Identification of a novel MAP3K1 variant in a family with 46, XY DSD and partial growth hormone deficiency

YIPING CHENG1-4, CHAO XU1-4, JIANGFEI YANG5, XINLI ZHOU1-4 and NAN CHEN6

1Department of Endocrinology and Metabolism, Shandong Provincial Hospital, Cheeulo College of Medicine, Shandong University; 2Department of Endocrinology and Metabolism, Shandong Provincial Hospital Affiliated to Shandong First Medical University; 3Institute of Endocrinology, Shandong Academy of Clinical Medicine; 4Shandong Clinical Medical Center of Endocrinology and Metabolism; Departments of 5Radiology and 6Dermatology, Shandong Provincial Hospital, Cheeulo College of Medicine, Shandong University, Jinan, Shandong 250021, P.R. China

Received July 27, 2021; Accepted March 15, 2022

DOI: 10.3892/mmr.2022.12854

Abstract. The 46, XY disorder of sex development (DSD) is the main cause of birth defects; however, as it is a group of highly heterogeneous diseases, >50% of cases are not accurately diagnosed. Identification of more cases will improve understanding of the relationship between genotype and phenotype for DSD. The present study conducted a systematic analysis of the clinical characteristics of a proband with 46, XY DSD, applied genetic analysis by whole-exome sequencing to this pedigree and performed bioinformatics analysis of the identified variant. The proband presented with a short penis, lack of testicles and partial growth hormone (GH) deficiency at 1 year old. Histopathological examination revealed there were oviduct, testicles and partial growth hormone (GH) deficiency at 1 year old. The abnormal external genitalia of patients with 46, XY DSD are usually born with birth defects and are at a high risk of gonadal tumors, which not only increases physical and mental harm to patients, but also subsequently burdens the family. Notably, an early diagnosis is important for a positive outcome for patients with 46, XY DSD (2). However, the clinical phenotype of 46, XY DSD is highly heterogeneous. The abnormal external genitalia of patients with 46, XY DSD can be manifested as completely male or female; however, more commonly, a phenotype between male and female is presented, with characteristics including hypospadias, cryptorchidism, clitoral enlargement and labia majora fusion (3). Clinical diagnosis of 46, XY DSD is difficult and >50% of cases are not accurately diagnosed (4).

The most frequent causes of 46, XY DSD are androgen insensitivity and gonadal dysgenesis (5), which may involve mutations in numerous genes, including sex-determining region Y (SRY), androgen receptor (AR), SRY-related HMG-box gene (SOX9), steroid 5α-reductase 2, nuclear receptor subfamily 5 group A member 1 and mitogen-activated protein kinase kinase kinase 1 (MAP3K1) (6). Among them, MAP3K1 (NM_005921.1) variants are a common cause and account for 15-20% of 46, XY DSD [Online Mendelian Inheritance in Man (OMIM) 400044] cases (7). These variants may lead to protein function acquisition that changes co-factor binding and increases phosphorylation of targets in the downstream

Correspondence to: Professor Nan Chen, Department of Dermatology, Shandong Provincial Hospital, Cheeulo College of Medicine, Shandong University, 324 Jing 5 Road, Jinan, Shandong 250021, P.R. China
E-mail: rhythm_two@sina.com

Abbreviations: DSD, disorder of sex development; SRY, sex-determining region Y; SOX9, SRY-related HMG-box gene 9; MAP3K1, mitogen-activated protein kinase kinase 1; MR, magnetic resonance; GnomAD, Genome Aggregation Database; TOPMED, Trans-Omics for Precision Medicine; ExAC, Exome Aggregation Consortium; HGMD, Human Gene Mutation Database; PCR, polymerase chain reaction; ACMG, American College of Medical Genetics and Genomics; IGF-1, insulin-like growth factor 1; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; AMH, anti-Müllerian hormone

Key words: 46, XY DSD, MAP3K1, diagnosis, heterogeneity
MAPK pathway, which can lead to an imbalance between SOX9/FGF9 and WNT/β-catenin signaling and abnormal human sex determination (8). Although these findings strongly suggest that MAP3K1 variants are involved in the etiology of testicular dysgenesis, it is still necessary to better understand the mechanism underlying the effects of the MAPK pathway on the gene regulatory network of human testicular development (6,9). Therefore, identification of more cases will help to better understand the relationship between genotype and phenotype in DSD caused by MAP3K1 mutations.

The present study detected a novel pathogenic MAP3K1 variant (c.3020a>G) in a Chinese patient with 46, XY DSD and partial growth hormone (GH) deficiency. The variant was revealed to be pathogenic and p.Gln1007 in MAP3K1 was shown to be conserved across various species in bioinformatics analysis. The present research expands the mutation spectrum of MAP3K1 and may be conducive to improved genetic diagnosis and counseling in the future.

