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Title: A cross-sectional study of the relationship between *CYP2D6* and *CYP2C19* variations and depression symptoms, for women taking SSRIs during pregnancy

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No authors have any relevant conflicts of interest to declare.

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These studies were performed in line with the principles of the Declaration of Helsinki. Studies were approved by the UBC/Children's and Women's Hospital ethics boards (cohort A: H06–70145; cohort O1: H00-70500; cohort O2: H05-70629).

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Informed consent was obtained from all individual participants included in the studies.

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Not applicable (no identifying information for any participant is included in the manuscript)

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Title: A cross-sectional study of the relationship between *CYP2D6* and *CYP2C19* variations and depression symptoms, for women taking SSRIs during pregnancy

Abstract

Purpose: Depression during pregnancy affects 10-15% of women, and 5% of women take antidepressants during pregnancy. Clinical guidelines provide recommendations for selective serotonin reuptake inhibitor (SSRI) drug choice and dose based on *CYP2D6* and *CYP2C19* genotype; however, they are based on evidence from non-pregnant cohorts. This study aimed to test the hypothesis that women with function-altering variants (increased, decreased, or no function) in these pharmacogenes, taking SSRIs prenatally, would have more depression symptoms than women whose pharmacogenetic variants are associated with normal SSRI metabolism.

Methods: Comprehensive *CYP2D6* and *CYP2C19* genotyping using a range of methods, including gene copy number analysis, was performed as secondary analyses on two longitudinal cohorts of pregnant women (*N*=83) taking the SSRIs paroxetine, citalopram, escitalopram, or sertraline. The Kruskal-Wallis test compared mean depression scores across four predicted metabolizer groups: poor (*n*=5), intermediate (*n*=10), normal (*n*=53), and ultrarapid (*n*=15).

Results: There were no significant differences between mean depression scores across the four metabolizer groups (H(3)=.73, *p*=.87, eta-squared= .029, epsilon-squared= .0089).

Conclusions: This is the first study of the relationship in pregnancy between *CYP2C19* pharmacogenetic variations and depression symptoms in the context of SSRI use. Findings from this initial study do not support the clinical use of pharmacogenetic testing for SSRI use during the second or third trimesters of pregnancy, but these findings should be confirmed in larger

cohorts. There is an urgent need for further research to clarify the utility of pharmacogenetic testing for pregnant women, especially as companies offering direct-to-consumer genetic testing expand their marketing efforts.

Keywords: Depression; Pregnancy; Pharmacogenetics; Treatment; SSRI

Introduction

Depression is common during the perinatal period, affecting 10-15% of women (O'Hara & Swain, 1996). Further, suicide is a leading cause of perinatal death (Knight et al., 2016; Lindahl et al., 2005). Untreated prenatal depression also negatively impacts maternal quality of life, and increases risk for preterm birth (Grigoriadis, VonderPorten, Mamisashvili, Tomlinson, et al., 2013). Antidepressants (e.g., selective serotonin reuptake inhibitors (SSRIs)) effectively treat depression (Cohen et al., 2006), and are used by ~5% of pregnant women (Daw et al., 2012; Hanley & Mintzes, 2014). However, prenatal antidepressant use may have risks for both mother (e.g., postpartum hemorrhage (Hanley et al., 2016)) and baby (e.g., poor neonatal adaptation syndrome (Grigoriadis, VonderPorten, Mamisashvili, Eady, et al., 2013)). Thus, the decision regarding whether to take antidepressants during pregnancy is complex.

Part of the complexity of this decision-making process stems from the need for women to evaluate the consequences of treatment options not only for themselves, but also for their fetuses (Hippman & Balneaves, 2018). Frequently, women report feeling that they have to make a trade-off between their own health and their baby's health. When attempting to weigh these risks against potential benefits, more information about the likelihood that antidepressants will alleviate and/or prevent their symptoms at given doses would be particularly helpful. Pharmacogenetic testing is a possible source of such insight.

There are clinical practice guidelines for pharmacogenetically-guided SSRI prescribing for the genes *CYP2D6* and *CYP2C19* (Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP), 2018; Hicks et al., 2015a). These highly polymorphic genes (Nofziger et al., 2020) produce enzymes of the same names, which are involved in the metabolism of many medications, including SSRIs. For the SSRI paroxetine, the

121 enzyme CYP2D6 is primarily responsible for metabolism (with CYP3A4 and CYP1A2 playing
122 secondary roles), while for the SSRIs citalopram, escitalopram and sertraline, the enzyme
123 CYP2C19 is primarily responsible for metabolism (with CYP2D6, CYP3A4, CYP2C9, and
124 CYP2B6 playing secondary roles). Some variants in these genes may impair function – causing
125 poor metabolism, and consequently, an increased risk of side effects and drug discontinuation.
126 Other variants may cause rapid or ultrarapid metabolism, whereby a drug breaks down more
127 quickly than normal metabolism, and – in the case of SSRIs – may leave inadequate levels for
128 symptom control. There is some evidence that such function-altering variants associated with
129 poor, rapid or ultrarapid metabolism can impact effective SSRI dose(Altar et al., 2013; Brandl et
130 al., 2014; Tsai et al., 2010), with prescribing recommendations that take these variants into
131 account articulated in the guidelines. However, these guidelines were based on research
132 conducted in non-pregnant cohorts.

