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Identifying genomic and developmental causes of adverse drug reactions in children

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Abstract

Adverse drug reactions are a concern for all clinicians who utilize medications to treat adults and children; however, the frequency of adult and pediatric adverse drug reactions is likely to be under-reported. In this age of genomics and personalized medicine, identifying genetic variation that results in differences in drug biotransformation and response has contributed to significant advances in the utilization of several commonly used medications in adults. In order to better understand the variability of drug response in children however, we must not only consider differences in genotype, but also variation in gene expression during growth and development, namely ontogeny. In this article, recommendations for systematically approaching pharmacogenomic studies in children are discussed, and several examples of studies that investigate the genomic and developmental contribution to adverse drug reactions in children are reviewed.

Keywords

adverse drug reactions; genetic variants; methotrexate; pediatrics; pharmacogenomics

Adverse drug reactions (ADRs) remain an ever present concern for all clinicians who utilize medications in adults and children. ADRs have been defined by the WHO as “a response to a drug that is noxious and unintended, and occurs at doses normally used in man for the prophylaxis, diagnosis or therapy of disease, or for modification of physiological function” [1]. Edwards and Aronson have broadened this definition to “an appreciably harmful or unpleasant reaction resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product” [2].

There is no question that ADRs represent a major burden to patients, the healthcare system and the drug-development process. The magnitude of the problem has been the subject of many publications, particularly over the past 10 years. A widely cited (and criticized) meta-

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analysis suggested that severe ADRs ranked between the fourth and sixth leading cause of death in the USA in 1994, affecting an estimated 2 million hospitalized patients [3]. A more recent study from Sweden implicated ADRs as the seventh-most common cause of death [4], supporting the unexpectedly high findings of the earlier study. In addition, ADRs can occur in patients once admitted to hospital, incurring increased costs owing to additional interventions and prolongation of hospital stays [5]. Despite the limited data available, in this nonsystematic article, we would like to highlight the importance of ADRs specifically in children, focusing on the contribution of development and genetic variation related to ADRs. We will discuss approaches to pharmacogenomic studies in children and utilize examples from the literature and our center (Children's Mercy Hospitals & Clinics, MO, USA) to illustrate these points.

ADR reporting in children: general considerations & challenges

Adverse drug reactions have not been as thoroughly studied in children as they have in adults, despite the significance of ADRs in children having been recognized for more than 25 years [6,7]. Of the 30 studies included in the original meta-analysis conducted by Lazarou *et al.*, only four contained pediatric data [3]. Several factors contribute to this phenomenon. Most drugs used in children have never been formally studied in the pediatric population, nor have they been approved by the US FDA for use in pediatric populations of different ages or developmental stages. Thus, there is little pediatric 'experience' from which knowledge of incidence and risk factors can be derived. Furthermore, the youngest of children may be at an increased risk owing to an inability to effectively communicate to caregivers the subjective symptoms attributable to a medication.

Nevertheless, ADRs in children represent a significant public health concern, and there is some literature that reports the frequency of ADRs in children. ADRs have been reported to be responsible for 4.3% of admissions to a pediatric hospital in children less than 2 years of age. This same group conducted a survey of ADRs in already-hospitalized children, reporting an incidence of 16.6%, with most reactions classified as mild to moderate [8]. Similar results have been reported by others, noting a rate of ADRs of 13.7% found in a prospective study in hospitalized children in Chile [9]. The risk of ADRs is even higher for infants in an intensive care setting, who are often critically ill, suffering from multiple organ system dysfunction and who require multiple drugs [10]. A meta-analysis of 17 published reports of pediatric ADRs [11] concluded that 2.1% of admissions to children's hospitals were due to ADRs, with 39% of those ADRs being life-threatening; and in children already hospitalized, the incidence of ADRs was higher at 9.5%, with 12.3% of those reactions being classified as severe.

These estimates almost certainly underestimate the true incidence of ADRs in children as there is no standard reporting system, and available primary data is derived from assessments conducted in individual pediatric hospitals. Furthermore, the under-reporting of ADRs is more likely to occur when data is collected for more general reasons in the first place, such as the need to meet the requirements of regulatory bodies. Concerns related to the 'discoverability' of ADR data may also limit the extent to which events are identified and recorded [12]. It is not surprising then that ADR detection strategies utilizing targeted trigger tools elicit higher reporting rates compared with strategies relying on voluntary or verbally solicited reports from hospital staff or unfocused retrospective chart reviews [13]. Finally, an overview of ADRs reported to the FDA's Adverse Event Reporting System revealed that the number of pediatric reports to the Adverse Event Reporting System over the past 5 years has remained steady (Figure 1), while reports for adults have increased fivefold over the same time period. The authors concluded that improvement in the passive surveillance system is needed from a pediatric perspective, and recommended that

investments be made in active surveillance systems to better capture risk information in children [14].