Subjects and methods

Patients. The proband was a premature infant who was delivered by caesarean section at 29 weeks’ gestation with no testicles detected at birth. At the age of 1 year, the parents of the proband came to Shandong Provincial Hospital (Shandong, China) to receive a diagnosis. The non-consanguineous parents of the patient had a normal phenotype and denied a family history of genetic diseases. All family members received careful clinical examinations and peripheral blood samples were obtained for genetic analyses. The hormone levels of the patient were detected; ultrasonography and pituitary magnetic resonance (MR), histopathological examination and laparoscopy were applied to evaluate the patient's genital development and pituitary development, respectively. The Ethics Committee of Shandong Provincial Hospital Affiliated to Shandong University approved the present study (approval no. 2019-147) and written informed consent was obtained from the parents of the patient. The GH stimulation was conducted as follows: The patient was followed up by regular visits to determine their specific characteristics and prognosis. In particular, their intellectual and urogenital development, treatment options and potential complications, such as gender anxiety, psychological and cognitive impairment, and the presence of gonadal tumors, were followed up on over 4 years.

Clinical follow-up. The patient was followed up by regular visits to determine their specific characteristics and prognosis. In particular, their intellectual and urogenital development, treatment options and potential complications, such as gender anxiety, psychological and cognitive impairment, and the presence of gonadal tumors, were followed up on over 4 years.

Whole-exome sequencing (WES) and Sanger sequencing. Using QIAamp DNA Mini kit (Qiagen GmbH), genomic DNA was isolated from peripheral blood leukocytes for genetic analysis of the pedigree (II-1, II-2 and III-1). Subsequently, DNA from peripheral blood was used for WES; the genes associated with GH deficiency were also studied by WES. Genomic DNA fragmentation, paired-end adaptor ligation, amplification and purification were performed, and human exons were then captured using SeqCap EZ Med Exome Enrichment Kit (Roche Diagnostics). Following post-capture amplification and purification, a DNA library was generated and sequenced using the Illumina HiSeq sequencing platform (Illumina, Inc.). To obtain the coverage and mean read depth of target regions, sequence data alignment to the human reference genome (hg19) and variant calling were performed using NextGene V2.3.4 software (SofGenetics). The average coverage of the exome was >100x, which allowed a deep enough examination of the target region to accurately match >99% of the target exons. Mutations with low coverage in the target area were screened and filtered to ensure the accuracy of data analysis. The quality/integrity of the processed samples were verified by QuBit4 Fluorometer (Thermo Fisher Scientific, Inc.) and Nanodrop one (Thermo Fisher Scientific, Inc.); the type of sequencing nucleotide length was 300 bp (PE150) and the direction of sequencing was paired end; the catalogue number of the sequencing kit was NovaSeq 6000 S4 Reagent kit v1.5 (300 cycles; cat. no. 20028312; Illumina Inc.). The loading concentration of the final library were ~10-20 pM for DNA sequencing.

In addition, the frequency of normal populations (whether there are variants included in the normal population database) were obtained by NextGene V2.3.4 and our in-house scripts from data from Genome Aggregation Database (GnomAD, https://gnomad.broadinstitute.org/), Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org), Trans-Omics for Precision Medicine (TOPMED, https://topmed.nhlbi.nih.gov/), Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), Clinvar and OMIM databases (http://www.omim.org).

A variant was identified as a mutation when it was not found in 500 Chinese controls with data from sequencing companies in dbSNP (https://www.ncbi.nlm.nih.gov/snp/) and in the exome variant server (https://evs.gs.washington.edu/EVS/), or when the allele frequency was ≤0.001 in the aforementioned databases. According to Standards and Guidelines for the Interpretation of Sequence Variants published by the American College of Medical Genetics and Genomics (ACMG) in 2015 (10), pathogenic variants were determined with Human Genome Variation Society nomenclature. Candidate variants were detected by WES. Subsequently, detected pathogenic or suspected pathogenic variants were verified by Sanger sequencing. The following tagged sequencing primers were designed for MAP3K1: Forward primer, 5'-CAACGAAACGACACAG¬3'; reverse primer, 5'-TGAGGAGAGTGACAGT¬3'. Polymerase chain reaction (PCR) was carried out in a 50-µl reaction system including 5 µl 10X PCR buffer, 4 µl genomic DNA obtained as aforementioned, 4 µl dNTPs, 1 µl forward and reverse primers, and 0.3 µl Taq Hot Start (all from Takara Bio, Inc.). The PCR conditions consisted of an initial denaturation step (95°C for 5 min), followed by 40 cycles of denaturation (95°C, 30 sec), annealing (65°C, 30 sec) and elongation (72°C, 30 sec). Using an ABI 3730 system (Applied Biosystems; Thermo Fisher Scientific, Inc.), amplicons were sequenced. Sequence analysis was performed using the autoassembler software Chromas 2.6.6 (Technelysium Pty., Ltd.).
Bioinformatics analysis. To confirm the conservation of amino acids at mutated positions, Clustal W (UCD) software (https://www.ebi.ac.uk/Tools/msa/clustalo/) was applied to compare 20 species. In addition, online software, such as Mutation Taster (https://www.mutationtaster.org/), PolyPhen v.2 (http://genetics.bwh.harvard.edu/pph2/) and PROVEAN (http://provean.jcvi.org/index.php), was used to predict the potential pathogenic effects of the variants. The Humvar model in PolyPhen v.2 is adapted to assess variations associated with Mendelian genetic diseases and can distinguish severely harmful variations from other human variants. The HumDiv model is suitable for evaluating rare variants that may be involved in complex diseases or in high-density regions of GWAS studies. Therefore, this study is more suitable for Humvar model.

