ORIGINAL ARTICLE

ASSOCIATIONS BETWEEN VARIATIONS IN *TPH1*, *TPH2* AND *SLC6A4* GENES AND POSTPARTUM DEPRESSION: A STUDY IN THE JORDANIAN POPULATION

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ABSTRACT

In this study, we investigated the association between *tryptophan hydroxylase-1 (TPH1)* (218A>C), *tryptophan hydroxylase-2* (*TPH2*) (1463G>A) and *serotonin carrier family 6, member 4 (SLC6A4)* [long (L) *vs.* short (S)] gene polymorphisms with postpartum depression (PPD) in women from Jordan. A total of 370 postpartum (130 depressed and 240 non depressed) women volunteered for the study. Genotyping was carried out using restriction fragment length polymorphism (RFLP) for *TPH1*, amplification refractory mutation system (ARMS) for *TPH2* and polymerase chain reaction (PCR) for *SLC6A4* S and L. The Edinburgh postnatal depression scale was used to screen postpartum women. Both S and L alleles of *SLC6A4* are common in Jordanian women (about 51.0 and 49.0%, respectively), while allele *TPH1*-218C is more common (64.0%) than allele A (37.0%). Regarding *TPH2*, allele A is absent from the examined women. None of the examined polymorphisms were found to be associated with PPD (*p* >0.05). However, depression history, pregnancy problems and economic status were found to be significantly associated with PPD (*p* <0.05). The results suggest that *TPH1*, *TPH2* and *SLC6A4* S and L polymorphisms do not seem to be important in Jordan for predisposing to PPD.

Keywords: Depression; Gene; Polymorphism; Post-partum depression (PPD); Serotonin.

INTRODUCTION

Postpartum depression (PPD) occurs in 20.0-50.0% of women who have recently given birth, and may extend during the first year after delivery [1,2]. The symptoms of PPD include feeling of being alone, sleep deprivation, decreased desire for food, regular occurrence of unstable, disproportional emotional displays, and to a lesser extent, recurrent thoughts of harming oneself and/or the child [1,2]. Depression is a complex disorder, such that environmental as well as genetic factors, may play a role in developing the disease.

The neurotransmitter serotonin plays a role in sleep, appetite, emotions, body temperature and blood pressure [3]. Disruption of serotonergic neurotransmission is implicated in the development of many psychiatric diseases such as depression and bipolar disorder [4]. Therefore, variations in genes involved in biosynthesis, transmission and metabolism of serotonin are candidates for predisposing to PPD. Among such candidates are *tryptophan hydroxylase-1* (*TPH1*) (218A>C), and *tryptophan hydroxylase-2*

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(*TPH2*) (1463G>A) genes that code for tryptophan hydroxylase, the rate limiting enzyme in the conversion of tryptophan into serotonin [5]. The *TPH1* gene is located on chromosome 11, spans 29 kb, and has 11 exons [6]. The gene is expressed mainly in the pineal gland as well as in the peripheral tissues [7]. Among the polymorphisms of the *TPH1* gene is the 218A>C (rs1800532) variation in intron 7 that has been shown to affect the expression of the gene [8,9]. Variations in *TPH1* including 218A>C polymorphism, have been shown to be associated with bipolar disorder, suicidal behavior, alcoholism, and aggression-related traits [10,11]. The *TPH2* gene is located on chromosome 12 and spans 93.5 kb with 11 exons. It is predominantly expressed in the brainstem [7]. Zhang *et al.* [12] identified a functional a polymorphism (1463G>A) in the *TPH2* gene that results in substitution of a highly conserved arginine by a histidine (Arg441His) and the subsequent alterations in enzyme activity [12]. Genetic variations in *TPH2* have been shown to be associated with suicide, attention-deficit and hyperactivity disorder, autism and depression [13,14]. Another candidate is serotonin transporter gene solute carrier, *serotonin carrier family 6, member 4 (SLC6A4)* which encodes the human serotonin transporter that plays an important role in terminating the synaptic action of serotonin and recycles it into the presynaptic neurotransmitter pool [15]. A long (L) and a short (S) variant in the transcriptional control region upstream of the *SLC6A4* coding sequence was reported [16]. In *vitro* analysis showed that the basal activity of the L form was about three-fold higher than that of the S variant [16]. This variant has been shown to be associated with neuroticism [17] and depression [18-20].

In this study, we investigated the association between *TPH1*, *TPH2* and the *SLC6A4* (L/S variant) with PPD in the Jordanian population. In addition, we examined the possible correlation between socio-demographic, intra-partum and psychosocial variables with PPD.

