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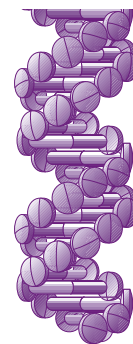
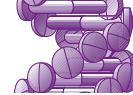
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Prenatal exposure to serotonin reuptake inhibitors and congenital heart anomalies: an exploratory pharmacogenetics study

Aim: To explore the role of pharmacogenetics in determining the risk of congenital heart anomalies (CHA) with prenatal use of serotonin reuptake inhibitors. **Methods:** We included 33 case-mother dyads and 2 mother-only (child deceased) cases of CHA in a case-only study. Ten genes important in determining fetal exposure to serotonin reuptake inhibitors were examined: *CYP1A2*, *CYP2C9*, *CYP2C19*, *CYP2D6*, *ABCB1*, *SLC6A4*, *HTR1A*, *HTR1B*, *HTR2A* and *HTR3B*. **Results:** Among the exposed cases, polymorphisms that tended to be associated with an increased risk of CHA were *SLC6A4* 5-HTTLPR and 5-HTTVNTR, *HTR1A* rs1364043, *HTR1B* rs6296 and rs6298 and *HTR3B* rs1176744, but none reached statistical significance due to our limited sample sizes. **Conclusion:** We identified several polymorphisms that might potentially affect the risk of CHA among exposed fetuses, which warrants further investigation.

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Keywords: gene–environment interaction • heart defects • pharmacogenetics • serotonin reuptake inhibitors • teratogenicity

One of the most prescribed antidepressant groups is the selective serotonin reuptake inhibitors (SSRIs), with up to 4 in 100 pregnant women being prescribed with this group of antidepressants [1–3]. SSRIs are generally well tolerated with the exception of concerns about increased risk of fetal congenital anomalies following prenatal exposure to these drugs. Following the US 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 the SSRIs combined [7]. Because clinical trials are not an option for measuring the risk of an exposure during pregnancy, most studies

were done retrospectively using data from pregnancy and/or prescription registries. The conflicting study results in 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*, *CYP2C19* 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 SNPs were found to reduce P-gp function [11]. In addition, a number of polymorphisms of the serotonin

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transporter (SERT) and the serotonin receptor 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 \times environment (G \times E) interaction between pharmacogenetic predictors of the SRIs and prenatal exposure to these drugs on the risk of CHA.

Methods

Study design

We performed an exploratory G \times 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. In our case, we considered CHA as a relatively rare disease, and G \times E interaction studies have been commonly performed to investigate the genetic and environmental risk factors for congenital anomalies. One of the assumptions made is that the genotype and environment are independent of each other [15–17].

Patient sampling

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, persistent foramen ovale) and ICD10 Q20–Q26 (excluding Q2111, persistent foramen ovale), which include common arterial truncus, transposition of great vessels, single ventricle, ventricular/atrial/atrioventricular septal defects, tetralogy of Fallot, tricuspid atresia and stenosis, Ebstein's anomaly, pulmonary valve stenosis, pulmonary valve atresia, aortic valve atresia/stenosis, hypoplastic

left/right heart syndrome, coarctation of aorta, total anomalous pulmonary venous return and patent ductus arteriosus. Cases born between 1 January 1997 and 31 December 2013 were eligible for this study. Exclusion criteria were: cases with genetic disorders, including chromosomal anomalies, microdeletions, monogenic disorders and those with known teratogenic causes; case mothers with a previous history of a malformed child or history unknown; and 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 telephonic 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 & SNPs

We selected ten 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* and *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 SERT and 5-HT signals

through serotonin receptors. 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’ [22]. The selection of polymorphisms in *ABCB1*, *SLC6A4* and serotonin receptor genes was based on their clinical effects on the SRI treatment. We selected eight SNPs in *ABCB1*, two repeat markers in *SLC6A4*, two SNPs in *HTR1A*, two SNPs in *HTR1B*, five SNPs in *HTR2A* and two SNPs in *HTR3B* (Supplementary Table 1) [8,14,23–24]. 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 4 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). Clear instructions about how to use the sample collection kit were provided, together with a link to an instructional video (in Dutch). Mothers (and children) were asked to return the cytobrushes to the researchers in prepaid mail envelopes, with a silica gel enclosed. A reminder letter was sent if we did not receive the samples after 4 weeks. Each collection tube containing the samples was labeled with the identifier code and with ‘Mother’ or ‘Child’. For the unexposed cases, DNA samples were retrieved from CHA patients from the Department of Genetics, UMCG who had consented to the use of their residual materials in future research. DNA was obtained from the blood and the isolation process was performed in the same facility as the samples from exposed cases.

Genotyping

DNA samples received from the exposed cases were labeled and stored until they were genotyped. DNA from exposed cases was extracted from the buccal cells using Isohelix DNA isolation kit (DDK-50/DDK-3, Cell Projects Ltd, UK). 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 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 the 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 and 486 bp, respectively) and 5-HTTVNTR STin2.9, STin2.10 and STin2.12 (250, 271 and 302 bp, respectively). Details on primer sequences are available upon request.

Phenotype & 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 [25,26]. 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 [27–29]. The score is associated with the transport of SRIs through P-gp. In the mother, P-gp is expressed in the intestines, 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 zero, one or two 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 four.

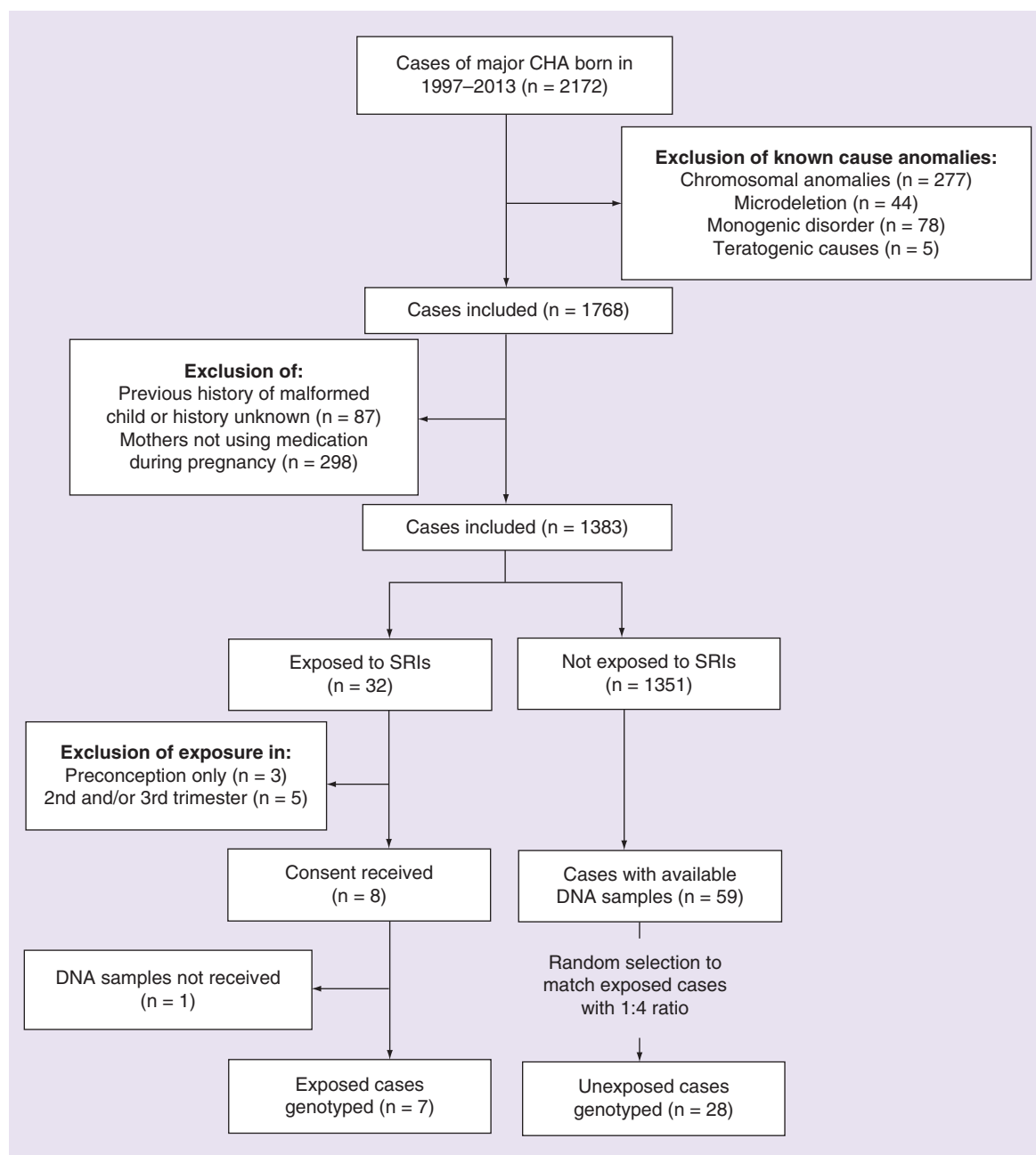


Figure 1. Case sampling.