ADRs in children: the impact of age on ADRs

Untoward events occurring in children have been pivotal in history for the subsequent development of the FDA and legislation aimed at protecting the public [4,15]. Clinical experience has revealed that children differ from adults in terms of risk for particular ADRs. For example, delayed maturation of drug-metabolizing enzymes may contribute to concentration-dependent toxicities, particularly in newborns where drug biotransformation capacity is most limited [16]. Furthermore, compelling data has been presented indicating that several severe idiosyncratic ADRs, such as valproic acid (VPA) hepatotoxicity [17–19], activation cluster adverse events with fluvoxamine [20] and cutaneous toxicity associated with lamotrigine, [21,22] occur more frequently in children than in adults. ADRs occurring early in life can additionally be accompanied by life-long morbidity with furosemide-induced ototoxicity in newborns and inappropriate weight gain due to atypical anti-psychotics, being just two examples. Similarly, there are situations where drug exposures or chronic drug therapy initiated early in life may be associated with consequences that do not become apparent until later on in life. Examples include the development of secondary malignancies following treatment for various childhood cancers [23,24], and the effect of steroid therapy on linear growth [25].

Several severe idiosyncratic ADRs, such as aspirin and Reye's syndrome [26], and cefaclor serum-sickness-like reactions [27] occur more frequently in children than in adults. The mechanisms of toxicity are poorly understood at best, and as a result, explanations for the apparent increased risk of these events in children is unknown. *In vivo* pharmacokinetic and therapeutic drug monitoring data implies that some drug biotransformation pathways exceed adult capacity, at least when dosage requirements/drug clearance between children and adults are compared and expressed relative to bodyweight. This issue is far from resolved, but developmentally increased CYP activity during childhood could conceivably result in increased formation of reactive, potentially toxic metabolites. If combined with pharmacogenetic variation in detoxification pathways (e.g., glucuronosyl transferases and sulfotransferases of glutathione transferases), net increases in reactive metabolite formation could be a significant determinant of risk for the development of idiosyncratic ADRs in young children. For example, experimental evidence suggests that chemically reactive metabolites generated from the potentially toxic metabolite 4-ene-VPA, such as 2,4-diene-VPA, have the potential to deplete mitochondrial glutathione pools [28], and through formation of conjugates with coenzyme A [29], inhibit enzymes in the β -oxidation pathway [30,31].

The phenomenon of behavioral adverse events termed 'activation cluster adverse event' (AC-AEs), manifested as an increased activity level, impulsivity, disinhibition and insomnia, have been associated uniquely in children receiving certain medications, specifically serotonin selective reuptake inhibitors (SSRIs) [20,32]. Reinblatt and colleagues reported AC-AEs in 45% of children receiving fluvoxamine compared with 4% in the placebo group as part of a retrospective analysis of a double-blind placebo-controlled trial. Elevated mean blood levels of fluvoxamine in patients who exhibited these behaviors and an apparent resolution of symptoms with dose reduction suggested that dose and metabolism may play an important role [20]. A pharmacokinetic study by Labellarte and colleagues has demonstrated a higher exposure to fluvoxamine in children than in adolescents and adults [33]. Fluvoxamine is metabolized by the cytochrome P450 enzymes, primarily CYP1A2. Delayed acquisition of catalytic activity may account for the differences in activation seen between age groups, as CYP1A2 is one of the last CYPs to be expressed postnatally.

However, one cannot exclude the possibility that susceptibility of younger children to AC-AEs is owing to developmental differences in the expression of the drug target, the serotonin reuptake pump (SLC6A4), or the presence of 'off-target' interactions that do not occur at older ages. Unfortunately, few, if any data characterizing the ontogeny of drug targets and downstream signal transduction pathways are presently available.

