HHS Public Access

Author manuscript

Clin Genet. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

Clin Genet. 2017 August ; 92(2): 213–216. doi:10.1111/cge.12996.

Genetic testing facilitates prepubertal diagnosis of congenital hypogonadotropic hypogonadism

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Abstract

Neonatal micropenis and cryptorchidism raise the suspicion of congenital hypogonadotropic hypogonadism (CHH), a rare genetic disorder caused by GnRH deficiency. Low plasma testosterone levels and low gonadotropins during minipuberty provide a clinical diagnostic clue, yet these tests are seldomly performed in general practice. We report a male neonate with no family history of reproductive disorders who was born with micropenis and cryptorchidism. Hormonal testing at age 2.5 months showed low testosterone (0.3 nmol/L) and undetectable gonadotropins (luteinizing hormone and follicle-stimulating hormone both < 0.5 U/L), suggestive of CHH. Genetic testing identified a *de novo*, heterozygous mutation in fibroblast growth factor receptor 1 (*FGFR1* p.L630P). L630 resides on the ATP binding cleft of the FGFR1 tyrosine kinase domain, and L630P is predicted to cause a complete loss of receptor function. Cell-based assays confirmed that L630P abolishes FGF8 signaling activity. Identification of a loss-of-function *de novo FGFR1* mutation in this patient confirms the diagnosis of CHH, allowing for a timely hormonal treatment to induce pubertal development. Therefore genetic testing can complement clinical and hormonal assessment for a timely diagnosis of CHH in childhood.

Graphical Abstract

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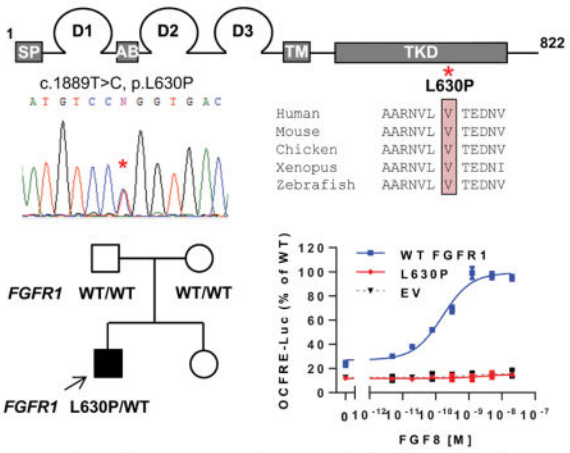
Conflict of Interest

The authors have declared that no conflict of interest exists.



Genetic testing can complement clinical investigations for a timely diagnosis of congenital hypogonadotropic hypogonadism in childhood.

Keywords

congenital hypogonadotropic hypogonadism; minipuberty; exome sequencing; fibroblast growth factor receptor 1

Introduction

Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic disorder characterized by absent or partial puberty and infertility due to defective secretion or action of gonadotropin-releasing hormone (GnRH). Mutations in > 30 genes are implicated in the disease (1). During early adolescence, CHH is difficult to differentiate from constitutional delay of growth and puberty (CDGP), because both present with absent or stalled puberty with low sex steroid and low/normal gonadotropins (i.e. hypogonadotropic hypogonadism) (1). Therefore CHH is often diagnosed in late adolescence or early adulthood, which delays hormonal treatment and causes psychosocial distress in patients and their families (1).

Neonatal micropenis and cryptorchidism raise the suspicion of CHH in male infants. These signs are thought to reflect the lack of activation of the hypothalamic-pituitary-gonadal (HPG) axis in these infants during prenatal development and minipuberty (2 weeks to 6 months of life) (2, 3). A biochemical profile of hypogonadotropic hypogonadism during minipuberty provides a clinical clue for CHH; however such hormonal testing is seldom performed in general practice. Further, the natural history of neonatal hypogonadotropic hypogonadism is unclear, because there are no reported long-term studies following these infants through puberty and adulthood. We report a case of early diagnosis of CHH in childhood, demonstrating the utility of genetic testing as a complement to clinical and hormonal assessment.

