Pediatric RESEARCH

www.nature.com/pr

REVIEW ARTICLE

Toward precision medicine in pediatric population using cytochrome P450 phenotyping approaches and physiologically based pharmacokinetic modeling

Gaëlle Magliocco[1,2], Frédérique Rodieux[1], Jules Desmeules[1,2,3,4], Caroline Flora Samer[1,3,4] and Youssef Daali[1,2,3,4]

The activity of drug-metabolizing enzymes (DME) shows high inter- and intra-individual variability. Genetic polymorphisms, exposure to drugs, and environmental toxins are known to significantly alter DME expression. In addition, the activity of these enzymes is highly age-dependent due to maturation processes that occur during development. Currently, there is a vast choice of phenotyping methods in adults using exogenous probes to characterize the activity of these enzymes. However, this can hardly be applied to children since it requires the intake of non-therapeutic xenobiotics. In addition, sampling may be challenging in the pediatric population for a variety of reasons: limited volume (e.g., blood), inappropriate sampling methods for age (e.g., urine), and metric requiring invasive or multiple blood samples. This review covers the main existing methods that can be used in the pediatric population to determine DME activity, with a particular focus on cytochrome P450 enzymes. Less invasive tools are described, including phenotyping using endogenous probes. Finally, the potential of pediatric model-informed precision dosing using physiologically based pharmacokinetic modeling is discussed.

Pediatric Research (2020) 87:441–449; https://doi.org/10.1038/s41390-019-0609-z

INTRODUCTION

Drug responses in children may differ from those in adults due to age-related differences, which are not limited to changes in weight and/or height. Childhood is characterized by growth in height, weight, and organs, and also by changes in body composition, as well as by important functional maturation of organs, and enzymatic and metabolic processes.[1] The term ontogeny includes all the developmental events of an individual organism.

Physiological changes in children during ontogeny are rapid, complex, and non-linear.[1,2] They occur at different rates according to age and individual, being more pronounced during the first 2 years of life, especially during the neonatal period. They explain the great heterogeneity of the pediatric population.[3,4] The impact of physiological transformations on the pharmacokinetics (PKs) and pharmacodynamics (PDs) of drugs makes pediatric doses extrapolated from adult data (simply adjusted to the child's body weight) inaccurate and makes it difficult to determine optimal dose in children.[2]

It is not yet perfectly known how the maturation affects the absorption, distribution, metabolism, and excretion of drugs. In newborns and young children, oral absorption may be delayed and decreased for many drugs due to slow gastric emptying and transit, relative hypochloremia, and incomplete intestinal flora in the newborns.[5,6] Dermal absorption is increased, mainly because of a higher body surface to weight ratio and the thinness of the stratum corneum.[7] The water-rich body composition of the young child influences the volume of distribution of hydrophilic molecules, while the variation in fat mass influences the distribution of lipophilic molecules.[8] The reduced plasma protein binding capacity in the first year of life may explain an increase in the free fraction and sensitivity to some drugs.[9] Immature renal function contributes to decreased clearance (CL), increased half-life, and the risk of accumulation of a large number of water-soluble drugs and drug metabolites.[10,11] However, of all these changes, the main determinants of overall pharmacokinetic differences observed between adults and children appear to be related to changes in drug metabolism.

Although the latter occurs in various organs, such as the intestine, lungs, kidney, skin, and brain, it is generally assumed that it occurs mainly in the liver.[12] For many drugs, metabolism is carried out in two phases: phase I reactions, mediated essentially by oxidative cytochrome P450 enzymes (CYP450), and phase II reactions, such as glucuroconjugation by uridine 5′-diphospho-glucuronosyltransferase (UGT) and sulfonation by sulfotransferase (SULT). All these enzymes are grouped under the term of drug-metabolizing enzymes (DMEs).[13]

CYP450 plays major role in drug metabolism. More than 50 functional human CYP450 have been described, about 10 of which are responsible for the biotransformation of more than 70 to 80% of all currently marketed drugs.[14] The most expressed isoforms in the liver are CYP3A4 and CYP2C9, whereas CYP2C8, CYP2E1, CYP1A2, CYP2D6, CYP2B6, CYP2C19, and 3A5 are less abundant but play a significant role in drug metabolism. The isoforms may

[1]Department of Acute Medicine, Division of Clinical Pharmacology and Toxicology, Geneva University Hospitals, Geneva, Switzerland; [2]Faculty of Sciences, School of Pharmacy, University of Geneva and Lausanne, Geneva, Switzerland; [3]Swiss Center for Applied Human Toxicology, Geneva, Switzerland and [4]Faculty of Medicine, University of Geneva, Geneva, Switzerland
Correspondence: Youssef Daali (youssef.daali@hcuge.ch)
These authors contributed equally: Gaëlle Magliocco, Frédérique Rodieux

Received: 12 July 2019 Revised: 4 September 2019 Accepted: 22 September 2019
Published online: 10 October 2019

have overlapping substrate specificities.[15] The second contributing factor to drug metabolism are the UGT enzymes,[16] mainly UGT2B7 (metabolizing up to 35% of medications on the market) followed by UGT1A4 and UGT1A1.[17]

The activity of most DME is highly variable from one individual to another but also within the same individual. The variability in DME activity can be caused by various factors, including exogenous factors, such as co-medications, food components, smoking habits, and environmental factors, as well as endogenous factors, such as genetic polymorphisms, gender, co-morbidity, and age. The activity can be reduced or increased, and result in a modification of the drug CL.[18] A decrease in DME activity may lead to reduced CL of drug substrate and increased plasma concentrations, resulting in increased effect and potential drug-related toxicity due to overexposure. If the drug is a prodrug that needs to be metabolically activated, the opposite effect is expected, which is a decrease in effectiveness. Increased DME activity may result in increased CL, decreased plasma concentrations, and potentially decreased effect, except for prodrugs for which there is a risk of toxicity.

With respect to endogenous factors, in recent years, significant work has been done to understand the impact of genetic polymorphisms on DME activity. For some DME, many allelic variants, with different degrees of functional significance, have been described. With more than 100 polymorphisms described, CYP2D6 is the most studied DME in relation to drug metabolism. The impact of its variability can be clinically significant and even fatal. Around the world, government agencies, research teams, and clinicians are working on the implementation of pharmacogenetic testing for routine medical care and the development of clinical dosing recommendations based on pre-emptive DME activity assessment.[19]

