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Running title: p53 codon 72 polymorphism in endometriosis

~~**Prognostic significance of proline form of p53 codon 72 polymorphism in endometriosis**~~

Proline form of p53 codon 72 polymorphism: associated with endometriosis

~~Chi-Chen Chang, MD, Yao-Yuan Hsieh, MD, Fuu-Jen Tsai, MD, PhD*, Chang-Hai Tsai, MD, PhD*, Horng-Der Tsai, MD, Cheng-Chieh Lin, MD†~~

Chi-Chen Chang, M.D.[a, d], Yao-Yuan Hsieh, M.D.[a], Fuu-Jen Tsai, M.D., Ph.D.[b], Chang-Hai Tsai, M.D., Ph.D.[b] , Horng-Der Tsai, M.D.[a], Cheng-Chieh Lin, M.D.[c]

[a] Department of Obstetrics and Gynecology, [b] Department of Pediatrics and Medical Genetics, [c] Department of Family Medicine, China Medical College Hospital, Taichung, Taiwan

[d] Department of Obstetrics and Gynecology, Lin Shin Hospital, Taichung, Taiwan

~~Department of Obstetrics and Gynecology, * Department of Pediatrics and Medical Genetics, † Department of Family Medicine, China Medical College Hospital, Taichung, Taiwan~~

Reprints and correspondence torequests: Fuu-Jen Tsai, MD, PhD.

Department of Pediatrics and Medical Genetics, China Medical College Hospital,

No.2 Yuh-Der Road, Taichung, Taiwan

Telephone: 886-4-22052121 Ext 1566

Fax: 886-4-22033295, E-mail: d0704@hpd.cmch.org.tw

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Yao-Yuan Hsieh

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Condensation: The p53 Arg homozygotes are related associated with a –lower risk of endometriosis development. The heterozygotes or Pro homozygotes are associatedrelated with a higher risk of endometriosis formationendometriosis development.

Objective: The mutation of p53 is related with the cell progression and malignancy. We aimed to evaluate the association between the endometriosis and the p53 polymorphism.

Design: Prospective study.

Setting: Department of gynecology and genetics in a medical center.

Patients: Women with or without ~~surgically diagnosed~~ endometriosis were included.

Interventions: Women were divided into two groups: (1) moderate/severe endometriosis (n=118); (2) non-endometriosis groups (n=140).

Main outcome measurements: Their p53 codon 72 polymorphisms [arginine (Arg) homozygotes, heterozygotes, proline (Pro) homozygotes] were detected by polymerase chain reaction. Associations between the endometriosis and p53 polymorphism were evaluated.

Results: The distributions of different p53 polymorphisms in both groups were significantly different. The proportions of Arg homozygotes/heterozygotes/Pro homozygotes in endometriosis and non-endometriois populations were 10.2/66.9/22.9% and 30.7/50/19.3%, respectively. We noted the dominant presentations of heterozygotes and Pro homozygotes in the endometriosis population than those in non-endometriosis population.

Conclusion: The association between endometriosis and p53 polymorphism exists. The p53 Arg homozygotes are related with lower risk of endometriosis development. The heterozygotes or Pro homozygotes are related with higher risk of ~~endometriosis formation~~endometriosis development.

Key words: Arginine form, endometriosis, polymorphism, proline form, p53

Introduction

Endometriosis, a common polygenic/multifactorial disease, might be caused by an

interaction between multiple genes as well as the environment [1]. Endometriosis displays features similar to malignancy, including local invasion and aggressive spread to distant organs. Tumor suppressor genes play a role in the regulation of cell growth and prevention of carcinogenesis. Genomic instability of p53 plays a role in the development and progression of various tumor types. The altered tumor suppressor genes might be related with the development of endometriosis [2].

