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Review Article

Mind the Gaps: Ontogeny of Human Brain P-gp and Its Impact on Drug Toxicity

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Abstract. Available data on human brain P-glycoprotein ontogeny during infancy and childhood are limited. This review discusses the current body of data relating to maturation of human brain P-glycoprotein including transporter expression levels in post-mortem human brain samples, *in vivo* transporter activity using probe substrates, surrogate marker endpoints, and extrapolations from animal models. Overall, the data tend to confirm that human brain P-glycoprotein activity keeps developing after birth, although with a developmental time frame that remains unclear. This knowledge gap is a concern given the critical role of brain P-glycoprotein in drug safety and efficacy, and the vulnerable nature of the pediatric population. Future research could include the measurement of brain P-glycoprotein activity across age groups using positron emission tomography or central pharmacodynamic responses. For now, caution is advised when extrapolating adult data to children aged younger than 2 years for drugs with P-glycoprotein-dependent central nervous system activity.

KEY WORDS: blood-brain barrier; brain; ontogeny; pediatric; P-glycoprotein.

INTRODUCTION

Cellular plasma membrane transporters are expressed in various tissues and ensure the uptake of endogenous and exogenous substances into cells, as well as their efflux. Hundreds of transporters have been identified, with about 20 being recognized as playing a role in drug absorption, distribution, and excretion (1). The multi-drug efflux pump P-glycoprotein (P-gp; also termed multi-drug resistance protein 1 [MDR1]; encoded by the *ABCB1* gene) belongs to the ATP-binding cassette (ABC) superfamily. Since its discovery more than 40 years ago (2), the function, localization, and regulation of P-gp have been extensively studied. P-gp is expressed throughout the body, typically at the apical surface of polarized cells from the liver and the kidneys, where it contributes to drug elimination. At physiological barriers such as the intestine, blood-brain barrier (BBB), and others (e.g., placenta and testis), P-gp is expressed at the luminal surface of cells, where it effluxes substrates and restricts their absorption or distribution into tissues.

P-gp is remarkable in its ability to transport a wide range of substances owing to its multiple substrate-binding sites. Changes in P-gp function have a profound effect on the pharmacokinetics, tissue distribution, efficacy, and toxicity of drugs that are substrates for this transporter. As an example, the over-the-counter anti-diarrheal drug loperamide is a potent synthetic μ-opioid agonist which, under normal circumstances, is devoid of central opioid side effects as a result of P-gp-mediated efflux and thus has limited brain distribution (3). However, concomitant administration of loperamide with strong P-gp inhibitors (e.g., quinidine and omeprazole) may lead to respiratory depression (3) and euphoria with abuse potential (4). Secondly, the etexilate prodrug of the anti-coagulant dabigatran shows poor oral absorption because of intestinal P-gp efflux (4). Co-administration with P-gp inhibitors has been reported to increase dabigatran exposure and therefore risk of severe hemorrhage (5).

Although less studied, the activity of the various drug transporters can change during growth and development, with potential impact on drug pharmacokinetics, pharmacodynamics, and safety in children (6–8). The International Transporter Consortium (ITC) recently issued a white paper about the ontogeny of drug transporters, reviewing the limited existing data and their clinical relevance for pediatrics, and making some recommendations (9). Notably, only intestinal-, hepatic-, and renal-transporter activities were discussed, without any mention of brain tissue. This underscores the paucity of information about brain P-gp ontogeny, which itself probably reflects the numerous technical and ethical challenges impeding investigations. However, this knowledge gap contrasts with the pressing need to better understand changes in drug kinetics during childhood, in order to determine appropriate pediatric dosing (10). Indeed, most of the drugs prescribed to children are off-

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label or unlicensed, with few (if any) clinical data available, and with rudimentary dose adjustment (11), which leaves this vulnerable population at risk of adverse safety reactions and efficacy failures.

This review discusses the available literature relating to brain P-gp ontogeny in humans and its relevance to drug safety in children. Immunohistochemistry data obtained in post-mortem human brain samples are reviewed, as well as information derived from alternate readouts such as CSF sampling, brain PET imaging, central nervous system (CNS) pharmacodynamic effects, and animal studies.

Immunohistochemical Investigations Using Post-mortem Human Brain Samples

A pioneering study performed in the late 1990s demonstrated P-gp expression in brain microvessels as early as 8 weeks of gestation (12). A subsequent study showed a gradual increase in brain microvessel P-gp expression with gestational age (13). The developmental pattern showed regional differences with an earlier P-gp expression in posterior forebrain when compared to cerebral cortex. Importantly, P-gp expression levels measured in post-mortem samples from term newborns were lower than those in adults, leaving open the question of post-natal maturation of P-gp.

