Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Date

Serum Dopamine Beta Hydroxylase (DβH) enzyme activity changes within individual women according to stage of pregnancy: Evidence that regulation of expression of DβH differs during pregnancy in women carrying different genotypes at rs1611115.

By
Dannie Lynn Perdomo
Master of Science

Graduate Division of Biology and Biomedical Science

Genetics and Molecular Biology

Joseph F. Cubells, M.D. Ph.D.
Advisor

Michael P Epstein, Ph.D.
Committee Member

Judith Fridovich-Keil, Ph.D.
Committee Member

David Weinshenker, Ph.D.
Committee Member

Michael E Zwick, Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

July, 19th, 2012
Date

Serum Dopamine Beta Hydroxylase (DβH) enzyme activity changes within individual women according to stage of pregnancy: Evidence that regulation of expression of DβH differs during pregnancy in women carrying different genotypes at rs1611115.

By

Dannie L. Perdomo

Bachelor of Science

Advisor: Joseph F. Cubells, M.D., Ph.D.

An abstract of

A thesis submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Master of Science

in the Graduate Division of Biology and Biomedical Science

Genetics and Molecular Biology

2012

Abstract

Serum Dopamine Beta Hydroxylase (DβH) enzyme activity changes within individual women according to stage of pregnancy: Evidence that regulation of expression of DβH differs during pregnancy in women carrying different genotypes at rs1611115.

By Dannie L. Perdomo

Dopamine Beta Hydroxylase (DβH) is a vesicular enzyme that plays a crucial role in the catecholaminergic pathway by converting dopamine into norepinephrine. DβH activity levels are easily assayed in serum due to the release of DβH along with NE from noradrenergic neurons as well as from chromaffin granules of the adrenal medulla. Early efforts to use serum DβH as an index of sympathetic activity rapidly led to the finding that serum DβH activity is regulated largely by genetic inheritance. Subsequent work has demonstrated conclusively that *DBH* is the major quantitative trait locus regulating serum DβH.

Dbh -/- mouse embryos die *in utero* and mothers of persons born with the rare Mendelian disorder of DβH deficiency report high rates of miscarriage (Senard and Rouet 2006). In the current study, we examine serum DβH activity across pregnancy in women seeking care for mental illness. We hypothesized that: (1) serum DβH activity will change over the course of pregnancy; (2) those changes will be affected by the main effect SNP, rs1611115, a well-documented functional SNP at *DBH* that accounts or ~50% of the variance in serum DβH activity. Serum samples were drawn during the pre-conception period, the 1st, 2nd, and 3rd trimester, and the early (first 8 weeks) and late (9- 25 weeks) post partum period. Serum DβH activity decreases across each trimester in pregnancy with its lowest point in the 3rd Trimester (3) ($t=-10.95$, $p<.0001$; Figure 1). It then begins to recover after delivery to Preconception (0) levels ($t=.94$; p=NS). Consistent with previous reports, the CC genotype associated with higher serum DβH activity than the CT or TT genotypes ($F=51.18$; $p<.0001$). There is a significant interaction between genotype at 1611115 and time in pregnancy ($F=3.39$, $p<0.008$), suggesting that women who carry the TT (low-serum DβH-associated) genotype regulate expression of the *DBH* gene differently across pregnancy than those carrying at least one C allele.

Serum Dopamine Beta Hydroxylase (DβH) enzyme activity changes within individual women according to stage of pregnancy: Evidence that regulation of expression of DβH differs during pregnancy in women carrying different genotypes at rs1611115.

By: Dannie L. Perdomo

Bethel University, B.S. 2006

Advisor:

Joseph F. Cubells, MD, Ph.D.

A Thesis submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of

Master of Science

Graduate Division of Biological and Biomedical Sciences

Genetics and Molecular Biology

May 2012

Acknowledgements

Before all else I must thank the LORD my God, the Father, the Son, and the Holy Ghost. No one has loved and guided me the way He has; any Good I do in my life is for His Glory. Next, I would like to thank my parents Roger C. and Johnnie Sue Perdomo whose faith in me never wavered. My friends and family in San Diego and Minnesota, who I have greatly missed here in Atlanta, thank you for supporting me. To the salsa community for giving me a release, and to all of my brothers and sisters in Christ, you have been my family while I was far from home. To my PI and committee, thank you for your patience and guidance. To my fellow classmates, thank you for your company and your humility. Some special thanks go to those who have trained and mentored me in science (and sometimes in life): Wenbiao Li, Adriana Lori, Yilang Tang, and Alicia Smith. In no way could I have done this without you; teachers like you are worth their weight in gold.

Table of Contents

Chapter 1

Introduction: Serum Dopamine β-Hydroxylase (DβH) Activity During Pregnancy: genotype-dependent regulation of a heritable biochemical trait in humans.

Dopamine β hydroxylase (DβH) is the catecholamine synthetic enzyme that catalyzes the conversion of dopamine to norepinephrine (Figure 1). The major form of the active DβH enzyme is a ~ 300,000-Dalton homo-tetramer, with each monomer bound to an atom of copper (Cu; Frigon et al. 1987). Also known as Dopamine β-monooxygenase, DβH is a member of the monooxygenase family of enzymes. DβH is able to split molecular oxygen using ascorbic acid as an electron donor, and attaches a hydroxyl group to the beta carbon of dopamine to create norepinephrine. Copper is an essential *in vivo* co-factor, but Pyrroloquinoline quinone (PQQ), and Fumarate may also act as co-factors (Friedman and Kaufman 1965). In its active form DβH may exist as a dimer, but more commonly, as a tetramer, each unit of which exists as a 72kDa subunit with a covalent Cu attached (Ash, Papadopoulos et al. 1984). Interestingly, a mutation in the mouse ATP7B Cu transporter, without any change in levels of DBH RNA production, caused a reduction in dopamine beta-hydroxylase in mouse adrenals; thus, the essential relationship between DβH and Cu is very apparent (Gerbasi, Lutsenko et al. 2003). DβH is naturally active at a pH of 5.0 in vesicles of noradrenergic neurons, chromaffin granules of the adrenal medulla, and endocrine cells. It is a heavily glycosylated enzyme with mannose, glucose, fructose, glucosamine, and galactose as well as sialic acid covalent attachments. It is an exceptionally stable enzyme outside of the body. In fact, in liquid solution at 37˚ C it is stable for 7 days without a significant drop in activity (Brimijoin 1975).

In the periphery, DβH enzyme is found in chromaffin granules of adrenal medulla neurosecretory cells and in sympathetic neurons (all of which are derived from neural crest). In the central nervous system, DβH is expressed in several small groups of

noradrenergic and adrenergic neurons located in the pons and medulla oblongata. The largest of these cell groups, which accounts for ~60% of the noradrenergic neurons in the brain, is the locus coeruleus. The enzyme occurs in membrane-bound and soluble forms within vesicles, and it is the soluble fraction that is released into the circulation during exocytosis.

The levels of DβH in circulation do not correlate with the levels of epinephrine or norepinephrine (which are released in a 17:3 ratio, respectively) in the sympathetic nervous system (SNS) "fight or flight" response with increased heart rate, blood pressure, metabolism, and vasodilation of skeletal muscles. Instead, DβH serum levels build up to a steady state that is reached in early childhood (~5 years of age), remain remarkably stable through most of adulthood (Weinshilboum 1978), and then decline slowly with advancing age in the later decades of life (Shores, White et al. 1999). One of the most interesting attributes of serum DβH is that it is a quantitative trait that varies widely (~100-fold) across individuals. Strong evidence suggests that ~80% of the variance in serum DβH activity can be attributed to inheritance at a single locus on chromosome 9q24 at the gene *DBH* (Cubells and Zabetian, 2004; Cubells et al., 2011). Although cold presser stimulation, exercise and other perturbations of the SNS result in dramatic changes in catecholamines including norepinephrine and epinephrine, DβH Activity is not significantly altered. In our closest relatives, monkeys and great apes, as well as other mammals, serum DβH activity tends to be 10 to 100 times lower than in humans. Therefore serum DβH activity is a unique, measurable, and highly heritable human quantitative trait.

Serum DβH has natural inhibitors in the blood stream including, but not limited to: peroxides, Copper chelators, Copper sulfate, Nitric Oxide (NO), monoamine oxidase inhibitors (MAO-Is), thiol-containing substances such as cystamine, and fusaric acid.

Human serum DβH activity: an accessible human genetic trait regulated largely by a single gene.

