Genetic profiles and three-year follow-up study of Chinese males with congenital hypogonadotropic hypogonadism

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Abstract

Genotypes-phenotypes correlation and treatment outcomes for 73 Chinese CHH male patients was performed in this study. Patients self-selected one of the four treatments: pulsatile Gonadorelin® pump, cyclical gonadotropins therapy, human menopausal gonadotropin monotherapy, or testosterone replacement treatment. Clinical assessments were performed every 3 months for 3 years. Baseline clinical features, spermatogenesis and secondary sexual development outcomes were analyzed. Whole exome sequencing identified 63 variants in 52 patients (70%), 18 of which were novel. Variants on FGFR1, PROKR2, CHD7, ANOS1 and NSMF gene were 10 (15.87%), 10 (15.87%), 7(11.11%), 5(7.93%) and 5(7.93%) respectively. Some null variants could lead to severe clinical manifestations than missense variants on FGFR1 and CHD7. The Lasso regression model for spermatogenic failure risk showed that cryptorchidism history, abnormal epididymis or prostate, lower basal LH and peak-LH post Triptorelin® stimulation were significant predictors. Approximately, 30% normosmic patients defined by simple olfactory assessment showed olfactory nerve center maldevelopment with nasal sinus MRI. The severity of reproductive system was attributed to spermatogenesis that could be predicted by nomogram model. No direct correlation was observed between candidate genes and spermatogenic outcome, however, the clinical severity is partially related with specific variants, and clinical features might in turn affect the treatment efficacy.

2. MATERIAL AND METHODS

The study was approved by the Ethics Committee of the First Affiliated Hospital, Sun Yat-sen University (approval number [2013] C-112). Informed consents were obtained from patients.

2.1 Subjects

Clinical data of 73 unrelated CHH males of Chinese Han ethnicity who were treated in first affiliated hospital of Sun Yat-sen University from June 2013 to June 2018, were reviewed and analyzed. CHH was confirmed according to Chinese Consensus Statement on Idiopathic Hypogonadotropic Hypogonadism (Chinese Society of Endocrinology, 2015). The inclusion criteria were as follows: (a) males aged over 15-year who failed to enter spontaneous puberty with partial or no development of secondary sex characteristics; (b) baseline serum testosterone level (TL) <1 ng/ml accompanied with low or inappropriate normal gonadotropins; (c) chromosome karyotype of 46,XY; (d) normal prolactin level; (e) available baseline and follow-up data. Exclusion criteria include: (a) pan-adenohypophysis hypofunction; (b) pituitary stalk interruption syndrome; (c) pituitary and hypothalamic lesions identified by MRI; (d) acquired hypogonadotropic hypogonadism caused by other reasons such as malnutrition, chronic systemic diseases or trauma; (e) sex reversal syndrome; (f) hypogonadotropic hypogonadism reversed before age 18. Family members were advised to receive genetic sequencing with the probands.

2.2Whole exome sequencing and variant selection

Detailed method of whole exome sequencing is described in**Supplementary Material** .

Variants selection criteria: (a) allele frequency with a cut-off value of <0.05 in four databases (dbSNP, HapMap,1000 Genome Project and in-house Chinese local database); (b) sequencing depth>30; (c) non-synonymous mutations on CHH-related genes; (d) heterozygous variants with autosomal dominant or X-linked (Hemi) inheritance; homozygous variants with autosomal recessive inheritance; (e) the previously reported pathogenic or disease-causing variants.

Classification of variants [pathogenic, likely pathogenic, variants of uncertain significance (VUS)] was done according to the variant interpretation guidelines of American College of Medical Genetics and Genomics (ACMG) (Richards et al., 2015).

2.3 Sanger sequencing

The primer pairs for Sanger sequencing are listed in**Supplementary Data** .