This question was addressed in a work recently published by Lam *et al.* that examined samples from gestational age 20–26 weeks to post-natal age 3–6 months and compared them to adults (14) (Table I). P-gp protein levels were measured by immunohistochemistry in formalin-fixed, paraffin-embedded, post-mortem brain samples. The authors reported that P-gp expression was limited at birth and increased to adult levels at post-natal age 3–6 months. The authors acknowledged several limitations in their study. Post-mortem interval, method of tissue fixation, and donor P-gp genotype were unknown, which all may affect P-gp level determination. Tissue autofluorescence (e.g., involving lipofuscin) was discussed as another potential confounding variable in the P-gp immunoassay, as well as the fact that only cortex samples were examined. These concerns are not unique to this study: previous immunohistochemical studies measuring P-gp in tissues have produced conflicting results that were ascribed to similar methodological challenges (23,24). One could also add that the pediatric samples in Lam's report were compared with a rather old adult population (mean age of 53.6 years), which may complicate data interpretation as brain P-gp levels are known to decline with aging (25,26). Finally, caution is warranted when interpreting P-gp protein expression data, which may not directly correlate with transporter activity (27–31). Discrepancies would not be unexpected as P-gp activity is influenced by numerous factors beyond the expression of the protein itself, e.g., transporter conformation (31), membrane lipid environment (29), and extracellular pH (32).

However, it should be emphasized that the availability of quality human pediatric tissues is scarce and does not allow for P-gp investigations with larger sample sizes and better-controlled conditions (9). Despite all the above-mentioned limitations, Lam's study is cited by many review articles stating that human brain P-gp is fully mature at 3–6 months of age (7,35–39).

In Vivo Functional Activity of P-gp in Human BBB

As discussed above, P-gp expression levels in post-mortem or surgical samples may not be predictive of functional activity *in vivo*. However, assessing brain P-gp activity in human subjects is challenging, particularly in young children. An *in vivo* probe substrate for brain P-gp should combine selectivity towards the transporter, efficient efflux, low metabolism, high tolerability, and sensitive bioanalytics. Performing pharmacokinetic studies in pediatrics represents an additional challenge given the numerous technical, ethical, and regulatory hurdles. A recent review of 1081 registered clinical trials in children revealed that only 24% incorporated pharmacokinetic measurements and that 74% were conducted in children aged over 2 years (40), while changes in drug pharmacokinetics tend to predominate in younger children. From an ethical and regulatory perspective, studies are not generally acceptable in healthy children, and drugs investigated in ill children should either provide a direct benefit to the subjects or be used at a dose where risks are minimal (41).

The methods for estimating brain pharmacokinetics in the clinic are very few and mostly restricted to cerebrospinal fluid sampling and positron emission tomography.

Cerebrospinal Fluid Sampling

Although the blood-CSF barrier differs from the BBB, especially with respect to active transporters such as P-gp (13,42–45), drug concentration in lumbar, cisternal, and ventricular CSF may provide an indirect indication of brain levels. CSF sampling has been widely used in adolescents and adults to determine human brain P-gp activity towards centrally acting drugs, and its changes in disease states or resulting from P-gp/ABCB1 polymorphism (46–49). However, data in children are scarce and CSF sampling has never been used to specifically investigate the post-natal maturation of brain P-gp activity.

Vincristine (VCR) is a plant alkaloid widely used as an intravenous infusion for the treatment of childhood and adult malignancies. A study in 17 pediatric patients (aged 2.5–14 years) measured paired VCR levels in plasma and CSF after intravenous dosing (15) (Table I). No measurable VCR was detected in CSF samples, with CSF/plasma ratios < 5% irrespective of patient age. CSF-to-free plasma-level ratios were estimated to be < 0.16 (detection limit 0.1 ng/mL, VCR plasma protein binding 71% (50)). Since VCR is a P-gp substrate (51), the data above could be interpreted as brain P-gp being fully mature from 2.5 years onwards. These findings should be taken with caution as the study did not investigate younger ages. Also, the data were from patients treated for acute lymphoblastic leukemia or non-Hodgkin lymphoma, disease states often associated with overexpression of P-gp (52,53). Finally, VCR also interacts with other transporters such as multi-drug resistance-associated proteins (MRPs) (54,55) and organic anion transporting polypeptides (OATPs) (56).

