Taiwanese Journal of Obstetrics & Gynecology 57 (2018) 270–275

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Original Article

Associations between a single nucleotide polymorphism of stress-induced phosphoprotein 1 and endometriosis/adenomyosis

Chia-Lung Tsai [a, 1], Yun-Shien Lee [b, 1], Angel Chao [c, d], Chih-Feng Yen [c], Hsin-Shih Wang [c], Tzu-Hao Wang [a, c, e, *]

[a] Genomic Medicine Core Laboratory, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[b] Department of Biotechnology, Ming-Chuan University, Taoyuan, Taiwan
[c] Department of Obstetrics and Gynecology, LinKou Medical Center, Chang Gung Memorial Hospital and Chang Gung University, Linkou, Taiwan
[d] Gynecologic Cancer Research Centre, LinKou Medical Center, Chang Gung Memorial Hospital, Linkou, Taiwan
[e] Graduate Institutes of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Accepted 14 March 2018

Keywords:
Endometriosis
SNP
STIP1

ABSTRACT

Objective: We have recently reported that stress-induced phosphoprotein 1 (STIP1) is over-expressed in endometriosis/adenomyosis tissues. STIP1 may also be involved in immune regulation, thus we attempted to study the association between STIP1 single nucleotide polymorphisms (SNPs) and endometriosis/adenomyosis.

Materials and methods: Five STIP1 SNPs (rs7941773, rs2845597, rs4980524, rs2282490, and rs2236647) were selected for genotyping with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) in 286 patients with endometriosis/adenomyosis and 288 healthy postmenopausal controls. *In vitro* studies included luciferase promoter reporter assays and western blot analysis for STIP1 and MMP9 proteins.

Results: The frequency of the G allele at rs4980524 was significantly higher in patients with endometriosis/adenomyosis than in control women. The promoter reporter with rs4980524 GG genotype significantly increased luciferase activity than that with TT genotype in endometrial cancer RL95-2 cells, and the primary endometrial stromal cells carrying rs4980524 GG genotype expressed higher protein levels of STIP1 and MMP9 than those carrying the TT one.

Conclusion: The G/G allele of STIP1 SNP rs4980524 is associated with the increased expression of STIP1 and MMP9 in endometriosis. Further validation in independent cohorts of endometriosis patients may prove its usefulness as a genetic risk maker for endometriosis/adenomyosis.

Introduction

Endometriosis is characterized by growth of ectopic endometrial tissues outside the endometrial cavity, affecting 5–10% of women of reproductive age [1]. Clinically, dysmenorrhea, deep dyspareunia, chronic pelvic pain, and infertility are commonly seen symptoms of endometriosis [2]. Despite its devastating effects on women's quality of life and reproductive potential, the etiology of endometriosis remains inconclusive. Historically, Sampson proposed that reflux menstruation might result in endometriosis [3], which is now known to be true only in the presence of impaired immunological surveillance. Meyer proposed the theory of coelomic metaplasia, predicting the pluripotency of certain groups of peritoneal mesothelium [4]. Meigs suggested that Mullerian metaplasia could be induced by abnormal hormonal influence [5]. Since these theories were proposed decades ago, thousands of studies, including immune dysfunction [1], have provided evidence that support or dispute against each theory.

Case-control association studies using genetic markers are based on the “common disease, common variant” assumption, which means that the genetic risk for a common disease can usually be attributed to a relatively small number of common genetic variants [6]. Single nucleotide polymorphisms (SNPs) are the preferred genetic markers for such studies because of their

* Corresponding author. Genomic Medicine Core Laboratory, Chang Gung Memorial Hospital, Taoyuan, Taiwan.
E-mail address: knoxtn@cgmh.org.tw (T.-H. Wang).
[1] C.L.T. and Y.S.L. contributed equally to this work.

https://doi.org/10.1016/j.tjog.2018.03.001

abundance; about 12 million unique human SNPs have been assigned a reference SNP number in the National Center for Biotechnology Information's SNP database [7]. The development of endometriosis is regulated by enzymes and receptors that are involved in biosynthesis and metabolism of many hormones, including estrogens [1,8]. Various SNPs have been associated with the severity of and the susceptibility to endometriosis [9,10]. Recently, we have also reported that both the GG and GA genotypes of the FSH receptor gene (Asn680Ser) are associated with a significantly lower risk of endometriosis [2].