2.4 Clinical assessment, study design and spermatogenesis treatment method

The baseline pituitary adrenal/thyroid/ gonadal /growth hormone functions were generally assessed. The protocol of Triptorelin® (a GnRH analog) stimulation test (T®-stimulation) and HCG stimulation test were described in our previous article (Zhang et al., 2019). The patients revisited hospital to get laboratory tests and physical examinations in every three months. The testicular volume (TV) was calculated as 0.71 × length × width × height measured by ultrasound. The simple smell sense assessment was done by letting patients to distinguish the smell of alcohol, white vinegar, shampoo and water. The nasal sinus MRI examinations were performed in order to allow optimal evaluation of the olfactory nerve center (ONC) development (Yousem et al., 1996). The presence of at least one of the following abnormalities was considered abnormal olfactory structural development: absent or hypoplastic olfactory bulbs, olfactory sulci, or olfactory tracts (Lewkowitz-Shpuntoff et al., 2012). FSH, LH, TL and other hormones were measured with chemiluminescent microparticle immunoassay (CMIA) (Abbott Co.).

Patients selected one of the four methods based on their personal choice or preferences: pulsatile Gonadorelin® (a GnRH medication) pump (PGP), cyclical gonadotropins [human chorionic gonadotropin (HCG) / human menopausal gonadotropin (HMG)] therapy (CGT), HCG-monotherapy and testosterone replacement treatment (TRT). The protocol of PGP and CGT were described in our previous article (Zhang et al., 2019). As for HCG-monotherapy, 2000iu HCG intramuscular injection was given one to three times every week. In TRT, the testosterone undecanoate was given in the form of oral administration or intramuscular injection. The follow-up assessments were done in every three months until spermatogenesis was documented, their sexual partner became pregnant, or the 36-month time point was reached, whichever came first.

2.5 Treatment outcomes measurement

The primary outcome was defined as the occurrence of spermatogenesis (sperm density [?] 0.1 million/ml) within three years treatment. Male patients who achieved spermatogenesis with PGP, CGT or HCG-monotherapy were classified as 'successful treatment subgroup', while the 'failure treatment subgroup' included patients who remained azoospermia under either PGP or CGT. Patients were classified as 'undetermined' if they remained non-spermatogenic under HCG-monotherapy or received TRT. As we know, the HCG-monotherapy might induce spermatogenesis in just some mild CHH patients, not a standard efficient spermatogenesis therapy. TRT could not induce spermatogenesis. Other indicators evaluating treatment outcomes included the serum TL and TV after treatment.

2.6 Statistical analysis

Statistical analysis was conducted with R software (version 3.6.3; http://www.Rproject.org). Least absolute shrinkage and selection operator (Lasso) method was used to select significant features and then a regression

model was built (Tibshirani et al., 1997) with package 'glmnet'. The 'rms' package was used for the prediction nomogram. The R packages used in this study please refer to **Supplementary Material** . The developed models were evaluated by their discrimination ability using C-statistics. Data were presented as *mean +- standard deviation* for the continuous variables in SPSS version 25. Student's t -test was performed to compare two groups of the continuous variables. Chi-square analysis was used for the comparison of rates. All statistical tests were two-sided, and P <0.05 was considered statistically significant.

3. RESULTS

3.1 Whole exome sequencing identified mutations

Based on selection criteria, sixty-three variants were identified in 52 patients (70%), among which eighteen variants were novel. Among these mutations, variants on *FGFR1, PROKR2, CHD7, ANOS1, NSMF* and*HS6ST1* gene were 10 (15.87%), 10 (15.87%), 7(11.11%), 5(7.93%), 5(7.93%), 4(6.35%) respectively. Eleven patients had digenic variants. All the identified gene variants are listed in**Table 1** .

3.2 Clinical features

46 cases (63%) were KS and 27 cases (37%) were nCHH based on simple smell sense assessment. Clinical features were shown in **Figure 1** . Detailed phenotypes are described in the **Supplementary Data** .

The mean TL and TV increased significantly after treatment. In contrast, penile lengths and circumferences were improved slowly after treatment. In particular, Patient 14 reached spontaneous spermatogenesis after treatment at age 27, who was the only patient with reversal HH in the study (**Table 1**).

3.3 Phenotypes and spermatogenic outcomes associated with individual gene variants

3.3.1 Patients harbouring *FGFR1* Mutation

Ten *FGFR1* variants were found in ten patients (**Table 1**), eight of which were novel. Sanger sequencing of the parents confirmed the *de novo FGFR1* variants in Patient 1, 2 and 3.