Amphotericin B (AmB) is a potent anti-fungal known for showing poor brain penetration and extensive P-gp-mediated efflux (57). Although several studies reported undetectable AmB levels in CSF across various age groups

Table I. Effect of Age on Various Potential Readouts of Human Brain P-gp Activity

Species	Experimental approach	*N*	Age[a]	Conclusion
Human	Protein expression of brain P-gp by immunohistochemistry of post-mortem human brain cortex samples (13)	9 10 9 3	22–26 weeks[b] 27–32 weeks[b] 33–42 weeks[b] adults	Positive P-gp immunostaining of microvessel endothelial cells from 22 to 26 weeks gestation (brainstem, hindbrain, and thalamus) and from 27 to 32 weeks gestation (other regions of the forebrain). Lower signal at birth than in adults.
	Protein expression of brain P-gp by immunohistochemistry of post-mortem human brain cortex samples (14)	8 7 8	0–3 months 3–6 months 54 ± 7 years	Brain P-gp expression mature at 3–6 months of age
	VCR concentration in CSF samples (15)	17	2.5–14 years	Brain P-gp function mature at ≤ 2.5 years
	Meta-analysis of CNS toxicity with loperamide (16)	1788	1 month–12 years	Increased frequency of CNS side effects with loperamide in children aged < 3 years vs older children or placebo
	CNS toxicity with CsA (17)	146	5 months–18 years	Regression analysis identified age < 6 years as a risk factor for CNS side effects with CsA
Rat[c]	Brain distribution of oseltamivir, brain P-gp protein and mRNA levels (18)	na	3–42 days	Brain P-gp expression and function mature at 21 days
	Brain PET imaging with [^{11}C]-oseltamivir (19)	na	7–44 days	Brain P-gp function mature at 21–24 days
	Brain distribution of digoxin, brain P-gp mRNA levels (20)	na	14–56 days	Brain P-gp expression and function mature at 21 days
Monkey[c]	Brain PET imaging with [^{11}C]-verapamil (21)	na	9 months–7 years	Brain P-gp function not fully mature in infant (9 months) or adolescent (24–27 months) monkeys
	Protein expression of brain P-gp levels by LC-MS/MS (22)	na	20-week fetus–50 years	Brain P-gp expression mature at 3–6 months

CNS central nervous system, *CsA* cyclosporine A, *CSF* cerebrospinal fluid, *LC-MS/MS* liquid chromatography-tandem mass spectrometry, *mRNA* messenger ribonucleic acid, *na* not applicable, *PET* positron emission tomography, *P-gp* P-glycoprotein, *VCR* vincristine

[a] Unless otherwise stated, postnatal age

[b] Gestational age

[c] 21 days of rat age reported to correspond to around 1–2 years of human age; 9 months of Rhesus monkey age reported to correspond to around 4 years of human age (33,34)

(58–60), the methods used were not sensitive enough to quantify the low concentrations expected due to high plasma protein binding of AmB. A more recent study in subjects approximately 8 years old used a highly sensitive AmB assay and established that CSF concentrations corresponded to 0.13% of serum levels (61); this translates into a CSF-to-free serum-level ratio of approximately 0.26 (assuming 99.5% plasma protein binding (62)), confirming restricted BBB penetration. AmB CSF concentrations reported in pre-term neonates are surprisingly high, i.e., up to 40–90% of the concentration measured in paired serum samples (63). It could be hypothesized that the higher CSF levels in neonates relate to immature brain P-gp. However, as for VCR, these data should be interpreted with caution since the role of P-gp in AmB distribution is still debated (64,65) and age-related changes in plasma protein binding may have also contributed to the higher AmB CSF concentrations in neonates (66). Moreover, it should be realized that CSF concentrations do not directly reflect brain extracellular fluid (ECF) concentrations. The relationship between brain ECF and CSF is drug dependent, system dependent, and time dependent, and it is the brain ECF concentrations that are needed to understand P-gp activity at the level of the BBB (45).

Positron Emission Tomography

PET imaging has been proposed as an alternative to CSF sampling to investigate drug distribution across the BBB. PET has the advantages of being non-invasive and allowing regional distribution studies (67). On the other hand, PET remains expensive and technically challenging. In addition, the PET signal is not easy to interpret since it shows the total concentration in the tissue of interest; it does not allow discrimination between the intact tracer and its labeled metabolites, nor between free and bound material. The PET signal also does not differentiate between the intracellular, extracellular, and intravascular compartments (68). PET has been applied to measure brain P-gp functional activity in animals and humans using [^{11}C]-labeled probe markers, e.g., [^{11}C]-verapamil and [^{11}C]-desmethyl-loperamide. So far, human PET studies have been restricted to adults, measuring brain P-gp activity in disease states and/or following co-administration with P-gp inhibitors (68). There are no reports of PET studies investigating brain P-gp maturation in children.

P-gp Activity in Peripheral Tissues as a Surrogate Marker of Activity in the Brain

Given the difficulties in quantifying *in vivo* brain P-gp functional activity in young children, a surrogate marker with easier access would be useful. P-gp is present in various tissues, particularly blood cells that are easy to collect. However, available literature suggests that P-gp functional activity varies across tissues, making blood cell assays unlikely to quantitatively predict the BBB situation. *In vivo*, brain P-gp is more resistant to inhibition than P-gp in blood lymphocytes (69–71). Similarly, *in vitro* data showed that circulating lymphocytes and brain endothelial cells respond differently to P-gp inducers, suggesting different mechanisms of transporter regulation (72). It has also been suggested that the location of P-gp within the cell membrane, its lipid microenvironment, and its expression level might differ between blood lymphocytes and brain endothelial cells, which could account for the observed differences in activity (69). In circulating T lymphocytes and NK cells, P-gp activity is maximal at birth and gradually decreases to adult levels at 6 months of age (73,74), suggesting that blood lymphocytes and brain have an opposite time frame with respect to P-gp maturation.

The organ-specific pattern of P-gp development has been already discussed for other peripheral tissues (7,38). Based on protein expression data, hepatic P-gp reaches adult levels at 1 year of age (75), duodenal P-gp appears fully mature from birth (76,77), while placenta P-gp decreases with advancing gestation (78). The mechanisms and physiological reasons for these tissue differences remain unclear. Overall, it is unlikely that P-gp maturation in peripheral tissues or blood cells will be informative for the situation at the BBB level.

CNS Pharmacodynamic Response as a Surrogate Marker of Brain P-gp Activity

Under certain circumstances, central effects of P-gp substrates on the brain may be considered as a potential surrogate for measuring the transporter activity at the BBB. This necessitates a drug with sizable P-gp-mediated efflux and easily measurable CNS pharmacological or toxicological response.

When taken as advised, loperamide does not elicit central opioid activity because of restricted brain penetration, a consequence of P-gp-mediated efflux (79). However, cases of neurological side effects have been reported in young children given loperamide (80–82). A large meta-analysis of 13 selected, randomized, controlled trials in children (total of 1788 children aged 1 month to 12 years) (16) (Table I) confirmed that loperamide is mostly efficacious and safe. However, serious adverse events were reported among loperamide-treated children aged < 3 years, compared with none in older children or those allocated to placebo. It has been suggested that decreased brain P-gp function in children might account for their increased vulnerability to loperamide CNS side effects (83).

Buprenorphine is a partial agonist of μ-opioid receptors used in adults for treatment of pain and narcotic addiction. It is also a valuable treatment option for neonatal abstinence syndrome, a condition affecting newborns that were exposed to opioids in utero (84). Norbuprenorphine, the major active metabolite, is a substrate of P-gp, which limits its brain distribution and partly masks its potential to provoke CNS depression (85). Buprenorphine has been rarely linked to overdose in adults and children. However, children under 2–3 years do not respond well to buprenorphine overdosage showing CNS toxicity such as respiratory and CNS depression, altered mental status and miosis (84,86–88). In theory, immature P-gp in children could allow higher brain exposure to norbuprenorphine (83,87). Other mechanisms could also account for the observed findings, for example a “ceiling” pharmacodynamics effect might limit respiration depression in adults but not in very young children (86).

Cyclosporin A (CsA) is a potent, amino acid-containing immunosuppressant. It is a P-gp substrate (89) that usually shows restricted distribution to the brain (90). However, severe neurotoxicity is a recognized complication of CsA

that can manifest as seizures, acute encephalopathy, coma, cerebral hemorrhage, and cortical blindness. It is not directly linked to CsA dose or plasma exposure (91); the exact underlying mechanisms are poorly understood and likely to be multi-factorial (92). Many risk factors have been identified, including co-administration with anti-cancer drugs and glucocorticoids, hypertension, hepatic and renal dysfunction, cerebral ischemia or hemorrhage, and low serum cholesterol (93,94). A retrospective study in 146 children (from 0.4 to 18 years of age) demonstrated that age is another risk factor for CsA neurotoxicity (17). Age < 6 years was found to be significantly associated with CsA encephalopathy (Table I), possibly because of immature brain P-gp.

