Pharmacogenomics in Pediatric Patients: Towards Personalized Medicine

Hedy Maagdenberg, Susanne J. H. Vijverberg, Marc B. Bierings, Bruce C. Carleton, Hubertus G. M. Arets, Anthonius de Boer, Anke H. Maitland-van der Zee

Abstract It is well known that drug responses differ among patients with regard to dose requirements, efficacy, and adverse drug reactions (ADRs). The differences in drug responses are partially explained by genetic variation. This paper highlights some examples of areas in which the different responses (dose, efficacy, and ADRs) are studied in children, including cancer (cisplatin), thrombosis (vitamin K antagonists), and asthma (long-acting β2 agonists). For childhood cancer, the replication of data is challenging due to a high heterogeneity in study populations, which is mostly due to all the different treatment protocols. For example, the replication cohorts of the association of variants in TPMT and COMT with cisplatin-induced ototoxicity gave conflicting results, possibly as a result of this heterogeneity. For the vitamin K antagonists, the evidence of the association between variants in VKORC1 and CYP2C9 and the dose is clear. Genetic dosing models have been developed, but the implementation is held back by the impossibility of conducting a randomized controlled trial with such a small and diverse population. For the long-acting β2 agonists, there is enough evidence for the association between variant ADRB2 Arg16 and treatment response to start clinical trials to assess clinical value and cost effectiveness of genotyping. However, further research is still needed to define the different asthma phenotypes to study associations in comparable cohorts. These examples show the challenges which are encountered in pediatric pharmacogenomic studies. They also display the importance of collaborations to obtain good quality evidence for the implementation of genetic testing in clinical practice to optimize and personalize treatment.

Key Points

- Implementation of pharmacogenomic testing in pediatric care is still scarce.
- To enable implementation of pharmacogenomic testing in clinical practice, consensus should be reached on the criteria that should be met before implementation.
- Heterogeneity of study populations is an important factor for hindering the replication of pharmacogenomic associations.
1 Introduction

Individuals with the same disease will often respond differently to the same drug. Some individuals will have a good response to the drug, while others experience little or no effect. Some patients will experience severe adverse drug reactions (ADRs), whereas others will not. In addition, some patients require a higher or lower dose compared with the standard dose defined in clinical trials to benefit optimally from the drug. In other words, personalizing drug treatment is required. Pharmacogenomics studies the relationship between genetic variation and drug responses. Single nucleotide polymorphisms (SNPs) can lead to changes in the function or the amount of proteins (e.g., enzymes, receptors, ion channels) and therefore in the drug response [1]. Pharmacogenomics covers associations with both germline and somatic mutations. In this review only the influence of germline mutations will be discussed.

The first pharmacogenomic studies were designed as candidate gene studies. The candidate genes are selected based on potential involvement with drug response, such as genes coding for metabolic enzymes and drug target proteins. However, the cause of a different drug response is not always in the potentially involved genes, which makes it difficult to choose candidate genes.

The design of a genome-wide association study (GWAS) is more data driven than the hypothesis-driven candidate gene association studies. In a GWAS, the whole genome of participants is screened for all frequently occurring SNPs. With GWAS, besides SNPs in candidate genes, also previously unknown associations between a specific SNP and a certain response to a drug can be found. The newest innovations in pharmacogenomics, enabled by the rapid improvement of genomics technology, are phenotype-wide association studies (PheWAS), whole exome sequencing (WES), and whole genome sequencing (WGS), which bring new opportunities to study the association between response and genetic variants [2].

Most pharmacogenomic research has been performed in adults. However, it is important to realize that findings in the adult population cannot be applied directly to the pediatric population [3]. Processes and systems (such as the metabolic system and hemostasis and drug biotransformation) are still under development in children [3, 4]. Therefore, drugs may act differently in children compared with adults. Although genetic variations remain stable, the contribution to treatment heterogeneity may be different at a younger age. In this article, we highlight examples of pharmacogenomic studies in pediatric patients. Pharmacogenomic research in childhood cancer is, apart from the focus on tumor genetics, focused on predicting which patients will suffer from severe ADRs. In the treatment of thrombosis, the studies have focused on predicting the right anticoagulant dose for each pediatric patient; and in asthma the main issue is to predict the efficacy of a bronchodilator drug. These are representative and extensively studied examples of the earlier mentioned sorts of differences in drug responses (ADRs, dose, and efficacy). These examples will give an insight into the challenges of pharmacogenomic research in children, but will also address the potential of pharmacogenomics to optimize and personalize treatment for children.

2 Pharmacogenomics in Children

2.1 Childhood Cancer

In 2012, the worldwide estimated number of children under the age of 15 years diagnosed with cancer was 163,300 [5]. The mean 5-year survival rates in the US are just above 80 %, but it largely depends on the type of cancer [6]. With the increase in survival rates, the ADRs, which can cause lifelong damage, are becoming increasingly important during and after treatment. Anticancer drugs that are well known for their ADRs are cisplatin (ototoxicity, renal toxicity), anthracyclines (cardiotoxicity), and vincristine (neurotoxicity). These ADRs can have a large impact on quality of life. Many pharmacogenomic studies in the field of childhood cancer have focused on the toxicity of treatment. However, clinical implementation of pharmacogenomic testing is still pending in many centers because of inconclusive study results or uncertainty about whether and for which patients implementation is clinically relevant. We will discuss cisplatin as an example. This drug has been associated with a risk of ototoxicity, which can be very impairing, especially for children who are developing their speech skills [7]. Several candidate gene studies have been conducted to investigate specific SNPs which are associated with an increased or decreased risk of ototoxicity. Variations in the following genes were found to influence the risk of cisplatin-induced ototoxicity: TPMT, COMT, ABCC3, SOD2, GSTT1*1, GSTP1, XPC, LRP2, Otos, SLC22A2, CTR1 and GSTM3*B [8–18]. However, a major issue is the reproducibility of these initial findings. Several groups have conducted relatively small candidate gene studies on the association between ototoxicity and variations in COMT and TPMT in different cohorts [9, 19–21]. The cohorts are very heterogeneous (Table 1) and some lack statistical power. For TPMT, the association was replicated in two similar cohorts [8, 9]. One small Spanish cohort (n = 38) also showed an association for TPMT; however, because of the lack of power it was not statistically significant (rs12201199, odds ratio (OR) 6.79, 95 % confidence interval (CI) 0.34–13.71) [20]. The association...
Table 1 Overview of the characteristics of cisplatin-induced ototoxicity studies