Stress-induced phosphoprotein (STIP1, Gene ID 10963; HPRD 05454) is also known as heat shock protein (HSP)-organizing protein (HOP) [11]. STIP1 modulates the chaperone activities of HSP 90 and HSP 70 [11] and overexpresses in several malignant tissues, including melanoma [12], hepatocellular carcinoma [13], glioma [14], ovarian cancer [15] and pancreatic cancer [16]. Knockout STIP1 in mice shows embryonic lethal, activates caspase 3 and impairs cell proliferation [17]. Knocking down endogenous STIP1 with siRNA decreased the expression of HSP90 client oncoproteins (e.g., HER2, Bcr-Abl, c-MET, and v-Src) [18]. STIP1 is also involved in regulation of cell migration through modulating matrix metalloproteinase-2,9 [18,19] and RhoC GTPase [20].

Several protein interaction domains of STIP1 are identified, including three tetratricopeptide repeat (TPR) domains (1,2A, 2B) and two rich in aspartate and proline (DP) domains (1 and 2) [21]. TPR1 and 2B are essential for HSP70 binding, whereas TPR2A, TPR2B and DP2 domains involve HSP90 interaction [22–24]. STIP1 also maintains protein stability of JAK2, CDK3, survivin and AKT [24,25] through these interactions. A glioblastoma cell line has been shown to secrete STIP1 into culture medium, and recombinant STIP1 can induce proliferation of glioma cells by activating the ERK, PI3K and BMP pathways [14,26]. Treatment of ovarian cancer cells with STIP1 significantly increases ERK phosphorylation, promotes DNA synthesis, and increases Ki-67 immunoreactivity in ovarian cancer cells, extending the pro-proliferative role of STIP1 in the tumorigenesis of ovarian cancer [15].

Recently, SNPs of STIP1 were found to correlate with lung function in asthmatic subjects treated with inhaled corticosteroids, suggesting its role as a prediction marker for glucocorticosteroid responses in patients with reduced lung function [27]. Given the implication of STIP1 involvement in immune regulation, we attempted to study the association between STIP1 SNPs and endometriosis. Our results showed that STIP1 over-expression in endometriosis [19] was associated with a higher allele frequency of G in the STIP1 SNP (rs4980524) in patients with endometriosis. Further *in vitro* studies supported that the G/G allele of rs4980524 indeed stimulated STIP1 expression.

Materials and methods

Ethics statement

All procedures complied with the tenets of the Helsinki declaration and were approved by the Institutional Review Board of the Chang Gung Memorial Hospital (IRB approval #94-975B and #97-0753B).

Culture and treatment of cell lines

Human endometrial cancer cell RL95-2 was obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in DEME/F12 with 10% fetal bovine serum and appropriate amounts of penicillin and streptomycin.

Extraction of genomic DNA

The procedure for genomic DNA extraction and the samples subjected to STIP1 SNPs genotyping have been previously described in detail [28]. In brief, cases were women with a pathological diagnosis of endometriosis who had undergone surgical treatment. Postmenopausal women with a negative history of infertility and dysmenorrhea who were free of endometriosis and/or adenomyosis were selected as controls. All controls did not previously undergo surgery for obstetric and/or gynecologic conditions. We purposely selected postmenopausal women as controls for genotyping to reduce the likelihood of including subjects at risk of developing endometriosis later in life. All of the patients and controls included in the study were of Taiwanese descent. Genomic DNA was extracted from peripheral leukocytes using a QIAmp DNA blood Midi Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's protocol.

DNA construction

STIP1 luciferase reporter vectors containing the rs4980524 SNP were amplified from the patients' genomic DNA based on their known genotypes. The following primers for rs4980524 were used: forward, 5′-ACGAAGCTTGCCGTGAGGCAAGATTGTGC-3′, and reverse, 5′-ACGAAGCTTCCGGTGGGATACAGCAGTTT3′. PCR conditions were as follows: initial step at 95 °C for 5 min, followed by 40 cycles of 1 min at 95 °C, 1 min at 55 °C, and 72 °C for 10 min. PCR products were digested with the restriction enzyme *Hind* III and then ligated with a *Hind* III/CIP-treated pGL4.26 vector (Promega, Madison, WI, USA). DNA sequences were confirmed with an ABI DNA autosequencer (Applied Biosystems, Foster City, CA, USA).