Ontogeny & ADRs in children

The developmental continuum between birth and adolescence is a very dynamic, complex period of life. The effects of development can be applied to all steps of drug disposition and response. These effects range from differences in gastric pH [34,35] and gastric emptying (affecting the absorption of compounds) [36], to changes in circulating plasma proteins with age, potentially affecting drug distribution [37]. Developmental changes in phase I drug biotransformation and phase II conjugating enzyme expression have the potential to alter drug metabolism [38]. In addition, developmental differences in glomerular filtration rates will affect drug excretion in children [39]. Common drug biotransformation pathways are also known to be shared with endogenous compounds involved in growth and development, a nonexhaustive list including: testosterone, progesterone, prostaglandins, cortisol and vitamin D3 [16]. Therefore, it may not be surprising that some of these drug biotransformation pathways may be affected by rapid growth and maturation, for example, during infancy and puberty. The developmental expression of these pathways at different rates may also lead to further variability in drug disposition and response.

Age-dependent predisposition to ADRs may also be a function of developmental changes in the expression of drug targets, including transporters, ion channels, receptors and downstream signal transduction pathways [40]. For example, the serotonergic system plays an important role in postnatal brain development, a period of considerable plasticity. However, very little research has actually been conducted in the area of ontogeny of the response to medications targeting the serotonergic pathway. In fact, the ontogeny of many important drug targets for medications widely used in human adults, such as warfarin (vitamin K oxido-reductase complex 1 [VKORC1]), HMG Co-A reductase inhibitors, angiotensin-converting enzyme inhibitors and atypical antipsychotics (dopaminergic pathway) remains virtually unknown.

Based on available *in vitro* data and *in vivo* pediatric pharmacokinetic studies, drug clearance pathways are known to undergo dramatic changes throughout the maturation process [37,38]. The activities of many enzymes involved in drug biotransformation are absent or very limited at birth, raising the possibility that there may be periods of relatively increased vulnerability to concentration-dependent drug toxicity. Cardiovascular collapse from chloramphenicol, associated with delayed maturation of glucuronidation and accumulation of the parent drug, is a classic example of this phenomenon [41–43]. The specific UGT isoform responsible for glucuronidation of chloramphenicol has now been identified as UGT2B7 [44]. The ontogeny of UGT2B7, both *in vitro* and *in vivo*, is reasonably well understood owing to the considerable number of studies on the ontogeny of morphine, a CYP2B7 substrate, in newborns, infants and young children [45]. Thus, delayed development of chloramphenicol glucuronidation is consistent with the ontogeny of UGT2B7 as inferred from the morphine data. However, genetic variation also contributes to variability in UGT2B7 activity and morphine glucuronidation [46], which may account for the apparent dose-dependent toxicity of chloramphenicol reported in adults [47].

Another example of the potential role of ontogeny in pediatric ADRs is the syndrome of irritability, tachypnea, tremors, jitteriness, increased muscle tone and temperature instability in neonates born to mothers receiving SSRIs during pregnancy. Controversy currently exists

as to whether these symptoms reflect a neonatal withdrawal (hyposerotonergic) state [48], or whether they represent manifestations of serotonin toxicity [49,50] analogous to the hyperserotonergic state attributed to SSRI-induced serotonin syndrome in adults [51]. Currently available data reveals that CYP2D6 and CYP3A4 are acquired in the first weeks of life. They support a hyperserotonergic state owing to delayed clearance of paroxetine and fluoxetine (CYP2D6), or sertraline (CYP3A4) in neonates exposed to these compounds *in utero*. Furthermore, decreases in plasma SSRI concentrations and resolution of symptoms would be expected to be present with increasing postnatal age and maturation of these pathways. In addition, genetic variation, especially in *CYP2D6*, may contribute to susceptibility to these reactions [52]. Given that treatment of a 'withdrawal' reaction may include administration of an SSRI, there is considerable potential for increased toxicity in affected neonates if this course of action is taken when they are at risk for delayed clearance. However, the relative contribution of ontogeny and genetic variation in genes involved in serotonin biosynthesis, catabolism, transport and response for risk of SSRI-induced neonatal adaptation syndrome and its appropriate management is less well understood. Initial data from a study investigating the role of genetic variation in the serotonin reuptake pump, *SLC6A4*, implies a complex interaction between genotype and adverse neonatal outcomes following maternal SSRI exposure [53].