Two patients (Patient 5 and 9) remained non-spermatogenic after CGT and PGP treatment. With a novel missense (p.Leu781Pro) mutation, Patient 9 showed small prostate, left varicocele and right-sided hydrocele of spermatic cord sheath (**Supplementary Data**). These clinical features could be the reasons of leading to spermatogenesis failure. Patient 5 had two variants, including a novel heterozygous*loss-of-function FGFR1* mutation and a novel hemizygous*ANOS1* splice-donor site mutation, and he was characterized by bilateral cryptorchidism. Six male patients who achieved spermatogenesis presented no/unilateral cryptorchidism and no bilateral varicocele. Interestingly, when comparing with males harbouring missense variants (Patient 7-10), those with *loss-of-function* mutations (Patient 1,2,3,6) tended to have lower LH after Triptorelin$^{(r)}$-stimulation (**Supplementary Data**).

3.3.2 Patients harbouring *PROKR2* Mutation

Four patients (Patient 14-16,18) harbouring the same heterozygous*PROKR2* mutation (p.Tyr113His) showed different clinical baseline with various treatment outcomes. Three of them achieved spermatogenesis (**Table 1**). Specifically, Patient 14 experienced HH reversal with spontaneous recovery of reproductive functions. Patient 18 achieved spermatogenesis within 2 months under HCG-monotherapy. Patient 15 failed to reach spermatogenesis with bilateral varicocele developed during treatment. Different smell senses and ONC development were also observed in these four cases. Patient 16 and 18 showed hyposmia with ONC underdevelopment. Patient 14 and 15 showed normal smell sense with MRI confirming normal ONC development in Patient 15. Patient 17 harbouring homozygous mutation (p.Tyr113His) lost follow-up.

Three patients (Patient 11-13) carrying the same heterozygous*PROKR2* mutation (p.Trp178Ser) all achieved successful spermatogenesis in short treatment period (**Table 1**). They presented similar baseline and peak-LH levels with similar ONC development conditions. Patient 13 showed hyposmia. Patient 11 and 12 manifested normal smell senses in simple olfactory test but MRI indicated ONC hypoplasia (**Supplementary**

Data). In addition, two patients (Patient 16,20) were diagnosed with diabetes and one patient identified with fatty liver (**Supplementary Data**).

3.3.3 Patients with *CHD7* Mutation

Among the seven patients with *CHD7* mutations, six (Patient 22-27) presented either hearing impairment, or external/ inner ear abnormalities, which meet the new diagnosis criteria of CHARGE Syndrome (Hale et al., 2016), therefore they were classified as CHARGE syndrome. Patient 22 and 24 had typical ear pavilion abnormalities (**Figure 1A, 2A**). Patient 22 harbouring *de novo CHD7* frameshift variant showed classical symptoms of CHARGE, including multiple organ deformities, left cryptorchidism, small prostate, poor testes condition, left ear deafness, bilateral auricular deformity, serious myopia, concomitant exotropia, bilateral uveitis and bilateral optic atrophy, mild thoracic kyphoscoliosis, bilateral cubitus valgus, dental dysplasia and heart valves disease. Five patients with CHARGE received spermatogenic treatment (Patient 22-23, 25-27), and three of them failed to reach spermatogenesis (**Table 1**). Besides, six patients presented severe olfaction defects. Patient 25 showed normal smell sense, unfortunately he did not receive nasal sinus MRI.

3.3.4 Patients with *ANOS1* Mutation

Five *ANOS1* variants were found, including two truncating, two splice-site and one missense variant (**Table 1**). These patients had different non-reproductive presentations: two of them (Patient 5,33) demonstrated unilateral renal dysplasia, and other clinical signs included fatty liver, hearing loss, and impaired glucose tolerance (**Supplementary Data**). These two patients remained non-spermatogenic after treatment. Specifically, patient 5 was digenic with a pathogenic mutation in *FGFR1* and a splice-donor site mutation in *ANOS1* gene, and he manifested worse testes condition of bilateral cryptorchidism (**Supplementary Data**).

3.3.5 Patients with digenic variants

Eleven patients had digenic variants (15%) (**Table 1**). Oligogenic inheritance involving *FGFR1* or *PROKR2* variants was found in six patients, five of them had successful induction of spermatogenesis (**Table 1**). In particular, Patient 5 with likely pathogenic *FGFR1/ANOS1* digenic variants showed renal agenesis, and his treatment for spermatogenesis was failed. Besides, digenic variants involving *CHD7* were identified in three patients, two of whom reached sperm production. *ANOS1/NSMF, WDR11/SEMA3A* mutations were found in 2 patients **(Table 1)** .