As described earlier, VCR is a plant alkaloid widely used as an intravenous infusion for the treatment of childhood and adult malignancies such as lymphoma, leukemia, and rhabdomyosarcoma. Severe and often fatal CNS toxicity has been reported following accidental intrathecal administration of VCR (95). Under normal circumstances, central side effects are rare since the drug does not distribute into the brain (15,96) as a consequence of its extensive efflux by P-gp and MRPs (55). Disruption of the BBB, for example due to brain tumor (55), osmotic opening (97), or impaired brain P-gp activity (98,99), potentially increases distribution of VCR to the brain and increases its central toxicity. Presumably, immature brain P-gp in young children could also translate into increased VCR central side effects. However, to our knowledge, there are no published reports suggesting a higher incidence of VCR-induced central side effects in young children *versus* older age groups.

Most of the above examples tend to confirm post-natal development of brain P-gp activity. However, these reports do not allow a precise clarification of the time frame of the transporter maturation, as some important variables were not controlled, e.g., P-gp genotype, environmental exposure, potential age-related changes in systemic drug levels, or in the expression of the target(s) driving the CNS response (such as for dopamine neurotransmission (100), $GABA_A$ (101,102), or SV2A (103)). Measuring CNS pharmacodynamics response as a surrogate marker of P-gp activity remains a viable future option for controlled prospective studies.

Extrapolations from Animal Models to Humans

Measuring protein expression levels and/or functional activity of BBB transporters in laboratory animals at various post-natal ages might potentially provide an indication of the human situation (9,104,105).

Age-related changes in brain disposition of various P-gp probe substrates have been thoroughly investigated in rodents. After intravenous infusion to male Wistar rats, the brain-to-plasma concentration ratio of the P-gp substrate oseltamivir was highest in 3-day-old pups and decreased to adult levels from 21 days onwards (Table I) (18). These data were confirmed in a subsequent PET imaging study with [^{11}C]-oseltamivir (19) and in a tissue distribution study using digoxin, another P-gp substrate (20). The above age-related changes in brain P-gp function broadly parallel the changes in mRNA (18,20,106,107) and protein (108–110) expression in brain microvessels, with adult levels being reached at postnatal weeks 3–4. In rats, the expression profile of P-gp protein appears to be comparable in the capillaries of cerebral cortex, hippocampus, and cerebellum (108). The developmental pattern of brain P-gp in mice is similar than in rat. A report investigating the brain disposition of the P-gp substrate CsA in mice demonstrated around 4-fold higher brain P-gp activity in adults compared with 1-day-old animals, and mature levels were achieved at around 19 days of age (111). Similarly, P-gp expression in mouse brain was found to be limited during embryogenesis and to increase with postnatal maturation. By day 21, brain P-gp protein expression approximates adult levels (112).

The numerous and well-aligned datasets in mice and rat contrast with very few and conflicting reports in non-human primates. In a comprehensive PET study in Rhesus monkeys, the brain-uptake clearance of R-[^{11}C]-verapamil, a parameter that is inversely proportional to P-gp activity at the BBB, was significantly higher in infant animals (9 months) compared with adolescents (24–27 months) or adults (5.6–6.6 years): 0.14 ± 0.04, 0.09 ± 0.02, and 0.06 ± 0.01 mL/min/g, respectively (mean ± standard deviation of five animals per group) (21) (Table I). This finding suggests a rather delayed maturation of brain P-gp, which contrasts with a concurrent study reported in cynomolgus monkeys (22). In this later study, brain P-gp was claimed to be mature at birth since similar P-gp protein levels were measured in 1-day-, 16-month-, and 4-year-old animals. However, these data should be interpreted with caution given the small sampling size (i.e., one animal per group) and potential disconnect between protein expression and functional activity.

