ORIGINAL RESEARCH
Key Words: depression, venlafaxine, pregnancy, real time PCR

Effects of Venlafaxine on the Size of Brain and Expression of *SHANK3*, *TUBB5* and *DDC* Genes in BALB/c Mice

By Ramesh Ranjbar, Zahra Zamanzadeh, Ali Mohammad Ahadi

ABSTRACT ~ ***Objectives:*** *A growing body of evidence has recently suggested that taking venlafaxine during pregnancy may be linked to increased risk of certain congenital defects. The study aimed to address the effects of venlafaxine use during pregnancy on the development of the brain in mice.* ***Experimental design:*** *Fourteen female BALB/c mice were randomly divided into two equally-sized groups: venlafaxine-treated and control. After mating, pregnant mice of venlafaxine-treated group were orally received the venlafaxine 35 mg/kg/day throughout pregnancy, while pregnant control mice did not receive any treatment. All pups were killed on postnatal day 21 and brain images were quantified using ImageJ software. The mRNA expression levels of SHANK3, TUBB5 and DDC of genes in pups' brain tissue samples were evaluated using quantitative real-time PCR method.* ***Principal observations:*** *The mean brain size of pups was significantly smaller in the venlafaxine-treated group than in the control group. Results showed that the mRNA expression levels of SHANK3 and TUBB5 was significantly downregulated in venlafaxine-treated mice compared to control group. Expression of DDC gene didn't showed significant differences between two groups.* ***Conclusions:*** *These results provide evidence that use of venlafaxine during pregnancy may affect the brain development in mice and altered the expression of SHANK3 and TUBB5 genes in brain tissue.* Psychopharmacology Bulletin. *2023;53(3):22–34.*

Ranjbar, PhD candidate, Department of Genetics, Faculty of Basic Sciences, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran. Zamanzadeh, PhD, Department of Genetics, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Isfahan, Iran. Ahadi, PhD, Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran.

To whom correspondence should be addressed: Zahra Zamanzadeh, PhD, Assistant Professor, Department of Genetics, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani University, Isfahan, Iran. E-mail: zamanzadehzahra2010@gmail.com.

Ali mohammad Ahadi, PhD, Associate Professor, Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran. E-mail: ahadi_ali@sku.ac.ir.

Introduction

Depression, also known as major depressive disorder or clinical depression, is a serious psychiatric illness that detrimentally affects the mental, emotional and physical health.[1] Depression is typically characterized by depressed mood, diminished interest or pleasure, impaired cognitive performance, and disturbed neurovegetative functions.[2,3] Globally, it is a highly prevalent psychiatric disorder in both sexes and is now considered to be a leading cause of disease-related disability with a lifetime risk of 10 to 20%. [4,5] Depression is a multifactorial disorder that arises from a complex interplay between genetic, psychosocial, lifestyle and environmental factors.[6,7] Despite the high prevalence of depression, the underlying etiology and pathophysiological mechanisms of disease remain still elusive.[8,9]

The lifetime risk of depression for women is nearly twice as high as for men, with a peak prevalence during reproductive years.[10] The prevalence of depression in pregnancy has been estimated in the range of 6.5–26.7%.[11,12] Evidence suggests that depression affects almost 15.5% of women in the first and second trimester of pregnancy, 11.1% in late pregnancy, and 8.7% in the postnatal period.[13,14] It is well established that untreated antenatal depression may have serious adverse effects on the health of both mother and fetus, and may be associated with increased risk of preterm birth, low birth weight, and intra-uterine growth restriction.[15] On the other hand, taking antidepressants while pregnancy may cause certain birth defects.[16,17] So, treatment or not treatment of depression during pregnancy remains a major dilemma for obstetricians and gynecologists. It is however estimated that almost 2–3% of pregnant women use antidepressants during pregnancy.[18] Serotonin and norepinephrine reuptake inhibitors (SNRIs) are a class of antidepressants that increase the quantities of serotonin (5-hydroxytryptamine; 5-HT) and norepinephrine (NE) and consequently their neurotransmission in the brain by blocking or delaying the reuptake of both neurotransmitters after being released into synapses.[19] Venlafaxine, also known as Effexor, is one of the most commonly used SNRI antidepressants to treat depression, anxiety and other mood disorders.[20] Venlafaxine, a bicyclic phenylethylamine derivative, is classified by the FDA as pregnancy-risk category C, denoting that there are no adequate and well-controlled studies in humans but animal studies have shown an adverse effect on the developing fetus.[21,22] The presence of venlafaxine in the amniotic fluid indicates that the placenta cannot be an impenetrable barrier for fetal venlafaxine exposure.[23] Because venlafaxine easily crosses the placenta, it may have a range of negative effects on the developing fetus and cause certain types of birth

