Нарушение формирования пола 46,XY, ассоциированное с мутациями в гене *MAP3K1*. Описание клинических случаев

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Причинами нарушения формирования пола (НФП) 46,XY могут быть мутации ряда генов, вовлеченных в процесс дифференцировки гонад. XY-инверсия пола может являться также следствием нарушений на уровне гена митоген-активированной протеинкиназы (MAPK) киназы киназы 1 (*MAP3K1)* и MAPK-сигнального пути. В последнее десятилетие было доказано участие MAPK-пути в инициации экспрессии гена *SRY* при формировании мужского гонадного пола у млекопитающих. Роль MAPK-сигнального пути в формировании пола у людей изучена недостаточно. Вероятно, *MAP3K1* и MAPK-сигнальный путь являются одним из генетических путей, контролирующих нормальное развитие яичек. В настоящее время в литературе описано несколько семей и спорадических случаев НФП 46,XY вследствие мутаций в гене *MAP3K1*. Клиническая картина НФП у этих пациентов различна и варьирует от женского фенотипа с правильным строением наружных гениталий до мужского фенотипа с гипоспадией. Мы приводим описания редких клинических случаев нарушений формирования пола 46,XY (семейный случай НФП у единоутробных сестер и спорадический случай) с не описанными ранее мутациями в гене MAP3K1. В статье также кратко анализируется литература по данной патологии.

Ключевые слова: нарушение формирования пола, дисгенезия гонад, ген MAP3K1, клинический случай.

Disorder of sex development 46,XY associated with mutations in the gene *MAP3K1*. The report of clinical cases

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The disorders of sex development (DSD) 46,XY may be caused by mutations in a number of genes involved in the gonadal differentiation. The XY sex inversion can be also due to disturbances at the level of mitogen-activated protein kinase (MAPK) kinase kinase 1 gene (MAP3K1) and MAPK-signaling pathway. During the last decade, the involvement of the MAPK pathway in the *SRY* gene up-regulation during the formation of male gonadal sex in mammals has been demonstrated. The role of MAPK-signaling pathway in the human sex determination is not fully understood. Probably, MAP3K1 and the MAPK-signaling pathway are one of the genetic pathways controlling normal development of human testis. So far, several families and sporadic cases of 46,XY DSD due to mutations in *MAP3K1* gene have been reported in the literature. Clinical presentation of DSD in these patients varies from female phenotype with normal externalia to male phenotype with hypospadias. We describe rare cases of the DSD 46,XY (a family case of DSD in uterine sisters and a sporadic case) with mutations in the *MAP3K1* gene that haven't been previously described. The article also presents brief literature review on this pathology.

Keywords: disorders of sex development, gonadal dysgenesis, MAP3K1 gene, case report.

INTRODUCTION

Disorder of sex development is a group of congenital defects involving atypical development of chromosomal, gonadal, or anatomical sex [1]. Development of male sex is primarily determined by expression of the *SRY* gene in Y-chromosome, facilitating the development of undifferentiated gonad to form testicles [2]. Along with the *SRY* gene, a number of genes and signaling pathways involved in sex determination and associated with a wide phenotypic spectrum of the disorders of sex development (DSD) have been identified [3]. Recent studies have found mutations in the gene of mitogen-activated protein kinase (MAPK) kinase kinase 1 *(MAP3K1,* also known as *MEKK1)* associated with 46,XY disorders of sex development [4, 5]. The role of the MAPK signaling pathway in sex determination in humans has not been studied. *MAP3K1* and MAPK-signaling pathway is probably one of the genetic pathways that control normal development of the testes. Current literature reports several familial and sporadic cases of XY,46 DSD caused by mutations in this gene [4, 5]. Known cases of 46,XY DSD associated with *MAP3K1* gene are characterized by different clinical presentation ranging from a female phenotype with normal structure of external genitalia to male phenotype with micropenis and varying degrees of hypospadias.

