Mutation profiles and clinical characteristics of Chinese males with isolated hypogonadotropic hypogonadism

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Objective: To investigate the mutation profiles and clinical characteristics of Chinese males with isolated hypogonadotropic hypogonadism (IHH) and discover new pathogenic genes that cause IHH.
Design: A gene panel, including 31 known IHH genes and 52 candidate genes, was used to perform semiconductor next-generation sequencing.
Setting: University hospital.
Patients: One hundred thirty-eight sporadic male IHH patients and 10 IHH families; 100 healthy men with normal fertility served as control subjects.
Interventions(s): None.
Main Outcome Measure(s): Targeted next-generation sequencing, polymerase chain reaction and sequencing, pedigree analysis, and bioinformatics analysis.
Result(s): Variants were distributed uniformly throughout 52 genes (52/83, 62.65%), including 16 (16/31, 51.61%) causal genes and 36 (36/52, 69.23%) candidate genes. Six new pathogenic variants and 52 likely pathogenic variants were identified in 16 genes known to cause nIHH/KS (normosmic IHH/Kallmann syndrome). In the 148 probands, *PROKR2* (22/148, 14.86%), *CHD7*, *FGFR1*, and *KAL1* had high mutation rates, and 8.78% (13/148) of the patients carried at least two variants in known genes. In addition, variants were identified in 36 candidate genes, and *EGFR*, *ERBB4*, *PAX6*, *IGF1*, *SEMA4D*, and *SEMA7A* should be prioritized for further research and genetic testing in IHH.
Conclusion(s): The mutation frequency of IHH-causal genes in Chinese HAN males was different from the data reported in white populations. Oligogenic inheritance was a common phenomenon in IHH. Our study expands the mutation profile for IHH, and the new likely pathogenic genes identified in our study warrant further research in GnRH neuronal networks. (Fertil Steril® 2018;110: 486–95. ©2018 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Isolated hypogonadotropic hypogonadism, novel variants, oligogenic genetic diagnosis, targeted next sequencing

Isolated hypogonadotropic hypogonadism (IHH) is a rare genetic disease characterized by hypogonadotropic hypogonadism with or without anosmia or hyposmia (1). Kallmann syndrome (KS) is a form of IHH with anosmia or hyposmia and accounts for approximately 60% of IHH cases. Another form of IHH characterized by a normal sense of smell is defined as normosmic IHH (nIHH). An additional rare form of IHH, adult-onset hypogonadotropic hypogonadism (AHH), is observed in patients who go through normal puberty and subsequently develop GnRH deficiency after achieving sexual maturity. IHH mainly affects pubertal development and reproductive functions and differs in prevalence between males and females (1:30,000 and 1:125,000,

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respectively) (2). IHH is a heterogeneous disease that is characterized by varying extents of abnormalities related to puberty in addition to infertility, which is caused by the deficient production, secretion, or action of GnRH. IHH is often accompanied by manifestations in other systems, such as renal agenesis or hypoplasia, cleft lip/palate, hearing loss, and cryptorchidism (3). Currently, at least 31 genes have reported associations with IHH. These include *ANOS1*, *FGFR1/FGF8*, and *PROK2/PROKR2* (4–8). However, only approximately 40% of IHH patients have mutations in these genes (9), indicating that many genes underlying the pathogenesis of IHH remain to be discovered. In addition, the mutation profiles of IHH patients may vary among different ethnic groups, and few genetic studies have been reported in Chinese patients with IHH (10, 11).

The key to diagnosing IHH is excluding differential diagnoses, such as pituitary tumors or functional causes. Currently, a diagnosis of IHH is based on clinical, biochemical, and imaging investigations. It is especially challenging to differentiate IHH and constitutional delay of growth and puberty in early adolescence (12). Genetic testing is a good method for diagnosing IHH and is necessary to determine an IHH prognosis and provide related genetic counseling (13). Thus, seeking an efficient and economical tool for genetically testing for this condition is very important for clinicians and patients.