3.4 The predictive model for spermatogenic failure risk

Sixteen clinical predictors (cryptorchidism history, varicocele, Tanner stage of pubic hair development, previous sex hormones exposure, calcification, abnormal epididymis or prostate on ultrasound, KS or nCHH, treatment method, age, BMI, average TV, basal LH, peak-LH after $T^{(r)}$-stimulation, basal FSH, peak FSH after $T^{(r)}$-stimulation, basal testosterone) were tested by Lasso regression model, they were normalized by transforming into new scores with a mean of 0 and a standard deviation of 1.

After variable selection, four predictors were selected as risk factors, including cryptorchidism history, abnormal epididymis or prostate on ultrasound, basal LH and peak-LH after $T^{(r)}$-stimulation were selected for calculating the spermatogenesis-failure risk score and risk model (nomogram) (**Figure 2**) (**Supplementary Material**). The nomogram had excellent discriminative power with a C-statistic of 0.783 (95%CI 0.076-0.746). The predicted probabilities of spermatogenesis-failure rate were shown in **Figure 2** .

The comparisons of clinical factors between patients achieving spermatogenesis with non-spermatogenesis were shown in **Table 2** . It indicated similar results to Lasso Model, which suggested that clinical predictors in favour of spermatogenesis included negative cryptorchidism, negative epididymis/ prostate disorders, nCHH, higher baseline basal-LH and peak-LH after $T^{(r)}$-stimulation.

3.5 Smell sense and olfactory nerve center (ONC) development

Almost all KS patients who underwent nasal sinus MRI exhibited ONC dysplasia except for Patient 3 who

acquired hyposmia from rhinitis with normal bilateral olfactory development. Among ten nCHH patients, three (Patient 11, 12 and 44) showed ONC maldevelopment in nasal sinus MRI, which suggested that the true olfactory deficit was underestimated by means of simple olfactory assessment (**Supplementary Data)** .

4. DISCUSSION

This study indicated that the spermatogenesis-failure is associated with clinical predictors including cryptorchidism, abnormal epididymis or prostate, lower basal and triptorelin-stimulated LH levels. However, some null variants (e.g. nonsense, frameshift, canonical +/-1 or 2 splice sites) could lead to severer clinical manifestations than missense variants (eg. *FGFR1, CHD7*), and the severe clinical symptoms would in turn affect the therapeutic efficacy.

4.1 CHH patients and disease-related mutations

As relatively strict criteria were adopted when selecting the variants generated by WES (see **Methods**), numerous heterozygous variants on *TACR3, POLR3A, KISS1, POLR3A, POLR3B, RNF216, WDR11* genes with autosomal recessive mode of inheritance were excluded. Under these criterion, disease-related mutations on CHH candidate genes were identified in 52 out of 75 patients. Forty-one patients were identified with monogenic inheritance and eleven patients were found oligogenic.

The *FGFR1* mutation prevalence of 17.24% in this study is consistent with previous reports (Pitteloud et al., 2005; Pitteloud et al., 2006). Frameshift variant could impair the function of FGFR-1 protein as it leads to amino acid substitution and creates a premature stop codon of the new reading frame, resulting in the formation of a truncated protein. Novel *FGFR1* frameshift variants were detected in five CHH patients located on exon 5, 7, 10, 16 and 17, respectively. A canonical splice site variant was detected in the intron 7 of*FGFR1* that destroyed the conserved splice site consensus sequence for exon 7. Based on sanger sequencing results of the asymptomatic parents of three probands, three *de novo* variants were identified and categorized as pathogenic. In these patients, we found that*loss-of-function FGFR1* variants can cause more severe clinical manifestations in CHH, and the clinical features could further affect treatment outcomes. Besides, CHH patients with *FGFR1* mutations were more likely to have dental, limbs, or kidney abnormalities. The non-reproductive signs in patients with *FGFR1* variants were consistent with several independent reports (Tsai et al., 2006; Costa-Barbosa et al., 2013; Dode et al., 2003).