Numerous cancers are related the abnormal p53 presentation, including the cervical carcinoma [3,4], ovarian carcnioma [5], bladder cancer [6], prostate cancer [7], hepatoma [8], gastric cancer [9], lung cancer [10], brain tumor [11], oral carcinoma [12], nasopharyngeal carcinoma [13], esophageal carcinoma [14], breast cancer [15], lymphoma [16], etc. Scanty literature presented the association between the endometriosis and p53 polymorphism. Some investigators have demonstrated the undetectable expression of p53 in the endometriosis specimens [17-19]. No literature revealed the association between the endometriosis and the genotype of p53 codon 72 polymorphism.

There is discrepancy about this presentation of p53 polymorphism and various tumors. The p53 Arg homozygote is considered to be a risk factor in the development of cancer [20]. In contrast, some investigators demonstrated the non-association between the different p53 polymorphism and cancer development [3,21]; other

studies revealed the higher risks in the Pro homozygotes [(10,22]). To resolve this issue, we firstly aimed to detect the p53 codon 72 polymorphism in Chinese women with or without endometriosis. To our knowledge, this report is the largest survey in this aspect.

Material and methods

Pre-menopausal Taiwan Chinese women with surgically diagnosed moderate/severe endometriosis and non-endometriosis were included. All ~~patients~~ individuals were divided into two groups: (1) endometriosis (n=118); (2) non-endometriosis groups (n=140). The non-endometriosis statuses were confirmed during the cesarean section or diagnostic laparoscopy. All operations were performed by three surgeons (Hsieh YY, Chang CC, Tsai HD).~~All women accepted the peripheral blood sampling for genotype analyses.~~ The stud~~ies wer~~y wase approved by the ethical committee and institutional review board of the China Medical College Hospital. Informed consents were signed by all women who donated their blood.

All women accepted the peripheral blood sampling for genotype analyses after the surgery. Genomic DNA was prepared from the peripheral blood by use of a DNA Extractor WB kit (Wako, Japan). Polymerase chain reactions (PCRs) were carried out in a total volume of 25 μl, containing genomic DNA; 2-6 *p*mole of each primer; 1X Taq polymerase buffer (1.5 mM MgCl); and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer). The primer Pro72 was designed for p53 codon 72 in proline (Pro) form and Arg72 for arginine (Arg) form, according to the procedure described by Storey et al ~~[~~(20~~]~~).

PCR amplification was performed in a programmable thermal cycler GeneAmp

PCR System 2400 (Perkin Elmer). Cycling condition for Pro72 was set as following: one cycle at 94˚C for 5 min, 35 cycles of 94˚ C for 15 sec, 52˚C for 20 sec, and 72˚C for 30 sec, and one final cycle of extension at 72˚C for 7 min; conditions for Arg72 were the same as Pro72 except 50˚C for annealing. The PCR products from Arg72 and Pro72 from the same individual were mixed together and 10 μl of this solution was loaded into 3% Agarose gel containing ethidium bromide for electrophoresis.

The distributions of p53 polymorphisms in both groups were examined. Correlation between the p53 genotype and endometriosis was evaluated. The SAS system with χ^2 and logistic regression were utilized for statistical analyses. A p-value of <0.05 was considered statistically significant.

Results

The PCR products of Arg and Pro forms were 141 and 177 bp, respectively. The proportions of different p53 polymorphisms in both groups were significantly different (χ^2 value=16.1, p<0.001). The proportions of Arg homozygote/heterozygote/Pro homozygote in endometriosis and non-endometriois populations were 10.2/66.9/22.9% and 30.7/50/19.3%, respectively (Table 1). There were non-significant differences between both groups in age, weight, and height.

We observed the dominant Arg presentation in the non-endometriosis population. There were increasing number of heterozygote and Pro homozygotes in the endometriosis group compared with the non-endometriosis group. The odds of

individuals with Arg/Pro or Pro/Pro gene experiencing endometriosis are 3.9 times greater than the odds of individuals with Arg/Arg. The odds of individuals with heterozygotes and Pro homozygotes experiencing endometriosis are 4.0 and 3.6 times greater than the odds of individuals with Arg homozygotes (Table 1).

