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ARTICLE

PAX2 in 192 Chinese women with Müllerian duct abnormalities: mutation analysis

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Abstract The paired box gene 2 (*PAX2*) has been proven to be a crucial gene during organogenesis of the urogenital system in mice models. This study was aimed to explore the relationship between *PAX2* mutations and human Müllerian duct abnormalities (MDA). A total of 192 Chinese MDA patients (15 cases of uterine aplasia and 177 of incomplete Müllerian fusion) and 192 ethnic-matched controls were recruited from 2009 to 2011. Coding regions of *PAX2* of MDA cases were amplified and sequenced. One rare novel synonymous variant (c.320G>A) was discovered in one patient with uterus didelphys, whereas this variant was not found in the control group. Mutations in *PAX2* may be not a common cause of MDA.

KEYWORDS: MRKH syndrome, mutation, Müllerian duct abnormalities, paired box gene 2, polymerase chain reaction, single-nucleotide polymorphism

Introduction

The upper part of the vagina, the uterus and the Fallopian tubes is derived from the embryonic Müllerian duct, also named paramesonephric duct. Müllerian duct malformation, also termed Müllerian duct abnormalities (MDA), consists of a miscellaneous group of congenital anomalies of the female reproductive duct, such as uterine agenesis,

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unicornuate uterus, uterus didelphys, bicornuate uterus and septate uterus (American Fertility Society, 1988), which might result in compromised fecundity, like infertility, recurrent pregnancy loss and other obstetric complications (Vallerie and Breech, 2010). These congenital abnormalities of the female reproductive duct might be the result of developmental arrest, abnormal development or Müllerian improper fusions.

To date, researchers have done a large amount of work to define the aetiology of MDA; however, the cause remains a mystery. Previous studies proposed that environmental and iatrogenic factors, such as exposure to ionizing radiation, intrauterine infections and teratogenic drugs such as thalidomide (Hoffmann et al., 1976) and diethylstilbestrol (DES) (McLachlan et al., 1975) may contribute to the malformation. Some evidence showed that there is a strong familial aggregation of MDA among female first-degree relatives (Hammoud et al., 2008), and this phenomenon supports the hypothesis of the genetic causation of this disease. Researchers have advanced genetic exploration to elucidate this disease; however, most of the hypothetical candidate genes finally failed to be proven to be the pathogenic gene of MDA (Sultan et al., 2009).

PAX2 is a member of the paired box gene family (Gruss and Walther, 1992). To date, this gene family consists of nine members, *PAX1* to *PAX9*, and each of them encodes a nuclear transcriptional regulators specifically expressed during the development of a wide range of structures and organs. *PAX2* is widely expressed during the development of both ductal and mesenchymal components of the urogenital system. It has been proven a crucial gene during organogenesis of urogenital systems in mice models. The homozygous ($PAX2^{-/-}$) mutant female mouse lack oviducts, uterus, vagina and kidneys. At day 14.5 of embryonic development (E14.5), Müllerian ducts are only present at the upper most levels of the genital ridge in $PAX2^{-/-}$, while in the wild type, Müllerian ducts could be found along the entire length of the genital ridge. By E16.5, the part of the Müllerian duct initially formed has degenerated (Torres et al., 1995). On the other hand, in human, mutations in *PAX2* have been identified as the cause of renal-coloboma syndrome, which is featured by kidney hypoplasia and colobomas of the optic nerve (Schimmenti, 2011). Therefore *PAX2* seems a good candidate. The current study thus screened coding regions of 192 Han Chinese MDA patients to identify any correlation between *PAX2* and MDA.

Materials and methods

The study recruited 192 patients who had visited the Centre for Reproductive Medicine, Provincial Hospital affiliated with Shandong University, China from 2009 to 2011. All the patients were of Han Chinese descent.

Five types of Müllerian duct malformation, including uterine agenesis ($n = 15$), unicornuate uterus ($n = 55$), uterus didelphys ($n = 38$), bicornuate uterus ($n = 43$) and septate uterus ($n = 41$), were screened by transvaginal ultrasonography and/or by hysteroscopy and hysterosalpingogram, whilst patients diagnosed as abnormal chromosome karyotype were excluded. Other related defects, such as renal, skeletal and auditory defects were also recorded. As control, 192 peripheral blood samples of reproductive age women with a normal reproductive tract, which were examined by transvaginal ultrasonography and hysterosalpingogram, were also obtained. Under the permission of the Reproductive Medicine Ethical Committee of Shandong University, each participant signed informed consent that her sample could be utilized for molecular genetic analysis and that her medical history could be analysed and published anonymously.

Methods of candidate gene mutation screening were articulated before (Ma et al., 2011). In general, genomic DNA was obtained from peripheral blood (QIAamp DNA Blood Mini Kit; Qiagen, Germany). Ten pairs of specific primers for *PAX2* coding region were designed from the human sequence (transcript ID ENST00000355243) and were numbered 1 to 10, while exons 9—10 and 11—12 were amplified by primers 9 and 10, respectively. PCR was performed to amplify the coding region of *PAX2* (Supplementary Table 1, available online only) and the amplification products were then loaded on to a capillary electrophoresis sequencer (ABI 3730 XL; Applied Biosystems, USA) for automatic sequencing. Any novel findings were validated by two additional PCR amplification and subsequent sequencing. Chi-squared test was performed and a *P*-value <0.05 was considered statistically significant.

Results

Among the 12 exons and exon—intron boundaries of *PAX2*, one rare heterozygous novel synonymous variant (c.320G>A) was found in exon 3 from a 31-year-old woman, who was nulligravida with a 5-year history of infertility. Semen analyses revealed azoospermia of her husband. The woman had regular menstrual cycles without dysmenorrhoea. Pelvic examination revealed a normal-sized, mobile uterus and cervix. Uterus didelphys was revealed by hysterosalpingography, whilst bilateral Fallopian tubes were not visualized. Aside from iron-deficiency anaemia, past medical history was not contributory. The patient had no siblings. Blood samples of her parents were not available and their medical history was not contributory. In the control group, this synonymous variation was not found. One known synonymous single-nucleotide polymorphism (SNP) (rs1800897 in exon 8) was also identified. This SNP was compared with the International HapMap project database for Han Chinese in Beijing (HapMap-CHB), which involved 42 Chinese women. The distribution of the SNP is not significantly different between the MDA group and the HapMap-CHB data (C/C 88% versus 83.3%; C/T 12% versus 16.7%). The genotype frequencies of the variations in the MDA and control groups are shown in **Table 1**.

Discussion

The prevalence of MDA can be up to 15% in women being evaluated with hysterosalpingography because of recurrent spontaneous abortion. Apart from environmental and iatrogenic causes, the aetiology of MDA has remained generally undefined. At the level of the karyotype, chromosome abnormalities, especially sex chromosome mosaicism, are

Table 1 Genotype frequencies of single-nucleotide polymorphisms (SNP) found in women with Müllerian duct abnormalities (MDA) and controls.

Location	*Variation (dbSNP ID)*	*Population*	
		MDA (n = 192)	*Control (n = 192)*
Exon 3	c.320G>A (novel)		
	GG	191 (99.5)	192 (100)
	GA	1 (0.5)	0 (0)
Exon 8	c.797C>T (rs1800897)		
	CC	169 (88.0)	35 (83.3)[a]
	CT	23 (12.0)	7 (16.7)[a]

Values are *n* (%).
[a]International HapMap project database.

found in 8% of MDA patients. Because MDA is so far considered of sporadic occurrence, it is hard to localize and identify candidate genes. Previous studies have suggested that mutations or polymorphisms of genes involved in the growth and differentiation of Müllerian duct might be the cause of MDA, such as anti-Müllerian hormone and its receptor (Resendes et al., 2001), homeobox genes (Burel et al., 2006) and the WNT family (Biason-Lauber et al., 2004). However, except for WNT4, none of them have been clearly implicated with the congenital anomalies (Sultan et al., 2009). Although the MDA are considered sporadic and multifactorial, genetic factors are still an important facet to elucidate this uncharted disease.

