Clinical Endocrinology (2015) **82**, 429–438 doi: 10.1111/cen.12618

ORIGINAL ARTICLE

Normosmic idiopathic hypogonadotropic hypogonadism due to a novel homozygous nonsense c.C969A (p.Y323X) mutation in the *KISS1R* gene in three unrelated families

Huseyin Demirbilek*, M. Nuri Ozbek*, Korcan Demir†, L. Damla Kotan‡, Yasar Cesur§, Murat Dogan§, Fatih Temiz¶, Eda Mengen¶, Fatih Gurbuz¶, Bilgin Yuksel¶ and A. Kemal Topaloglu‡,¶

Division of Pediatric Endocrinology, Children's State Hospital, Diyarbakir, †Division of Pediatric Endocrinology, Children's Hospital, Gaziantep, ‡ Institute of Sciences, Department of Biotechnology, Cukurova University, Adana, §Division of Pediatric Endocrinology, Yuzuncu Yil University, Van and ¶ Faculty of Medicine, Division of Pediatric Endocrinology, Cukurova University, Adana, Turkey

Summary

Objective The spectrum of genetic alterations in cases of hypogonadotropic hypogonadism continue to expand. However, *KISS1R* mutations remain rare. The aim of this study was to understand the molecular basis of normosmic idiopathic hypogonadotropic hypogonadism.

Methods Clinical characteristics, hormonal studies and genetic analyses of seven cases with idiopathic normosmic hypogonadotropic hypogonadism (nIHH) from three unrelated consanguineous families are presented.

Results One male presented with absence of pubertal onset and required surgery for severe penoscrotal hypospadias and cryptorchidism, while other two males had absence of pubertal onset. Two of four female cases required replacement therapy for pubertal onset and maintenance, whereas the other two had spontaneous pubertal onset but incomplete maturation. In sequence analysis, we identified a novel homozygous nonsense (p.Y323X) mutation (c.C969A) in the last exon of the *KISS1R* gene in all clinically affected cases.

Conclusions We identified a homozygous nonsense mutation in the *KISS1R* gene in three unrelated families with nIHH, which enabled us to observe the phenotypic consequences of this rare condition. Escape from nonsense-mediated decay, and thus production of abnormal proteins, may account for the variable severity of the phenotype. Although *KISS1R* mutations are extremely rare and can cause a heterogeneous phenotype, analysis of the *KISS1R* gene should be a part of genetic analysis of patients with nIHH, to allow better understanding of phenotype–genotype relationship of *KISS1R* mutations and the underlying genetic basis of patients with nIHH.

Correspondence: Huseyin Demirbilek, Diyarbakir Children's State Hospital, Division of Pediatric Endocrinology, 21100 Diyarbakir, Turkey. Tel.: +90 (412) 2245751; Fax: ++90 (412) 2245752; E-mail: dr_huseyin@hotmail.com

(Received 13 July 2014; returned for revision 19 August 2014; finally revised 2 September 2014; accepted 13 September 2014)

Introduction

Idiopathic hypogonadotropic hypogonadism (IHH) is characterised by a defect in the onset or maintenance of puberty caused by hypothalamic–pituitary–gonadal axis dysfunction with the absence of an organic lesion. IHH causes a lack of secondary sexual characteristics and a mature reproductive system. Its prevalence is estimated to be around 1–10/100 000.[1,2] Based on the presence or absence of anosmia, IHH is defined as anosmic or normosmic.[3] The prototype of anosmic IHH is Kallmann syndrome which is characterised by hypogonadotropic hypogonadism and hyposmia/anosmia due to abnormal embryonic migration of hypothalamic GnRH neurons. Normosmic idiopathic hypogonadotropic hypogonadism (nIHH) denotes those IHH cases that are not associated with anosmia/hyposmia.[3] To date, a number of genetic defects causing nIHH have been identified at various sites,[4–6] including the GnRH receptor (*GnRHR*),[7] *KISS1R*,[8,9] the fibroblast growth factor receptor 1 (*FGFR1*),[10,11] fibroblast growth factor 8 (*FGF8*),[12] GnRH-1 (*GNRH1*),[13] *TAC3*, *TACR3*[14] and *KISS1*.[15] However, these genetic defects account for only 30–50% of all cases with nIHH.[1,16] *KISS1R* (NM_032551, formerly *GPR54*) encodes the kisspeptin receptor (KISS1R) which is essential for the secretion of GnRH and induction of puberty.[6,8] Loss of function mutations in the *KISS1R* constitutes a rare cause of nIHH.[2,6] Herein, we present a novel homozygous nonsense mutation in the *KISS1R* gene causing nIHH in seven members of three unrelated families from different cities.

