Paving ways for personalizing drug therapy during pregnancy

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chapter
General Discussion
Pregnant women comprise a special group with unmet needs when it comes to drug therapy. It is a big challenge to perform studies related to drug teratogenicity and the safety of drug use during pregnancy, as was mentioned in Chapter 1. Ideally, before new drugs are approved for their use on the market, their safety and efficacy profile should also be tested in the pregnant population. Clinical trials should be designed to appropriately capture the time-dependent physiological changes throughout pregnancy, where each patient could serve as their own control (1). This will enable clinicians and patients to make an informed choice of a treatment based on evidence from clinical data. However, clinical trials are not usually done in pregnant women due to ethical and legal reasons. Recently, experts have proposed the involvement of pregnant women in clinical trials, provided that minimization of risk for achieving the objectives of the research was considered (1-5).

At present, the knowledge of human teratogenic risks is still limited. This emphasizes the need for increasing the quality of current study designs, and using complementary study designs (which will be discussed further) to assess the potential risk of drug exposures during pregnancy. Research generates more information continuously, but it is a challenge to select relevant information for clinical decision making. In this chapter, I will discuss our main findings and future perspectives towards personalization of drug therapy during pregnancy, with a focus on the risk of drug teratogenicity.

**The role of transporter proteins in fetal drug exposure**

*Drug interactions mediated by transporter proteins*

Drugs taken by pregnant women may be transferred to the fetus. Transporter proteins expressed in the placenta are known to be the gates that facilitate or limit the transport of substrate drugs from the maternal circulation to the fetal side (6-9). In Chapter 2 and 3, we used literature data to classify different drugs per specific transporter protein; they can either be substrates, inhibitors, inducers, substrate/inhibitors, or substrate/inducers. About 1 in 10 mothers in our case and reference populations had used drugs which are substrates of P-glycoprotein (P-gp) (Chapter 2). Meanwhile, the user rates of drugs which are substrates of other transporters were much lower (for example around 6-8% for multidrug resistance-associated protein 1 (MRP1) and 2-3% for breast cancer resistance protein (BCRP)) (Chapter 3). As P-gp is considered to be the most relevant transporter protein in drug transport and elimination, it is studied much more extensively than the other transporters (10).

Fetal drug exposure can be modulated by drug-drug interactions mediated by placental transporters. We used the risk of congenital anomalies as a proxy for fetal drug
exposure as the outcome in our studies. In Chapter 2, we identified women who used drugs transported by P-gp, and with possible negative effects on the fetus. We found that the women who took these drugs concurrently with an inhibitor of P-gp, had an increased risk of having children with congenital anomalies. This finding was supported in the literature, mainly in animal and in vitro/ex vivo studies which showed that P-gp has a role in limiting fetal exposure to toxicants (10,11). These preclinical studies, however, mainly used placental models obtained in late pregnancy, leaving us with a knowledge gap on the drug transport mechanism during early pregnancy (12-15). The expression of placental P-gp is assumed to be much higher in early pregnancy, as compared to late pregnancy (16,17), suggesting that the placenta’s ability to protect the fetus from drugs is greater in early pregnancy. Therefore the effect of the P-gp inhibition on fetal drug transfer might have been more pronounced during this period.

The role of other placental transporters in fetal drug transfer is still understudied. Our study in Chapter 3 did not find any association between drug interactions mediated by these transporters and the risk of congenital anomalies (i.e. breast cancer resistance protein (BCRP) and multidrug resistance-associated protein 1 (MRP1) as efflux transporters; and organic cation transporter 3 (OCT3), equilibrative nucleoside transporter (ENT1), organic anion transporter 4 (OAT4), organic anion transporting polypeptide 2B1 (OATP2B1) and monocarboxylate transporters 1, 4, 8 and 10 (MCT1, MCT4, MCT8, MCT10) as solute carrier proteins). One major point we learnt from this study is that there is a lack of data with many drugs that are not yet characterized clinically as substrates, inhibitors or inducers of these transporter proteins.