Unique among the catecholamine-synthetic enzymes in its localization within catecholamine secreting synaptic vesicles (or chromaffin granules of the adrenal medulla), DβH is released by exocytosis into the extracellular space upon release of norepinephrine or epinephrine from those vesicles (de Potter, PW, et al. 1969). Exocytotic release of soluble DβH is thought to account for its appearance in the plasma and serum, although constitutive release of DβH protein has also been reported in cultured cells (Oyarce and Fleming, 1991).

Human serum DβH activity is a highly heritable trait, the genetic variation of which is predominantly regulated by the structural gene *DBH*. Approximately 80% of the variance in serum DβH activity is regulated by genetic inheritance; furthermore, SNPs at *DBH* that account for ~ 50% of the total variance and ~ 60% of the genetic variance have been described in prior studies (Cubells and Zabetian 2004; Chen et al., 2010). However, more work is probably necessary to catalogue all the functional genetic variation at *DBH* (and possibly in other loci) impacting serum DβH activity. A re-sequencing study of DNA from individuals exhibiting extreme values of plasma DβH activity by Zabetian et al (2001) revealed that a single nucleotide polymorphism (SNP), rs1611115, located approximately 1kb upstream of the translation start site, strongly

associated with variation in serum DβH enzyme activity. Specifically, those with an rs1611115 genotype of CC showed high DBH activity, the CT individuals showed intermediate activity, and the TT individuals showed very low but still measureable DβH levels (Zabetian et al., 2001). In that study, genotype at rs1611115 accounted for 31-52% of the variance in serum DβH activity, depending on the predominant ancestry of the research participants (European-American, African-American, or Japanese).

Many studies have replicated the strong association of rs1611115 to variation in serum DβH activity, and more specifically, of the T allele of that SNP with lower serum DβH activity (Cubells and Zabetian 2004) (Tang, Anderson et al. 2005) (Chen, Wen et al. 2010). Given its location in 5' region of the *DBH* gene, the strong association of the T allele at rs1611115 with low serum DβH activity suggests the obvious hypothesis that it must also associate with lower expression of the *DBH* gene due to diminished transcription as compared to that driven by the C allele-containing sequence. However, recent transient transfection of reporter-gene constructs in rat pheochromocytoma-derived PC-12 cells suggested that the T allele at rs1611115 associates with *higher* transcriptional activity than the (high-serum DβH activity-associated) C allele (Chen, Wen et al. 2010) and (Chen, Zhang et al. 2011). It remains unclear why the *in vitro* and *in cella* experiments of Chen and colleagues indicate that the T allele at rs1611115 associates with a transcriptional effect that apparently opposes its strong association with human serum DβH activity. The authors of the paper speculated that the association of the T allele with lower serum DβH activity might reflect a neural regulatory effect, in which greater transcription of central *DBH* might lead to lower expression in the periphery. However, that hypothesis has yet to be tested. Experiments are currently under way to use

bacterial-artificial chromosome (BAC) transgenesis to express the C and T alleles of rs1611115 in separate lines of *Dbh -/-* mice, in order to examine the *in vivo* effect of that polymorphism on central and peripheral expression of DβH (Cubells, Weinshenker, Perdomo, unpublished data). Regardless of the mechanism of the apparently opposite effects of rs1611115 on *in cella* transcription and *in vivo* protein levels, the observation of a strong effect of rs1611115 on transcription of reporter constructs provides important evidence that the SNP exerts a direct regulatory effect on transcription, rather than merely being in linkage disequilibrium with another functional SNP.

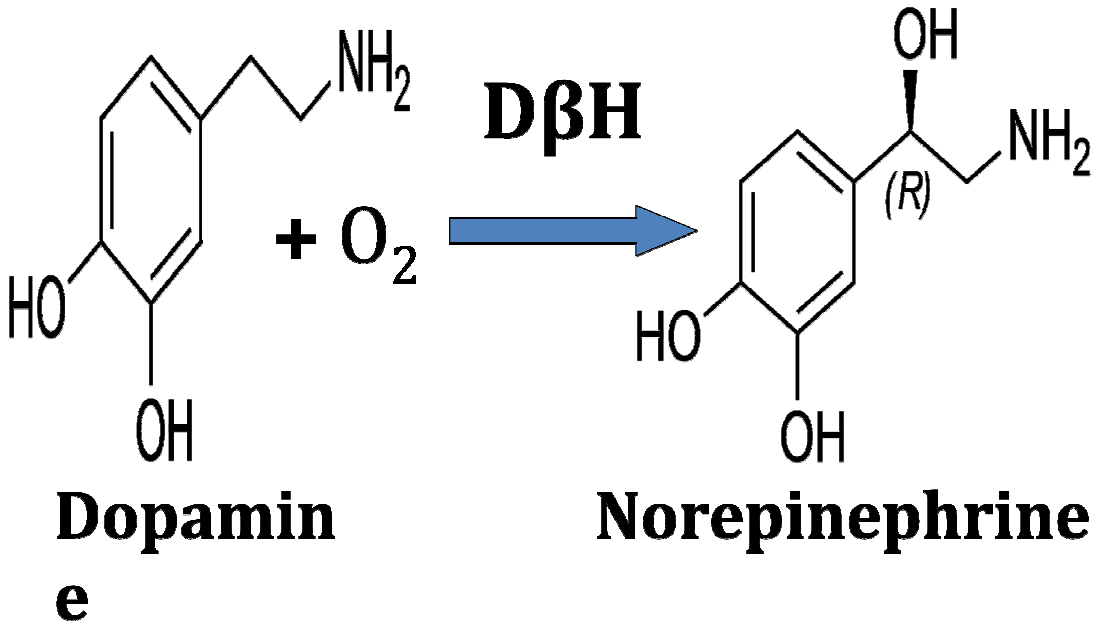


Figure 1: Dopamine Beta Hydroxylase (DβH) is the catecholamine synthetic enzyme that catalyzes the conversion of Dopamine and Molecular Oxygen into Norepinephrine.

Chapter 2

Genotype-controlled analysis of serum dopamine β-hydroxylase (DβH) activity during pregnancy and the post-partum period in women with psychiatric disorders

Information presented in this chapter will be submitted for publication as cited below:

Dannie L. Perdomo, W. Li, R.W. Bonsall, Y.L., Tang, D.J. Newport, B. Knight, Z.N. Stowe, and J.F. Cubells. Genotype-controlled analysis of serum dopamine β-hydroxylase (DβH) activity during pregnancy and the post-partum period in women with psychiatric disorders. Target Journal: American Journal of Medical Genetics, Part B (Neuropsychiatric Genetics).

Introduction

Dopamine β-Hydroxylase (DβH) is the catecholamine synthetic enzyme that catalyzes the conversion of dopamine (DA) to norepinephrine (NE). DβH is expressed in noradrenergic and adrenergic neurons, as well as in catecholamine-secreting endocrine

cells. It is unique among the catecholamine synthetic enzymes by virtue of its localization inside secretory vesicles (synaptic vesicles or chromaffin granules) from which it is released via exocytosis into the extracellular space during synaptic release of norepinephrine from those vesicles [8]. As a result, soluble intravesicular DβH protein is released along with NE from noradrenergic neurons of the peripheral nervous system into the circulation, where it can readily be assayed in plasma or serum, either as enzyme activity under saturating co-factor and substrate conditions, or as levels of DβH protein detected by immuno-assay methods; the two types of measures are highly correlated and to a first approximation, serum DβH activity (sDβH) represents levels of circulating DβH protein (Weinshilboum 1978; O'Connor, Cervenka et al. 1994).

Early studies, which attempted to use serum DβH activity (sDβH) as an index of acute changes in sympathetic tone revealed that sDβH is in fact remarkably stable within individuals, despite wide variation (~100-fold) in sDβH across individuals within the human population. Even extreme stimuli such as exercise or cold-presser stimulation which dramatically acute release of NE from sympathetic nerve terminals result in little or no changes in sDβH within individuals (Reid and Kopin 1974). Similarly, prolonged pharmacological modification of plasma NE and sympathetic nervous activity in the rat did not alter sDβH (Horwitz, Alexander et al. 1973; Reid and Kopin 1974).

Family and twin studies showed that sDβH is a genetic trait, with approximately 80% of the variance in sDβH activity accounted for by genetic inheritance (Ross 1973) (Weinshilboum 1978). Linkage (Elston et al 1979; Goldin et al., 1982; Wilson et al. 1988; Cubells et al., 2011), and association studies (Wei et al., 1997; Cubells et al., 1998; Zabetian et al., 2001) subsequently identified the DBH locus as the major quantitative

trait locus regulating sDβH. One particular single nucleotide polymorphism (SNP), rs1611115, residing 970 bp upstream of the transcriptional start site of DBH, accounts for 30-50% of the variance in sDβH in humans of diverse geographical ancestry (Cubells, van Kammen et al. 1998; Cubells, Kranzler et al. 2000; Zabetian, Anderson et al. 2001). Studies of rs1611115 in transient-transfection assays using reporter constructs for expression, and of protein-DNA interactions in gel-mobility-shift experiments, support the hypothesis that rs1611115 is a functional variant that alters the binding of transcription factors to DBH, leading to differences in transcription (Chen et al., 2010).