Age is also an important endogenous factor in the variability of DME activity. Different studies have shown that most DME are present at birth, although not mature (i.e., with decreased activity compared to adulthood).[2] With age, the specific activity of these different DME evolves to reach adult activity, each one with a specific pattern. It is generally accepted that CYP1A2 activity appears to be very low at birth (5% of adult activity) and develop slowly after birth; activity reaching 25% at the end of early childhood, then about 50% at 6 years. In comparison, CYP2B6 activity increases rapidly, that is, twice as much, in the first month of life. The activity of CYP2C9 and 2C19 increases mainly during the first 2 years of life, while CYP3A4 activity reaches 50% of that of an adult at 1 year. CYP2D6 activity in newborns is about 20% of that in adults. It reaches 90% of adult values around the age of 1 year and adult values between 2 and 3 years old. CYP3A7, on the other hand, is a predominant isoform during fetal life and in children; its activity decreases significantly from birth and disappears almost around the age of 1 year in the liver tissue.[1,2] The ontogeny of phase II enzymes is less well described. The activity of UGTs is reduced at birth and also increases gradually, with different profiles depending on the isoforms. Their activity reaches their adult values around 3 months.[20] DME with higher activity in fetal life than in postnatal life are rare, but described for SULT1E1, SULT1A3, and flavin-containing monooxygenase.[21] The immaturity of DME can have significant clinical effects. The best-known example is the classic historical case of Gray Baby syndrome in chloramphenicol-treated neonates when doses were extrapolated from adult because of the immature UGT.[22]

However, although the importance of ontogeny and its effect on DME activity was highlighted several years ago, data on DME activity in children are still sporadic, the specific rate of evolution of enzymes activity is still poorly understood, and it is not clear when adult activity is reached.

In children, the phenomenon of maturation (ontogeny) must be taken into account among the factors that modify DME activity. It must be considered when interpreting a lack of clinical response or the occurrence of adverse drug reactions despite appropriate weight-based dosing, and also to allow a priori drug dosing adjustment accordingly. Given the number of covariates involved in DME activity, it should be kept in mind that DME activity, in particular in children, is determined at a given point in time and may evolve over time and in parallel with the maturation of enzymes. It is essential to develop accurate approaches to assess the effective DME activity and the effect of these covariates on drug PKs in children.

This review aims to describe:

Existing approaches to predict DME activity: CYP450 genotyping and phenotyping procedures.
Modeling and simulation methods, such as physiologically based pharmacokinetics (PBPKs), based on physiological compartments and integrating database of drugs and systems, such as genetics, ethnicity, pathologies, and organs function[23] to achieve the goal of individualized therapy by predicting the effect of several factors on drug PKs.

GENOTYPING

CYP450 genotyping is based on DNA analysis, including the detection of genetic polymorphisms, gene deletion, and the assessment of copy number variation.[24] If there is a relationship between genotype and phenotype, genotyping allows the prediction of enzyme activity from the identified alleles.[25] CYP2C9, CYP2C19, CYP2D6, and CYP3A5 polymorphisms are associated with a corresponding well-established phenotype (e.g., poor or extensive metabolizer).

The advantage of a genotyping test is that it represents a trait marker. The test can be performed in any situation and its result lasts a lifetime

It should however be pointed out that genotyping may not screen for some rare variants and an allele may be erroneously considered functional.[26] In addition, it does not measure the ontogeny and the influence of some environmental factors on CYP450 activity, which may limit its use in several cases.[27] Drug–drug interactions (DDI), for instance, can induce or inhibit CYP450 activity, leading to sub-therapeutic or increased drug concentrations, respectively.

Currently, genotype-guided therapy is increasingly being applied clinically in adults. Its use is still limited in pediatrics, even if CYP3A5 genotype is increasingly assessed in pediatric transplant recipients receiving tacrolimus.[27] Studies have shown that age and CYP3A5 expresser genotype in children receiving tacrolimus are independently associated with higher dose requirements and lower tacrolimus concentration/dose ratios.[28,29] In addition, CYP3A5 genotype-guided dosing may lead to earlier therapeutic tacrolimus concentrations and fewer out-of-range concentrations.[30]

Similarly, there is a growing interest in pharmacogenetic testing in children with attention-deficit hyperactivity disorder, depression, and anxiety disorders to predict therapeutic response, adverse drug reaction, and psychotropic drugs selection,[31] as well as CYP2C19 genotype-guided proton-pump inhibitor therapy in children.[32]

In order to facilitate sampling in children, saliva sample, which is clearly less invasive and more easily collected in children, can be used in this population to perform genotyping.[33]

PHENOTYPING

Determining the in vivo metabolic activity, that is, phenotyping, is the most accurate way to document the combined effects of genetic, environmental, endogenous, ontogenetic, and other developmental factors on CYP450 activity, and also, when this

Table 1. Summary of pediatric studies performed to measure CYP1A2 activity using non-therapeutic exogenous probes

References	Age[a]	Subjects (number)	Probe	Range (mean ± SD)	Sampling
Caffeine dose exhaled as labeled carbon dioxide (%)					
Parker et al.[51]	4–15 years	6	Oral labeled caffeine (^{13}C) 3 mg/kg	9.37 ± 2.60 (before ciprofloxacin therapy) 4.17 ± 1.33 (after ciprofloxacin therapy)	Breath sampling at −20, −10, −1, 15, 30, 45, 60, 75, 90, 105, and 120 min after drug administration
Parker et al.[52]	6–17 years	5		3.47 ± 1.79 (before carbamazepine therapy) 7.65 ± 2.86 (after carbamazepine therapy)	
Oshikoya et al.[53]	7.6 ± 2.9 years	15		7.56 ± 4.01 (prenutritional rehabilitation) 7.95 ± 3.68 (postnutritional rehabilitation)	
Oshikoya and Smith[54]	3–8 years	12		8.59 ± 1.11 (before Casilan® ingestion) 8.58 ± 1.33 (after Casilan® ingestion)	
(AFMU + 1X+ 1U)/17U					
Masimirembwa et al.[46]	12–16 years (black rural Shona children)	45	Oral caffeine 40 mg (soft drink)	3.78 ± 2.90	24-h urine collection after drug administration
	6–11 years (white children from Harare City)	10		8.86 ± 3.36	
	3–11 years (children from Toronto)	21		7.92 ± 1.88	
el-Yazigi[43]	2–13 years	9 (hepatocellular failure patients) 9 (control)	Oral caffeine 2 mg/kg (soft drink)	0.67 ± 1.36 ($n = 2$) 3.13 ± 1.35 ($n = 9$)	48-h urine collection after drug administration
Mayayo-Sinués et al.[41]	4.1–13.1 years	31	Oral caffeine 1.5 mg/kg (Nescafe)	5.17 (3.87–5.59)[b] (before rhGH therapy) 4.57 (3.90–5.97)[b] (after 4 rhGH therapy)	6-h urine collection after drug administration
Kennedy et al.[47]	3–8 years	12 (CF patients) 12 (control)	Oral caffeine 35 mg (soft drink)	6.1 (5.4–6.7)[c] 6.3 (5.3–7.3)[c]	8-h urine collection after drug administration
Kennedy et al.[48]	4–14 years	12	Oral caffeine 11.5 mg (soft drink)	4.8 (3.5, 6.3)[d] (before rhGH therapy) 5.0 (3.3, 8.9)[d] (after 3 months rhGH therapy) 4.3 (3.2, 13.3)[d] (after 6 months rhGH therapy)	8-h urine collection after drug administration
Kennedy et al.[49]	3–8 years	24		6.2 ± 1.3	
Chiney et al.[50]	8–10 6–10	7 (obese) 13 (lean)		5.4 ± 2.1 6.7 ± 1.7	
Caffeine CL (saliva) (mL/min/kg)					
el-Yazigi[43]	2–13 years	9 (hepatocellular failure patients) 9 (control)	Oral caffeine 2 mg/kg (soft drink)	0.75 ± 0.53 1.36 ± 0.64	Saliva sampling at 4–5 and 16–17 h after drug administration