Discussion

The p53 gene and its encoded protein are related with the regulation of cell cycle, cellular growth, and apoptosis. It is a gatekeeper or guardian of the cell division [(23,24)]. The p53 mutations are associated with instability of cell development and cycle progression [(25)]. Alterations of p53 are related to the induction of apoptosis in malignant tumors. Individuals lacking functional p53 are at an increased risk of tumor development and a mutated p53 gene or malfunctioned p53 protein has often observed in patients with most types of malignancies [(25)].

Kosugi et al. [(26)] demonstrated the increased heterogeneity and aneuploidy of chromosome 17 in endometriosis specimen. Because p53 is located in chromosome

17, the chromosome 17 aneuploidy might impaired the function of p53, which influences the further progression of endometriosis. These residue 72~~structural features~~ of p53 ~~(residues 61–94) have been well preserved throughout evolution except residue 72, which~~ has been recognized as a polymorphism that Arg residue substituted the Pro form ~~[~~(27~~]~~). A single base change (from CGC to CCC) caused the alteration of amino acid residue 72 from Arg to Pro ~~[~~(27~~]~~).

There is discrepancy about the distribution of p53 polymorphism in different malignancy. The p53 Arg homozygote is a significant risk factor in the development of invasive form of human papilloma virus-associated cancers ~~[~~(4,20,28~~]~~). In contrast, some investigators demonstrated the non-association between the cervical cancer and different p53 polymorphisms ~~[~~(3,21~~]~~). Some reports also revealed that Pro allele homozygote is a risk factor of lung and hepatocellular carcinoma ~~[~~(10,22~~]~~). In the study of lung carcinoma, Wang et al. ~~[~~(10~~]~~) found those patients with p53 Arg/Arg or Pro/Pro homozygous had worse prognoses compared with those with the heterozygous form. This discrepancy may be due to the different cell nature and racial variation.

In this series, we observed that p53 Arg homozygotes is related with lower risk of endometriosis development. The Pro forms of codon 72 in p53 (Pro homozygotes or heterozygotes) are related with the higher risk of ~~endometriosis~~

~~formation~~endometriosis development. Our finding was compatible with Wang et al. ~~[~~(10~~]~~) and Yu et al. ~~[~~(22~~]~~), who demonstrated the association between the Pro homozygotes and lung or hepatocellular carcinoma. Our another study also appeared the high association between Pro homozygotes and invasive bladder cancer ~~[~~(29~~]~~). We noted that more than 70% of the non-invasive bladder cancers were Arg homozygotes. Combined these above studies, it suggested the dominant p53 Pro forms is a risk factor for the development of cancer in Chinese population.

In conclusion, the association between endometriosis and p53 polymorphism exists. The p53 Arg homozygotes are related with lower risk of endometriosis development. The Pro homozygotes or heterozygotes are related with higher risk of ~~endometriosis formation~~endometriosis development. The p53 polymorphisms may become a useful marker to predict the endometriosis development. Although the real role of p53 polymorphism has not been clarified, it deserves more attention in the study of endometriosis and the development of gene therapy.

References

1. Bischoff FZ, Simpson JL. Heritability and molecular genetic studies of endometriosis. Hum Reprod Update 2000;6:37-44.

2. Jiang X, Hitchcock A, Bryan EJ, Watson RH, Englefield P, Thomas EJ, Campbell IG. Microsatellite analysis of endometriosis reveals loss of heterozygosity at candidate ovarian tumor suppressor gene loci. Cancer Res 1996;56:3534-9.