Acute alterations of the sympathetic nervous system (SNS) such as exercise or cold-pressor paradigms do not appreciably alter short term sDβH. Therefore, sDβH probably represents a steady state of circulating DβH protein, in which ongoing sympathetic release of minute quantities of DβH protein adds DβH while slow degradation of the protein removes it (Weinshilboum 1978). Severe chronic alcoholism, in which the noradrenergic nervous system is known to sustain damage, results in diminished levels of sDβH independent of the effects of rs1611115 (Kohnke, Zabetian et al. 2002), and decreases in sDβH have also been observed in neurodegenerative diseases such as Alzheimer disease (Miyata, Nagata et al. 1984). Major depression with psychotic features also results in a genotype-independent decrease of sDβH, possibly representing chronic functional down-regulation of DβH expression (Cubells, Price et al. 2002). We hypothesized that long-term alterations in sympathetic function, such as those that occur during pregnancy, would similarly result in alterations of sDβH that would also associate with changes in sDβH that are independent of DBH genotype.

Pregnancy alters maternal hemodynamics in complex ways, including increased cardiac output, decreased peripheral vascular resistance, and increased plasma volume. These changes return to baseline shortly after parturition (Fu and Levine 2009) thus providing an opportunity to examine the consequences of a relatively long-term increase in sympathetic outflow (during pregnancy), as well as its return to pre-gravid baseline (during the post-partum period), on sDβH. We hypothesized that pregnancy would result in altered sDβH that would subsequently return to pre-gravid baseline in the post-partum period. Furthermore, we hypothesized that women of differing genotype at rs1611115 would exhibit differences in the pattern of change in sDβH, because evidence suggests that the SNP alters regulation of transcription of DBH. The present study tested these hypotheses by characterizing DβH activity from pre-conception, across pregnancy and into the post-partum period. Our hypothesis is that DβH activity levels change during pregnancy and that those changes vary according to genotype at DBH, as well as by the physiological stages of pregnancy, and that some of the variation in sDβH would be attributable to an interaction between those main predictors.

Methods

Biological samples and Human Subjects

All biological samples were donated by participants in research projects of the Emory Women's Mental Health Program (WMHP), a referral center for the treatment of and research on peri-natal psychiatric illness. The referral sources include: 1) mental health professionals (psychiatrists, psychologists and other health based centers); 2) obstetrical care providers (obstetricians, nurse midwives, doulas, etc.); 3) community based health centers and fliers. Women with a personal or family history of psychiatric illness (n=205) were enrolled prior to 14 weeks gestation and followed through delivery. Written informed consent was obtained for each woman following procedures approved by the Emory University Institutional Review Board. Research procedures included gathering of demographic, psychiatric and medical data, as well as collection of venous blood for separation of serum and extraction of DNA. All subjects completed an intake questionnaire collecting demographic and clinical data, and 160 (78%) of the subjects completed the Structured Clinical Interview for *DSM-IV* (SCID (First, Spitzer et al. 2002). Since prior studies have shown that psychiatric diagnosis as well as a large variety of medications exert little to no influence on sDβH (reviewed by Cubells and Zabetian, 2004), we did not attempt to select samples for analysis on the basis of those factors. DNA and serum samples from women carrying singleton pregnancies were chosen for analysis according to the following criteria: 1) the donor was of European descent, 2) serum samples of at least 0.1 ml per sample were available from at least three of the following periods associated with pregnancy: pre-conception, the first second or third trimester, early (<8 weeks) or late (≥ 9 weeks) post-partum period, 3) blood-derived

genomic DNA available for genotyping and 4) the pregnancy resulted in delivery of a live infant. The study procedures involving contact with human subjects were performed following procedures approved by the Emory University Institutional Review Board.

Data were collected, and biological samples stored at -80^{o} C, from the years 2000-2010. Demographic and clinical data that were recorded to be used as potential associated variables in the analysis included: age, parity, gravida, bdi score, smoking status. Biochemical and genotyping assays were performed on large batches of samples to minimize assay-to-assay variation.

DβH Enzyme Assay

Venous blood was collected into non-heparinized tubes, and serum was prepared by centrifugation. All samples were stored frozen at -80˚C until analyzed. Serum DβH enzyme activity levels were assessed using high performance liquid chromatography with electrochemical detection (HPLC-ECD) using an enzymatic assay described by (Nagatsu and Udenfriend 1972) (see review). This assay was modified to isolate and quantify the reaction product, octopamine, and to determine oxidized octopamine levels from only a 5µL of serum. DβH activity was determined in 5-µl aliquots of serum as the rate of conversion of tyramine to octopamine (Nagatsu and Udenfriend 1972; Cubells, van Kammen et al. 1998).

Genotyping

Blood was drawn into EDTA-containing tubes, and DNA was extracted using the Invitrogen pure clean genomic DNA blood extraction kit. Genotyping of DBH rs1611115 was performed using a validated Taqman SNP assay (C-2535789_10) from Applied

Biosystems. All genotypes were in Hardy-Weinberg Equilibrium $\chi^2 = 0.53$, $p > 0.25$ (Supplementary Table 1).

Statistical analysis

Data were normalized using a square root transformation (Zabetian et al., 2001), after which there were no gross violations of the linearity, homoscedasticity, or normality assumptions. Serum DβH Activity (μmoL·min-1·L-1) for 151 women was the dependent continuous variable. Predictor variables included time in pregnancy by Trimester (Table 1) and rs1611115 genotype under the assumption of a co-dominant model.

The relationship between the dependent variable and each predictor variable was evaluated using linear mixed models. All analyses were performed using SAS 9.2 (Cary, NC); mixed models were run using PROC MIXED.

Results

Table 2 summarizes the mean and standard deviation of sDβH, age of mother in years, parity, gravida and the number and percent of each categorical value genotype, smoking status for all the mothers in the study. Because it was a clinical cohort, psychiatric diagnosis and treatment varies among subjects. For example, 48 of 129 women took an SSRI as monotherapy (Supplementary Table 2). Mean and standard deviations of sDβH Activity levels are given in Table 3; since transformation was necessary to normalize DβH Activity values (Supplementary Figure 1) square-root values are also given (Table 3). Serum DβH Activity levels associate with genotype and pregnancy time point. Examined without accounting for genotype, sDβH decreases across each trimester in pregnancy with its lowest point in the 3rd Trimester (3) ($t=-10.95$, $p<.0001$; Figure 1), then begins to recover after delivery to Preconception (0) levels ($t=.94$; p=NS) (Figure 1). Consistent with previous reports, the CC genotype associated with higher serum DBH activity than the CT or TT genotypes ($F=51.18$; $p<.0001$) (Figure 2). Notably, there is a significant interaction between genotype and time in pregnancy ($F=3.39$, $p<0.008$) (Figure 3).

Discussion

In this study, we evaluated the relationship between serum DβH Activity and time points before, during, and after pregnancy. Consistent with previous studies, we found that sDβH strongly associated with genotype at rs1611115 (Zabetian, Anderson et al. 2001) (Tang, Buxbaum et al. 2006) (Tang, Epstein et al. 2007). Furthermore, the overall pattern of genotype-dependence of sDβH was similar at each time point throughout the study, with the rank order of mean sDβH remaining CC>CT>TT for the three genotypes at rs1611115. However, in addition to the effect of genotype at rs1611115, time in pregnancy also associated with a main effect on sDβH. The data therefore confirmed our hypothesis that sDβH changes dramatically during pregnancy, but contrary to our expectation that sDβH would rise during pregnancy as a result of increased sympathetic activity, the overall measure declined, probably reflecting a dilutional effect due the large increase in plasma volume known to occur during pregnancy. Finally, the presence of a significant genotype x time-in-pregnancy interaction indicates that the pregnancy-related change in sDβH differs in women of different genotype. This latter observation is notable, because (i) it cannot be explained by a plasma-volume effect, and (ii) it suggests that regulation of sDβH differs across pregnancy depending on genotype at rs1611115.