Materials and Methods

Clinical features

This male patient of European descent was born with micropenis (2 × 0.5 cm) and bilateral non-palpable inguinal cryptorchidism without other visible malformations. At age 2.5 months, blood testing revealed low testosterone (T 0.3 nmol/l, normal range 0.43–7.71 nmol/l) (4), undetectable gonadotropins (LH and FSH < 0.5 U/l, normal range LH 0.65–2.69 IU/l, FSH 0.86–2.52 IU/l) (4), and otherwise normal pituitary function. The karyotype was normal. The patient received 3 testosterone injections (25 mg i.m. monthly) to induce penile growth (3 × 1.5 cm). Surgery was performed at age 2.5 years for atrial septal defect, and at age 3 years for bilateral orchidopexy, which showed a testicular volume of 0.2–0.3 ml bilaterally. During childhood, he exhibited normal growth and development. He reports normal sense of smell, confirmed by formal smell testing (Sniffin' Stick, 10/16, 10th percentile). He describes normal hearing and does not exhibit other CHH-associated phenotypes such as skeletal or dental defects. He has no family history of consanguinity, delayed puberty, infertility or anosmia.

Genetic analysis

After obtaining written informed consent for genetic studies, exome sequencing was performed in the proband and his parents using previously described methods (5) and data was filtered for mutations in known CHH genes (1). A putative mutation is defined if a variant: (i) has a minor allele frequency (MAF) of <1% in non-Finnish European controls from the Exome Aggregation Consortium (ExAC) database, and (ii) is a nonsense, frameshift, or missense variant predicted to be damaging by SIFT and/or PolyPhen-2. Identified mutations were further tested by Sanger sequencing (6). Paternity was confirmed using all exome variants to calculate relatedness (7) using the *relatedness2* function of VCFtools (8). The clinical and genetic studies were approved by the ethics committee of the University of Lausanne.

Functionality studies of FGFR1 L630P

The crystal structure of the tyrosine phosphorylated kinase domain of human FGFR1 complexed with non-hydrolysable ATP analog (PDB ID 3GQI) was used as template to predict the functional consequences of the FGFR1 L630P mutation, as previously reported (6).

HEK 293T cells were transfected with FGFR1 wide type (WT) and L630P constructs. Cell lysates were subjected to endoglycosidase digestion and then western blot analysis to determine the overall expression and maturation levels (6). Cell surface abundance was measured by radiolabeled antibody binding assay using COS7 cells (6). The activation of downstream signaling pathways by FGFR1 WT and L630P was interrogated using the osteocalcin-FGF response element (OCFRE) reporter, which reports activity of the MAPK pathway. Transient transfection and luciferase assays in L6 myoblasts stimulated by FGF8 were performed as previously described (6).

Results

Genetic testing identified a de novo FGFR1 p.L630P mutation

The proband harbors a *de novo* heterozygous mutation (c.1889T>C, p.L630P) in *FGFR1* (NM_023110) (Figure 1A). This mutation is not found in ExAC controls and is predicted to be deleterious by both SIFT and Polyphen2. No additional mutation in other known CHH genes was found.

FGFR1 L630P mutant is loss-of-function in vitro

The L630 amino acid residue maps to the highly-conserved tyrosine kinase domain of FGFR1 (Figure 1B). L630 resides on β7 strand at the base of the ATP binding cleft between the N and C-lobes of kinase where it packs against the aromatic adenine ring of the ATP analog, thereby playing a major role in ATP coordination. Proline is incompatible with β strand formation, and hence the L630P mutation should have a negative impact on the proper tertiary fold of the kinase domain (Figure 1C), leading to a complete loss of receptor function.

