Paving ways for personalizing drug therapy during pregnancy

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chapter 7

Prenatal exposure to serotonin reuptake inhibitors and congenital heart anomalies:

An exploratory gene-environment interaction study

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ABSTRACT

Background: Prenatal use of serotonin reuptake inhibitors (SRIs) was previously associated with congenital heart anomalies (CHA). We aimed to explore whether pharmacogenetics has a role in this fetal outcome.

Methods: A total of 33 case-mother dyads and 2 mother-only (children deceased) cases registered in EUROCAT Northern Netherlands were included in a case-only study. Of these, five case-mother dyads and two mother-only (children deceased) were exposed to SRIs in the first trimester of pregnancy. Ten genes encoding enzymes or proteins important in determining fetal exposure to SRIs or its mechanism were selected: genes coding for CYP450 enzymes (*CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6)*, a P-glycoprotein coding gene (*ABCB1*), a serotonin transporter gene (*SLC6A4*) and serotonin receptor genes (*HTR1A*, *HTR1B*, *HTR2A*, and *HTR3B*). All subjects were genotyped for 58 genetic variations in these genes.

Results: Among exposed cases, several polymorphisms tended to be associated with an increased risk of CHA: *ABCB1* rs1128503, *SLC6A4* 5-HTTLPR and 5-HTTVNTR, *HTR1A* rs1364043, *HTR1B* rs6296 & rs6298 and *HTR3B* rs1176744. However, the sample sizes of this exploratory study were limited and our results did not reach statistical significance.

Conclusion: This is the first study to explore the CHA risk associated with potential gene-environment interaction between pharmacogenetic determinants and SRI use. We did find indications of a role for serotonin receptor polymorphisms in fetuses exposed to SRIs that warrants further investigation.

INTRODUCTION

One of the most prescribed antidepressant groups is the selective serotonin reuptake inhibitors (SSRIs), with up to four in 100 pregnant women being prescribed with this group of antidepressants (1-3). SSRIs are generally well tolerated with the exception of concerns about the increased risk of fetal congenital anomalies following prenatal exposure to these drugs. Following the US Food and Drug Administration (FDA) warning about this risk in 2005, many studies have been performed to elucidate the magnitude and effect of this association. However, the results of these studies have been inconsistent. Meta-analyses by two groups of researchers reported around a 40% increase in the risk of fetal congenital heart anomalies (CHA) following prenatal exposure to paroxetine (4-6), but a similar risk increment was not found for all SSRIs combined (7). Because clinical trials are not an option to measure the risk of an exposure during pregnancy, most studies were done retrospectively using data from pregnancy and/or prescription registries. The conflicting study results impede decision-making among clinicians on a safe and effective therapy for their patients, and best practice at present is to assess individual risk factors before any treatment recommendation.

We previously identified several genes that might be important in the metabolism and mechanism of action of SRIs that may also potentially play a role in the development of SRI-related CHA (8). Several polymorphisms of metabolic enzymes (CYP1A2, CYP2C9, CYPC19 and CYP2D6) were reported to affect the pharmacokinetics and the risk of side effects of SRIs (9,10). P-glycoprotein (P-gp) expressed in the placenta plays a role in limiting fetal exposure to SRIs, and several single nucleotide polymorphisms (SNPs) were found to reduce P-gp function (11). In addition, a number of polymorphisms of the serotonin transporter (SERT) and the serotonin receptors these genes were associated with variation in the clinical response to SRIs and the severity of side effects (12-14).

We therefore aimed to explore the genetic variations that may be involved in fetal exposure to SRIs, and their mechanism of action, to further understand why some children exposed to SRIs in the first trimester of pregnancy develop CHA while others do not. Our objective was to determine the effect of the gene x environment (G x E) interaction between pharmacogenetic predictors of the SRIs and prenatal exposure to these drugs on the risk of CHA.

METHODS

Study design and patient sampling

We performed an exploratory G x E interaction study using case-only design. This design can detect the effect of genotype and exposure in a group of cases when the disease is rare. One of the assumptions made is that the genotype and environment are independent of each other (15-17). The study population includes children with CHA registered in the EUROCAT Northern Netherlands (NNL) database, a population-based birth defect registry covering the three northern provinces of the Netherlands. EUROCAT NNL registers fetuses or children diagnosed with major congenital anomalies before or after birth, and up to 10 years old, upon consent of their parents. For cases registered up to 2001, the types of CHA were classified according to the EUROCAT Subgroup of Congenital Anomalies version 2012 (18) and the International Classification of Diseases (ICD) coding system 9th revision. For cases registered from 2002 onwards, the ICD coding system 10th revision was used for classification. We included only major CHA cases, either as single heart anomalies, as part of complex heart anomalies (including cardiovascular anomalies), or as part of complex anomalies involving other organ systems. Diagnosis codes included were ICD9 745-746, 7470-7474 (excluding 74550) and ICD10 Q20-Q26 (excluding Q2111).

Cases born between January 1, 1997 and December 31, 2013 were eligible for this study. Exclusion criteria were: 1) cases with genetic disorders, including chromosomal anomalies, microdeletions, monogenic disorders and with known teratogenic causes; 2) case mothers with a previous history of a malformed child or history unknown; and 3) cases in which the mother never used any medication during pregnancy in order to reduce the selection bias of including mothers among the unexposed group who were generally 'healthy'. Cases were invited to participate in this study via the Pediatric Cardiology Clinic, University Medical Center Groningen (UMCG), and were asked to provide DNA samples. This study received a waiver from ethical clearance consideration by the Medical Ethical Committee of the UMCG.

Drug exposure

Exposed cases were defined as CHA cases whose mothers had used at least one of the following SRIs (ATC codes) at some point between 30 days before conception and 90 days of gestation: fluoxetine (N06AB03), citalopram (N06AB04), paroxetine (N06AB05), sertraline (N06AB06), fluvoxamine (N06AB08), escitalopram (N06AB10), venlafaxine (N06AX16) and duloxetine (N06AX21). The information on drug use in EUROCAT NNL was obtained primarily via pharmacy records, upon consent of the mother, and later verified by telephone interviews to ensure the validity

of the information obtained. The unexposed cases were CHA cases whose mothers had used any drugs other than SRIs during pregnancy. Variables like smoking during the pregnancy, alcohol intake during the pregnancy, maternal medical history and folic acid supplementation were obtained from a questionnaire given upon registration with EUROCAT NNL.

Selection of candidate genes and SNPs

We selected 10 genes that encode enzymes or proteins important in determining fetal exposure to SRIs: the CYP450 enzymes (*CYP1A2*, *CYP2C9, CYP2C19* and *CYP2D6*), P-gp (*ABCB1*), SERT (*SLC6A4*), and serotonin receptors (*HTR1A, HTR1B, HTR2A, HTR3B*). The CYP450 metabolic enzymes are involved in the pharmacokinetics of SRIs and influence the drug concentration in the maternal circulation. Since all the SRIs examined in this study are substrates of P-gp, changes in P-gp expression or activity may alter the fetal exposure to SRIs (19,20). SRIs inhibit the uptake of serotonin (5-HT) through the SERT, and 5-HT signals through serotonin receptors. A normal 5-HT signaling is important for normal development of fetal heart cells (21).