Previous studies of DβH and pregnancy have been limited, but it seems that DβH activity does fluctuate subtly in response to hormonal variation (Rockson, Stone et al. 1975), such as during the menstrual cycle or during contraceptive use (Lamprecht, Matta et al. 1974; Redmond, Murphy et al. 1975). Research in the 1970s suggested that women who experienced contraceptive-induced increases in mean arterial pressure (MAP) also exhibited parallel increases in DβH activity; suggesting that in susceptible individuals,

oral contraceptive-induced stimulus to the sympathetic nervous system lead to increases in MAP (Rockson, Stone et al. 1975). However, those findings should be viewed cautiously, considering the small sample sizes (and resulting limited statistical power), and the fact that none of those studies accounted for the strong effect of *DBH* genotype on sDβH. They were therefore confounded to an known degree by unmeasured genetic variation among the subjects.

Possible explanations for sDβH variation during pregnancy are: hormonal influences, stress pathways such as the classic HPA-axis or other pathways are altered, a direct influence of fetal molecules (including the fetuses' own DβH enzyme, their stress systems, their hormonal changes, etc). Another important factor that almost certainly influences the measurement of sDβH during pregnancy is dilution of the enzyme activity due to physiological expansion of plasma volume during pregnancy. Beginning as early as the fourth week of pregnancy, plasma and red blood cell volumes increase by 40% and 25%, respectively and peak around the 28th week (Jim, Sharma et al. 2010). The volume of blood filtered by the kidneys reaches a maximum between 16 and 24 weeks and remains at the maximum until immediately before delivery (Karabulut, Baki Yagci et al. 2003). During pregnancy, the amount of blood pumped by the heart (cardiac output) increases by 30 to 50% (Longo 1983). By the end of pregnancy, the uterus is receiving one fifth of the woman's pre-pregnancy blood supply. Blood pressure usually decreases during the 2nd trimester but may return to a normal pre-pregnancy level in the 3rd trimester (Nama, Antonios et al. 2011).

The observation of a significant genotype x time-in-pregnancy interaction in our analysis of sDβH is the most noteworthy observation of the current study. This

interaction provides evidence that regulation of *DBH* expression in TT individuals might differ from that of C-carrying individuals in the setting of prolonged substantial alterations of sympathetic function. Moreover, there was a large difference between the baseline means of sDβH in CC, CT and TT genotyped individuals. An interaction between genotype and changes in sDβH is unlikely to represent a dilution effect. If anything, assuming an equivalent dilution affects across all genotype groups, one might speculate that TT women up-regulate sDβH more robustly relative to baseline compared to C carriers. TT individuals have means that, if anything, rise slightly over pregnancy, while they clearly dip in CC and CT women (Figure 3). One caveat of course, is that because the TT group is smallest, and their sDβH is the lowest to begin with, the measurement of sDβH was the least precise in the TT group (Table 3). Therefore, additional genotype-adjusted studies of sDβH across pregnancy are necessary before we firmly conclude the pregnancy-genotype interaction observed in this study is true.

Our analyses were conducted in samples gathered from European American women seeking care for psychiatric illness during pregnancy; and thus cannot be generalized neither to healthy pregnancy, nor to women with other medical disorders during pregnancy. Since the study depended on available banked samples, our data set was incomplete: only 20 out of 129 women had serum samples at all six time points examined (preconception through late Post-Partum). Although the mixed model analysis we performed was able to accommodate missing data, future studies should ideally involve more complete sample collection across pregnancy, and sampling of healthy women.

Supplementary Table 1: Hardy-Weinberg Equilibrium				
Genotype	**Count**	**Frequency**	**Allele**	**Frequency**
CC	68	.53	C	.72
CT	49	.38	T	.28
TT	12	.09		χ^2= 0.53
Total	129			p >0.25

χ^2= Chi-squared, p- probability

Table 1: Defined Time Periods of Pregnancy	
Time Period (Trimester)	**Definition In Weeks**
Preconception (0)	-40 - -0.1
1st Trimester (1)	0-13.9
2nd Trimester (2)	14-27.9
3rd Trimester (3)	28-40+
1st Post-partum (4)	0-8.9
2nd Post-partum (5)	9-40

Trimester: 0= Preconception, 1= 1st Trimester, 2= 2nd Trimester, 3= 3rd Trimester, 4= 1st Post-Partum, 5= 2nd Post-Partum (Note: through out paper, unless otherwise mentioned, all references to time across pregnancy are similarly coded).

Table 2. Descriptive statistics including age, parity, and BDI for each mother.	
Genotyped (N=129)	Mean (SD) or N (%)
rs1611115: CC = 2	68
CT = 1	49
TT = 0	12
BDI score	15.5 (9.6)
smoking while pregnant	11 (0.9%)
Age of Mother (in years)	

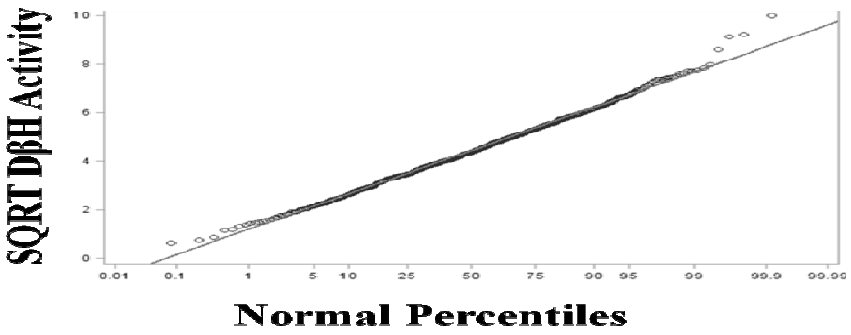
Supplementary Table 2: Medication Regime For the Moms	
Medication	N
SSRI[1] Only	48
SSRI[1] Plus Other Psychotropics	31
SSRI[1] Plus Other Antidepressants	6
SSRI[1] Plus Other Antidepressants & Psychotropics	6
NO SSRI[1]/ Other Antidepressants	16
NO SSRI[1]/ Other Psychotropics	8
NO SSRI[1]/ Other Antidepressants & Psychotropics	6
No SSRI[1] Or Psychotropics	8

SSRI- Selective Serotonin Reuptake Inhibitor

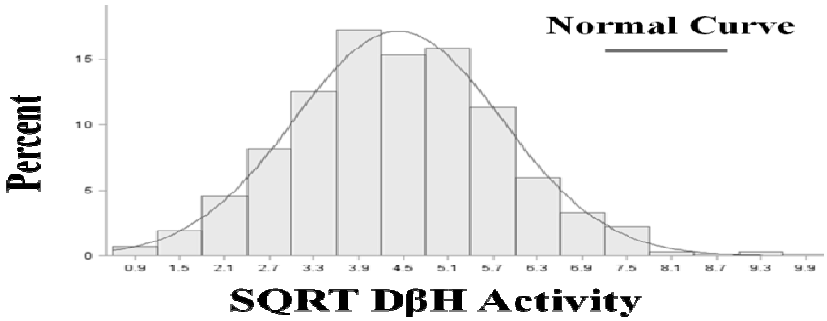
Table 3: Descriptive Statistics of DβH Activity for the all samples			
Time	Count	Mean (Stdev)	**SQRT** Mean (Stdev)
Preconception (0)	33	29.2 (15.6)	5.2 (1.6)
1st trimester (1)	146	6.2 (14.8)	4.9 (1.6)
2nd trimester (2)	145	19.5 (10.9)	4.3 (1.3)
3rd trimester (3)	148	15.6 (8.6)	3.8 (1.1)
1st post-partum (4)	126	21.3 (11.8)	4.5 (1.4)
2nd post-partum (5)	140	24.2 (15)	4.7 (1.5)
All Times (0-5)	738	21.7 (13.1)	4.4 (1.4)

Where Square-root DβH Activity is defined as the SQRT of **µmols of octopamine formed/ minute/liter of serum at 37°C [√(µmoL·min-1·L-1)].**

a.



b.



Supplementary Figure 1 a, b.

a. Q-Q Plot for the Distribution of all values of SQRT DBH Activity. B. Histogram of all values of SQRT DBH Activity with a superimposed normal distribution curve.