|                      | Ross et al. [8] | Pussegoda et al. [9] | Yang et al. [19] | Lanvers-Kaminsky et al. [21] | Hagleitner et al. [20] | Xu et al. [22] |
|----------------------|----------------|----------------------|------------------|-----------------------------|------------------------|---------------|
|                      | Discovery      | Replication          | Radiation        | No radiation                | No radiation           |               |
| Number of patients   | 53            | 109                  | 155              | 213                         | 41                     | 63            |
| Treatment protocols  | NS            | NS                   | NS               | SJMB-96; SJMB-03             | SJNB-97; SJNB-05;      | No radiation |
|                      |               |                      |                  |                             | SJOS-08; COSS-82/86/91;| NS            |
|                      |               |                      |                  |                             | NB-90; MAHO-94;        |               |
|                      |               |                      |                  |                             | MAKEI-89; HIT-91       |               |
| Age [y], median (range) |              |                      |                  |                             |                        |               |
| Cases                | 5 (0–16)      | 6 (0–16)             | 6 (1–25)         | 7.6 (3.1–21.6)              | 3–12 (0.8–18)          | 11.8 (5–22) |
| Controls             | 9 (0–16)      | 9.5 (1–19)           | 11 (0–18)        | 10.1 (3.3–19.8)             | 10 (2–13)              | 13.9 (5–20) |
| Sex, male [%]        | 67.9          | 57.8                 | 49.7             | 66.2                        | 70.7                   | 60.3          |
| Ethnicity [%]        | Caucasian    | Caucasian            | white 80.0       | White 78.9; non-white 21.1  | NS                     | European ancestry |
|                      | 79.0a         |                      |                  |                             |                        |               |
| Cancer type          | Various cancers | Various cancers    | Various cancers  | Medulloblastoma              | Neuroblastoma;         | Various cancers (mainly osteosarcoma) |
|                      |               |                      |                  |                             | osteosarcoma           |               |
| Follow up duration [y], median (range) or fixed number of years | Cases: 3 (0–18); controls: 2 (0–15) | Cases: 5 (0–25); controls: 2 (0–16) | 1.7 | NS | 2.5 | NS |
| Craniospinal irradiation [%] | 17.0 | 19.3 | 18.1 | 100 | 0 | NS |
| Concurrent drug therapy | Yes | Yes | Yes | NS | NS | Yes |
| Use of ototoxic antibiotics | Yes | Yes | Yes | NS | NS | No |
| Vincristine (%)      | 39.5a         | 49.7                 | 100              | 0                           | NS                     | 15.8          |
| Otoprotectants (%)   | NS            | NS                   | 90.6             | 0                           | NS                     | 4.5           |
| Cumulative cisplatin dose [mg/m²], median (range) | Cases | Controls | Cases | Controls |
|                      | 360 (180–630) | 410 (100–720)        | 400 (92–800)     | 400 (20–768)                | 300 (77–313)           | 300 (77–313) |
|                      | 390–618 (113–1105) | 412 (120–644)        | 412 (120–644)    | 418 (161–560)               | 504 (120–870)          | 500 (100–600) |
|                      | 254 (225–815) | 515 (140–720)        | 480 (200–600)    |                             |                        |               |
|                      | 287 ± 35b     | ±300                  |                   |                             |                        |               |

a, b, c Statistical significance as compared to control group.
From these studies we can conclude that the mutation in ACYP2 seems to be an important predictor of ototoxicity in children, but that it explains only a small part (12.4%) of ototoxicity [22]. More research is needed to replicate these findings and to find practical solutions for the implementation of ACYP2 testing in clinical practice. Studies of the mechanism for TPMT and COMT involvement in cisplatin-induced ototoxicity are involved in cisplatin-induced ototoxicity.

Table 1 continued

| Outcome | Association COMT | Association TPMT |
|---------|------------------|------------------|
| Discovery | Replication | Discovery | Replication | Discovery | Replication | Discovery | Replication |
| Ototoxicity grading scale and comparison groups | | | | | | | |
| CTCAE >1 vs CTCAE = 0 | CTCAE >1 vs CTCAE = 0 | CTCAE >0 vs CTCAE = 0 + ordinal and Chang score = 0 vs >0; 2a vs ≥2a and ordinal | CTCAE ordinal | NS | CTCAE >1 vs CTCAE = 0 | Chang score >0 vs Chang score = 0 |

**CTCAE** common terminology criteria for adverse events, **NS** not specified, **SIOP** international society of pediatric oncology, y years

a Based on combined cohort (discovery + replication) [9]
b Mean and standard deviation given instead of median and range
c Based on combined cohort (cases + controls)

ACYP2 encodes for an acylphosphatase which is among other places, expressed in the cochlea [23]. The exact mechanism by which this mutation in ACYP2 increases the risk of ototoxicity is still unclear. The replication of the results found in the different candidate studies has led to an extensive discussion about the underlying reasons [24–28]. The replication of the results found in the discovery cohort in another independent cohort also existed within the studies [22]. ACYP2 was replicated twice [8, 20], but in one of the studies the association was in the opposite direction [20].