The overall abundance, protein maturation levels and cell surface expression are similar between the WT and L630P (Figure 1D–E). We further studied the effect of FGFR1 L630P on downstream MAPK signaling. Stimulation of WT FGFR1 with increasing doses of FGF8 yielded a typical sigmoidal dose-response curve (Figure 1F). In contrast, the L630P mutant was completely silent, reflecting the defective kinase activity as predicted by structural modeling (Figure 1F).

Confirmation of CHH diagnosis leads to optimal clinical management

The combined clinical, genetic, and functional investigations confirmed the CHH diagnosis and directly impacted the clinical management of this patient. Testosterone replacement therapy was initiated at age 13 years to induce secondary sexual development concurrent with his peers. The genetic counseling was provided to the family and the *de novo* nature of the mutation alleviated the parental concerns regarding a reproductive disorder in their other child, as well as future children.

Discussion

In this study, we describe a prepubertal male patient with micropenis, cryptorchidism and a biochemical profile of hypogonadotropic hypogonadism during minipuberty, suggestive of CHH. The identification of a *de novo FGFR1* heterozygous mutation (p.L630P) coupled with functional analyses provided genetic confirmation for the diagnosis of CHH.

Micropenis and/or cryptorchidism are evident in 30–50% of male CHH patients, indicating a severe form of GnRH deficiency (1). Minipuberty provides a key window to detect hypogonadotropic hypogonadism, as the HPG axis is transiently activated during this short period and then becomes quiescent until the onset of puberty (2, 3). Therefore male infants born with micropenis and/or cryptorchidism should be tested during this short window for an early screening of CHH. Further, combined gonadotropin treatment can be initiated

during the first year of life to correct micropenis and stimulate testicular growth, as well as to improve Sertoli cell number/function and induce testis decent (3, 9).

Genetic testing is of particular utility in cases of neonatal suspicion in order to strengthen the diagnostic of CHH as well as to provide genetic counseling to the family. Previous reports described genetic testing in familial cases using Sanger sequencing (10, 11). Here, we report genetic diagnosis of sporadic CHH in a prepubertal child using exome sequencing. Because more than 30 genes are implicated in CHH and each accounts for 1–10% of cases, testing multiple genes is needed to increase the diagnostic yield. High-throughput panel gene sequencing is more advantageous than Sanger for the cost and time effectiveness. In the genetic diagnostic setting, taking Switzerland for example, Sanger sequencing of a single gene of *FGFR1* would cost 2580 CHF and this gene is mutated only in 10% of CHH patients; while a panel testing of ten CHH genes costs 2900 CHF (including bioinformatic support), which will lead to a much higher diagnostic yield. In this current study, we performed exome sequencing because the patient participated in a larger research project and exome sequencing provides a further advantage for novel gene discovery in case of no mutation in known CHH genes can be identified.

As emphasized in recent guidelines, one major challenge of genetic testing is the interpretation of the identified variant(s) (12). In the present case, several lines of evidence support that the *FGFR1* p.L630P mutation is pathogenic: (i) this mutation is absent in ExAC controls and has been previously identified in an unrelated female patient with Kallmann Syndrome (CHH and ansomia) (13); (ii) the *de novo* nature of this mutation is consistent with the absence of family history; and (iii) functional studies confirmed that FGFR1 L630P is loss-of-function and abolishes FGF8/FGFR1 signaling, a major pathway in GnRH neuron development (14).

In conclusion, optimal diagnosis of CHH at a prepubertal age requires a high degree of clinical suspicion, and timely hormonal profile during minipuberty, ideally complemented by genetic testing with functional studies.

Acknowledgments

This work was supported by Swiss National Science Foundation Project SNF 31003A 153328 (to N. P.) and National Institute of Dental and Craniofacial Research at the National Institutes of Health Grant R01 DE-13686 (to M. M.).