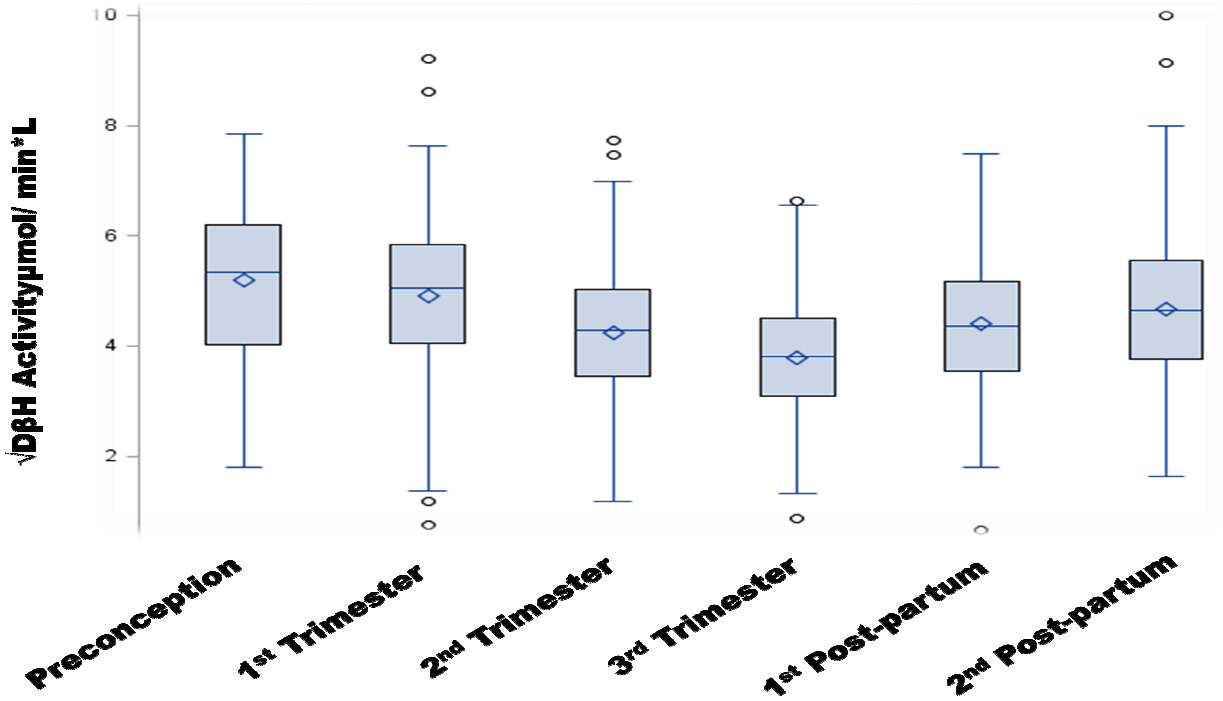


Figure 1: Box-plots of SQRT DBH Activity across Pregnancy. Y- axis: Square root (SQRT) of DβH Activity, X- Axis: Trimester where 0= Preconception, 1= 1st Trimester, 2= 2nd Trimester, 3= 3rd Trimester, 4= 1st Post-Partum, 5= 2nd Post-Partum (Note: through-out this paper, unless otherwise mentioned, all references to time across pregnancy are similarly coded).

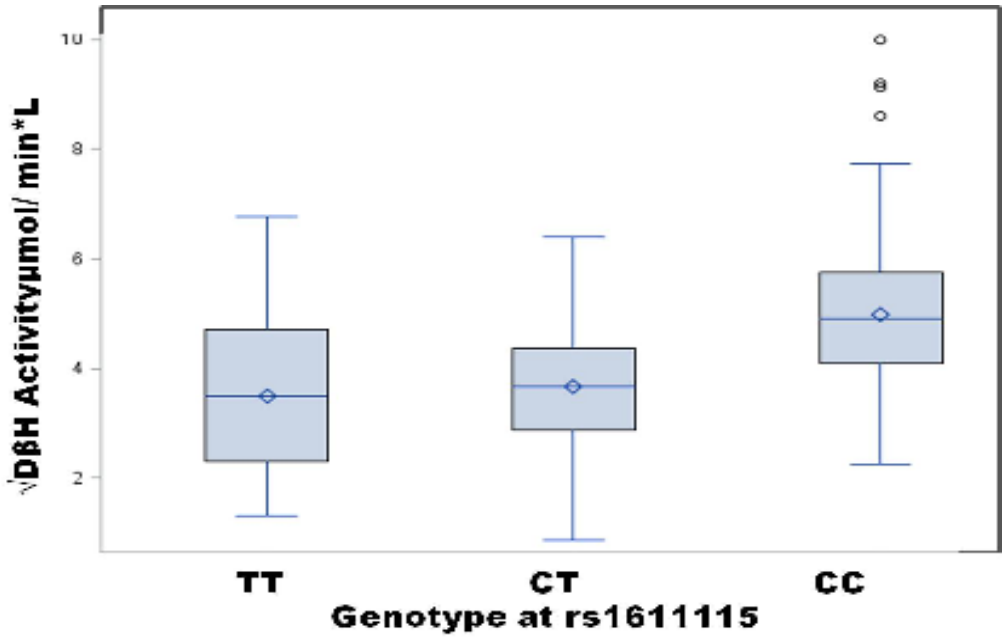


Figure 2: Box-plots of SQRT DBH Activity Relative to Genotype. Y- axis: Square root (SQRT) of DβH Activity, X- Axis: Genotype at rs1611115 (approximately -970 bp ahead of the DβH transcription start site).

Table 4: Descriptive Statistics

Genotype	Preconception	1st Trimester	2nd Trimester	3rd Trimester	1st Post-Partum	2nd Post-Partum
CC	18	84	65	66	52	56
CT	9	45	46	48	41	43
TT	3	12	12	12	10	10
Total	**30**	**123**	**123**	**126**	**129**	**129**

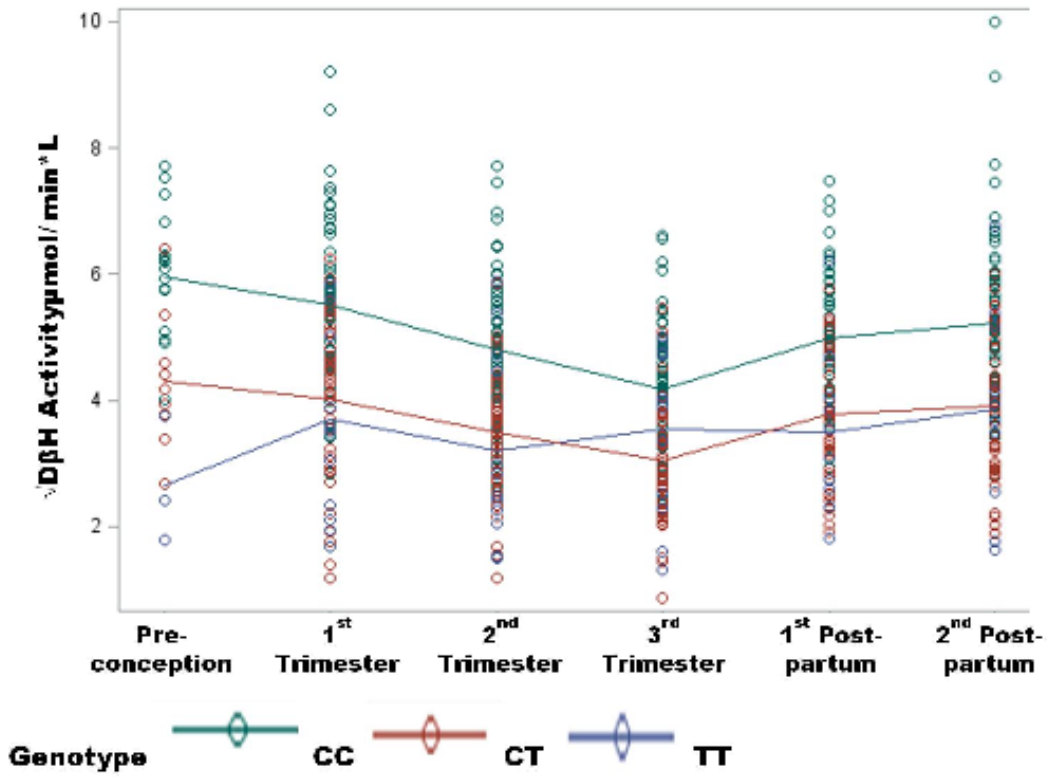


Figure 3. Genotype Dependent Serum DβH Activity across Pregnancy. Mean serum DβH Activity in μmol/ minutes*liter, is dependent both on Genotype at rs1611115 as well as Time in pregnancy. Our statistics show a significant interaction between time in pregnancy and genotype ($F=3.39$, $p>0.008$). Bars are +/- standard error.

Chapter 3: Supplemental Methods

Additional Notes on Methods

DβH Activity levels by HPLC-ECD: After incubating serum in reaction buffer, DßH converts the substrate Tyramine to Octopamine, High Performance Liquid Chromatography with Electrochemical Detection separates the reactants; activity is calculated according to the resultant electrochemical shift (Figure 1).

