Supporting Information

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SI Materials and Methods

Study Subjects Selection. IGD in this cohort was defined by: (*i*) absent or incomplete puberty by age 18 y; (*ii*) serum testosterone <100 ng/dL in men or estradiol <20 pg/mL in women in the face of low or normal levels of serum gonadotropins; (*iii*) otherwise normal anterior pituitary function; (*iv*) normal serum ferritin concentrations; and (*v*) normal MRI of the hypothalamic-pituitary region (1). Both self-report of olfaction as well as University of Pennsylvania Smell Identification Test (UPSIT) scores were used to classify KS and nIGD (2). A diagnosis of typical CHARGE requires involvement of three major organ systems (coloboma, choanal atresia, and hypoplastic semicircular canals) or two major and two of five minor criteria (cranial nerve abnormalities, abnormal external/middle ear, intellectual disability, hypothalamic-pituitary dysfunction, and malformation of mediastinal organs). Partial/incomplete CHARGE syndrome requires two major and one minor criteria whereas atypical CHARGE requires two major or one major and three minor criteria by Verloes (3).

In Silico Prediction of Novel RSVs. In silico prediction of the likely effects of RSVs was performed using web-based software programs to analyze novel missense and splice site variants: PolyPhen-2 (genetics.bwh.harvard.edu/pph2/), Sorting Intolerant From Tolerant (SIFT) (sift.jcvi.org/), PMUT (mmb2.pcb.ub.es:8080/PMut/) and MutationTaster (www.mutationtaster.org/) for missense changes and the NNSPLICE (www.fruitfly.org/seq_tools/splice.html), EX-SKIP (ex-skip.img.cas.cz/), and Human Splicing Finder (www.umd.be/HSF/) for splice-site variants. Multiple protein-sequence alignment of CHD7 with its orthologs was investigated among nine species, including *Homo sapiens* (human), *Pan troglodytes* (chimpanzee), *Mus musculus* (mouse), *Canis families* (dog), *Bos taurus* (cow), *Gallus gallus* (chicken), *Xenopus* (frog), and *Danio rerio* (zebrafish) using ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2/).

Three-Dimensional Structural Modeling. There are no experimentally derived structures of each domain of the CHD7 protein as yet. Therefore, an automated homology structural model was constructed using SWISS-MODEL (swissmodel.expasy.org/) (4). The homology models of the CHD7 protein were constructed using YASARA v12.6.3 (www.yasara.org/). Modeling of the *CHD7* RSVs and the assessment of the effect on CHD7 stability was performed using the FoldX protein design algorithm to analyze the effect of RSVs on CHD7 stability (5). Protein stabilities were calculated as ΔG values that were estimated as the difference between the energy of the wild type protein and that of the mutant. RSVs increasing the calculated ΔG values more than 1 kcal/mol were considered to be potentially "destabilizing," as described previously (6).

1. Pitteloud N, et al. (2002) The role of prior pubertal development, biochemical markers of testicular maturation, and genetics in elucidating the phenotypic heterogeneity of idiopathic hypogonadotropic hypogonadism. *J Clin Endocrinol Metab* 87(1):152–160.
2. Lewkowitz-Shpuntoff HM, et al. (2012) Olfactory phenotypic spectrum in idiopathic hypogonadotropic hypogonadism: Pathophysiological and genetic implications. *J Clin Endocrinol Metab* 97(1):E136–E144.
3. Verloes A (2005) Updated diagnostic criteria for CHARGE syndrome: A proposal. *Am J Med Genet A* 133A(3):306–308.
4. Bordoli L, et al. (2009) Protein structure homology modeling using SWISS-MODEL workspace. *Nat Protoc* 4(1):1–13.
5. Van Durme J, et al. (2011) A graphical interface for the FoldX forcefield. *Bioinformatics* 27(12):1711–1712.
6. Bergman JE, et al. (2012) A novel classification system to predict the pathogenic effects of CHD7 missense variants in CHARGE syndrome. *Hum Mutat* 33(8):1251–1260.