Using I-TASSER software (https://zhanglab.ccmb.med.umich.edu/I-TASSER/), modeling of wild type and mutant proteins was performed. PyMOL Viewer 2.0 (http://pymol.sourceforge.net). was employed to observe the effect of the variants on the protein.

Results

Clinical manifestation. Due to reduced fetal movement during pregnancy, the proband of the present study was delivered by cesarean section at 29 weeks’ gestation with a birth weight of 1.26 kg. The newborn classification was low birth weight and small for gestational age. After birth, the infant was found to have a short penis, low muscle tension and no testicles. Growth and development lagged behind those of their peers; however, the proband’s intellectual development was basically normal. The lineage of the patient is shown in Fig. 1.

The patient was assessed at our hospital at the age of 1 year. Physical examination showed a small penis, lack of bilateral testicles, slightly reduced muscle tension in the limbs and short stature. Their height was 65.7 cm (target height, 76.5±2.6 cm) and body weight was 8.9 kg (target weight, 10.05±1.05 kg); in addition, their parents' height and body weight were relatively low (father: height, 176 cm and weight, 65 kg; mother: height, 159 cm and weight, 54 kg). Bone age test showed an age of 6 months. According to an abdominal ultrasound, the patient presented with bilateral scrotum emptiness, and no testicular tissue was detected in groin and pelvic regions.

The karyotype analysis revealed 46, XY in 50 metaphases. The estradiol, inhibin B and insulin-like growth factor I levels were low for a child, whereas the luteinizing hormone (lH), follicle-stimulating hormone (FSH) and cortisol levels were high. Furthermore, testosterone, prolactin and adrenocortico‑tropic hormone levels were normal for a child (Table i).

GH stimulation test indicated that in response to stimuli, a conserved caspase‑3 cleavage site and a ubiquitin interaction motif. Two zinc finger structures, including one RING structure and one SWIM region, are located at the N-terminus of MAP3K1 (11). The heterozygous variant was located at position 1,007 (Gln1007Arg; Fig. 2A). Since no substitution was detected in TOPMED, GnomAD and ExAC, this variant was not considered polymorphic. The C-terminus of MAP3K1 has a serine/threonine kinase domain; upstream of this domain is a conserved caspase-3 cleavage site and a ubiquitin interaction motif. Two zinc finger structures, including one RING structure and one SWIM region, are located at the N-terminus of MAP3K1 (11). The heterozygous variant was located at position 1,007 (Gln1007Arg), upstream of the serine/threonine kinase domain (Fig. 2B). PolyPhen v.2 predicted the hemizygous MAP3K1 variant to be potentially damaging with a score of 0.953 (Fig. 2C), and this variant was predicted to be

Follow up. The patient underwent laparoscopy at the age of 2.0 years and no testes were detected in the abdominal cavity and inguinal region. Their height was 80.2 cm (target height, 88.5±3.4 cm) and body weight was 11.40 kg (target weight, 12.54±1.23 kg). Histopathological examination revealed there were oviduct, epididymis and fibrous vascular tissue on both sides of the abdomen, but no obvious convoluted tubule and follicular tissue. Hormone measurements at 2.0 years indicated low estradiol AMH and inhibin B-levels for a child, whereas the levels of LH and FSH were high. Testosterone levels was decreased from year 1-year 2 (Table I).

The patient identified as male at the last follow-up when they were 5 years of age. Their height was 110.2 cm (target height, 111.3±4.4 cm) and body weight was 16.5 kg (target weight, 18.98±1.97 kg). Gynecological examination confirmed male external sexual organs with a lack of testicles. The length of the penis was 1.5 cm.

The mental development of the patient was normal. During the follow-up period, no medication was used, and psychological or cognitive impairment, gender anxiety disorder and gonadal tumors were not observed.