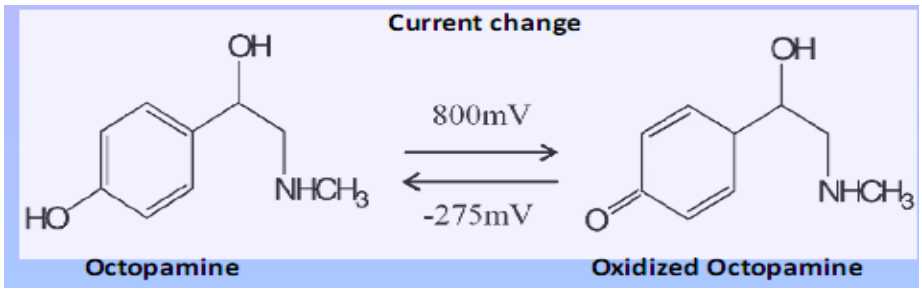
The assay uses tyramine as a substrate, and measures the production of octopamine, using electrochemical detection after separation of product from reactants by HPLC was modified to isolate and quantify the reaction product, octopamine, and to determine oxidized octopamine levels.

Briefly, octopamine was measured by a column-switching, reverse phase HPLC-ECD system, using coulometric electrochemical detection (a method which is ~ 10-15 fold more sensitive than fluorometric detection), with synephrine as an internal standard. DβH activity was reported as μmols of octopamine formed per minute from a solution of tyramine (0.2 mol/L) by one liter of serum at 37˚C (μmols/min*L) (Nagatsu and Udenfriend 1972). The DβH enzyme is optimal at a pH of 5.0; we therefore used Sodium Acetate as a Buffer. DβH is Cu2+ dependent, and requires both molecular oxygen and Ascorbic Acid (which was provided at an optimal amount of 3~5μmoL/L) as co-factors. In the reaction O_2 serves as an oxygen donor and Ascorbic Acid serves as a Proton donor. In addition, endogenous inhibitors of the enzyme exist in the serum. In our reaction, N-ethylmaleimide is used for the inhibition of endogenous inhibitors, while pargyline is used to specifically inhibit endogenous monoamine oxidases. Vmax occurs at 60min with

incubation at 37°C, when tyramine is used as substrate. Both Sodium Fumarate and Catalase serve in enzyme activation as well as a means to remove any hydrogen peroxide (a chemical to which DβH is sensitive).Tyramine was used as a substrate for the reaction instead of DβH's *in vivo* substrate Dopamine, because both tyramine and its product octopamine are less reactive than DA and NE and therefore are more convenient metabolites for analytic purposes. This approach markedly simplifies the assay and allows measurement of enzyme activity in 5 μL or less of serum. An ESA Coulochem II electrochemical detector was used to provide 'on-column sensitivity' for octopamine at10 pg. All assays were run in duplicate, with quality-control samples ensuring a coefficient of variation between assays of < 10%.

Statistical tests

We used SAS version 9.2 to conduct statistical analysis including linear regressions and correlations, etc. Because we were measuring both between separate women as well as at different times within the same women, a mixed model using with repeated measures approach was used.



a.

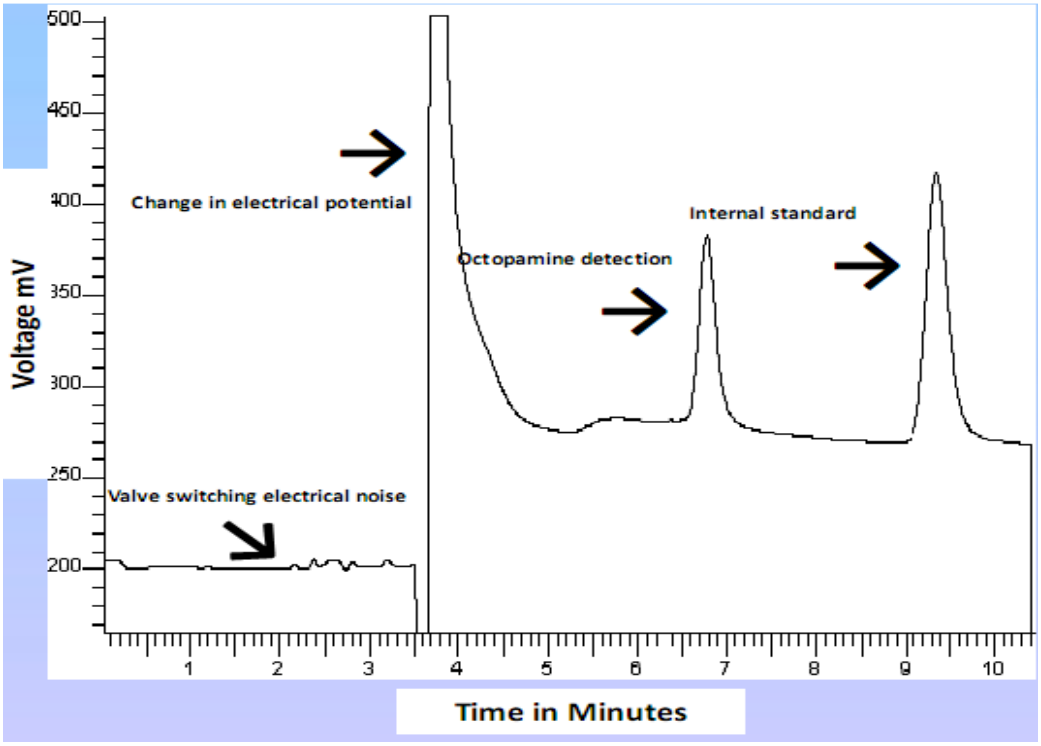


Figure 1: HPLC-ECD: Chromatography of Octopamine and Synephrine by column-switching, reverse phase HPLC system, using coulometric electrochemical detection, and synephrine as an internal standard. (µmoL·min-1·L-1). DβH activity = µmols of octopamine formed/ minute/liter of plasma at 37˚C **[µmoL·min-1·L-1].**

References

For DβH Review

Ash, D. E., N. J. Papadopoulos, et al. (1984). "Kinetic and spectroscopic studies of the interaction of copper with dopamine beta-hydroxylase." J Biol Chem **259**(6): 3395-3398.

Bartko, G., E. Frecska, et al. (1990). "Predicting neuroleptic response from a combination of multilevel variables in acute schizophrenic patients." Acta Psychiatr Scand **82**(6): 408-412.

Bennett, M. R. (1998). "Monoaminergic synapses and schizophrenia: 45 years of neuroleptics." J Psychopharmacol **12**(3): 289-304.

Biaggioni, I. and D. Robertson (1987). "Endogenous restoration of noradrenaline by precursor therapy in dopamine-beta-hydroxylase deficiency." Lancet **2**(8569): 1170-1172.

Bourdelat-Parks, B. N., G. M. Anderson, et al. (2005). "Effects of dopamine beta-hydroxylase genotype and disulfiram inhibition on catecholamine homeostasis in mice." Psychopharmacology (Berl) **183**(1): 72-80.

Chen, Y., G. Wen, et al. (2010). "Human dopamine beta-hydroxylase (DBH) regulatory polymorphism that influences enzymatic activity, autonomic function, and blood pressure." J Hypertens **28**(1): 76-86.

Chen, Y., K. Zhang, et al. (2011). "Human dopamine beta-hydroxylase promoter variant alters transcription in chromaffin cells, enzyme secretion, and blood pressure." Am J Hypertens **24**(1): 24-32.

Cheng, S. Y., L. I. Serova, et al. (2008). "Regulation of rat dopamine beta-hydroxylase gene transcription by early growth response gene 1 (Egr1)." Brain Res **1193**: 1-11.

Cubells, J. F., H. R. Kranzler, et al. (2000). "A haplotype at the DBH locus, associated with low plasma dopamine beta-hydroxylase activity, also associates with cocaine-induced paranoia." Mol Psychiatry **5**(1): 56-63.

Cubells, J. F., X. Sun, et al. (2011). "Linkage analysis of plasma dopamine beta-hydroxylase activity in families of patients with schizophrenia." Hum Genet **130**(5): 635-643.

Cubells, J. F., D. P. van Kammen, et al. (1998). "Dopamine beta-hydroxylase: two polymorphisms in linkage disequilibrium at the structural gene DBH associate with biochemical phenotypic variation." Hum Genet **102**(5): 533-540.

Cubells, J. F. and C. P. Zabetian (2004). "Human genetics of plasma dopamine beta-hydroxylase activity: applications to research in psychiatry and neurology." Psychopharmacology (Berl) **174**(4): 463-476.