Table S1. *CHD7* RSVs identified in IGD patients

Patient count	Pedigree no.	Sex	Age	Origin	Diagnosis	Nucleotide change	Amino acid change	Exon/Intron
1	1	M	23	Caucasian	KS	599T > A	M200K	2
2	2	M	27	Caucasian	KS	1046A > G	N349S	2
3	3	F	27	Caucasian	KS	1117C > T	L373F	2
4	4	M	44	Caucasian	KS	1175C > T	S392F	2
5	5	F	22	Caucasian	KS	1188G > T	M396I	2
6	6*	M	31	Caucasian	KS	2095A > G	S699G	3
7	7	F	71	NA	KS	2185A > G	K729E	3
8	9	M	NA	Caucasian	nIHH	2680A > G	T894A	9
9	10*	M	40	African American	nIHH	2780C > G	A927G	10
10	11*	M	NA	Caucasian	KS	2819C > T	P940L	10
11	12	M	NA	Caucasian	nIHH	2819C > T	P940L	10
12	13	M	NA	Caucasian	nIHH	2835+8T > C	Splice site	10
13	15	F	29	Asian	KS	3202–5T > C	Splice site	12
14	16*	F	24	NA	KS	3202–5T > C	Splice site	12
15	18*	M	27	Caucasian	KS	4084T > C	F1362L	19
16	19*	M	26	Caucasian	KS	4354–3C > G	Splice site	19
17	20	M	57	Caucasian	nIHH, adult-onset	120A > C/4565A > T	Q40H/D1522V	2/20
18	21	F	NA	Caucasian	KS	4847A > G	Y1616C	21
19	22	M	36	Caucasian	nIHH, adult-onset	5300+8C > T	Splice site	25
20	23	M	19	Caucasian	nIHH	5300+8C > T	Splice site	25
21	24	F	35	Caucasian	KS	5533G > A	G1845R	26
22	25	M	34	Asian	nIHH	5689G > A	E1897K	29
23	26*	F	45	Hispanic	KS	5858C > T	A1953V	29
24	27	M	67	Caucasian	KS	5895+2G > C	Splice site	29
25	28	M	NA	Caucasian	KS	5945G > A	G1982E	30
26	29	F	49	Caucasian	nIHH	6103+6T > C	Splice site	30
27	30	M	62	Caucasian	nIHH, adult-onset	6103+6T > C	Splice site	30
28	31	M	NA	Caucasian	KS	6103+7A > G	Splice site	30
29	32	M	28	African American	KS	6190A > G	I2064V	31
30	33*	F	NA	Caucasian	KS	6193C > G	R2065G	31
31	34	M	NA	Caucasian	nIHH	6411C > G	A2137G	31
32	35	M	67	Asian	nIHH, adult onset	6694A > G	I2232V	31
33	36	M	23	Caucasian	nIHH, adult onset	7043G > A	G2348D	33
34	37	F	34	Caucasian	KS	7592G > A	R2531Q	34
35	38	F	NA	African American	nIHH	7595C > T	T2532M	34
36	39	M	24	Caucasian	KS	7727A > T	D2576V	35
37	40	M	56	Caucasian	KS	7971+7G > T	Splice site	36
38	41	F	23	Caucasian	nIHH	8122G > A	V2708I	38
39	42	M	63	Caucasian	nIHH	8366C > T	A2789V	38
40	43*	F	NA	NA	KS	8366C > T	A2789V	38
41	44	M	63	Caucasian	KS	8405G > A/7861C > G	G2802E/Q2621E	38/36

F, female; KS, Kallmann syndrome; nIHH, normosmic idiopathic hypogonadotropic hypogonadism; M, male; NA, not assessed/not available.
*Probands with additional CHARGE features but not fulfilling Verloes CHARGE syndrome criteria.

