Homozygous DMRT1 mutation c.967G>A found in a Chinese patient with 46,XY complete gonadal dysgenesis and review of literature

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Abstract

A Chinese woman with 46,XY CGD was diagnosed by clinicopathologic findings . A homozygous mutation c.967G>A(p. Val323Ile) in the DMRT1 gene was detected in the patient by WES. As far as we know, this is the first case of 46,XY CGD caused by a homozygous mutation in the DMRT1 gene.

Introduction:

46,XY gonadal dysgenesis (GD) is one kind of disorders of sex development (DSD) caused by alterations occurring at the process of gonadal determination, which requires coordinated expression and regulation of many genes(1). The clinical features of 46,XY GD ranged from 46,XY partial gonadal dysgenesis (PGD) to 46,XY complete gonadal dysgenesis (CGD). Genetic heterogeneity for 46,XY GD has been demonstrated in recent studies. A number of chromosomal structural aberrations and gene mutations have been found to be associated with 46,XY GD, such as 9p24.3 deletion, Xp21.2 duplication, 10q26.1 deletion, mutation of SRY, SOX9, DHH, DMRT1 and NR5A1 genes(2, 3). About 20-30% 46,XY gonadal dysgenesis patients have mutations in SRY or NR5A1 gene(4).

The DMRT1 (Doublesex and mab-3 related transcription factor 1) gene encodes a male-specific transcriptional regulator with a conserved zinc finger-like DNA-binding domain which plays an important role in sex determination and differentiation by controlling testis development and male germ cell proliferation (5, 6). The deletions and mutations of DMRT1 gene have been reported to cause 46,XY GD(7, 8). Here, we identified a homozygous DMRT1 mutation c.967G>A (p.Val323Ile) in a Chinese patient of CGD, to the best of our knowledge, this is the first case of homozygous mutation in the DMRT1 gene which could provide insight into the disease-causing mechanism by a hypomorphic mutation in an autosomal recessive pattern.

Clinical and pathologic findings

The patient presented to our clinic at 18 years of age because of primary amenorrhea and hypoplasia of uterus found in local hospital. Physical examine revealed normal height (168cm) and weight (45kg), phenotypically female external genitalia, hypoplastic uterus, sparse axillary hairs (Tanner II), undeveloped breast (Tanner I-II), normal vaginal depth (6cm) (Fig. 1A/B). Ultrasonography exams showed a small uterus (2.22*2.14*1.33cm), smaller gonads than normal (left 1.7*0.9, right 2.7*2.3 containing 2.1*1.8cm ambiguous swelling).

Endocrinologic investigations of blood serum showed hypergonadotropic hypogonadism with elevated FSH 109.58 (3.85-8.78 IU/L) and LH 53.25 (2.12-10.98U/L), minor reduced E2 32 (27-122 pg/ml), and normal female testosterone 0.33ug/L (0.1-0.75 ug/L), normal Prog 0.70 (0.31-1.52 ug/L) and PRL 9.00 (3.34-26.72ug/L).

Exploratory laparoscopy was performed to determine the nature of the gonadal tissues. The result showed a naive uterus, normal bilateral fallopian tubes, dysplastic gonadal tissues (left 1.5*1*1cm, right 3*2.5*2cm) (Fig. 1C/D/E).

Excised gonadal tissues were examined by microscopic histomorphology combined with immunohistopathology. The results revealed normal bilateral fallopian tube tissue, gonadoblastoma and local dysgerminoma (Fig. 1F/G/H).

Results from pedigree and genetic analyses

The phenotypic female proband was the fifth child of a healthy woman. The pedigree of the family as shown in Figure 2A indicated that the parents (III-1/2) of the proband (IV-5) are first cousins. All elder sisters (IV-1/2/3) and the brother (IV-4) of the proband were phenotypically normal, the eldest sister (IV-1) had given birth to two female babies (V-1/2)(Fig. 2A). Informed consent for genetic analysis was obtained from the patient (IV-5), the eldest sister (IV-1), and the mother (III-2), but the other family numbers refused to accept the genetic analysis.

Routine chromosome analysis performed on the proband found a normal male karyotype of 46, XY. Genomic DNA was extracted from peripheral blood samples of the patient, the eldest sister and the mother, using QIAamp DNA Blood Mini Kit (Qiagen). The concentration and quality of genomic DNA was analyzed by Nano-drop ND-1000 (Thermo Scientific, Wilmington, DE, USA).