Genotyping an individual for variations that affect function is an important step in understanding variability in outcomes. However, knowing if and when that gene is expressed at a given point in the developmental continuum is a concept specific to genotype–phenotype relationships in children [54,55]. An approach to investigating hypotheses related to drug outcomes in children can be guided by the following questions [54]:

- What gene products are quantitatively important in the disposition (absorption, distribution, metabolism and excretion) of the drug in question?
- For each gene product, what is the ontogeny for the acquisition of functional activities?
- Is allelic variation in the gene(s) of interest associated with any functional consequences *in vivo*?
- Does allelic variation affect the ontogeny of the drug disposition phenotype?
- What is the developmental context in which the gene(s) of interest is/are operating?

It may be impossible to address all of these questions simultaneously, and there may be several unknown areas requiring further investigation to fill a specific knowledge deficit. However, by keeping these questions in mind, one can systematically investigate many of the potential sources of variability in drug responses experienced in children. From this, additional new research opportunities may arise. The entire process results in a more global approach to investigating outcomes, such as drug response and toxicity. Several examples of investigative approaches evaluating drug responses in children in various stages of development are discussed in the next section.

Examples of genotype–phenotype correlations in children: dextromethorphan & CYP2D6

Several medications commonly used in pediatrics are dependent upon CYP2D6 activity for their elimination from the body, including dextromethorphan (DM). DM has been an area of investigation at our center for several years. Subjects participating in these studies range from 2 weeks postnatal age to adolescents (16 years of age). The youngest subjects participated in a longitudinal study in which phenotyping was conducted at time points

coinciding with well-baby visits at 2 weeks, 1 month, 2 months, 4 months, 6 months and 12 months of age [56]. In this study, a total of 193 male and female newborns (more than 32 weeks gestation and birthweight of more than 1500 g) received an oral dose (0.3 mg/kg) of DM 1 h after their evening feeding at each time point (882 total visits). Urine was expressed from diapers collected overnight for up to 12 h after dosing and analyzed for DM and its metabolites; dextrophan, 3-methoxymorphinan and 3-hydroxymorphinan, by HPLC. Results from this study revealed that CYP2D6 phenotype was consistent with genotype from as young as 2 weeks of age, and remained consistent throughout the first year of life. Thus, genetic variation in *CYP2D6* is a more important determinant of variability in CYP2D6 activity than ontogeny over the first year of life (Figure 2). These *in vivo* phenotyping data are consistent with a recent *in vitro* investigation involving a larger number (>220) of fetal and postnatal liver microsomal samples. This *in vitro* investigation revealed that CYP2D6 protein and activity were similar between third trimester samples and those from infants in the first week of life. Furthermore, both protein and activity remained relatively constant after 1 week of age up to 18 years [57]. A greater role for genetic variation over developmental changes in CYP2D6 activity is supported by studies of tramadol pharmacokinetics in newborns [58]. An important secondary observation from the longitudinal DM phenotyping study was the increasing proportion of *N*-demethylated metabolites (predominantly 3-hydroxymorphinan) in the total DM and metabolites recovered in the urine collections, increasing from approximately 20% at 2 weeks of age to approximately 50–60% at 12 months, higher than the approximately 30% observed in adults [59,60].

This information has direct relevance to investigating the several advisories issued over the past 5 years concerning the use of several substrates in childhood (e.g., fatal respiratory depression and promethazine in children less than 2 years of age; DM and other over the counter cough and cold remedies in children less than 2 years of age; SSRIs and the risk of self-harm in adolescents). Given the data indicating that genetic variability is a greater source of variability in observed CYP2D6 activity than ontogeny, pharmacokinetic studies conducted in a relatively small number of children drawn from the population extremes – children with no functional alleles or genotypic poor metabolizers at one extreme and those with two or more functional alleles or genotypic ultrarapid metabolizers at the other – would be expected to efficiently capture the full range of drug exposures anticipated in the target population following a fixed dose of drug [55]. Such knowledge would be extremely helpful in predicting the risk of concentration-dependent toxicity involving drugs that are CYP2D6 substrates in both children and adults.

6-mercaptopurine & thiopurine S-methyltransferase

The proposed paradigm can also be applied to assess the relative roles of ontogeny and genetic variation as determinants of toxicity to 6-mercaptopurine (6-MP), azathioprine and 6-thioguanine. Thiopurine S-methyltransferase (TPMT) is quantitatively important in the disposition of 6-MP and 6-thioguanine. TPMT activity is usually measured in erythrocytes, which is thought to reflect activity found in other tissues, including liver and leukemic blasts. In newborn infants, peripheral blood TPMT activity is reported to be 50% greater than in race-matched adults and shows a distribution of activity that is consistent with the polymorphism characterized in adults. