**Peer Review File**

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**Reviewer A**

**Comment 1:** This paper is clear and well written, and generally ready for publication with one or two small details to review. One thing that is lacking is the population background from which your 40 index patients are drawn. This should be clarified and stipulated so that it is evident.

**Reply 1:** Thanks for the reviewer's suggestion. We are very sorry for the inconvenience caused by our negligence to your reading of the manuscript. We have downloaded several articles recently published by your magazine according to your opinions and carefully read and compared our revised manuscripts. We have tried our best to edit this manuscript.

All the patients were Han Chinese males who came to our hospital for gonadal dysplasia or fertility problems. Sample collection criteria: (1) Reproductive phenotype: small penis, small testis or cryptorchidism or penis testis were childish, secondary sex characteristics were not prominent, etc. Patients with or without anosmia; A family history of related diseases; (2) Sex hormone tests: low testosterone (T), very low follicle-stimulating hormone (FSH) and follicle-stimulating hormone (LH) levels or below the upper limit of normal [(in general: T< 3.47 nmol/L, LH< 0.7 IU/L), while prolactin (PRL) values were average (to exclude Prolactinoma)]; (3) Brain MRL/CT showing normal or underdeveloped olfactory lamina and/or olfactory sulci (to exclude pituitary tumors); (4) Chromosome karyotype 46, XY without Y chromosome microdeletion; (5) Other related hormone tests showed no abnormalities.

Readers have learned a lot from all the papers published in your journal. We hope to have our article considered for publication in your journal. Should there be any other corrections we could make, don't hesitate to get in touch with us. Special thanks to you for your good comments.

**Changes in the text:** Line 81-92: “All the patients were Han Chinese males who came to our hospital for gonadal dysplasia or fertility problems. Sample collection criteria: (1) Reproductive phenotype: small penis, small testis or cryptorchidism or penis testis were childish, secondary sex characteristics were not prominent, etc. Patients with or without anosmia; A family history of related diseases; (2) Sex hormone tests: low

testosterone (T), very low follicle-stimulating hormone (FSH) and follicle-stimulating hormone (LH) levels or below the upper limit of normal [(in general: T< 3.47 nmol/L, LH< 0.7 IU/L), while prolactin (PRL) values were average (to exclude Prolactinoma)]; (3) Brain MRL/CT showing normal or underdeveloped olfactory lamina and/or olfactory sulci (to exclude pituitary tumors); (4) Chromosome karyotype 46, XY without Y chromosome microdeletion; (5) Other related hormone tests showed no abnormalities."

**Comment 2:** I am surprised to find such a high number of presentations with HH in a four-year period. In 30 years in one university centre covering a city population of three million and a regional population of upwards of 20 million, I encountered no more than a handful of women or men with HH, certainly less than 10.

**Reply 2:** Thanks for the reviewer's suggestion. We are sorry to make readers confused. We have tried to revise all data in our manuscript according to the comments. We diagnosed 40 IHH patients (22 KS and 18 nIHH) from 2014 to 2021 in Nanjing Jinling Hospital, Jiangsu Provincial People's Hospital, and the First Affiliated Hospital of the University of Science and Technology of China.

In addition, we have carefully studied the reviewer's words and have made a correction which we hope meets with approval. Special thanks to you for your good comments.

**Changes in the text:** Line 78-81: "We diagnosed 40 IHH patients (22 KS and 18 nIHH) from 2014 to 2021 in Nanjing Jinling Hospital, Jiangsu Provincial People's Hospital, and the First Affiliated Hospital of the University of Science and Technology of China."

**Comment 3:** The quality of the writing is occasionally less than expected and I would urge you to read through the paper very carefully to exclude and correct these. Two examples are here:
Four of the patients carried out a pedigree investigation - well, they didn't but you did so on them - please correct

The sister of proband four was concerned with sensorineural hearing - I imagine you mean "by" not "with"

**Reply 3:** Based on these comments and suggestions, we have carefully modified the original manuscript and carefully read the manuscript to minimize errors. In addition, we have carefully studied the reviewer's suggestions and have made a correction which we hope meets with approval. We believe the manuscript has been dramatically

improved and hope it has reached your magazine's standard. Special thanks to you for your good comments.

**Changes in the text:** Line144: "We carried out a pedigree investigation in four of the patients."
Line147-148: "The sister of proband four was concerned by sensorineural hearing and olfactory impairment."

**Reviewer B**

**Major points:**

**Comment 1:** Several important details are missing from this manuscript. For example, "whole exon sequencing" (maybe should be "whole exome sequencing") details (both experimental and computational), which are the key foundation of this study, are missing from the Material and method section. Such details are critical and necessary to understand this study.

**Reply 1:** We are very sorry for the confusion caused to your reading due to the clarity of the pictures. According to your suggestion, we have rewritten this part of the manuscript. We hope you can be satisfied with this modification. WES experiments for this study were conducted with the assistance of Beijing Nuohe Zhiyuan BioInformation Technology Co., LTD. Specific steps: First, we extracted 3mL of peripheral blood from all participants to extract gDNA. We tested the sample quality again; the total amount of DNA was ≥2ng, and the concentration was ≥40ng/μL. Then the library construction and quality inspection. Then the samples were sequenced on the machine, and we analyzed the sequencing results informatically. Based on your comments, we also attached a point-by-point letter to you. We have made extensive revisions to our previous draft. We hope the revised manuscript could be acceptable to you. Special thanks to you for your good comments.