SNPs genotyping

Five STIP1 SNPs (rs7941773, rs2845597, rs4980524, rs2282490, and rs2236647) were selected for genotyping [27], which was conducted by MALDI-TOF MS in a 96-well format [29,30]. PCR primers and mini-primer sets are reported in Table 1. PCR amplification was performed in a final volume of 10 μL containing 5 ng of genomic DNA, 1 × PCR buffer, 100 μM of each dNTP, 1 μM of each primer, and 1U Taq DNA polymerase. PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 30 s denaturation at 95 °C, annealing for 30 s at Tm of each primer set, and extension for 30 s at 72 °C, with a final extension at 72 °C for 2 min. PCR products were purified using a GenoPure DS purification kit (Bruker Daltonics, Bremen, Germany). Allele-specific primer extension reactions were catalyzed by Thermo Sequenase™ DNA polymerase (Amersham Pharmacia, Amersham, UK) for 50 cycles of 8 s at 94 °C, 8 s at 52 °C, and 8 s at 72 °C. Primer extension products were treated with the GenoPure Oligo purification kit (Bruker Daltonics) to remove salts from the reaction buffer. Matrix 3-hydroxypicolinic acid (3-HPA) (Fluka, Buchs, Switzerland) was used at a concentration of 10 mg/mL containing 1 mg/mL diammonium hydrogen citrate. We initially spotted 0.5 μL of the matrix on the Anchor Chip using the map II/8 MALDI AutoPrep system (Bruker Daltonics). After drying, 0.5 μL of the primer extension product was loaded onto the dried matrix. Finally, we added 75% acetonitrile (0.5 μL) to the sample which was subsequently subjected to MALDI-TOF MS (Autoflex, Bruker Daltonics) analysis.

DNA transfection and luciferase reporter assay

The procedures used for DNA transfection and construction of the luciferase reporter assays have been previously described

Table 1
Genotyping of STIP1 SNPs using mini-sequencing primer extension: sequences of primers and mini-primers.

SNP	Forward primer	Reverse primer	Mini-primer for extension
rs7941773	5′-CACAGGCGGTCAACTACTGA-3′	5′GCCTCCTGGCTGTTAGGTTC-3′	5′-CACGAATTCCCCTCGGCC-3′
rs2845597	5′-CTTAGCCACGAGGAACTTGG-3′	5′-CACCTTTCGGAAGTGTTGGT-3′	5′-ACCGACTCTACCTGATGC-3′
rs2282490	5′-CATGAACCCTTTCAACATGC-3′	5′-TGAAATATGCTCCCCTGACC-3′	5′-TGTTATGCTTTCTTCCTG-3′
rs2236647	5′-TGCTTCAAGTCGAAGGGATT-3′	5′-ATCGTTCCCCAGCTCTTTTT-3′	5′-ACTTTATTGTCTATAGCTGA-3′
rs4980524	5′-CAACTGACTGGGGAGAGAGG-3′	5′-ATGAGCTTCTCAAGGGGTTG-3′	5′-TGCATTTTCCACATTAAA-3′

Abbreviation: SNP, single nucleotide polymorphism.

[31,32]. Briefly, endometrial cancer RL95-2 cells were trypsinized and resuspended in serum-free RPMI at concentration of 10×10^6 cells/mL. Cell suspensions (200 μL) were mixed with the reporter DNA (5 μg) and the renilla plasmid (20 ng; Promega, Madison, WI, USA) and subsequently transferred to a 2 mm-gap electroporation cuvette, where they were pulsed at 120 V for 70 ms with an ECM2001 instrument (BTX Instrument Division Harvard Apparatus, Inc., Holliston, MA, USA). Cells were re-seeded into a 6-well plate and maintained in DMEM/F12 containing 10% fetal bovine serum for 24 h. Luciferase activity was measured using a Dual luciferase reporter assay system (Promega, Madison, WI, USA) according the manufacturer's instructions.

Primary endometrial stroma cell culture

Primary endometrial stroma cells were isolated and maintained as follows. In brief, tissue chopped into small pieces was digested in serum-free DMDM/F12 medium containing 0.1% collagenase type IV, 0.01% DNAse, 20 μg/mL penicillin/streptomycin, and 1 μL/mL amphotericin B for 30 min at 37 °C in a shaking water bath. After washing three times with PBS, stroma cells were prepared by filtering through a 40-μm cell strainer and cultured in DMEM/F12 containing 10% fetal bovine serum as well as penicillin and streptomycin at appropriate levels.

Western blot analysis

Cell lysates were prepared with RIPA buffer (150 mM NaCl, 20 mM Tris-Cl pH7.5, 1% Triton X-100, 1% NP40, 0.1% SDS, 0.5% deoxycholate) containing freshly added proteinase and phosphatase inhibitors (Bionovas, Toronto, Canada). Protein concentrations were assayed with the Bradford method. One hundred microgram of each sample were subjected to electrophoresis in 10% SDS-polyacrylamide gels and subsequently transferred onto nitrocellulose membranes. All antibodies were obtained from commercial sources, as follows: MMP-9 (Cell Signaling Technology, Danvers, MA, USA), calmodulin binding peptide tag (CBP) (Millipore, Billerica, MA, USA), halo tag (Promega), actin and STIP1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and corresponding horseradish peroxidase-conjugated antibodies (Santa Cruz Biotechnology). Enhanced chemiluminescence reagents were from Millipore. The autoradiogram signal intensity was quantified with the UN-SCAN-IT graph digitizing software (Silk Scientific, Orem, UT, USA). The relative intensity of each sample was normalized by the corresponding actin intensity.