Table S2. IGD patients with *CHD7* RSVs who displayed CHARGE features but without fulfilling full CHARGE syndrome based on Verloes criteria

Pedigree no.	Diagnosis	Mutation in *CHD7*	Second hits	C	H	A	RG	RD	G	HL	E	CLP	FA
6	KS	S699G	None	NA	—	—	NA	—	+	+	+	+	—
10	nIHH	A927G	None	—	+	—	—	—	+	—	—	—	—
11	KS	P940L	None	—	—	—	—	—	+	+	—	—	—
16	KS	c.3202–5T > C	None	—	—	—	—	—	+	—	—	+	—
18	KS	F1362L	None	—	—	—	NA	+	+	+	—	—	—
19	KS	c.4354–3C > G	None	—	—	—	NA	+	+	—	—	NA	NA
26	KS	A1953V	None	NA	—	—	NA	—	+	+	—	—	—
33	KS	R2065G	None	—	—	—	—	—	—	+	—	—	—
43	KS	A2789V	None	NA	NA	—	NA	—	+	+	NA	—	NA

A, atresia of choanae; C, coloboma; CLP, cleft lip/plate; E, external ear defect; FA, facial asymmetry; G, genital defects; H, heart defects; HL, hearing loss; NA, not assessed; RG, retarded growth; RD, retarded development.

Table S3. IGD patients with phenotypic evaluation showing full CHARGE syndrome based on Verloes criteria

Pedigree no.	Sex	Age	Diagnosis	Mutation in *CHD7*	Second mutation	C	H	A	RG	RD	G	HL	E	CLP	FA	Verloes criteria
8	F	33	CHARGE with KS	Q814X	None	+	+	NA	NA	+	+	+	NA	+	NA	Atypical
14	F	NA	CHARGE with KS	V1021G	None	+	NA	+	NA	NA	+	NA	+	+	NA	Typical
17	F	56	CHARGE with KS	A1289V	None	NA	+	+	NA	+	+	+	NA	NA	NA	Atypical
45	M	27	CHARGE with KS	D2988GfsX1	None	+	—	+	—	+	+	+	+	+	—	Typical

A, atresia of choanae; C, coloboma; CLP, cleft lip/plate; E, external ear defect; FA, facial asymmetry; G, genital defects; H, heart defects; HL, hearing loss; RG, retarded growth; RD, retarded development; NA, not assessed.

Table S4. Prediction of splice variants on protein function according to web-based prediction software programs and conservation across species

		NNSPLICE	EX-SKIP			Human splicing finder	
Splice-site variants	Wild-type/mutation	Splice score	ESS (total)	ESE (total)	ESS/ESE ratio	CV	ΔCV (%)
2835+8T > C	Wild-type	0.90	48	153	0.31	91.15	No difference
	Mutation	0.95	48	153	0.31	91.15	
3202–5T > C	Wild-type	0.80	34	19	1.79	86.82	−1.00
	Mutation	0.80	22	19	1.16	85.93	
4354–3C > G	Wild-type	0.95	34	192	0.18	77.20	−2.47
	Mutation	0.91	34	192	0.18	75.29	
5300+8C > T	Wild-type	1.00	11	6	1.83	94.02	No difference
	Mutation	0.99	18	6	3.00	94.02	
5895+2G > C	Wild-type	0.92	26	42	0.62	86.60	−7.9
	Mutation	0.92	25	36	0.69	79.79	
6103+6T > C	**Wild-type**	**0.98**	**139**	**202**	**0.69**	**77.05**	**−34.83 Site broken**
	Mutation	**0.71**	**139**	**202**	**0.69**	**50.21**	
6103+7A > G	Wild-type	0.98	139	202	0.69	77.05	−1.5
	Mutation	0.94	139	202	0.69	75.89	
7971+7G > T	Wild-type	0.85	19	43	0.44	84.71	No difference
	Mutation	0.71	19	47	0.40	84.71	

Variant shown in boldface indicates variant predicted to be deleterious in at least two programs. CV, consensus value; ESE, exonic splice enhancer; ESS, exonic splice silencer.