Overall, from the above data, brain P-gp activity appears to be mature in rodents from ca. 3-week post-natal age and in Rhesus monkeys aged > 9 months. Translating these data to humans should consider potential species differences in organ and function maturation. Using a "neuroinformatic" approach combining various neurodevelopmental endpoints (33), 3 weeks of rat age would correspond to 1–2 years of human age, with 9 months in Rhesus monkey age corresponding to approximately 4 years in humans. Broadly similar schedules can be derived from the popular age-comparative categories described by Buelke-Sam, which are based on overall CNS and reproductive development (113). One major weakness of this approach is that it assumes that brain P-gp development parallels other CNS endpoints such as neurogenesis, myelination, sensimotor function, and brain growth. Such assumptions might be flawed, as illustrated by intestinal P-gp. Indeed, human intestinal P-gp is fully mature at birth (76,114) while other intestinal functions, such as duodenal CYP3A4 activity, intestinal cell morphology, and Peyer's patch development, show delayed maturation (115–117). Translating brain P-gp maturation data from animals to humans is made even more complex by species differences in BBB transporter expression and activity, especially between rodents and primates (118–120). Overall, although strongly indicating maturation of BBB P-gp after birth, the actual animal data are somewhat conflicting and difficult to extrapolate to humans, especially in refining the maturation time frame of the transporter.

Physiologically Based Pharmacokinetic Modeling of CNS

PBPK modeling is increasingly used to investigate drug pharmacokinetics in children (121–123). PBPK is a

mechanism-based modeling approach where all drug metabolism and pharmacokinetic processes and their interconnections are described mathematically. A broad range of input data (*in silico*, *in vitro*, pre-clinical and clinical data) can be incorporated to parameterize the PBPK model for both system-specific (e.g., anatomy, physiology, flow rates, tissue volume and composition, or metabolizing enzyme activity) and drug-specific (e.g., solubility, permeability, metabolic clearance, or fraction bound to plasma proteins) parameters. Typically, an adult PBPK model is first built, then refined and verified against measured clinical data. Later, system parameters can be adjusted to account for maturational changes in pharmacokinetic processes, and the model can be used to predict pharmacokinetics in children (121).

So far, pediatric PBPK modeling has primarily been applied *a posteriori* using data for well-known reference drugs, in order to explore the maturation of renal and metabolic clearance processes (124–129). *A priori* pediatric PBPK studies for guiding dose selection and trial design are far fewer (130–132). The prospective use of pediatric PBPK models is hindered by limited understanding of age-related changes in some pharmacokinetic mechanisms. The developmental patterns of processes involved in oral drug absorption remain particularly poorly characterized (114,133). The information gap around the ontogeny of P-gp activity is another critical limitation, especially when the PBPK model is intended to predict drug concentrations in the brain and associated CNS therapeutic or toxic activities. To our knowledge, there are no published PBPK reports investigating the CNS distribution of P-gp substrates in pediatric vs adult populations. Predicting the distribution of P-gp substrates across adult BBB, i.e., ignoring age-related changes, remains a challenging task (134–137). Recently, Yamamoto *et al.* reported a generic CNS PBPK model to predict the concentration-time profile of drugs in various compartments of adult rat (138) and human (139) brain. The model was validated against multiple drugs with varying physicochemical properties and active transport activities. Moreover, this model was instrumental in predicting morphine brain concentrations in children with traumatic brain injury (140). Hopefully, as knowledge of ontogeny of brain P-gp improves, it will become possible to extend PBPK models to predict brain disposition in children.

CONCLUSIONS

Reliable data on the timing of postnatal maturation of human brain P-gp activity are scarce. Our review of data from a variety of sources tends to confirm that post-natal changes in human brain P-gp activity occur, but does not allow a precise determination of the time schedule (Table I). Depending on the data considered, maturation could be achieved either at term birth or not until several years of age. Unfortunately, measuring brain P-gp functional activity in pediatric subjects is associated with considerable technical, ethical, and regulatory challenges. In the future, prospective studies using PET imaging or assessment of CNS pharmacodynamic responses may provide more robust insights into the normal human brain P-gp maturation pattern. Undoubtedly, a better understanding of the mechanisms underlying the maturation of P-gp in brain microvessels would also contribute filling the knowledge gaps and/or identifying other biomarkers. For instance, astrocytes have been suggested to modulate P-gp through multiple signaling pathways (e.g., TGF-β1, lipids) that are sensitive to developmental changes and disease effects (141–143). Once available, all these data could be used to refine the currently available human CNS PBPK model and to predict brain-drug disposition in pediatric patients. However, for now, caution is advised when extrapolating adult data to children aged younger than 2 years for drugs with P-gp-dependent CNS activity.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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