PROKR2 is related to GnRH neuron migration and bulb morphogenesis. Interestingly, four patients with heterozygous missense mutation (p.Tyr113His) (Cole et al., 2008) showed widely different clinical features and therapeutic response, while the three patients with p.Trp178Ser variant presented similar baseline conditions and treatment outcomes. We speculate that the similar baseline testes conditions, ONC abnormalities among patients harbouring p.Trp178Ser mutation showed well genotype-phenotype correlation and were associated with similar outcomes. Diabetes and fatty liver were found in this cohort with *PROKR2* variants (**Supplementary Data**), which is consistent with the previous reports that some metabolic disorders were observed in association with *PROKR2* mutations (Sarfati et al., 2010; Sarfati et al., 2013). One patient carrying *PROKR2* variant presented CHH reversal. CHH reversal was observed in 10-15% CHH patients (Lewkowitz-Shpuntoff et al., 2012; Dwyer et al., 2016) and some were reported to have pathogenic variants in related genes, including*GNRHR* , *FGFR1* , *CHD7* and *PROKR2* (Mitchell et al., 2011).

CHD7 mutations were identified in approximately 60-70% patients with CHARGE syndrome, which is characterized by coloboma, heart defect, atresia choanae, retarded growth and development, genital hypoplasia, ear anomalies and deafness (Lalani et al., 2006). Among the six patients diagnosed with CHARGE Syndrome, one patient harbouring *de novo CHD7* frameshift variant showed classical clinical manifestations, while the remaining five with missense variants showed only partial signs listed above. This finding was consistent with recent work confirming that patients with *CHD7* variants could also have a milder phenotype, presenting KS or nCHH, and that milder forms were usually associated with missense variants on *CHD7* (Marcos et al., 2014; Balasubramanian et al., 2014). The structural and functional outer/inner ear, or hearing problems in patients with CHARGE syndrome need to be recognized with caution through Pure Tone Audiometry

examination, or Ear Mastoid CT scan, because the mild form could be ignored sometimes. Here we reported that the ear/hearing problem was the most common sign and was a clue for CHARGE Syndrome. It still remained controversial regarding the possibility that *CHD7* variants could cause CHH with normal olfaction (Kim et al., 2008). In this study, six *CHD7*- mutated patients appeared severe olfaction defects, the remaining one patient had normal smell sense but his precise ONC evaluation was missing. We recommend detailed assessment of ear structure and hearing function especially for those with *CHD7*mutations as mild hearing/ear problems were frequently neglected or under-reported. Besides, there were twelve CHH patients who presented ear or hearing problem harbouring different variants on genes including *PROKR2, FGFR1, ANOS1, SOX3* (**Supplementary Data**). Hence, ear / hearing problem is one of the common non-reproductive features in CHH patients.

Most of the reported *ANOS1* variants were frameshift, only a few missense alterations were reported (Tsai et al., 2006; Goncalves et al., 2017). The frequently associated non-reproductive signs included bimanual synkinesis and renal agenesis (Goncalves et al., 2017). Three out five *ANOS1* mutations identified in CHH were novel, including one frame-shift, and two splice site variants. Non-reproductive symptoms in these patients included unilateral renal dysplasia, fatty liver, hearing loss, and impaired glucose tolerance (**Supplementary Data**). These features broaden the clinical spectrum of patients with *ANOS1* mutations.

Moreover, several patients were also identified with variants on *WDR11* , *LHB* , *NR5A1, LHX4, SPRY4, etc.,* which were also reported as CHH-related genes (Miraoui et al., 2013; Cho et al., 2019; Bashamboo et al., 2010). The clinical baseline and treatment results indicated genotype-phenotypes correlations but the spermatogenic treatment efficacy was not directly correlated with the specific candidate genes. The severity of clinical symptoms is partially related with the gene variants may due to different extent of damage caused by the variants to the gene functions, and the severe clinical symptoms would in turn affect the therapeutic efficacy.