3. Rosenthal AN, Ryan A, Al-Jehani RM, Storey A, Harwood CA, Jacobs IJ. P53

codon 72 polymorphism and risk of cervical cancer in UK. Lancet 1998;352:871-2

4. Zehbe I, Voglino G, Wilander E, Genta F, Tommasion M. Codon 72 polymorphism of p53 and its association with cervical cancer. Lancet 1999;354:218-9.
5. Kupryjanczyk J, Bell DA, Yandell DW, Scully RE, Thor AD. p53 expression in ovarian borderline tumors and stage I carcinomas. Am J Clin Pathol 1994;102:671-6.
6. Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC, Nichols PW, Skinner DG, Jones PA, Cote RJ. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Eng J Med 1994;331:1259-64.
7. Steiner MS, Zhang X, Wang Y, Lu Y. Growth inhibition of prostate cancer by an adenovirus expressing a novel tumor suppressor gene, pHyde. Cancer Res 2000;60:4419-25.
8. Wang NM, Tsai CH, Yeh KT, Chen SJ, Chang JG. P53 codon 72 arg polymorphism is not a risk factor for carcinogenesis in the Chinese. Int J Mol Med 1999;4:249-52.
9. Takeda A, Shimada H, Nakajima K, Suzuki T, Hori S, Hayashi H, Arima M, Kouzu T, Ochiai T, Isono K. Impact of circulating p53 autoantibody monitoring

after endoscopic resection in mucosal gastric cancer. Endoscopy 2000;32:740-1.

10. Wang YC, Lee HS, Chen SK, Chang YY, Chen CY. Prognostic significance of p53 codon 72 polymorphism in lung carcinomas. Eur J Cancer 1999;35:226-30
11. Nutt CL, Noble M, Chambers AF, Cairncross JG. Differential expression of drug resistance genes and chemosensitivity in glial cell lineages correlate with differential response of oligodendrogliomas and astrocytomas to chemotherapy. Cancer Res 2000;60:4812-8.
12. Chang KW, Lin SC, Mangold KA, Jean MS, Yuan TC, Lin SN, Chang CS. Alterations of adenomatous polyposis Coli (APC) gene in oral squamous cell carcinoma. Int J Oral Maxillofac Surg 2000;29:223-6.
13. Crook T, Nicholls JM, Brooks L, O'Nions J, Allday MJ. High level expression of deltaN-p63: a mechanism for the inactivation of p53 in undifferentiated nasopharyngeal carcinoma (NPC)? Oncogene 2000;19:3439-44.
14. Miyazaki T, Kato H, Shitara Y, Yoshikawa M, Tajima K, Masuda N, Shouji H, Tsukada K, Nakajima T, Kuwano H. Mutation and expression of the metastasis suppressor gene KAI1 in esophageal squamous cell carcinoma. Cancer 2000;89:955-62.
15. Pich A, Margaria E, Chiusa L. Oncogenes and male breast carcinoma: c-erbB-2 and p53 coexpression predicts a poor survival. J Clin Oncol 2000;18:2948-56.

16. Boley SE, Anderson EE, French JE, Donehower LA, Walker DB, Recio L. Loss of p53 in benzene-induced thymic lymphomas in p53+/- mice: evidence of chromosomal recombination. Cancer Res 2000;60:2831-5.
17. Vercellini P, Trecca D, Oldani S, Fracchiolla NS, Neri A, Crosignani PG. Analysis of p53 and ras gene mutations in endometriosis. Gynecol Obstet Invest 1994;38:70-1.
18. Schneider J, Jimenez E, Rodriguez F, del Tanago JG. c-myc, c-erb-B2, nm23 and p53 expression in human endometriosis. Oncol Rep 1998;5:49-52.
19. Horiuchi A, Osada R, Nakayama K, Toki T, Nikaido T, Fujii S. Ovarian yolk sac tumor with endometrioid carcinoma arising from endometriosis in a postmenopausal woman, with special reference to expression of alpha-fetoprotein, sex steroid receptors, and p53. Gynecol Oncol 1998;70:295-9.
20. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. Role of a p53 polymorphism in the development of human papilloma virus-associated cancer. Nature 1998;393: 229-34.
21. Helland A, Langerod A, Johnsen H, Olsen AO, Skovlund E, Borresen-Dale AL. p53 polymorphism and risk of cervical cancer. Nature 1998;396:530-1.
22. Yu MW, Yang SY, Chiu YH, Chiang YC, Liaw YF, Chen CJ. A p53 genetic

polymorphism as a modulator of hepatocellular carcinoma in relation to chronic liver disease, familial tendency, and cigarette smoking in hepatitis B carriers. Hepatology 1999;29:697-702.

