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Original Article

Correlation research on Estrogen receptor α gene and Xba I Pvu II and PPD

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Abstract: Objective: To explore the correlation of polymorphism of Estrogen Receptor-α (ERα) and postpartum depression (PPD) and provide genetic evidence for the etiology and pathogeny of PPD. And then provide the theory basis to diagnosis and therapy patients in postpartum depression genetically. Methods: Forty-five cases (depressed women postpartum) and forty-five control (non-depressed women postpartum) were selected by the questionnaires. PCR-RFLP was used to examine the polymorphism of Pvu II and Xba I on estrogen receptor α (ERα), adopt the genetic Numeration to record the allele frequency of depressed group and control group. To analyze the correlations between the polymorphism of Pvu II and Xba I and by Hardy-Welnbery and postpartum depression SPSS18.0. Results: The chi-square test revealed that The genotype distribution of ERα and Pvu II in depressed group and control group was significant difference, the difference of allele frequencies (P and p) was significant between depressed group and control group (P<0.05); The chi-square test revealed that the genotype distribution of ERα and Xba I in depressed group and control group was no significant difference, The difference of allele frequencies (X and x) was no significant between depressed group and control group (P>0.05), chi-square test indicated that joint genotype distribution ERα Pvu II and ERα Xba I was no significant between depressed group and control group (P>0.05). Conclusion: There is relativity between polymorphism of ERα Pvu II and postpartum depression (PPD), there isn't relativity between genetic mutation of Xba I and postpartum depression, the analysis of the joint genotype shows that the general genotype distributions of depressed group and control group are similar.

Keywords: Postpartum depression, estrogen receptor-α, gene polymorphisms, Xba I, Pvu II

Introduction

PPD (postpartum depression disorder) is one of the common complications found after childbirth. After childbirth, the mantel body and mind of the puerperant have been changed greatly; the women suffering [1] PPD influence their social function and produce a series of severe problems [2]. PPD has become an important research direction in the field of mental health [3, 4]. As a subtype of general depression and its pathogenesis, PPD is similar to that of common depression. There are a lot of studies on the genetic polymorphism about depression, to show the genetic susceptibility of depressive patients. This study mainly discusses the level of genetic polymorphism in the etiology. An estrogen receptor includes two subtypes: ERα and ERβ, where ERα plays an important role in the pregnancy and in the course of postpartum hormones [5, 6]. There are three common ERα polymorphism genes, and these three mutations occur in the restriction enzymes Pvu II, XBa I, and Bat UI recognition sites, to form a corresponding gene polymorphism. Based on estrogen receptor ERα with the ER RFLP (PCR) technique used for the first time, the research studied the relationship between estrogen receptor α gene Pvu II and Xba 1 and PPD, as well as to analyze the distribution of genotypes, to further explore the association between ERα genetic polymorphism and PPD. This allows us to further understand the causes of postpartum depression, as well as to further study the etiology and pathogenesis of PPD, the gene diagnosis and treatment to provide a theoretical basis.

Data and methods

General information is agreed by the patient and the family members, selected pregnant women between 23-35 years of age in perinatal care between January 2012 and December 2012 in Guiyang Medical College Hospital. There were 45 preliminary select cases in the control group and the experimental group. Criteria of PPD patient groups: (1), 1 week postpartum women of 23-35 years old; (2), with the United States classification and diagnostic criteria of mental disorders fourth edition (DSM-IV) diagnosis standards and assessment scale: Edinburgh postnatal Depression Scale (EPDS) ≥10 points, general hospitals with anxiety/depression scale (HAD) ≥9 points; (3), no previous personal history of mental disorders and psychiatric family history, no other serious physical organic diseases, such as nervous system, endocrine system diseases; no previous history of severe pregnancy complications; (4), excluding non-addictive psychoactive substances and non-addictive substance-induced depression; (5) signed informed consent, can cooperate with the completion of the scale.

Research tools

The edinburgh postnatal depression scale (EPDS): The scale was drawn up by Cox in order to evaluate the current major depressive symptoms (within one week). The scale is a self-rating scale, totally including l0 entries, with each entry divided into 4 grades (0 to 3 points). The whole score is 0~30 points, and the higher the score is, the more severe the degree of depression is. 13 points would indicate severe depression, and 9 or 10 points mild or severe depression. This study takes 10 points as the critical point. In this study, the coefficient of scale is 0.76.