defects, especially congenital brain defects.[20,24,25] Venlafaxine can enter the developing brain and disrupt the key processes of brain development through interfering with monoaminergic neurotransmission.[26] Besides, it has previously been shown that venlafaxine activates the expression of genes involved in neurotrophic signaling, glutamatergic transmission, neuroplasticity, synaptogenesis and cognitive processes.[27] We therefore hypothesized that taking venlafaxine during pregnancy may interfere with normal brain development by alterations in the patterns of gene expression. To test this hypothesis, the present study was carried out to evaluate the adverse effect of venlafaxine use during pregnancy on the development of the brain in the BALB/c mice by quantifying the expression of *SHANK3, TUBB5* and *DDC* genes at mRNA levels in pups' brain tissue samples of venlafaxine-treated and control groups using quantitative real-time polymerase chain reaction (qPCR) procedure.

Materials and Methods

Experimental Animals and Venlafaxine Treatment

A total of 21 BALB/c mice (14 females and 7 males) (eight to ten-week-old and weighing 25–30 g) were purchased from Royan Institute, Tehran, Iran and maintained at the Animal Center of the Shahid Ashrafi Esfahani University, Isfahan, Iran. They were housed in groups of three animals in the standard Makrolon type III cages (Tecniplast, Milan, Italy) and allowed to acclimate to new environments for one week prior to the experiment. Experimental animals were maintained under standard laboratory conditions at temperature of 20–25°C, relative humidity of 40–60%, 10–15 air changes per hour and 12-hour phase shift in the light/dark cycle with free access to standard rodent diet and sterile water and libitum throughout the study. Female mice were randomly assigned into two equally-sized study groups: venlafaxine-treated (n = 7) and control (n = 7) groups. Venlafaxine was completely dissolved in deionized water and orally administered. The dose of venlafaxine was adjusted based on body weight and given orally to treated mice with food. The venlafaxine-treated group received daily administration of venlafaxine (0.015 mg/kg/day) for three days to allow their body to adjust to the medication. To obtain pregnant mice, two females and one male were placed simultaneously into each cage until a copulation occurred, and then pregnant mice were placed in their own cage. Successful mating was confirmed by the presence of vaginal plug and/or sperm in the vaginal smear. Female mice who had previously been treated with venlafaxine were received daily administration

of the drug (35 mg/kg/day) during pregnancy for three weeks. In the control group, pregnant female mice did not receive any drug treatment. Pregnant mice were allowed to deliver spontaneously and nurse their pups until three weeks postpartum. The live pups were counted, sexed, weighed, and examined externally for gross malformations on the day of birth. At postnatal day 21, the pups were anesthetized by chloroform-impregnated cotton and sacrificed through rapid decapitation and then their brains were removed cautiously from the skulls. For subsequent analysis, the brain of pups were immediately rinsed several times in phosphate-buffered saline (PBS) solution to remove blood and tissue debris. All animal care and handling procedures was conducted in accordance with the recommendations of the guidelines for care and use of laboratory animals of the National Institutes of Health (NIH). Animal experiments were approved by the Animal Ethics Committee of the Razi Vaccine and Serum Research Institute (No. 515.92 GD, 26.1.2010).

Brain Image Analysis

After washing with PBS, images of brains were captured and quantitative image analysis was performed using ImageJ software to evaluate the difference in brain size between venlafaxine-treated and control groups pups.

RNA Extraction and cDNA Synthesis

The fresh brain of pups was sliced into small pieces and then immediately submerged in RNAlater® RNA Stabilization Reagent (Qiagen, Hilden, Germany) and stored at −20°C until RNA extraction. Brain tissue samples (~100 mg) were slowly homogenized by grinding in liquid nitrogen with mortar and pestle, and followed by the extraction of total RNA using RNX-Plus reagent (Sinaclon BioScience Co, Tehran, Iran) according to manufacturer's instructions. To eliminate genomic DNA contamination, RNA samples were treated with RNase-free DNase I (Thermo Scientific, USA). The purity and concentration of extracted RNA samples were assessed by NanoDrop® ND-1000 UV-Vis Spectrophotometer (ThermoFisher Scientific™, Waltham, MA, USA). Subsequently, 1 μl of extracted RNA was reverse transcribed into complementary DNA (cDNA) using Viva cDNA Synthesis Kit (Vivantis Technologies, Selangor Darul Ehsan, Malaysia) following the manufacturer's protocol. Briefly, total RNA was added to reaction mixture containing random hexamer primers (50 ng/μl), Oligo d(T)18

(40 μm), 10 mM dNTPs mix, 10X Buffer M-MuLV and M-MuLV Reverse Transcriptase, and incubated at 25 °C for 10 min and 42 °C for 50 min, followed by an inactivation step at 80 °C for 1 min.