**Changes in the text:** Line104-109: "WES experiments for this study were conducted with the assistance of Beijing Nuohe Zhiyuan BioInformation Technology Co., LTD. Specific steps: First, we extracted 3mL of peripheral blood from all participants to extract gDNA. We tested the sample quality again; the total amount of DNA was ≥2ng, and the concentration was ≥40ng/μL. Then the library construction and quality inspection. Then the samples were sequenced on the machine, and we analyzed the sequencing results informatically."

**Comment 2:** Figure 1 is not relevant to this particular study and should be removed from the manuscript.

**Reply 2:** Thank you for your lovely comments on our article. Following your suggestions, we have removed Figure 1 from the previous manuscript and corrected several errors. Based on your comments, we also attached a point-by-point letter to you. We have made extensive revisions to our last draft. We hope the revised manuscript could be acceptable to you.

Readers have been learning a lot from all the papers published in your journal. We hope to have our article considered for publication in your journal. Should there be any other corrections we could make, please contact us. Special thanks to you for your good comments.

**Changes in the text:** Following your suggestions, we have removed Figure 1 from the previous manuscript.

**Comment 3:** There are a lot of errors in the manuscript, even in the Abstract and Keywords section. For example, "Kallmann syndrome" in line 15 and line 35 (should be Kallmann syndrome) and "whole exon sequencing" in line 20 (maybe should be "whole exome sequencing").

**Reply 3:** Thanks for the reviewer's suggestion. Sorry for the confusion. We have tried our best to edit this manuscript. In addition, we carefully studied the reviewer's words and made corrections to be recognized. Thank you very much for your kind comments.

Readers have learned a lot from all the papers published in your journal. We hope to have our article considered for publication in your journal. Should there be any other corrections we could make, don't hesitate to get in touch with us. Special thanks to you for your good comments.

**Changes in the text:** Line 15: "Male idiopathic hypogonadotropic hypogonadism (IHH) is a heterogeneous clinical rare genetic disorder that can be divided into two forms: Kallmann syndrome (KS) and olfactory normal IHH (nIHH)."
Line36: "Keywords: Idiopathic hypogonadotropic hypogonadism; Kallmann syndrome; Whole exome sequencing; Sanger sequencing; IHH"
Line20: "The proband genomic DNA (gDNA) was confirmed by whole exome sequencing (WES) and Sanger sequencing."

**Comment 4:** The conclusion in the Abstract in line 28, "Once destroyed, the function of the protein is seriously affected", needs to be revised or experimental data to support it.

**Reply 4:** Thank you for the your suggestion. Based on your suggestion, we rechecked this part of the manuscript, and after careful consideration, we deleted it. We have tried our best to revise our manuscript according to the comments. In addition, we have carefully studied the Editor's words and have made a correction which we hope meet with approval. Once again, thank you very much for your comments and suggestions.

**Changes in the text:** Line 27-30: "The comparison of MEGA5 software showed that all the variants had extremely high homology among different species and were extremely conservative in evolution. The total positive detection rate of 40 patients was 30% (nIHH 8/18 + KS 4/22), and the FGFR1 mutation rate accounted for 7.5% (3/40)."

**Minor points**

**Comment 1:** The text in Figure 3 is too small to be seen.

**Reply 1:** We are sorry for your careless mistakes. Thank you for your reminder. Thank you again for your positive comments on our manuscript. We reworked the Figure 3, split it into two parts and enlarged it. We believe that the manuscript has been greatly improved and hope it has reached your magazine's standard.

**Changes in the text:** We reworked the Figure 3, split it into two parts and enlarged it.

**Comment 2:** The writing of the manuscript needs improvement.
For example:
Line 76: "From a genetic point of view, determine the cause of the patient's illness."
Line 103: "We designed primer 5.0 primer design software to extend"
Line 136: "Four of the patients carried out a pedigree investigation". I think that the authors carried out the investigation, not the patients.
Line 165: "EMA3E gene mutations" (should be SEMA3E)
Line 227: "In 1991, ANOS1, a candidate gene for X-linked KS, encoded a protein related to adhesion molecules."
Line 260: "test-tube babies"
Line 261: "We screen for abnormalities in embryos to prevent genomic abnormalities in fetuses and achieve the goal of eugenics."

**Reply 2:** Thank you for the Reviewer's suggestion, as the Reviewer suggested that we have tried our best to revise our manuscript according to the comments. In addition, we have carefully studied the Reviewer's words and have made a correction which we hope meet with approval. If there are any other modifications we could make, we would like to modify them very much, and we appreciate your help. We hope that our manuscript can be considered for publication in your journal. Thank you very much for your support.

**Changes in the text:** Line 76: "From a genetic point of view, determine the cause of the patient's illness." We have carefully read the reviewer's suggestion and decided to delete this part of the manuscript.
Line116-117: "We designed primer using primer 5.0 software and amplified the exome regions of possible disease gene mutation sites analyzed by the proband and some families from the WES results."
Line 149: "We carried out a pedigree investigation in four of the patients"
Line 178: "*SEMA3E* gene mutations are primarily related to KS and affect the survival of GnRH neurons."
Line 240: "In 1991, ANOS1, a candidate gene for X-linked KS, encoded a protein related to adhesion molecules." We have carefully read the reviewer's suggestion and decided to delete this part of the manuscript.
Line 270-274: "IHH patients to treat (for example, patients with autosomal dominant inheritance patterns of disease genes, in theory, there are risks of 1/2 passed on to the next-generation), can be utilized to assisted reproductive technology to produce healthy babies, recommended before transplantation genetic diagnosis technology."
Line 261: "We screen for abnormalities in embryos to prevent genomic abnormalities in fetuses and achieve the goal of eugenics." We have carefully read the reviewer's suggestion and decided to delete this part of the manuscript.