IHH is classically categorized as a monogenic disorder, meaning that one defective gene is sufficient to cause the disease phenotype. However, the bigenic and oligogenic inheritance has been observed in many genetic diseases, including IHH (14). In addition, multiple gene defects could synergize to produce a more severe IHH phenotype. Thus, it is very important to use genetic models to explore genotype-phenotype correlations.

In recent years, next-generation sequencing has emerged as an effective tool for sequencing large numbers of genes (15). Considering its efficiency and cost, targeted sequencing is effective for detecting mutations within candidate genes for well-characterized diseases with known causal genes. In addition, targeted sequencing can yield more than a 99% coverage rate and 500 × depth data (16), ensuring ample coverage and precision when used to detect mutations in correctly diagnosed patients.

The objective of our study was to investigate the mutation profiles of known pathogenic genes in Chinese males with IHH and to use targeted next-generation sequencing to identify new candidate causal genes for IHH. In addition, the clinical features of IHH patients carrying specific gene mutations were observed and noted. Furthermore, the data presented here suggest that targeted sequencing could act as a tool for genetic testing for IHH, which is important for the diagnosis, prognosis, and genetic counseling of IHH patients.

MATERIALS AND METHODS

Study Subjects

This study included 138 sporadic male IHH patients and 10 IHH families. All patients were admitted to the outpatient department of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, from 2005 to 2013. All of the included patients were diagnosed with IHH and classified as KS or nIHH according to standard procedures and previously described diagnostic criteria (2, 17).

The patients recruited in the cohort or their adult parents signed an informed consent form. The experiments performed on the tissue obtained from the patients and 100 controls were approved by the ethics committee of Tongji Hospital.

Clinical Measurements

Body weight, height, Tanner stage, testicular volume, and penis length were evaluated at each visit. Body height was measured as the distance between the sole and the highest point of the head. Body weight was measured after the patient removed articles and clothing under comfortable conditions. Testicular volume (including the scrotum) was measured using a Prada orchidometer. Tanner stages were evaluated by a senior physician. Penis length was measured in the standing position after emptying the bladder.

Laboratory Test

Serum FSH, LH, T, and E_2 levels were measured by chemiluminescence immunoassays (UniCel DXI 800, Beckman Coulter). The normal ranges for serum FSH, LH, T, and E_2 levels were 1.27–19.26 mIU/mL, 1.24–8.62 mIU/mL, 1.75–7.81 ng/mL, and 20–75 pg/mL, respectively.

Designing Gene Panel and Sequencing

A search of well-known databases (OMIM, GTR-NCBI, and HGMD) resulted in the identification of 83 genes that were used as targets in the next step of the study. These genes included 31 known disease-causing genes and 52 candidate genes. All the known disease-causing genes were reported to be discovered in KS or nIHH patients and were validated by functional experiments. The candidate genes were classified into two modules: [1] genes that were confirmed in mouse models to affect GnRH neuron migration and function and [2] family members or paralogs of known genes that were predicted to result in GnRH neuron abnormality. All the genes and their corresponding references are shown in Supplemental Table 1. Next-generation sequencing was conducted on the IonTorrent PGM platform (18). Detailed quality control information is summarized in Supplemental Table 2.

Bioinformatics Analysis

All variants were compared with entries in known databases (dbSNP, UCSC, ESP, and ExAC) to remove common single nucleotide polymorphisms (minor allele frequency $\geq$ 1%). Then all the intron variants were discarded. The retained variants were searched in the control samples, resulting in a collection of potential pathogenic variants. The putative disease-causing single nucleotide variants were then imported into online scoring databases (SIFT, Proven, Polyphen2, and MutationTaster) to evaluate the possibility of pathogenicity (in SIFT, score $<$ 0.05; in Proven, score $<$ –2.5; in Polyphen2, score $>$ 0.5; and in MutationTaster, disease causing or disease causing automatically; shown in Supplemental Table 3).