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Original Research

A rare variant in the *MARVELD2* gene is associated with Chinese samples with ovarian endometriosis

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Abstract

Objectives: Endometriosis is a common gynecological disease affecting up to ~10% of women at reproductive age. Prior combined studies implied that *MARVELD2* might be involved in the pathogenesis of certain malignancies. Here, 211 Han Chinese samples with ovarian endometriosis were analyzed for the presence of *MARVELD2* mutations. **Methods**: We analyze the potential presence of *MARVELD2* mutations by direct DNA sequencing. **Results**: A total of 7 variants, 5 missense and 2 synonymous variants, were identified in our 211 ovarian endometriosis samples with different frequencies. Among the 5 missense variant, a missense rare variant p.V198M (c.592G>A), was identified in 10 out of our 211 samples (4.74%). This rare variant was identified with extremely low frequency in 766 control samples from 766 Chinese women without endometriosis (0.13%, 1/766) and control samples in the public databases. The evolutionary conservation analysis results suggested that the *MARVELD2* rare variant lead to highly conserved amino acid substitutions among 14 vertebrate species from *Human* to *Snake*. Furthermore, both the SIFT and Polyphen-2 programs predicted this rare variant to be 'disease causing'. However, we failed to observe any statistical significance between the *MARVELD2* rare variant and the available clinical data. **Conclusions**: We identified a potential pathogenic rare variant in the *MARVELD2* gene in Chinese samples with ovarian endometriosis, indicating the *MARVELD2* rare variant might play an active role in the pathogenesis of endometriosis.

Keywords: *MARVELD2*; Rare variant; Ovarian endometriosis; Han Chinese

1. Introduction

Endometriosis is a common gynecological disease, in spite of the subject of many scientific researches, the detailed molecular etiology remains still unclear [1]. Among which, the 'Sampsons hypothesis' is the most extensively admissive interpretation, which implies that endometriosis takes place owing to retrograde menstruation, where endometrial tissue passes through the fallopian tube into the peritoneal and pelvic cavity where it implants [2]. The implantation theory indicates the formation of endometriosis in the peritoneal cavity and ovary needs endometrial tissue or cells fulfilling a process of adhesion, invasion and proliferation [3].

Tricellulin, a major component of tight junctions, encoded by the *MARVELD2* gene [4], was the first identified protein to be uniquely localized at the tri-epithelial junctions [5]. Prior studies have revealed that *MARVELD2* mutations could cause nonsyndromic deafness, and these *MARVELD2* mutations were shown to cause tricellulin defects and deletion, thereby affecting the adhesion and the tight junction function between epithelial cells [6–8]. Furthermore, dysregulated *MARVELD2* expression was associated with patients' prognosis in certain cancer types [9–11] and could promote cell migration [12]. Endometriosis is similar to cancer and exhibits enhanced invasion and migration [13–15]. This disorder is considered as a precancerous lesion and harbored a variety of mutations in certain oncogene and tumor suppressor genes [16–19]. A large-scale sequencing effort has revealed that epithelial cells harbored cancer-associated mutations promoting the pathogenesis of endometriosis [20]. In addition, increased expression of tricellulin could promote the invasions and metastasis of certain human tumor [21].

Prior studies have revealed that certain germline mutations, including single nucleotide polymorphism (SNP), played important roles in the pathogenesis of endometriosis via candidate gene or large-scale sequencing strategies [22,23]. In addition, somatic mutations in certain genes, were also shown to confer the risk of endometriosis [16–18,24]. Prior studies have found that dysregulated expression of *MARVELD2* was associated with patients' prognosis in certain cancer types [9–11] and could promote cell migra-

Table 1. The potential association of *MARVELD2* rare variant with clinical data in 211 Chinese samples with ovarian endometriosis.