Statistical analysis

Deviations from Hardy–Weinberg equilibrium were tested using Pearson’s χ^2 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 as interaction odds ratio (OR) and 95% 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 2172 CHA cases born between 1997 and 2013 and registered in EUROCAT NNL, we selected 1383 cases that matched the inclusion criteria (Figure 1). For the exposed cases, 24 case mothers were invited to participate in the study and 8 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 chosen to be four-times the number of exposed cases because increasing the ratio would not have added to the significance of the results. Therefore, 28 unexposed case-mother dyads were randomly selected from the available DNA samples.

The characteristics of all the cases and mothers can be found in **Table 1**. Majority of our cases and case mothers are Caucasians (91.4%). Of the seven exposed case-mothers, three were recorded to have depression. No chronic illness was registered of the other four mothers, but we can assume they will all have had depression or anxiety symptoms. Of the 28 unexposed case-mothers, none had a registered psychiatric illness. The SRIs used by the case-mothers were paroxetine (three), fluoxetine (two), venlafaxine (one), and paroxetine and venlafaxine (one). None of the exposed mothers were using psychoactive drugs other than SRIs and none of the unexposed mothers were using psychoactive drugs.

Other types of medications used by these mothers in the first trimester are: antiemetics (six), analgesics (six), hormone preparations (five), antacids (four), laxatives (four), antibiotics (three), antihistamines (two), thyroid preparation (one), cholesterol lowering agent (one) and cough preparation (one).

Genotyping

A total of 65 DNA samples were obtained and genotyped: 12 samples from the exposed cases (five children and seven 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, five SNPs (*CYP2D6* *4, *6, *8,*17 and *41) failed to be genotyped. Out of 53 SNPs, four 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 × E interaction analysis.

CYP enzyme & P-glycoprotein phenotypes

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 the case-mothers were normal *CYP2D6* metabolizers (**Supplementary Table 2**). Two cases were exposed to venlafaxine and one of them was an intermediate *CYP2C19* metabolizer. Fluoxetine was used by two case-mothers who were both normal

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

Characteristics	n	%
Child's 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

[†]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.
 CHA: Congenital heart anomaly; ICD-10: 10th revision of the International Statistical Classification of Diseases and Related Health Problems; SRI: Serotonin reuptake inhibitor.

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
ABCB1										
	rs1128503	C/T	19	29	14	3	95.4	0.46	0.42	0.65

[†]Allele frequency of the European population [30,31].

[‡]Allele frequency of population worldwide. Also, see **Supplementary Table 2** for genotype frequencies in exposed and unexposed case-mothers.

Fq: Frequency; HWE: Hardy–Weinberg equilibrium; NA: Not available; VT: Variant; WT: Wild-type.

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

[†]Allele frequency of the European population [30,31].
[‡]Allele frequency of population worldwide. Also, see **Supplementary Table 2** for genotype frequencies in exposed and unexposed case-mothers.
Fq: Frequency; HWE: Hardy–Weinberg equilibrium; NA: Not available; VT: Variant; WT: Wild-type.

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.

For *ABCB1*, there was no change in the risk of CHA with any of the *ABCB1* SNPs in the mothers and the children, except for maternal *rs1128503* (not significant, **Table 3**). Due to the low number of sample, the risks were not corrected for maternal risk factors, such as maternal obesity, history of alcohol and illicit drug use. 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 for the unexposed cases ($p = 0.47$).