Quantitative Real-Time PCR (qPCR)

The expression of *SHANK3, TUBB5* and *DDC* genes at mRNA levels in pups' brain tissue samples of venlafaxine-treated and control groups was quantified by SYBR Green-I based qPCR method on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). All qPCR assays were conducted in triplicate using BioFACT™ 2X Real-Time PCR Master Mix (BioFact, Daejeon, South Korea) and gene-specific primer pairs designed by Gene Runner Software (Hastings Software Inc., Hastings, NY, USA) (Table 1). The PCR reactions were carried out in a final volume of 20 μL, containing 10 μL of BioFACT™ 2X Real-Time PCR Master Mix (BioFact, Daejeon, South Korea), 2 μl of each primer pair (10 pmol/μL) and 100 ng cDNA. The thermal cycling conditions were 95 °C for 10 min, 40 cycles of 95 °C for 20 s and annealing at temperatures shown in Table 1 for each of the primer pairs for 30 s, followed by 72 °C for 30 s. The mRNA expression levels of *SHANK3, TUBB5* and *DDC* genes were normalized to *Actb* as a reference gene. No template control (NTC) was used as a negative control.

Statistical Analysis

The data were statistically analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, N.Y., USA) and p value of $\leqslant 0.05$ was regarded as the threshold of statistical significance. The Kolmogorov-Smirnov test was used to determine the normality of the data and continuous variables with normal distribution were expressed as mean ± standard deviation (SD). The Student's t-test was used to compare the mean expression of *SHANK3, TUBB5* and *DDC* genes at mRNA levels in pups' brain tissue samples of venlafaxine-treated and control groups.

Gene Network Analysis

KEGG, Reactome and STRING servers were used for collecting of primary data for interaction of the genes that studied in this research. Finally, Cytoscape software was used to analyze the gene network.

TABLE 1

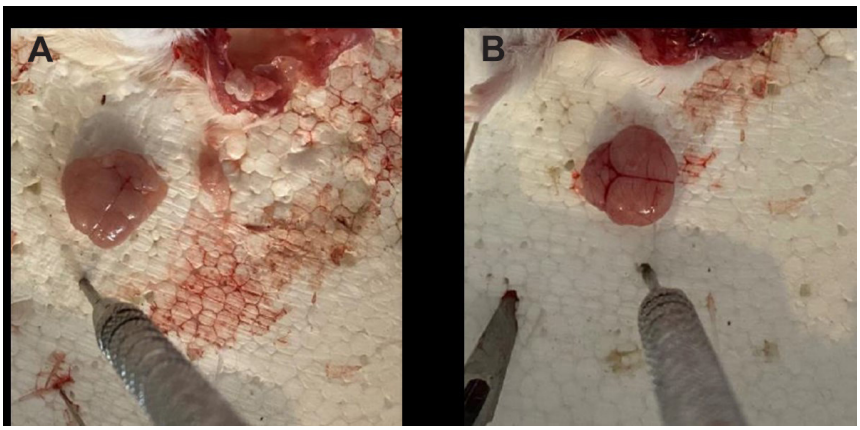
List of Primers Used for qPCR Assays

GENE	GENBANK ACCESSION NO.	PRIMER SEQUENCE	ANNEALING TEMPERATURE (°C)	AMPLICON SIZE (BP)
SHANK3	NM_001190448.1	F 5′-AGCTACACACAAAGGCAAACC-3′ R 5′-AGTCAGGGTCATGGAAATTGG-3′	61	116
TUBB5	NM_011655.5	F 5′-TCGGTGCTAAGTTCTGGGAG-3′ R 5′-GGCTTCATTATAGTACACAGAG-3′	61	117
DDC	NM_021423.4	F 5′-GAGTGATCCAGGGTTTCTCC-3′ R 5′-CCCTTCACCAGCTCTTCCAG-3′	61	128
Actb	NM_007393.5	F 5′-GGACTCCTATGTGGGTGACG-3′ R 5′-AGGTGTGGTGCCAGATCTTC-3′	61	119

Abbreviation: Quantitative real-time polymerase chain reaction, qPCR; *SHANK3*, SH3 and multiple ankyrin repeat domains 3; *TUBB5*, Tubulin beta-5 chain; *DDC*, 3,4-dihydroxyphenylalanine (DOPA) decarboxylase; *Actb*, β-actin.