4.2 Oligogenic Pattern

Eleven CHH patients may follow the oligogenic pattern in the disease development, including *FGFR1/ANOS1/LHB/NR0B1* ,*PROKR2/IL17RD/SOX10* , *CHD7/SEMA3A/NSMF* , *ANOS1/NSMF, WDR11/SEMA3A,* suggesting that these variants acted in synergy to bring to the phenotypes of CHH. In particular, Patient 5 with *FGFR1/ANOS1* digenic variants showed renal agenesis. However, as almost no case of renal abnormalities was reported with *FGFR1* variants, the renal malformation is more likely caused by *ANOS1* variant. Both *FGFR1* and *PROKR2* were found to coincide with the same *LHB* variant (Gly122Ser) in 2 patients (**Table 1**). This *LHB* variant have been reported to significantly affect the binding ability of luteinizing hormone to its receptor and greatly reduce the biopotency for steroidogenesis of luteinizing hormone (Liao et al., 2002). No direct obvious correlation was observed between number of variants in oligogenic inheritance and spermatogenesis outcomes, partially because of relatively small number of patients.

4.3 Treatment Outcome pattern

The primary outcome was defined as sperm density [?]0.1 million/ml, as it is known that low sperm concentration does not preclude conception (Burris et al., 1988). Sixteen clinical predictors of spermatogenesis were firstly included in Lasso model based on previous reports (Liu et al., 2009; Liu et al., 2016; Pitteloud et al., 2002; French et al., 2008; Mao et al., 37-41) and the availability of clinical baseline data. Lasso regression model was firstly applied to CHH predictive study. It demonstrated that significant predictors of spermatogenesis-failure included cryptorchidism history, abnormal epididymis or prostate development, lower basal and triptorelin-stimulated peak LH level. Our results are in consistent with previous studies (Liu et al., 2009; King et al., 2012).

4.4 Smell sense and olfactory nerve center (ONC) development

Simple olfactory assessment might underestimate the true olfactory deficit rate in CHH patients. MRI examination will be more accurate (Lewkowitz-Shpuntoff et al., 2012). Here, 30% of nCHH patients showed

ONC maldevelopment under nasal sinus MRI examination, which indicated that these patients should be re-classified as KS. KS is different from nCHH in pathophysiology. So, it is necessary to have some precision method, such as nasal sinus MR or olfactory evoked potential examination with olfactometer, to differentiate KS from nCHH.

This study provided informative long-term treatment data on CHH patients genetically screened with WES sequencing. However, limitations should be noted. No direct correlation was observed between certain responsible genes and spermatogenic outcomes, which may due to small number of subgroups with different gene variants. Besides, the heterogeneity of fertility-inducing treatment may underestimate the correlations between genetic alterations and outcomes as it was not a controlled trial. Further, the eighteen novel mutations identified worth experimental validation in the future.

This study used whole exome sequencing in a large number of CHH cohorts and correlated genotypes with detailed treatment results, providing comprehensive evaluation of all associated gene deficiencies. With comprehensive evaluation of all associated/candidate gene deficiencies, this research provides new sights into correlation between clinical treatment outcomes and genotypes, giving more evidence for CHH genetic counselling. Also, a predictive model for spermatogenic failure risk was created. The precision assessment of ONC development was advised.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