Statistical analysis

Continuous variables were compared with the Student's *t*-tests, whereas categorical variables were analyzed with Yates-corrected χ^2 tests. Goodness-of-fit χ^2 test values were examined for significant deviation from Hardy–Weinberg equilibrium for each SNP under investigation. Genotypes were determined by direct counting followed by allele frequency calculations. Results of logistic regression analyses are presented as odds ratios (ORs) with their 95% confidence intervals (CIs). All calculations were performed with Statistical Analysis System (SAS) software (version 8.1 for Windows; SAS Institute Inc., Cary, NC, USA).

Table 2
Genotype and allele frequencies of STIP1 SNPs in patients with endometriosis and control women.

SNP	Position	Genotype	Number of patients	Number of controls	OR (95% CI)	p Value
rs7941773	Promoter	CC	234	248		
		CT	50	40		
		TT	2	0		
		C allele frequency	0.91	0.93	Reference	
		T allele frequency	0.09	0.07	1.39 (0.89–2.22)	0.13
rs2845597	Intron 1	CC	25	20		
		CG	131	114		
		GG	128	152		
		G allele frequency	0.68	0.27	Reference	
		C allele frequency	0.32	0.73	0.78 (0.60–1.02)	0.07
rs4980524	Intron 1	GG	37	26		
		GT	137	124		
		TT	112	137		
		T allele frequency	0.63	0.69	Reference	
		G allele frequency	**0.37**	**0.31**	**1.31 (1.03–1.72)**	**0.03**
rs2282490	Intron 4	CC	12	12		
		CT	94	83		
		TT	178	187		
		T allele frequency	0.79	0.81	Reference	
		C allele frequency	0.21	0.19	1.12 (0.83–1.54)	0.46
s2236647	Intron 5	CC	101	121		
		CT	138	127		
		TT	44	39		
		C allele frequency	0.60	0.64	Reference	
		T allele frequency	0.40	0.36	1.19 (0.93–1.54)	0.14

Bold values signifies Chi-square test.
Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