D'Andrea, G., R. Ostuzzi, et al. (2008). "Study of tyrosine metabolism in eating disorders. Possible correlation with migraine." Neurol Sci **29 Suppl 1**: S88-92.

Das, P. C., W. K. McElroy, et al. (2003). "Potential mechanisms responsible for chlorotriazine-induced alterations in catecholamines in pheochromocytoma (PC12) cells." Life Sci **73**(24): 3123-3138.

Dunnette, J. and R. Weinshilboum (1979). "Human plasma dopamine-beta-hydroxylase: variation in thermal stability." Mol Pharmacol **15**(3): 649-660.

Friedman, S. and S. Kaufman (1965). "3,4-dihydroxyphenylethylamine beta-hydroxylase. Physical properties, copper content, and role of copper in the catalytic acttivity." J Biol Chem **240**(12): 4763-4773.

Ghosh, J., S. Pradhan, et al. (2011). "Role of dopaminergic gene polymorphisms (DBH 19 bp indel and DRD2 Nco I) in genetic susceptibility to migraine in North Indian population." Pain Med **12**(7): 1109-1111.

Grimm, J., A. Mueller, et al. (2004). "Molecular basis for catecholaminergic neuron diversity." Proc Natl Acad Sci U S A **101**(38): 13891-13896.

Gross, H. A., C. R. Lake, et al. (1979). "Catecholamine metabolism in primary anorexia nervosa." J Clin Endocrinol Metab **49**(6): 805-809.

Horwitz, D., R. W. Alexander, et al. (1973). "Human serum dopamine- -hydroxylase. Relationship to hypertension and sympathetic activity." Circ Res **32**(5): 594-599.

Jasmin, L., D. Tien, et al. (2002). "The NK1 receptor mediates both the hyperalgesia and the resistance to morphine in mice lacking noradrenaline." Proc Natl Acad Sci U S A **99**(2): 1029-1034.

Jepma, M., J. Deinum, et al. (2011). "Neurocognitive function in dopamine-beta-hydroxylase deficiency." Neuropsychopharmacology **36**(8): 1608-1619.

Ji, N., L. Shuai, et al. (2011). "Dopamine beta-hydroxylase gene associates with stroop color-word task performance in Han Chinese children with attention deficit/hyperactivity disorder." Am J Med Genet B Neuropsychiatr Genet **156**(6): 730-736.

Kalinin, S., V. Gavrilyuk, et al. (2007). "Noradrenaline deficiency in brain increases beta-amyloid plaque burden in an animal model of Alzheimer's disease." Neurobiol Aging **28**(8): 1206-1214.

Kim, C. H., A. Leung, et al. (2011). "Norepinephrine deficiency is caused by combined abnormal mRNA processing and defective protein trafficking of dopamine beta-hydroxylase." J Biol Chem **286**(11): 9196-9204.

Kohnke, M. D., C. P. Zabetian, et al. (2002). "A genotype-controlled analysis of plasma dopamine beta-hydroxylase in healthy and alcoholic subjects: evidence for alcohol-related differences in noradrenergic function." Biol Psychiatry **52**(12): 1151-1158.

Lea, R. A., A. Dohy, et al. (2000). "Evidence for allelic association of the dopamine beta-hydroxylase gene (DBH) with susceptibility to typical migraine." Neurogenetics **3**(1): 35-40.

Leslie, F. M., S. E. Loughlin, et al. (1985). "Noradrenergic changes and memory loss in aged mice." Brain Res **359**(1-2): 292-299.

Man in 't Veld, A. J., F. Boomsma, et al. (1987). "Congenital dopamine-beta-hydroxylase deficiency. A novel orthostatic syndrome." Lancet **1**(8526): 183-188.

Marino, M. D., B. N. Bourdelat-Parks, et al. (2005). "Genetic reduction of noradrenergic function alters social memory and reduces aggression in mice." Behav Brain Res **161**(2): 197-203.

Mathias, C. J., R. B. Bannister, et al. (1990). "Clinical, autonomic and therapeutic observations in two siblings with postural hypotension and sympathetic failure

due to an inability to synthesize noradrenaline from dopamine because of a deficiency of dopamine beta hydroxylase." Q J Med **75**(278): 617-633.

Mitchell, H. A., J. W. Bogenpohl, et al. (2008). "Behavioral responses of dopamine beta-hydroxylase knockout mice to modafinil suggest a dual noradrenergic-dopaminergic mechanism of action." Pharmacol Biochem Behav **91**(2): 217-222.

Morin, X., H. Cremer, et al. (1997). "Defects in sensory and autonomic ganglia and absence of locus coeruleus in mice deficient for the homeobox gene Phox2a." Neuron **18**(3): 411-423.

Mufson, E. J., J. Wuu, et al. (2010). "Preservation of cortical sortilin protein levels in MCI and Alzheimer's disease." Neurosci Lett **471**(3): 129-133.

Murchison, C. F., X. Y. Zhang, et al. (2004). "A distinct role for norepinephrine in memory retrieval." Cell **117**(1): 131-143.

O'Connor, D. T., J. H. Cervenka, et al. (1994). "Dopamine beta-hydroxylase immunoreactivity in human cerebrospinal fluid: properties, relationship to central noradrenergic neuronal activity and variation in Parkinson's disease and congenital dopamine beta-hydroxylase deficiency." Clin Sci (Lond) **86**(2): 149-158.

Olson, V. G., C. L. Heusner, et al. (2006). "Role of noradrenergic signaling by the nucleus tractus solitarius in mediating opiate reward." Science **311**(5763): 1017-1020.

Pace-Schott, E. F. and J. A. Hobson (2002). "The neurobiology of sleep: genetics, cellular physiology and subcortical networks." Nat Rev Neurosci **3**(8): 591-605.

Pattyn, A., X. Morin, et al. (1999). "The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives." Nature **399**(6734): 366-370.

Perez-Nievas, B. G., J. L. Madrigal, et al. (2010). "Corticosterone basal levels and vulnerability to LPS-induced neuroinflammation in the rat brain." Brain Res **1315**: 159-168.

Portbury, A. L., R. Chandra, et al. (2003). "Catecholamines act via a beta-adrenergic receptor to maintain fetal heart rate and survival." Am J Physiol Heart Circ Physiol **284**(6): H2069-2077.

Reimold, M., C. Solbach, et al. (2007). "Occupancy of dopamine D(1), D (2) and serotonin (2A) receptors in schizophrenic patients treated with flupentixol in comparison with risperidone and haloperidol." Psychopharmacology (Berl) **190**(2): 241-249.

Robertson, D., M. R. Goldberg, et al. (1986). "Isolated failure of autonomic noradrenergic neurotransmission. Evidence for impaired beta-hydroxylation of dopamine." N Engl J Med **314**(23): 1494-1497.

Robertson, D., V. Haile, et al. (1991). "Dopamine beta-hydroxylase deficiency. A genetic disorder of cardiovascular regulation." Hypertension **18**(1): 1-8.

Robinson, P. D., C. K. Schutz, et al. (2001). "Genetically determined low maternal serum dopamine beta-hydroxylase levels and the etiology of autism spectrum disorders." Am J Med Genet **100**(1): 30-36.

Rogeness, G. A., J. M. Hernandez, et al. (1982). "Biochemical differences in children with conduct disorder socialized and undersocialized." Am J Psychiatry **139**(3): 307-311.

Sapru, M. K., B. S. Rao, et al. (1989). "Serum dopamine-beta-hydroxylase activity in clinical subtypes of depression." Acta Psychiatr Scand **80**(5): 474-478.

Schank, J. R., R. Ventura, et al. (2006). "Dopamine beta-hydroxylase knockout mice have alterations in dopamine signaling and are hypersensitive to cocaine." Neuropsychopharmacology **31**(10): 2221-2230.

Shores, M. M., S. S. White, et al. (1999). "Tyrosine hydroxylase mRNA is increased in old age and norepinephrine uptake transporter mRNA is decreased in middle age in locus coeruleus of Brown-Norway rats." Brain Res **826**(1): 143-147.

Spitzer, R. L., J. Endicott, et al. (1975). "Research diagnostic criteria." Psychopharmacol Bull **11**(3): 22-25.

Sternberg, D. E., D. P. van Kammen, et al. (1983). "CSF dopamine beta-hydroxylase in schizophrenia." Arch Gen Psychiatry **40**(7): 743-747.

Sternberg, D. E., D. P. VanKammen, et al. (1982). "Schizophrenia: dopamine beta-hydroxylase activity and treatment response." Science **216**(4553): 1423-1425.

Stubbusch, J., A. Majdazari, et al. (2011). "Generation of the tamoxifen-inducible DBH-Cre transgenic mouse line DBH-CT." Genesis.

Szot, P., D. Weinshenker, et al. (1999). "Norepinephrine-deficient mice have increased susceptibility to seizure-inducing stimuli." J Neurosci **19**(24): 10985-10992.

Tang, Y., G. M. Anderson, et al. (2005). "Haplotype-controlled analysis of the association of a non-synonymous single nucleotide polymorphism at DBH (+ 1603C --> T) with plasma dopamine beta-hydroxylase activity." Am J Med Genet B Neuropsychiatr Genet **139B**(1): 88-90.

Tang, Y., S. G. Buxbaum, et al. (2006). "A single nucleotide polymorphism at DBH, possibly associated with attention-deficit/hyperactivity disorder, associates with lower plasma dopamine beta-hydroxylase activity and is in linkage disequilibrium with two putative functional single nucleotide polymorphisms." Biol Psychiatry **60**(10): 1034-1038.

Tang, Y. L., M. P. Epstein, et al. (2007). "Genotypic and haplotypic associations of the DBH gene with plasma dopamine beta-hydroxylase activity in African Americans." Eur J Hum Genet **15**(8): 878-883.

Thomas, D. W., R. B. Mannon, et al. (1998). "Coagulation defects and altered hemodynamic responses in mice lacking receptors for thromboxane A2." J Clin Invest **102**(11): 1994-2001.

Thomas, K. R. (1995). "The knockout mouse: six years old and growing stronger." Am J Respir Cell Mol Biol **12**(5): 461-463.

Thomas, S. A., B. T. Marck, et al. (1998). "Restoration of norepinephrine and reversal of phenotypes in mice lacking dopamine beta-hydroxylase." J Neurochem **70**(6): 2468-2476.

Thomas, S. A. and R. D. Palmiter (1997). "Impaired maternal behavior in mice lacking norepinephrine and epinephrine." Cell **91**(5): 583-592.

Thomas, S. A. and R. D. Palmiter (1997). "Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline." Nature **387**(6628): 94-97.

Viggiano, D., D. Vallone, et al. (2003). "Behavioural, pharmacological, morpho-functional molecular studies reveal a hyperfunctioning mesocortical dopamine

system in an animal model of attention deficit and hyperactivity disorder." Neurosci Biobehav Rev **27**(7): 683-689.

Weinshenker, D., N. S. Miller, et al. (2002). "Mice with chronic norepinephrine deficiency resemble amphetamine-sensitized animals." Proc Natl Acad Sci U S A **99**(21): 13873-13877.

Weinshenker, D., N. C. Rust, et al. (2000). "Ethanol-associated behaviors of mice lacking norepinephrine." J Neurosci **20**(9): 3157-3164.

Weinshenker, D. and J. P. Schroeder (2007). "There and back again: a tale of norepinephrine and drug addiction." Neuropsychopharmacology **32**(7): 1433-1451.

Weinshenker, D., P. Szot, et al. (2001). "Alpha(1) and beta(2) adrenoreceptor agonists inhibit pentylenetetrazole-induced seizures in mice lacking norepinephrine." J Pharmacol Exp Ther **298**(3): 1042-1048.

Weinshilboum, R. M. (1978). "Serum dopamine beta-hydroxylase." Pharmacol Rev **30**(2): 133-166.

Yamamoto, K., J. F. Cubells, et al. (2003). "Dopamine beta-hydroxylase (DBH) gene and schizophrenia phenotypic variability: a genetic association study." Am J Med Genet B Neuropsychiatr Genet **117B**(1): 33-38.

Zabetian, C. P., S. G. Buxbaum, et al. (2003). "The structure of linkage disequilibrium at the DBH locus strongly influences the magnitude of association between diallelic markers and plasma dopamine beta-hydroxylase activity." Am J Hum Genet **72**(6): 1389-1400.

Zhou, X., M. G. Espey, et al. (2000). "Inhibitory effects of nitric oxide and nitrosative stress on dopamine-beta-hydroxylase." J Biol Chem **275**(28): 21241-21246.

Zhu, M. Y., W. P. Wang, et al. (2005). "Age-associated changes in mRNA levels of Phox2, norepinephrine transporter and dopamine beta-hydroxylase in the locus coeruleus and adrenal glands of rats." J Neurochem **94**(3): 828-838.

For DβH & Pregnancy Paper

Ash, D. E., N. J. Papadopoulos, et al. (1984). "Kinetic and spectroscopic studies of the interaction of copper with dopamine beta-hydroxylase." J Biol Chem **259**(6): 3395-3398.

Chen, Y., G. Wen, et al. (2010). "Human dopamine beta-hydroxylase (DBH) regulatory polymorphism that influences enzymatic activity, autonomic function, and blood pressure." J Hypertens **28**(1): 76-86.

Chen, Y., K. Zhang, et al. (2011). "Human dopamine beta-hydroxylase promoter variant alters transcription in chromaffin cells, enzyme secretion, and blood pressure." Am J Hypertens **24**(1): 24-32.

Cubells, J. F., D. P. van Kammen, et al. (1998). "Dopamine beta-hydroxylase: two polymorphisms in linkage disequilibrium at the structural gene DBH associate with biochemical phenotypic variation." Hum Genet **102**(5): 533-540.

Cubells, J. F. and C. P. Zabetian (2004). "Human genetics of plasma dopamine beta-hydroxylase activity: applications to research in psychiatry and neurology." Psychopharmacology (Berl) **174**(4): 463-476.

First, M. B., R. L. Spitzer, et al. (2002). Structured Clinical Interview for DSM-IV-TR Axis I disorders, Research Version, Patient Edition (SCID-I/P). New York, New York State Psychiatric Institute.

Friedman, S. and S. Kaufman (1965). "3,4-dihydroxyphenylethylamine beta-hydroxylase. Physical properties, copper content, and role of copper in the catalytic acttivity." J Biol Chem **240**(12): 4763-4773.

Jim, B., S. Sharma, et al. (2010). "Hypertension in pregnancy: a comprehensive update." Cardiol Rev **18**(4): 178-189.

Karabulut, N., A. Baki Yagci, et al. (2003). "Renal vein Doppler ultrasound of maternal kidneys in normal second and third trimester pregnancy." Br J Radiol **76**(907): 444-447.

Lamprecht, F., R. J. Matta, et al. (1974). "Plasma dopamine-beta-hydroxylase (DBH) activity during the menstrual cycle." Psychosom Med **36**(4): 304-310.

Longo, L. D. (1983). "Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control." Am J Physiol **245**(5 Pt 1): R720-729.

Nagatsu, T. and S. Udenfriend (1972). "Photometric assay of dopamine- -hydroxylase activity in human blood." Clin Chem **18**(9): 980-983.

Nama, V., T. F. Antonios, et al. (2011). "Mid-trimester blood pressure drop in normal pregnancy: myth or reality?" J Hypertens **29**(4): 763-768.

Redmond, D. E., Jr., D. L. Murphy, et al. (1975). "Menstrual cycle and ovarian hormone effects on plasma and platelet monoamine oxidase (MAO) and plasma dopamine-beta-hydroxylase (DBH) activities in the rhesus monkey." Psychosom Med **37**(5): 417-428.

Rockson, S. G., R. A. Stone, et al. (1975). "Plasma dopamine-beta-hydroxylase activity in oral contraceptive hypertension." Circulation **51**(5): 916-923.

Shores, M. M., S. S. White, et al. (1999). "Tyrosine hydroxylase mRNA is increased in old age and norepinephrine uptake transporter mRNA is decreased in middle age in locus coeruleus of Brown-Norway rats." Brain Res **826**(1): 143-147.

Tang, Y., G. M. Anderson, et al. (2005). "Haplotype-controlled analysis of the association of a non-synonymous single nucleotide polymorphism at DBH (+ 1603C --> T) with plasma dopamine beta-hydroxylase activity." Am J Med Genet B Neuropsychiatr Genet **139B**(1): 88-90.

Tang, Y., S. G. Buxbaum, et al. (2006). "A single nucleotide polymorphism at DBH, possibly associated with attention-deficit/hyperactivity disorder, associates with lower plasma dopamine beta-hydroxylase activity and is in linkage disequilibrium with two putative functional single nucleotide polymorphisms." Biol Psychiatry **60**(10): 1034-1038.

Weinshilboum, R. M. (1978). "Serum dopamine beta-hydroxylase." Pharmacol Rev **30**(2): 133-166.

Zabetian, C. P., G. M. Anderson, et al. (2001). "A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus." Am J Hum Genet **68**(2): 515-522.