CASE 1

Familial form of sex development disorder in uterine sisters with 46,XY karyotype

Patient O. was registered at birth as a female and brought up as a girl. According to medical records, hypertrophy of the clitoris was found during the primary patronage, the patient was not examined and did not visit endocrinologist. The patient had spontaneous late puberty characterized by abnormal order of the development of secondary sexual characteristics (adrenarche in 14 years, thelarche in 17 years), primary amenorrhea. The patient was first examined at the place of residence at the age of 16 years. Hypergonadotropic hypogonadism was detected (LH 23.4 mIU/ml, FSH 99.1 mIU/ml), 46,XY karyotype.

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The girl was admitted to the children's department of the Endocrinology Research Center at the age of 17 years. An objective examination showed normal height (164.3 cm, height SDS +0.35), sexual development corresponded to Tanner stage 2 (V2R2). Maldevelopment of external genitalia was observed [hypertrophied clitoris (2.5—3 cm) with balanus, poorly developed cavernous bodies, meatus at the bottom of the clitoris, split scrotolabial fold, narrowed vaginal orifice]. There were high levels of gonadotropins [LH 88 IU/l (2.6—12), FSH 104 IU/l (1.9—11.7)], estradiol [70 pmol/L (97—592)], and testosterone [3.11 nmol/l (0.1—2.7)]. The levels of dihydroepiandrosterone-sulfate (DHEA-S) and 17-hydroxyprogesterone (17-OHP) were within the reference range [7.52 μmol/L (0.92—7.6), and 3.6 nmol/l (0.1—7.0), respectively]. MRI of pelvic organs showed strand-shaped uterus sized 2.3 × 0.9 cm and gonads (2×1 cm on the right, 0.7×1.4 cm on the left). The patient was diagnosed with 46,XY disorder of sex development and diagnostic laparoscopy was recommended, which showed hypoplastic bicornuate uterus, two fallopian tubes, and dysgenetic gonads on both sides in the pelvis. Morphological examination of surgical specimens at the Endocrinology Research Center verified bilateral gonadoblastoma. Re-examination of histological preparations at the Dmitriy Rogachev National Research Center of Pediatric Hematology, Oncology, and Immunology confirmed bilateral gonadoblastoma accompanied by transformation to dysgerminoma on the right side. The girl was consulted by oncologist. Given the results of chest CT scan (neither focal nor infiltrative changes were detected), histological type of the tumor, and the stage of the disease, chemotherapy was not administered. Continuous replacement therapy with female sex hormones was recommended. Feminizing plastic repair of external genitalia was then carried out.

Patient B., 13.5 years old, the half-sister of the patient O., was examined due to the absence of spontaneous puberty and diagnosed 46,XY DSD in her older sister (Fig. 1).

At birth, normal structure of external genitalia was observed. The results of examination at the age of 13 showed 46,XY karyotype, pelvic ultrasound showed aplasia of the uterus and ovaries. During clinical examination, the girl's height was 155.9 cm (height SDS = −0.64), reproductive status corresponded to Tanner stage 1. Female external genitalia were formed. Laboratory examination confirmed hypergonadotropic hypogonadism [LH 39.9 IU/l (2.6—12), FSH 137 IU/l (1.9—11.7), estradiol 43.3 pmol/l (97—592)], there was normal testosterone level [0.6 nmol/l (0.1—2.7)]. Pelvic MRI scan showed strand-shaped uterus, gonads were not visualized. Diagnostic pelvic laparoscopy detected hypoplastic uterus, two fallopian tubes, and streak gonads. Morphological examination of surgical specimens verified ovotesticular gonadal dysgenesis.

Both patients underwent molecular genetic testing using massive parallel sequencing with an oligonucleotide panel designed at the Endocrinology Research Center for analysis of 45 genes associated with various forms of disorders of sex development. Heterozygous mutation p.C691R in *MAP3K1* gene was detected in both girls**.** This mutation has not been previously described. To date, no molecular genetic testing of patients' mother was carried out. However, given the presence of identical heterozygous mutation of *MAP3K1* in uterine sisters conceived by different fathers, we can conclude that the missense mutation was inherited from the mother and probably caused 46,XY DSD.