Features	Total sample	Wild type (n = 201)	Mutation (n = 10)	*p* value
Age (years)	211	29.56 ± 7.33	31.5 ± 4.72	0.42
Age of menarche (years)	211	12.53 ± 1.49	13.2 ± 2.14	0.37
Hemoglobin (g/L)	211	122.35 ± 11.34	130.1 ± 4.23	0.16
TSH (mIU/mL)	211	2.12 ± 1.23	1.8 ± 1.18	0.12
FT3 (pg/mL)	211	3.15 ± 0.18	3.06 ± 0.26	0.09
FT4 (ng/dL)	211	1.31 ± 0.13	1.26 ± 0.19	0.08
AFP (ng/mL)	211	2.62 ± 1.36	3.11 ± 0.93	0.35
CEA (ng/mL)	211	1.03 ± 0.42	0.96 ± 0.26	0.22
CA125 (U/mL)	211	115.33 ± 166.23	103.25 ± 78.39	0.56
SCCA (ng/mL)	211	1.47 ± 0.56	1.52 ± 0.86	0.37
Whitebloodcellcount ($\times 10^9$)	211	6.26 ± 2.12	6.12 ± 0.58	0.39
Lymphocyte cell count ($\times 10^9$)	211	1.88 ± 0.63	1.46 ± 0.39	0.34
Eosinophil granulocyte ($\times 10^9$)	211	0.15 ± 0.07	0.08 ± 0.06	0.55
Mononuclear cell count ($\times 10^9$)	211	0.47 ± 0.07	0.42 ± 0.09	0.11
Neutrophil cell count ($\times 10^9$)	211	3.88 ± 1.62	3.96 ± 1.22	0.77
Platelet ($\times 10^9$)	211	201.38 ± 52.63	213.66 ± 45.38	0.45
Neutrophil cell proportion (%)	211	58.37 ± 7.66	65.38 ± 3.38	0.27

TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; AFP, α-fetoprotein; CEA, carcinoembryonic antigen; CA125, cancer antigen 125; SCCA, squamous cell carcinoma antigen.

Table 2. The primer information for *MARVELD2* gene (NM_001038603) amplification.

Gene	Exon	Annealing	PCR amplicon	Forward primers (5′-3′)	Reverse primers (5′-3′)
MARVELD2	2	56 °C	1260 bp	atcagcatcattgagagga	acatacacacacaaatgag
MARVELD2	3	52 °C	335 bp	atcaacctcttaaaattgag	ggtcttgaaattctggtctc
MARVELD2	4, 5	55 °C	706 bp	ccacctgatcttcttcctc	ctctagatttcagtgctcct
MARVELD2	6	58 °C	339 bp	tctcagtgtgctttgagata	aatgctcatttctctaggtt
MARVELD2	7	53 °C	286 bp	tgtagagagcttaactgttac	tggtttcaaataagcgtta

tion [12]. Considering the important role of tight junction played in cell invasion and migration [24], and endometriosis was a premalignant disorder and harbored a number of mutations [25,26], we thus hypothesized that *MARVELD2* mutations might also existed in endometriosis samples.

Here, we recruited and analyzed a total of 211 Chinese patients with ovarian endometriosis for the presence of *MARVELD2* mutations. A rare variant in the *MARVELD2* gene was identified in 10 out of 211 samples (4.74%).

2. Materials and methods

2.1 Samples

A total of 211 Chinese patients with ovarian endometriosis, as well as control samples from 766 Chinese women without endometriosis were also collected from Jiangxi Provincial Maternal and Child Health Hospital (Nanchang, China). Written informed consent was obtained from each sample prior to this study, and the present study was performed according to the tenets of the Helsinki Declaration and was approved by the Institutional Review Board of Jiangxi Provincial Maternal and Child Health Hospital.