FIGURE 1

Remove The Pups' Brains From The Skull



Notes: A) Pups' brain of venlafaxine-treated group. B) Pups' brain of control group.

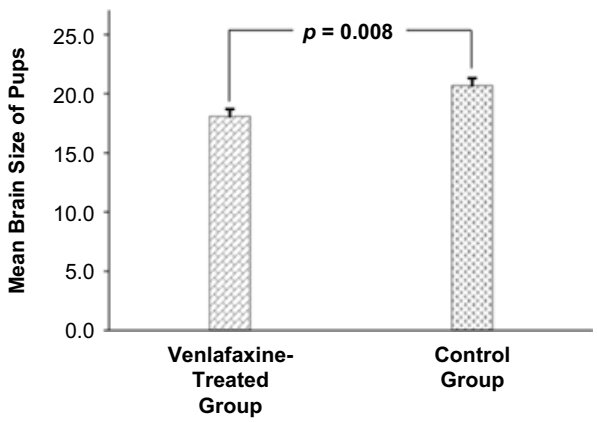
Results

The Mean Size of BALB/c Mice Pups' Brain

After removing the pups' brains from the skulls, the images of the brain were captured and analyzed using ImageJ software (Figure 1). The findings revealed that the mean size of pups' brain was significantly smaller in the venlafaxine-treated group (18.03 ± 0.616 Kilopixel (KPx)) compared to control group (20.66 ± 0.548 KPx) ($P < 0.05$) (Figure 2).

FIGURE 2

The Mean Size of Brain in Pups' of Venlafaxine-Treated and Control Groups



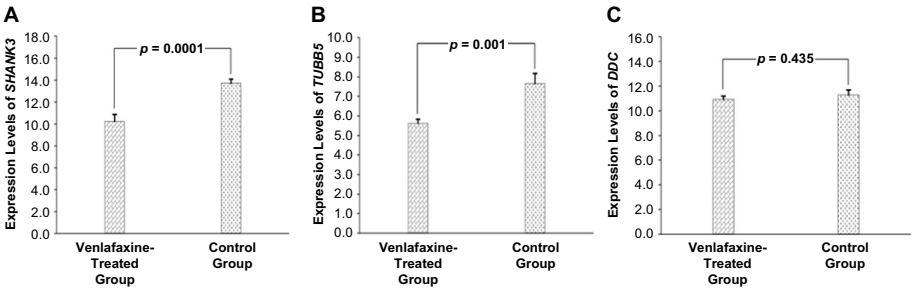
Notes: The mean size of brain in pups' of venlafaxine-treated group (18.03 ± 0.616 kilopixel (KPx)) was significantly smaller than that of the control group (20.66 ± 0.548 KPx) ($P < 0.05$).
Source: Data are expressed as the mean ± SEM.

Expression Levels of SHANK3, TUBB5 and DDC Genes in Pups' Brain Tissue Samples

The expression of *SHANK3, TUBB5* and *DDC* genes at mRNA levels in pups' brain tissue samples of venlafaxine-treated and control groups was quantified using qPCR assays. The Kolmogorov-Smirnov test was used to determine the normality of data, which showed that the mRNA expression levels of *SHANK3, TUBB5* and *DDC* genes were normally distributed ($P > 0.05$). Based on the cycle threshold (Ct) values, the relative expression levels of *SHANK3, TUBB5* and *DDC* genes were determined using the $2^{-\Delta\Delta CT}$ method. Our results showed that the mRNA expression of *SHANK3* was significantly down-regulated in pups' brain tissue samples of the venlafaxine-treated group compared to the control group (fold change (FC): 1.342; 95% confidence interval (95% CI): −4.988 to −2.019; $P = 0.0001$) (Figure 3a). As shown in Figure 3b, the expression levels of *TUBB5* were found to be significantly reduced in brain tissue samples obtained from venlafaxine-treated group pups compared to control group (FC: 1.360; 95% CI: −3.186 to −0.868; $P = 0.001$) (Figure 3b). A decreased mRNA level of *DDC* in pups' brain tissue samples of venlafaxine-treated group was observed when compared to control group, but not statistically significant (FC: 1.035; 95% CI: −1.386 to 0.614; $P = 0.435$) (Figure 3c).