Table S5. Prediction of missense variants on protein function according to web-based prediction software programs and conservation across species

Nucleotide change	Amino acid change	Exon	Polyphen-2	SIFT	PMUT	Mutation taster	Conservation	Structural modeling
120A > C	Q40H	2	Damaging	Benign	Benign	Damaging	Partially conserved (7/8)	Undetermined
599T > A	M200K	2	Benign	Benign	Benign	Benign	Partially conserved (7/8)	Undetermined
1046A > G	N349S	2	Benign	Damaging	Benign	Benign	Partially conserved (6/8)	Undetermined
1117C > T	L373F	2	Benign	Benign	Benign	Benign	Partially conserved (7/8)	Undetermined
1175C > T	S392F	2	Damaging	Damaging	Damaging	Benign	Fully conserved (8/8)	Undetermined
1188G > T	M396I	2	Damaging	Benign	Benign	Benign	Fully conserved (8/8)	Undetermined
2095A > G	S699G	3	Benign	Damaging	Benign	Benign	Fully conserved (8/8)	Undetermined
2185A > G	K729E	3	Damaging	Damaging	Benign	Benign	Fully conserved (8/8)	Undetermined
2680A > G	T894A	9	Benign	Benign	Benign	Damaging	Partially conserved (6/8)	Stabilizing
2780C > G	A927G	10	Benign	Benign	Benign	Benign	Partially conserved (5/8)	Stabilizing
2819C > T	P940L	10	Benign	Damaging	Damaging	Damaging	Partially conserved (7/8)	Destabilizing
3062T > G	V1021G	12	Damaging	Damaging	Benign	Damaging	Fully conserved (8/8)	Destabilizing
3866C > T	A1289V	16	Damaging	Damaging	Benign	Damaging	Partially conserved (7/8)	Destabilizing
4084T > C	F1362L	19	Damaging	Damaging	Damaging	Damaging	Fully conserved (8/8)	Stabilizing
4565A > T	D1522V	20	Damaging	Damaging	Damaging	Damaging	Partially conserved (7/8)	Undetermined
4847A > G	Y1616C	21	Damaging	Damaging	Damaging	Damaging	Fully conserved (8/8)	Stabilizing
5533G > A	G1845R	26	Damaging	Benign	Damaging	Damaging	Partially conserved (7/8)	Undetermined
5689G > A	E1897K	29	Benign	Benign	Benign	Damaging	Partially conserved (7/8)	Undetermined
5858C > T	A1953V	29	Damaging	Benign	Benign	Benign	Partially conserved (7/8)	Undetermined
5945G > A	G1982E	30	Damaging	Damaging	Damaging	Damaging	Fully conserved (8/8)	Destabilizing
6190A > G	I2064V	31	Benign	Benign	Benign	Benign	Fully conserved (8/8)	Stabilizing
6193C > G	R2065G	31	Damaging	Damaging	Damaging	Damaging	Fully conserved (8/8)	Stabilizing
6411C > G	A2137G	31	Benign	Benign	Benign	Benign	Partially conserved (5/8)	Undetermined
6694A > G	I2232V	31	Benign	Benign	Benign	Benign	Partially conserved (6/8)	Undetermined
7043G > A	G2348D	33	Damaging	Benign	Benign	Damaging	Partially conserved (6/8)	Undetermined
7592G > A	R2531Q	34	Benign	Benign	Benign	Damaging	Fully conserved (8/8)	Undetermined
7595C > T	T2532M	34	Damaging	Benign	Benign	Damaging	Partially conserved (6/8)	Undetermined
7727A > T	D2576V	35	Damaging	Benign	Benign	Benign	Partially conserved (7/8)	Stabilizing
7861C > G	Q2621E	36	Benign	Benign	Benign	Damaging	Partially conserved (7/8)	Stabilizing
8122G > A	V2708I	38	Damaging	Benign	Benign	Benign	Fully conserved (8/8)	Stabilizing
8366C > T	A2789V	38	Damaging	Damaging	Benign	Damaging	Fully conserved (8/8)	Undetermined