133 Pregnancy impacts drug-metabolizing enzyme function regardless of genotype, with the
134 activity of CYP2D6 increasing by 25-200% (Ke et al., 2013; Tracy et al., 2005) and CYP2C19
135 decreasing by ~50% (McGready et al., 2003), particularly in the third trimester. Though higher
136 SSRI doses are generally required in late pregnancy to achieve pre-pregnancy serum
137 concentrations (Hostetter et al., 2000; Sit et al., 2008), the relative contribution of all factors
138 contributing to this need for a higher dose (e.g., changes in volume of distribution, glomerular
139 filtration rate, glucuronidation, enzyme function) is unclear, and the effect of genotype in
140 pregnancy is largely unknown. Impact of *CYP2D6* genotype on depression symptoms amongst
141 women taking SSRIs prenatally has been explored in only two studies(Bérard, Gaedigk, Sheehy,
142 Chambers, Roth, Bozzo, Johnson, Kao, Lavigne, Wolfe, Quinn, Dieter, & Zhao, 2017; Ververs

et al., 2009). There are no studies that have evaluated the impact of *CYP2C19* genotype during pregnancy.

Purpose

This study tested the hypothesis that women with function-altering variants in the pharmacogenes *CYP2D6* or *CYP2C19,* who took the SSRIs paroxetine, citalopram, escitalopram, or sertraline prenatally, would have more depression symptoms than women whose pharmacogenetic variants have been associated with normal SSRI metabolism.

Materials and Methods

This study was a secondary analysis of data collected in previous cohort studies.

Recruitment & study procedure for previous cohorts

Participants were recruited as part of two prospective, longitudinal cohort studies: the Austin cohort - cohort A (Hanley et al., 2013), and the Oberlander cohort - cohort O (Hanley et al., 2013) (Kennedy et al., 2016)(Text box 1 for details). Studies were approved by the UBC/Children's and Women's Hospital ethics boards (cohort A: H06–70145; cohort O1: H00-70500; cohort O2: H05-70629).

From these cohorts, participants were eligible for analysis if they: had a lifetime diagnosis of depression; were regularly taking paroxetine, sertraline, citalopram, or escitalopram for a minimum of two weeks prior to enrollment (to allow sufficient time for therapeutic response (Cox et al., 1987a); and had provided a DNA sample and an Edinburgh Postnatal Depression Scale (EPDS) score during pregnancy (*N*=83). Blood or extracted DNA samples from both cohorts were stored in -80° freezers between collection and analysis.

Depression symptom measurement

The EPDS is a self-report instrument with strong reliability (α=0.87)(Cox et al., 1987b), which was designed for perinatal use to assess symptoms of depression(Murray & Cox, 1990). Higher EPDS scores indicate more depression symptoms (range: 0-30).

Pharmacogenetic analyses

DNA was extracted from blood samples and quantified according to published protocol (Shukla et al., 2015). The following alleles were genotyped: *CYP2D6 *2 through *11, *13, *14, *17, *29, *36, *41, CYP2C19 *2, *3, *4, *17* (See Supplemental Table S1 for further details). Cohort A was analyzed using a custom pharmacogenetic panel consisting of pre-plated TaqMan assays (Applied Biosystems, now Thermo Fisher Scientific, Waltham, MA). Reactions (10 µl with 10 ng DNA per reaction) were performed in 384-well plates on the QuantStudio 7.0 Real Time PCR System (Thermo-Scientific). Cohort O1 was genotyped using restriction fragment length polymorphism (RFLP) assays carried out on a 6.6 kb long-range PCR (XL-PCR) fragment encompassing the *CYP2D6* gene. Cohort O2 was genotyped using commercially available TaqMan assays (Applied Biosystems, now Thermo Fisher Scientific, Waltham, MA) directly on gDNA. Eight µl reactions were performed in 96-well plates under conditions recommended by the manufacturer. Both cohorts were also interrogated for the presence of *CYP2D6* copy number variation (deletions and duplications). For variant classification procedures, see Text box 1.

Statistical analyses

We used descriptive statistics to summarize demographic variables, EPDS scores, metabolizer phenotypes, and standardized daily SSRI doses (prescribed daily dose (PDD)/defined daily dose (DDD) - the international classification system for drug utilization research recommended by the World Health Organization (WHO Collaborating Centre for Drug

Statistics Methodology, 2019)). We compared cohorts A and O using parametric or non-parametric tests, as appropriate.

To test the main hypothesis, we compared mean depression scores across the four groups (ultrarapid metabolizer (UM), normal metabolizer (NM), intermediate metabolizer (IM), and poor metabolizer (PM)) using the Kruskal-Wallis test. Because this was a secondary analysis of available data and considering the complexity of performing power analyses for non-parametric tests, we did not perform an *a priori* power calculation. The eta-squared measure and epsilon-squared estimate of effect sizes were calculated for the main comparison (Tomczak & Tomczak, 2014). Given the single hypothesis, the threshold for statistical significance was set at $\alpha=0.05$. Data analyses were conducted using SPSS version 25 (IBM Corp., Armonk, NY).

Results

Descriptive statistics

Participant characteristics and descriptive statistics are presented in Table 1. A range of EPDS scores were observed across standardized SSRI daily doses (Figure 1) and for each metabolizer group. Predictions of phenotype from genotype were as follows: 53 normal metabolizers, 15 ultrarapid metabolizers, 10 intermediate metabolizers, and 5 poor metabolizers (Table 2), which aligns with similar populations (Fricke-Galindo et al., 2016; LLerena et al., 2014).

Group comparisons

The Kruskal-Wallis test was selected for the main hypothesis because assumptions underlying ANCOVA (the preferred analysis) were found to be violated (all variables of interest were found to violate the assumption of normality, and none of the potential covariates of interest correlated with EPDS score). There was no statistically significant difference between

EPDS scores across the four predicted metabolizer groups (H(3)=.73, *p*=.87, eta-squared=.029 (Lenhard & Lenhard, 2016), epsilon-squared=.0089 (Tomczak & Tomczak, 2014))(Table 3; Figure 2). Results of further exploratory analyses to enable more precise comparisons to previous literature can be found in Supplemental results.