Serotonin transporter & receptor polymorphisms

The *LL* genotype of the *SLC6A4* 5-HTTLPR and 12/12 genotype of 5-HTTVNTR indicated an increase in the risk of CHA among the cases exposed to SRIs, but our sample size was too small to reach statistical

significance (Table 3). The mean genetic scores of the exposed mothers tended to be higher than those of the unexposed mothers (2.5 ± 0.8 vs 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 increase in the

interaction OR, although none achieved statistical significance (Table 3). We then calculated the genetic scores for these SNPs, which included *HTR1A* rs1364043, *HTR1B* rs6296, *HTR1B* rs6298 and *HTR3B* rs1176744 (maximum score of 8). The mean genetic score for exposed cases tended to be higher compared with unexposed cases (3.4 ± 2.2 vs 1.9 ± 1.6 , respectively; $p = 0.065$), and the distribution was skewed toward higher genetic scores (Figure 4).

Table 3. Interaction odds ratio of the multiplicative interaction between each serotonin reuptake inhibitors of the *ABCB1* gene (coding for P-glycoprotein) and *HTR* genes (coding for the serotonin receptors) and serotonin reuptake inhibitors exposure on the risk of congenital heart anomalies.

Gene/SNPs	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 (0.48–41.82)	3 (60.0)	21 (75.0)	0.43 (0.058–053.14)
rs1128503	6 (85.7)	14 (56.0)	3.86 (0.4–37.58)	3 (60.0)	20 (71.4)	0.53 (0.072–073.82)
rs1882478	2 (28.6)	9 (36.0)	0.64 (0.13–13.06)	2 (40.0)	9 (32.1)	0.80 (0.32–31.99)
rs2032582	6 (58.7)	15 (60.0)	0.95 (0.52–51.76)	3 (60.0)	20 (71.4)	0.52 (0.086–083.59)
rs2235040	2 (28.6)	8 (32.0)	0.71 (0.19–12.67)	2 (40.0)	8 (28.6)	0.89 (0.38–32.10)
rs4148739	2 (28.6)	7 (28.0)	0.71 (0.24–22.09)	2 (40.0)	8 (28.6)	0.89 (0.38–32.10)
rs9282564	0	7 (28.0)	-	1 (20.0)	8 (28.6)	0.58 (0.07–05.08)
rs10256836	7 (100)	22 (88.0)	0.84 (0.32–32.18)	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 (0.15–17.19)
HTR1A						
rs1364043				3 (60.0)	9 (32.1)	3.0 (0.42–21.30)
rs6295				5 (100)	21 (75.0)	-
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 (0.10–15.01)
rs6314				1 (20.0)	6 (21.4)	0.88 (0.082–089.38)
rs1928040				3 (60.0)	20 (74.1)	0.45 (0.06–03.35)
rs6311				2 (40.0)	14 (50.0)	0.67 (0.10–14.62)
HTR3B						
rs1176744				4 (80.0)	11 (39.3)	5.82 (0.57–59.32)
rs3831455				0	1 (3.6)	-

OR: Odds ratio.

