Genetic association between PAX2 and mullerian duct anomalies in Han Chinese females

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Received: 3 April 2016 / Accepted: 30 August 2016 / Published online: 8 October 2016
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Abstract

Purpose The study aims to investigate the genetic association between paired box gene 2 (PAX2) and mullerian duct anomalies (MDA) in Chinese Han females.

Methods Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to identify the genotypes of three tag single nucleotide polymorphisms (SNPs) in PAX2 in 362 MDA cases and 406 controls.

Results We found that one tag SNP (rs12266644) of PAX2 was associated with susceptibility to MDA. The genotype distributions of the SNP rs12266644 have a statistically significant difference in the MDA patients and controls with

a p value = 0.008. In the dominant model, we also observed that the GT + TT genotype increased the risk for MDA (p = 0.015, OR = 1.637, 95 % CI = 1.096–2.443).

Conclusion The polymorphism rs12266644 of PAX2 might be a risk factor for MDA in Chinese Han females.

Keywords PAX2 · Mullerian duct anomalies · Single nucleotide polymorphism · Association study

Capsule The PAX2 variant rs12266644 might increase the risk for mullerian duct anomalies in Chinese women.

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Introduction

Mullerian duct anomalies (MDA) are characterized by congenital complex malformation of the female reproductive tract, presenting the absence or agenesis of the oviduct, uterus, cervix, and upper part of the vagina. According to the variety of clinical phenotypes, MDA consists of congenital loss of the uterus and vagina which is also called Mayer–Rokitansky–Küster–Hause syndrome (MRKH), bicornuate uterus, unicornuate uterus, uterus didelphys, arcuate uterus, septate uterus (complete or partial), and vaginal septum [1]. Patients affected by MDA generally have normal female karyotype, and they also have normal hormone levels and secondary sexual characteristics because of functional ovaries. MDA may occur alone or sometimes be accompanied with renal agenesis and skeletal malformations [2]. To some extent, MDA could lead to infertility, recurrent spontaneous abortion, and other complications [3], which have a serious impact on women's life.

Till now, the etiology of MDA remains unknown. Apart from the known reasons such as environmental factors, harmful hormones, ionizing radiation, and drug effects, genetic factors may act as the major cause of MDA [4–6]. In the recent two decades, a lot of essential candidate genes related to the development of the mullerian duct have been studied, such

as the WNT and HOX families, LHX1, and TBX6 [7–14]. However, most of the researches failed to explain the relationship between the genes and MDA.

As a crucial factor, PAX2 is widely expressed in the epithelial cells of the mullerian-derived reproductive tract and the condensing mesenchyme of the kidney, which participates in the development and differentiation of the vertebrate urogenital system [15, 16]. The important function of PAX2 in the urogenital system has been further demonstrated through animal models. In mice models, a homozygous mutant of PAX2 leads to a loss of the ureters, uterus, oviducts, and kidneys [17]. In humans, spontaneous heterozygosity mutations of PAX2 were considered to have a close relationship with phenotypic spectrums like bilateral optic nerve colobomas and renal hypoplasia, which is now called renal-coloboma syndrome [18, 19].

According to the above researches, it is reasonable to presume that PAX2 might be involved in the development of MDA. Therefore, in the present study, we perform an association study to explore the potential relationship between PAX2 and MDA in Chinese Han females.

Materials and methods

A total of 362 Chinese Han women diagnosed with MDA by transvaginal ultrasonography, hysteroscopy, laparoscopy, and hysterosalpingogram at the Reproductive Medicine Center, The First Affiliated Hospital of Anhui Medical University, and 406 ethnicity-matched controls who requested for fertility treatment on account of oviduct obstruction or male factors were recruited in this study. The MDA patients had normal female karyotypes (46, XX) and also had normal secondary sexual characteristics, and two of them were accompanied with renal agenesis. According to a new classification method announced by the ESHRE/ESGE (European Society of Human Reproduction and Embryology/European Society for Gynaecological Endoscopy), which is based on the anatomy of the female reproductive duct, the MDA patients were classified into different categories (Table 1). All the participants signed written informed consents. Our experiment was approved by the local ethics committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Genomic DNA from peripheral blood leukocytes of all the participants was extracted using QIAamp DNA Blood Mini Kit (QIAGEN, Germany) based on the standard methods. The concentration and purity of the DNA were measured by a NanoDrop2000 spectrophotometer. Tag single nucleotide polymorphisms (SNPs) of PAX2 were selected from the International HapMap Project Web site (<http://hapmap.ncbi.nlm.nih.gov/>) for Han Chinese people in Beijing (CHB) with the criteria of minor allele frequency (MAF) >0.05 and R^2 >0.8. Genotyping was performed by matrix-

Table 1 Classification of the MDA patients according to the ESHRE/ESGE classification

Classification	Number
Uterus	
Normal uterus (U0)	5
Dysmorphic uterus (U1)	19
Septate uterus (U2)	150
Bicorporeal uterus (U3)	107
Hemi-uterus (U4)	35
Aplastic uterus (U5)	17
Unclassified malformations (U6)	29
Cervix	
Normal cervix (C0)	325
Septate cervix (C1)	9
Double cervix (C2)	24
Unilateral cervical aplasia (C3)	3
Cervical aplasia (C4)	1
Vagina	
Normal vagina (V0)	335
Longitudinal non-obstructing vaginal septum (V1)	11
Longitudinal obstructing vaginal septum (V2)	4
Transverse vaginal septum and/or imperforate hymen (V3)	9
Vaginal aplasia (V4)	3

assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using Sequenom MassARRAY, and the data we collected were analyzed by MassARRAY Typer software (Sequenom, USA).

Genotype distributions and allele frequencies of the three tag SNPs between the MDA cases and controls were analyzed with chi-square test by using the Statistical Product and Service Solutions software version 13 (SPSS 13.0). Fisher's exact test was applied when the number of cells was <5. The

Fig. 1 Linkage disequilibrium plot of the three SNPs (rs12266644, rs17113442, rs4244341) of the PAX2 intron region

Table 2 The genotype distributions and allele frequencies of the three tag SNPs between the MDA patients and controls

SNP ID	Genotype				Missing		HWE		<i>p</i> value	Allele	<i>p</i> value	OR (95 % CI)		Dominant model		Recessive model	
							χ^2	<i>p</i>						<i>p</i> value	OR (95 % CI)	<i>p</i> value	OR (95 % CI)
rs12266644	GG	GT	TT						0.008	G	T	0.034	1.506 (1.030–2.203)	0.015	1.637 (1.096–2.443)	0.501	0.995 (0.988–1.002)
	Case	293	66	0	3		3.68	0.159		652	66						
rs17113442	CC	CT	TT						0.593	C	T	0.394	0.823 (0.525–1.290)	0.441	0.833 (0.524–1.326)	1.000	0.998 (0.993–1.002)
	Case	326	35	0	1		0.94	0.625		687	35						
rs4244341	GG	GT	TT						0.610	G	T	0.357	1.170 (0.837–1.636)	0.337	1.193 (0.832–1.712)	1.000	0.890 (0.179–4.439)
	Case	286	71	3	2		0.38	0.827		643	77						
	Control	356	47	2	1		0.11	0.946		759	51						
	Control	357	45	1	3		0.11	0.946		759	47						
	Control	332	69	3	2		0.08	0.961		733	75						

odds ratios (OR) and 95 % confidence intervals (95 % CI) were calculated to evaluate the risk levels of the allele distributions. And we used Online Encyclopedia for Genetic Epidemiology studies (OEGE) [20] to test for the Hardy–Weinberg equilibriums of each tag SNP in the case and control groups. Linkage disequilibrium plot and haplotype association analysis were performed via SHEsis software [21], and haplotypes with frequencies <0.05 were excluded from our study. To decrease statistical errors, we adjusted the data for multiple comparisons applied with Bonferroni correction. *p* values < 0.0167 were considered statistically significant.

Results

Three tag SNPs (rs12266644, rs17113442, and rs4244341) were explored from the HapMap database in the PAX2 region for genotyping. All the three SNPs were located in the introns of PAX2. The linkage disequilibrium (LD) plot of the three SNPs in PAX2 is displayed in Fig. 1. Two pairs of the SNPs (rs12266644, rs17113442 and rs17113442, rs4244341) were in strong LD. Genotyping was performed in 362 MDA patients and 406 controls, and the results of allele frequencies and genotype distributions between the two groups are listed in Table 2. All the three SNPs have consistent HWE ($p > 0.05$) in the patients and controls.