For the *CYP1A2*, *CYP2C9*, *CYP2C19* and *CYP2D6* genes, we selected 37 SNPs with known phenotypes of either "ultrarapid metabolizer", "rapid metabolizer", "extensive metabolizer", "intermediate metabolizer" or "poor metabolizer" " (http://www.cypalleles.ki.se/). The selection of polymorphisms in *ABCB1*, *SLC6A4* and serotonin receptor genes was based on their clinical effects on SRIs treatment: 8 SNPs in *ABCB1*, 2 repeat markers in *SLC6A4*, 2 SNPs in *HTR1A,* 2 SNPs in *HTR1B,* 5 SNPs in *HTR2A,* and 2 SNPs in *HTR3B* (**Appendix 6.1**) (8,14,22,23). SNPs with call rates of <90% were excluded from the analysis.

DNA collection

An invitation letter and package was sent to the mother of each exposed case, followed by a reminder letter after four weeks, if necessary. Once written informed consent was received from the mothers (and children), we sent them the sample collection kit including cytobrushes to collect buccal cell samples (Isohelix SK-1 swab kits with Isohelix Dri-capsules, Cell Projects Ltd, UK). A clear instruction on how to use the sample collection kit was provided, together with the link to an instruction video (in Dutch). Mothers (and children) were asked to return the cytobrushes, with a silica gel enclosed, to the researchers in prepaid mail envelopes. A reminder letter was sent if we did not receive the samples after four weeks. Each collection tube containing the samples was labelled with the identifier code and with 'Mother' or 'Child'. DNA samples received from the exposed cases were labelled and stored until

they were genotyped. The DNA from exposed cases was extracted from the buccal cells using Isohelix DNA isolation kit (DDK-50/DDK-3, Cell Projects Ltd, UK). For the unexposed cases, DNA samples were retrieved from CHA patients from the Department of Genetics, UMCG who consented with the use of residual materials. The DNA was obtained from blood and the isolation process was performed in the same facility as the samples from exposed cases.

Genotyping

SNP genotyping for *CYPs*, *ABCB1* and *HTR* genes was performed using 10 ng of DNA samples using the iPLEX® Gold platform (Agena Bioscience GmbH, Hamburg, Germany) according to the standard protocol. The region of interest was amplified by polymerase chain reaction (PCR) using gene-specific primers, followed by single base extension using the iPLEX Gold cocktail of primer, enzyme, buffer and terminator nucleotides, resulting in extended fragments with a specific mass for each allele. The mass was detected by the MassARRAY® System and genotype calling was performed using the MassARRAY® Typer Analyzer 4.0 software tools (Agena Bioscience GmbH or Sequenom, Hamburg, Germany). Manual inspection and adjustment of the genotype classifications was also performed by authors on all SNPs with call rates of less than 90%. For the *SLC6A4* repeat markers, the regions of 5-HTTLPR and 5-HTTVNTR were amplified by PCR using specific primers. Amplified DNA fragments were separated by electrophoresis: 5-HTTLPR long and short alleles (530 bp and 486 bp, respectively) and 5-HTTVNTR STin2.9, STin2.10 and STin2.12 (250 bp, 271 bp, and 302 bp, respectively).

Phenotype and genotype scoring

The genotypes of CYP enzyme polymorphisms were grouped into phenotypes that depict the functionality of the enzymes (i.e. normal metabolizer, poor metabolizer or rapid metabolizer etc.), and were reported according to the standardized terms from the Clinical Pharmacogenomics Implementation Consortium (24,25). Since the CYP enzymes in the fetus are not fully developed during the first trimester, only the genotype from the mothers was analyzed.

The risk of CHA was determined for each genetic variation of the *ABCB1*, *HTR1A*, *HTR1B*, *HTR2A* and *HTR2B* genes using a recessive model and for the *SLC6A4* gene using a dominant model, based on the number of exposed cases to perform the analysis. To further explore the cumulative effect of *ABCB1* SNPs, we calculated a genetic score per individual based on the number of risk alleles present as done previously (26-28). The score is associated with the transport of SRIs through P-gp. In the mother, P-gp is expressed in the intestine, liver and kidney where it helps to

eliminate substrate drugs, while P-gp in the placenta limits drug transport into the fetal circulation. A maternal *ABCB1* genotype encoding for reduced P-gp function increases the plasma drug concentration available for transfer through the placenta, while the same genotype in the fetus increases the transfer of the drug into the fetus. Seven SNPs in the *ABCB1* gene previously associated with reduced expression or function were included in the scoring: rs1045642, rs1128503, rs1882478, rs2032582, rs2235040, rs4148739 and rs9282564. The risk alleles can occur in a homozygous or heterozygous form; therefore each individual could have 0, 1, or 2 alleles for each SNP, resulting in a cumulative risk score up to 14. For the *SLC6A4* 5-HTTLPR and 5-HTTVNTR polymorphisms, the cumulative score was up to 4.

Statistical analysis

Deviations from Hardy-Weinberg equilibrium were tested using Pearson's chi-square test. To test for the effect of pharmacogenetic predictors (genotype) and prenatal exposure to SRIs (environment) on the risk of fetal CHA, we determined the departure from multiplicative interaction between gene and environment using multivariable logistic regression and expressed with interaction odds ratio (OR) and 95% confidence interval (CI). An OR of more than 1 indicates that the presence of both pharmacogenetic predictors and SRI-use increases the risk of CHA.

RESULTS

Case sampling

From 2,172 CHA cases born between 1997 and 2013 and registered in EUROCAT NNL, we selected 1,383 cases that matched the inclusion criteria (**Figure 1**). For the exposed cases, twenty-four case mothers were invited to participate in the study, and eight case-mother dyads gave their consent. For cases under the age of 12, written informed consent was obtained from their mothers. The DNA samples were available for five exposed dyads and two mothers-only (with deceased child) cases; four exposed case-mother dyads and two mothers-only cases provided their DNA samples, and the samples of one case-mother dyad were retrieved from the clinical diagnostic laboratory. The number of unexposed cases was decided to be four times the number of exposed cases, therefore 28 unexposed case-mother dyads were randomly selected from the available DNA samples.