Discussion

This is the first study of *CYP2C19* variation in relation to depression symptoms in pregnancy, and the second interrogating pharmacogenetic variation in relation to depression symptoms and citalopram, escitalopram, and sertraline use in pregnancy, which highlights the dearth of research connecting genotype to phenotype within the context of SSRI use in pregnancy. We found no statistical difference between metabolizer group and EPDS scores amongst cohorts of women taking SSRIs in the second and third trimesters of pregnancy.

Our sample size was relatively small, which could suggest lack of power as an explanation for our finding of no significant difference. However, it is also important to consider effect size and clinical significance. The eta-squared effect size we observed is typically interpreted as being "small" in magnitude (Pedersen, n.d.). While eta-squared is a more commonly used measure of effect size, it is uncorrected and positively biased. Given that, we also calculated the epsilon-squared effect size, which is a corrected measure of effect size. The epsilon-squared effect size we found was less than .01, which has been characterized as "negligible" in magnitude (Rea & Parker, 1992). Detecting an effect of this magnitude would require a sample size of 216,769. Accordingly, the observed difference between groups does not appear to be of clinical significance; it has been proposed that a difference of clinical significance can be approximated by ½ the standard deviation of the mean score (Norman et al., 2003). In this case, ½ the standard deviation of the mean EPDS score (*M*=8.51; *SD*=5.56) is 2.78.

The largest difference between mean EPDS scores for the four metabolizer groups in our study is 1.87 (between PM and NM groups).

There are other possibilities that could explain our finding of no significant difference between metabolizer group and EPDS scores. Our metabolizer predictions were based on available data, synthesized in the CPIC and DPWG guidelines regarding genotype-guided dosing for SSRIs (Hicks et al., 2015b). However, these guidelines are intended for use in the general, non-pregnant population, and based on evidence using non-pregnant samples. Evidence shows that the activity of CYP450 enzymes differs during pregnancy (Pariente et al., 2016), specifically that pregnancy induces CYP2D6 expression levels which leads to an increase in activity (Ke et al., 2013; Tracy et al., 2005), and a decrease in CYP2C19 activity (McGready et al., 2003). Thus, metabolizer predictions based on data collected outside the prenatal context are likely not appropriate to apply during pregnancy.

There is also insufficient evidence at present to combine genotype information from *CYP2D6* and *CYP2C19* to predict SSRI metabolic capacity based on genotypes for both genes – even outside the perinatal context. It is possible that differences between EPDS scores would emerge for our sample if it becomes possible to use a holistic phenotype prediction algorithm incorporating genotype information for all genes in the SSRI metabolic pathways. However, this algorithm would likely also need to be modified for use in pregnancy, as suggested by pharmacokinetic studies (Deligiannidis et al., 2014). In particular, one study evaluated pharmacokinetic changes during pregnancy for citalopram, escitalopram, and sertraline, and corresponding depression symptoms, and found increased metabolism and increased depression symptoms in the third trimester of pregnancy (Sit et al., 2008). The authors suggested that the

pregnancy-induced activation of CYP2D6 overrides the pregnancy-induced inhibition of CYP2C19.

It is difficult to conclude for certain how our findings compare to the results of the first study that evaluated pharmacogenetic variation in relation to depression symptoms and the use of paroxetine in pregnancy (*N*=74). This study found that depression symptoms increased for their extensive/ultrarapid metabolizer group, but remained steady for their intermediate/poor metabolizer group (Ververs et al., 2009), however, the statistical analysis used to reach this conclusion was not specified, nor were any statistical values. This study only collected data in the second and third trimesters and found no significant differences in the proportions of women scoring above an EPDS cut-off score of 12 or more in their extensive/ultrarapid metabolizer group compared to their intermediate/poor metabolizer group at any time-point. They also reported higher depression scores overall for those in the intermediate/poor metabolizer group compared to the extensive/ultrarapid metabolizer group, which is contrary to theoretical expectations. We do note that this study obtained data at three prenatal time-points, and therefore was able to make comparisons of predicted phenotype, observed phenotype (plasma paroxetine concentrations), and EPDS scores across the second and third trimesters of pregnancy. Thus, it is possible that an influence of *CYP2D6* variation on depression symptoms in pregnancy amongst individuals taking paroxetine are only apprehensible intra-individually, over the course of pregnancy, although we also note that the previous study did not report a significant interaction term for depression symptoms in their model.

It is also difficult to conclude for certain how our findings compare to the results of the second study that evaluated pharmacogenetic variation in relation to depression symptoms and the use of antidepressants in pregnancy (*N*=246) because, while the article reports that EPDS

scores were collected in the third trimester, it does not report any results for the third trimester (Bérard, Gaedigk, Sheehy, Chambers, Roth, Bozzo, Johnson, Kao, Lavigne, Wolfe, Quinn, Dieter, Zhao, et al., 2017). The study does report a significantly higher proportion of women in the "faster" metabolizer group compared to "slow" metabolizers with depression scores in the first trimester above an EPDS cut-off of 13 or more (19.81 vs. 5.88%, p=0.049), but that this difference disappears in the second trimester.

Limitations

As already discussed, our sample size was small, however, the observed effect sizes and evaluation of clinical significance suggest that any potential differences that may exist between groups would be very small. We posit that it is more likely that predictions for metabolizer phenotype (that were based on available data from non-pregnant cohorts) were not appropriate for use during pregnancy. With an improved understanding of CYP2D6 and CYP2C19 enzyme activity in pregnancy, and the impact of *CYP2D6* and *CYP2C19* genetic variation on their activity in pregnancy, it would be possible to refine a prediction algorithm and re-test our hypothesis.