PAX2 binds to the enhancer of the DNA sequence to modify the subsequent gene transcription. *PAX2* is specifically expressed in the development of the central nervous system, eye, ear, mammary glands and urogenital tract (Wolffian and Müllerian ducts and also the mesonephros) in a spatial and temporal pattern and has been proved to be crucial for this organogenesis (Torres et al., 1995). *PAX2* is encoded by 12 exons and resides on chromosome 10q22.1—24.3 (Eccles et al., 2002). The first four exons encode the paired box domain, which is a DNA-binding paired domain. The current study identified a heterozygous 320G>A variation in exon 3, which resides in centre of this domain. The variation is synonymous, so does not affect the coding sequences or alter expression. However, it may impact the transcriptional efficiency. Experimental studies in prokaryotes suggest that codon usage is not random. This phenomenon, termed codon-usage bias, is now recognized as crucial in shaping gene expression and cellular function (Plotkin and Kudla, 2011). The CCG to CCA codon alteration might alter *PAX2* in MDA patients through its effects on RNA processing, protein translation and protein folding.

This study also identified one known SNP (rs1800897 in exon 8). The distribution did not differ from that of the HapMap-CHB data. It is inferred that this allele is not related to MDA.

In conclusion, the one synonymous novel and rare variation in one uterus didelphys patient indicates that mutations in *PAX2* may not be a common cause of MDA in the Han Chinese population. This study should be considered the prelude for further studies of larger scale in other ethnic groups.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.rbmo.2012.04.010.

References

American Fertility Society, 1988. The American Fertility Society classification of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, Müllerian anomalies and intrauterine adhesions. Fertil. Steril. 49, 944—955.

Biason-Lauber, A., Konrad, D., Navratil, F., Schoenle, E.J., 2004. A WNT4 mutation associated with Mullerian-duct regression and virilization in a 46, XX woman. N. Engl. J. Med. 351, 792—798.

Burel, A., Mouchel, T., Odent, S., Tiker, F., Knebelmann, B., Pellerin, I., Guerrier, D., 2006. Role of HOXA7 to HOXA13 and PBX1 genes in various forms of MRKH syndrome (congenital absence of uterus and vagina). J. Negat. Results Biomed. 23, 4.

Eccles, M.R., He, S., Legge, M., Kumar, R., Fox, J., Zhou, C., French, M., Tsai, R.W., 2002. PAX genes in development and disease: the role of *PAX2* in urogenital tract development. Int. J. Dev. Biol. 46, 535—544.

Gruss, P., Walther, C., 1992. Pax in development. Cell 29, 719—722.

Hammoud, A.O., Gibson, M., Peterson, C.M., Kerber, R.A., Mineau, G.P., Hatasaka, H., 2008. Quantification of the familial contribution to Müllerian anomalies. Obstet. Gynecol. 111, 378—384.

Hoffmann, W., Grospietsch, G., Kuhn, W., 1976. Thalidomide and female genital malformations. Lancet 2, 794.

Ma, J., Qin, Y., Liu, W., Duan, H., Xia, M., Chen, Z.J., 2011. Analysis of PBX1 mutations in 192 Chinese women with Müllerian duct abnormalities. Fertil. Steril. 30, 2615—2617.

McLachlan, J.A., Newbold, R.R., Bullock, B., 1975. Reproductive tract lesions in male mice exposed prenatally to diethylstilbestrol. Science 190, 991—992.

Plotkin, J.B., Kudla, G., 2011. Synonymous but not the same: the causes and consequences of codon bias. Nat. Rev. Genet. 12, 32—42.

Resendes, B.L., Sohn, S.H., Stelling, J.R., Tineo, R., Davis, A.J., Gray, M.R., Reindollar, R.H., 2001. Role for anti-Müllerian hormone in congenital absence of the uterus and vagina. Am. J. Med. Genet. 98, 129—136.

Schimmenti, L.A., 2011. Renal coloboma syndrome. Eur. J. Hum. Genet. 19, 1207—1212.

Sultan, C., Biason-Lauber, A., Philibert, P., 2009. Mayer—Rokitansky—Kuster—Hauser syndrome: recent clinical and genetic findings. Gynecol. Endocrinol. 25, 8—11.

Torres, M., Gómez-Pardo, E., Dressler, G.R., Gruss, P., 1995. Pax-2 controls multiple steps of urogenital development. Development 121, 4057—4065.

Vallerie, A.M., Breech, L.L., 2010. Update in Müllerian anomalies: diagnosis, management, and outcomes. Curr. Opin. Obstet. Gynecol. 22, 381—387.

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