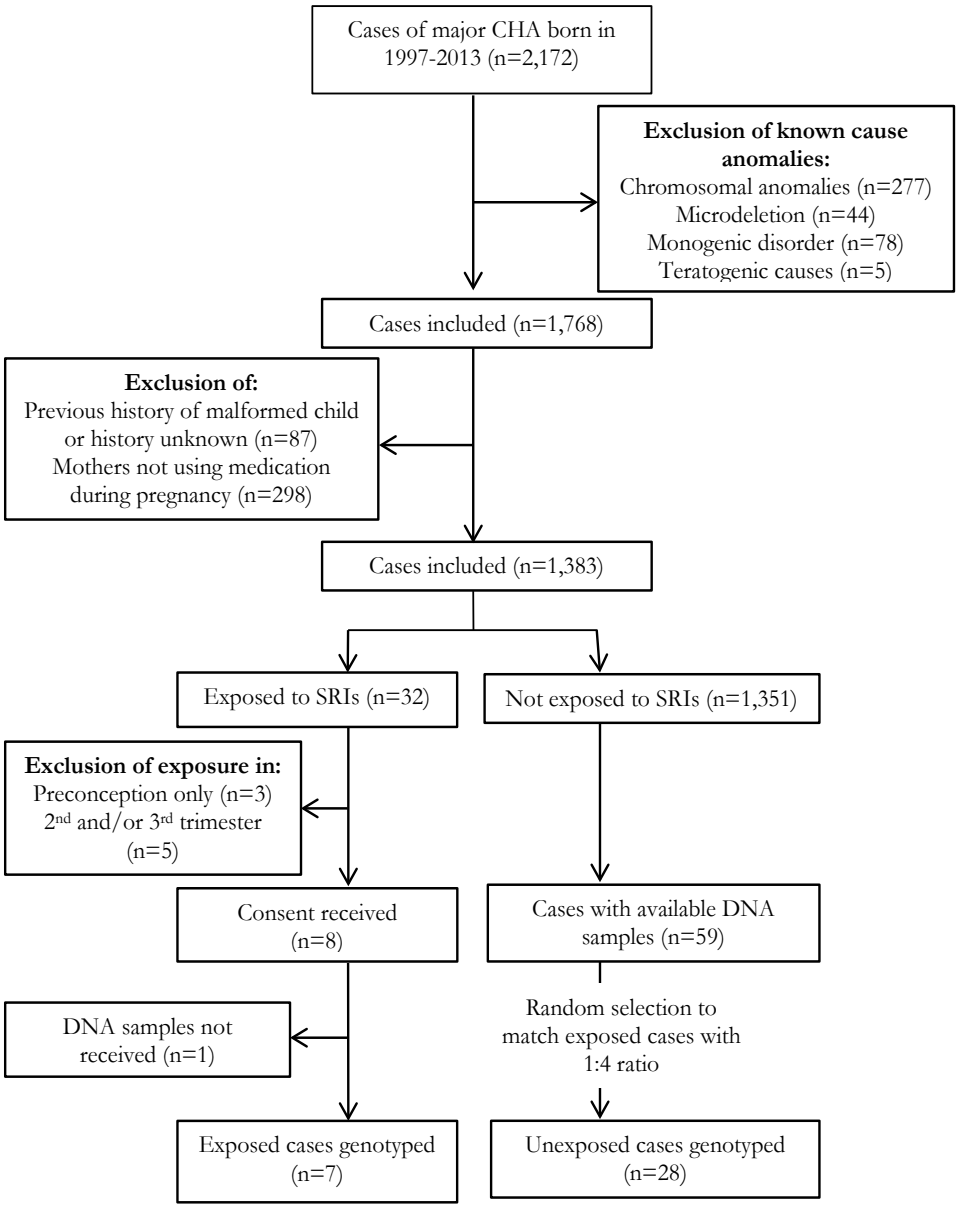

Figure 1: Case sampling

The characteristics of all cases and mothers can be found in **Table 1**. The majority of our cases and case mothers are Caucasians (91.4%). The SRIs used by the case-mothers were paroxetine (3), fluoxetine (2), venlafaxine (1), and paroxetine and venlafaxine (1). Among all cases, the types of medications used in the first trimester are: antiemetics (6), analgesics (6), hormone preparations (5), antacids (4), laxatives (4), antibiotics (3), antihistamines (2), thyroid preparation (1), cholesterol lowering agent (1) and cough preparation (1).

Genotyping

A total of 65 DNA samples were obtained and genotyped: 12 samples from the exposed cases (5 children and 7 mothers) and 53 samples from the unexposed cases (28 children and 25 mothers). Genotype and allele frequencies for all case-mother dyads are listed in **Table 2**. Out of 58 polymorphisms analyzed, 5 SNPs (*CYP2D6 *4, *6, *8,*17, *41*) failed to be genotyped. Out of 53 SNPs, 4 SNPs (*CYP2D6*2, *9, *11* and **12*) had allele calling rates of less than 90% and the genotype frequency for *HTR2A* rs7997012 was not in Hardy Weinberg equilibrium (p=0.03). Due to the different call rates among SNPs, the number of case-mother dyads differed for each G x E interaction analysis.

CYP enzyme and P-glycoprotein phenotypes, SRI exposure and CHA

The interaction between maternal CYP enzyme phenotypes and SRI exposure does not indicate an effect on the risk of CHA. Among cases exposed to paroxetine (n=4), all of the case-mothers were normal CYP2D6 metabolizers (**Appendix 6.2**). Two cases were exposed to venlafaxine, and one of them is an intermediate CYP2C19 metabolizer. Fluoxetine was used by two case-mothers who are both normal CYP2C9 and CYP2D6 metabolizers. Therefore, we cannot determine the effect of metabolic enzyme phenotypes on the risk of CHA associated with the use of SRIs among our case-mothers.

Table 1: Characteristics of case-mother dyads included in the study (N=35)

Characteristics	n	%
Child sex		
Boy	27	77.1
Girl	8	22.9
Year of birth		
2003-2007	24	68.6
2008-2013	11	31.4
First pregnancy	8	22.9
Types of birth		
Live birth	33	94.3
Termination of pregnancy	2	5.7
Types of CHA		
Single	22	62.9
Complex	13	37.1
Subtypes of CHA**		
Cardiac cambers and connections, ICD10 Q20	5	14.3
Cardiac septa, ICD10 Q21	11	31.4
Pulmonary and tricuspid valves, ICD10 Q22	2	5.7
Aortic and mitral valves, ICD10 Q23	22	62.9
Great arteries, ICD10 Q25	12	34.3
Maternal age at delivery, mean years (range)	31	24-39
Maternal education level		
Low	2	5.7
Middle	18	51.4
High	15	42.9
Folic acid use during pregnancy	33	94.3
Smoking during first trimester	6	17.1
Alcohol intake in the first trimester	14	40.0
Medication use in the first trimester		
SRIs	7	20
Other medication***	23	65.7
Maternal medical history		
Gestational diabetes	3	8.6
Congenital anomalies	4	11.4
Chronic disease	6	17.1

CHA: congenital heart anomalies; SRIs: serotonin reuptake inhibitors; **more than one subtype is counted for cases of complex CHA; ***other than SRIs (for exposed group) and folic acid/supplements (for unexposed group); within 30 days before conception and 90 days in the first trimester

For *ABCB1*, there is no indication of changes in the risk of CHA with any of the *ABCB1* SNPs in the mothers and the children, except possibly for maternal rs1128503 (**Table 3**). However, the sample size was too small to reach statistical significance. For the maternal genotype, the mean score among the exposed case mothers was 3.9 ± 0.7, while the mean scores of the unexposed case mothers was 4.3 ± 1.9 (p=0.41). The distribution of the genetic scores of the exposed and unexposed cases is shown in Figure 2. The mean genetic score of the exposed cases (children) was 5.0 ± 1.9 and 4.4 ± 1.8 among the unexposed cases (p=0.47).