Patients and methods

Our study included three families which have more than one member with nIHH. The diagnosis of HH was based on the

absence of spontaneous pubertal development by age 13 in girls (Tanner breast stage 1) and 14 in boys (testicular volumes of <4 ml) while attaining a bone age of 11·5 or greater and hypogonadal sex steroid concentrations of testosterone <0·7 nmol/l or oestradiol <73 pmol/l in the setting of inappropriately normal or low gonadotrophin levels. Other pituitary hormones including TSH, prolactin, ACTH, cortisol and IGF-1 were measured to rule out multiple pituitary hormone deficiency. There was no evidence of structural lesions on imaging of the hypothalamic–pituitary region. Patients with chronic systemic diseases (e.g. chronic renal failure, thalassaemia, poorly controlled diabetes mellitus, chronic inflammatory diseases), eating disorders (e.g. anorexia nervosa, bulimia nervosa) or protein energy malnutrition that can cause hypogonadotropic hypogonadism were excluded. All subjects had a normal sense of smell on conventional testing. Cases with typical features of Bardet–Biedl, Biemond, Prader–Willi or any other syndromes associated with hypogonadism were also excluded. The study was approved by the Ethics Committee of Cukurova University Faculty of Medicine. Written informed consent was obtained from all patients or their legal guardians.

Assays and hormonal Studies

An Access Immunoassay System (Beckman Coulter, Inc. Fullerton, CA, USA) was used to measure FSH (the lowest detectable level: 0·2 IU/l, intra-assay CVs: 3·1–4·3%), LH (the lowest detectable level: 0·2 IU/l, intra-assay CVs: 3·6–5·4%), testosterone (the lowest detectable level: 0·3 nmol/l, intra-assay CVs: 1·67–3·93%) and oestradiol (the lowest detectable level: 73 pmol/l, intra-assay CVs: 12–21%). A GnRH stimulation test (2·5 μg/kg, maximum 100 μg, i.v.) was performed. Serum LH and FSH levels were measured at 0, 15, 30, 45 and 60 min after GnRH stimulation. Olfactory bulbs and sulci and hypothalamic–pituitary structures were analysed by magnetic resonance imaging.

DNA Sequencing

DNA was extracted from peripheral blood leucocytes using standard methods. The coding regions and neighbouring intronic regions of the known genes for nIHH (*GNRHR, GNRH1, TACR3, TAC3, KISS1R* and *KISS1*) were amplified by PCR. The PCR products were purified and directly sequenced using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA, USA) in an ABI PRISM 310 automatic sequencer. Any deviations from reference sequence were checked in dbSNP database, 1000 Genomes Project database and Exome Variant Server database. To analyse for co-segregation with disease phenotype, the identified variant was subsequently screened in other family members.

Results

Family 1

The proband was a 15-year-old boy, who presented with delayed pubertal onset. His parents were first cousins. He had a history of surgery for bilateral cryptorchidism and severe penoscrotal hypospadias. At the time of admission, his height was 1·475 m (<3rd percentile) and weight 38 kg (<3rd percentile). The testes were scrotal and 1 ml in size bilaterally with a stretched penile size of 3·2 cm (<−2SD). There was a chordee, suggesting surgical scarring or abnormal differentiation of the phallus. Bone age was 11·5 years. Hormonal evaluation revealed a low testosterone (<0·7 nmol/l) and inappropriately low LH (1·1 IU/l) and FSH (2·6 IU/l) levels. At first presentation, his older sister had no secondary sexual characteristics, an older brother had normal pubertal development and two younger sisters were at prepubertal age. Other anterior pituitary hormones of all subjects were within normal ranges.

Sequencing analysis of the *KISS1R* gene identified a novel nonsense (p.Y323X) mutation (c.C969A) in exon 5 of the *KISS1R* gene in homozygous state in three siblings and heterozygous in both parents, the clinically unaffected brother and one prepubertal sister (Figs. 1 and 2). This mutation was not present in the Human Gene Mutation Database (HGMD), dbSNP database, 1000 Genomes Project database or Exome Variant Server database. It causes a premature stop codon in translation at position 323 located in the transmembrane domain of *KISS1R* protein and was predicted to result in a truncated protein with abnormal function. Bioinformatic analysis with Mutation Taster revealed that the mutation was capable to cause disease (probability, 0·999999999999524). Sequencing analysis of other candidate genes (*GNRHR, GNRH1, TACR3, TAC3* and *KISS1)* involved in the development of nIHH did not show any pathogenic variant.

Testosterone replacement therapy was commenced in the index case. However, testosterone was not administered regularly. The older sister was given oestrogen replacement for pubertal induction and subsequent oral contraceptive therapy for regular menstrual cycles. To elucidate the long-term effects of the mutation on clinical and hormonal characteristics of affected members, all family members were re-evaluated, at a follow-up visit, 2 years after the first presentation (Table 1).