Another problem with COMT and TPMT is the lack of information on the mechanism in which these two enzymes are involved in cisplatin-induced ototoxicity.

Hagleitner et al. conducted a meta-analysis for COMT and TPMT in 2014 and found only a small association for COMT (rs4646316) (OR 1.52, 95% CI 1.16–1.99, \( p = 0.003 \)). For the analyzed TPMT mutations there was a trend towards increased risk (rs12201199; OR 2.15, 95% CI 1.16–1.99, \( p = 0.003 \)) [20]. However, it is debatable if these results give an accurate effect estimation, because of the heterogeneity in populations of the included studies.

Recently, a GWAS failed to find any association for TPMT, COMT or any of the other genes studied in the candidate gene studies [22]. The GWAS study was conducted in 238 pediatric patients with newly diagnosed brain tumors. A strong association was found for a mutation in ACYP2 (rs1872328, hazard ratio 4.5, 95% CI 2.63–7.69, \( p = 3.9 \times 10^{-8} \)), which was replicated in a new cohort of 68 patients that was almost similarly treated as the discovery cohort. In the discovery and the replication cohorts, 100% of the patients that carried at least one mutated allele developed ototoxicity. In the patients with no mutated allele, still more than 70% developed ototoxicity [22]. ACYP2 encodes for an acylphosphatase which is, among other places, expressed in the cochlea. The exact mechanism by which this mutation in ACYP2 increases the risk of ototoxicity is still unclear.

The problems with replication of the results found in the different candidate studies has led to an extensive discussion about the underlying reasons [24–28]. The replication of the results found in the discovery cohort in another independent cohort also existed within the studies [22]. The exact mechanism by which this mutation in ACYP2 increases the risk of ototoxicity is still unclear. The replication of the results found in the different candidate studies has led to an extensive discussion about the underlying reasons [24–28]. The replication of the results found in the discovery cohort in another independent cohort also existed within the studies [22].
induced ototoxicity and independent replication in similar cohorts are required.

For some patients the toxicity is unacceptable (e.g., ototoxicity for a patient who is blind). In such patients, decisions on therapy will be influenced by genetic polymorphisms that enhance the risk of developing toxicity. With the identification of significant risk variants, patients who are at an increased risk can be identified and might be given alternative treatments and/or undergo closer monitoring during treatment and the follow-up period. Adapting complex treatment regimens in an attempt to reduce side effects is complicated since efficacy must remain intact. Different approaches maybe explored: identifying a protecting agent against ototoxicity is an attractive option. The knowledge gained from the identification of variants that influence the risk of cisplatin-induced ototoxicity can be used to identify new drug targets for protecting agents. This research is promising and will eventually lead to a more personalized anticancer treatment.

2.2 Thrombosis

In recent years there has been a higher incidence of thrombosis in children [30], mainly due to intensified medical treatments and increased awareness of the risk of thrombosis. Currently, low molecular weight hepans (LMWHs) and vitamin K antagonists (VKAs) are the only two drugs approved for the treatment or prevention of thrombosis in pediatric patients. The relatively new direct oral anticoagulants (DOACs) are currently being tested in pediatric patients. In 2018, the first phase III studies with DOACs in pediatric patients will be completed. At this time, the VKAs are the only oral drugs which are approved for the treatment of thrombosis in pediatric patients.

VKAs inhibit the action of vitamin K epoxide reductase (VKORC1), which leads to lower levels of active vitamin K-dependent clotting factors, and thus to inhibition of the coagulation cascade [31]. In clinical practice, a large variability in dose requirement of VKAs is seen [32]. This is problematic because VKAs also have a narrow therapeutic window. Dosing all patients equally leads to an increased risk of bleeding and thrombotic events. In children, this problem is even more compelling because of the developing hemostatic system and the growing body. In the last decade, many studies have been carried out to explain the large interindividual dose variability in children and adults [33, 34]. In addition to clinical factors such as age, weight, and gender, genetic factors play an important role [34]. Mutations in VKORC1 lead to less enzyme production and to a lower dose requirement. Loss-of-function mutations in CYP2C9 (*2 and *3) lead to a decrease in the required dose [31, 35]. To a lesser extent, mutations in CYP4F2 and CYP2C18 have also been found to be (possibly) contributing to the dose variability [33, 35–37].

Seven regression dosing models have been constructed for pediatric patients, almost all for warfarin [38–44]. No pediatric dosing model is available for acenocoumarol. What these pediatric models have in common is that factors related to ontogeny (i.e., age, weight, and height) explain roughly one-third of the dosing variability. The variability explained by the CYP2C9 and VKORC1 genotypes fluctuates between the different models. The CYP2C9 genotype explained 0.4 [38] to 12.8 % [39] of the variability in dose requirement, the VKORC1 genotype 3.7 [38] to 47 % [41]. One of the possible explanations is the small sample size of the cohorts ranging from 37 to 120 children. Only two studies included at least 100 patients [39, 44].

Also, two pharmacokinetic/pharmacodynamic (PK/PD) dosing models have been built for pediatric patients [45, 46]. Hamberg and Wadelius evaluated the regression and PK/PD models in a retrospective pediatric cohort [34]. Of the evaluated models, the PK/PD model of Hamberg et al. [46] performed best with regards to the proportion of patients for whom the predicted maintenance dose was within ±20 % of the observed dose. Hamberg et al. developed a tool for their model which can run on every computer without licensing for a program and is easy to use [47]. The best performing regression model incorporates the CYP2C9 and VKORC1 genotype, height, and indication and can be used with a simple pocket calculator [39].