23. Lane DP. Cancer. p53, guardian of the genome. Nature 1992;358:15-6.
24. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323-31.
25. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. N Eng J Med 1993;329:1318-27.
26. Kosugi Y, Elias S, Malinak LR, Nagata J, Isaka K, Takayama M, Simpson JL, Bischoff FZ. Increased heterogeneity of chromosome 17 aneuploidy in endometriosis. Am J Obstet Gynecol 1999;180:792-7.
27. Ara S, Lee PSY, Hansen MF, Saya H. Codon 72 polymorphism of the TP53 gene. Nucleic Acids Res 1990;18:4961.
28. Walker KK, Levine AJ. Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc Natl Sci USA 1996;93:15335 -40.
29. Chen WC, Tsai FJ, Wu JY, Wu HC, Lu HF, Li CW. Distributions of p53 codon 72 polymorphism in bladder cancer-proline form is prominent in invasive tumor. Urol Res 2000, in press

Table 1. Distributions of p53 codon 72 polymorphisms in populations with and without endometriosis.

	Endometriosis (n=118)	Non-Endometriosis (n=140)	Chi-square Statistics	Odds Ratio (95% Confidence Interval)
Arg/Arg	12 (10.2%)	43 (30.7%)	16.1*	1.0
Arg /Pro or Pro/Pro	106 (89.8%)	97 (69.3%)		3.9 (2.0, 7.6) †
Arg /Pro	79 (66.9%)	70 (50%)		4.0 (2.0, 8.3) *
Pro/Pro	27 (22.9%)	27 (19.3%)		3.6 (1.6, 8.2) †

*: $p<0.001$; †:$p<0.01$

Fuu-Jen Tsai, MD, PhD
Department of Pediatrics and Medical Genetics, China Medical College Hospital,
NO. 2 Yuh Der Road, Taichung, Taiwan
Telephone: 886-4-22052121 Ext 1566, Fax: 886-4-22033295
E-mail: d0704@hpd.cmch.org.tw

Jun 15, 2001

Dear Professor Alan H. DeCherney:
Thank you very much for your reviewing of my manuscript "Androgen receptor trinucleotide polymorphism in endometriosis".

Thank you very much for your consideration of publishing my revised manuscript "**Androgen receptor trinucleotide polymorphism in endometriosis** ". Concerning the comments of all reviewers, my addresses were made as following:

Comments for Editor:

1. The condensation has been revised according the editor’s suggestion.
2. After the consultation with the native speaker of English, we made some revision to clarify syntax.
3. The second paragraphy in page 7 has been divided into two new paragraphs.
4. The parentheses were revised.

The elucidation about referee 1:

1. We acknowledge the reviewer's comment. All operations were performed by three surgeons (Hsieh YY, Chang CC, Tsai HD). The blood samplings were obtained after

the laparoscopy and pathologic examination. These statements have been added in the revised text.

2. Only the cases with the severe endometriosis and endometrial cysts were included. The stage for these cases were stage III/IV. All cases with endometriosis were confirmed by the laparoscopy and pathologic examination.
3. The term “endometriosis development” has been chosed to used in the whole text.
4. Some revision were made according the reviewer’s suggestion.

The elucidation about referee 2:

1. Only the cases with the moderate/severe endometriosis and endometrial cysts were included. The stages for these cases were stage III/IV. All cases with endometriosis were confirmed by the laparoscopy and pathologic examination.
2. The non-endometriosis statuses were confirmed during the cesarean section. Some patients with idiopathic infertility and accepting the diagnostic laparoscopy were included.
3. Only the stage III/IV endometriosis were included. Therefore, it is not feasible to evaluate their association.
4. Most patients accepted the blood sampling during the postoperative outpatients visiting. Because the non-change of the gene polymorphism, we think it will not change the final result of this study.
5. The title has been revised according to the reviewer’s suggestion.

Finally, I appreciate your kindly suggestion and revision of our manuscripts.

Thank you very much.

With best regard

Yao-Yuan Hsieh, M.D.