Serotonin transporter and receptor polymorphisms, SRI exposure and CHA

The LL genotype of the *SLC6A4* 5-HTTLPR and 12/12 genotype of the 5-HTTVNTR indicated an increase in the risk of CHA among cases exposed to SRIs, although not significant (**Table 3**). The mean genetic scores of the exposed mothers tended to be higher than the unexposed mothers (2.5 ± 0.8 versus 1.88 ± 0.7, respectively; p=0.061) (**Figure 3**). Meanwhile, the mean genetic scores of the exposed and unexposed cases (children) were comparable (2.4 ± 0.5 and 2.18 ± 0.8, respectively; p=0.57).

For fetal 5-HT receptors, the SNPs in *HTR1A*, *HTR1B* and *HTR3B* showed increases in the interaction OR, although none achieved statistical significance (**Table 3**). We then calculated the genetic scores for these SNPs, which include *HTR1A* rs1364043, *HTR1B* rs6296, rs6298 and *HTR3B* rs1176744 (maximum score of 8).The mean genetic score for exposed cases tended to be higher as compared to unexposed cases (3.4 ± 2.2 versus 1.9 ± 1.6, respectively; p=0.065), and the distribution was skewed towards higher genetic scores (**Figure 4**).

Table 2: Genotype frequency of study SNPs in case-mother dyad samples (N=65)

Gene/ SNPs	rs number	wt/vt allele	wt/wt	wt/vt	vt/vt	NA	Allele calling rate	Variant allele fq	Variant allele fq (European)*	HWE p value
CYP1A2										
	rs2069521	G/A	62	1	0	2	96.9	0.01	0.02	0.95
	rs2069526	T/G	62	1	0	2	96.9	0.01	0.02	0.95
	rs4646425	C/T	62	1	0	2	96.9	0.01	0.02	0.95
	rs4646427	T/C	63	1	0	1	98.5	0.01	0.02	0.95
	rs2472304	G/A	4	27	32	2	96.9	0.72	0.6	0.59
	rs2470890	C/T	5	26	33	1	98.5	0.72	0.6	0.97
CYP2C9										
***2**	rs1799853	C/T	65	0	0	0	100	0	0.12	NA
***3**	rs1057910	A/C	57	7	0	1	98.5	0.05	0.07	0.64
***4**	rs56165452	T/A	62	1	0	2	96.9	0.01	0†	0.95
***6**	rs9332131	A/DEL	62	0	0	3	95.4	0	0	NA
***5**	rs28371686	C/G	65	0	0	0	100	0	0	NA
***8**	rs7900194	G/A	64	0	0	1	98.5	0	0	NA
***11**	rs28371685	C/T	65	0	0	0	100	0	0	NA
***13**	rs72558187	T/C	65	0	0	0	100	0	0	NA
***15**	rs72558190	C/A	65	0	0	0	100	0	0†	NA
CYP2C19										
***2**	rs4244285	G/A	51	12	2	0	100	0.12	0.15	0.24
***3**	rs4986893	G/A	65	0	0	0	100	0	0	NA
***4**	rs28399504	A/G	59	1	0	5	92.3	0.01	0	0.95
***5**	rs56337013	C/T	65	0	0	0	100	0	0†	NA

***6**	rs72552267	G/A	65	0	0	0	100	0	0	NA
***7**	rs72558186	T/A	64	0	0	1	98.5	0	0†	NA
***8**	rs41291556	T/C	64	0	0	1	98.5	0	0	NA
***9**	rs17884712	G/C	65	0	0	0	100	0	0	NA
***10**	rs6413438	C/T	65	0	0	0	100	0	0	NA
***17**	rs12248560	C/T	35	23	3	4	93.8	0.24	0.22	0.75
CYP2D6										
***2**	rs16947	G/A	27	18	9	11	83.1	0.33	0.34	0.06
***3A**	rs35742686	A/DEL	61	0	0	4	93.8	0	0.02	NA
***7**	rs5030867	A/C	64	0	0	1	98.5	0	0	NA
***9**	rs5030656	AAG/ DEL	13	0	0	52	20	0	0.03	NA
***10**	rs1065852	C/T	39	22	4	0	100	0.23	0.2	0.71
***11**	rs5030863	G/C	44	0	0	21	67.7	0	NA	NA
***12**	rs5030862	G/A	56	0	0	9	86.2	0	0	NA

Fq, frequency; wt, wild type; vt, variant; HWE, Hardy Weinberg equilibrium; NA, not available; * allele fq of the European population (http://www.ncbi.nlm.nih.gov/SNP/ , http://www.ensembl.org/index.html); † allele frequency of population worldwide.

Table 2 (cont.): Genotype frequency of study SNPs in case-mother dyad samples (N=65)

Gene/ SNPs	rs number	wt/vt allele	wt/ wt	wt/vt	vt/vt	NA	Allele calling rate	Variant allele fq	Variant allele fq (European)*	HWE p value
ABCB1	rs1128503	C/T	19	29	14	3	95.4	0.46	0.42	0.65
	rs2032582	G/T/A	19	29	14 (TT), 1 (TA)	2	96.9	0.46(T),0.01(A)	0.41 (T), 0.02(A)	0.71
	rs1045642	C/T	13	27	23	2	96.9	0.58	0.52	0.34
	rs2235040	G/A	43	19	1	2	96.9	0.17	0.13	0.5
	rs4148739	A/G	43	18	1	3	95.4	0.16	0.13	0.57
	rs1882478	G/A	40	20	2	3	95.4	0.19	0.26	0.79
	rs9282564	A/G	46	14	2	3	95.4	0.15	0.08	0.48
	rs10256836	G/C	3	26	34	2	96.9	0.75	0.3	0.48
SLC6A4										
5HTTLPR	rs4795541	S/L	18	32	13	2	96.9	0.46 (L)	0.40	0.86
5HTTVNT	rs57098334	STin2.9, 10,12	-	4 (9/10), 5 (9/12), 22 (10/12)	8 (10/10), 25 (12/12)	1	98.5	0.07 (9) 0.33 (10) 0.6 (12)	0.47 (10)	0.69
HTR1A	rs1364043	A/C	38	21	4	2	96.9	0.23	0.21	0.64
	rs6295	G/C	16	34	14	1	98.5	0.48	0.54	0.61
HTR1B	rs6296	G/C	30	24	9	2	96.9	0.33	0.74	0.26
	rs6298	C/T	30	24	9	2	96.9	0.33	0.26	0.26
HTR2A	rs7997012	C/T	25	23	16	1	98.5	0.43	0.43	0.03
	rs6313	C/T	26	29	8	2	96.9	0.36	0.44	0.98
	rs6314	C/T	51	12	0	2	96.9	0.09	0.08	0.4
	rs1928040	C/T	16	30	17	2	96.9	0.38	0.49	0.71
	rs6311	G/A	26	28	10	1	98.5	0.37	0.44	0.59
HTR3B	rs1176744	A/C	37	23	3	2	96.9	0.23	0.31	0.81
	rs3831455	TCC/DEL	63	0	0	2	96.9	0	NA	NA