Family 2

The proband was a 16·5-year-old boy, presenting with delayed pubertal onset. One younger brother had the same clinical phenotype. Their parents were first cousins. The proband measured 1·684 m (25th percentile) in height and 56 kg (75th percentile) in weight. At the time of admission, he was on testosterone therapy (Sustanon® 250 mg, monthly, Merck Sharp & Dohme (MSD) Pharmaceuticals, Istanbul, Turkey). Testes were 1·5 ml bilaterally, located in the scrotum. Stretched penile length was 11·5 cm. Development of pubic and axillary hair was Tanner stage 3. Bone age was 14·5 years. He had a basal testosterone level of 4·4 nmol/l with undetectable gonadotrophin levels (LH: <0·2 IU/l and FSH: <0·2 IU/l). His $12^{11/12}$-year-old brother was admitted for investigation.

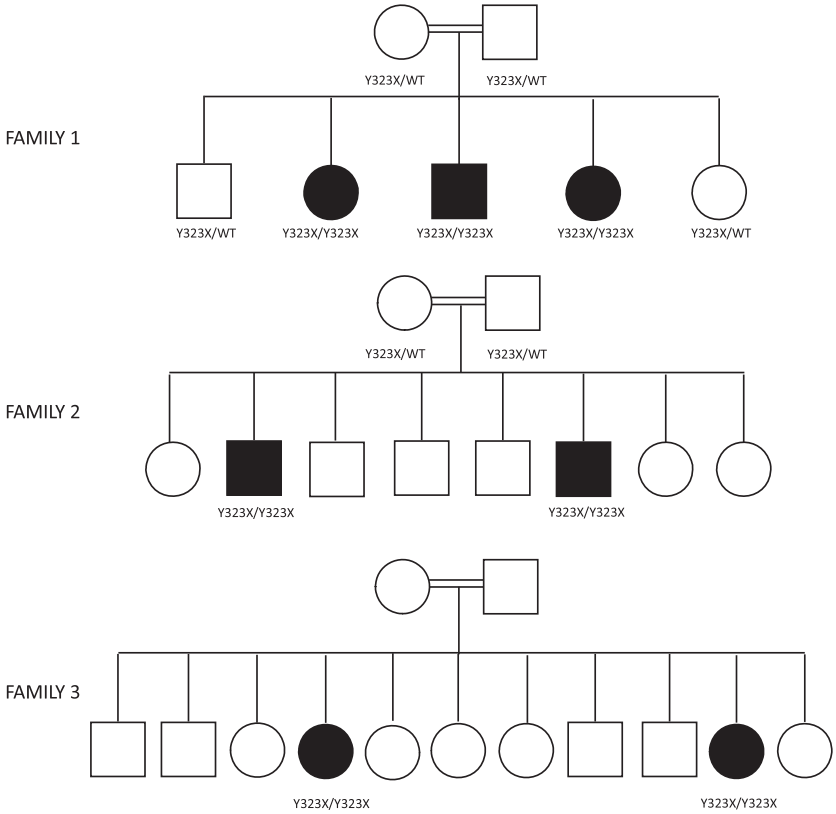

Fig. 1 Pedigrees of three unrelated families with the diagnosis of normosmic idiopathic hypogonadotropic hypogonadism due to an identical homozygous nonsense mutation in *KISS1R* gene.

His height was 1·353 m (10th percentile), and weight was 31·5 kg (<3rd percentile). He had Tanner stage 1 axillary and pubic hair, stretched penile size was 3·5 × 1·5 cm, and testes volume was 1 ml located in the scrotum bilaterally. Bone age was 10 years. Sequencing of genes associated with nIHH revealed a homozygous nonsense Y323X (c.C969A) mutation in *KISS1R* and normal sequences in other candidate genes. The parents were heterozygous for this mutation. Although it was learnt that there were two additional male siblings with nIHH phenotype, genetic analysis could not been performed due to unavailability of DNA sample. No females have been referred from this family. The long-term effects of the mutation in affected members were evaluated on a follow-up visit (Table 1).

Family 3

The proband was a 15·7-year-old female who was admitted due to lack of breast development. She had a 36-year-old sister with the same complaints during adolescence who had required oestrogen replacement and was on cyclical oral contraceptive for regular menstrual bleeding. The parents were