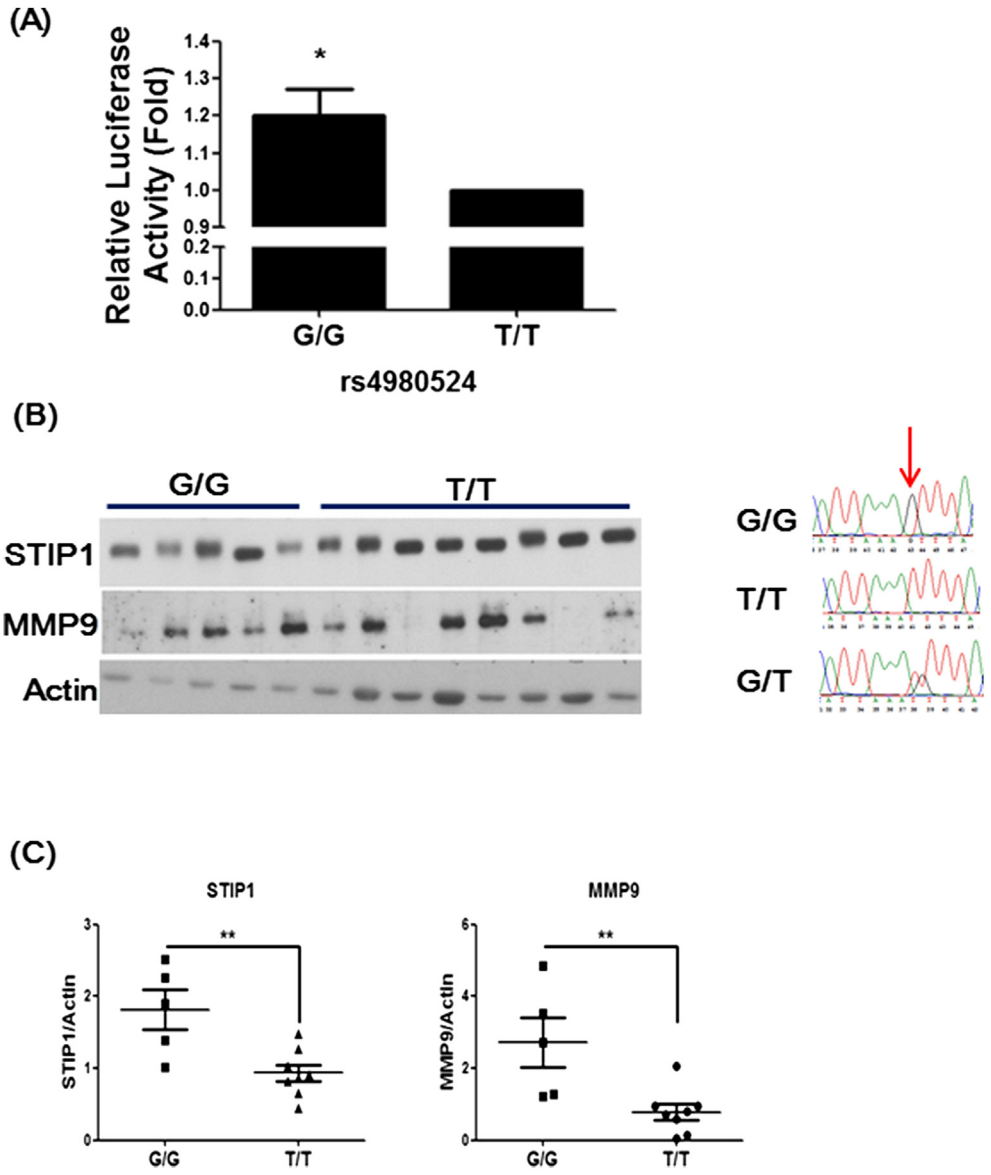

Fig. 1. STIP1 overexpression in endometriosis and functional significance of the rs4980524 STIP1 SNP. (A) RL 95-2 cells were transfected with reporter constructs of rs4980524 GG and TT genotypes. The reporter carrying the GG genotype showed a significantly higher luciferase activity compared with the TT genotype construct. (B) STIP1 and MMP-9 protein levels (left panel) were quantified by western blot in primary endometrial stroma cells carrying the GG or TT genotypes. rs4980524 GG and TT genotypes of the primary stroma cells were identified with sequence (right panel) (C) STIP1 and MMP-9 protein levels in panel (B) were normalized to actin expression. Signal intensities of STIP1, MMP-9, and actin were calculated with the UN-SCAN-IT graph digitizing software (Silk Scientific, Orem, UT, USA). Results in panels (A) and (C) are shown as means ± standard errors from three independent experiments. Statistical differences were calculated with the Student's *t*-test. *$p < 0.05$ and **$p < 0.01$.

Two-tailed p values < 0.05 were considered statistically significant.

Results

The STIP1 SNP rs4980524 is associated with endometriosis

Five STIP1 SNPs (rs7941773, rs2845597, rs4980524, rs2282490, and rs2236647) that are associated with immune regulation [27] were genotyped with MALDI-TOF mass spectrometry in 286 patients with endometriosis and 288 healthy postmenopausal control women. The most common allele for each SNP in the control group was taken as reference (OR = 1). Distributions of genotypes in cases and controls did not deviate from those predicted by the Hardy–Weinberg equilibrium for all of the investigated SNPs. Of the five SNPs analyzed, the frequency of the G allele at rs4980524 was significantly higher in patients with endometriosis than in control women (OR for endometriosis in carriers of the G allele = 1.31; 95% CI = 1.03–1.72, p = 0.03; Table 2).

Functional significance of the rs4980524 STIP1 SNP

Owing to its location in the first STIP1 intron, we reasoned that the rs4980524 STIP1 SNP could affect transcriptional levels. To test this hypothesis, we constructed a reporter assay in which the rs4980524 STIP1 SNP was inserted in the enhancer region of a pGL4.26 vector (containing a basal TATA-box promoter before the luciferase gene). Compared to that with TT genotype, the vector with rs4980524 GG genotype resulted in a 21% increase in relative luciferase activity when transfected in endometrial cancer RL95-2

cells (Fig. 1A). When primary endometrial stromal cells were dichotomized into those carrying the GG *versus* the TT genotype, STIP1 and MMP9 expression as assessed by western blot was higher in the former (Fig. 1B). These results were confirmed after normalization for endogenous actin expression (Fig. 1C). These results indicate that (i) the rs4980524 STIP1 SNP has a functional significance and (ii) the G allele of the rs4980524 STIP1 SNP promotes STIP1 expression. These results were also in agreement with our recent report on STIP1 and MMP9 [19].

Discussion

Results of this study indicate that STIP1 SNP (rs4980524) is associated with the risk of endometriosis. We recently reported that STIP1 regulates the expression of MMP9 in endometrial cancer cell and endometriosis tissue [19]. Results of this study suggest that the G/G allele of STIP1 SNP (rs4980524) promotes endometrial STIP1 expression, which in turn regulates cell migration via increased MMP-9 levels. If this association can be validated in additional cohorts of endometriosis patients, the G/G allele of STIP1 SNP (rs4980524) may be used as a risk genetic marker for endometriosis.