Fq, frequency; wt, wild type; vt, variant; HWE, Hardy Weinberg equilibrium; NA, not available; S, short allele; L, long allele; * allele fq of the European population (http://www.ncbi.nlm.nih.gov/SNP/ , http://www.ensembl.org/indexhtml)

Table 3: The G x E interaction effect of several SRIs pharmacogenetic predictors on the risk of CHA

SNPs/Genetic variations	Case mothers with variant alleles, n (%)			Cases with variant alleles, n (%)		
	Exposed n (%) N=7	Unexposed n (%) N=25	OR (95% CI)	Exposed n (%) N=5	Unexposed n (%) N=28	OR (95% CI)
ABCB1						
rs1045642	7 (100.0)	19 (76.0)	0.94 (0.48-1.82)	3 (60.0)	21 (75.0)	0.43 (0.058-3.14)
rs1128503	6 (85.7)	14 (56.0)	3.86 (0.4-37.58)	3 (60.0)	20 (71.4)	0.53 (0.072-3.82)
rs1882478	2 (28.6)	9 (36.0)	0.64 (0.13-3.06)	2 (40.0)	9 (32.1)	0.80 (0.32-1.99)
rs2032582	6 (58.7)	15 (60.0)	0.95 (0.52-1.76)	3 (60.0)	20 (71.4)	0.52 (0.086-3.59)
rs2235040	2 (28.6)	8 (32.0)	0.71 (0.19-2.67)	2 (40.0)	8 (28.6)	0.89 (0.38-2.10)
rs4148739	2 (28.6)	7 (28.0)	0.71 (0.24-2.09)	2 (40.0)	8 (28.6)	0.89 (0.38-2.10)
rs9282564	0	7 (28.0)	-	1 (20.0)	8 (28.6)	0.58 (0.07-5.08)
rs10256836	7 (100)	22 (88.0)	0.84 (0.32-2.18)	5 (100)	26 (92.9)	-
SLC6A4	*[N=6]*	*[N=24]*		*[N=5]*	*[N=28]*	
5-HTTLPR (*LL*)	2 (33.3)	5 (20.8)	1.90 (0.27-13.52)	1 (20)	5 (17.9)	1.15 (0.11-12.62)
	[N=6]	*[N=25]*		*[N=5]*	*[N=28]*	
5HTTVNTR (12/12)	3 (50)	9 (36)	1.78 (0.3-10.72)	2 (40)	11 (39.3)	1.03 (0.15-7.19)
HTR1B						
rs6296				3 (60.0)	11 (39.3)	2.18 (0.31-15.29)
rs6298				3 (60.0)	11 (39.3)	2.18 (0.31-15.29)
HTR2A						
rs6313				2 (40.0)	13 (46.4)	0.72 (0.10-5.01)
rs6314				1 (20.0)	6 (21.4)	0.88 (0.082-9.38)
rs1928040				3 (60.0)	20 (74.1)	0.45 (0.06-3.35)
rs6311				2 (40.0)	14 (50.0)	0.67 (0.10-4.62)
HTR3B						
rs1176744				4 (80.0)	11 (39.3)	5.82 (0.57-59.32)
rs3831455				0	(3.6)	-

N, total number of cases; * not in Hardy Weinberg equilibrium

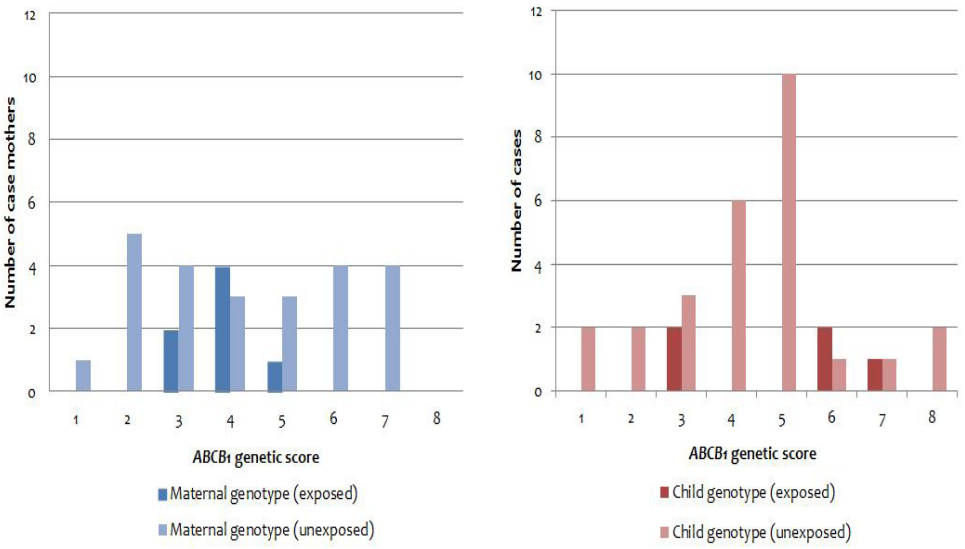

Figure 2: Distribution of maternal and child ABCB1 genetic scoring associated with reduced P-gp function

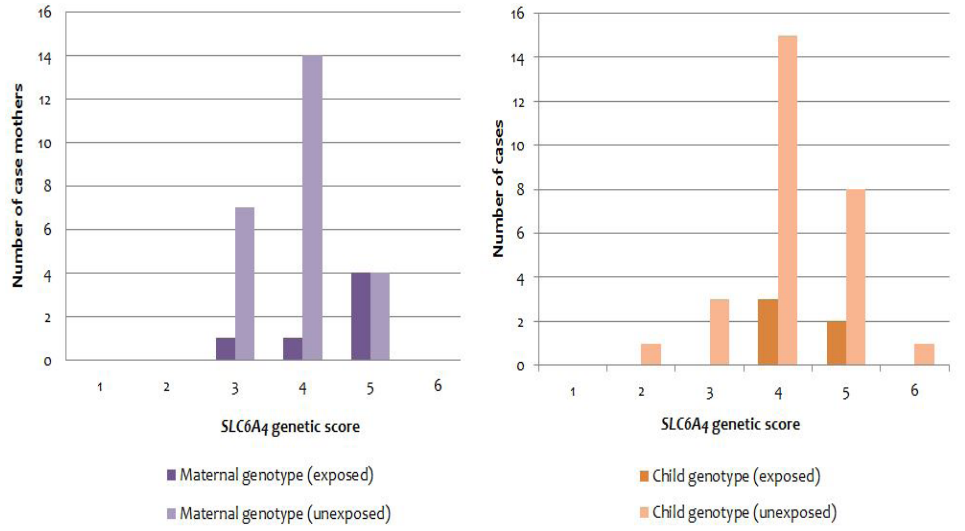

Figure 3: Distribution of maternal and child SLC6A4 genetic scoring associated with increased serotonin transporter function