Chromosomal Microarray Analysis (CMA) was performed to detect the copy-number variant (CNV) and the region of homozygosity (ROH) using a CytoScan 750 array (Affymetrix, Santa Clara, CA, USA) by following the manufacturer's instructions. No causative deletion or duplication was found, while 19 ROH regions amounting to 175Mb (6.31% of total autosomal length) were noticed on the proband, which was consistent with the consanguinity of the parents. Actually, the patient initially concealed the genetic relationship of her parents, until we reminded the indication of the CMA result.

To detect the causative gene mutation, next generation sequencing was performed on two gene panels, the first one including more than 200 genes for DSD was analyzed at BGI (Shenzhen, Guangdong, PRC), the second one including more 2800 genes by clinical whole-exome sequencing (WES) was performed at our laboratory. A homozygous mutation c.967G>A (p. Val323Ile) in the DMRT1 gene was detected in both panels. No other pathogenic variants were found in other DSD-related genes. Further analysis using Sanger sequencing confirmed this mutation in the patient and detected the mother and the eldest sister as heterozygous carrier for the c.967G>A mutation (Fig. 2B). Because the father refuse the test,the CMA test were done on the sister and mother. A haplotyping analysis on the SNP nearby the DMRT1 gene to dissect mother's and father's haplotype from the proband and her sister was done by using the CMA results of proband and her sister and mother to provide an indicated evidence the father should an obligate carrier of this mutation(Fig. 2A).

To rule out the polymorphism of this variant c.967G>A, we screened 64 normal fertile Chinese male individuals by Sanger sequencing, compared the conservatism of the amino acid among different species, and searched online databases including ExAC (http://exac.broadinstitute.org/) and ClinVar (https://www.ncbi.nlm.nih.gov/ClinVar). We did not find c.967G>A (p.Val323Ile) variant among 64 normal fertile male individuals. Val323 amino acid of AR protein is highly conserved in compared mammals. The variant was presents in ExAC (Exome Aggregation Consortium) database (Chr9:916907 G/A, rs746758951) as a singleton state (heterogeneous), so the frequency of the allele is extremely low (1 in total 121394 alleles, 8.238e-06), and the carrier was a European (Non-Finnish) individual, the gender and phenotype of the carrier was not mentioned.

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Considering that the c.967G is the last nucleotide of DMRT1 exon 4, we performed Minigene assay to evaluate the effect of c.967G>A to the pre-mRNA splicing. PCAS2 plasmid was used as a vector for this Minigene assay following the protocol of Gaildrat P, et al [9]. The PCAS2 plasmid was generated through a collaboration with Zhang Xue's Laboratory of Peking Union Medical College. The Minigene assay result showed that the c.967G>A variant had no effect on splicing in comparison with wild type.

To evaluate the genotype-phenotype correlations for the DMRT1 gene, a review of literature on PubMed and of cases on ClinVar database was performed. A total of 12 mutations and five intragenic deletions in the DMRT1 gene was identified from 11 reports (7, 10, 11, 12, 13-19). These mutations and deletions and their associated phenotypes were summarized in Table 1 and Fig 2C.

Discussion

The DMRT1 gene encodes a DNA-binding DM domain transcription factor and is widely conserved across animals. The DMRT1 protein can binds and regulates genes which were known to play an important role in sexual development. It can active male sex–determining gene Sox9 and repress female sex–determining genes Wnt4 and Rspo1 (10). It has been demonstrated that the mutation or loss of DMRT1 gene can disturb the normal male sexual development in several non-mammalian vertebrates like zebrafish (20-22), medaka (23), chicken (24), largemouth bass (25). In mammals, the DMRT1 gene is not the male sex determinant, but overexpression of Dmrt1 gene in XX mice can cause female-to-male sex reversal, indicating that this gene can initiate male development (26). In human fetal testis, repressing the expression of DMRT1 gene can change the expression of key genes which were necessary in gonadal development, leading to a move away from a male phenotype to a more ovarian-like phenotype (27).