CASE 2

Patient M. had abnormal structure of the external genitalia at birth. Examination at the place of residence identified 46,XY karyotype. Hormonal profile at the age of 8.5 months was characterized by slightly elevated levels of FSH (5.28 mIU/ml), low level of testosterone, LH, and 17-OHP. US examination showed testicles localized in the scrotum (right 0.75 × 0.52 cm, left 0.87 × 0.59 cm). Prostate and seminal vesicles were not visualized during pelvic CT scan.

The child was first examined at the Endocrinology Research Center at the age of 11 months. Examination detected abnormal structure of the external genitalia [testes were palpable in the split scrotum, there was about 3-cm-long curved penis, dense cavernous bodies, developed balanus, dissecting sulcus of the penis on the dorsal surface; narrow urogenital sinus opening on the scrotum (scrotal hypospadia); the penis was recessed into the scrotum due to its curvature]. There was slight increase in the level of FSH [3.8 IU/l (0—2)], low level of testosterone [(0.17 nmol/l (0.3—0.6)], LH [0.2 IU/l (0—1.5), and anti-Müllerian hormone [12.8 ng/ml (63—132)]. Multisteroid blood test showed no data indicative of impaired steroidogenesis. Chorionic gonadotrophin test (4 injections of 1000 IU) showed increase in testosterone level to 5.7 nmol/l, which was indicative of normal functioning of Leydig cells. The child was diagnosed with 46,XY disorder of sex development and later on underwent corrective surgery to remove penile curvature and hypospadias. Molecular genetic study ("disorder of sex development" gene panel) detected heterozygous mutation s.2858 2872del CAACAACAACAACAA p.944 948del in *MAR3K1* gene. This mutation has not been previously described as well. Molecular genetic study of *MAR3K1* gene aimed at searching for similar deletion is planned.

DISCUSSION

MAPK is activated by evolutionarily conservative ternary signaling cascade consisting of mitogen-activated protein kinase (MAP3K1) kinase kinase 1, MAP2K, and MAPK [3]. MAPK-signaling pathways are the key pathways regulating cell proliferation and differentiation [6, 7]. Abnormalities in the regulation of MAPK-cascade