Until now, no randomized controlled trial (RCT) has been conducted with a regression dosing model in children. One trial has just started, in which a PK/PD dosing model is tested against standard dosing [48]. In adults, 12 RCTs have been carried out to evaluate the dosing algorithms [49]. These trials gave conflicting results with regards to improving the time within therapeutic range (TTR) and outcomes such as bleeding and thromboembolic complications. In a recent meta-analysis, a statistically significant increase in TTR and decrease in minor bleeding was found when comparing fixed standard dosing with genotype-guided dosing [49].

Currently, the American College of Chest Physicians (ACCP) guideline for antithrombotic therapy and prevention of thrombosis does not recommend genotyping before starting VKAs in adults [50]. The FDA follows this recommendation, while still including information on the impact of pharmacogenomics in the drug label [51]. When genetic information is available, the physician can use this to adjust the dose. In pediatric patients, this information should not be used. Studies showed that the adult models overestimate the VKA dose in children [39, 42]. Therefore, pediatric models should be used when genetic information...
is available. An RCT for examining a pediatric regression model does not seem to be a realistic option for determining the usefulness of genotyping before starting a VKA. The numbers of children using these drugs are very low, and therefore such a trial would be very costly and time consuming. We think the pediatric algorithms should be implemented and evaluated in a clinical setting. Using a dosing model can only lead to an increase in the quality of treatment. There are no risks involved, because adjustments of the dose can still be made based on the International Normalized Ratio (INR). The costs of using a model only consist of the price of genetic testing and these costs are already quite reasonable compared with other medical tests, and will probably decrease further over time. It might be possible that genotyping becomes cost effective, because when INR stability increases it is likely that fewer INR measurements will be needed, and fewer bleeding and thrombotic events will occur. Evaluations should be carried out during implementation in order to determine if the genetic testing is increasing the quality of treatment and/or lowering the costs.

### 2.3 Asthma

Asthma is the most common chronic disease in children. Asthma is treated with a stepwise approach [52]. Short-acting β2 agonists (SABA) as needed are prescribed initially to relieve symptoms of bronchoconstriction. Inhaled corticosteroids (ICS) are added to the regimen if asthma symptoms persist to reduce the airway inflammation and are considered to be the cornerstone of asthma treatment [52]. Additionally, long-acting β2 agonists (LABA) or leukotriene receptor agonists (LTRA) can be added if a child’s asthma remains insufficiently controlled. Although asthma treatment is effective in many patients, there is a large variability in the level of symptom control or lung function improvement. Already more than 15 years ago, Drazen et al. suggested that up to 80% of the interindividual variants in drug response in asthmatic patients could be due to genetic variations [53]. Since then, candidate gene approaches and a handful of GWAS studies have described several genetic variants associated with asthma treatment response, yet effect sizes are often small and a successful replication remains rare [54–56].

Pharmacogenomics of LABA seems closest to clinical implementation. An SNP of interest (ADRB2 Arg16) has been replicated and prospectively tested and the risk genotype is relatively frequent within the population. Variation in the gene that encodes the β2 receptor (ADRB2) is associated with LABA response in children [57–59], yet not all studies point in the same direction [60]. Nevertheless, a recent meta-analysis of 4226 children of white Northern European and Latino origin showed that this variant (ADRB2 Arg16) was associated with an increased risk of asthma exacerbation when treated with ICS + LABA (OR 1.52, 95% CI 1.17–1.99; p = 0.0021) [61]. In addition, further evidence has been provided by a small prospective study of 62 children with the genetic variation randomized to ICS + LABA or ICS + LTRA. The trial showed that children treated in the ICS + LTRA arm had fewer exacerbations (exacerbation score of −0.39, 95% CI −0.15 to −0.64; p = 0.049) and school absences (difference in scores of 0.40, 95% CI −0.22 to −0.58; p = 0.005) compared with the group treated with ICS + LABA [62]. Approximately 16% of the children with asthma are homozygous for this variant [57], and may benefit from genotyping before initiation of LABA treatment. Larger trials are necessary to assess the clinical value and cost effectiveness of ADRB2 genotyping.

Defining treatment response in asthma is complicated. Symptoms vary over time and different dimensions of response (lung function, exacerbations, and symptoms) can be associated with different genetic risk profiles [63]. Furthermore, asthma consists of a heterogeneous population of various distinct phenotypes (e.g., eosinophilic versus neutrophilic asthma), which seems to differ for children and adults. Performing studies in children is therefore of the utmost importance. Recently, the Pharmacogenomics in Childhood Asthma (PiCA) consortium has been formed to bring asthma researchers in this field together to perform meta-analyses in well-defined joined pediatric asthma cohorts [61, 64].

### 3 Challenges and Future Directions

Although the research field of pediatric pharmacogenomics is rapidly growing, few applications have made it to clinical practice. We have provided examples of three pediatric diseases where pharmacogenomics holds a promise to personalize treatment: childhood cancer, thrombosis, and asthma. These examples illustrate that gathering evidence for a pharmacogenomic association in children is challenging. Replication of genetic associations is complicated by the heterogeneity in both outcome measures and in small study populations in terms of ethnicity, disease phenotype, and age, which leads to underpowered biased studies. To overcome this obstacle, collaborations should be undertaken to enlarge the number of patients studied.

