural circuit formation, as well as repetitive behaviours, hyperactivity and social abnormalities typically observed in autism spectrum disorder (32). In this cohort, proband 2 was affected with ataxia in infancy, proband 4 manifested congenital anhidrosis and proband 11 showed delayed mental development. However, all patients with *CCDC141* RSVs in the previous study were otherwise healthy with normal anthropometric measurements (18), so our study greatly broadens the phenotypic spectrum of *CCDC141* more than originally anticipated.

Interestingly, some patients with *CCDC141* RSVs also showed vision-related symptoms. Proband 4 and 10 presented with congenital high myopia, and both proband 3 and the *CCDC141* RSVs' positive father of proband 2 had colour weakness. It will, therefore, be intriguing to investigate potential roles of *CCDC141* in vision development.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-19-1018>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

Funding

This project is financially supported by National Natural Science Foundation of China (81770780, 81728013, 31972913 and 81900948),

the Key Research and Development Programs from Hunan Province (2018DK2010, 2018DK2013), Training Program for Excellent Young Innovators of Changsha (kq1802019), Natural Science Foundation of Hunan Province (2018JJ3573) and Foundation of Education Bureau of Hunan Province (18A493), Strategic Priority Research Program of Central South University (ZLXD2017004), and Guangdong Key Project in 'Development of new tools for diagnosis and treatment of Autism' (2018B030335001).

Acknowledgements

The authors would like to thank the patients for their participation in the study as well as their family members for their collaboration.

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Received 14 December 2019

Revised version received 9 May 2020

Accepted 8 June 2020