Mutation detection. The WES technique was applied to the genetic analysis of the pedigree to further search for the disease-causing genes. Notably, a novel heterozygous MAP3K1 variant (c.3020A>G) was identified in the patient and their mother, which resulted in a change in the 1,007th amino acid of the encoded protein from glutamine to arginase (Gln1007Arg; Fig. 2A). Since no substitution was detected in TOPMED, GnomAD and ExAC, this variant was not considered polymorphic. The C-terminus of MAP3K1 has a serine/threonine kinase domain; upstream of this domain is a conserved caspase-3 cleavage site and a ubiquitin interaction motif. Two zinc finger structures, including one RING structure and one SWIM region, are located at the N-terminus of MAP3K1 (11). The heterozygous variant was located at position 1,007 (Gln1007Arg), upstream of the serine/threonine kinase domain (Fig. 2B). PolyPhen v.2 predicted the hemizygous MAP3K1 variant to be potentially damaging with a score of 0.953 (Fig. 2C), and this variant was predicted to be
neutral by PROVEAN with a score of -0.557. Mutation Taster showed that the pathogenic variant could influence protein properties. According to ACMG criteria, the MAP3K1 variant had ‘unknown significance’. Using the Clustal W tool, multiple amino acid sequence alignments suggested that p.Gln1007 is conserved across various species (Fig. 3). Currently, the 3D MAP3K1 protein cannot be crystalized; therefore, structural analysis could not be performed. In addition, there were no other mutations associated with hypogonadism in the exome of the patient. Therefore, it was hypothesized that this rare MAP3K1 mutation (c.3020A>G) may be associated with the occurrence of 46, XY DSD. The presence of allelic variants in the main genes associated with GH deficiency were excluded.

Discussion

The present study identified a novel heterozygous MAP3K1 variant (c.3020A>G, p.Gln1007Arg) in a patient with 46, XY DSD. The GH levels of the patient were evaluated and a partial GH deficiency was detected. Follow-up for 4 years indicated that the patient had a good prognosis except for developmental delay. The present findings enrich the MAP3K1 variant spectrum and contribute to the study of genotype-phenotype relationships in patients with 46, XY DSD.

The expression of the SRY gene (MIM 480000) on the Y chromosome controls male sex determination, leading to the development of undifferentiated gonads into testes (12,13). Sertoli cells in the testis produce anti-Müllerian hormone, which causes the degradation of Müllerian ducts, whereas Leydig cells secrete testosterone to promote Wolffian duct differentiation into seminal vesicles, vas deferens and epididymis. In the absence of the Y chromosome and SRY expression, undifferentiated gonads develop into ovaries, Müllerian ducts form the uterus, fallopian tubes and the upper third of the vagina, and Wolffian ducts degenerate. This process occurs approximately from the 4th week to the 12th week of pregnancy, and is performed in a strictly timed and gene dose-dependent manner (12). If there is a problem with gonad development, it can lead to DSDs (14,15). Notably, 46, XY DSD may be caused by functional mutations in genes (16,17) and a missense mutation of the MAP3K1 gene is a common cause of 46, XY DSD (18). In the present study, a missense mutation, c.3020A>G, was found in exon 14, which changed the 1,007th amino acid of the encoded protein from glutamine to arginine; glutamine is an uncharged neutral amino acid in wild type MAP3K1, which in this mutation is replaced by the

| Patient | age | L.H., mIU/l | FSH, mIU/l | T, ng/ml | E, pg/ml | AMH, ng/ml | ACTH, pg/ml | MSH, mIU/l | COR, ng/ml | PRL, pg/ml | IGF-I, ng/ml | Inhibin B, pg/ml | AMH, anti-Müllerian hormone; ACTH, adrenocorticotropic hormone; COR, cortisol; E, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MSH, melanocyte-stimulating hormone |}
|---------|-----|-------------|------------|--------|-------|----------|------------|----------|---------|--------|------------|----------------|-------|
| 1/2 year| 20.76 | 160.51 | 0.78 | 1.54 | 0.01 | 33.09 | 503.90 | <25.0 | <25.0 | 11.21 | <25.0 | 14.21 | AMH, anti-Müllerian hormone; ACTH, adrenocorticotropic hormone; COR, cortisol; E, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone; MSH, melanocyte-stimulating hormone |
| 2/0 year| 35.68 | 141.59 | 0.11 | 1.68 | 0.12 | 1.20 | 4.79 | 4.97 | 4.79 | 172 | 4.97 | 0.79 | 0.79 | Table I. Hormone measurements of the patient. |
positively charged basic amino acid arginine. The secondary structure of the protein may be affected by this change in charge. The conservation of the p.Gln1007 residue and a high pathogenic probability of this MAP3K1 mutation indicates the importance of the residue in the structure and function of the protein. Similarly, Das et al. (19) identified a missense mutation in exon 14 of the MAP3K1 gene (c.2416G>A, p.Asp806Asn) in four patients with hypospadias with gonadal abnormalities, which resulted in aspartic acid at position 806 of the amino acid sequence being replaced by asparagine; computer analysis indicated this missense mutation was a pathogenic mutation.