FIGURE 3

The Expression of *SHANK3*, *TUBB5* and *DDC* Genes at mRNA Levels in Pups' Brain Tissue Samples of Venlafaxine-Treated and control Groups



Notes: A) The expression of *SHANK* in pups' brain tissue samples was significantly down-regulated in venlafaxine-treated group compared with control group ($P = 0.0001$, 95% CI = −4.988 to −2.019). B) The expression of *TUBB5* in pups' brain tissue samples was significantly down-regulated in venlafaxine-treated group compared with control group ($P = 0.001$, 95% CI = −3.186 to −0.868). C) The expression of *DDC* in pups' brain tissue samples was decreased in venlafaxine-treated group compared with control group, but not statistically significant ($P = 0.435$, 95% CI = −1.386 to 0.614).
Source: Data are expressed as the mean ± SEM.

Gene Network Analysis

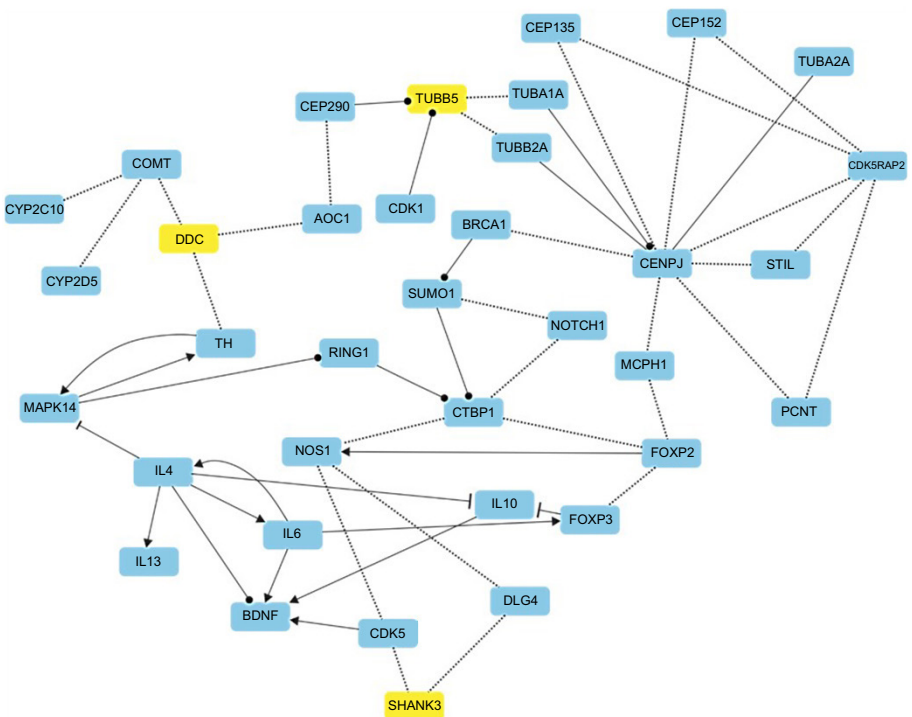
The gene network for *SHANK3*, *TUBB5* and *DDC* genes was plotted using cytoscape software (Figure 4). We found that *SHANK3*, *TUBB5* and *DDC* are linked to genes involved in brain development, including *BDNF*, *MCPH1* and *CENPJ*.

Discussion

The development of the brain during pregnancy is a highly complex dynamic process that involves proper timing and coordination of a series of cellular and molecular events.[28,29] These events are tightly integrated with regulation of gene expression over the course of the brain development.[30] It is thus not surprising that dysregulated expression of genes involved in the brain development can affect its structure and function. One such gene is Src homology 3 and multiple ankyrin repeat domains 3 *(SHANK3)*, which encodes a multi-domain, scaffolding protein found in the post-synaptic density complex of excitatory glutamatergic synapses on dendritic spines.[31,32] The *SHANK3* gene