Table S6. Minor allele frequency in study population and control cohorts

Nucleotide change	Amino acid change	Exon	MAF from IGD patients (%) (n = 783)	MAF from controls (n = 98)	MAF from NHLBI Exome Sequencing Project (ESP) Exome Variant Server (evs.gs.washington.edu/EVS/) (%) European American/African American/All
120A > C	Q40H	2	0.0639	0	ND
599T > A	M200K	2	0.0639	0	ND
1046A > G	N349S	2	0.0639	0	0.0293/0.0/0.0196
1117C > T	L373F	2	0.0639	0	0.0/0.028/0.0095
1175C > T	S392F	2	0.0639	0	0.0291/0.0/0.0191
1188G > T	M396I	2	0.0639	0	0.0875/0.00/0.0577
2095A > G	S699G	3	0.0639	0	ND
2185A > G	K729E	3	0.0639	0	0.0491/0.0545/0.0508
2440C > T	Q814X	6	0.0639	0	ND
2680A > G	T894A	9	0.0639	0	0.0/0.0322/0.0103
2780C > G	A927G	10	0.0639	0	ND
2819C > T	**P940L**	**10**	**0.1278**	**0**	**ND**
2835+8T > C	Splice site	10	0.0639	0	0.121/0.0263/0.0912
3202–5T > C	Splice site	12	0.2554	0	0.0122/0.0/0.0084
3062T > G	**V1021G**	**12**	**0.0639**	**0**	**ND**
3866C > T	**A1289V**	**16**	**0.0639**	**0**	**ND**
4084T > C	**F1362L**	**19**	**0.0639**	**0**	**ND**
4354–3C > G	Splice site	19	0.0639	0	ND
4565A > T	D1522V	20	0.0639	0	ND
4847A > G	**Y1616C**	**21**	**0.0639**	**0**	**ND**
5300+8C > T	Splice site	25	0.0639	0	0.0597/0.05/0.0566
5533G > A	**G1845R**	**26**	**0.0639**	**0**	**ND**
5689G > A	**E1897K**	**29**	**0.0639**	**0**	**0.0149/0.0/0.0101**
5858C > T	A1953V	29	0.0639	0	ND
5895+2G > C	Splice site	29	0.0639	0	ND
5945G > A	**G1982E**	**30**	**0.0639**	**0**	**ND**
6103+6T > C	Splice site	30	0.1278	0	0.0121/0.0/0.0083
6103+7A > G	Splice site	30	0.0639	0	ND
6190A > G	**I2064V**	**31**	**0.0639**	**0**	**ND**
6193C > G	**R2065G**	**31**	**0.0639**	**0**	**ND**
6411C > G	A2137G	31	0.0639	0	ND
6694A > G	**I2232V**	**31**	**0.0639**	**0**	**ND**
7043G > A	G2348D	33	0.0639	0	ND
7592G > A	R2531Q	34	0.0639	0	ND
7595C > T	**T2532M**	**34**	**0.0639**	**0**	**0.0/0.1963/0.0669**
7727A > T	D2576V	35	0.0639	0	ND
7861C > G	**Q2621E**	**36**	**0.0639**	**0**	**ND**
7971+7G > T	Splice site	36	0.0639	0	ND
8122G > A	V2708I	38	0.0639	0	ND
8366C > T	**A2789V**	**38**	**0.1278**	**0**	**0.0/0.0298/0.0099**
8405G > A	G2802E	38	0.0639	0	ND
8963insG	D2988GfsX1	38	0.0639	0	ND

MAF of European American were retrieved from NHLBI Exome Sequencing Project (ESP) Exome Variant Server (evs.gs.washington.edu/EVS/); ND, not detected. Variants in boldface represent alleles tested in Zebrafish assays.