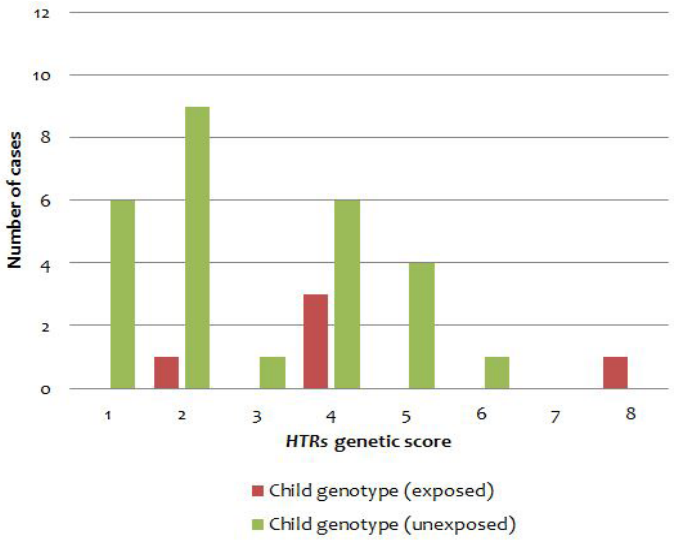

Figure 4: Distribution of child genetic scoring of *HTR* genes associated with increased interaction OR

DISCUSSION

In this exploratory study we tried to find associations of polymorphisms in 10 genes involved in the metabolism of drugs by pregnant women and the occurrence of CHA in their children. Concerning the *ABCB1* SNPs, only maternal rs1128503 had an increased, although non-significant, interaction OR and could therefore be associated with an increased risk of CHA following exposure to SRIs. This SNP, together with rs1045642 (*C3435T*) and rs2030582 (*G2677T/A*), were previously associated with reduced expression/function of placental P-gp and modulated the placental transfer of substrate drugs (29-32). This modulation may affect the protective barrier against xenobiotics in the early stage of pregnancy. It has also been suggested that these SNPs play a role in the clinical response of SRIs because P-gp regulates the transport of SSRIs through the blood-brain barrier (45–50). With regard to congenital anomalies, two previous observational studies reported that maternal and fetal *C3435T* increased fetal susceptibility to CHA and cleft lip following general medication use during pregnancy (39-41). This association was not found in our study, probably because of the different types of medication included in the exposure groups, as we have focused on SRIs use instead of any medication in general.

The L allele of the 5-HTTLPR and 12 repeats of the 5-HTTVNTR of *SLC6A4* had previously been associated with higher efficacy or side effects of SRI treatment, which was proposed to be caused by a higher expression of the SERT (42-46). In this study, the G x E interaction between these variants and SRI-use tended to cause an increase in the risk of CHA, but only for the maternal genotype interaction. Looking at the effect on the fetus, one would expect that the fetal *SLC6A4* variant would have a larger effect on SERT expression in the placenta that is of fetal origin. However, SERT mRNA was also detected in epithelial cells of early decidua, which is the uterine lining of the maternal endometrium (47). The increase in SERT expression may cause a higher response to SRIs, and is manifested by the increased inhibition of 5-HT uptake into the placenta. The exact mechanism seems to be intricate and unclear; however, we can hypothesize that the combination of SERT polymorphisms and SRI-exposure might cause a disruption in the normal 5-HT level available for the transport into the fetal circulation.

Our study found that four SNPs in *HTR* genes encoding for 5-HT receptors showed a possibly increased risk of CHA after the exposure to SRIs, although the effect was not significant. Two of the SNPs, *HTR1A* rs1364043 and *HTR1B* rs6296*,* had previously been associated with an increased response to citalopram (23), while *HTR3B* rs1176744 had been shown to reduce the side effects of paroxetine (48). However, these associations have not yet been replicated in larger studies. On the other hand, *HTR1B* rs6298 was associated with a reduced response to citalopram (23). The role of genetic variations in 5-HT receptors needs further investigation given the importance of 5-HT signaling during embryogenesis, particularly in cell division, differentiation, migration and synaptogenesis (49). Any alteration in the 5-HT level and receptor activity during this period could lead to susceptibility to faulty fetal heart development.

Strengths and limitations

One of the strengths of this study is that it is the first attempt at elucidating the role of pharmacogenetics in the development of CHA associated with prenatal use of SRIs. A further strength is the G x E interaction approach, which is a powerful design for determining the contribution of genetics to adverse drug events or teratogenicity. Previous studies have identified several genetic variations associated with CHA in the presence of environmental factors like maternal obesity, tobacco use and folic acid intake (50-53). A third strength is that the EUROCAT NNL database used in this study records complete information on maternal risk factors (i.e. smoking-, alcohol-, and medication use). Since all cases were selected from the same database, any misclassification of exposure would be non-differential among exposed and

unexposed cases. Finally, we also included cases of terminated pregnancies, which are usually missing from the health surveillance databases.

There are several limitations of this study. First, a case-only study can only measure the risk of the G x E interaction, not the separated risks of G or E. Second, this design is vulnerable to population stratification, although we can assume this effect is minor in this study since the majority of our population is Caucasian (54). Third, we cannot differentiate between different SRIs and doses in the analysis because of the limited number of cases exposed to SRIs and the exploratory nature of the study. The type of SRI might also be a relevant factor, since SRIs can have different pharmacokinetic and pharmacodynamic characteristics.

The use of pharmacogenetics as a tool in personalized drug therapy has been studied before, but the importance of this concept among pregnant patients is now taking the spotlight (55-58). The pharmacogenetic parameters explored in this study are part of a complex interplay between other genetic variants and environmental factors contributing to CHA. Potential gene-gene (G x G) or G x E interaction can occur within the maternal or fetal genotypes and also between maternal and fetal genotypes (59). Based on our current, still limited, knowledge on the pharmacogenetics of SRIs, we need more genetic studies among pregnant patients with depression in order to promote the safest treatment option for both the mothers and their unborn children.

CONCLUSION

Maternal use of SRIs during the first trimester of pregnancy has long been studied for its association with fetal CHA, although the results to date have been conflicting. In this exploratory study, we were not able to find significant genetic variations that may modulate the risk of CHA in fetuses that were exposed to SRIs in the first trimester of pregnancy. Nevertheless, we found that polymorphisms of 5-HT receptors may play a role. Future studies will need a larger number of exposed cases, and possibly incorporate the effect of maternal G x E and fetal G x E contribution to CHA.

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REFERENCES

1. Charlton R, Jordan S, Pierini A, Garne E, Neville A, Hansen A, et al. Selective serotonin reuptake inhibitor prescribing before, during and after pregnancy: a population-based study in six European regions. BJOG. 2014 Oct 28;(DOI 10.1111/1471-0528.13143).

2. Andrade SE, Raebel MA, Brown J, Lane K, Livingston J, Boudreau D, et al. Use of antidepressant medications during pregnancy: a multisite study. Am J Obstet Gynecol. 2008;198(2):194–5.