FIGURE 4

The Network of Brain Development Related Genes using Cytoscape Software



is located on chromosome 22q13.3 in human, and on chromosome 15E3 in mice.[32] Shank3 protein, also known as proline-rich synapse-associated protein 2 (ProSAP2), interacts with multiple signaling proteins and complexes to regulate the maintenance and maturation of neuronal dendritic spines and synapse formation.[33,34] Mutations in the *SHANK3* gene are strongly associated with neurodevelopmental disorders like autism spectrum disorders, and deletion of the *SHANK3* is likely to be a cause of the major neurological symptoms of 22q13.3 deletion/ Phelan-McDermid syndrome (PMS).[33–35] The other one is the *TUBB5* gene, which encodes Beta-5 tubulin that can polymerize to form microtubules. Because of the importance of microtubules (MTs) in regulating cell division, migration, polarity, differentiation, signaling and intracellular trafficking, they play a fundamental role in the developing mammalian nervous system.[36,37] Ngo *et al.* revealed that TUBB5 is critical for neuronal differentiation and dendritic spine formation in the murine cerebral cortex.[38] It has already been shown that defects in the MTs can lead to neurodevelopmental diseases such as lissencephaly, polymicrogyria, intellectual disabilities and autism spectrum disorders.[38] In 2012, Breuss *et al.* demonstrated that the *TUBB5* gene is highly expressed in the developing human and mouse brains, and mutation in this gene results in microcephaly.[39] On the other hand, the DDC gene encodes aromatic L-amino acid decarboxylase (AADC) enzyme (EC.4.1.1.28) is implicated in the biosynthesis of the several key neuroactive monoamine neurotransmitters, such as dopamine, epinephrine, norepinephrine, and serotonin.[40] AADC deficiency, a severe neurometabolic disorder, is caused by genetic variations in the DDC gene, which leads to the disturbance in cerebral monoamines metabolism.[40] AADC deficiency is clinically characterized by severe developmental delay, muscular hypotonia, dystonia, oculogyric crises, autonomic dysfunction and other neurological disorders.[40,41] Meanwhile, mutations in the DDC gene have been reported to be associated with autism, schizophrenia, bipolar affective disorders and attention deficit hyperactivity disorder.[42] These data suggest that *SHANK3*, *TUBB5* and *DDC* genes profoundly influence the development of brain architecture and the function of a subset of the nerve cells. On the other hand, there is now compelling evidence to show that taking venlafaxine during pregnancy may lead to disruption of essential neurodevelopmental processes by altering the expression of brain development-related genes.[20,24–27] Accordingly, we investigated the effect of venlafaxine on brain development in the BALB/c mice and mRNA expression levels of *SHANK3*, *TUBB5* and *DDC* in pups' brain tissue samples of venlafaxine-treated and control groups. Our findings showed that the mRNA expression levels of *SHANK3*, *TUBB5* were significantly down-regulated in pups'

brain tissue samples of venlafaxine-treated group compared to control group ($P < 0.05$) (Figure 3). The *DDC* expression was also lower in pups' brain tissue samples of venlafaxine-treated group compared to control group, but not statistically significant ($P > 0.05$) (Figure 3). Based on the literature review, no studies thus far have investigated the effects of venlafaxine on the expression levels of *SHANK3*, *TUBB5* and *DDC* genes, which serve critical roles in brain development. As far as we know, this is the first report showing that administration of venlafaxine to pregnant BALB/c mice during the gestation period can cause a reduction in brain size of pups and down-regulation of *SHANK3*, *TUBB5* genes expression in pups' brain tissue. However, these findings should be interpreted with caution due to a lack of previous studies in the field.

Gene network analysis by Cytoscape software suggests that *SHANK3*, *TUBB5* and *DDC* are linked to genes involved in brain development, including brain-derived neurotrophic factor (*BDNF*), Microcephalin 1 (*MCPH1*) and Centromere Protein J (*CENPJ*).[43–45] BDNF, a highly conserved gene, is expressed in the developing and mature brain, and plays an important role in neuronal survival, synaptic plasticity and brain development.[43] A statistically significant difference in the mean brain size of pups between the venlafaxine-treated and control groups was also found ($P < 0.05$) (Figure 2). In line with our findings, it has been reported that *MCPH1* (also known as *BRIT1*) is expressed during fetal brain development and mutations in this gene can cause primary microcephaly that is characterized by severely reduced brain size.[44,45] Mutations in the *CENPJ* (also known as *CPAP*, *MCPH6*, *SCKL4*), a highly conserved and ubiquitously expressed gene, have been also found to cause primary microcephaly.[45] Despite these preliminary promising results, further studies are warranted to validate the current findings.

In conclusion, this study provides evidence to support the hypothesis that taking venlafaxine during pregnancy adversely affects the developing brain in BALB/c mice, at least in terms of brain size. Besides, venlafaxine significantly decreases the expression of *SHANK3* and *TUBB5* genes in BALB/c mice pups' brain tissue and future studies can focus on identifying the mechanisms by which the drug reduces the expression of these genes. ✤

Acknowledgments

We would like to express our sincere appreciation to all collaborators in Shahid Ashrafi Esfahani University, Isfahan, Iran for their generous help to conduct this research work.

This work was performed at Shahid Ashrafi Esfahani University, Isfahan, Iran.

Declarations of Interest

None.