General hospital anxiety/depression scale (HAD): This scale was created by Zigmond AS and Snaith RP, composed with 14 entries, and widely used in assessing and screening anxiety and depression in general hospitals. The mainland version of Weifei YE and others has carried out rigorous tests in general hospitals and found out nine points work out best as the threshold for depression assessment with 100% sensitivity and specificity toward depression. They have also proved that HAD is better at screening depression than anxiety. The coefficient of the scale in this research is 0.85.

Research methods

Use unified guidance language, assess at any time point among the three postpartum follow-up time points (postnatal 3rd day, 42nd day, and 3rd month), and use the measurement tools of the general questionnaire, Edinburgh Postnatal Depression Scale (EPDS). Collect 5 ml of every research subject's fasting venous blood in the early morning on postpartum 3rd day, and then strictly store it in -80°C refrigerator according to domestic reports and literature. Extract DNA with blood DNA extraction kit provided by ComWin Company, and apply the primers designed by DNAstar software: forward primer 5'-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3' and reverse primer 5'-TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3', PCR instrument (provided by American Bio-Rad Company) conditions: initial denaturation, 94°C, 5 min, 1 cycle; denaturation, 94°C, 50 s, annealing 63°C, 60 s, extension 72°C, 90 s, a total of 35 cycles; the last extension, 72°C, 10 min. PCR products were digested with restriction enzymes Pvu II and Xba I respectively, then use l% agarose gel electrophoresis after the digestion is determined. Finally, observe the result and take photography with UV gel imaging analyzer after the completion of electrophoresis. RFLP results are indicated with P or p and x or X, with the presence of restriction sites marked by lowercase letters and deletions by uppercase letters.

Statistical methods

Count genotype and allele frequencies separately between the case group and the control group with the method of gene notation, and make a statistical analysis using SPSS18.0 statistical software after that Hardy-We1nberg balance test. Genotype and allele frequency differences between the two groups are tested with c2, with $P<0.05$ considered statistically significant and $P<0.01$ highly statistically significant.

Results

Estrogen receptor α gene's Pvu II and Xba I restriction enzyme digestion results

ERα gene with fragment of 1300 bp has been obtained through PCR amplification reactions. Three kinds of genotypes could be distinguished through Pvu II restriction enzyme di-

Table 1. The ERα gene Pvu II site distribution of the genotype in the postpartum depression group and control group

Group	Pvu II Genotype n (%)			Pvu II Allele gene n (%)	
	PP	Pp	pp	P	p
Case group (n=44)	16 (36.37)	19 (43.18)	9(20.45)	51 (57.95)	37 (42.05)
Control group (n=43)	11 (25.58)	15 (34.88)	17(39.54)	37 (43.02)	49 (56.98)
χ^2 Value		6.95*		7.08*	

Note: *P<0.05.

Table 2. The ERα Xba I site distribution of the genotype in the postpartum depression group and control group

Group	Xba I Genotype n (%)			Xba I Allele gene n (%)	
	XX	Xx	xx	X	x
Case group (n=44)	10 (23.26)	14 (32.56)	20 (46.51)	34 (38.64)	54 (61.36)
Control group (n=43)	9 (20.93)	11 (25.58)	23 (53.49)	29 (33.72)	57 (66.28)
χ^2 Value		3.89		2.73	

Note: P>0.05.

gestion on ERα gene: PP genotype (1300 bp), Pp genotype (1300, 850, 450 bp), and pp genotype (850, 450 bp). Three kinds of genotypes could be distinguished through Xba I enzyme digestion: XX genotype (1300 bp), Xx genotype (1300, 910, 390 bp), and xx genotype (910, two bands of 390 bp) [7-9].

Estrogen receptor α gene's polymorphism results

PP genotype distribution of case group and control group, through *2 test, genotype frequency: the distribution difference between the two groups has a statistical significance (*12=4.18, P=0.019, P<0.05); Pp genotype, through *2 test: the distribution difference between the two groups has a statistical significance (*12=3.149, P=0.005, P<0.05); pp genotype, through *2 test: the distribution difference between the two groups has a statistical significance (*12=3.778, P=0.043, P<0.05); the whole distribution of these three genotypes of two groups through *2 test genotype frequency in distribution difference between the two groups has a statistical significance (*12=6.95, P<0.05). P allele gene frequency of case group and control group were 57.95% and 43.02%, through *2 test, with the allele gene of two groups in distribution difference between the two groups having a statistical significance (*22=7.08, P<0.05) (See **Table 1**).