More studies have been performed on pharmacogenomic associations in adults, including a couple of RCTs, but unfortunately these results in adults cannot be simply extrapolated to children. Pharmacogenomic studies in pediatric populations remain essential. The therapeutic goal of a certain treatment is often different for adults and children. In addition, differences in co-medication, diet,
and duration of drug use can also lead to dissimilar results. Before data can be extrapolated to children it should be clear if the association is not influenced by ontogeny. Children not only differ from adults in body size, but also in the dynamic expression of metabolic enzymes, drug transporters, and drug targets [3, 65]. Furthermore, the organs involved in drug metabolism and elimination (liver and kidney) are under the influence of developmental processes during childhood [3]. Besides these physical differences, the disease can also manifest itself differently in children, as seen, for example, in asthma [66]. These differences make it hard to predict the PK/PD of a drug in children. The drug response can differ between children, but also within one child over time. Therefore, the extrapolation of results between children of different ages should be done with the same caution as the extrapolation of adult data to children. Pediatric patients span a period from birth to adulthood by most definitions. An RCT is still considered the gold standard to collect evidence. However, performing RTCs in children is complicated by the large sample sizes which are required, especially in rare diseases such as cancer and thrombosis. For example, in the case of VKAs, obtaining the required sample size is a large problem. For the EU-PACT (European Pharmacogenetics of Anticoagulant Therapy) trial in adults, investigating the effectiveness of the pharmacogenomic dosing models for acenocoumarol, phenprocoumon, and warfarin, the calculated sample size was 400 per VKA [67, 68]. To put this in perspective, in the Netherlands, currently only 226 children under the age of 15 years use VKAs [69]. To obtain the number of patients needed, international collaborations are essential. Besides the large sample size, the high costs of an RCT need to be considered. This type of research is usually not in the direct interest of pharmaceutical companies, especially if it concerns off-patent drugs. Therefore, it is difficult to find funding for these kinds of trials, and specific financial or other incentives might be required to bridge this obstacle [70].

As stated in the introduction, the improvement of genomics technology creates opportunities to study pharmacogenomics in new ways. The newest is PheWAS, which is the opposite of GWAS. Instead of studying genetic associations with a predefined phenotype, patients with a certain mutation are the starting point to search for the matching phenotype. Other examples are WES/WGS in which all DNA mutations will be considered, in contrast to GWAS, which is directed to known (frequently occurring) SNPs.

There is no one method better than the others. Which method or combination of methods is the most appropriate depends largely on the research question/situation (e.g., knowledge about drug mechanism, available budget). Findings of a GWAS, for instance, can be subsequently replicated in a candidate gene study, which requires far fewer patients and is less expensive than an additional GWAS.

The progression from gathering evidence to clinical relevance is not easy. Even when an association is strong it does not mean that it is clinically relevant. For example, in the case of ACYP2 and ototoxicity, the association was quite strong, but it still could explain only 12.4% of the ototoxicity cases. The clinical relevance largely depends on the relative frequency of the risk allele in the population of interest, the disease phenotype, the severity of the outcome, and the risk attribution of the risk-allele to the outcome. Cost effectiveness of a pharmacogenomic test is inevitably necessary to reach clinical implementation. Even when the costs of genetic testing decline, other costs such as the costs of the possible alternative treatment, use of protective agents, and/or extra monitoring should be considered.

To be able to proceed with implementation of pharmacogenomic testing in children, consensus should be reached about what evidence is needed to implement a pharmacogenetic test into clinical practice if RTCs are not feasible. Furthermore, in some cases performing an RCT could be considered unethical. An important example of this is the risk of codeine-induced infant mortality based on a CYP2D6 genotype of breastfeeding mothers [71]. This has led to a change in the registration of codeine. Codeine is no longer approved for pediatric use in the EU and is contraindicated in women during breastfeeding [72].

When an RCT is impossible, at least worldwide replication studies are needed to support the generalizability of the association. This is only possible with international collaboration. However, the healthcare systems and availability of treatment options (e.g., differences in authorized VKAs) differ largely between countries and treatment protocols vary between countries, study populations, and over time. This makes finding a comparable replication cohort challenging. Therefore, international treatment harmonization would ease the process of worldwide replication studies.

Strong evidence in adults might support the associations found in pediatric patients. However, because of differences related to ontogeny, adult-derived information should be considered with caution and is not essential. This caution should also be applied when using the dosing guidelines available for adults. As seen in the example for VKAs, using the adult models would lead to an overestimation of the required dose. Pharmacogenomics needs to be considered as valuable information in addition to clinical parameters to guide treatment decisions.

It is important that consensus is reached about the evidence needed for implementation and that healthcare professionals also support these criteria; published, peer-reviewed clinical practice guidelines could be of particular
help here. Clinicians need to be appropriately educated on the value of pharmacogenomic testing. Only then will pharmacogenomics be implemented in pediatric clinical practice.

4 Conclusion

Pharmacogenomics is a promising research field, but has not reached the pediatric clinic yet. International collaborations are needed to gain a more structured approach for pharmacogenomic research in children. When heterogeneity is reduced and research groups work together in order to obtain larger numbers of patients, it is possible to get stronger evidence, both qualitatively and quantitatively. The criteria for implementing a pharmacogenomic test without the presence of a supporting pediatric RCT should be further elaborated by healthcare professionals and researchers. Reaching consensus could lead to easier acceptance by healthcare professionals to the use of these tests in daily clinical practice.

Compliance with Ethical Standards

Conflict of interest Bruce C. Carleton is employed by the Pharmaceutical Outcomes Programme (POPi). POPi has received financial support for the presentation of its pharmacogenomics research from Canada Foundation for Innovation (CFRI), Canadian Institutes of Health Research, Genome Canada, Genome British Columbia and the Provincial Health Services Authority. POPi has also received support from the University of British Columbia and the Child and Family Research Institute, and Pfizer Canada (unrestricted). Anke H. Maitland-van der Zee has received an unrestricted research grant from GSK, and she has recently received a research grant from ERANET (Eracosysmed) and ZonMw called SysPharmPedia (Systems Pharmacology Approach to Pediatric Asthma). Hedy Maagdenberg, Susanne J.H. Vijverberg, Marc B. Bierings, Hubertus G.M. Arets, and Anthonius de Boer declare that they have no conflict of interest.

Funding No funding was received for this manuscript.

Acknowledgments We would like to thank the children and families who participated in this study. We are especially grateful to our two research assistants, Kortney Gauvreau and Jordan Waymouth.

Open Access This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits any noncommercial use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. McLeod HL, Evans WE. Pharmacogenomics: unlocking the human genome for better drug therapy. Annu Rev Pharmacol Toxicol. 2001;41:101–21.
2. Mooney SD. Progress towards the integration of pharmacogenomics in practice. Hum Genet. 2015;134:459–65.
3. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology—drug disposition, action, and therapy in infants and children. N Engl J Med. 2003;349:1157–67.
4. Andrew M, Vegh P, Johnston M, Bowker J, Ofose F, Mitchell L. Maturation of the hemostatic system during childhood. Blood. 1992;80:1998–2005.
5. American Cancer Society. Global cancer facts & figures. 3rd ed. Atlanta: American Cancer Society; 2015.
6. Howlander N, Noone A, Krapcho M, Grashell J, Miller D, Altekruse S, et al. Cancer Statistics Review, 1975–2012 National Cancer Institute, Bethesda, MD, http://seer.cancer.gov/csr/1975_2012/, based on November 2014 SEER data submission, posted to the SEER web site, April 2015.
7. Bass JK, Knight KR, Yock TI, Chang KW, Cipkala D, Grewal SS. Evaluation and management of hearing loss in survivors of childhood and adolescent cancers: a report from the Children’s Oncology Group. Pediatr Blood Cancer. 2016. doi:10.1002/pbc.25951.
8. Ross CJ, Katzov-Eckert H, Dubé M-P, Brooks B, Rashek SR, Barhdadi A, et al. Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. Nat. Genet. 2009;41:1345–9.
9. Pussegoda K, Ross CJ, Visscher H, Yazdanpanah M, Brooks B, Rashek SR, et al. Replication of TPMT and ABCC3 genetic variants highly associated with cisplatin-induced hearing loss in children. Clin Pharmacol Ther. 2013;94:243–51.
10. Brown AL, Lupo PJ, Okcu MF, Lau CC, Rednam S, Scheurer ME. SOD2 genetic variant associated with treatment-related ototoxicity in cisplatin-treated pediatric medulloblastoma. Cancer Med. 2015;4:1679–86.
11. Choeyprasert W, Sawangpanich R, Lertsukprasert K, Udombpayakul U, Songdej U, Unurathapan H, et al. Cisplatin-induced ototoxicity in pediatric solid tumors: the role of glutathione S-transferases and megalin genetic polymorphisms. J Pediatr Hematol Oncol. 2013;35:e138–43.
12. Oldenburg J, Kraggerud SM, Cvanarova M, Lothe RA, Fossa SD. Cisplatin-induced long-term hearing impairment is associated with specific glutathione S-transferase genotypes in testicular cancer survivors. J Clin Oncol Off J Am Soc Clin Oncol. 2007;25:708–14.
13. Caronia D, Patiño-García A, Milne RL, Zalacain-Díez M, Pita G, Alonso MR, et al. Common variations in ERCC2 are associated with response to cisplatin chemotherapy and clinical outcome in osteosarcoma patients. Pharmacogenomics J. 2009;9:347–53.
14. Riedemann L, Lanvers C, Deuster D, Peters U, Boos J, Jürgens H, et al. Megalin genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Pharmacogenomics J. 2008;8:23–8.
15. Spracklen TF, Whitehorn H, Vorster AA, Ramma L, Dalvie S, Ramesar RS. Genetic variation in Otos is associated with cisplatin-induced ototoxicity. Pharmacogenomics. 2014;15:1667–76.
16. Lanvers-Kaminsky C, Sprowl JA, Malath I, Deuster D, Eveslage M, Schlatter E, et al. Human OCT2 variant c.808G>T confers protection effect against cisplatin-induced ototoxicity. Pharmacogenomics. 2015;16:323–32.
17. Xu X, Ren H, Zhou B, Zhao Y, Yuan R, Ma R, et al. Prediction of copper transport protein 1 (CTR1) genotype on severe cisplatin-induced ototoxicity in non-small cell lung cancer (NSCLC) patients. Lung Cancer Amst. Neth. 2012;77:438–42.
18. Peters U, Preisler-Adams S, Hebeisen A, Hahn M, Seifert E, Lanvers C, et al. Glutathione S-transferase genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. Anticancer Drugs. 2000;11:639–43.
19. Yang JJ, Lim JYS, Huang J, Bass J, Wu J, Wang C, et al. The role of inherited TPMT and COMT genetic variation in cisplatin-induced ototoxicity in children with cancer. Clin Pharmacol Ther. 2013;94:252–9.
20. Hagleitner MM, Coenen MJH, Patino-Garcia A, de Bont ESJM, Gonzalez-Neira A, Vos HL, et al. Influence of genetic variants in TPMT and COMT associated with cisplatin induced hearing loss in patients with cancer: two new cohorts and a meta-analysis reveal significant heterogeneity between cohorts. PloS One. 2014;9:e115869.

21. Lanvers-Kaminsky C, Malath I, Deuster D, Ciarimboli G, Boos J, Am Zehnhoff-Dinnenes AG. Evaluation of pharmacogenomic markers to predict the risk of Cisplatin-induced ototoxicity. Clin Pharmacol Ther. 2014;96:156–7.

22. Xu H, Robinson GW, Huang J, Lim JY-S, Zhang H, Bass JK, et al. Common variants in ACYP2 influence susceptibility to cisplatin-induced hearing loss. Nat Genet. 2015;47:263–6.

23. Scheffer DI, Shen J, Corey DP, Chen Z-Y. Gene expression by mouse inner ear hair cells during development. J Neurosci Off J Soc Neurosci. 2015;35:6366–80.