To date, numerous mutations have been identified in the open reading frame or non-coding region of the MAP3K1 gene from the HGMD database; however, only 15 missense mutations of the MAP3K1 gene have been reported to cause 46, XY DSD (Table III). Pearlman et al. (20) identified three missense mutations of MAP3K1 in a family from New Zealand, which included five women with complete gonadal dysfunction (c.1846G>A, p.Gly616Arg) and two patients with sporadic 46, XY gonadal dysgenesis (c.566T>C, p.Leu189Pro and c.566T>G, p.Leu189Arg). PolyPhen and SIFT revealed that p.Leu189Pro and p.Leu189Arg mutations
may have interference functions, whereas p.Gly616Arg mutations did not; however, this mutation caused the conservative neutral amino acid at this position to be replaced by a basic amino acid. Further experiments revealed that the missense mutations identified in the two sporadic cases changed the phosphorylation of downstream targets p38 and ERK1/2, and enhanced the binding of RHOA to the MAP3K1 complex; the findings of Loke et al (21) also identified a p.Pro153Leu variant in a patient with gonadal dysgenesis. Baxter et al (16) reported on two cases that had the p.Gly616Arg variant, of which one was a female with complete gonadal dysgenesis and another was a male with ovotesticular disorder of sex development; furthermore, a patient with complete gonadal dysgenesis had a de novo p.Arg339Gln missense variant and a male patient with complex ambiguous genitalia but without gonad hypoplasia had a p.Pro257Leu missense variant. Eggers et al (22) inferred 10% prevalence of MAP3K1 variants amongst patients with 46, XY DSD in a smaller cohort and this could be up to 18% if the MAP3K1 phenotypic spectrum was expanded. In addition, two MAP3K1 variants (p.Met312Leu and p.Ala1443Val) were identified in multiple patients with a variety of phenotypes, including complete or partial gonadal dysgenesis, hypospadias and under-virilization. Granados et al (23) performed clinical evaluation, endocrine evaluation and genetic analysis of six individuals with 46, XY DSD from four unrelated families. Three of these patients exhibited complete gonadal dysgenesis with MAP3K1 variants (c.1760T>A, p.Leu587His; c.2291T>G, p.Leu764Arg; c.566T>A, p.Leu189Gln). In addition, Xue et al (24) detected a missense mutation (c.2117T>G; p.Leu706Arg) in exon 12 of the MAP3K1 gene in a patient with 46, XY DSD and a missense mutation (c.2416G>A; p.Asp806Asn) was identified in exon 14 of the MAP3K1 gene in patients with 46, XY DSD by Das et al (19). Notably, Pearlman et al (20) found only one

### Table III. Reported missense mutations of the MAP3K1 gene in 46, XY DSD.

| First author, year | Coding change | Protein change | Zygosity | Exon | Phenotype | Functional study | (Refs.) |
|-------------------|---------------|----------------|----------|------|-----------|------------------|--------|
| Eggers, 2016      | G → C         | p.Asp132His    | Heterozygous | 1    | DSD       | No               | (22)   |
| Loke, 2014        | C → T         | p.Pro153Leu    | Heterozygous | 1    | GD        | Yes              | (21)   |
| Pearlman, 2010;   | T → C         | p.Leu189Pro    | Heterozygous | 2    | CGD       | Yes              | (20)   |
| Loke, 2014;       |               |                |           |      |           |                  |        |
| Granados, 2017    | T → A         | p.Leu189Gln    | Heterozygous | 2    | CGD       | No               | (23)   |
| Eggers, 2016      | A → G         | p.Gln237Arg    | Heterozygous | 3    | 46, XY DSD | No               | (22)   |
| Baxter, 2015      | C → T         | p.Pro257Leu    | Heterozygous | 3    | DSD       | No               | (16)   |
| Eggers, 2016      | A → T         | p.Met312Leu    | Heterozygous | 4    | 46, XY DSD | No               | (22)   |
| Baxter, 2015      | G → A         | p.Arg339Gln    | Heterozygous | 4    | CGD       | No               | (16)   |
| Granados, 2017    | T → A         | p.Leu587His    | Heterozygous | 10   | CGD       | No               | (23)   |
| Pearlman, 2010;   | G → A         | p.Gly616Arg    | Heterozygous | 10   | CGD       | No               | (20)   |
| Baxter, 2015      |               |                |           |      |           |                  | (16)   |
| Eggers, 2016      | T → C         | p.Cys691Arg    | Heterozygous | 11   | CGD       | No               | (22)   |
| Xue, 2019         | T → G         | p.Leu706Arg    | Heterozygous | 12   | CGD       | Yes              | (24)   |
| Granados, 2017    | T → G         | p.Leu764Arg    | Heterozygous | 13   | PGD       | No               | (23)   |
| Das, 2013         | G → A         | p.Asp806Asn    | Heterozygous | 14   | 46, XY DSD | No               | (19)   |
| The present study | A → G         | p.Gln1007Arg   | Heterozygous | 14   | 46, XY DSD | No               | (22)   |
| Eggers, 2016      | C → T         | p.Ala1443Val   | Heterozygous | 19   | 46, XY DSD | No               | (22)   |