3. Alwan S, Reefhuis J, Rasmussen SA, Friedman JM. Patterns of antidepressant medication use among pregnant women in a United States population. J Clin Pharmacol. 2011;51(2):264–70.

4. Wurst KE, Poole C, Ephross SA, Olshan AF. First trimester paroxetine use and the prevalence of congenital, specifically cardiac, defects: a meta-analysis of epidemiological studies. Birth defects Res A, Clin Mol Teratol. 2010;88(3):159–70.

5. Grigoriadis S, VonderPorten EH, Mamisashvili L, Roerecke M, Rehm J, Dennis C-L, et al. Antidepressant exposure during pregnancy and congenital malformations: is there an association? A systematic review and meta-analysis of the best evidence. J Clin Psychiatry. 2013 Apr;74(4):e293-308.

6. Myles N, Newall H, Ward H, Large M. Systematic meta-analysis of individual selective serotonin reuptake inhibitor medications and congenital malformations. Aust N Z J Psychiatry. 2013;47(11):1002–12.

7. Wang S, Yang L, Wang L, Gao L, Xu B, Xiong Y. Selective Serotonin Reuptake Inhibitors (SSRIs) and the Risk of Congenital Heart Defects: A Meta-Analysis of Prospective Cohort Studies. J Am Heart Assoc. 2015;4(e001681 doi: 10.1161/JAHA.114.001681)).

8. Daud A, Bergman J, Kerstjens-Frederikse W, Groen H, Wilffert B. The Risk of Congenital Heart Anomalies Following Prenatal Exposure to Serotonin Reuptake Inhibitors—Is Pharmacogenetics the Key? Int J Mol Sci. 2016;17(8):1333; doi:10.3390/ijms17081333.

9. Swen JJ, Wilting I, de Goede AL, Grandia L, Mulder H, Touw DJ, et al. Pharmacogenetics: from bench to byte. Clin Pharmacol Ther. 2008;83(5):781–7.

10. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, et al. Pharmacogenetics: from bench to byte-an update of guidelines. Clin Pharmacol Ther. 2011;89(5):662–73.

11. Daud AN, Bergman JE, Bakker MK, Wang H, de Walle HEK, Plösch T, et al. Pharmacogenetics of drug-induced birth defects: the role of polymorphisms of placental transporter proteins. Pharmacogenomics. 2014;15(7):1029–41.

12. Fabbri C, Minarini A, Niitsu T, Serretti A. Understanding the pharmacogenetics of selective serotonin reuptake inhibitors. Expert Opin Drug Metab Toxicol. 2014;10(8):1093–118.

13. Kroeze Y, Zhou H, Homberg JR. The genetics of selective serotonin reuptake inhibitors. Pharmacol Ther. 2012 Dec;136(3):375–400.

14. Wilkie MJ, Smith G, Day RK, Matthews K, Smith D, Blackwood D, et al. Polymorphisms in the SLC6A4 and HTR2A genes influence treatment outcome following antidepressant therapy. Pharmacogenomics J. 2009 Feb;9(1):61–70.

15. Hassanzadeh J, Moradzadeh R, Rajaeefard A, Tahmasebi S, Golmohammadi P. A Comparison of Case-control and Case-only Designs to Investigate Gene-environment Interactions using Breast Cancer Data. Iran J

Med Sci. 2012;37(2):112–8.

16. Li D, Conti D V. Detecting gene-environment interactions using a combined case-only and case-control approach. Am J Epidemiol. 2009;169(4):497–504.

17. Albert P, Ratnasinghe D, Tangrea J, Wacholder S. Limitations of the case-only design for identifying gene-environment interactions. Am J Epidemiol. 2001;154(8):687–93.

18. European Surveillance of Congenital Anomalies (EUROCAT). Subgroups of Congenital Anomalies (Version 2012) . http://www.eurocat-network.eu/content/EUROCAT-Guide-1.4-Section-3.3.pdf. Last accessed 10 June 2016.

19. Akamine Y, Yasui-Furukori N, Ieiri I, Uno T. Psychotropic drug-drug interactions involving P-glycoprotein. CNS Drugs. 2012 Nov;26(11):959–73.

20. Hodges LM, Markova SM, Chinn LW, Gow JM, Kroetz DL, Klein TE, et al. Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein). Pharmacogenet Genomics. 2011 Mar;21(3):152–61.

21. Sadler TW. Selective serotonin reuptake inhibitors (SSRIs) and heart defects: potential mechanisms for the observed associations. Reprod Toxicol. 2011 Dec;32(4):484–9.

22. Gex-Fabry M, Eap CB, Oneda B, Gervasoni N, Aubry J-M, Bondolfi G, et al. CYP2D6 and ABCB1 genetic variability: influence on paroxetine plasma level and therapeutic response. Ther Drug Monit. 2008;30(4):474–82.

23. Villafuerte SM, Vallabhaneni K, Sliwerska E, McMahon FJ, Young EA, Burmeister M. SSRI response in depression may be influenced by SNPs in HTR1B and HTR1A. Psychiatr Genet. 2009 Dec;19(6):281–91.

24. Cariaso M, Lennon G. SNPedia: A wiki supporting personal genome annotation, interpretationand analysis. Nucleic Acids Res. 2012;40:1308–12.

25. Caudle KE, Dunnenberger HM, Freimuth RR, Peterson JF, Burlison JD, Whirl-Carrillo M, et al. Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC). Genet Med. 2016;(January):1–9.

26. Swen JJ, Guchelaar H-JJ, Baak-Pablo RF, Assendelft WJJ, Wessels JAM. Genetic risk factors for type 2 diabetes mellitus and response to sulfonylurea treatment. Pharmacogenet Genomics. 2011;21(8):461–8.

27. Nelveg-Kristensen KE, Madsen MB, Torp-Pedersen C, Køber L, Egfjord M, Rasmussen HB, et al. Pharmacogenetic risk stratification in angiotensin-converting enzyme inhibitor-treated patients with congestive heart failure: A retrospective cohort study. PLoS One. 2015;10(12):e0144195. doi:10.1371/journal.pone.0144195.

28. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008;359(21):2208–19.

29. Hitzl M, Schaeffeler E, Hocher B, Slowinski T, Halle H, Eichelbaum M, et al. Variable expression of P-glycoprotein in the human placenta and its association with mutations of the multidrug resistance 1 gene (MDR1, ABCB1). Pharmacogenetics. 2004;14(5):309–18.

30. Molsa M, Heikkinen T, Hakkola J, Hakala K, Wallerman O, Wadelius M, et al. Functional role of P-glycoprotein in the human blood-placental barrier. Clin Pharmacol Ther. 2005 Aug;78(2):123–31.

31. Rahi M, Heikkinen T, Härtter S, Hakkola J, Hakala K, Wallerman O, et al. Placental transfer of quetiapine in relation to

P-glycoprotein activity. J Psychopharmacol. 2007 Sep;21(7):751–6.