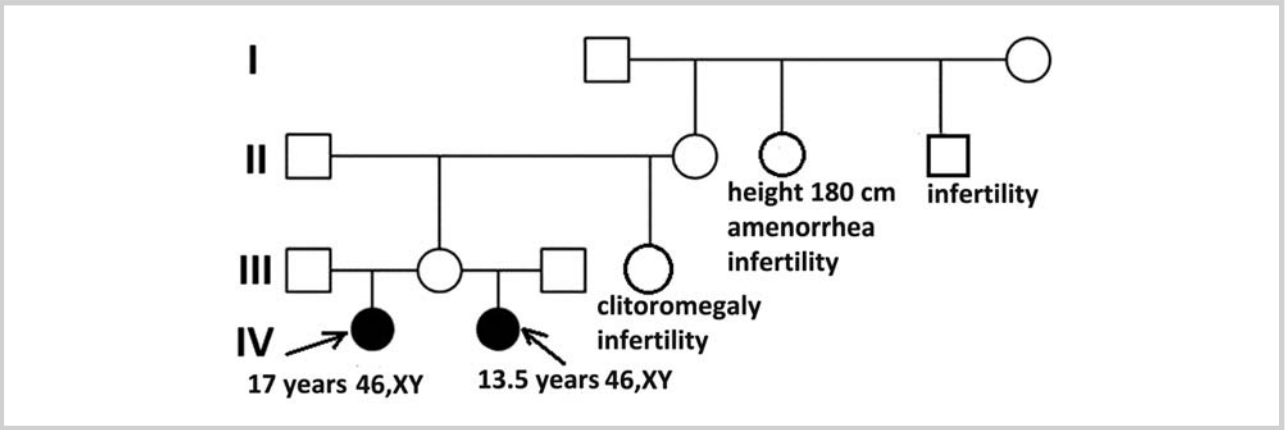

***Figure 1*. Family history of patients with 46,XY disorder of sex development.**

contribute to the development of several oncological diseases [8]. Association of *MAP3K1* with XY,46 disorders of sex development has been recently found, but the role of MAPK-signaling pathway in the development of this disease has not yet been studied in humans. Expression of *MAP3K1* is observed in murine embryonic gonad on the 11th day after conception, which corresponds to the stage of gonad development, and on the 13th day after conception in the testicular tubules [4]. The role of MAPK-pathway in sex determination in mammals has been determined by identifying *MAP3K4* gene mutations in mice, where sex reversal was presumably related to the inability to activate expression of the *SRY* gene [9, 10]. It was shown that in the absence of two isoforms of the mitogen-activated protein kinase kinase kinase-1 (p38a and p38b), XY sex reversal is formed, which is also caused by disorders at the level of *SRY* expression [11]. Thus, the available literature data are indicative of the involvement of MAPK pathway in the initiation of corresponding expression of *SRY* during the development of male gonadal sex in mammals. In humans, the relationship between mutations in *MAP3K1* gene and 46,XY disorder of sex development was first detected during analysis of genes linkage in the long arm of the chromosome 5 in DSD patients from two families and 11 sporadic cases [4]. Six mutations were identified in the *MAP3K1* gene and their functional analysis was carried out. In the aforementioned cased, clinical manifestations varied, ranging from completely female phenotype without virilization of external genitalia to males with micropenis and/or hypospadias. Different phenotypes associated with the same mutation were also observed in patients within the same family. Another study involving 4 patients with 46,XY DSD, predominantly male phenotype, and varying degrees of external genitalia development disorders identified mutations in *MAP3K1* gene [5]. Comparison of genotype and phenotype associated with one of the identified missense mutation showed that all patients with this mutation were characterized by male phenotype with hypospadias. This missense mutation is also associated with bronchial asthma [12]; malformations of external genitalia have not been previously described. Thus, its role still remains unknown.

Interestingly, sex reversal is not observed in *MAP3K1* knockout mice [13, 14]. These animals remain viable with intact reproductive function, but with a reduced amount of Leydig cells and increased length of embryonic gonads. For this reason, the authors suggested that *MAP3K1* does not play significant role in the development of testicles in mice. This may indicate that the signaling pathways of MAP-kinase are not identical in human and mouse [3]. To date, the role of *MAP3K1* in sex determination in humans remains poorly understood. Phenotype of complete 46,XY gonadal dysgenesis is similar to that with mutations in the *SRY* gene, i.e. *MAP3K1* mutations could affect the early stages of testis development.

Molecular genetic study of our patients with 46,XY DSD using the panel that included 45 genes associated with various disorders of sex development detected mutations only in the *MAP3K1* gene. In the aforementioned cases of patients with identified mutations in the *MAR3K1* gene, various clinical presentations of XY,46 DSD were observed, which were characterized by various degrees of gonadal differentiation and development of external genitalia. In the first family described in our report, heterozygous mutation inherited from patient's mother is suggested, which is characteristic of families with 46,XY DSD due to *MAP3K1* gene mutations *[4].* Identified heterozygous mutations have not been previously reported in the literature. Molecular genetic studies of patients' parents is planned in order to confirm their pathological significance.

CONCLUSION

46,XY disorder of sex development may be caused by pathology of a number of genes involved in the gonad differentiation. Participation of the *MAP3K1* gene and MAP-kinase signaling pathway in the development of DSD has been found recently and it is currently poorly understood. Clinical manifestations in patients with 46,XY DSD presumably caused by mutations in the *MAP3K1* gene are characterized by pronounced hetero-

geneity. There is no literature data about significant correlation between genotypic and phenotypic characteristics. Given that *MAP3K1* and MAPK-signaling pathway may be involved in normal testicular development, further study of the pathological significance of mutations in this gene in the development of various clinical presentations of 46,XY DSD is required.

ADDITIONAL INFORMATION

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