A genome-wide association study (GWAS) usually tests thousands of diseased cases versus controls to identify disease-associated SNPs with a DNA microarray platform. The candidate SNPs from GWAS analysis are selected at p value lower than 6×10^{-8} (multiple hypothesis testing, Bonferroni correction) [33]. In our case, if we extend our data to one thousands cases versus five thousands controls at odds ratio 1.31, the p value will be estimated at 2×10^{-4}, and rs4980524 would have been ignored in GWAS studies. Therefore, we believe that some disease-associated SNPs that would have not been identified by GWAS may only be identified by a knowledge-based approach and confirmed with a MALDI-TOF or QPCR platform.

Intronic SNPs can regulate mRNA expression through several mechanisms, including control transcription, RNA splicing and maturation [34]. In this study, rs4980524 is located in first intron of STIP1 and considered as an enhancer to regulate STIP1 expression in transcription and protein levels. Most of this single nucleotide change on enhancer can only induce 1.2–2 fold difference in target gene expression [34,35], which is the range of our results of luciferase reporter assay. Our results also support that the G/G allele of rs4980524 is associated with increased STIP1 expression and consequently increased MMP9 levels in endometrial stromal cells.

In summary, our data suggest that the G/G allele of STIP1 SNP rs4980524 is associated with the increased expression of STIP1 and MMP9 in endometriosis. Further validation in independent cohorts of endometriosis patients may prove its usefulness as a genetic risk maker for endometriosis.

Author contributions

C.L.T., Y.S.L and T.H.W. designed the study; C.L.T. and Y.S.L. performed and interpreted the experiments; A.C., C.F.Y., and H.S.W. recruited participants, collected clinical data, and were involved in patient care; C.L.T., Y.S.L., and T.H.W. wrote the manuscript. All authors read and approved the final manuscript.

Competing financial interests

The authors declare no competing financial interests.

Acknowledgements

This research was financially supported by grants from the Chang Gung Medical Research Foundation (CMRPG3F1621/2 to CLT and CMRPG3H0511 to THW) and the Ministry of Science and Technology (MOST106-2314-B-182A-087-MY3 to CLT and MOST103-2314-B-182-057-MY3 to THW).