WEB RESOURCES

dbSNP: https://www.ncbi.nlm.nih.gov/snp/

HapMap: https://www.genome.gov/10001688/international-hapmap-project

1000 Genome Project: www.internationalgenome.org

Exome Aggregation Consortium: http://exac.broadinstitute.org/

gnomAD: https://gnomad.broadinstitute.org/

Burrows Wheeler Aligner software (version 0.59): bio-bwa.sourceforge.net

GATK Indel Realigner: gatk.broadinstitute.org

GATK Base Recalibrator: gatk.broadinstitute.org

GATK UnifiedGenotyper: gatk.broadinstitute.org

SIFT: http://sift.jcvi.org/

PolyPhen2: http://genetics.bwh.harvard.edu/pph2/

Mutation Taster: http://www.mutationtaster.org/

OMIM database: www.omim.org

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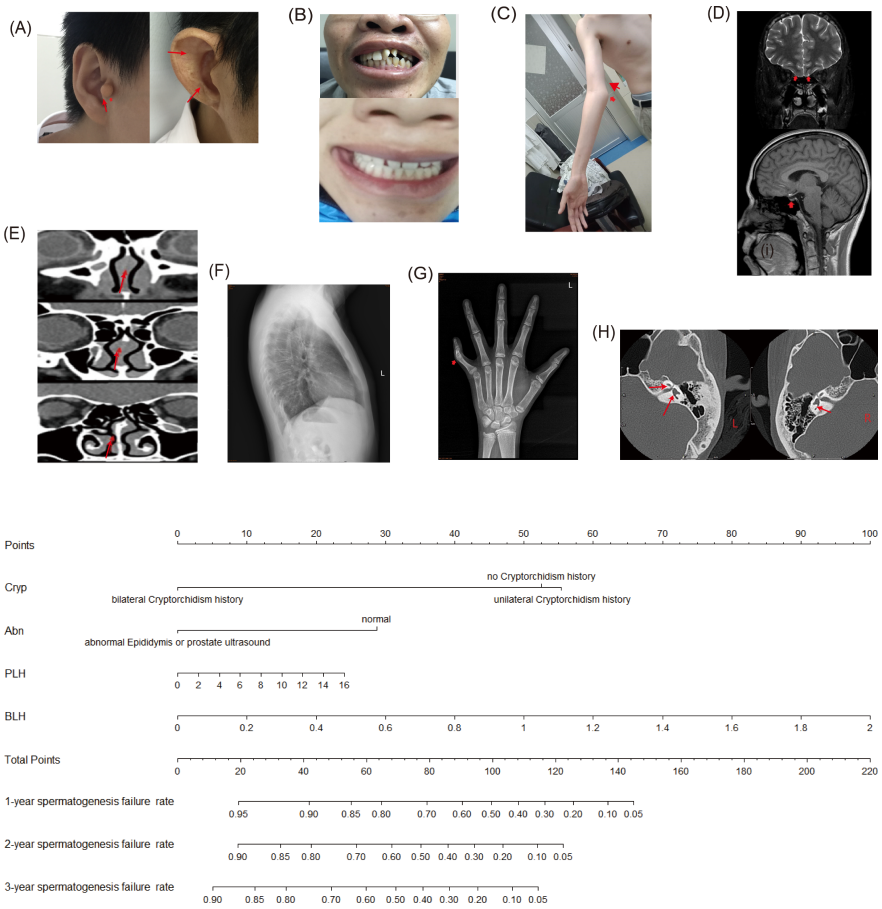
Figure legends

Figure 1. Some clinical abnormalities of CHH.

A. The arrow points to a verrucous soft tissue neoplasm in front of the anterior notch of right ear (Patient 22 and 24 with CHARGE Syndrome). B. The upper picture: cleft lip post operation and abnormal teeth alignment (Patient 2). The lower picture indicates small teeth (Patient 4). C. The arrow indicates cubitus valgus (the carriage angle increased), slender hands with short and curved tail finger (clinodactyly) (Patient 11). D. The upper picture showed abnormal nasal sinus MRI: arrows indicate dysplasia of left olfactory groove, absence of right olfactory groove, bilateral olfactory bulbs and tracts (Patient 49). The lower picture showed sagittal position of pituitary MRI: pituitary dysplasia at height of 2.5mm (Patient 40). E. The arrows in the MRI image shows aplasia of bilateral olfactory bulbs, tracts and sulci (Patient 22). F. X-ray examination indicates scoliosis (Patient 23). G. X-ray examination shows the deformity of little finger (clinodactyly) (Patient 11). H. Mastoid CT shows abnormal inner ear. Left ear: cochlear nerve foramen was atresia, and internal auditory canal was stenotic; Both ears: the arrows indicate semi-circular canal were dysplasia (Patient 22).

Figure 2. A prediction nomogram integrated the predictors selected by Lasso method, including Cryptorchidism history (Cryp), abnormal epididymis or prostate with ultrasound (Abn), basal LH level (BLH) and peak LH level post Triptorelin$^{(r)}$ stimulation test (PLH). For each predictor axis, the score of different conditions located on axis is determined by corresponding Points numerical value (a straight line could be drawn upward to the top Points axis to determine numerical value of each predictor condition). For each patient, the scores of different predictors could be added together to generate total points, and the 1-, 2- or 3-year spermatogenesis-failure risk could be determined by the corresponding Total Points (a straight line could be drawn downward from Total Points axis to the three axes below). For example, Patient 52 had no cryptorchidism history (52 points), normal epididymis / prostate (52 points), with basal LH level being 0.84 IU/L (41 points) and triptorelin-stimulated peak LH level 7.97 IU/L (12 points), so his total point was 134 and corresponding 1-year, 2-year and 3-year spermatogenic failure risk was 15%, $< 5\%$ and $<5\%$ respectively.



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