MATERIALS AND METHODS

Subjects. This prospective cross-sectional study involved 370 randomly selected postpartum women (240 controls and 130 depressed cases) from five maternity centers in Irbid, Jordan. Random selection was carried out based on locations of the clinics in the city

and type of clinic (public *vs.* private). Women with pre existing psychiatric disorders were excluded from the study. Women were recruited 4 to 6 weeks after child delivery. Informed written consent was obtained from all subjects in accordance with the requirements of the Institutional Review Boards of Jordan University of Science and Technology, Irbid, Jordan.

Edinburgh Postnatal Depression Scale Questionnaire. An Arabic version of the Edinburgh Postnatal Depression Scale (EPDS) was used to screen postpartum women [21]. An EPDS score >13 was used as a criterion to indicate the presence of depression illness(es) [21]. This cut-off value has been reported to have high sensitivity and specificity. Several demographic variables were also included in the questionnaire to assess the possible correlation between PPD and some environmental factors such as maternal age, educational level, income satisfaction, number of children, delivery mode, depression history and pregnancy problems.

DNA Extraction. Venous blood samples were collected in EDTA tubes and obtained from all subjects. DNA was extracted using Promega wizard genomic DNA purification kit (Promega, Madison, WI, USA) according to the protocol provided by the manufacturer. DNA concentrations were measured using Smart-SpectTM3000 (Bio-Rad Laboratories, Hemel Hempstead, Hertfordshire, UK). Extracted DNA was stored at –20°C until used.

Genotyping of the *TPH1* (218A>C) Polymorphism. *TPH1* was analyzed using polymerase chain reaction (PCR) followed by the restriction fragment length polymorphism (RFLP) method. The *TPH1* gene fragment was amplified using primers described previously [22]. The reaction mixture of 25 μL contained 5 ng of genomic DNA, 1 μM of each primer, green master mix (Promega), 1 mM of spermidine and H_2O. The reaction mixture was initially denatured at 95°C for 5 min., followed by 30 cycles at 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds. The PCR was completed by a final extension cycle at 72°C for 5 min. Successful amplification of the fragment was confirmed by detection of a 847 bp band on a 2.0% agarose gel. The restriction enzyme digestion was carried out overnight at 37°C in a 20 μL volume reaction mixture containing 10 μL of the amplified PCR product and 1 unit of *Bfa*1. The digested fragments were then separated using 2.0% agarose gel electrophoresis and detected by ethidium bromide

staining. The PCR product with the A allele remained uncut (847 bp), whereas the digested PCR product with the C allele gave fragments of 599 and 248 bp.

Genotyping of the *TPH2* (1463G>A) Polymorphism. Allele-specific PCR using amplification refractory mutation system (ARMS) method was used for analyzing the *TPH2* G1463A polymorphism as described by Zhang *et al.* [12]. Only the G allele PCR-specific product was detected in the DNA obtained from participating women, while the A allele product was not detected. Validity of the ARMS results was achieved by sequencing of more than 10.0% of the PCR products using ABI PRISM™ 3.1 automated sequencer (Life Technologies, Applied Biosystems, Foster City, CA, USA) as previously described [23].

Genotyping of the *SLC6A4* (L/S) Polymorphism. The *SLC6A4* (L/S) polymorphism was analyzed using PCR. Desired DNA target sequences, 469 bp for the S allele and 513 bp for the L allele, were amplified as described by Middledorp *et al.* [24]. The reaction mixture of 20 μL contained 5 ng of genomic DNA, 1 μM of each primer, Promega green master mix, 2.0% DMSO and H_2O. The reaction mixture was initially denatured at 94°C for 3 min., followed by 35 cycles at 94°C for 30 seconds, 59°C for 30 seconds and 72°C for 90 seconds. The PCR procedure was terminated by a final extension at 72°C for 6 min. Amplified 469 or 513 bp fragments were electrophoresed through 2.0% agarose and visualized by ultraviolet illumination upon ethidium bromide staining.

Statistical Analyses. Data analysis was carried out using the statistical package for social studies (SPSS) version 17 (SPSS Inc., Chicago, IL, USA) to compute all de-scriptive statistics. The chi-squate (χ^2) test was used to identify the correlation between the studied demographic variables and PPD. If $n <5$, exact Fisher statistic test was used. In addition, the same tests were used to evaluate the genotype distribution and allele frequencies of the three studied polymorphisms. The test power was calculated for allele and genotype frequencies using Power and Sample Size Calculation Program (PS version 3.0.1; Vanderbilt University Medical Center, Nashville, TN, USA). For all analyses, the power was more than 75.0%. A p value <0.05 was considered significant. The Hardy-Weinberg equilibrium was assessed using the χ^2 test.

RESULTS

This study included 370 postpartum women aged between 17 to 45 years old. Almost all the women were married (99.5%), more than half of them (62.2%) were aged less than or equal to 30 years, and about 80.0% had completed their education with at least a high school certificate. The majority of women were homemakers (86.6%) and most were not satisfied with their income adequacy (74.1%). Demographic variables are shown in Tables 1, 2 and 3.