Table 1 continued

References	Age[a]	Subjects (number)	Probe	Range (mean ± SD)	Sampling
Caffeine CL (blood) (mL/min/kg)					
Akinyinka et al.[44]	1.3–4.5 years (Kwashiorkor patients)	7	Caffeine 40 mg (through a nasogastric tube)	1.6 ± 1.0	Blood sampling at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, and 36 h after drug administration
	3–9 years (malaria patients)	5	Oral caffeine 100 mg	2.0 ± 0.9	
	7–9.9 years (healthy patients)	5		4.4 ± 1.9	
Paraxanthine/caffeine plasma molar ratio					
ten Tusscher et al.[42]	7–12 years	37	Oral caffeine 3 mg/kg (soft drink)	1.4 ± 0.5	Blood sampling 6 h after drug administration
Senggunprai et al.[45]	12.2 ± 2.7 years (thalassemia patients)	23	Oral caffeine 2 mg/kg (soft dink)	0.75 (0.63–0.87)[c]	Blood sampling 6 h after drug administration
	11.0 ± 1.3 years (control)	24		0.76 (0.66–0.85)[c]	
Caffeine elimination rate constant					
Blake et al.[67]	0.5–6 months	38	Oral caffeine citrate 7 mg/kg	NA	Blood sampling 2 and 24 h after drug administration (drawn by heelstick)
Caffeine/(Caffeine + 1X + 1U + 17X + 13X + 3X)					
Blake et al.[67]	0.5–6 months	38	Oral caffeine citrate 7 mg/kg	NA	24-h urine collection after drug administration
AFMU/1×					
Evans et al.[59]	3–21 years	26	Oral caffeine 25 to 46 mg	0.672 (0.095, 3.947)[d] (caffeine given alone) 0.59 (0.137, 2.646)[d] (caffeine given with dextromethorphan)	4-h urine collection after drug administration
el-Yazigi[43]	2–13 years	9 (hepatocellular failure patients)	Oral caffeine 2 mg/kg (soft drink)	0.51 ± 0.49 ($n = 5$)	48-h urine collection after drug administration
		9 (control)		0.53 ± 0.56 ($n = 9$)	

1U 1-methyluric acid, *1X* 1-methylxanthine, *1U* 1-methyluracil, *3X* 3-methylxanthine, *13X* theophylline, *17U* 1,7-dimethyluric acid, *17X* 1,7-dimethylxanthine, *AFMU* 5-acetylamino-formylamino-3-methyluracil, *CF cystic fibrosis*, *CL* clearance, *NA* not available, *rhGH* recombinant human growth hormone, *SD* standard deviation
[a] Either mean plus or minus SD or ranges are presented based on the availability from the original paper
[b]Median (95% confidence interval)
[c]Mean (95% confidence interval)
[d]Median (minimum, maximum)

Table 2. Summary of pediatric studies performed to measure CYP2C19 activity using non-therapeutic exogenous probes

References	Age[a]	Subjects (number)	Probe	Range (mean ± SD)	Sampling
Omeprazole/5-hydroxyomeprazole plasma metabolic ratio					
Gumus et al.[56]	2–18 years	19	Oral omeprazole 20 mg	NA	Blood sampling 3 h after drug administration
Favela-Mendoza et al.[57]	10.2 ± 0.9 years	118		NA	Blood sampling between 0.5 and 4.5 h after drug administration

NA not available, *SD* standard deviation
[a]Either mean plus or minus SD or ranges are presented based on the availability from the original paper

assessment is made in a specific individual, the most accurate way to predict a personalized therapeutic dosage.[34]

CYP450 phenotyping consists in administering a probe, which is usually a drug specifically metabolized by an isoenzyme. In most cases, this probe is given orally, but it can also be administered intravenously. The probe must be specific, safe for humans, assessable in biological fluids together with its major metabolite (s), and available as a therapeutic drug for exogenous phenotyping.[35] After the intake or injection of the probe, a phenotyping metric is determined most of the time with blood or urinary samples. Ideally, systemic or partial CL are considered as gold standards for CYP450 activity phenotyping.

Different criteria must be met to validate the use of a phenotyping metric, including correlation with a validated metric and changes during treatment with CYP450 inhibitors or inducers.[35]

Phenotyping can be performed by administering a probe (selective phenotyping) or multiple probes (mixed phenotyping) at a time, also known as the "cocktail phenotyping" method.[36] For some isoenzymes, it is also possible to quantify endogenous biomarkers (e.g., cortisol and its metabolite 6β-hydroxycortisol for CYP3A), which is less invasive.[37]

When phenotyping in children, it is necessary to take into account the difficulties that are added to this population compared to adults. The use of non-toxic probes, validated in children with an appropriate microdosing approach in order to avoid pharmacological effects during phenotyping, is absolutely necessary. The choice of the probe is therefore particularly important, as well as its conditions of administration. The preparation of micro-dose forms being challenging, fast dissolving buccal films have been studied by Kiene et al.[38] and appear to be a very promising diagnostic tool for the pediatric population.[38] The measurement methods are also important to consider in children, for whom blood tests can be tedious, blood volume collected is limited, and urine collection at a given time point impossible. For these reasons, measurement methods requiring as little sampling as possible, a microsize sampling volume or even alternative methods, such as saliva samples, are necessary. The dried blood spots (DBS) sampling method consisting of collecting a drop of capillary blood (obtained from a finger or heel prick) on blotting paper is applied to phenotyping. Although this method is minimally invasive, it remains painful and difficult to generalize in children.[39]

In this review, the different exogenous probes that have been used in children to phenotype CYP450 enzymes, including CYP1A2, CYP2C19, CYP2D6, and CYP3A, are discussed. Despite its importance, CYP2C9 is not discussed in this article as data are very limited in children regarding phenotyping.