2.2 Clinical data

The clinical data for the participating women with ovarian endometriosis was collected at the time of sampling, including age, age at menarche, the laboratorial data included serum hemoglobin, free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), squamous cell carcinoma antigen (SCCA) and α-fetoprotein (AFP) were determined on day 3 of the menstrual cycle by radioimmunoassay, as described previously [17,18]. In addition, the number of white blood cells, lymphocytes, eosinophil granulocytes, neutrophil granulocytes, mononuclear cells, platelet, and neutrophil granulocyte proportion was analyzed by an automated hematology analyzer XN-3000 (Sysmex Corporation, Kobe, Japan) (Table 1), as described previously [17,18].

2.3 DNA extraction and mutation analysis

The genomic DNA was extracted from the peripheral blood of our samples. Omega Blood DNA kit (OMEGA Bio-tek Inc., Doraville, GA) was used to isolate the genomic DNA for our samples, as described previously [17]. The entire coding regions of the *MARVELD2* gene

Table 3. The identified variants of the *MARVELD2* gene (NM_001038603) in our patients.

SNP	Amino acid /Nucleotide change	Frequency	SIFT prediction	Polyphen-2 prediction
rs1185246	p.T33I/c.98C>T	43.60% (92/211)	Tolerated	Benign
rs111458976	p.Y62Y/c.186C>T	55.45% (117/211)	-	-
rs181575833	p.Pro102Pro/c.306G>A	0.48% (1/211)	-	-
rs575942430	p.Y159C/c.476A>G	0.48% (1/211)	Tolerated	Benign
rs201914751	p.V198M/c.592G>A	4.74% (10/211)	Damaging	Damaging
rs771384203	p.G237A/c.710G>C	0.48% (1/211)	Tolerated	Benign
rs1198930354	p.V489M/c.1465G>A	1.42% (3/211)	Tolerated	Benign

Table 4. The allele frequency comparison of *MARVELD2* rare variant among our patients and control samples in the present study and public databases.

SNP ID	Case (N = 211)	Normal control (N = 766)	*p* value [a]	Allele frequency in EXAC	*p* value [a]	Allele frequency in 1000 genome	*p* value [a]
rs201914751	10/422	1/1532	2.36×10^{-6}	22/121412	2.2×10^{-16}	3/5008	2.31×10^{-9}

[a] *p* value, Fisher's exact test.

(NM_001038603) were amplified with certain PCR primer pairs (Table 2), and subjected to direct DNA sequencing. The PCR reactions were performed as follows, 30 ng DNA, 1.5 μL 10 $\times$ PCR buffer, 250 μM dNTPs mix, 0.20 μM each primer, 2.5 mM $MgCl_2$, 0.5 U Taq DNA polymerase (Takara, Dalian, China). The PCR reaction was performed as follows: 95 °C premature for 5 min, 35 cycles including 94 °C for 30 sec, 52–58 °C for 45 sec and 72 °C for 30 sec, ended with a 10 min extension at 72 °C. The obtained PCR products were sequenced on an ABI 3730XL DNASequencer (Applied Biosystems, Waltham, MA, USA). The potential *MARVELD2* mutations were analyzed and aligned with standard DNA sequences of the Human *MARVELD2* gene via DNAStar Lasergene software (Madison, WI, USA).

2.4 Evolutionary conservation analysis of the rare variant of MARVELD2

We downloaded the *MARVELD2* proteins from 14 vertebrate species from GenBank database (www.ncbi.nlm.nih.gov/gene), including Human (NP_001033692), Chimpanzee (XP_003310745), Monkey (XP_001094419), Mouse (NP_001033691), Rat (NP_001102406), Cattle (XP_002696327), Dog (XP_019690741), Horse (XP_023474013), Pig (NP_001230877), Cat (XP_019690741), Tree shrew (XP_006169576), Chicken (XP_424965), Bat (XP_006762445) and Snake (XP_039176681). The conservation of the mutated *MARVELD2* residue was analyzed with MEGA4 software (Tokyo, Japan) [27].