In Table 2, we compared the genotype distributions and the variety of genetic models of the three tag SNPs between the

MDA patients and controls. The genotype distribution of rs12266644 showed a statistically significant difference between the MDA patients and controls with a *p* value = 0.008. Furthermore, the rs12266644 GT + TT variants increased the risk for susceptibility to MDA in the dominant model ($p = 0.015$, OR = 1.637, 95 % CI = 1.096–2.443). The haplotype analysis of the three SNPs of PAX2 is shown in Table 3, but there was no significant association between the haplotypes and MDA.

Discussion

Mullerian duct anomalies is a rare reproductive tract disease with an incidence between 0.1 and 3 % of female live births [22]. And in women who have suffered from recurrent spontaneous abortion, the rate of MDA could rise to 15 %. Most MDA cases are sporadic, but family aggregates in MDA, especially in MRKH, exhibit a strong relationship to recurrence in female first-degree relatives [23]. Although the genetic etiologies and inheritance patterns of MDA are still obscure, it has been postulated that MDA is a multifactor/polygenic disorder [6].

PAX2 belongs to the paired-box gene family, which contains nine family members (PAX1–PAX9) [24]. The PAX genes constitute a 128-amino acid paired domain, and each of them encodes for a transcription factor regulating specific DNA binding during vertebrate organogenesis [15]. Pax2, as a crucial transcription factor with specific expression patterns, is involved in the development of structure and organs during the organogenesis of several systems [15]. A serine/threonine-rich transactivation domain is located at the C-terminal region end of the PAX2 proteins, which is important for transactivation of target genes [25]. Pax2 was first detected in the intermediate mesoderm, which would evolve into the mullerian duct, Wolffian duct, kidney, and other derivatives of the urogenital system afterwards. Subsequently, Pax2 was found in both ductal and

Table 3 Haplotype analysis of the tag SNPs of PAX2 between the MDA cases and controls

Haplotype	Case frequency	Control frequency	<i>p</i> value	OR (95 % CI)
GCG	0.818	0.834	0.513	0.896 (0.646–1.244)
GTG	0.047	0.055	0.478	0.847 (0.534–1.342)
TCT	0.064	0.046	0.116	1.426 (0.914–2.226)

mesenchymal components and played a vital role in mesenchyme-to-epithelium transition during genital tract and kidney development [16, 17, 26]. As we mentioned before, the function of PAX2 in the urogenital system was also further investigated in mice models. In Pax2-knockout mice, the mullerian duct starts to invaginate but then degenerates quickly, proving that PAX2 is required for the elongation and maintenance of the mullerian duct [27]. But until now, the precise molecular mechanisms of Pax2 in the mullerian duct development remain obscure. Early studies showed that the Pax2 protein could interact with some repressor proteins to change the positive regulators to negative during the period of transcription [28]. In recent works, scholars identified that the Pax2 transactivation domain is phosphorylated by the c-Jun N-terminal kinase (JNK) [29]. Research proves that the JNK can regulate downstream of the WNT signaling pathway, which is essential for the development of female reproductive ducts [30]. Other MDA candidate genes, such as LHX1, which also encodes for a transcription factor, participate in the period of mullerian duct formation [31]. And it is possible that PAX2 can adjust upstream of or in parallel with LHX1 and cooperatively regulate other factors in the development of the mullerian duct [22].

However, research on the PAX2 gene in human MDA is scarce so far. A case–control study was conducted to investigate the connection between MDA and PAX2 in 192 Chinese Han patients, but only a novel synonymous mutant and a known SNP were identified. In our study, we selected three tag SNPs of PAX2 and desired to explore the association between the PAX2 gene and MDA. The results showed that the rs12266644 significantly conferred a risk for MDA susceptibility. Genetic studies which research on MDA mostly concentrated on finding novel mutations in the candidate genes, while there have not been much positive results gained until now. Contrary to our study, previous research shows that PAX2 may not be a major gene which directly causes female reproductive tract malformation; the positive polymorphism changes may interact with other causative factors, such as environmental factor, increasing the risk for susceptibility to MDA.

In conclusion, we investigate the relationship between the tag SNPs of PAX2 and MDA in Chinese Han women. The results indicated that the SNP rs12266644 is associated with the risk for MDA susceptibility. Further explorations and functional experiments would be needed to validate the importance of PAX2 in the development of the female reproductive tract.

Acknowledgments The authors thank all the participants in this study; without them, the research could not have been performed. This study was supported by the National Natural Science Foundation of China (81370691), the National Basic Research Program of China (2012CB944704), and the Research Fund of National Health and Family Planning Commission of China (201402004).

Compliance with ethical standards All the participants signed written informed consents. Our experiment was approved by the local ethics committee and conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

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