XX Genotype distribution of case group and control group, through *2 test, genotype frequency in distribution difference between the two groups has no statistical significance (*12=0.041, P=0.523, P>0.05); similarly, Xx genotype through *2 test in distribution difference between the two groups has a statistical significance (*12=3.462, P=0.036, P<0.05); xx genotype through *2 test in distribution difference between the two groups has no statistical significance (*12=0.562, P=0.297, P>0.05); the whole distribution of three genotypes of two groups through *2 test genotype frequency in distribution difference between the two groups has no statistical significance (*12=3.89, P>0.05). The case group and control group X allele gene frequency were 38.64% and 33.72%, through *2 tests between two groups. The allele gene in distribution difference between the two groups has no statistical significance (*22=2.73, P>0.05). (See **Table 2**).

Analysis on combined genotypes are tested with 2, which showed no significant difference between the case and control groups (2=7.85, P>0.05). (See **Table 3**).

Discussion

The cause of postpartum depression is very complex, because its pathogenesis has not been clear so far, and most scholars confirm the existence of a genetic predisposition for its occurrence [10, 11]. Estrogen and estrogen receptor (ER) are both closely related to postpartum depression. The role that estrogen

Table 3. The ERα gene Pvu II and Xba I polymorphism distribution in postpartum depression group and control group

Group	PPXX	PPXx	PPxx	PpXX	PpXx	Ppxx	ppXX	ppXx	ppxx
Case group (n_1=44)	4	5	7	3	6	10	3	3	3
Gene frequency n_1%	9.09	11.36	15.91	6.82	13.64	22.73	6.82	6.82	6.82
Case group (n_2=43)	5	6	0	3	3	9	1	2	14
Gene frequency n_2%	11.63	13.95	0.00	6.98	6.98	20.93	2.33	4.65	32.56
χ^2 Value					7.85				

Note: *P*>0.05.

plays in the occurrence of depression has been confirmed by multiple experiments [12, 13], and estrogen exerts biological effects mainly through combining with the estrogen receptor (mostly located within the nucleus). Binding the estrogen receptor in the upstream of a target gene together with estrogen will lead to conformational changes within the receptor, which further regulates target gene transcription, translates corresponding functional protein through mRNA and sends accurately to the target cell. This facilitates the appropriate cellular, protein etc. to exercise their corresponding functions [14-16]. Obviously the physiological or pathological effects that estrogen is having on target cells depends not only on the secretion and metabolism of estrogen itself, but also on the regulation and expression of ER, which have been affected by ER gene polymorphisms. ER gene polymorphism may influence the transcription and translation approach of genes and result in the dysfunction of ER expression, which has been confirmed by domestic and foreign scholars. ER includes two subtypes: ERα and ERβ, to which the estrogen has primarily bound and exerted effects [17]. Yuefen TANG, Shenxun SHI and other scholars in our country have clearly demonstrated the fact that estrogen β (ERβ) receptor gene polymorphism is related with the occurrence of PPD [18], while Julia K. Pinsonneault and other foreign scholars' studies have shown: ERα should have a particular form of impact on the formation of 5-serotonin signal and thus plays an important role in the etiology of PPD [5, 6]. The results of this study show that: ERα Pvu II gene polymorphism is correlated with the incidence of postpartum depression. However, this experiment has not dealt with the issue of whether Pvu II gene is involved in the formation of 5-serotonin signal, which is expected to be further studied and confirmed in the future. A variety of genetic polymorphisms are existing in ERα for so many different phenotypes or genotypes in the population, which can be expressed in many forms, such as the characters, chromosome structure, amino acid sequence and DNA sequence (Genes and polymorphism of DNA). While the polymorphism of gene and DNA is basic, it can determine the other forms of polymorphism. Genetic polymorphism of ERα is the restriction fragment length polymorphism, whose mutation happens in first intron, which exists respectively in the recognition site of restriction enzymes Pvu II, Xba I and BstUI. Nowadays, research on genetic polymorphism [19] of ERα shows that there are three types of gene in genetic polymorphism of Pvu II and Xba I, and the gene types of Pvu II restriction sites were: PP, Pp and PP, while gene types of Xba I restriction sites were: XX, Xx and XX. Owing to the fact that because of the important sequence that is in the regulation of gene transcription such as enhancer and promoter in the first intron in ERα, we speculated that the occurrence of point mutation of gene polymorphism may affect the expression and function of the gene, which will finally affect estrogen. To our delight, some subtypes of gene ER have been reported to be related to some diseases such as breast cancer, Alzheimer's disease, systemic lupus erythematosus and so on, and that can be expressed differently in clinical manifestations such as severity, type and incidence [20-22]. Accordingly, we also speculated that there are also relationships between genetic polymorphism of ERα and the incidence of postpartum depression, the severity of the disease and the risk of recurrence, etc. It was found that in the case group, the Ppxx genotype is the highest frequency in whole genotypes, while it was higher in the normal control, and statistical analysis also showed that mutation of ERα and Pvu II may increase the risk of maternal of postpartum depression. There was a correlation between gene polymorphism and postpartum