It is also possible that confounding variables masked the impact of *CYP2D6* and *CYP2C19* genetic variation in our sample, such as SSRI dose, maternal weight, cigarette smoking, and co-medication with substances that have competing or interacting impacts on the CYP system. Unfortunately, including these covariates in our analysis was not possible given either the limitations of secondary data analyses, or violations of the assumptions underlying our preferred analytic approach - ANCOVA. In particular, it is worth noting that no relationship was observed between predicted metabolizer status and standardized daily dose. Theoretically, it might be expected that individuals who are poor metabolizers might end up titrating to a lower

dose, compared to normal metabolizers, through trial and error, while ultra-rapid metabolizers might titrate to a higher dose. No such relationship was observed in this sample, which further meant that it wasn't possible to include standardized daily dose as a covariate in an ANCOVA. Additionally, SSRI plasma concentrations were not available to explore the impact of these potential confounders more directly.

Data on SSRI side effects and adherence were not available (again, due to limitations of secondary data analyses). However, this would be a greater concern for a Type I error if there were a statistically significant difference between the groups. Theoretically, individuals who are poor metabolizers have the greatest risk for side effects and – consequently – low adherence. Individuals who are ultra-rapid metabolizers have the lowest risk for side effects and low adherence. This could impact depression scores such that there would be a larger difference between poor metabolizers and ultra-rapid metabolizers, that would be partially due to the differences in SSRI adherence (with side effects as a mediating variable). This risk is mitigated because no difference in EPDS scores across the groups was observed in these data.

Further, it is possible that participants may have been miscategorized in terms of predicted phenotype because we did not fully sequence *CYP2D6* and *CYP2C19* for all participants. However, the alleles that were not tested for all participants are rare, and the testing that was completed for all participants was chosen based on observed population allele frequencies (greater than 1% minor allele frequency in one or more in the 1000 Genomes Project major continental population groups). These assays have been validated to ensure 99.5% genotyping accuracy.

Clinical implications & future research

Results from this study do not support the clinical use of pharmacogenetic testing for SSRI prescribing during the second and third trimesters of pregnancy. Our results, in the context of previous findings and the body of literature documenting changes in drug-metabolizing enzyme function in pregnancy (Ke et al., 2013; McGready et al., 2003; Tracy et al., 2005), suggest that there is insufficient evidence at this time for the application of the practice guidelines for *CYP2D6/CYP2C19*-guided SSRI dosing in the second or third trimesters of pregnancy. However, it is important for clinicians to be aware that women might seek this testing from companies that offer it directly to consumers. Clinicians could proactively explore women's illness and medication necessity beliefs and share what is currently known regarding the causes of perinatal depression, ideally referring to a psychiatric genetic counsellor to best support this discussion (Inglis et al., 2017). Further, clinicians could consider sharing information about available direct-to-consumer pharmacogenetic testing, along with current limits regarding its interpretation and clinical application.

Additional research is warranted before pharmacogenetic testing can offer women guidance for the personalization of antidepressant medication choice and dose during pregnancy. Historically, clinical trials evaluating antidepressant medications have not prioritized the inclusion of women, and – in fact – have specifically excluded pregnant women (Galea et al., 2019; van der Zande et al., 2017; Yonkers & Brawman-Mintzer, 2002). Thus, our knowledge of the function of antidepressants in pregnant women is woefully inadequate. Even our knowledge of the function of antidepressants in general populations does not currently allow for combining results of pharmacogenetic testing of different genes, such as *CYP2D6* and *CYP2C19*, in a holistic phenotype prediction algorithm. Avenues for future research include: 1) the impact of pharmacogenetic variants of multiple genes together on phenotype (e.g., polygenic risk scores

346 for the contributions of pharmacogenetic variation to SSRI metabolism); 2) the function of
347 enzymes relevant to SSRI metabolism during pregnancy; 3) the function of metabolic pathways
348 responsible for antidepressants other than SSRIs and their relationships to underlying genomic
349 variation; and 4) the impact of pharmacogene variation (via metabolic activity) on maternal and
350 infant outcomes, including for women taking multiple antidepressants at the same time.

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Figure Captions

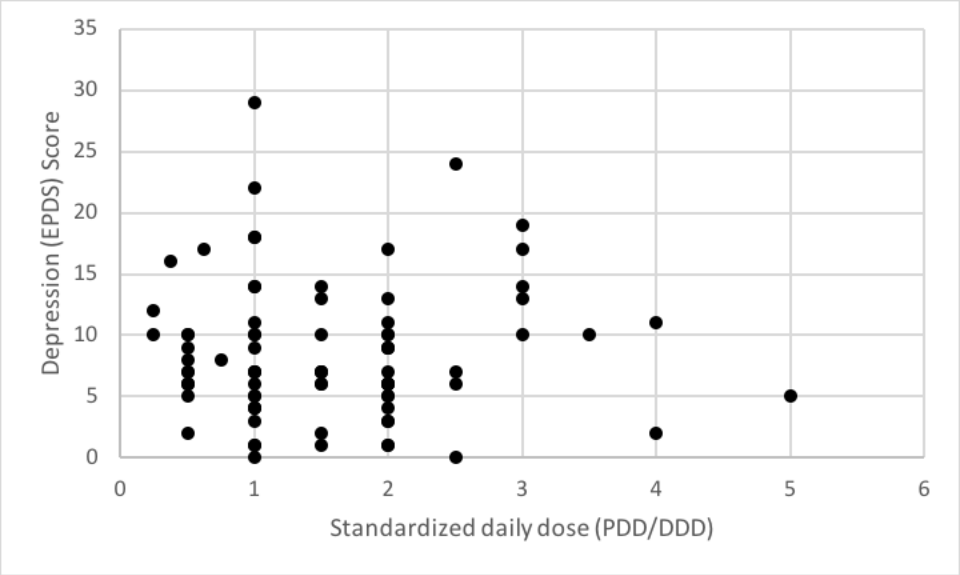
Fig. 1 Depression (EPDS) scores across standardized selective serotonin reuptake inhibitor (SSRI) daily doses (prescribed daily dose (PDD) / defined daily dose (DDD))

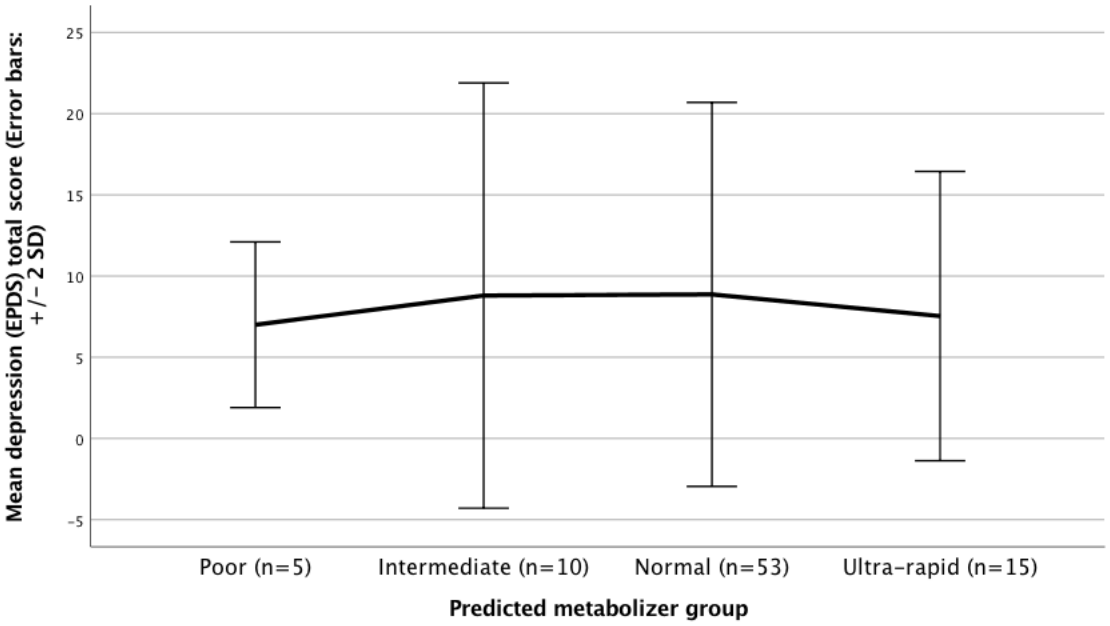
Fig. 2 Mean depression (EPDS) scores (+/- 2 standard deviations (SD)) for each predicted metabolizer group

Supplementary Information Captions

Online resource 1: Table S1 Genotyping summary

Online resource 2: Supplemental results


Depression (EPDS) Score
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
Standardized daily dose (PDD/DDD)


Mean depression (EPDS) total score (Error bars: +/− 2 SD)
25
20
15
10
5
0
-5
Poor (n=5)
Intermediate (n=10)
Normal (n=53)
Ultra-rapid (n=15)
Predicted metabolizer group

Text box 1. Methodology details: Data collection and interpretation procedures

Recruitment and study procedures for the two cohorts

Cohort A was recruited between 2007 – 2016. Prenatal data collection for this cohort involved one visit for enrollment, occurring at 15 weeks gestation or later. This enrollment visit occurred either at participants' homes or at the BC Children's and Women's Hospital. Cohort O was comprised of two sub-cohorts, with very similar procedures and characteristics – cohort O1 recruited between 2002 – 2005, cohort O2 from 2006 – 2010. Prenatal data collection for this cohort involved an enrollment visit in the second trimester of pregnancy, and a visit between 33-36 weeks gestation. These visits occurred at the BC Children's and Women's Hospital. All cohorts were pregnant, English-speaking women recruited from the Greater Vancouver area through community advertising or from the British Columbia (BC) Reproductive Mental Health specialty clinic. As part of extensive data collection including clinical interviews, questionnaires, and blood draws, participants completed the Edinburgh Postnatal Depression Scale (EPDS) to measure symptoms of depression and provided details in terms of SSRI dose (if applicable), weight, and gestational age (self-reported) at least once during pregnancy. For cohort O, data from the 33-36 week visit were used for this study.

Pharmacogenetic variant classification

To predict metabolizer phenotype from participant genotype, it was first necessary to translate genotype to star allele classification. For both *CYP2D6* and *CYP2C19,* classification of genotype to star allele were made as recommended by the Pharmacogene Variation Consortium (PharmVar) at https://www.pharmvar.org/ (Gaedigk et al., 2018). From star alleles, it was then possible to classify by metabolic functional status using the supplemental data available as a companion to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for

CYP2D6 and *CYP2C19* genotype-guided SSRI dosing (Hicks et al., 2015)(*CYP2D6*-Supplemental Table S2; *CYP2C19*-Supplemental Table S5). For *CYP2D6* genotype, activity scores were assigned as follows: ultrarapid metabolizer (UM)>2; normal metabolizer (NM)=2, 1.5, 1; intermediate metabolizer (IM)=0.5; poor metabolizer (PM)=0. The functional status classifications for each allele were then combined (two alleles per gene per participant) into two predicted metabolizer phenotypes (one for each gene) for each participant (*CYP2D6*-Supplemental Table S14; *CYP2C19*-Supplemental Table S15). Given the lack of published protocol for combining functional status classifications from multiple genes into one holistic metabolizer phenotype prediction for each participant, the predicted metabolizer phenotype group that was used for analysis for each participant was assigned based on the primary metabolic pathway for the SSRI used. In accordance with both CPIC and Dutch Pharmacogenetics Working Group (DPWG) guidelines (Dutch Pharmacogenetics Working Group (DPWG) of the Royal Dutch Pharmacists Association (KNMP), 2018; Hicks et al., 2015), predicted metabolizer group was assigned based on *CYP2D6* genotype for participants taking paroxetine, and based on *CYP2C19* genotype for participants taking sertraline, citalopram, or escitalopram.

Table 1. Participant characteristics and descriptive statistics (*N*=83)

Characteristic	**Number of participants (%) or Mean (SD; Range)**			
	Total	Cohort A (*n*=46)	Cohort O (*n*=37)	Difference between Cohorts A and O?
SSRI taken				
Paroxetine	28 (33.7%)	9 (19.6)	19 (51.4)	(p = .003, Fisher's Exact Test)
Citalopram	25 (30.1%)	16 (34.8)	9 (24.3)	
Sertraline	18 (21.7%)	10 (21.7)	8 (21.6)	
Escitalopram	12 (14.5%)	11 (23.9)	1 (2.7)	
SSRI standardized daily dose	1.56 (.93; .25-5)	1.53 (.87; .25-4)	1.59 (1.01; .25-5)	ns
Age (years)	31.69 (5.41; 19-44)	31.29 (6.13; 19-44)	32.16 (4.43; 24-40)	ns
Gestational age (weeks)[a]	32.28 (5.19; 15-39)	30.65 (6.44; 15.86-39.14)	34.31 (1.37; 32.85-37.28)	$t(49.98) = 3.75$, $p < .001$
Second trimester	12 (14.5)	12 (26.1)	0 (0)	$\chi^2(1) = 11.28$, $p = .001$
Third trimester	71 (85.5)	34 (73.9)	37 (100)	
Weight (kg)	78.44 (12.5; 53-126.10)	80.26 (13.58; 53-126.10)	76.28 (10.87; 59.4-103.5)	ns
Education (years)	15.91 (2.96; 10-29)	16.05 (2.43; 10-20)	15.76 (3.48; 11-29)	ns
EPDS score	8.51 (5.56; 0–29)	9.74 (6.2; 1-29)	6.97 (4.25; 0-17)	$t(79.17) = -2.40$, $p = .019$
Score of 15 or more	10 (12)	8 (17.4)	2 (5.4)	ns
Score of 13 or more	17 (21)	13 (28.3)	4 (10.8)	ns

Note. EPDS = Edinburgh Postnatal Depression Scale; SSRI = selective serotonin reuptake inhibitor; SD = standard deviation

[a]Gestational age at time of EPDS score used in analysis for this study

Table 2. Summary of genotype results and predicted metabolizer phenotypes (*N*=83)

Number of participants with combined genotype	***CYP2D6* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	***CYP2C19* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	**SSRI taken (number of participants)**	**Predicted metabolizer phenotype[a] assigned for analysis**
8	**1/*1*	NM	**1/*1*	NM	Escitalopram (2)/ Sertraline (4)/ Citalopram (1)/ Paroxetine (1)	NM
2	**1/*1*	NM	**1/*2*	IM	Paroxetine (1)	NM
					Sertraline (1)	IM
4	**1/*1*	NM	**1/*17*	UM	Paroxetine (3)	NM
					Citalopram (1)	UM
1	**1/*1*	NM	**2/*17*	IM	Citalopram	IM
1	**1/*1*	NM	**17/*17*	UM	Citalopram	UM
1	Unknown[b]	--	**1/*1*	NM	Escitalopram	NM
2	**1/*2*	NM	**1/*2*	IM	Paroxetine	NM
1	**1/*2*	NM	**2/*2*	PM	Citalopram	PM
2	**1x2/*4*	NM	**1/*1*	NM	Sertraline (1)/ Citalopram (1)	NM
8	**1/*4*	NM	**1/*1*	NM	Citalopram (5)/ Sertraline (1)/ Escitalopram (1)/ Paroxetine (1)	NM

Number of participants with combined genotype	***CYP2D6* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	***CYP2C19* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	**SSRI taken (number of participants)**	**Predicted metabolizer phenotype[a]**
2	**1/*4*	NM	**1/*2*	IM	Paroxetine	NM
8	**1/*4*	NM	**1/*17*	UM	Paroxetine	NM
					Citalopram (2)/ Escitalopram (4)/ Sertraline (1)	UM
1	**1/*5*	NM	**1/*1*	NM	Escitalopram	NM
1	**1/*5*	NM	**1/*2*	IM	Escitalopram	IM
1	**1/*6*	NM	**1/*17*	UM	Citalopram	UM
2	**1/*9*	NM	**1/*1*	NM	Paroxetine (1)/ Citalopram (1)	NM
1	**1/*9*	NM	**1/*17*	UM	Citalopram	UM
1	**1/*10*	NM	**1/*2*	IM	Paroxetine	NM
1	**1/*10*	NM	**2/*2*	PM	Paroxetine	NM
4	**1/*41*	NM	**1/*1*	NM	Sertraline (2)/ Citalopram (2)	NM
1	**1/*41*	NM	**1/*17*	UM	Paroxetine	NM
2	**2/*2*	NM	**1/*1*	NM	Paroxetine	NM
1	**2/*3*	NM	**1/*2*	IM	Citalopram	IM

Number of participants with combined genotype	***CYP2D6* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	***CYP2C19* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	**SSRI taken (number of participants)**	**Predicted metabolizer phenotype[a]**
1	**2/*4*	NM	**1/*1*	NM	Paroxetine	NM
1	**2/*4*	NM	**1/*17*	UM	Sertraline	UM
1	**2/*5*	NM	**1/*17*	UM	Citalopram	UM
1	**2/*10*	NM	**2/*2*	PM	Paroxetine	NM
1	**2/*10*	NM	**1/*17*	UM	Sertraline	UM
3	**2/*41*	NM	**1/*1*	NM	Citalopram (2)/ Escitalopram (1)	NM
1	**2/*41*	NM	**2/*17*	IM	Paroxetine	NM
3	**4/*4*	PM	**1/*1*	NM	Sertraline	NM
1	**4/*4*	PM	**1/*2*	IM	Escitalopram	IM
1	**4/*4*	PM	**2/*17*	IM	Citalopram	IM
1	**4/*4*	PM	**1/*17*	UM	Paroxetine	PM
1	**4/*5*	PM	**1/*2*	IM	Paroxetine	PM
1	**4/*9*	IM	**1/*1*	NM	Sertraline	NM
1	**4/*10*	IM	**1/*17*	UM	Paroxetine	IM
1	**4/*41*	IM	**2/*2*	PM	Citalopram	PM
1	**4/*41*	IM	**1/*1*	NM	Citalopram	NM

Number of participants with combined genotype	***CYP2D6* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	***CYP2C19* genotype (star alleles)**	**Predicted metabolizer phenotype[a]**	**SSRI taken (number of participants)**	**Predicted metabolizer phenotype[a]**
1	**4/*41*	IM	**2/*17*	IM	Paroxetine	IM
1	**4/*41*	IM	**1/*17*	UM	Paroxetine	IM
1	**4/*41*	IM	**17/*17*	UM	Sertraline	UM
1	**5/*9*	IM	**1/*1*	NM	Paroxetine	IM
1	**10/*36+10*	NM	**2/*2*	PM	Sertraline	PM
1	**10/*10*	NM	**1/*2*	IM	Paroxetine	NM
1	**41/*41*	NM	**1/*2*	IM	Paroxetine	NM

[a]PM = poor metabolizer, IM = intermediate metabolizer, NM = normal metabolizer, UM = ultrarapid metabolizer

[b]Positive for the deletion, *CYP2D6*5*, but heterozygous for the **10*-defining variant (rs1065852: C/T). Same result on repeat analysis. No further analysis attempted, given that the participant was taking escitalopram.

Table 3. EPDS total score, with associated standardized SSRI daily dose, maternal weight, and gestational age, for each predicted phenotype group (*N*=83)

Predicted Phenotype Group	**EPDS total score (Mean, SD)**	**Standardized SSRI daily dose (PDD/DDD; Median)**	**Maternal weight (kg; Mean, SD)**	**Gestational age (weeks; Mean, SD)**
Poor Metabolizer (*n*=5)	7.00 (2.55)	1.5	78.61 (14.08)	34.17 (1.37)
Intermediate Metabolizer (*n*=10)	8.80 (6.55)	1.5	75.88 (15.90)	31.11 (6.19)
Normal Metabolizer (*n*=53)	8.87 (5.91)	1.5	77.85 (12.44)	32.29 (5.31)
Ultrarapid metabolizer (*n*=15)	7.53 (4.45)	1.0	82.27 (10.19)	32.41 (5.04)

Note. EPDS = Edinburgh Postnatal Depression Scale; SSRI = selective serotonin reuptake inhibitor; SD = standard deviation; PDD = prescribed daily dose; DDD = defined daily dose

Title: A cross-sectional study of the relationship between *CYP2D6* and *CYP2C19* variations and depression symptoms, for women taking SSRIs during pregnancy

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Journal name:

Archives of Women's Mental Health

Table S1. Genotyping summary

Allele[a]	Genotyping approach used	rs ID[b]	Genetic variation (e.g., SNP)	Region targeted[c]	Participants tested		
					Cohort O1	Cohort O2	Cohort A
			CYP2D6				
**2, *17, *29, *41*	RFLP or TaqMan	rs16947	C>T	2851	Yes	Yes	No
**3*	RFLP or TaqMan	rs35742686	A-del	2550	Yes	Yes	Yes
**4*	RFLP or TaqMan	rs3892097	G>A	1847	Yes	Yes	Yes
**6*	RFLP or TaqMan	rs5030655	T-del	1708	Yes	Yes	Yes
**7*	RFLP or TaqMan	rs5030867	A>C	2936	Yes	Yes	No
**8, *14*	RFLP	rs5030865	G>T G>A	1759	A subset	No	No
**9*	TaqMan	rs5030656	AAG-del	2616	No	No	Yes
**4, *10, *36*	RFLP or TaqMan	rs1065852	C>T	100	Yes	Yes	Yes

Allele[a]	Genotyping approach used	rs ID[b]	Genetic variation (e.g., SNP)	Region targeted[c]	Participants tested		
					Cohort O1	Cohort O2	Cohort A
**11*	RFLP or TaqMan	rs201377835	G>C	882	A subset[d]	A subset	No[e]
**17*	RFLP or TaqMan	rs28371706	C>T	1022	Yes	Yes	Yes
**29*	RFLP or TaqMan	rs59421388	G>A	3184	Yes	Yes	Yes
**41*	RFLP or TaqMan	rs28371725	G>A	2989	Yes	Yes	Yes
**5*	XL-PCR or TaqMan[f]	N/A	Gene deletion		A subset	A subset	Yes
xN	XL-PCR or TaqMan[g]	N/A	Gene duplication or multiplication		Yes	Yes	Yes
**36*	RFLP or TaqMan	N/A	Exon 9 conversion		A subset	A subset	Yes
**13*	XL-PCR or TaqMan	N/A	*CYP2D7-2D6* hybrid genes		No	Yes	Yes
			CYP2C19				
**2*	TaqMan	rs4244285	G>A	c.681	Yes	Yes	Yes
**3*	TaqMan	rs4986893	G>A	c.636	Yes	Yes	Yes
**4*	TaqMan	rs28399504	A>G	c.1	Yes	Yes	No
**17*	TaqMan	rs12248560	C>T	g. -806	Yes	Yes	Yes

[a] Some single nucleotide polymorphisms (SNPs) are part of multiple allele definitions (haplotypes) and may occur on alleles not shown here. The table lists only those alleles identified.