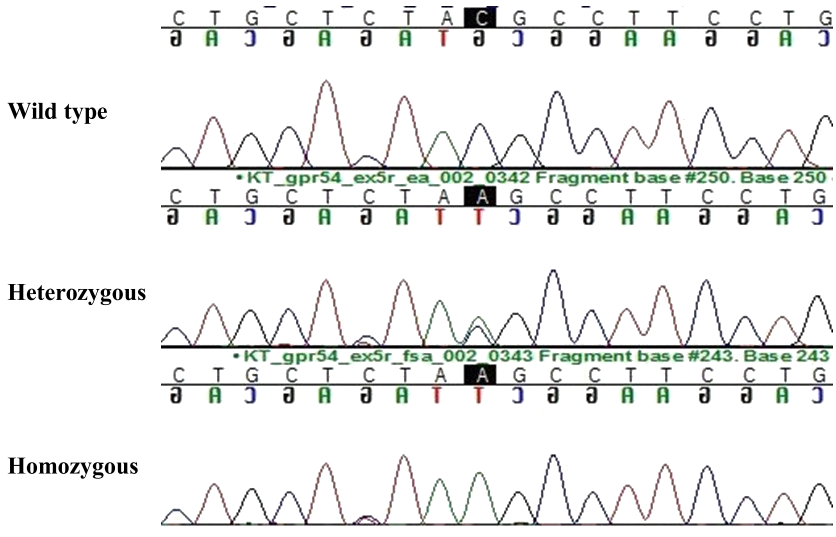

Fig. 2 Mutation analysis of exon 5 of the *KISS1R* gene in three unrelated families revealed a C-to-A change at c.969 leading to p.Y323X.

Table 1. Follow-up characteristics of patients with homozygous and heterozygous *KISS1R* mutation

Age/sex	Genetics	2^0 SC	SPL	TS	BD	PH	AH	FSH	LH	T	E	Follow-up characteristics
17 year/M proband (F1)	Homozygous	No	3 cm	1/1 ml	–	T 2		3·2	0·4	<0·3	–	No erection, ejaculation and libido. Chordee (+) required reconstructive surgery for chordee release. Testosterone replacement was commenced, however not administered regularly
21 year/F (F1)	Homozygous	Yes	–	–	T5	T5	+	NA	NA	–	NA	Spontaneous onset although incomplete pubertal progression, required oestrogen replacement and subsequent OC therapy. Regular menarche on OC therapy. Had got married. Two attempts at fertilisation with ovulation induction by rhFSH failed
13·7 year/F (F1)	Homozygous	Incomplete	–	–	T2	T2	+	3·7	0·9	–	37	Spontaneous onset of puberty for 1 year and incomplete pubertal progression. Pelvic ultrasonography findings were prepubertal. Oestrogen replacement commenced for full pubertal maturation and subsequent OC therapy for regular menstrual cycles
20 years/M (F1)	Heterozygous	Yes	12 cm	25/25 ml	–	T5	+	4·0	2·87	14·4	–	Spontaneous onset and normal pubertal maturation
9·2 years/F (F1)	Heterozygous	No	–	–	T1	T1	–	NA	NA	–	NA	Has not been further evaluated since was prepubertal
19 years/M, (proband) (F2)	Homozygous	Yes	12 cm	3/3 ml	–	T4	+	0·69	0·74	1·0	–	Was on testosterone replacement (250 mg per month). Not administered regularly
16·1 years/M (F2)	Homozygous	Yes	12 cm	3/3 ml	–	T4	+	0·74	0·69	2·4		Was on testosterone replacement (250 mg per month). Not administered regularly
15·7 years/F Index case (F3)	Homozygous	Yes	–	–	T5	T5	+	NA	NA	–	NA	Required oestrogen replacement and subsequent OC therapy. Regular menarche on OC therapy
36 years/F (F3)	Homozygous	Yes	–	–	T5	T3	+	NA	NA	–	NA	Required oestrogen replacement and subsequent OC therapy. Regular menarche on OC therapy. Had got married. No fertility

2^0 SC, secondary sexual characteristics; SPL, stretched penile length; TS, testis size; BD, breast development; PH, pubic hair, AH, axillary hair; FSH, follicle-stimulating hormone; LH, luteinizing hormone; T, testosterone (nmol/l); E, oestradiol (pmol/l); T1,2,3,4,5 refers Tanner stages; M, male; F, female; NA, not available; F1, F2, F3 refer to families 1, 2 and 3; OC, oral contraceptive.

second cousins. Physical examination at first admission revealed a weight of 51 kg (25th–50th percentiles) and a height of 1·547 m (10th percentile). Breast development and pubic hair development were consistent with Tanner stages 1 and 4, respectively. Basal LH level was <0·2 IU/l, FSH 2·25 IU/l and oestradiol <73 pmol/l. Pelvic ultrasonography revealed a uterine length of 20 mm. Peak LH and FSH levels in GnRH stimulation test were 1·4 IU/l and 8·13 IU/l, respectively. Breast development of the 36-year-old sister was Tanner stage V, while pubic hair was scarce. Her FSH was 6·27 IU/l, LH 1·54 IU/l and oestradiol <73 pmol/l. Mutation analysis of both cases revealed a homozygous nonsense Y323X (c.C969A) mutation in *KISS1R* and normal sequences in the other candidate genes for nIHH.

Discussion

In the present study, we have described a novel homozygous nonsense mutation in the *KISS1R* gene, causing nIHH in three male and four female cases from three families of Kurdish origin, from different provinces in Turkey. To our knowledge, this is the first reported homozygous nonsense mutation in the *KISS1R* gene.

Table 2. Description, ethnic distribution, clinical expression and follow-up characteristics of mutations detected in *KISS1R* in patients with nIHH

Type	Exon/intron	DNA description	Protein description	Zygosity	Consanguinity	Ethnicity	Sex, number of affected cases, phenotypic characteristics, treatment, fertility and follow-up	Reference
MS	Exon 5	c.C969A	p.Y323X	HM	First cousin	Family 1 Kurdish	Male (n = 1): Cryptorchidism, micropenis, hypospadias and absence of pubertal onset. Required testosterone replacement for pubertal onset, maturation and maintenance. Surgical repair and testosterone replacement therapy. Female (n = 2): Delayed spontaneous onset of puberty, however incomplete pubertal progression. Required replacement therapy for full pubertal maturation and subsequent OC therapy for regular menstrual bleeding. Two attempts to achieve fertility with rhFSH injection failed in the eldest one.	Present study
					First cousin	Family 2 Kurdish	Male (n = 2): Absence of spontaneous pubertal onset in two affected males. Required testosterone replacement for pubertal onset, maturation and maintenance	
					Second cousin	Family 3 Kurdish	Female (n = 2): Failure in onset and maturation of puberty. Required oestrogen replacement for pubertal induction, maturation and subsequent OC therapy for regular menstrual bleeding	
Ins/del	Intron 2	(IVS2-4_-2del/ ins ACCGGCT)	p.?	HM	NA	Brazilian	M (n = 2): Micropenis and cryptorchidism at birth Absence of spontaneous pubertal onset.	Teles[22]
Del	Intron 4 Exon 5	155-bp deletion	p.247X	HM		Caucasian	Male (n = 4): No pubertal development Female (n = 1): Incomplete pubertal development	de Roux[9]
Ins		c.1001-1002insC	p.334fsinC.	HM	Second cousin	German	Male (n = 1): Cryptorchidism, no pubertal development and	Lanfranco[26]

(continued)

Table 2. *(Continued)*

Type	Exon/intron	DNA description	Protein description	Zygosity	Consanguinity	Ethnicity	Sex, number of affected cases, phenotypic characteristics, treatment, fertility and follow-up	Reference
							mild hypospadias. Pulsatile GnRH therapy achieved fertility.	
Ins	Insertion of 9 nucleotide at position 1023	c.1023Ins9	p.?	HT	No	French	Male (n = 1): Absence of pubertal development	Chevrier[27]
MS	Exon 5	c.754G>C/N	p.E252Q/-	HT	Yes	Brazilian	Male (n = 1): Micropenis, cryptorchidism and no pubertal development	Teles[22]
MS	Exon 2	c. 305 C >T	p.L102P	HM	First cousin	Family 1 Syrian	Female (n = 1): Amenorrhoea and incomplete pubertal development. Treated with oestrogen and progestin. Two successful pregnancies and delivery by ovulation induction using pulsatile GnRH. Required replacement for menarche after deliveries	Tenenbeum[28]
						Family 2 Israeli-Arabic	Male (n = 2): Micropenis, cryptorchidism and failure in pubertal onset. Orchiopexy and testosterone therapy. No improvement in testicular size to 6 months SC hCG therapy in one and infertility and azoospermia in other one. Female (n = 2): Primary amenorrhoea and incomplete pubertal development. OC therapy for cyclic menstrual bleeding. One had failed fertility for 5 years.	
MS	Exon 5	c.815T>C	p.F272S	HM	Yes	Arab-Muslim from Israeli	Male (n = 5): Micropenis, cryptorchidism and no pubertal development. Testosterone replacement. Azoospermia in one at the age of 27 years. Female (n = 1): No pubertal development and primary amenorrhoea. Recombinant	Nimri[6]

(continued)

Table 2. *(Continued)*

Type	Exon/intron	DNA description	Protein description	Zygosity	Consanguinity	Ethnicity	Sex, number of affected cases, phenotypic characteristics, treatment, fertility and follow-up	Reference
							gonadotropin therapy induced menarche with subsequent ovulation, although fertility has not yet been achieved.	
MS	Exon 5	c.1079A >T	p.H360L	HT		Caucasian	Male (n = 1): No pubertal development	Cerrato[21]
MS	Exon 4 Exon 5	c.667T >C/ c.891G >T	p.C223R/p.R297 L	CHT	No	Turkish-Cypriot/ Jamaican	Male (n = 1): Micropenis and cryptorchidism. Absence of pubertal development. Testosterone replacement therapy	Sample[29]
NS/NST	Exon 5	c.991C >T/ c.1195T >A	p.R331X/p.X399R	CHT		Black	Male (n = 1): No pubertal development. Treated with GnRH injection. No further detail.	Seminara[8]
MS	Exon 3	c.443T >C	p.L148S	HM		Saudi Arabian	Male (n = 4): Delayed pubertal onset and micropenis. LH/FSH therapy increased testis size and fertility achieved in 3 of 4. Female (n = 2): Primary amenorrhoea and incomplete pubertal development. Oestrogen replacement. Fertility achieved with LH/FSH and GnRH therapy in one case.	Seminara (8)
MS	Intron 1 (Exon 1–2)	c.2451G>A	p.A82Gfs*151	HM	First cousin	Palestinian	Male (n = 1): Absence of secondary sexual characteristics and pubertal onset. No micropenis and no cryptorchidism. Pubertal induction by testosterone replacement.Female (n = 2): Absence of pubertal development and primary amenorrhoea. Pubertal induction and menarche achieved by sex hormone replacement	Breuer et al[30]
MS	TMD	c.937T>C	p.Tyr313His	HM	First cousin	Portuguese	Male (n = 1): Micropenis and absence of pubertal development. Female (n = 2): Primary amenorrhoea and absence of pubertal onset	Frederic Brioude[25]

(continued)

Table 2. *(Continued)*

Type	Exon/intron	DNA description	Protein description	Zygosity	Consanguinity	Ethnicity	Sex, number of affected cases, phenotypic characteristics, treatment, fertility and follow-up	Reference
MS/NST	TMD	c.305T.C and c.1195T.A	p.L102P/p.X399R	CHT	No	French Caucasian	Male (n = 1): Micropenis and absence of pubertal development. Testosterone therapy leading to satisfactory libido. Combination of rhFSH and hCG therapy increased testis size to 10 ml/12 ml, normal testosterone and inhibin B level, normal sperm count and achieved fertility.	Frederic Brioude[25]

MS: missense; NS: nonsense; NST: nonstop; Ins: insertion; Del: deletion; TMD: trans-membrane domain; F: familial; S: sporadic; HM: homozygous; HT: heterozygous; CHT: compound heterozygous; NA: not available; SC: subcutaneous.

Kisspeptin and its receptor (KISS1R) play an essential role in pubertal onset, maturation and reproductive function by triggering the GnRH pulse generator in the hypothalamus.[8,17–19] According to the current understanding, KNDy (kisspeptin, neurokinin B, dynorphin)-expressing cells located in the infundibular nucleus in humans are thought to act as the GnRH pulse generator which generates pulsatile kisspeptin release. Kisspeptin molecules bind their receptors on the axons of GnRH neurons in the median eminence and cause pulsatile GnRH release into the portal system. Therefore, a loss of function mutation in the *KISS1R* gene will result in the failure of release of GnRH, hence absent pubertal development.[20]

Mutations in *KISS1R* are extremely rare causes of nIHH.[21] In a study evaluating 22 families with multiplex nIHH, Gurbuz *et al.* detected mutations in 17. Of these, *KISS1R* mutations accounted for only 4·1% of all mutations.[2] Similarly, Teles *et al.* reported a rate of *KISS1R* gene mutations in <5% among 312 patients with normosmic IHH.[22] In another study from Italy evaluating 53 patient with nIHH, no mutation was detected in *KISS1R*.[23] Quaynor *et al.* analysed 37 patients with IHH/KS, and none of 24 mutations detected were in the *KISS1R* gene.[24] Therefore, inactivating *KISS1R* mutations have been shown to be responsible for only a small proportion of nIHH.

To our knowledge to date, only 15 different variants causing loss of function in *KISS1R* have been reported: 11 point mutations,[6,8,21,22,25] one partial deletion of the coding sequence,[9] two insertions in the coding sequence[26,27] and one insertion/deletion within the three-splice acceptor site of intron 2[22] (Table 2). All point mutations reported so far are missense mutations, except for a compound heterozygous mutation in which one allele includes a nonsense (R331X) and other a nonstop (X399R) variant.[8]

Despite the fact that *KISS1R* mutations have been reported extremely rarely, we identified a novel homozygous mutation in three distinct families. The families were from different provinces and were not knowingly related. The detection of an identical mutation and similar phenotypic characteristics may suggest either a mutational hot spot or founder effect for this mutation in the Kurdish population. Analysis of further Kurdish cases with nIHH and *KISS1R* would provide additional information to clarify this issue.

In the present study, despite an identical mutation being detected in homozygous state, marked phenotypic heterogeneity was observed among affected cases (Table 2). This heterogeneity has also been reported in previous studies.[6,28] The phenotypic characteristics of the index male case from family 1, including hypospadias, micropenis and cryptorchidism, were considered to be a reflection of severe insufficiency of androgen exposure during intra-uterine life.[29] Lanfranco *et al.* (25) previously reported an 18-year-old male with mild hypospadias who became fertile with pulsatile GnRH therapy. The index case in family 1, who presented with severe penoscrotal hypospadias, is the second case presenting with a disorder in differentiation of the external genitalia. Although KISS1/KISS1R is known to play a role in triggering the GnRH pulse generator, their potential role in intra-uterine testicular function and development of the external genitalia are still unknown. In addition, although we have

excluded hormonal reasons that can cause abnormal sexual differentiation, we did not study genetics for disorders of sex development (DSD). Therefore, our patient with the finding of DSD may have either an unknown effect of *KISS1R* or a mutation in other genes that play a role in sexual differentiation.

The presenting and follow-up characteristics of the cases from our three families such as failure of pubertal onset and absence of sexual maturation in both sexes were considered as evidence for complete hypogonadotropic hypogonadism. However, like our female cases from family 1, who had spontaneous onset and incomplete pubertal maturation, patients with *KISS1R* mutations have been reported in previous publications to undergo spontaneous though incomplete pubertal development, consistent with clinical features and hormonal profile of partial hypogonadotropic hypogonadism.[9,28,30] Although in previous studies, like our index case from family 1, micropenis and cryptorchidism were reported in male patients with *KISS1R* mutations,[26,28,31] some male patients displayed intact external genitalia.[8,9] These findings suggest that some mutations, including the present mutation, may have more detrimental effect on the hypothalamic–pituitary axis and differentiation of male external genitalia.

Depending on whether or not a premature stop codon triggers 'nonsense-mediated decay' (NMD), the consequences of a nonsense mutation can be very different. NMD is a mechanism that clears all mRNA that has stop codon. This is to ensure that no abnormal protein is produced. When a stop codon is in the last exon, NMD does not function and an abnormal protein is produced. This could be more harmful than the total absence of the native protein. For example, mutations in the *SOX10* gene that trigger NMD result in Waardenburg syndrome type 4 (hearing loss, pigmentary abnormalities; Hirschsprung disease; OMIM 277580), while nonsense mutations that escape NMD cause a much more severe neurological phenotype.[32] In the case of mutation p.Y323X, the nucleotide change is in the last exon of *KISS1R*. Therefore, it should escape NMD and cause an abnormal kisspeptin receptor protein. Variable severity of function of this abnormal protein in different biological settings may explain the phenotypic variability despite the same genotype. In other words, if this mRNA had undergone NMD, a more uniform phenotype may have been expected.

In conclusion, we have identified a novel homozygous nonsense mutation in the *KISS1R* gene in seven members of three apparently unrelated Kurdish families with the diagnosis of nIHH. Variable phenotypic expression of this mutation among males from two families and male and female patients from family 1 suggests complex genetic interactions. For better understanding of the phenotype–genotype relationship of *KISS1R* mutations and the underlying genetic basis of patients with nIHH, analysis of *KISS1R* gene should be a part of genetic analysis of patients with nIHH.

Funding

This study was supported (Grant number 1095455) by Turkish Scientific and Technical Research Council (TUBITAK).

Disclosure Statement

The authors have nothing to discloseLegends.

References

1 Bianco, S.D. & Kaiser, U.B. (2009) The genetic and molecular basis of idiopathic hypogonadotropic hypogonadism. *Nature Reviews Endocrinology*, **5**, 569–576.
2 Gurbuz, F., Kotan, L.D., Mengen, E. *et al.* (2012) Distribution of gene mutations associated with familial normosmic idiopathic hypogonadotropic hypogonadism. *Journal of Clinical Research in Pediatric Endocrinology*, **4**, 121–126.
3 Semple, R.K. & Topaloglu, A.K. (2010) The recent genetics of hypogonadotrophic hypogonadism - novel insights and new questions. *Clinical Endocrinology (Oxf)*, **72**, 427–435.
4 Pitteloud, N., Durrani, S., Raivio, T. *et al.* (2010) Complex genetics in idiopathic hypogonadotropic hypogonadism. *Frontiers of Hormone Research*, **39**, 142–153.
5 Brioude, F., Bouligand, J., Trabado, S. *et al.* (2010) Non-syndromic congenital hypogonadotropic hypogonadism: clinical presentation and genotype-phenotype relationships. *European Journal of Endocrinology*, **162**, 835–851.
6 Nimri, R., Lebenthal, Y., Lazar, L. *et al.* (2011) A novel loss-of-function mutation in GPR54/KISS1R leads to hypogonadotropic hypogonadism in a highly consanguineous family. *Journal of Clinical Endocrinology and Metabolism*, **96**, E536–E545.
7 de Roux, N., Young, J., Misrahi, M. *et al.* (1997) A family with hypogonadotropic hypogonadism and mutations in the gonadotropin-releasing hormone receptor. *New England Journal of Medicine*, **337**, 1597–1602.
8 Seminara, S.B., Messager, S., Chatzidaki, E.E. *et al.* (2003) The GPR54 gene as a regulator of puberty. *New England Journal of Medicine*, **349**, 1614–1627.
9 de Roux, N., Genin, E., Carel, J.C. *et al.* (2003) Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 10972–10976.
10 Miura, K., Acierno, J.S. Jr & Seminara, S.B. (2004) Characterization of the human nasal embryonic LHRH factor gene, NELF, and a mutation screening among 65 patients with idiopathic hypogonadotropic hypogonadism (IHH). *Journal of Human Genetics*, **49**, 265–268.
11 Pitteloud, N., Acierno, J.S. Jr, Meysing, A. *et al.* (2006) Mutations in fibroblast growth factor receptor 1 cause both Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 6281–6286.
12 Falardeau, J., Chung, W.C., Beenken, A. *et al.* (2008) Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. *The Journal of Clinical Investigation*, **118**, 2822–2831.
13 Bouligand, J., Ghervan, C., Tello, J.A. *et al.* (2009) Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation. *New England Journal of Medicine*, **360**, 2742–2748.
14 Topaloglu, A.K., Reimann, F., Guclu, M. *et al.* (2009) TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for Neurokinin B in the central control of reproduction. *Nature Genetics*, **41**, 354–358.