depression, and the Pp genotype was the most common in the patients as well as it was in the normal control group. The distribution of each genotype had a significant difference, which also showed that it is high in risk for postpartum depression in patients with Pvu II Pp genotype. At the same time, Allele P in the case group and normal control group is significantly different to such a point that it can be speculated that the allele P may be the protective gene of PPD, which can reduce the incidence of PPD. However, we haven't found the correlation between PPD and gene polymorphism of ER and Xba. And from the statistical analysis in allele frequencies, we know there were no significant differences between the case group and normal control group of allele X. All these results are consistent with the findings of the study that the risk of allele P carriers in the patients was higher than that of p allele carriers; two receptor gene expression of estrogen is a variety of factors, but not a single factor. Gene polymorphism of Pvu II and Xba I is important in receptor ER.

ERα is involved in the regulation of various physiological functions of the human body [23, 24]. The different levels of ER gene expression will affect functional differences and further affect the level of estrogen in vivo, and ultimately affect the incidence of certain diseases, progress and prognosis. The study hasn't found the correlation between gene polymorphism Xba I and the existence of PPD, and it is worthwhile to further explore the content of the sample. Furthermore, attention is needed because we choose the blood sample time as 3 days after the birth, which is shorter than the expected onset time. There may then exist a sampling error, thus further study on a large number of samples is needed. Presently, domestic related research reports about PPD and ER Pvu II and Xba I gene polymorphism are still rare. We expect more research and to further expand the sample size to get more reliable conclusions.

Disclosure of conflict of interest

None.

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References

[1] Shrivastava SR, Shrivastava PS, Ramasamy J. Antenatal and postnatal depression: A public health perspective. J Neurosci Rural Pract 2015; 6: 116-9.

[2] O'Hara MW, McCabe JE. Postpartum depression: current status and future directions. Annu Rev Clin Psychol 2013; 9: 379-407.

[3] Liu CH, Tronick E. Rates and predictors of postpartum depression by race and ethnicity: results from the 2004 to 2007 New York City PRAMS survey (Pregnancy Risk Assessment Monitoring System). Matern Child Health J 2013; 17: 1599-610.

[4] Banker JE, Lacoursiere DY. Postpartum depression: risks, protective factors, and the couple's relationship. Issues Ment Health Nurs 2014; 35: 503-8.

[5] Shapiro GD, Fraser WD, Seguin JR. Emerging risk factors for postpartum depression: serotonin transporter genotype and omega-3 fatty acid status. Can J Psychiatry 2012; 57: 704-12.

[6] Pinsonneau JK, Sullivan D, Sadee W, Soares CN, Hampson E, Steiner M. Association study of the estrogen receptor gene ESR1 with postpartum depression - a pilot study. Arch Womens Ment Health 2013; 16: 499-509.

[7] Spitzer JA. Gender different in some host defense mechanisms. Lupus 1999; 8: 380-383.

[8] Bebo BF Jr, Schuster JC, Vandenbark AA, Offner H. Androgens alter the cytokine profile and reduce encephali to genecity of myelin reactive T cells. Immunol 1999; 162: 35-40.

[9] Hogancamp WE, Rodrigues M, Weinshenker BG. The Epidemiology of Multiple Sclerosis. Mayo Clin Proc 1997; 72: 871-878.