2.5 In silico analysis of the MARVELD2 rare variant

We used SIFT [28] and Polyphen-2 [29] online programs to predict the potential pathogenicity of these missense variants of *MARVELD2*. These programs could automatically assess this rare variant to be damaging or benign.

2.6 Statistical analysis

We used two-sided Student's *t*-test and Mann-Whitney's method to analyze the potential association of numerical and continuous variables between ovarian endometriosis samples with and without *MARVELD2* mutations, respectively; Fisher's exact test was used to analyze the allele frequency of *MARVELD2* rare variant between the cases and controls; *p* value less than 0.05 was considered statistically significant. All statistical analyses were performed by the software SPSS 19.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1 Sample characteristics

The median age of the samples was 32 years (range, 20–52) and the median age at menarche was 14 years (range, 10–19). The detailed clinical data, including hemoglobin, FT3, FT4, TSH, CEA, CA125, SCCA, AFP, and blood cell counts, are summarized in Table 1.

3.2 MARVELD2 rare variant in ovarian endometriosis

A total of 7 variants, 5 missense and 2 synonymous variants, were identified in our 211 samples with ovarian endometriosis with different frequencies (Table 3). Among the 5 missense variant, a rare variant (rs201914751) p.V198M (c.592G>A), was identified with high frequency in our samples with ovarian endometriosis (10/211, 4.74%) (Fig. 1) (Table 3). This rare variant was found in 766 control samples from 766 Chinese women without endometriosis with extremely low frequency (0.13%, 1/766) ($p < 0.01$); furthermore, this rare variant existed in extremely low frequencies in control samples in the 1000 genome (www.ncbi.nlm.nih.gov/variation/tools/1000genomes) and EXAC (www.exac.broadinstitute.org) databases, which included 2504 and 60706 samples, respectively ($p < 0.01$) (Table 4). The average age and age of menarche of the 10 sample with *MARVELD2* rare variant (p.V198M) was 31.5

and 13.2 years old, respectively.

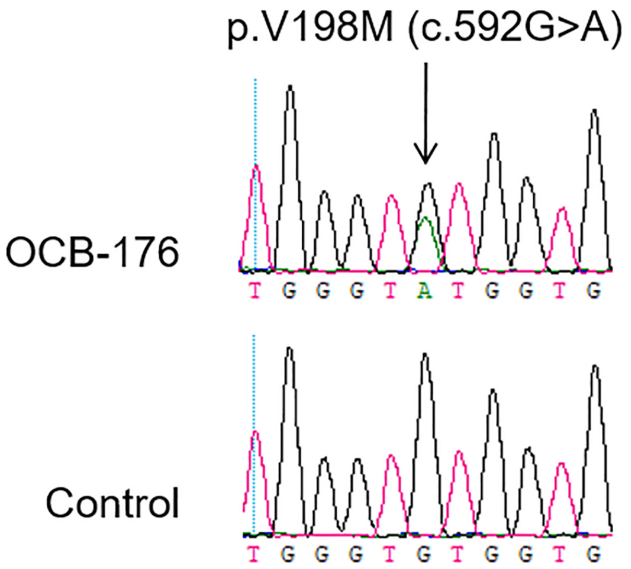

Fig. 1. The representative sequencing electropherograms of *MARVELD2* p.V198M (c.592G>A) mutation, the arrow refers to locations of the mutations. "OCB-176" was an endometriosis samples with *MARVELD2* mutation, while "Control" was a control sample without *MARVELD2* mutation.

Fig. 2. The evolutionary conservation analysis result of *MARVELD2* p.V198Mmutation from *Human* to *Snake*.

3.3 In silico and Evolutionary conservation analyses of the MARVELD2 missensevariants

Both the SIFT and Polyphen-2 programs were used to predict the potential pathogenicities of these variants, the predicted results showed that the p.V198M variant was 'disease causing'; while other four variants were benign. The evolutionary conservation analysis result suggested that the *MARVELD2* rare variant lead to highly conserved amino acid substitution from valine to methionine at the 198th codon (V198M), among the 14 vertebrate species from *Human* to *Snake* (Fig. 2).