References

[1] Bulun SE. Endometriosis. N Engl J Med 2009;360(3):268–79. https://doi.org/10.1056/NEJMra0804690. Epub 2009/01/16. 360/3/268 [pii]. PubMed PMID: 19144942.
[2] Wang HS, Cheng BH, Wu HM, Yen CF, Liu CT, Chao A, et al. A mutant single nucleotide polymorphism of follicle-stimulating hormone receptor is associated with a lower risk of endometriosis. Fertil Steril 2010. https://doi.org/10.1016/j.fertnstert.2010.07.1092. Epub 2010/09/08. S0015-0282(10)02219-3 [pii]. PubMed PMID: 20817169.
[3] Sampson JA. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927:14.
[4] Ridley JH. Coelomic metaplasia. Am J Obstet Gynecol 1981;140(2):233–4. https://doi.org/10.1016/002-9378(81)90113-7. Epub 1981/05/15. [pii]. PubMed PMID: 7234920.
[5] Meigs JV. Endometriosis. Ann Surg 1948;127(5):795–809. Epub 1948/05/01. PubMed PMID: 18865342.
[6] Wang TH, Wang HS. A genome-wide association study primer for clinicians. Taiwan J Obstet Gynecol 2009;48(2):89–95. https://doi.org/10.1016/S1028-4559(09)60265-5. Epub 2009/07/04. S1028-4559(09)60265-5 [pii]. PubMed PMID: 19574166.
[7] Wheeler DL, Barrett T, Benson DA, Bryant SH, Canese K, Chetvernin V, et al. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 2008;36(Database issue):D13–21. https://doi.org/10.1093/nar/gkm1000. Epub 2007/11/30. gkm1000 [pii]. PubMed PMID: 18045790; PubMed Central PMCID: PMC2238880.
[8] Huber A, Keck CC, Hefler LA, Schneeberger C, Huber JC, Bentz EK, et al. Ten estrogen-related polymorphisms and endometriosis: a study of multiple gene-gene interactions. Obstet Gynecol 2005;106(5 Pt 1):1025–31. https://doi.org/10.1097/01.AOG.0000185259.01648.41. Epub 2005/11/02. 106/5/1025 [pii]. PubMed PMID: 16260521.
[9] Tempfer CB, Simoni M, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part II–endometriosis. Hum Reprod Update 2009;15(1):97–118. https://doi.org/10.1093/humupd/dmn040. Epub 2008/09/23. dmn040 [pii]. PubMed PMID: 18805939; PubMed Central PMCID: PMC2639061.
[10] Simoni M, Tempfer CB, Destenaves B, Fauser BC. Functional genetic polymorphisms and female reproductive disorders: part I: polycystic ovary syndrome and ovarian response. Hum Reprod Update 2008;14(5):459–84. https://doi.org/10.1093/humupd/dmn024. Epub 2008/07/08. dmn024 [pii]. PubMed PMID: 18603647; PubMed Central PMCID: PMC2515090.
[11] Odunuga OO, Longshaw VM, Blatch GL. Hop: more than an Hsp70/Hsp90 adaptor protein. Bioessays 2004;26(10):1058–68. PubMed PMID: 15382137.
[12] Carta F, Demuro PP, Zanini C, Santona A, Castiglia D, D'Atri S, et al. Analysis of candidate genes through a proteomics-based approach in primary cell lines from malignant melanomas and their metastases. Melanoma Res 2005;15(4):235–44. PubMed PMID: 16034300.
[13] Sun W, Xing B, Sun Y, Du X, Lu M, Hao C, et al. Proteome analysis of hepatocellular carcinoma by two-dimensional difference gel electrophoresis: novel protein markers in hepatocellular carcinoma tissues. Mol Cell Proteom 2007;6(10):1798–808. PubMed PMID: 17627933.
[14] Erlich RB, Kahn SA, Lima FR, Muras AG, Martins RA, Linden R, et al. STI1 promotes glioma proliferation through MAPK and PI3K pathways. Glia 2007;55(16):1690–8. PubMed PMID: 17886292.
[15] Wang TH, Chao A, Tsai CL, Chang CL, Chen SH, Lee YS, et al. Stress-induced phosphoprotein 1 as a secreted biomarker for human ovarian cancer promotes cancer cell proliferation. Mol Cell Proteom 2010;9(9):1873–84. https://doi.org/10.1074/mcp.M110.000802. Epub 2010/05/27. M110.000802 [pii]. PubMed PMID: 20501939; PubMed Central PMCID: PMC2938116.
[16] Walsh N, O'Donovan N, Kennedy S, Henry M, Meleady P, Clynes M, et al. Identification of pancreatic cancer invasion-related proteins by proteomic analysis. Proteome Sci 2009;7:3. https://doi.org/10.1186/1477-5956-7-3. Epub 2009/02/17. 1477-5956-7-3 [pii]. PubMed PMID: 19216797.
[17] Beraldo FH, Soares IN, Goncalves DF, Fan J, Thomas AA, Santos TG, et al. Stress-inducible phosphoprotein 1 has unique cochaperone activity during development and regulates cellular response to ischemia via the prion protein. FASEB J 2013;27(9):3594–607. https://doi.org/10.1096/fj.13-232280. PubMed PMID: 23729591.
[18] Walsh N, Larkin A, Swan N, Conlon K, Dowling P, McDermott R, et al. RNAi knockdown of Hop (Hsp70/Hsp90 organising protein) decreases invasion via MMP-2 down regulation. Cancer Lett 2011;306(2):180–9. https://doi.org/10.1016/j.canlet.2011.03.004. Epub 2011/04/08. S0304-3835(11)00153-4 [pii]. PubMed PMID: 21470770.
[19] Wang HS, Tsai CL, Chang PY, Chao A, Wu RC, Chen SH, et al. Positive associations between upregulated levels of stress-induced phosphoprotein 1 and matrix metalloproteinase-9 in endometriosis/adenomyosis. PLoS One 2018;13(1):e0190573. https://doi.org/10.1371/journal.pone.0190573. PubMed PMID: 29304094.

[20] Willmer T, Contu L, Blatch GL, Edkins AL. Knockdown of Hop downregulates RhoC expression, and decreases pseudopodia formation and migration in cancer cell lines. Cancer Lett 2013;328(2):252–60. https://doi.org/10.1016/j.canlet.2012.09.021. PubMed PMID: 23036489.

[21] Carrigan PE, Riggs DL, Chinkers M, Smith DF. Functional comparison of human and Drosophila Hop reveals novel role in steroid receptor maturation. J Biol Chem 2005;280(10):8906–11. https://doi.org/10.1074/jbc.M414245200. Epub 2005/01/06. M414245200 [pii]. PubMed PMID: 15632128.

[22] Scheufler C, Brinker A, Bourenkov G, Pegoraro S, Moroder L, Bartunik H, et al. Structure of TPR domain-peptide complexes: critical elements in the assembly of the Hsp70-Hsp90 multichaperone machine. Cell 2000;101(2):199–210. https://doi.org/10.1016/S0092-8674(00)80830-2. PubMed PMID: 10786835.