[10] Byrne EM, Carrillo-Roa T, Penninx BW, Sallis HM, Viktorin A, Chapman B, Henders AK; Psychiatric Genomic Consortium Major Depressive Disorder Working Group, Pergadia ML, Heath AC, Madden PA, Sullivan PF, Boschloo L, van Grootheest G, McMahon G, Lawlor DA, Landén M, Lichtenstein P, Magnusson PK, Evans DM, Montgomery GW, Boomsma DI, Martin NG, Meltzer-Brody S, Wray NR. Applying polygenic risk scores to postpartum depression. Arch Womens Ment Health 2014; 17: 519-28.

[11] Figueiredo FP, Parada AP, Araujo LF, Silva Jr WA, Del Ben CM. The Influence of genetic factors on peripartum depression: A systematic review. Affect Disord 2014; 172C: 265-273.

[12] Ryan J, Burger HG, Szoeke C, Lehert P, Ancelin ML, Henderson VW, Dennerstein L. A prospec-

tive study of the association between endogenous hormones and depressivesymptoms in postmenopausal women. Menopause 2009; 16: 509-17.
[13] Newhouse P, Albert K. Estrogen, Stress, and Depression: A Neurocognitive Model. JAMA Psychiatry 2015; 72: 727-9.
[14] Mehta D, Newport DJ, Frishman G, Kraus L, Rex-Haffner M, Ritchie JC, Lori A, Knight BT, Stagnaro E, Ruepp A, Stowe ZN, Binder EB. Early predictive biomarkers for postpartum depression point to a role for estrogen receptor signaling. Psychol Med 2014; 5: 1-14.
[15] Ohsawa M, Mizunuma H, Kagami I, Miyamoto S, Kanuma T, Ibuki Y. Effeet of I, 25(OH)2D3 on expression of estrogen receptor-alpha mRNA on rat osteosarcoma cell line. Life Sci 2000; 66: 2465-2469.
[16] Huang X, Li Y. Research Progress on Estrogen Receptor Gene Polymorphism. Section of Foreign Medical Clinical Biochemistry and Laboratory Medicine 2000; 21: 254-25.
[17] Miller KK, Al-Rayyan N, Ivanova MM, Mattingly KA, Ripp SL, Klinge CM, Prough RA. DHEA metabolites activate estrogen receptors alpha and beta. Steroids 2013; 78: 15-25.
[18] Tang Y, Chen Y, Shi S. Study on The Association between Single Nucleotide Polymorphisms of Estrogen Receptor β gene and postpartum depression. Archives of Psychiatry in Shanghai 2008; 20: 193-239.
[19] Rvan J, Scali J, Carriere I, Peres K, Rouaud O, Scarabin PY, Ritchie K, Ancelin ML. Estrogen receptor alpha gene variants and major depressive episodes. J Affect Disord 2012; 136: 1222-6.
[20] Veeraraqhan J, Tan Y, Cao XX, Kim JA, Wang X, Chamness GC, Maiti SN, Cooper LJ, Edwards DP, Contreras A, Hilsenbeck SG, Chang EC, Schiff R, Wang XS. Recurrent ESR1-CCDC 170 rearrangements in an aggressive subset of oestrogen receptor-positive breast cancers. Nat Commun 2014; 7: 4577.
[21] Li F, Che Z, Liu Y. Study on Estrogen Receptor α Gene Polymorphism in female SLE patients. Chinese Journal of Clinical Laboratory Science 2008; 2: 76-78.
[22] Boada M, Antunez C, Lopez-Arrieta J, Caruz A, Moreno-Rey C, Ramírez-Lorca R, Morón FJ, Hernández I, Mauleón A, Rosende-Roca M, Martínez-Lage P, Marín J, Tárraga L, Alegret M, Pedrajas JR, Urda N, Royo JL, Saez ME, Gayán J, González-Pérez A, Real LM, Ruiz A, Galán JJ. Estrogen receptor alpha gene variants are associated with Alzheimer's disease. Neurobiol Aging 2012; 33: 198.e15-24.
[23] Henning P, Ohlsson C, Engdahl C, Farman H, Windahl SH, Carlsten H, Lagerquist MK. The effect of estrogen on bone requires ERα in nonhematopoietic cells but is enhanced by ERα in hematopoietic cells. Am J Physiol Endocrinol Metab 2014; 307: E589-95.
[24] Li L, Wang Q, Lv X, Sha L, Qin H, Wang L, Li L. Expression and localization of estrogen receptor in human breast cancer and its clinical significance. Cell Biochem Biophys 2015; 71: 63-8.