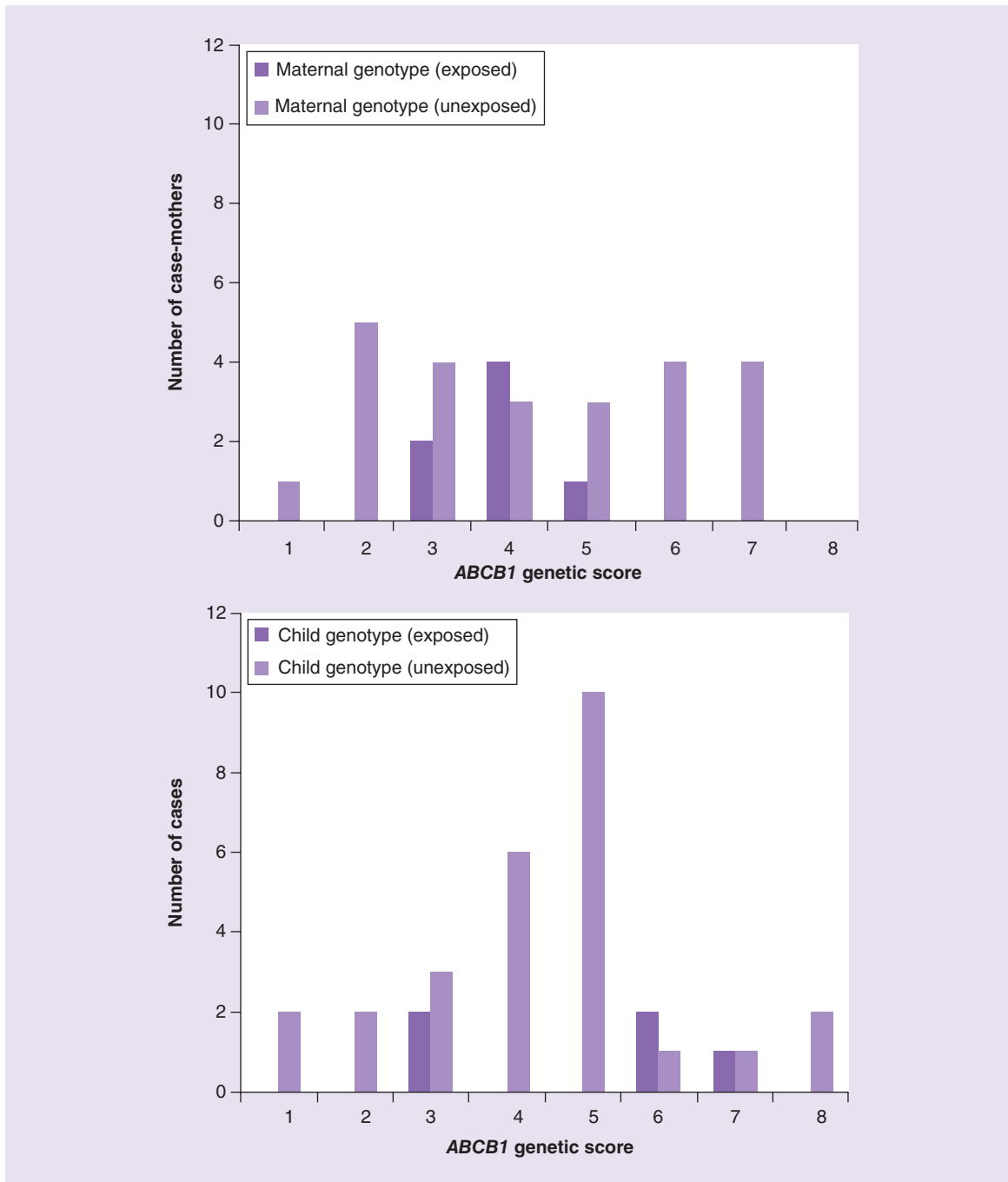


Figure 2. Distribution of maternal and child *ABCB1* genetic scoring associated with reduced P-glycoprotein function.

Discussion

In this exploratory study, we aimed to find associations between polymorphisms in ten genes involved in the metabolism of drugs in pregnant women and the occurrence of CHA in their children. Concerning the *ABCB1* SNPs, only maternal rs1128503 had an increased, although nonsignificant, 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), was previously associated with reduced expression/function of placental P-gp and shown to modulate the placental transfer of substrate drugs [32–35]. 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 [36–41]. 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 [42–44]. 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 SRI use instead of medication use 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, effects proposed to be caused by a higher expression of SERT [45–49]. In this study, the G × E interaction between these variants and SRI use tended