CYP1A2

Despite a complex metabolism involving several enzymes, caffeine is the most widely accepted probe to phenotype CYP1A2 activity since this enzyme contributes to about 95% of the total caffeine CL.[40] In children, it may be relatively easily measured after ingestion of a caffeinated beverage (usually 1.5 to 3 mg/kg).[41,42] Children as young as 2 weeks old have been phenotyped in this manner using blood, urine, or saliva after caffeine intake.[41–43] Different phenotyping metrics can be used to characterize this enzyme in children, including the plasma paraxanthine/caffeine metabolite ratio (MR) and caffeine plasma or salivary CL.[42–45] 5-Acetylamino-6-formylamino-3-methyluracil (AFMU), 1-methyluric acid (1U), 1-methylxanthine (1X), and 1,7-dimethyluric acid (17U) can also be quantified in urine in order to measure the following urinary metabolic ratio (UMR): (AFMU + 1X + 1U)/17U, which is representative of the activity of CYP1A2.[41,43,46–50] Finally, to phenotype CYP1A2 activity, it is also possible to give orally labeled ^{13}C-caffeine with a nonradioactive stable isotope and to measure caffeine dose exhaled as $^{13}CO_2$ over a 2-h period.[51–54] None of these studies identified side effects. Table 1 summarizes the different studies conducted in pediatric populations on CYP1A2 phenotyping using caffeine.

CYP2C19

Currently, due to the limited availability of mephenytoin, omeprazole is the most common used probe in the adult population to phenotype CYP2C19 activity.[34] Data regarding CYP2C19 phenotyping in pediatric population is relatively poor. Proton-pump inhibitors (PPIs) are relatively safe and frequently used drugs in children during gastroesophageal reflux disease.[55] This class of drugs is metabolized by CYP450 and mostly by CYP2C19 into hydroxylated metabolites.[34] Most of the time, phenotyping using PPIs is done when children have a therapeutic need to take a PPI. For instance, in 94 children aged 2 to 18 years, CYP2C19 enzyme was phenotyped using plasma MR ratio of lansoprazole/5-hydroxy lansoprazole after administration of 15 or 30 mg lansoprazole (15 mg when body weight ≤30 kg and 30 mg when >30 kg) for therapeutic purposes in order to assess genotype–phenotype correlation.[56] No serious adverse effects were observed during the study. Nineteen of these children were then phenotyped with a single dose of 20 mg omeprazole in order to determine the plasma MR of omeprazole/5-hydroxyomeprazole and to establish a potential correlation with lansoprazole. In another study, 118 healthy Mexican children (mean age 10.2 ± 0.9 years) received 20 mg omeprazole.[57] Plasma MR of omeprazole/5-hydroxyomeprazole was determined 0.5 to 4.5 h after dosing. Table 2 summarizes the two studies described above.

CYP2D6

The best current probe for measuring CYP2D6 activity is dextromethorphan (DEM), an opioid, which is predominantly biotransformed by this enzyme into dextrorphan (DOR).[58] It is the reference probe in adults to characterize the activity of this enzyme. DEM can be used at sub-therapeutic doses to phenotype CYP2D6 activity in children.[58] For this purpose, a dose of 0.5 mg/kg of DEM (maximum dose of 30 mg) has already been administered in different clinical trials to children as young as 3 years old.[47–49] The urine is then collected for 8 h in most studies (ranging from 4 to 12 h[59,60]) and the UMR DEM/DOR is established.

Table 3. Summary of pediatric studies performed to measure CYP2D6 activity using non-therapeutic exogenous probes

References	Age	Subjects (number)	Probe	Range (mean ± SD)	Sampling
DEM/DOR urinary metabolic ratio					
Kennedy et al.[47]	3–8 years	12 (CF patients)	Oral DEM 0.5 mg/kg (max 30 mg)	0.0095 (0.0008–0.0182)[a]	8-h urine collection after drug administration
		12 (control)		0.0096 (0.0021–0.0172)[a]	
Kennedy et al.[48]	4–14 years	11		0.003 (0.001, 0.117)[b] (before rhGH therapy) 0.004 (0.001, 0.090)[b] (after 3 months rhGH therapy) 0.006 (0.001, 0.083)[b] (after 6 months rhGH therapy)	
Kennedy et al.[49]	3–8 years	24		0.01 ± 0.011	
Findling et al.[86]	5–17 years	30	Oral DEM 30 mg	0.077 ± 0.325	8-h urine collection after drug administration
Evans et al.[59]	3–21 years	26	Oral DEM 30 mg	0.021 (0.002, 3.283)[b] (caffeine given alone) 0.017 (0.002, 2.680)[b] (caffeine given with dextromethorphan)	4-h urine collection after drug administration
Blake et al.[60]	0.5–12 months	193	Oral DEM 0.3 mg/kg	NA	12-h urine collection after drug administration

DEM dextromethorphan, *DOR* dextrorphan, *NA* not available, *rhGH* recombinant human growth hormone, *SD* standard deviation
[a]Mean (95% confidence interval)
[b]Median (minimum, maximum)

In one study, 0.3 mg/kg of DEM was administered to children in their first year of life: 2 weeks, 1, 2, 4, 6, and 12 months. Urine was collected for 12 or 24 h using fiber-based diapers to understand the influence of developmental maturation on drug biotransformation.[60] Evans et al.[59] and Kennedy et al.[47–49] reported that no side effects were observed during the studies. The different studies conducted in infants and children to phenotype CYP2D6 using DEM are described in Table 3.

CYP3A

In adulthood, midazolam, a benzodiazepine, appears to be the reference probe for CYP3A activity since it is metabolized selectively to 1′-hydroxymidazolam by this enzyme.[61] In children, assessment of CYP3A activity is somehow more challenging for ethical reasons. Indeed, the risks associated with midazolam intake could be significant in this population. For these reasons, CYP3A phenotyping using midazolam in children was only performed when this drug was used for therapeutic purposes (i.e., sedation).[62] One report used non-therapeutic midazolam to phenotype 50 obese and 50 non-obese children, but they were mainly close to adulthood (i.e., 11–18 years).[63] They received an intravenous micro-dose of midazolam infused within 5 min (1 µg). The ^{13}C-erythromycin breath test, another widely accepted and validated CYP3A probe, has also been used to phenotype CYP3A activity of newborn infants, but only in the context of an ureaplasma infection.[64]

Both omeprazole and DEM are biotransformed by CYP3A (minor metabolic pathway) into omeprazole-sulfone and 3-methoxymorphinan (3HM), respectively.[65,66] Thus, in addition to CYP2C19 and CYP2D6, omeprazole and DEM can be used to phenotype CYP3A activity. However, it is somewhat controversial as modulation of the other metabolic pathway (e.g., inhibition of CYP2D6 or CYP2C19) may impact their metabolism by CYP3A and skew the results.

Omeprazole 20 mg has been used in a recent study to characterize CYP3A activity by using plasma MR of omeprazole/sulfone-omeprazole in a pediatrics' study involving 118 healthy volunteers at a mean age of 10.2 ± 0.9 years.[57]

Regarding DEM, a dose of 0.3 mg/kg was administered to newborns at 0.5, 1, 2, 3, 4, and 6 months of age. The UMR 3HM/DOR was then used to determine CYP3A activity to measure the effect of infant diet on enzymatic activity.[67]

No information was found on the possible side effects of these probes in these two studies. Table 4 summarizes the clinical trials conducted when DEM and omeprazole were used to phenotype CYP3A activity.

As explained above, phenotyping of CYP450 can also use endogenous probes and/or simultaneous probes (phenotyping cocktail). It seems also possible to use pupillometry as a non-invasive method for CYP2D6 phenotyping.[68] Finally, PBPK modeling is another promising tool for the prediction of pediatric PK. These methods are described thereafter.

ENDOGENOUS CYP450 BIOMARKERS

There is a growing interest in CYP450 phenotyping using endogenous markers. In the adult population, cortisol/6β-hydroxycortisol and cholesterol/4β-hydroxycholesterol are currently the most commonly described endogenous probes for CYP3A.[37] In childhood, it is a very promising area but poorly explored.

As explained above, changes in the maturation of CYP3A may influence the overall metabolic capacity of the drug through this enzyme sub-family. This has been also demonstrated using the 6β-hydroxycortisol/cortisol UMR in a neonatal population. Nakamura et al.[69,70] showed that the ratio decreased significantly from day 0 to day 7 after birth in mature neonates, probably due to a decrease in CYP3A7 expression. In addition, the 6β-hydroxycortisol/cortisol UMR was significantly higher in mature neonates than in adults on day of

Table 4. Summary of pediatric studies performed to measure CY3A activity using non-therapeutic exogenous probes

References	Age[a]	Subjects (number)	Probe	Range (mean ± SD)	Sampling
3HM/DOR plasma metabolic ratio					
Blake et al.[67]	0.5–6 months	38	Oral DEM 0.3 mg/kg	NA	24-h urine collection after drug administration
Omeprazole/sulfone-omeprazole plasma metabolic ratio					
Favela-Mendoza et al.[57]	10.2 ± 0.9 years	118	Oral omeprazole 20 mg	NA	Blood sampling between 0.5 and 4.5 h after drug administration

3HM 3-methoxymorphinane, *DEM* dextromethorphan, *DOR* dextrorphan, *NA* not available, *SD* standard deviation
[a]Either mean plus or minus SD or ranges are presented based on the availability from the original paper

birth, but significantly lower than in adults within 5 days after birth. Similarly, Vauzelle-Kervroedan et al.[71] observed a progressive and significant decline of 6β-hydroxycortisol/cortisol UMR in 7 preterm neonates, 13 term neonates, and 30 infants from 7.2 to 5.0 ($p = 0.048$), the ratio being the highest in the first group. They suggested that the decrease in CYP3A7 expression is faster than the increase in CYP3A4 content after birth.

Tay-Sontheimer et al.[72] observed a significant down-regulation (or even undetectable levels) of an unidentified compound, M1 (*m/z* 444.3102), in urine samples of poor metabolizer (PM) subjects compared to non-PMs subjects in a pediatric population ($n = 189$) using metabolomics.[72] The creatinine/M1 UMR was more than 100-fold higher in PMs than non-PMs ($p < 0.0001$). This endogenous urinary biomarker seems very promising to phenotype CYP2D6 activity and needs to be identified structurally.

PHENOTYPING COCKTAIL

To our knowledge, only one report used an oral cocktail to phenotype CYP450 activity in children. Li et al.[73] gave simultaneously four probe drugs to 12 children/adolescents aged 12 to 21 years with a non-alcoholic fatty liver disease and 17 control subjects aged 18 to 21 years: caffeine (100 mg, CYP1A2), omeprazole (20 mg, CYP2C19), losartan (25 mg, CYP2C9), and midazolam (2 mg, CYP3A). Venous blood and urine samples were then collected at 1, 2, 4, and 6 h.

Although not widely used, phenotyping cocktails remain potentially interesting for use in pediatric, especially with the development of micro-dose cocktails and non-invasive sampling methods.

PUPILLOMETRY

Pupillometry, that is, measurement of pupil size and reactivity using a handheld pupilometer, was studied as a method of evaluating opioid PD. Although the extent of pupil dilatation may provide an index of nociceptive input via autonomic innervation of the iris muscles, the extent of attenuation of the pupillary response upon exposure to analgesics opioids may reflect the extent of μ opioid receptors occupancy in the central nervous system. In adults, a correlation between pupil response and PKs of tramadol has been demonstrated.[74,75] The pupil response was different between ultrarapid metabolizer and extensive metabolizer patients, and correlated with the tramadol/M1 MR.[74,76] Studies are needed to determine whether the measurement of pupillary response and/or pupil diameter could be a useful biomarker for determining CYP2D6 activity in children and thus determine whether pupillometry could be a rapid and non-invasive method to phenotype CYP2D6 activity after opioid administration such as tramadol, codeine, or oxycodone.

Physiologically based pharmacokinetics

Model Informed Prediction Dosing (MIPD) may be of value in a pediatric clinical setting, particularly for drugs with a narrow therapeutic window or drugs highly metabolized or subject to DDI. Over the past decade, PBPK approaches integrating developmental physiology, ontogeny, organs failure, and DDI have been developed and used in drug development.[77–79] However, their routine use in adjusting doses in children remains limited.

PBPK models are multi-compartment models capable of predicting the PKs and PDs of drugs, taking into account realistic anatomical and physiological properties of the body as well as physicochemical properties of the drugs. Predictions are based on in vitro and in vivo input data for the drugs. Various softwares are available for PBPK modeling, such as Simcyp®, Gastro-Plus®, and PK-Sim®. In-house models can also be developed using mathematical softwares such as Matlab®. They include different models of absorption, distribution, and elimination processes. Prior to clinical use, models have to be fully validated in accordance with regulatory guidelines.[80] For further details regarding the use of PBPK in children, the authors refer to a previous published review by Marsousi et al.[81]

Recently, Templeton et al.[82] conducted a 10-year literature review for pediatric drugs with PK data. They built PBPK models for 35 drug models and compared predicted and observed concentrations in different age groups. Overall, PBPK modeling adequately predicted observed data for all drugs tested in all simulated age groups, except in neonates where only 55% predicted concentrations were within 1.5 time the observed concentrations. Diestelhorst et al.[83] proposed a PBPK model to improve prescribing practices of the alkylating agent busulfan in children. Glutathione *S*-transferase activity was implemented in 11 organs and an age-dependent enzyme activity and maturation ratio was fixed compared to adults (ratios of 0.8, 0.6, and 0.9 for children up to 2 years, >2–6 years, and >6–18 years, respectively). The study was conducted in 36 children and the developed PBPK model successfully described the observed concentrations. In another study, a PBPK model was set up to predict tramadol PKs during pediatric life.[84] Tramadol metabolic pathways, including CYP2B6, CYP2D6, and CYP3A4, were optimized in vitro and in vivo, considering the ontogenetic profiles and maturation functions. Renal maturation in pediatric age groups was also considered for renal CL of tramadol and its metabolites. Overall, the model predicted well tramadol CL in early infancy.