24. Ratain MJ, Cox NJ, Henderson TO. Challenges in interpreting the evidence for genetic predictors of ototoxicity. Clin Pharmacol Ther. 2013;94:631–5.

25. Boddy AV. Genetics of cisplatin ototoxicity: confirming the unexplained? Clin Pharmacol Ther. 2013;94:198–200.

26. Carleton BC, Ross CJ, Bhavsar AP, Amstutz U, Pussegoda K, Visscher H, et al. Role of TPMT and COMT genetic variation in cisplatin-induced ototoxicity. Clin Pharmacol Ther. 2014;95:253.

27. Carleton BC, Ross CJ, Hayden MR. Genetic markers of cisplatin-induced hearing loss in children. Clin Adv Hematol Oncol. 2014;12:527–8.

28. Carleton BC, Ross CJ, Bhavsar AP, Lee JW, Visscher H, Rassek SR, et al. Response to “evaluation of pharmacogenetic markers to predict the risk of cisplatin-induced ototoxicity”. Clin Pharmacol Ther. 2014;96:158.

29. U.S. Food and Drug Administration (FDA); centre for drug evaluation and research. Cisplatin label information. 2015. http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/018057s083lbl.pdf. Accessed 02 Feb 2016.

30. Raffini L, Huang YS, Wittmer C, Feudtner C. Dramatic increase in venous thromboembolism in children’s hospitals in the United States from 2001 to 2007. Pediatrics. 2009;124:1001–8.

31. Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, et al. Vitamin K antagonists in children with heart disease: height and VKORC1 genotype are the main determinants of the warfarin dose requirement. Blood. 2012;119:861–7.

32. Shaw K, Amstutz U, Hildebrand C, Rassek SR, Hosking M, Neville K, et al. VKORC1 and CYP2C9 genotypes are predictors of warfarin-related outcomes in children. Pediatr Blood Cancer. 2014;61:1055–62.

33. Verhoef TI, Redekop WK, Jansen J, Jonzon A, et al. Warfarin dose prediction in children using pharmacometric bridging—comparison with published pharmacogenetic dosing algorithms. Eur J Clin Pharmacol. 2013;69:1275–83.

34. De Montfort University; University hospitals, Leicester. Model-based versus traditional warfarin dosing in children. In: ClinicalTrials.gov. Bethesda(MD); National Library of Medicine (US). 2000. http://clinicaltrials.gov/show/NCT02475863. Accessed 26 Jan 2016.

35. Guyatt GH, Akh EIA, Crowther M, Gutterman DD, Schuennemann HJ. American College of Chest Physicians antithrombotic therapy and prevention of thrombosis, 9th ed: American college of chest physicians evidence-based clinical practice guidelines. Chest. 2012;141:445S–488S.

36. Streif W, Andrew M, Marzinotto V, Massicotte P, Chan AK, Julian JA, et al. Analysis of warfarin therapy in pediatric patients: a prospective cohort study of 319 patients. Blood. 1999;94:3007–14.

37. Verhoeft TJ, Redekop WK, Daly AK, van Schie RMF, de Boer A, Maitland-van der Zee A-H. Pharmacogenetic-guided dosing of coumarin anticoagulants: algorithms for warfarin, acenocoumarol and phenprocoumon. Br J Clin Pharmacol. 2014;77:626–41.

38. Nowak-Göttl U, Dietrich K, Schaffranek D, Eldin NS, Yasui Y, Geisen C, et al. In pediatric patients, age has more impact on dosing of vitamin K antagonists than VKORC1 or CYP2C9 genotypes. Blood. 2010;116:6101–5.

39. Biss TT, Avery PJ, Brandão LR, Chalmers EA, Williams MD, Grainger JD, et al. VKORC1 and CYP2C9 genotype and patient characteristics explain a large proportion of the variability in warfarin dose requirement among children. Blood. 2012;119:868–73.

40. Kato Y, Ichida F, Saito K, Watanabe K, Hirono K, Miyawaki T, et al. Effect of the VKORC1 genotype on warfarin dose requirements in Japanese pediatric patients. Drug Metab Pharmacokinet. 2011;26:295–9.

41. Guyatt GH, Akl EA, Crowther M, Gutterman DD, Schuennemann HJ. American College of Chest Physicians antithrombotic therapy and prevention of thrombosis panel. Executive summary: antithrombotic therapy and prevention of thrombosis, 9th ed: american college of chest physicians evidence-based clinical practice guidelines. Chest. 2012;141:7S–47S.

42. Shaw K, Amstutz U, Hildebrand C, Rassek SR, Hosking M, Neville K, et al. VKORC1 and CYP2C9 genotypes are predictors of warfarin-related outcomes in children. Pediatr Blood Cancer. 2014;61:1055–62.

43. Moreau C, Bajolle F, Siguret V, Lasne D, Golmard J-L, Elie C, et al. Vitamin K antagonists in children with heart disease: height and VKORC1 genotype are the main determinants of the warfarin dose requirement. Blood. 2012;119:861–7.

44. Vear SL, Ayers GD, Van Driest SL, Sidonio RF, Stein CM, Ho RH. The impact of age and CYP2C9 and VKORC1 variants on stable warfarin dose in the paediatric population. Br J Haematol. 2014;165:832–5.

45. Lala M, Burekatt GJ, Takao CM, Pravica V, Monper JD, Gobburu JVS. Genetics-based pediatric warfarin dosage regimen derived using pharmacometric bridging. J Pediatr Pharmacol Ther. 2013;18:209–19.

46. Hamberg A-K, Friberg LE, Hansvås K, Ekman-Joelsson B-M, Sunnegårdh J, Jonzon A, et al. Warfarin dose prediction in children using pharmacometric bridging—comparison with published pharmacogenetic dosing algorithms. Eur J Clin Pharmacol. 2013;69:1275–83.