DSD, disorder of sex development; GD, gonadal dysgenesis; CGD, complete gonadal dysgenesis; PGD, partial gonadal dysgenesis.

| Species                  | Alignment |
|--------------------------|-----------|
| Homo sapiens             | TATDVSKHRDKDFPCRIPSA |
| Pan troglodytes           | TATDVSKHRDKDFPCRIPSA |
| Hylabates moloch          | TATDVSKHRDKDFPCRIPSA |
| Chlorocebus sabaesus      | TATDVSKHRDKDFPCRIPSA |
| Eumetopias jubatus        | TGDVSKHRDKDFPCRIPSA |
| Muscota emerina           | TGDVSKHRDKDFPCRIPSA |
| Tupana chinensis          | TATDVSKHRDKDFPCRIPSA |
| Lipotes vexillifer        | TATDVSKHRDKDFPCRIPSA |
| Globocephala melas        | TATDVSKHRDKDFPCRIPSA |
| Ursus arctos horribilis   | TATDVSKHRDKDFPCRIPSA |
| Daspyus novemcinctus      | TATDVSKHRDKDFPCRIPSA |
| Ceratotherium simum       | TATDVSKHRDKDFPCRIPSA |
| Enhydra lutris kenyoni    | TATDVSKHRDKDFPCRIPSA |
| Chrysocloris asiatica     | TATDVSKHRDKDFPCRIPSA |

Figure 3. Cross-species conservation of mitogen-activated protein kinase kinase kinase 1 p.Gln1007Arg.
of these individuals in pedigrees from two families of interest exhibiting sex-limited autosomal-dominant mendelian inheritance of 46, XY DSD had micropenis and cryptorchidism (III-13). The absence of hypospadias was found in the proband of the present study, which is different from complete hypogonadism. The missense mutation of MAP3K1 is a common cause of 46, XY DSD. It was speculated that the location of missense mutations may affect the function of genes or encoded proteins, leading to differences in clinical phenotypes of patients. Alternatively, interactions between this gene and other genes may also be responsible for this phenomenon, but further research is needed.

It is worth noting that in addition to 46, XY DSD, the present proband exhibited partial GH deficiency. GH deficiency is the most common type of restricted growth and can present as a simple GH deficiency or with multiple pituitary hormone deficiencies. Previous studies have reported that genetic mutations are an important cause of idiopathic GH deficiency. Currently known pathogenic genes of GH deficiency include GH gene, GH-releasing hormone receptor gene, and POU1F1 gene (25,26). To date, no patients with 46, XY DSD have been reported to have GH deficiency and no research has identified a causal relationship between MAP3K1 and GH deficiency; this needs to be further explored in functional tests.

In the gene network responsible for gonadal development, MAP3K1 is an important component. MAP3K1 is involved in the regulation of the transcription of so many important genes, and serves a key role in cell proliferation, differentiation, apoptosis (27,28). Studies have revealed that variants in the MAP3K1 gene can lead to changes in cofactor binding and increase the phosphorylation of targets in the downstream MAPK pathway, including ERK1/2 and p38, ultimately leading to the occurrence of 46, XY DSD. Increased phosphorylation of ERK1/2 may lead to decreased expression of SOX9, whereas increased phosphorylation of p38 may increase the expression of CTNNB1, which are important signaling molecules in testicular or ovarian-promoting pathways (19,21,29-31). Chamberlin et al (29) showed that mutations in the MAP3K1 gene have different effects on protein binding, depending on the domain in which the mutation occurs. Generally, the net effect of increased binding of the pro-kinase RHOA and MAP3K4 proteins, and decreased binding of the inhibitory RAC1 protein results in the observed gain of function with MAP3K1 mutations. GH signaling is necessary for GH to function. Firstly, GH is combined with GH receptor (GHR) to establish the post-GHR process, and the most important GHR-mediated signaling pathways include GHR/JAK2/SHC/MAPK, GHR/JAK2/STATs and GHR/IRS/Pi3K/Akt pathways (32). Notably, Jin et al (33) demonstrated that JAK2 was required for ERK signaling in response to GH in preadipocytes and hepatoma cells. Therefore, it is reasonable to speculate that the MAP3K1 variant identified in the present study may cause changes in GHR-mediated signaling pathways and ultimately lead to the occurrence of partial GH deficiency.