References

1. Otte C, Gold SM, Penninx BW, Pariante CM, Etkin A, Fava M, et al. Major depressive disorder. *Nature Reviews Disease Primers*. 2016;2(1):1–20.
2. Fava M, Kendler KS. Major depressive disorder. *Neuron*. 2000;28(2):335–341.
3. Kennedy SH. Core symptoms of major depressive disorder: relevance to diagnosis and treatment. *Dialogues in Clinical Neuroscience*. 2022.
4. Sabic D, Sabic A, Bacic-Becirovic A. Major depressive disorder and difference between genders. *Materia Socio-Medica*. 2021;33(2):105.
5. Bertschy G, Velten M, Weibel S. Major depression: does gender influence the risk of recurrence? A systematic review. *The European Journal of Psychiatry*. 2016;30(1):7–27.
6. Remes O, Mendes JF, Templeton P. Biological, psychological, and social determinants of depression: a review of recent literature. *Brain Sciences*. 2021;11(12):1633.
7. Shadrina M, Bondarenko EA, Slominsky PA. Genetics factors in major depression disease. *Frontiers in Psychiatry*. 2018;9:334.
8. Ménard C, Hodes GE, Russo SJ. Pathogenesis of depression: Insights from human and rodent studies. *Neuroscience*. 2016;321:138–162.
9. Dean J, Keshavan M. The neurobiology of depression: An integrated view. *Asian Journal of Psychiatry*. 2017;27:101–111.
10. Alwan S, Reefhuis J, Rasmussen SA, Olney RS, Friedman JM. Use of selective serotonin-reuptake inhibitors in pregnancy and the risk of birth defects. *New England Journal of Medicine*. 2007;356(26):2684–92.
11. Okagbue HI, Adamu PI, Bishop SA, Oguntunde PE, Opanuga AA, Akhmetshin EM. Systematic review of prevalence of antepartum depression during the trimesters of pregnancy. *Open Access Macedonian Journal of Medical Sciences*. 2019;7(9):1555–1560.
12. Luong-Thanh B-Y, Nguyen LH, Murray L, Eisner M, Valdebenito S, Hoang TD, et al. Depression and its associated factors among pregnant women in central Vietnam. *Health Psychology Open*. 2021;8(1):2055102920988445.
13. Figueiredo B, Conde A. Anxiety and depression in women and men from early pregnancy to 3-months postpartum. *Archives of Women's Mental Health*. 2011;14(3):247–255.
14. Teixeira C, Figueiredo B, Conde A, Pacheco A, Costa R. Anxiety and depression during pregnancy in women and men. *Journal of Affective Disorders*. 2009;119(1–3):142–148.
15. Staneva A, Bogossian F, Pritchard M, Wittkowski A. The effects of maternal depression, anxiety, and perceived stress during pregnancy on preterm birth: A systematic review. *Women and Birth*. 2015;28(3):179–193.
16. Kornum JB, Nielsen RB, Pedersen L, Mortensen PB, Nørgaard M. Use of selective serotonin-reuptake inhibitors during early pregnancy and risk of congenital malformations: updated analysis. *Clinical Epidemiology*. 2010;2:29.
17. Bérard A, Zhao J-P, Sheehy O. Sertraline use during pregnancy and the risk of major malformations. *American Journal of Obstetrics and Gynecology*. 2015;212(6):795. e1–e12.
18. Dubovicky M, Belovicova K, Csatlosova K, Bogi E. Risks of using SSRI/SNRI antidepressants during pregnancy and lactation. *Interdisciplinary Toxicology.* 2017;10(1):30.
19. Shelton RC. Serotonin and norepinephrine reuptake inhibitors. Antidepressants: Springer; 2018 p. 145–180.
20. Polen KN, Rasmussen SA, Riehle-Colarusso T, Reefhuis J, National Birth Defects Prevention Study. Association between reported venlafaxine use in early pregnancy and birth defects, national birth defects prevention study, 1997–2007. Birth Defects Research Part A: *Clinical and Molecular Teratology*. 2013;97(1):28–35.
21. Patil AS, Kuller JA, Rhee EH. Antidepressants in pregnancy: a review of commonly prescribed medications. *Obstetrical & Gynecological Survey*. 2011;66(12):777–787.
22. Law R, Bozzo P, Koren G, Einarson A. FDA pregnancy risk categories and the CPS: do they help or are they a hindrance? *Canadian Family Physician*. 2010;56(3):239–241.