[23] Schmid AB, Lagleder S, Grawert MA, Rohl A, Hagn F, Wandinger SK, et al. The architecture of functional modules in the Hsp90 co-chaperone Sti1/Hop. EMBO J 2012;31(6):1506–17. https://doi.org/10.1038/emboj.2011.472. PubMed PMID: 22227520; PubMed Central PMCID: PMC3321170.

[24] Tsai CL, Chao A, Jung SM, Tsai CN, Lin CY, Chen SH, et al. Stress-induced phosphoprotein-1 maintains the stability of JAK2 in cancer cells. Oncotarget 2016;7(31):50548–63. https://doi.org/10.18632/oncotarget.10500. PubMed PMID: 27409672; PubMed Central PMCID: PMCPMC5226602.

[25] Horibe T, Kohno M, Haramoto M, Ohara K, Kawakami K. Designed hybrid TPR peptide targeting Hsp90 as a novel anticancer agent. J Transl Med 2011;9:8. https://doi.org/10.1186/1479-5876-9-8. PubMed PMID: 21235734; PubMed Central PMCID: PMC3032679.

[26] Tsai CL, Tsai CN, Lin CY, Chen HW, Lee YS, Chao A, et al. Secreted stress-induced phosphoprotein 1 activates the ALK2-SMAD signaling pathways and promotes cell proliferation of ovarian cancer cells. Cell Rep 2012;2(2):283–93. https://doi.org/10.1016/j.celrep.2012.07.002. PubMed PMID: 22884369.

[27] Hawkins GA, Lazarus R, Smith RS, Tantisira KG, Meyers DA, Peters SP, et al. The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. J Allergy Clin Immunol 2009;123(6):1376–1383 e7. https://doi.org/10.1016/j.jaci.2009.01.049. Epub 2009/03/04. S0091-6749(09)00170-5 [pii]. PubMed PMID: 19254810.

[28] Wang HS, Cheng BH, Wu HM, Yen CF, Liu CT, Chao A, et al. A mutant single nucleotide polymorphism of follicle-stimulating hormone receptor is associated with a lower risk of endometriosis. Fertil Steril 2011;95(1):455–7. https://doi.org/10.1016/j.fertnstert.2010.07.1092. PubMed PMID: 20817169.

[29] Chen JY, Wang CM, Ma CC, Luo SF, Edberg JC, Kimberly RP, et al. Association of a transmembrane polymorphism of Fcgamma receptor IIb (FCGR2B) with systemic lupus erythematosus in Taiwanese patients. Arthritis Rheum 2006;54(12):3908–17. https://doi.org/10.1002/art.22220. Epub 2006/11/30. PubMed PMID: 17133600.

[30] Chang KP, Hao SP, Tsang NM, Chang YL, Cheng MH, Liu CT, et al. Gene expression and promoter polymorphisms of DNA methyltransferase 3B in nasopharyngeal carcinomas in Taiwanese people: a case-control study. Oncol Rep 2008;19(1):217–22. Epub 2007/12/22. PubMed PMID: 18097598.

[31] Chao A, Wang TH, Lee YS, Hsueh S, Chao AS, Chang TC, et al. Molecular characterization of adenocarcinoma and squamous carcinoma of the uterine cervix using microarray analysis of gene expression. Int J Cancer 2006;119(1):91–8.

[32] Chao A, Tsai CL, Wei PC, Hsueh S, Chao AS, Wang CJ, et al. Decreased expression of microRNA-199b increases protein levels of SET (protein phosphatase 2A inhibitor) in human choriocarcinoma. Cancer Lett 2010;291:99–107.

[33] Chung WH, Chang WC, Lee YS, Wu YY, Yang CH, Ho HC, et al. Genetic variants associated with phenytoin-related severe cutaneous adverse reactions. JAMA 2014;312(5):525–34. https://doi.org/10.1001/jama.2014.7859. PubMed PMID: 25096692.

[34] Corradin O, Scacheri PC. Enhancer variants: evaluating functions in common disease. Genome Med 2014;6(10):85. https://doi.org/10.1186/s13073-014-0085-3. PubMed PMID: 25473424; PubMed Central PMCID: PMCPMC4254432.

[35] Patwardhan RP, Hiatt JB, Witten DM, Kim MJ, Smith RP, May D, et al. Massively parallel functional dissection of mammalian enhancers in vivo. Nat Biotechnol 2012;30(3):265–70. https://doi.org/10.1038/nbt.2136. PubMed PMID: 22371081; PubMed Central PMCID: PMCPMC3402344.