Several transporter-mediated drug interactions have been reported to cause clinical changes on the pharmacokinetics of several substrate drugs. For example, the inhibition of P-gp expressed in the renal tubules and the intestines increases the plasma concentration of digoxin, a P-gp substrate (18). For the OATP uptake transporters expressed in the hepatocytes (OATP1B1, OATP1B3, OATP2B1), co-administration of pravastatin (substrate) and gemfibrozil (inhibitor) leads to an increase in the plasma concentration of pravastatin (19). However, translating these findings for transporters expressed in the placenta can be challenging. Furthermore, previous preclinical and clinical studies investigated only selected substrate drugs. Digoxin, for example, is one of the most extensively used substrate in drug interaction studies mediated by P-gp. Due to narrow therapeutic index of digoxin and its negligible degree of metabolism, differences in the efflux activity of P-gp would have a major impact on its pharmacokinetics (18). However, extrapolating the results to our drugs of interest, which could have different physicochemical properties,
needed further evidence. It is also a big challenge to measure the changes in placental transport of drugs at the relevant concentration expected in patients. Furthermore, there are other parameters contributing to the levels of fetal drug exposure, including the metabolism of drugs by metabolic enzymes, drug interactions mediated by these enzymes, and pharmacokinetic changes of a drug throughout pregnancy (20). Also, large confidence intervals were found in our studies, pointing to a need for larger studies to replicate the findings and improve the clinical relevance.

*Genetic polymorphisms of transporter proteins*

Besides the effect of drug inhibition of the placental transporters, the expression and activity of a transporter can also be modulated by genetic polymorphisms. In Chapter 4 we reviewed all genetic polymorphisms that were reported to be related to the expression and transport activity of placental transporter proteins. We also proposed to group these relevant genetic variants into phenotype classifications, as previously used for metabolic enzymes (21). These phenotypes are based on their effect on transporter function in the placenta: ‘increased’, ‘normal’, ‘decreased’ and ‘abolished’ activities. Similar to the genetic risk scoring method, every risk allele counted is given the same absolute effect size on the phenotype scale, either as increased, normal, decreased or abolished transport (22,23). Another assumption is that the same phenotype applies to all possible substrates of the same transporter. The limitation of these assumptions is that it may not be a true reflection of the biological basis of transporter activity.

The usefulness of this phenotype scoring is yet to be demonstrated in a clinical setting. By using this phenotype grouping, it is possible to perform genetic association studies using smaller and more realistic sample sizes. Such studies might further explain the associations previously found between P-gp rs1045642 (3435C>T), maternal drug/toxicant exposures, and the risk of congenital anomalies (24-26). Taking into account the non-genetic factors, especially maternal drug use, gene-environment interaction studies are one of the options in investigating the safety of drugs used during the first trimester. Two common polymorphisms, OATP1B1 rs4149056 (521T>C) and BCRP rs2231142 (421C>A), have also been reported to have sufficient impact on medication disposition or response, which warrant their incorporation into the drug development process (27,28).

In general, our studies point out the relevance of P-gp as one of the protective mechanisms against the possible harmful effect of a drug on the developing fetus. However, it is unknown whether the role of P-gp mediated drug-drug interactions is as large as the role of the drug-drug interactions mediated by metabolic enzymes,
which are currently being optimized for the implementation in clinical decision supports (29,30). As outlined by The International Transporter Consortium, future studies on drug transporters could start from in vitro studies to determine whether the drug of interest is a substrate to any of the placental transporters (27). If drug-drug interactions mediated by these transporters contribute significantly to the drug’s pharmacokinetics, and if the drug has a small therapeutic window, pharmacogenomic studies should then be considered. A better understanding of the ontogeny in the expression of placental transporter proteins is also of clinical importance. To facilitate future research, the established public data repositories in drug transporter studies can be useful in knowledge sharing, e.g. the UCSF–FDA Transportal21 (http://bts.ucsf.edu/fdatransportal) and PharmGKB (http://www.pharmgkb.org) (31).

Pharmacogenetics as a tool towards personalized drug therapy

Pharmacogenetics can be a tool to identify patients who require changes in drug dosing or selection, in order to ensure the efficacy and safety of the drug therapy. Before investing in the implementation of this concept on a national scale, we need to educate the public on pharmacogenetics. Surveys assessing the knowledge and attitude towards pharmacogenetics, in the early 2000’s, were targeted to specific patient populations or racial groups (32,33). More recent studies have extended the focus towards the public and health care providers, and new insights of the challenges were gained from both population groups.

As our focus group is pregnant women, we assessed the knowledge and attitude of the concept of pharmacogenetics among women who recently became mothers (Chapter 5). We observed that many of the respondents (nearly 70%, N=219) are aware of the relation between their genes and the response of their body to medication, although only few of them knew the term ‘pharmacogenetics’. The use of this term might also be one of the reasons of the low response rate (22%) in our study, as it might be intimidating and might have caused the women to refrain from participating.

Our respondents were generally positive towards the implementation of pharmacogenetics in clinical care. However, some concerns were raised, which were mostly consistent with the ones reported in the literature. They included the privacy and anonymity of genetic information, possible misuse by employers or insurance companies and a lack of understanding of the concept (32,34-37). Our survey also found that the public expects to receive relevant information from their health care providers, while according to other surveys, the health care providers were concerned about their lack of knowledge of pharmacogenetics (38,39). Other
barriers perceived by the health care providers were the cost and reimbursement for the service, shortage of personnel, lack of clinical guidelines and time constraints (38-40). The gap between patients’ high expectation of information and health care providers’ limited knowledge highlights the need for better knowledge dissemination on pharmacogenetics. This calls for a more uniform educational program and training for the pharmacists and medical doctors in interpreting pharmacogenetic information and translating it into clinical care (41,42).

For future studies, it is important to improve the participation rates. When conducting surveys involving patient populations or the public, the choice of terms used might be important. Other terms within the context of pharmacogenetics include ‘personalized medicine/drug therapy’, ‘precision medicine’, ‘individualized medicine’, which are easier and more likely to be used in the general media. In addition, more effort should be given to educate the public on the concept of pharmacogenetics and what it can offer towards better drug therapy options, for example through seminars, brochures, websites or using social media platforms.

Pharmacogenetics and drug-induced teratogenicity

As an effort to pave the way to personalized drug therapy during pregnancy, we explored the use of pharmacogenetic research in determining fetal risk of teratogenicity. Finding pharmacogenetic markers relevant for the risk of congenital anomalies can be a tool in preventive measures against drug teratogenicity. We explored the use of this tool in assessing the risk of congenital heart anomalies (CHA) associated with the use of serotonin reuptake inhibitors (SRIs) in the first trimester of pregnancy. We first identified possible pharmacogenetic predictors in relation to the pharmacokinetics of SRIs and the proposed teratogenic effect on the fetal heart (Chapter 6). Important components in the mechanism of this purported teratogenicity include the maternal metabolic enzymes, placental transporter proteins, serotonin transporter and serotonin receptors. Although the human placenta and the developing fetus each have some minor metabolizing capacity, both seem unlikely to contribute to the total pharmacokinetics of drugs taken by the mother (43). The dominant metabolic enzyme in the fetal liver is CYP3A7, which participates in the synthesis of estrogens. However, the interindividual variability of CYP3A7 expression was high and data on its role in the metabolism of drugs was scarce (44,45).

In Chapter 7, we performed an exploratory gene-environment (G x E) interaction study to explore the G x E effect of the pharmacogenetic predictors and prenatal exposure to SRIs on the risk of CHA. Among several single nucleotide polymorphisms (SNPs) tested, fetal genotypes in serotonin receptors (HTR1A
rs1364043, HTR1B rs6296 & rs6298, HTR3B rs1176744) seemed to interact with SRI exposure to cause an increased risk of CHA. However, we had too limited sample sizes for these associations to reach statistical significance. The participation rate was low (30% among cases exposed to SRIs), despite our efforts of sending reminders and collecting DNA via buccal swabs instead of blood collection. We also offered them the results of their pharmacogenetic tests relevant for the dosing of certain drugs, which are quite costly. We understand that we are dealing with a rather difficult population, as congenital anomalies can be a significant medical and psychological burden to the families affected. In addition, the parents may not understand the benefit of such pharmacogenetic tests for themselves. To improve participation rate, it would be helpful to first educate them on the pertinent role of genetics in understanding the etiology and risk of congenital anomalies. Ways to approach them can be extended through the physician or specialist who treats the child, relevant support groups, or organizations that are directly involved in the well-being and support for these families.

Our exploratory study faced several challenges, which contribute to some limitations. CHA can range from very mild (unnoticed, undiagnosed) to very severe (leading to spontaneous abortion or termination of pregnancy). The range in severity creates a selection bias towards cases with more severe CHA which were detected within a few years of life. Also, G x E studies are prone to inadvertent bias in the selection of candidate genes, and there is the risk of finding false positive associations as a result from multiple testing (46). Therefore, it is crucial to replicate the findings with a large enough sample to identify the initial association. In general, sample sizes required to detect the G x E interaction are larger than those required to detect main genetic or environmental effects. For this, collaborations between registries of congenital anomalies are needed.

There are other aspects that we can improve in this study. First, it is important for future research to consider the effect of gene-gene interactions when identifying the cumulative effect of genetic variations on the abnormal phenotype (46). As an example, genes associated with folate, homocysteine and transsulfuration pathways (methylene tetrahydrofolate reductase (MTHFR) and thymidylate synthetase (TYMS)) are important in modulating plasma folic acid concentration in the fetus, which is also a factor in the development of CHA (47,48). Second, genetic predispositions from the paternal side may also be part of the complex etiology of drug teratogenicity, next to the effects of both the mother and the fetus (49). It might be useful to perform a case-parent triad design, which also includes the fathers as participants, to gain insight in the role of paternal genetic variants. Finally, it is important to acknowledge that multiple interaction effects may occur between maternal exposure to drugs with either
the mother's genes or the infant's genes, resulting in maternal G x E and infant G x E effects (50). Many considerations need to be taken into account in G x E studies of congenital anomalies highlighting the importance of a proper study design and prior knowledge of which type of genetic information to collect and which statistical approaches to use.

**Future perspectives**

The future of pharmacogenetics in drug therapy in pregnancy can be foreseen in terms of its clinical application in both future patient care and research possibilities. In future patient care, pregnant women may benefit from personalized drug therapy, which will deliver tailored drug dosing and selection to ensure drug efficacy with limited toxicities. This will enable medical practitioners to counsel on antenatal drug selection by recognizing those fetuses who are at a higher risk for drug teratogenicity. In research, identifying which children were susceptible for drug toxicity based on their pharmacogenetic risk factors may elucidate the mechanism involved in drug teratology (43).

The initial step towards personalized drug therapy for pregnant women is identifying important genetic variants, or ‘pharmacogenetic predictors’, associated with drug-induced teratogenicity. More pharmacogenetic studies are needed to enrich the existing knowledge, and they need to be replicated (Box 1). The pharmacogenetic predictors relevant for drug pharmacokinetics or pharmacodynamics shall be incorporated as a parameter in drug modeling approaches, e.g. pharmacokinetic/pharmacodynamic (PK/PD) and physiologically-based pharmacokinetic (PBPK) models in pregnancy (51-54). These models are being developed as a quantitative prediction model for drug pharmacokinetics and dosing in pregnant women, which may include fetal and/or amniotic compartments to better characterize the placental drug transfer (55). These models will then need validation before implementing it in clinical recommendations.

Although pharmacogenetics is gaining attention, the implementation of this knowledge in drug therapy is truly challenging. Many of the results from association studies are contradictory, or are performed in a small patient population that might not be homogenous in terms of drug treatment. As we strive to prevent drug-induced prenatal genetic screening. This screening is now focused on the detection of fetal chromosomal abnormalities (aneuploidy), using cell-free fetal DNA in the maternal circulation (56,57). The use of advanced molecular tools, including digital polymerase chain reaction and SNP genotyping microarray, has also increased the possibility of
a complete non-invasive fetal genotyping in utero (56). It would be a big challenge to run genetic tests in unborn babies as a routine clinical procedure, but it should be an option, at least for those with other known risk factors.

With the rapid growth of genomic analysis technology and continuously decreasing costs of genotyping, the ability to predict a fetus’s risk of teratogenicity may be achievable in the future. However, provided that we are able to detect fetal phenotypes associated with an increased risk of drug-induced teratogenicity, are we able to implement this knowledge in a preventive manner? Pharmacogenetic screening may be able to select high-risk subjects that may benefit from dose changes or alternative drugs. However, the contribution of pharmacogenetic predictors may explain only parts of the total prediction risk. Therefore, we need more data to establish a treatment plan aimed to provide an efficacious treatment to the mother with the lowest possible risk to the fetus.

Putting the context of this thesis into clinical practice seems quite distant, but it could be one step towards understanding the mechanistic pathway of the teratogenicity of a drug taken during pregnancy. Therefore, we need continuous research in the areas of epidemiology and genetics, as well as epigenetics, to shed light on the risk factors of teratogenicity, which will hopefully be valuable in preventive strategies in clinical practice.
**Box 1: Alternative approaches in genetic studies related to drug-induced teratogenicity**

- Genome-wide association studies (GWAS) have been useful in detecting common variants associated with congenital anomalies, especially CHA, cleft lip and/or palate (CLP) and hypospadias (49). These genetic risk variants could then be included in G x E as one of the predictors relevant in the development of drug teratogenicity.

- The G x E wide interaction studies (GEWIS), an extension from GWAS, is the approach of detecting G x E effects from the signals obtained from GWAS. It begins with the identification of SNPs which are associated with the outcome, and then evaluates the SNPs for their interactions with environmental exposures with the traditional case-control G x E studies (58,59).

- Epigenetic modifications, especially DNA methylation, were also found to be an underlying mechanism in the development of CHA (60,61). Various maternal factors implicated with abnormal fetal development have been shown to affect DNA methylation patterns. The combined effects of pharmacogenetics and epigenetics on fetal development are yet to be explored.
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