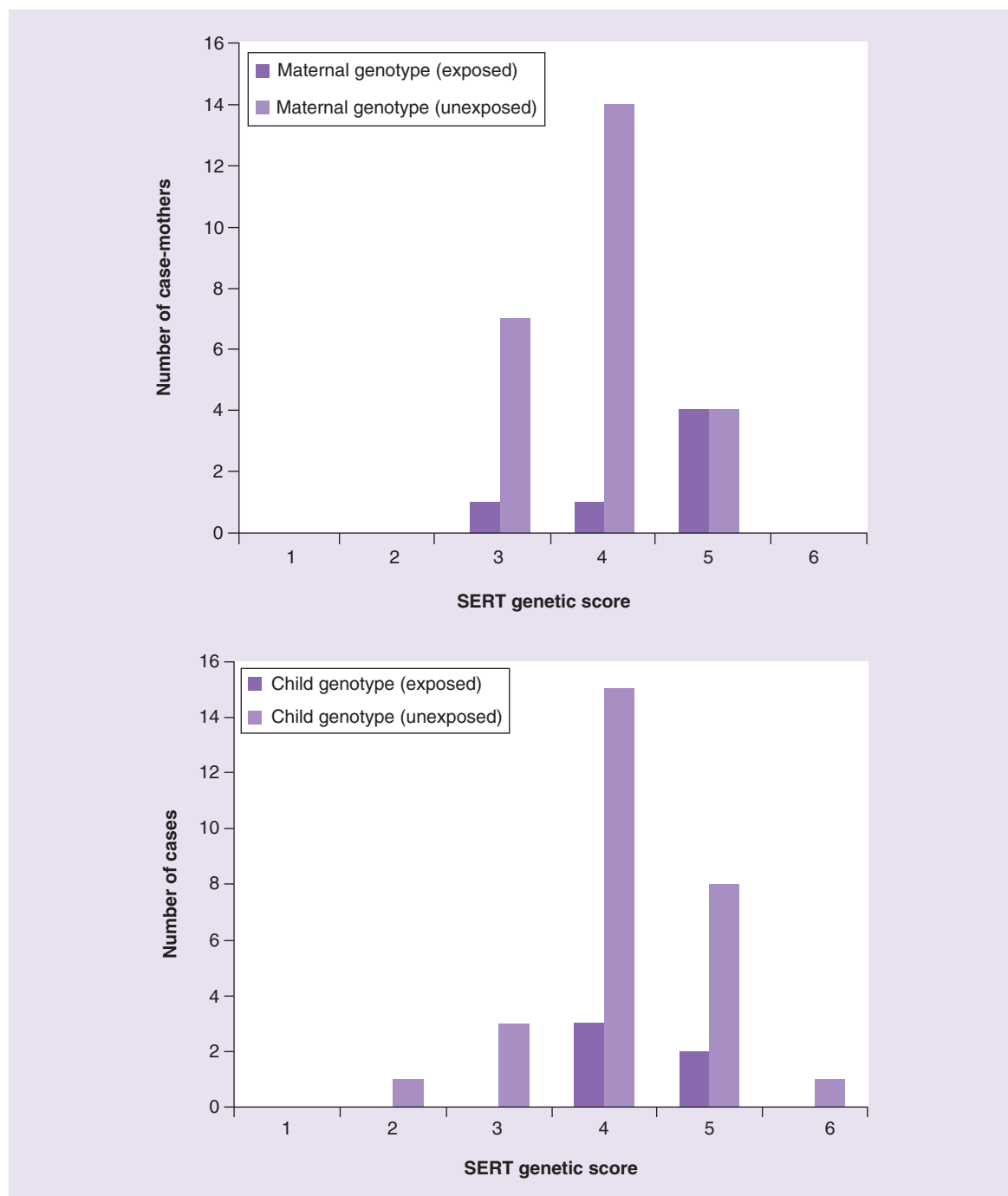


Figure 3. Distribution of maternal and child *SLC6A4* genetic scoring associated with increased serotonin transporter function.