The integration of DME activity data, assessed by genotyping or phenotyping, with PBPK modeling will certainly increase the predictability and accuracy of the models and thus improve dosage adjustments.

CONCLUSION

In clinical practice, the measurement of DME activity, by phenotyping or genotyping, is often performed a posteriori to

understand a lack of clinical response to treatment, low plasma concentrations discordant with the prescribed dosage, and doubt about observance, and also in case of adverse drug reactions at therapeutic dose.

Drug selection and dose adjustment based on the pre-emptive DME activity assessment should be evaluated in prospective randomized controlled trials. This would make sense, especially for children in whom the activity of the DME varies with their development and for drugs with a narrow therapeutic range, or whose efficacy and toxicity are clearly related to plasma concentrations.

To date, CYP2D6 genotyping is only required by the Food and Drug Administration (FDA) prior to administration and for genotype-specific dose adjustment of two drugs, pimozide and tetrabenazine, to avoid toxicity. The use of these two drugs in the pediatric population is limited to a few sporadic cases. Clinical dosing recommendations or drug selection based on DME activity predicted by genotype are already available in adults. They are published by independent international expert panels, such as the Clinical Pharmacogenetics Implementation Consortium (https://cpicpgx.org), on the Pharmacogenomics Knowledgebase (PharmGKB) website (https://www.pharmgkb.org/). Most CYP450 guidelines are for CYP2D6 and treatment with its substrates codeine, tramadol, and some antidepressants, as well as CYP3A and tacrolimus.

To date, no specific guideline has been specifically designed for the pediatric population. However, St. Jude Research Hospital is already using a clinical decision support system to guide the dosing and prescribing of codeine in children with sickle cell disease by the age of 9 months, in line with CPIC pharmacogenetics-based codeine prescribing recommendations in adults and warnings from the FDA.[85]

Other clinical decision support tools could be validated in children, in the same way as those validated in adults, and based on the actual DME activity, to allow for safer administration of drugs in the pediatric population.

In the age of personalized medicine, it seems necessary to be able to develop such dosing guidelines based on the enzyme activity in both adults and children. In addition, the development of PBPK models and MIPD should help clinicians to improve drugs' efficacy and safety in children.

AUTHOR CONTRIBUTIONS

Y.D. supervised the project. G.M., F.R., and Y.D. wrote the manuscript with support of C.F.S. and J.D. All authors provided critical feedback and helped shape the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES

1. Kearns, G. L. et al. Developmental pharmacology—drug disposition, action, and therapy in infants and children. *N. Engl. J. Med.* **349**, 1157–1167 (2003).
2. van den Anker, J., Reed, M. D., Allegaert, K. & Kearns, G. L. Developmental changes in pharmacokinetics and pharmacodynamics. *J. Clin. Pharm.* **58**, S10–S25 (2018).
3. Allegaert, K. & van den Anker, J. N. Clinical pharmacology in neonates: small size, huge variability. *Neonatology* **105**, 344–349 (2014).
4. Smits, A., Kulo, A., de Hoon, J. N. & Allegaert, K. Pharmacokinetics of drugs in neonates: pattern recognition beyond compound specific observations. *Curr. Pharm. Des.* **18**, 3119–3146 (2012).
5. Nicolas, J., Bouzom, F., Hugues, C. & Ungell, A. Oral drug absorption in pediatrics: the intestinal wall, its developmental changes and current tools for predictions. *Biopharm. Drug Dispos.* **38**, 209–230 (2017).
6. Merchant, H. A., Liu, F., Orlu Gul, M. & Basit, A. W. Age-mediated changes in the gastrointestinal tract. *Int. J. Pharm.* **512**, 382–395 (2016).
7. Rutter, N. Percutaneous drug absorption in the newborn: hazards and uses. *Clin. Perinatol.* **14**, 911–930 (1987).
8. Friis-Hansen, B. Body composition during growth. In vivo measurements and biochemical data correlated to differential anatomical growth. *Pediatrics* **47**, 264+ (1971).
9. Roberts, S. B. et al. Long-term variation in oxygen consumption rate in preterm infants. *Biol. Neonate* **52**, 1–8 (1987).
10. Rodieux, F., Wilbaux, M., van den Anker, J. N. & Pfister, M. Effect of kidney function on drug kinetics and dosing in neonates, infants, and children. *Clin. Pharmacokinet.* **54**, 1183–1204 (2015).
11. De Cock, R. F. W. et al. Simultaneous pharmacokinetic modeling of gentamicin, tobramycin and vancomycin clearance from neonates to adults: towards a semi-physiological function for maturation in glomerular filtration. *Pharm. Res.* **31**, 2643–2654 (2014).
12. Watkins, P. B. Drug metabolism by cytochromes P450 in the liver and small bowel. *Gastroenterol. Clin. N. Am.* **21**, 511–526 (1992).
13. Iyer, K. R. & Sinz, M. W. Characterization of phase I and phase II hepatic drug metabolism activities in a panel of human liver preparations. *Chem. Biol. Interact.* **118**, 151–169 (1999).
14. Bertz, R. J. & Granneman, G. R. Use of in vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin. Pharmacokinet.* **32**, 210–258 (1997).
15. van der Weide, J. & Steijns, L. S. Cytochrome P450 enzyme system: genetic polymorphisms and impact on clinical pharmacology. *Ann. Clin. Biochem.* **36**, 722–729 (1999).
16. Iyanagi, T. Molecular mechanism of phase I and phase II drug-metabolizing enzymes: implications for detoxification. *Int. Rev. Cytol.* **260**, 35–112 (2007).
17. Lv, X. et al. Recent progress and challenges in screening and characterization of UGT1A1 inhibitors. *Acta Pharm. Sin. B* **9**, 258–278 (2019).
18. Zanger, U. M. & Schwab, M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharm. Ther.* **138**, 103–141 (2013).
19. Swen, J. J. et al. Pharmacogenetics: from bench to byte—an update of guidelines. *Clin. Pharm. Ther.* **89**, 662–673 (2011).
20. Court, M. H. et al. Quantitative distribution of mRNAs encoding the 19 human UDP-glucuronosyltransferase enzymes in 26 adult and 3 fetal tissues. *Xenobiotica Fate Foreign Compd. Biol. Syst.* **42**, 266–277 (2012).
21. Hines, R. N. The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharm. Ther.* **118**, 250–267 (2008).
22. Knight, M. Adverse drug reactions in neonates. *J. Clin. Pharm.* **34**, 128–135 (1994).
23. Jones, H. & Rowland-Yeo, K. Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT Pharmacomet. Syst. Pharm.* **2**, e63 (2013).
24. Ingelman-Sundberg, M., Sim, S. C., Gomez, A. & Rodriguez-Antona, C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharm. Ther.* **116**, 496–526 (2007).
25. Samer, C. F., Lorenzini, K. I., Rollason, V., Daali, Y. & Desmeules, J. A. Applications of CYP450 testing in the clinical setting. *Mol. Diagn. Ther.* **17**, 165–184 (2013).
26. Hicks, J. K., Swen, J. J. & Gaedigk, A. Challenges in CYP2D6 phenotype assignment from genotype data: a critical assessment and call for standardization. *Curr. Drug Metab.* **15**, 218–232 (2014).
27. Korbel, L., George, M. & Kitzmiller, J. Clinically relevant pharmacogenomic testing in pediatric practice. *Clin. Pediatr. (Philos.)* **53**, 831–838 (2014).
28. Gijsen, V. et al. Age and CYP3A5 genotype affect tacrolimus dosing requirements after transplant in pediatric heart recipients. *J. Heart Lung Transpl.* **30**, 1352–1359 (2011).
29. Andrews, L. M. et al. A population pharmacokinetic model to predict the individual starting dose of tacrolimus following pediatric renal transplantation. *Clin. Pharmacokinet.* **57**, 475–489 (2018).
30. Min, S. et al. A randomized clinical trial of age and genotype-guided tacrolimus dosing after pediatric solid organ transplantation. *Pediatr. Transplant.* **22**, e13285 (2018).
31. Wehry, A. M., Ramsey, L., Dulemba, S. E., Mossman, S. A. & Strawn, J. R. Pharmacogenomic testing in child and adolescent psychiatry: an evidence-based review. *Curr. Probl. Pediatr. Adolesc. Health Care* **48**, 40–49 (2018).
32. Cicali, E. J. et al. Novel implementation of genotype-guided proton pump inhibitor medication therapy in children: A Pilot, Randomized, Multisite Pragmatic Trial. *Clin. Transl. Sci.* **12**, 172–179 (2019).
33. Stockmann, C. et al. Effect of CYP3A5*3 on asthma control among children treated with inhaled beclomethasone. *J. Allergy Clin. Immunol.* **136**, 505–507 (2015).
34. Streetman, D. S., Bertino, J. S. & Nafziger, A. N. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics* **10**, 187–216 (2000).