47. Hamberg A-K, Hellman J, Dahlberg J, Jonsson EN, Wadelius M. A Bayesian decision support tool for efficient dose individualization of warfarin in adults and children. BMC Med Inform Decis Mak. 2015;15:7.

48. De Montfort University; University hospitals, Leicester. Model-based versus traditional warfarin dosing in children. In: ClinicalTrials.gov. Bethesda(MD); National Library of Medicine (US). 2000. http://clinicaltrials.gov/show/NCT02475863. Accessed 26 Jan 2016.

49. Van Driest SL, Stein CM, Ho RH. The impact of age and CYP2C9 and VKORC1 variants on stable warfarin dose in the paediatric population. Br J Haematol. 2014;165:832–5.

50. Hagleitner MM, Coenen MJH, Patino-Garcia A, de Bont ESJM, Gonzalez-Neira A, Vos HL, et al. Influence of genetic variants in TPMT and COMT associated with cisplatin induced hearing loss in patients with cancer: two new cohorts and a meta-analysis reveal significant heterogeneity between cohorts. PloS One. 2014;9:e115869.
53. Drazen JM, Silverman EK, Lee TH. Heterogeneity of therapeutic responses in asthma. Br Med Bull. 2000;56:1054–70.
54. Tantisira KG, Lasky-Su J, Harada M, Murphy A, Litonjua AA, Himes BE, et al. Genomewide association between GLCCI1 and response to glucocorticoid therapy in asthma. N Engl J Med. 2011;365:1173–83.
55. Hosking L, Bleecker E, Ghosh S, Yeo A, Jacques L, Mosteller M, et al. GLCCI1 rs37973 does not influence treatment response to inhaled corticosteroids in white subjects with asthma. J Allergy Clin Immunol. 2014;133:587–9.
56. Vijverberg SJH, Tavendale R, Leusink M, Koenderman L, Raaijmakers JAM, Postma DS, et al. Pharmacogenetic analysis of GLCCI1 in three north European pediatric asthma populations with a reported use of inhaled corticosteroids. Pharmacogenomics. 2014;15:799–806.
57. Zuurhout MJL, Vijverberg SJH, Raaijmakers JAM, Koenderman L, Postma DS, Koppelman GH, et al. Arg16 ADRB2 genotype increases the risk of asthma exacerbation in children with a reported use of long-acting β2-agonists: results of the PACMAN cohort. Pharmacogenomics. 2013;14:1965–71.
58. Basu K, Palmer CNA, Tavendale R, Lipworth BJ, Mukhopadhyay S. Adrenergic β(2)-receptor genotype predisposes to exacerbations in steroid-treated asthmatic patients taking frequent albuterol or salmeterol. J Allergy Clin Immunol. 2009;124(1188–94):e3.
59. Palmer CNA, Lipworth BJ, Lee S, Ismail T, Macgregor DF, Mukhopadhyay S. Arginine-16 beta(2) adrenoceptor genotype predisposes to exacerbations in young asthmatics taking regular salmeterol. Thorax. 2006;61:940–4.
60. Giubergia V, Gravina L, Castaños C, Chertkoff L. Influence of β(2)-adrenergic receptor polymorphisms on asthma exacerbation in children with severe asthma regularly receiving salmeterol. Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol. 2013;110:156–60.
61. Turner S, Francis B, Vijverberg S, Pino-Yanes M, Maitland-van der Zee AH, Basu K, et al. Childhood asthma exacerbations and the Arg16 β2-receptor polymorphism: a meta-analysis stratified by treatment. Immunol: J Allergy Clin; 2016.
62. Lipworth BJ, Basu K, Donald HP, Tavendale R, Macgregor DF, Ogston SA, et al. Tailored second-line therapy in asthmatic children with the Arg(16) genotype. Clin Sci. 2013;124:521–8.
63. Wu AC, Tantisira K, Li L, Schuemann B, Weiss ST, Fuhlbrigge AL, et al. Predictors of symptoms are different from predictors of severe exacerbations from asthma in children. Chest. 2011;140:100–7.
64. Vijverberg SJH, Raaijmakers JAM, Maitland-van der Zee AH. ADRB2 Arg16 and the need for collaboration in childhood asthma pharmacogenomics. Pharmacogenomics. 2013;14:1937–9.
65. Mooij MG, Nies AT, Knibbe CAJ, Schaeffeler E, Tibboel D, Schwab M, et al. Development of human membrane transporters: drug disposition and pharmacogenetics. Clin Pharmacokinet. 2016;55:507–24.
66. Wang F, He XY, Baines KJ, Gunawardhana LP, Simpson JL, Li F, et al. Different inflammatory phenotypes in adults and children with acute asthma. Eur Respir J. 2011;38:567–74.
67. Verhoef TI, Ragia G, de Boer A, Barallon R, Kolovou G, Kolovou V, et al. A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. N Engl J Med. 2013;369:2304–12.
68. Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, et al. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med. 2013;369:2294–303.
69. Dutch National Health Care Institute. Drug Information System. 2014. https://www.gipdatabank.nl/databank.asp?tabel=03-lftgesl&geg=gebr&item=B01AA. Accessed 4 April 2016.
70. Koch VG. Incentivizing the utilization of pharmacogenomics in drug development. J Health Care L Pol’y. 2012;15:263–302.
71. Koren G, Cairns J, Chitayat D, Gaedigk A, Leeder SJ. Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother. Lancet Lond Engl. 2006;368:704.
72. European Medicine Agency. Codeine not to be used in children below 12 years for cough and cold. 2015. http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Codeine_cough_or_cold_in_children/Position_provided_by_CMDh/WC500186159.pdf. Accessed 16 Mar 2016.