Table S7. Clinical characteristics of IGD patients with benign *CHD7* RSVs by zebrafish model

Pedigree no.	Sex	Origin	De novo	Diagnosis	Olfaction	Nucleotide change	Amino acid change	Exon/ Intron	Mutations in other genes
21	F	Caucasian	No	KS	Self-reported anosmia	4847A > G	Y1616C	21	None
33*	F	Caucasian	NA	KS	Self-reported anosmia	6193C > G	R2065G	31	None
42	M	Caucasian	NA	nIHH	Self-reported normal	8366C > T	A2789V	38	*FGFR1*: 1854G > T (K618N); *GNRHR*: 785G > A (R262Q)
43*	F	NA	NA	KS	Self-reported anosmia	8366C > T	A2789V	38	None

F, female; KS, Kallmann syndrome; M, male; NA, not assessed; nIHH, normosmic idiopathic hypogonadotropic hypogonadism.
*Probands with additional CHARGE features but not fulfilling Verloes CHARGE syndrome criteria.

Table S8. Clinical characteristics of IGD patients with *CHD7* RSVs that were not tested in zebrafish model

IGD patients with or without minor CHARGE features

Pedigree no.	Sex	Origin	De novo	Diagnosis	Olfaction	Nucleotide change	Amino acid change	Exon/ Intron	Mutations in other genes
1	M	Caucasian	No	KS	Self-reported anosmia	599T > A	M200K	2	None
2	M	Caucasian	NA	KS	UPSIT hyposmia	1046A > G	N349S	2	None
3	F	Caucasian	NA	KS	Self-reported anosmia	1117C > T	L373F	2	None
4	M	Caucasian	NA	KS	Self-reported anosmia	1175C > T	S392F	2	None
5	F	Caucasian	No	KS	UPSIT anosmia	1188G > T	M396I	2	None
6*	M	Caucasian	NA	KS	Self-reported anosmia	2095A > G	S699G	3	None
7	F	NA	NA	KS	UPSIT anosmia	2185A > G	p.K729E	3	None
9	M	Caucasian	NA	nIHH	Self-reported normal	2680A > G	T894A	9	None
10*	M	African American	NA	nIHH	Self-reported normal	2780C > G	A927G	10	None
13	M	Caucasian	NA	nIHH	Self-reported normal	2835+8T > C	Splice site	10	None
15	F	Asian	NA	KS	Self-reported anosmia	3202−5T > C	Splice site	12	None
16*	F	NA	No	KS	Self-reported anosmia	3202−5T > C	Splice site	12	None
19*	M	Caucasian	NA	KS	Self-reported anosmia	4354−3C > G	Splice site	19	None
20	M	Caucasian	NA	nIHH, adult-onset	UPSIT normal	120A > C/4565A > T	Q40H/ D1522V	2/20	None
22	M	Caucasian	NA	nIHH, adult-onset	UPSIT normal	5300+8C > T	Splice site	25	None
23	M	Caucasian	NA	nIHH	UPSIT normal	5300+8C > T	Splice site	25	None
26*	F	Hispanic	NA	KS	UPSIT anosmia	5858C > T	A1953V	29	None
27	M	Caucasian	NA	KS	UPSIT anosmia	5895+2G > C	Splice site	29	None
29	F	Caucasian	NA	nIHH	UPSIT normal	6103+6T > C	Splice site	30	None
30	M	Caucasian	NA	nIHH, adult-onset	Self-reported normal	6103+6T > C	Splice site	30	None
31	M	Caucasian	NA	KS	Self-reported anosmia	6103+7A > G	Splice site	30	None
34	M	Caucasian	NA	nIHH	UPSIT normal	6411C > G	A2137G	31	None
36	M	Caucasian	NA	nIHH, adult onset	UPSIT normal	7043G > A	G2348D	33	None
37	F	Caucasian	NA	KS	Self-reported anosmia	7592G > A	R2531Q	34	None
39	M	Caucasian	NA	KS	UPSIT hyposmia	7727A > T	D2576V	35	None
40	M	Caucasian	NA	KS	UPSIT anosmia	7971+7G > T	Splice site	36	None
41	F	Caucasian	No	nIHH	UPSIT normal	8122G > A	V2708I	38	None

F, female; KS, Kallmann syndrome; M, male; NA, not assessed; nIHH, normosmic idiopathic hypogonadotropic hypogonadism.
*Probands with additional CHARGE features but not fulfilling Verloes CHARGE syndrome criteria.