32. Hemauer SJ, Nanovskaya TN, Abdel-Rahman SZ, Patrikeeva SL, Hankins GD V, Ahmed MS. Modulation of human placental P-glycoprotein expression and activity by MDR1 gene polymorphisms. Biochem Pharmacol. 2010 Mar 15;79(6):921–5.

33. Noordam R, Aarts N, Hofman A, van Schaik RHN, Stricker BH, Visser LE. Association between genetic variation in the ABCB1 gene and switching, discontinuation, and dosage of antidepressant therapy: results from the Rotterdam Study. J Clin Psychopharmacol. 2013;33(4):546–50.

34. Kato M, Fukuda T, Serretti A, Wakeno M, Okugawa G, Ikenaga Y, et al. ABCB1 (MDR1) gene polymorphisms are associated with the clinical response to paroxetine in patients with major depressive disorder. Prog Neuropsychopharmacol Biol Psychiatry. 2008 Feb;32(2):398–404.

35. Nikisch G, Eap CB, Baumann P. Citalopram enantiomers in plasma and cerebrospinal fluid of ABCB1 genotyped depressive patients and clinical response: a pilot study. Pharmacol Res. 2008;58(5–6):344–7.

36. Lin KM, Chiu YF, Tsai IJ, Chen CH, Shen WW, Liu SC, et al. ABCB1 gene polymorphisms are associated with the severity of major depressive disorder and its response to escitalopram treatment. Pharmacogenet Genomics. 2011 Apr;21(4):163–70.

37. Singh AB, Bousman CA, Ng CH, Byron K, Berk M. ABCB1 polymorphism predicts escitalopram dose needed for remission in major depression. Transl Psychiatry. 2012;2:e198.

38. Fukui N, Suzuki Y, Sawamura K, Sugai T,Watanabe J, Inoue Y, et al. Dose-dependent effects of the 3435 C>T genotype of ABCB1 gene on the steady-state plasma concentration of fluvoxamine in psychiatric patients. Ther Drug Monit. 2007 Apr;29(2):185–9.

39. Obermann-Borst SA, Isaacs A, Younes Z, van Schaik RHN, van der Heiden IP, van Duyn CM, et al. General maternal medication use, folic acid, the MDR1 C3435T polymorphism, and the risk of a child with a congenital heart defect. Am J Obstet Gynecol. 2011 Mar;204(3):236.e1-8.

40. Bliek BJB, van Schaik RHN, van der Heiden IP, Sayed-Tabatabaei FA, van Duijn CM, Steegers EAP, et al. Maternal medication use, carriership of the ABCB1 3435C > T polymorphism and the risk of a child with cleft lip with or without cleft palate. Am J Med Genet A. 2009 Oct;149A(10):2088–92.

41. Wang C, Zhou K, Xie L, Li Y, Zhan Y,Qiao L, et al. Maternal medication use, fetal 3435 C>T polymorphism of the ABCB1 gene, and risk of isolated septal defects in a Han Chinese population. Pediatr Cardiol. 2014;35:1132–41.

42. Kato M, Fukuda T, Wakeno M, Fukuda K, Okugawa G, Ikenaga Y, et al. Effects of the serotonin type 2A, 3A and 3B receptor and the serotonin transporter genes on paroxetine and fluvoxamine efficacy and adverse drug reactions in depressed Japanese patients. Neuropsychobiology. 2006;53:186–95.

43. Lee SH, Choi TK, Lee E, Seok JH, Lee HS, Kim SJ. Serotonin transporter gene polymorphism associated with short-term treatment response to venlafaxine. Neuropsychobiology. 2010 Aug;62(3):198–206.

44. Hu X-Z, Rush AJ, Charney D, WilsonAF, Sorant AJM, Papanicolaou GJ, et al. Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. Arch Gen Psychiatry. 2007 Jul;64(7):783–92.

45. Murphy GM, Hollander SB, Rodrigues

HE, Kremer C, Schatzberg AF. Effects of the serotonin transporter gene promoter polymorphism on mirtazapine and paroxetine efficacy and adverse events in geriatric major depression. Arch Gen Psychiatry. 2004;61(11):1163–9.

46. Staeker J, Leucht S, Laika B, Steimer W. Polymorphisms in serotonergic pathways influence the outcome of antidepressant therapy in psychiatric inpatients. Genet Test Mol Biomarkers. 2014 Jan;18(1):20–31.

47. Bottalico B, Pilka R, Larsson I, Casslen B, Marsal K, Hansson SR. Plasma membrane and vesicular monoamine transporters in normal endometrium and early pregnancy decidua. Mol Hum Reprod. 2003;9(7):389–94.

48. Sugai T, Suzuki Y, Sawamura K, Fukui N, Inoue Y, Someya T. The effect of 5-hydroxytryptamine 3A and 3B receptor genes on nausea induced by paroxetine. Pharmacogenomics J. 2006;6(5):351–6.

49. Bonnin A, Levitt P. Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. Neuroscience. 2011;197:1–7.

50. Hobbs CA, Cleves MA, Karim MA, Zhao W, MacLeod SL. Maternal folate-related gene environment interactions and congenital heart defects. Obs Gynecol. 2010;116:316–22.

51. Hobbs CA, Cleves MA, Macleod SL, Erickson SW, Tang X, Li J, et al. Conotruncal heart defects and common variants in maternal and fetal genes in folate, homocysteine, and transsulfuration pathways. Birth Defects Res Part A - Clin Mol Teratol. 2014;100(2):116–26.

52. Tang X, Nick TG, Cleves MA, Erickson SW, Li M, Li J, et al. Maternal obesity and tobacco use modify the impact of genetic variants on the occurrence of conotruncal heart defects. PLoS One. 2014;9(10):e108903. doi:10.1371/journal.pone.0108903.

53. Tang X, Hobbs CA, Cleves MA, Erickson SW, Macleod SL, Malik S. Genetic variation affects congenital heart defect susceptibility in offspring exposed to maternal tobacco use. Birth Defects Res Part A - Clin Mol Teratol. 2015;103(10):834–42.

54. Wang LY, Lee WC. Population stratification bias in the case-only study for gene-environment interactions. Am J Epidemiol. 2008;168(2):197–201.

55. Haas DM, D'Alton M. Pharmacogenetics and other reasons why drugs can fail in pregnancy: higher dose or different drug? Obstet Gynecol. 2012 Nov;120(5):1176–9.

56. Haas DM. Obstetric therapeutics-How pharmacogenetics may inform drug therapy for pregnant women in the future. Obstet Gynecol Surv. 2013;68(9):650–4.

57. Quinney SK, Patil AS, Flockhart DA. Is personalized medicine achievable in obstetrics? Semin Perinatol. 2014;38(8):534–40.

58. Dorfman EH, Cheng EY, Hebert MF, Thummel KE, Burke W. Prenatal pharmacogenomics: a promising area for research. Pharmacogenomics J. 2016;(May):1–2.

59. Li M, Li J, Wei C, Lu Q, Tang X, Erickson SW, et al. A Three-Way Interaction among Maternal and Fetal Variants Contributing to Congenital Heart Defects. Ann Hum Genet. 2016;80(1):20–31.