35. Fuhr, U., Jetter, A. & Kirchheiner, J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. *Clin. Pharm. Ther.* **81**, 270–283 (2007).
36. Tanaka, E., Kurata, N. & Yasuhara, H. How useful is the 'cocktail approach' for evaluating human hepatic drug metabolizing capacity using cytochrome P450 phenotyping probes in vivo? *J. Clin. Pharm. Ther.* **28**, 157–165 (2003).
37. Magliocco, G., Thomas, A., Desmeules, J. & Daali Y. Phenotyping of human CYP450 enzymes by endobiotics: current knowledge and methodological approaches. *Clin. Pharmacokinet.* (2019). https://doi.org/10.1007/s40262-019-00783-z.
38. Kiene, K. et al. Microdosed midazolam for the determination of cytochrome P450 3A activity: development and clinical evaluation of a buccal film. *Eur. J. Pharm. Sci. J. Eur. Fed. Pharm. Sci.* **135**, 77–82 (2019).
39. Bosilkovska, M. et al. Geneva cocktail for cytochrome p450 and P-glycoprotein activity assessment using dried blood spots. *Clin. Pharmacol. Ther.* **96**, 349–359 (2014).
40. Zhou, S.-F., Wang, B., Yang, L.-P. & Liu, J.-P. Structure, function, regulation and polymorphism and the clinical significance of human cytochrome P450 1A2. *Drug Metab. Rev.* **42**, 268–354 (2010).
41. Mayayo-Sinués, E. et al. Lack of effect of growth hormone replacement therapy on CYP1A2 and xanthine oxidase activities in growth hormone-deficient children. *Eur. J. Clin. Pharm.* **62**, 123–127 (2006).
42. ten Tusscher, G. W. et al. Perinatal dioxin exposure, cytochrome P-450 activity, liver functions and thyroid hormones at follow-up after 7–12 years. *Chemosphere* **70**, 1865–1872 (2008).
43. el-Yazigi, A. et al. Salivary clearance and urinary metabolic pattern of caffeine in healthy children and in pediatric patients with hepatocellular diseases. *J. Clin. Pharm.* **39**, 366–372 (1999).
44. Akinyinka, O. O., Sowunmi, A., Honeywell, R. & Renwick, A. G. The pharmacokinetics of caffeine in Nigerian children suffering from malaria and kwashiorkor. *Eur. J. Clin. Pharm.* **56**, 153–158 (2000).
45. Senggunprai, L., Kukongviriyapan, U., Jetsrisuparb, A. & Kukongviriyapan, V. Drug metabolizing enzyme CYP1A2 status in pediatric patients with hemoglobin E-beta thalassemia. *J. Med. Assoc. Thai.* **92**, 1675–1680 (2009).
46. Masimirembwa, C. M., Beke, M., Hasler, J. A., Tang, B. K. & Kalow, W. Low CYP1A2 activity in rural Shona children of Zimbabwe. *Clin. Pharm. Ther.* **57**, 25–31 (1995).
47. Kennedy, M. J. et al. Activities of cytochrome P450 1A2, *N*-acetyltransferase 2, xanthine oxidase, and cytochrome P450 2D6 are unaltered in children with cystic fibrosis. *Clin. Pharm. Ther.* **75**, 163–171 (2004).
48. Kennedy, M. J. et al. Six-month, prospective, longitudinal, open-label caffeine and dextromethorphan phenotyping study in children with growth hormone deficiency receiving recombinant human growth hormone replacement. *Clin. Ther.* **30**, 1687–1699 (2008).
49. Kennedy, M. J., Abdel-Rahman, S. M., Kashuba, A. D. M. & Leeder, J. S. Comparison of various urine collection intervals for caffeine and dextromethorphan phenotyping in children. *J. Clin. Pharm.* **44**, 708–714 (2004).
50. Chiney, M. S., Schwarzenberg, S. J. & Johnson, L. A. Altered xanthine oxidase and *N*-acetyltransferase activity in obese children. *Br. J. Clin. Pharm.* **72**, 109–115 (2011).
51. Parker, A. C., Preston, T., Heaf, D., Kitteringham, N. R. & Choonara, I. Inhibition of caffeine metabolism by ciprofloxacin in children with cystic fibrosis as measured by the caffeine breath test. *Br. J. Clin. Pharm.* **38**, 573–576 (1994).
52. Parker, A. C., Pritchard, P., Preston, T. & Choonara, I. Induction of CYP1A2 activity by carbamazepine in children using the caffeine breath test. *Br. J. Clin. Pharm.* **45**, 176–178 (1998).
53. Oshikoya, K. A., Sammons, H., Smith, K. & Choonara, I. Lack of a significant change in caffeine metabolism in underweight children as determined by the caffeine breath test. *Arch. Dis. Child* **100**, 689–693 (2015).
54. Oshikoya, K. A. & Smith, K. Effect of Casilan® on ^{13}C-caffeine metabolism in overnight-fasted healthy Nigerian children. *J. Pharm. Pharmacother.* **4**, 19–26 (2013).
55. Colletti, R. B. & Di Lorenzo, C. Overview of pediatric gastroesophageal reflux disease and proton pump inhibitor therapy. *J. Pediatr. Gastroenterol. Nutr.* **37**, S7–S11 (2003).
56. Gumus, E. et al. Evaluation of lansoprazole as a probe for assessing cytochrome P450 2C19 activity and genotype–phenotype correlation in childhood. *Eur. J. Clin. Pharm.* **68**, 629–636 (2012).
57. Favela-Mendoza, A. F. et al. Correspondence between the CYP2C19 and CYP3A4 genotypes with the inferred metabolizer phenotype by omeprazole administration in Mexican healthy children. *J. Clin. Pharm. Ther.* **43**, 656–663 (2018).
58. Frank, D., Jaehde, U. & Fuhr, U. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. *Eur. J. Clin. Pharm.* **63**, 321–333 (2007).