15 Topaloglu, A.K., Tello, J.A., Kotan, L.D. *et al.* (2012) Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *New England Journal of Medicine*, **366**, 629–635.
16 Crowley, W.F. Jr, Pitteloud, N. & Seminara, S.. (2008) New genes controlling human reproduction and how you find them. *Transactions of the American Clinical and Climatological Association*, **119**, 29–37; discussion 37-28.
17 Navarro, V.M. & Tena-Sempere, M. (2012) Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. *Nature Reviews Endocrinology*, **8**, 40–53.
18 Silveira, L.G., Latronico, A.C. & Seminara, S.B. (2013) Kisspeptin and clinical disorders. *Advances in Experimental Medicine and Biology*, **784**, 187–199.
19 Dedes, I. (2012) Kisspeptins and the control of gonadotrophin secretion. *Systems Biology in Reproductive Medicine*, **58**, 121–128.
20 Pinilla, L., Aguilar, E., Dieguez, C. *et al.* (2012) Kisspeptins and reproduction: physiological roles and regulatory mechanisms. *Physiological Reviews*, **92**, 1235–1316.
21 Cerrato, F., Shagoury, J., Kralickova, M. *et al.* (2006) Coding sequence analysis of GNRHR and GPR54 in patients with congenital and adult-onset forms of hypogonadotropic hypogonadism. *European Journal of Endocrinology*, **155**(Suppl 1), S3–s10.
22 Teles, M.G., Trarbach, E.B., Noel, S.D. *et al.* (2010) A novel homozygous splice acceptor site mutation of KISS1R in two siblings with normosmic isolated hypogonadotropic hypogonadism. *European Journal of Endocrinology*, **163**, 29–34.
23 Bonomi, M., Libri, D.V., Guizzardi, F. *et al.* (2012) New understandings of the genetic basis of isolated idiopathic central hypogonadism. *Asian Journal of Andrology*, **14**, 49–56.
24 Quaynor, S.D., Kim, H.G., Cappello, E.M. *et al.* (2011) The prevalence of digenic mutations in patients with normosmic hypogonadotropic hypogonadism and Kallmann syndrome. *Fertility and Sterility*, **96**, 1424–1430. e1426.
25 Brioude, F., Bouligand, J., Francou, B. *et al.* (2013) Two families with normosmic congenital hypogonadotropic hypogonadism and biallelic mutations in KISS1R (KISS1 receptor): clinical evaluation and molecular characterization of a novel mutation. *PLoS ONE*, **8**, e53896.
26 Lanfranco, F., Gromoll, J., von Eckardstein, S. *et al.* (2005) Role of sequence variations of the GnRH receptor and G protein-coupled receptor 54 gene in male idiopathic hypogonadotropic hypogonadism. *European Journal of Endocrinology*, **153**, 845–852.
27 Chevrier, L., de Brevern, A., Hernandez, E. *et al.* (2013) PRR repeats in the intracellular domain of KISS1R are important for its export to cell membrane. *Molecular Endocrinology*, **27**, 1004–1014.
28 Tenenbaum-Rakover, Y., Commenges-Ducos, M., Iovane, A. *et al.* (2007) Neuroendocrine phenotype analysis in five patients with isolated hypogonadotropic hypogonadism due to a L102P inactivating mutation of GPR54. *Journal of Clinical Endocrinology and Metabolism*, **92**, 1137–1144.
29 Grumbach, M.M. (2005) A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *Journal of Clinical Endocrinology and Metabolism*, **90**, 3122–3127.
30 Pallais, J.C., Bo-Abbas, Y., Pitteloud, N. *et al.* (2006) Neuroendocrine, gonadal, placental, and obstetric phenotypes in patients with IHH and mutations in the G-protein coupled receptor, GPR54. *Molecular and Cellular Endocrinology*, **254–255**, 70–77.
31 Semple, R.K., Achermann, J.C., Ellery, J. *et al.* (2005) Two novel missense mutations in g protein-coupled receptor 54 in a patient with hypogonadotropic hypogonadism. *Journal of Clinical Endocrinology and Metabolism*, **90**, 1849–1855.
32 Inoue, K., Khajavi, M., Ohyama, T. *et al.* (2004) Molecular mechanism for distinct neurological phenotypes conveyed by allelic truncating mutations. *Nature Genetics*, **36**, 361–369.