About 35.0% of postpartum women scored 13 or higher on the EPDS, thus a considerable fraction of the participating women had PPD, of whom 29.2% had a previous history of depression. Several psychosocial variables were examined for their association with PPD including: marital status, depression history and living status, whether with husband and children only or in the household of their husband's family. Among these factors, only the history of depression was found to be highly significantly associated with PPD ($p <0.001$, Table 1). However, divorced women were only found

Table 1. The correlation between psychosocial variables and postpartum depression.

Parameters	Normal n (%)	Depressed n (%)	Total n (%)	p Value
History of depression				
yes	31 (12.9)	38 (29.2)	69 (18.6)	
no	209 (87.1)	92 (70.8)	301 (81.4)	0.000
Marital status				
married	240 (100.0)	128 (98.5)	368 (99.5)	
divorced	0 (0.0)	2 (1.5)	2 (0.5)	0.054
Living status				
with husband's family	223 (92.9)	119 (91.5)	342 (92.5)	
with husband and children only	17 (7.1)	11 (8.5)	28 (7.5)	0.632

Table 2. The correlation between intra-partum variables and postpartum depression.

Parameters	Normal n (%)	Depressed n (%)	Total n (%)	p Value
Delivery mode				
normal	198 (82.5)	105 (80.8)	303 (81.9)	
Cesarean section	42 (17.5)	25 (19.2)	67 (18.1)	0.680
Pregnancy problems[a]				
yes	34 (14.2)	29 (23.3)	63 (17.0)	
no	206 (85.8)	101 (76.7)	307 (83.0)	0.047
Breast feeding				
yes	221 (92.1)	122 (93.8)	343 (92.7)	
no	19 (17.9)	8 (6.2)	27 (7.3)	0.534
Planning pregnancy				
yes	169 (70.4)	86 (66.2)	255 (68.9)	
no	71 (29.6)	44 (33.8)	115 (31.1)	0.398
Taking vitamins				
yes	233 (97.1)	125 (96.2)	358 (96.8)	
no	7 (2.9)	4 (3.8)	11 (3.2)	0.394
Number of children				
1-3	162 (67.5)	82 (63.1)	244 (65.9)	
4-6	63 (26.3)	39 (30.0)	102 (27.6)	
>6	15 (6.2)	9 (6.9)	24 (6.5)	0.691
Baby's sex				
males	125 (52.1)	63 (48.5)	188 (50.8)	
females	115 (47.9)	67 (51.5)	182 (49.2)	0.506

[a] Pregnancy problems included hypertension, hypo/hyperglycemia, bleeding, edema, problems in thyroid secretion and others.

Table 3. The correlation between some socio-demographic variables and postpartum depression.

Parameters	Normal n (%)	Depressed n (%)	Total n (%)	p Value
Age of mother				
<30 years old	152 (63.3)	78 (60.0)	230 (62.2)	
>30 years old	88 (36.7)	52 (40.0)	140 (27.8)	0.376
Education level				
<secondary	45 (18.7)	30 (23.1)	75 (20.3)	
secondary certificate	112 (46.7)	66 (50.8)	178 (48.1)	
college (2 years)	47 (19.6)	18 (13.8)	65 (17.6)	
university or more	36 (15.0)	16 (12.3)	52 (14.0)	0.378
Employment status				
working	31 (12.9)	18 (13.8)	49 (13.2)	
homemaker	209 (87.1)	112 (84.2)	321 (86.8)	0.459
Income satisfaction				
yes	71 (29.6)	25 (19.2)	96 (25.6)	
no	169 (71.4)	105 (80.8)	274 (74.4)	0.030

within the depressed women group (two cases), thus, marital status may be correlated to PPD but was only marginally significant (p value = 0.054).

Among the examined intra-partum variables, only the presence of maternal health problems (hypertension, hypo/ hyperglycemia, bleeding, edema, problems in thyroid secretion and others) was associated with PPD (p <0.05, Table 2). With respect to socio-demographic variables, income satisfaction was significantly related to PPD (p <0.05). Other socio-demographic variables including maternal age, educational level, and employment status were not associated with PPD (Table 3).

The genotype and allele frequencies of the *SLC6A4* (L/S) and *TPH1* polymorphisms are shown in Table 4. All genotypes of both polymorphisms

Table 4. Genotype and allele frequencies of polymorphism among the participating women.

Genotypes and Alleles	Control Group n (%)	Depressed Group n (%)	p Value
SLC6A4 (5-HTTLPR)			
LL	62 (25.8)	36 (27.7)	
SL	124 (51.7)	59 (45.4)	
SS	54 (22.5)	35 (26.9)	0.480
Allele L	248 (51.7)	131 (50.4)	
Allele S	232 (48.3)	129 (49.6)	0.887
TPH1 (A218C)			
AA	34 (14.2)	15 (11.5)	
AC	116 (48.3)	61 (46.9)	
CC	90 (37.5)	54 (41.5)	0.661
Allele A	184 (38.3)	91 (35.0)	
Allele C	296 (61.7)	169 (65.0)	0.768

were in Hardy-Weinberg equilibrium. The distribution of both S and L alleles of the *SLC6A4* (L/S) polymorphism is similar, and both are common among Jordanian women (about 51.0 and 49.0%, respectively). With respect to the *TPH1* polymorphism, allele C is more common than allele A (63.0 and 37.0%, respectively). There was no significant association between the genotypes of either *SLC6A4* (L/S) or *TPH1* polymor-phisms and PPD in the studied population (p >0.05, Table 4). Similarly, allele frequencies of the examined variations were not related to PPD (p >0.05, Table 4). Regarding *TPH2* polymorphism, the A allele was not detected in the investigated population, and therefore no analysis was performed.

DISCUSSION

Serotonin has been shown to be involved in many physiological and behavioral processes, including: mood, sleep and cognation [3]. In addition, alterations in the serotonergic system have been implicated in many neuropsychiatric disorders such as aggressive, suicidal behavior and depression [4,25]. In this study, we investigated the role of variations in *SLC6A4*, *TPH1* and *TPH2* genes with depression in Jordanian women during the postpartum period.

The results presented in this study reveal lack of association between the *TPH1* polymorphism and PPD in the examined sample. In agreement with our results, Frisch *et al.* [26] found no association between the *TPH1* polymorphism and unipolar affective disorder including depression. Similar results were reported by Serretti *et al.* [27] who failed to find any association between this polymorphism and

mood disorders. In contrast, Sun *et al.* [28] reported significant association between the *TPH1* gene polymorphism with depression, anxiety, and comorbid depression and anxiety in Taiwanese women during the postpartum period. In addition Porter *et al.* [22] reported an association between *TPH1* and reduced tryptophan levels in females but not in males and the subsequent predisposition to depression.

The results also showed absence of association between the *SLC6A4* (L/S) polymorphism and depression in postpartum Jordanian women. In agreement with our results, Middledorp *et al.* [24] suggested that *SLC6A4* (L/S) has no direct association with neuroticism, anxiety and depression in The Netherlands. In addition, another two studies comprising 5,262 subjects failed to find any association between the S allele and stressful life events of social adversity [29,30]. However, a Spanish study found association between the L allele and PPD [31]. Moreover, evidence pointing to a correlation between the S allele and increased risk of depression was found in females who had experienced chronic diseases [32]. Thus, association between examined polymorphisms and PPD might have a population-specific component being affected by genetic background and/or associated with certain conditions.

In the study sample, approximately 35.0% of women scored 13 or higher on the EPDS. Slightly lower prevalence rates were reported in other Arab countries including Dubai (17.8%) and Beirut (16.0%) [33,34]. A recent review of 143 studies comprising 40 countries has shown that the PPD rate may reach up to 60.0%, especially in a low income population [34]. Thus, Jordan is ranked in the middle in countries in the prevalence of PPD.

The result of the present study showed that depression history, income level and pregnancy complications were associated with PPD. This is in agreement with O'Hara *et al.* [35] and Beck *et al.* [2] who found a strong association between depression history and economic status with developing PPD. In addition Rich-Edward *et al.* [36] considered depression history as the most powerful risk factor in developing PPD. Moreover, high prevalence rates of PPD (25.0-50.0%) was reported in a low income population [37]. Finally, some studies related PPD to the mother's health [38] and pregnancy problems [35]. The lack of association with other examined factors is consistent with previous studies that failed to find an association between PPD and maternal age, educational level [2,35], marital status [39,40], baby gender [41], delivery mode [40], breast-feeding and employment [36]. These results could be explained by a stable marital relationships as almost all participants were married and most of them were educated. In addition, the majority of them were homemakers and had enough time to do their work at home and take care of their children.

Postpartum depression is a complex disorder, which likely results from a combination between genetics as well as environmental factors. The contribution of environmental factors has stronger influence on PPD than genetics as the degree of PPD heritability seems to be weak [42]. Thus, the stronger influence of environmental factors on PPD might cover or minimize the weaker genetic effect. In addition, it is possible that we have other polymorphisms across the genes in the Jordanian population, which in turn, might modulate the effect of the studied polymorphisms. Therefore, additional molecular genetic studies are needed to screen possible modifier polymorphisms under the influence of environmental and other hormonal effectors.

In conclusion, the results of this study indicate that *SLC6A4* (L/S) and *TPH1* variations are not associated with the development of PPD in Jordanian women. In addition, the *TPH2* A allele was absent in the examined population.

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