We thank the patient and his family for the participation of our study. We thank Dr. Yisrael Sidis for the guidance with the cellular studies, Dr. Nicolas Niederländer and Dr. Andrea Messina for help in manuscript preparation.

References

1. Boehm U, Bouloux PM, Dattani MT, et al. Expert consensus document: European Consensus Statement on congenital hypogonadotropic hypogonadism-pathogenesis, diagnosis and treatment. Nature reviews Endocrinology. 2015; 11:547–564.
2. Grumbach MM. Commentary: A window of opportunity: The diagnosis of gonadotropin deficiency in the male infant. Journal of Clinical Endocrinology and Metabolism. 2005; 90:3122–3127. [PubMed: 15728198]
3. Bouvattier C, Maione L, Bouligand J, et al. Neonatal gonadotropin therapy in male congenital hypogonadotropic hypogonadism. Nature reviews Endocrinology. 2012; 8:172–182.

4. Main KM, Schmidt IM, Skakkebaek NE. A possible role for reproductive hormones in newborn boys: progressive hypogonadism without the postnatal testosterone peak. The Journal of clinical endocrinology and metabolism. 2000; 85:4905–4907. [PubMed: 11134160]
5. Cariboni A, Andre V, Chauvet S, et al. Dysfunctional SEMA3E signaling underlies gonadotropin-releasing hormone neuron deficiency in Kallmann syndrome. The Journal of clinical investigation. 2015; 125:2413–2428. [PubMed: 25985275]
6. Raivio T, Sidis Y, Plummer L, et al. Impaired fibroblast growth factor receptor 1 signaling as a cause of normosmic idiopathic hypogonadotropic hypogonadism. The Journal of clinical endocrinology and metabolism. 2009; 94:4380–4390. [PubMed: 19820032]
7. Manichaikul A, Mychaleckyj JC, Rich SS, et al. Robust relationship inference in genome-wide association studies. Bioinformatics. 2010; 26:2867–2873. [PubMed: 20926424]
8. Danecek P, Auton A, Abecasis G, et al. The variant call format and VCFtools. Bioinformatics. 2011; 27:2156–2158. [PubMed: 21653522]
9. Lambert AS, Bougneres P. Growth and descent of the testes in infants with hypogonadotropic hypogonadism receiving subcutaneous gonadotropin infusion. International journal of pediatric endocrinology. 2016; 2016:13. [PubMed: 27379168]
10. Sarfati J, Bouvattier C, Bry-Gauillard H, et al. Kallmann syndrome with FGFR1 and KAL1 mutations detected during fetal life. Orphanet journal of rare diseases. 2015; 10:71. [PubMed: 26051373]
11. Villanueva C, Jacobson-Dickman E, Xu C, et al. Congenital hypogonadotropic hypogonadism with split hand/foot malformation: a clinical entity with a high frequency of FGFR1 mutations. Genetics in medicine: official journal of the American College of Medical Genetics. 2015; 17:651–659. [PubMed: 25394172]
12. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine: official journal of the American College of Medical Genetics. 2015; 17:405–424. [PubMed: 25741868]
13. Shaw ND, Seminara SB, Welt CK, et al. Expanding the phenotype and genotype of female GnRH deficiency. The Journal of clinical endocrinology and metabolism. 2011; 96:E566–576. [PubMed: 21209029]
14. Falardeau J, Chung WC, Beenken A, et al. Decreased FGF8 signaling causes deficiency of gonadotropin-releasing hormone in humans and mice. The Journal of clinical investigation. 2008; 118:2822–2831. [PubMed: 18596921]