In humans, the deletions or mutations of DMRT1 gene has been reported to cause DSD or sex-reversal (4, 8, 11, 28). Through literature review, we found twelve different kinds of mutations in the DMRT1 gene and the phenotypes are variable (Table1, Fig. 2C) (7, 13-18, 28). All of twelve mutations were heterozygous and no homozygous mutation was reported except the homozygous mutation c.967G>A detected in our study. Among these twelve mutations, seven mutations were related to sex determination. Four of these seven mutations (c.240G>C, c.251A>G, c.331A>G, c.332G>T) were located in the first exon of DMRT1 gene, and the other three mutations were mapped in exon 2 (c.416T>A), exon 4 (c.884C>T) and 3'UTR (3'UTR+11insT). The DMRT1 protein contains a highly conserved human DNA-binding domain (DM domain) located in the amino acid sequence 67 to 136 of the DMRT1 protein (DMRT167–136) (28). The four mutations c.240G>C, c.251A>G, c.331A>G, c.332G>T mapped in the DMRT167–136 caused DSD. The functional study of mutant protein (c.331A>G, R111G) showed that the mutant protein had strongly reduced DNA affinity and it also can interfere with the binding stoichiometry of wild-type DMRT1, suggesting that the mutation c.331A>G may combine haploinsufficiency with a dominant negative to cause 46,XY sex reversal (28). Deletions involving the DMRT1 gene have been reported in many individuals with GD, but most large deletions contain not only the entire DMRT1 gene but also other nearby genes. Review of literature found five intragenic deletions in the DMRT1 gene (Table 1, Fig. 3C). One case with a 103Kb deletion affecting exons 1 and 2 caused 46,XY GD (11); one case with a 35Kb deletion affecting exons 3 and 4 caused 46,XY ovotesticular disorder of sexual development (12); the other three cases including a 141Kb deletion affecting exons 3-5, a 150Kb deletion affecting exons 3-5 and a 55Kb deletion affecting exons 3 and 4, are all caused azoospermia (19). These cases demonstrated that intragenic deletions affecting exons 1 and 2 of the DM domain of the DMRT1 gene would disrupt the mRNA expression and exhibit haploinsufficiency for 46,XY GD, while the deletions affecting exons 3-5 might produce truncated proteins with reduced activity for a specific phenotype of azoospemia. The genotype-phenotype correlations for the DMRT1 gene from reported cases indicated that missense mutations and intragenic deletions affecting the DM domain would cause more severe phenotype of 46,XY GD to CGD. The variable phenotypes from azoospermia to sex reversal could be resulted from deletions and missense mutations involving exons 3, 4 and 5 of the DMRT1 gene.

In this study, we reported a homozygous mutation (c.967G>A, Val323Ile) of DMRT1 gene in a Chinese patient with 46,XY CGD. Her mother and old sister have a heterozygous c.967G>A mutation with a normal

phenotype. Her healthy father refused to provide samples for testing, but he is an obligate heterozygous carrier of this mutation theoretically. The c.967G>A mutation was a missense variant with very low frequency in GnomAD (1/246186) and ExAC (1/121394) database and not reported in ClinVar. The homozygotes of this variant were not found in normal populations or DSD patients. According to ACMG variant interpretation guidelines, this variant was categorized as uncertain significance (29). This mutation was located outside of the DMN domain at the last base of the fourth exon of DMRT1 gene, and the minigene test showed that the mutation has no effect on mRNA splicing. It is reasoned that this c.967G>A is most likely hypomorphic mutation with reduced level of activity and minimal impact on sex development in heterozygous. When this mutation presented in a homozygous state, the reduced activity from both alleles probably reach the sensitive threshold of haploinsufficiency and caused 46,XY CGD in an autosomal recessive pattern.

In conclusion, we found a homozygous c.967G>A mutation of DMRT1 gene in a patient with 46,XY CGD. This unique case suggested the presence of hypomorphic mutations in the DMRT1 gene which could be inherited in an autosomal recessive pattern and exhibit haploinsufficiency of the DMRT1 gene at homozygous state to cause disorders of sex development. This finding enriched the mutational spectrum of the DMRT1 gene and highlighted the importance of accurate genotype-phenotype correlations for the interpretation of genetic defects for 46,XY GD. Further investigation should be performed to clarify the molecular mechanisms causing disorders of sex development by deletion or mutation of DMRT1 gene.

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Figure 2_Family Pedigree and Genetic Results.ppt available at https://authorea.com/users/404568/articles/515730-homozygous-dmrt1-mutation-c-967g-a-found-in-a-chinese-patient-with-46-xy-complete-gonadal-dysgenesis-and-review-of-literature

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Table 1_The DMRT1 gene mutations and small deletions.pdf available at https://authorea.com/users/404568/articles/515730-homozygous-dmrt1-mutation-c-967g-a-found-in-a-chinese-patient-with-46-xy-complete-gonadal-dysgenesis-and-review-of-literature