The previously reported genetic pattern of pathogenic MAP3K1 variants is autosomal dominant and sex-limited (20,23,24). In two large families and two of 11 sporadic cases with 46, XY DSD, MAP3K1 variants were first reported with an autosomal dominant, sex-limited pattern of transmission (20). Phenotypic manifestations of the large family reported a range from hypospadias and cryptorchidism to complete hypogonadism; however, there were eight and four unaffected female carriers, respectively. Granados et al (23) reported that six individuals from four unrelated families with pathogenic MAP3K1 variants had both partial and complete gonadal dysgenesis. In addition, 46, XX females carrying MAP3K1 variants were not affected. Furthermore, Xue et al (24) speculated that the unaffected mother of a patient with 46, XY DSD may carry the same MAP3K1 mutation; however, the mother was not genetically tested. The present findings are consistent with the aforementioned studies; the mother of the proband had the same variant as the proband, but remained phenotypically healthy. These findings indicated that the genetic pattern of the novel pathogenic MAP3K1 variant (c.3020A>G, p.Gln1007Arg) may be based on autosomal dominant and sex-restricted patterns.

The present study has some limitations. The patient was diagnosed with 46, XY DSD based on clinical data, imaging characteristics and genetic testing results; nevertheless, the association between the phenotype and genotype of 46, XY DSD is not known, and more patients need to be studied to supply more comprehensive information. In addition, more functional tests are needed to verify the findings of the present study.

In conclusion, the present study detected a novel pathogenic MAP3K1 variant (c.3020A>G) in a patient with 46, XY DSD and partial GH deficiency. The present study expands the mutation spectrum of MAP3K1, and will be conducive to improved genetic diagnosis and counseling in the future. WES may find more genetic variants that expound the heterogeneity associated with MAP3K1 variants.

Acknowledgements

Not applicable.

Funding

The present study was supported by grants from the National Natural Science Foundation (grant nos. 81670720 and 81974124), special funds for Taishan Scholar Project (grant no. tsqn20161071) and the Shandong Key R&D Program (grant no. 2017GSF18129).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YC, CX and NC designed the present study. YC, JY and XZ performed the present study and analyzed the data. YC wrote the main manuscript. CX and NC confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study protocol was in line with the Declaration of Helsinki (as revised in Brazil 2013). The Ethics Committee
of Shandong Provincial Hospital affiliated to Shandong University approved this study (approval no. 2019-147), and written informed consent was obtained from the parents of the patient.

Patient consent for publication

Written informed consent was obtained from the parents of the patient.

Competing interests

The authors declare that they have no competing interests.

References

1. Allen L: Disorders of sexual development. Obstet Gynecol Clin North Am 36: 25-45, 2009.
2. Domenice S, Arnhold II, Costa EM and Mendonca BB: 46,XY Disorders of sexual development. In: Endotext. Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, Hershman JM, Kalsas G, Koch C, Kopf P, et al (eds). MDText.com, Inc., South Dartmouth, MA, 2000.
3. Hull CL and Fausto-Sterling A: How sexually dimorphic are we? Review and synthesis. Am J Hum Biol 15: 112-116, 2003.
4. Wertheim-Tysarowska K, Gos M, Sykut-Cegielska J and Bal J: Disorders of sexual development. In: Endotext. Feingold KR, Domenice S, Arnhold IJ, Costa EM and Mendonca BB: 46,XY north am 36: 25‑45, 2009.
5. Zheng Q, Ye J, Wu H, Yu Q and c ao J: a ssociation between
6. Li Q and Wang K: InterVar: Clinical interpretation of genetic
7. Jost A, Vigier B, Prépin J and Perchellet JP: Studies on sex differ
8. Granados A, Alaniz VI, Mohnach L, Barseghyan H, Vilain E, Ostrer H, Quint EH, Chen M and Keegan CE: MAP3K1-related gonadal dysgenesis: Six new cases and review of the literature. Am J Med Genet C Semin Med Genet 175: 253-259, 2017.
9. Fujita T, Watanabe M, Kato Y, Tsukahara K, Ota Y, Fujita M, Tani M, Usui K, Iriyama K, Kitagawa Y, Aoki H, Kurata S, Kato S, Terui K, Miura K, Kubo T, Hirohashi S, Nishikawa J and Ueda K: Clinical features of patients with mutations in MAP3K1, a gene encoding the MAPK8 kinase family member MAP3K1. Hum Genet 130: 1442-1452, 2012.
10. Li Q and Wang K: InterVar: Clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. Am J Hum Genet 100: E333-E344, 2015.
11. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Welker PA, Stevanovic M, Weissbach J, Mansour S, Young ID, Goodfellow PN, et al: Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 372: 525-530, 1994.
12. Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ER, Fraccaro M, et al: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7: 497-501, 1994.
13. Jost A, Vigier B, Prépin J and Perchellet JP: Studies on sex differentiation in mammals. Recent Prog Horm Res 29: 1‑41, 1973.
14. Gallager E: The MEKK1 PHD ubiquitins TAB1 to acti
15. Granados A, Alaniz VI, Mohnach L, Barseghyan H, Vilain E, Ostrer H, Quint EH, Chen M and Keegan CE: MAP3K1-related gonadal dysgenesis: Six new cases and review of the literature. Am J Med Genet C Semin Med Genet 175: 253-259, 2017.
16. Fujita T, Watanabe M, Kato Y, Tsukahara K, Ota Y, Fujita M, Tani M, Usui K, Iriyama K, Kitagawa Y, Aoki H, Kurata S, Kato S, Terui K, Miura K, Hirohashi S, Nishikawa J and Ueda K: Clinical features of patients with mutations in MAP3K1, a gene encoding the MAPK8 kinase family member MAP3K1. Hum Genet 130: 1442-1452, 2012.
17. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Welker PA, Stevanovic M, Weissbach J, Mansour S, Young ID, Goodfellow PN, et al: Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 372: 525-530, 1994.
18. Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ER, Fraccaro M, et al: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7: 497-501, 1994.
19. Jost A, Vigier B, Prépin J and Perchellet JP: Studies on sex differ
20. Granados A, Alaniz VI, Mohnach L, Barseghyan H, Vilain E, Ostrer H, Quint EH, Chen M and Keegan CE: MAP3K1-related gonadal dysgenesis: Six new cases and review of the literature. Am J Med Genet C Semin Med Genet 175: 253-259, 2017.
21. Fujita T, Watanabe M, Kato Y, Tsukahara K, Ota Y, Fujita M, Tani M, Usui K, Iriyama K, Kitagawa Y, Aoki H, Kurata S, Kato S, Terui K, Miura K, Hirohashi S, Nishikawa J and Ueda K: Clinical features of patients with mutations in MAP3K1, a gene encoding the MAPK8 kinase family member MAP3K1. Hum Genet 130: 1442-1452, 2012.
22. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Welker PA, Stevanovic M, Weissbach J, Mansour S, Young ID, Goodfellow PN, et al: Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 372: 525-530, 1994.
23. Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ER, Fraccaro M, et al: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7: 497-501, 1994.
24. Jost A, Vigier B, Prépin J and Perchellet JP: Studies on sex differ
25. Granados A, Alaniz VI, Mohnach L, Barseghyan H, Vilain E, Ostrer H, Quint EH, Chen M and Keegan CE: MAP3K1-related gonadal dysgenesis: Six new cases and review of the literature. Am J Med Genet C Semin Med Genet 175: 253-259, 2017.
26. Fujita T, Watanabe M, Kato Y, Tsukahara K, Ota Y, Fujita M, Tani M, Usui K, Iriyama K, Kitagawa Y, Aoki H, Kurata S, Kato S, Terui K, Miura K, Hirohashi S, Nishikawa J and Ueda K: Clinical features of patients with mutations in MAP3K1, a gene encoding the MAPK8 kinase family member MAP3K1. Hum Genet 130: 1442-1452, 2012.
27. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Welker PA, Stevanovic M, Weissbach J, Mansour S, Young ID, Goodfellow PN, et al: Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 372: 525-530, 1994.
28. Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ER, Fraccaro M, et al: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7: 497-501, 1994.
29. Jost A, Vigier B, Prépin J and Perchellet JP: Studies on sex differ
30. Granados A, Alaniz VI, Mohnach L, Barseghyan H, Vilain E, Ostrer H, Quint EH, Chen M and Keegan CE: MAP3K1-related gonadal dysgenesis: Six new cases and review of the literature. Am J Med Genet C Semin Med Genet 175: 253-259, 2017.
31. Fujita T, Watanabe M, Kato Y, Tsukahara K, Ota Y, Fujita M, Tani M, Usui K, Iriyama K, Kitagawa Y, Aoki H, Kurata S, Kato S, Terui K, Miura K, Hirohashi S, Nishikawa J and Ueda K: Clinical features of patients with mutations in MAP3K1, a gene encoding the MAPK8 kinase family member MAP3K1. Hum Genet 130: 1442-1452, 2012.
32. Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Welker PA, Stevanovic M, Weissbach J, Mansour S, Young ID, Goodfellow PN, et al: Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY-related gene. Nature 372: 525-530, 1994.
33. Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrante E, Chiumello G, McCabe ER, Fraccaro M, et al: A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7: 497-501, 1994.
34. Jost A, Vigier B, Prépin J and Perchellet JP: Studies on sex differ
35. Granados A, Alaniz VI, Mohnach L, Barseghyan H, Vilain E, Ostrer H, Quint EH, Chen M and Keegan CE: MAP3K1-related gonadal dysgenesis: Six new cases and review of the literature. Am J Med Genet C Semin Med Genet 175: 253-259, 2017.