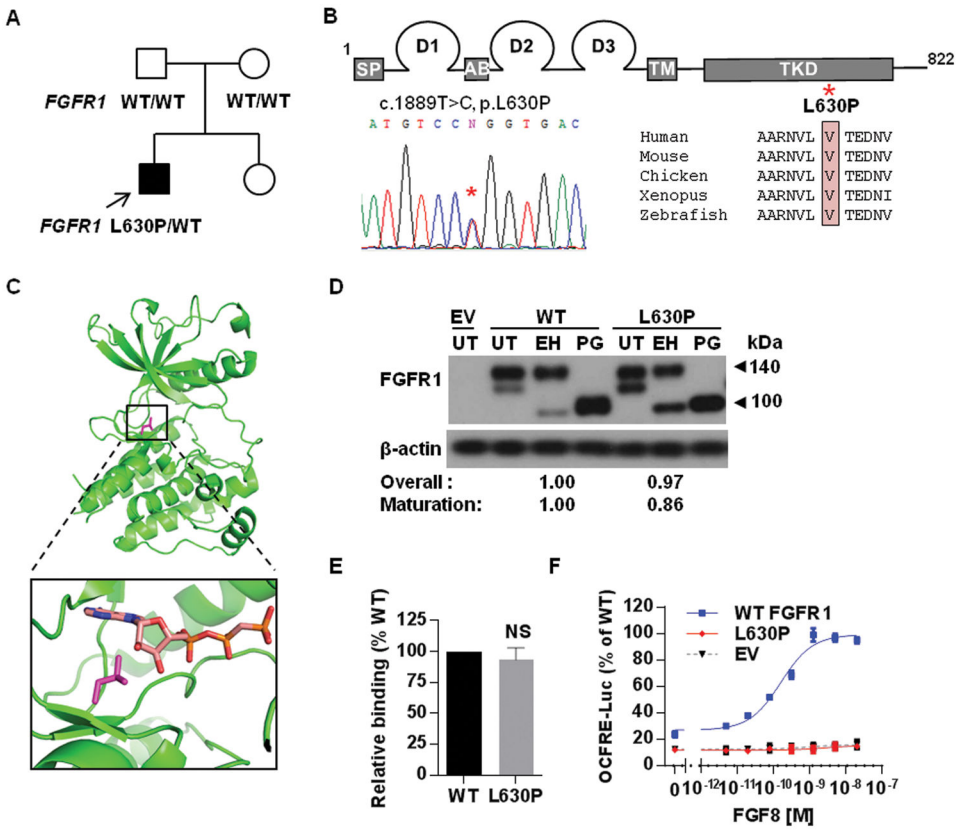


Figure 1. Loss-of-function *de novo* heterozygous *FGFR1* p.L630P mutation in the patient with neonatal suspicion of congenital hypogonadotropic hypogonadism

(A) Pedigree of the patient. WT: wild type; arrow: proband. **(B)** Schematic of FGFR1 with conservation data of L630 residue. Sanger sequencing chromatograms confirming *FGFR1* c. 1889T>C. SP: signal peptide; D1–D3: immunoglobulin like domains 1–3; AB: acidic box; TM: transmembrane domain; TKD: tyrosine kinase domain. **(C)** Structural modeling of FGFR1 L630P. Pink: L630 residue; green: FGFR1 kinase; brown stretch: ATP. **(D)** Endoglycosidase analysis of FGFR1 L630P. H293T cells were transfected with WT and L630P. Cell lysates underwent Peptide N-Glycosidase F (PG) or Endoglycosidase H (EH) deglycosidase digestion followed by western blot. Overall expression levels were determined by PG-treated samples, and receptor maturation levels were estimated by calculating the EH-resistent band (i.e. mature receptor) out of total FGFR1 immnuoreactivity of EH-treated samples. The mutant values were expressed as a ratio of WT. Experiments were repeated two times. EV: empty vector; UT: untreated. **(E)** Cell surface expression FGFR1 L630P. Values were plotted as a percentage of WT levels, shown are the means ± SEM of three experiments, and compared by unpair *t* test. NS: not significant. **(F)** Transcription reporter activity of FGFR1 WT and L630P. Experiments were repeated three times, and maximal FGF8 response was compared using F-test (Graphpad, Version 7).