3.4 Association between MARVELD2 rare variant and clinical data

The clinical data between the ovarian endometriosis samples with and without *MARVELD2* rare variant (p.V198M) was analyzed, including patients' age, age of menarche, FT3, FT4, TSH, hemoglobin, CA125, SCCA, AFP, CEA, white blood cell count, eosinophil granulocyte, lymphocyte cell count, neutrophil cell count, mononuclear cell count, platelet and neutrophil cell proportion. However, we failed to get any significant association between *MARVELD2* rare variant and these clinical parameters (Table 1).

4. Discussion

MARVELD2, formerly referred to as TRIC-a, encodes tricellulin, a tricellular tight junction protein [4]. Tricellulin is mainly localized in tricellular cell contacts, while in bicellular tight junctions to a lesser extent [30]. To date, it's deemed that tricellulin expression ubiquitously exists in epithelial junctions of tissues and organs throughout the body [6], as well as in ovarian epithelia [31].

Prior large-scale sequencing studies have revealed that common variants in certain genes played important role in the pathogenesis of endometriosis, including vascular endothelial growth factor receptor 2 (VEGFR2), mitogen-activated protein kinase kinase kinase 4 (MAP3K4), and Wnt family member 4 (WNT4) [32–34]. Recently, increasing evidences have suggested that rare variants also facilitate the initiation and development of endometriosis [35,36]. In the present study, we have screened a total of 211 Han Chinese samples with ovarian endometriosis for the presence of *MARVELD2* mutations, via sequencing the whole coding region and the exon-intron boundaries of the *MARVELD2* gene. Here, we identified a *MARVELD2* missense rare variant, p.V198M (c.592G>A), in 10 out of 211 Han Chinese samples with ovarian endometriosis (4.74%), the frequency of this rare variant is significant higher than that either in the local control women without endometriosis or in the control samples in the 1000 genome and EXAC databases ($p < 0.01$). The evolutionary conservation anal-

ysis result showed that the *MARVELD2* rare variant caused highly conserved amino acid substitution among 14 vertebrate species. Furthermore, the bioinformatic programs prediction result showed this rare variant might be damaging.

MARVELD2 mutations could cause nonsyndromic deafness [6–8] and the molecular mechanism involved in affecting the paracellular permeability, leading to a toxicity for cochlear hair cells [37]. Subsequent studies found that dysregulated expression of *MARVELD2* could change the capacities of cell invasion and migration in diverse cancer types, including colorectal and gastric cancers, and the potential underlying molecular mechanism involved in actin and cytoskeletal reorganization, as well as epithelial-mesenchymal transitions (EMT) process [21,38]. As a pre-malignant condition, endometriosis usually exhibited with dysregulation of cell invasion and migration, we thus speculated that the *MARVELD2* rare variant identified in the present study might play active role in the pathogenesis of endometriosis.

On the other hand, we failed to observe any positive association between the *MARVELD2* rare variant and the available clinical data. Of note, our sample size of ovarian endometriosis was relatively small, we will continue to obtain additional samples in order to re-analyze the potential association.

5. Conclusions

We identified a relatively high frequency of *MARVELD2* rare variant in our samples with ovarian endometriosis, indicating this rare variant might play positive role in the pathogenesis of this disease.

Author contributions

QW—investigation and manuscript preparation; RL—sample collection; YZ—investigation; YL—investigation; JZ—data analysis; YD—sample collection; XZ—data analysis; GG—sample collection; OH—methodology, manuscript revision.

Ethics approval and consent to participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Jiangxi Provincial Maternal and Child Health Hospital (approval number: JXSFYBJYY2020KJK015).

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Conflict of interest

The authors declare no conflict of interest.

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