23. Hostetter A, Ritchie JC, Stowe ZN. Amniotic fluid and umbilical cord blood concentrations of antidepressants in three women. *Biological Psychiatry*. 2000;48(10):1032–1034.
24. Rampono J, Simmer K, Ilett K, Hackett L, Doherty D, Elliot R, et al. Placental transfer of SSRI and SNRI antidepressants and effects on the neonate. *Pharmacopsychiatry*. 2009;42(03):95–100.
25. Anderson KN, Lind JN, Simeone RM, Bobo WV, Mitchell AA, Riehle-Colarusso T, et al. Maternal use of specific antidepressant medications during early pregnancy and the risk of selected birth defects. *JAMA Psychiatry*. 2020;77(12):1246–1255.
26. Dubovický M, Császárová E, Brnoliaková Z, Ujházy E, Navarová J, Mach M. Effect of prenatal administration of venlafaxine on postnatal development of rat offspring. *Interdisciplinary Toxicology*. 2012;5(2):92.
27. Tamási V, Petschner P, Adori C, Kirilly E, Ando RD, Tothfalusi L, et al. Transcriptional evidence for the role of chronic venlafaxine treatment in neurotrophic signaling and neuroplasticity including also glutatmatergic-and insulin-mediated neuronal processes. *PloS One*. 2014;9(11):e113662.
28. Stiles J, Jernigan TL. The basics of brain development. *Neuropsychology Review*. 2010;20(4):327–348.
29. Silbereis JC, Pochareddy S, Zhu Y, Li M, Sestan N. The cellular and molecular landscapes of the developing human central nervous system. *Neuron*. 2016;89(2):248–268.
30. Cardoso-Moreira M, Halbert J, Valloton D, Velten B, Chen C, Shao Y, et al. Gene expression across mammalian organ development. *Nature*. 2019;571(7766):505–509.
31. Kursula P. Shanks—multidomain molecular scaffolds of the postsynaptic density. *Current Opinion in Structural Biology*. 2019;54:122–128.
32. Delling JP, Boeckers TM. Comparison of SHANK3 deficiency in animal models: Phenotypes, treatment strategies, and translational implications. *Journal of Neurodevelopmental Disorders*. 2021;13(1):1–37.
33. Durand CM, Perroy J, Loll F, Perrais D, Fagni L, Bourgeron T, et al. SHANK3 mutations identified in autism lead to modification of dendritic spine morphology via an actin-dependent mechanism. *Molecular Psychiatry*. 2012;17(1):71–84.
34. Costales JL, Kolevzon A. Phelan–McDermid syndrome and SHANK3: implications for treatment. *Neurotherapeutics*. 2015;12(3):620–360.
35. Alexandrov PN, Zhao Y, Jaber V, Cong L, Lukiw WJ. Deficits in the proline-rich synapse-associated Shank3 protein in multiple neuropsychiatric disorders. *Frontiers in Neurology*. 2017:670.
36. Logan CM, Menko AS. Microtubules: Evolving roles and critical cellular interactions. *Experimental Biology and Medicine*. 2019;244(15):1240–1254.
37. Kapitein LC, Hoogenraad CC. Building the neuronal microtubule cytoskeleton. *Neuron*. 2015;873:492–506.
38. Ngo L, Haas M, Qu Z, Li SS, Zenker J, Teng KSL, et al. TUBB5 and its disease-associated mutations influence the terminal differentiation and dendritic spine densities of cerebral cortical neurons. *Human Molecular Genetics*. 2014;23(19):5147–5158.
39. Breuss M, Heng JI-T, Poirier K, Tian G, Jaglin XH, Qu Z, et al. Mutations in the β-tubulin gene TUBB5 cause microcephaly with structural brain abnormalities. *Cell Reports*. 2012;2(6):1554–1562.
40. Shih D-F, Hsiao C-D, Min M-Y, Lai W-S, Yang C-W, Lee W-T, et al. Aromatic L-amino acid decarboxylase (AADC) is crucial for brain development and motor functions. *PLoS One*. 2013;8(8):e71741.
41. Brun L, Ngu L, Keng W, Ch'Ng G, Choy Y, Hwu W, et al. Clinical and biochemical features of aromatic L-amino acid decarboxylase deficiency. *Neurology*. 2010;75(1):64–71.
42. Eisenberg DP, Kohn PD, Hegarty CE, Ianni AM, Kolachana B, Gregory MD, et al. Common variation in the DOPA decarboxylase (DDC) gene and human striatal DDC activity in vivo. *Neuropsychopharmacology*. 2016;41(9):2303–2308.
43. Cohen-Cory S, Kidane AH, Shirkey NJ, Marshak S. Brain-derived neurotrophic factor and the development of structural neuronal connectivity. *Developmental Neurobiology*. 2010;70(5):271–288.
44. Pulvers JN, Journiac N, Arai Y, Nardelli J. MCPH1: a window into brain development and evolution. *Frontiers in Cellular Neuroscience*. 2015;9:92.
45. Tang BL. Molecular genetic determinants of human brain size. *Biochemical and Biophysical Research Communications*. 2006;345(3):911–916.