[b] rs IDs are not available for gene deletions, duplications, or conversions.

[c] Position coordinates are for *CYP2D6*1* reference sequence NG_008376.3. Allele definitions are as described by the Pharmacogene Variation Consortium at www.PharmVar.org.

[d] For cohort O, participants positive for 2850T (variant), but negative for SNPs identifying **17*, **29*, or **41* were selected for testing for the presence of the rare **11* allele in cohorts O1 and O2, and the rare **8* and **14* alleles in cohort O1. Further, long-range polymerase chain reaction (XL-PCR) was performed on all cohort O samples to detect the presence of a gene duplication or multiplication (*xN*). All samples with an initial homozygous genotyping result were also tested by XL-PCR for the presence of the *CYP2D6*5* gene deletion. Participants carrying the 100C>T SNP (i.e., were heterozygous C/T or homozygous T/T) were selected for testing for the presence of the *CYP2D7*-derived exon 9 conversion indicative of **36*. For cohort O1, the presence of the exon 9 conversion was tested by restriction fragment length polymorphism (RFLP) analysis; for cohort O2, by a quantitative multiplex PCR method described elsewhere[1]. All samples were tested by XL-PCR for the presence of Fragment B, which targets the intergenic region between duplicated gene copies. Fragment B is only amplified if a duplication event is present, and the additional gene copy has a *CYP2D6*-derived downstream structure. For example, *CYP2D6*1xN, *2xN, *4xN* will amplify fragment B, while **36+*10* will not. All samples positive for Fragment B were selected for testing using Fragment D (an XL-PCR fragment encompassing the entire duplicated gene unit). Fragment D was amplified and subsequently genotyped to determine which allele was duplicated or multiplicated, to discriminate between *CYP2D6*1xN, *2xN, *4xN*, etc. This fragment is amplified regardless of whether the duplication event has a *CYP2D6* or *2D7*-derived downstream region.

[e] The following were not genotyped for all cohorts due to their low population frequencies: *CYP2D6*7, *8, *11, *13, *14,* and *CYP2C19*4*.

[f] TaqMan copy number variation (CNV) analysis performed for cohort A used assay IDs: Hs00010001_cn (targeting *CYP2D6* exon 9), Hs04083572_cn (*CYP2D6* intron 2), and an RNAseP control. All copy number assays were performed in quadruplicate.

[g] Ambiguous duplication events were resolved by amplifying the upstream duplicated gene, as described in Gaedigk *et al.*[2], and genotyping of key allele-defining variants with Sanger sequencing on nested PCR templates.

1. Gaedigk, A., Twist, G. P. & Leeder, J. S. CYP2D6, SULT1A1 and UGT2B17 copy number variation: quantitative detection by multiplex PCR. *Pharmacogenomics* **13**, 91–111 (2012).

2. Gaedigk, A. *et al.* Cytochrome P4502D6 (CYP2D6) gene locus heterogeneity: Characterization of gene duplication events. *Clin. Pharmacol. Ther.* **81**, 242–251 (2007).

Title: A cross-sectional study of the relationship between *CYP2D6* and *CYP2C19* variations and depression symptoms, for women taking SSRIs during pregnancy

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Supplemental results

Subsequent exploratory analyses revealed no significant differences between metabolizer groups for standardized daily dose (H(3)=1.88, *p*=.60), maternal weight (H(3)=.67, *p*=.88), or gestational age (H(3)=2.86, *p*=.41). There was also no statistically significant difference between EPDS scores across the four metabolizer groups for either cohort individually (Cohort A: H(2)=.90, *p*=.64; Cohort O: (H(3)=.95, *p*=.81)). Further, there was no statistically significant difference between EPDS scores across the four metabolizer groups when groups were re-categorized according to updated guidelines (Caudle et al., 2020) with *CYP2D6* genotypes translated into activity scores of 1 being classified as IM (rather than NM)(H(3)=.67, *p*=.88).

In an exploratory two-group comparison of 1) normal metabolizers (NM) and ultrarapid metabolizers (UM) to 2) intermediate metabolizers (IM) and poor metabolizers (PM), as done by Ververs et al., (2009) and Berard et al., (2017), there was no significant difference between EPDS scores of these two groups ($U = 485$, $p = .766$). In a further comparison of these two predicted metabolizer groups, there was no statistically significant difference between the percentage of participants scoring above an EPDS cut-off of 13 (as used by Berard et al., (2017)), with 22.1% in the NM/UM group and 13.3% in the IM/PM group ($p = .725$ - Fisher's exact test).

In an exploratory sub-analysis of the impact of *CYP2D6* variations for all participants (including those taking citalopram, escitalopram, sertraline, and paroxetine), we found no statistically significant differences when comparing EPDS scores across either the three available predicted metabolizer groups (there were no participants identified in the *CYP2D6* UM group; H(2)= 4.11, p= .13), or across the two predicted metabolizer groups (NM/UM vs. IM/PM; $U = 356$, $p = .08$).

In an exploratory sub-analysis of the impact of *CYP2D6* variations for only participants taking paroxetine, we found no statistically significant differences when comparing EPDS scores across either the three available predicted metabolizer groups (there were no participants identified in the *CYP2D6* UM group; H(2)= 2.73, p= .26), or across the two predicted metabolizer groups (NM/UM vs. IM/PM; $U = 49$, $p = .34$).

In an exploratory sub-analysis restricted to only *CYP2C19* variations for participants taking citalopram, escitalopram, or sertraline (participants taking paroxetine excluded because CYP2C19 isn't in the metabolic pathway for paroxetine), we found no statistically significant differences when comparing EPDS scores across either the four predicted metabolizer groups (H(3)= 1.48, p= .69), or across the two predicted metabolizer groups (NM/UM vs. IM/PM)($U = 191$, $p = .72$).