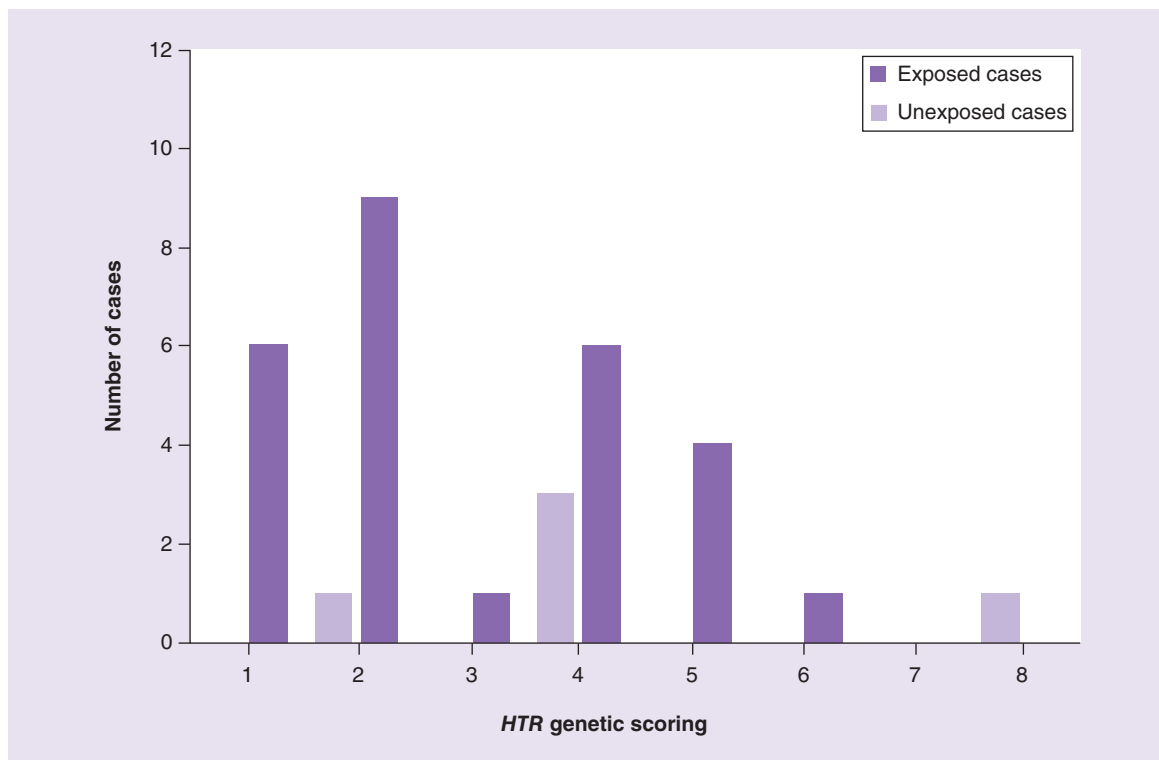


Figure 4. Distribution of child genetic scoring of *HTR* genes associated with increased interaction odds ratio.

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 [50]. 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 four SNPs in *HTR* genes encoding for 5-HT receptors that 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 [24], while *HTR3B* rs1176744 had been shown to reduce the side effects of paroxetine [51]. 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 [24]. 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 [52]. Any alteration in the 5-HT level and receptor activity during this period could lead to susceptibility to faulty fetal heart development.

Strengths & 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 × 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 [53–56]. 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 nondifferential 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 to this study. First, a case-only study can only measure the risk of the $G \times E$ 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 [57]. 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. Fourth, our participation rate was low (30% among cases exposed to SRIs), despite a concerted effort to reduce barriers to participation that included sending reminders to potential participants and use of a noninvasive method of DNA collection. It is possible that the low participation was because mothers were reluctant to know whether the medication they took had contributed to the development of a heart defect in their child. They may also not have understood the benefit of pharmacogenetic tests or the results of this study for themselves.

In 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 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 to incorporate the effect of maternal $G \times E$ and fetal $G \times E$ contribution to CHA.

Future perspective

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 [58–61]. The pharmacogenetic parameters explored in this study are a part of a complex interplay between other genetic variants and environmental factors contributing to CHA. Potential gene–gene ($G \times G$) or $G \times E$ interaction can occur within the maternal or fetal genotypes and also between maternal and fetal genotypes [62]. Based on our current, still limited knowledge about the pharmacogenetics of SRIs, we need more genetic studies among pregnant patients with depression in order to identify the safest treatment option for both the mothers and their unborn children.

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Summary points

Role of genetic variations in the risk of drug-induced teratogenicity

- There are discrepancies in the reported risk of CHA associated with prenatal exposure to SRIs.
- Genetic polymorphisms that cause clinical changes in SRI response and side effects, might affect the fetus's exposure to these drugs.
- Genetic differences among fetuses exposed to SRIs may potentially explain their risk of having CHA.

CYP enzyme & P-glycoprotein (*ABCB1*) phenotypes

- The effect of CYP enzyme phenotypes on the risk of CHA could not be determined due to limited sample sizes.
- Only the maternal *rs1128503* of the *ABCB1* gene was found to cause an increase in the risk of CHA, but the association was not significant.

Serotonin transporter (*SLC6A4*) & serotonin receptor polymorphisms

- The maternal *LL* genotype of the *SLC6A4* 5-HTTLPR and 12/12 genotype of the 5-HTTVNTR showed an increase in the risk of CHA among exposed cases (not significant).
- For fetal serotonin receptors, several SNPs in *HTR1A*, *HTR1B* and *HTR3B* showed an increase in the risk of CHA among exposed cases (not significant).

Conclusion

- A gene–environment interaction study can be used to explore the role of pharmacogenetics in drug teratogenicity.
- Genetic risk scoring can be helpful for summarizing the cumulative risk of different pharmacogenetic risk alleles.

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