59. Evans, W. E. et al. Dextromethorphan and caffeine as probes for simultaneous determination of debrisoquin-oxidation and N-acetylation phenotypes in children. *Clin. Pharm. Ther.* **45**, 568–573 (1989).
60. Blake, M. J. et al. Ontogeny of dextromethorphan O- and N-demethylation in the first year of life. *Clin. Pharm. Ther.* **81**, 510–516 (2007).
61. Thummel, K. E. et al. Use of midazolam as a human cytochrome P450 3A probe: I. In vitro–in vivo correlations in liver transplant patients. *J. Pharm. Exp. Ther.* **271**, 549–556 (1994).
62. de Wildt, S. N., Ito, S. & Koren, G. Challenges for drug studies in children: CYP3A phenotyping as example. *Drug Discov. Today* **14**, 6–15 (2009).
63. Gade, C. et al. The CYTONOX trial. *Dan. Med. J.* **63**, 1–5 (2016).
64. de Wildt, S. N., Berns, M. J. P. & van den Anker, J. N. 13C-erythromycin breath test as a noninvasive measure of CYP3A activity in newborn infants: a pilot study. *Ther. Drug Monit.* **29**, 225–230 (2007).
65. Böttiger, Y. Use of omeprazole sulfone in a single plasma sample as a probe for CYP3A4. *Eur. J. Clin. Pharm.* **62**, 621–625 (2006).
66. Funck-Brentano, C. et al. Measurement of CYP2D6 and CYP3A4 activity in vivo with dextromethorphan: sources of variability and predictors of adverse effects in 419 healthy subjects. *Eur. J. Clin. Pharm.* **61**, 821–829 (2005).
67. Blake, M. J., Abdel-Rahman, S. M., Pearce, R. E., Leeder, J. S. & Kearns, G. L. Effect of diet on the development of drug metabolism by cytochrome P-450 enzymes in healthy infants. *Pediatr. Res* **60**, 717 (2006).
68. Connelly, M. A. et al. Pupillometry: a non-invasive technique for pain assessment in paediatric patients. *Arch. Dis. Child* **99**, 1125–1131 (2014).
69. Nakamura, H. et al. Comparison of urinary 6beta-hydroxycortisol/cortisol ratio between neonates and their mothers. *Br. J. Clin. Pharm.* **47**, 31–34 (1999).
70. Nakamura, H. et al. Changes in urinary 6beta-hydroxycortisol/cortisol ratio after birth in human neonates. *Eur. J. Clin. Pharm.* **53**, 343–346 (1998).
71. Vauzelle-Kervroedan, F. et al. Non invasive in vivo study of the maturation of CYP IIIA in neonates and infants. *Eur. J. Clin. Pharm.* **51**, 69–72 (1996).
72. Tay-Sontheimer, J. et al. Detection of an endogenous urinary biomarker associated with CYP2D6 activity using global metabolomics. *Pharmacogenomics* **15**, 1947–1962 (2014).
73. Li, H. et al. Pediatric cytochrome P450 activity alterations in nonalcoholic steatohepatitis. *Drug Metab. Dispos. Biol. Fate Chem.* **45**, 1317–1325 (2017).
74. Fliegert, F., Kurth, B. & Göhler, K. The effects of tramadol on static and dynamic pupillometry in healthy subjects—the relationship between pharmacodynamics, pharmacokinetics and CYP2D6 metaboliser status. *Eur. J. Clin. Pharm.* **61**, 257–266 (2005).
75. Knaggs, R. D., Crighton, I. M., Cobby, T. F., Fletcher, A. J. P. & Hobbs, G. J. The pupillary effects of intravenous morphine, codeine, and tramadol in volunteers. *Anesth. Analg.* **99**, 108–112 (2004).
76. Slanar, O. et al. Miotic action of tramadol is determined by CYP2D6 genotype. *Physiol. Res.* **56**, 129–136 (2007).
77. Hornik, C. P. et al. Development of a pediatric physiologically-based pharmacokinetic model of clindamycin using opportunistic pharmacokinetic data. *Clin. Pharmacokinet.* **56**, 1343–1353 (2017).
78. Moj, D. et al. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model of the histone deacetylase (HDAC) inhibitor vorinostat for pediatric and adult patients and its application for dose specification. *Cancer Chemother. Pharm.* **80**, 1013–1026 (2017).
79. Rioux, N. & Waters, N. J. Physiologically based pharmacokinetic modeling in pediatric oncology drug development. *Drug Metab. Dispos. Biol. Fate Chem.* **44**, 934–943 (2016).
80. Shebley, M. et al. Physiologically based pharmacokinetic model qualification and reporting procedures for regulatory submissions: a consortium perspective. *Clin. Pharm. Ther.* **104**, 88–110 (2018).
81. Marsousi, N., Desmeules, J. A., Rudaz, S. & Daali, Y. Usefulness of PBPK modeling in incorporation of clinical conditions in personalized medicine. *J. Pharm. Sci.* **106**, 2380–2391 (2017).
82. Templeton, I. E., Jones, N. S. & Musib, L. Pediatric dose selection and utility of PBPK in determining dose. *AAPS J.* **20**, 31 (2018).
83. Diestelhorst, C. et al. Predictive performance of a physiologically based pharmacokinetic model of busulfan in children. *Pediatr. Hematol. Oncol.* **31**, 731–742 (2014).
84. T'jollyn, H. et al. Physiologically based pharmacokinetic predictions of tramadol exposure throughout pediatric life: an analysis of the different clearance contributors with emphasis on CYP2D6 maturation. *AAPS J.* **17**, 1376–1387 (2015).
85. Gammal, R. S. et al. Pharmacogenetics for safe codeine use in sickle cell disease. *Pediatrics* **138**, 1–12 (2016).
86. Findling, R. L. et al. Paroxetine pharmacokinetics in depressed children and adolescents. *J. Am. Acad. Child Adolesc. Psychiatry* **38**, 952–959 (1999).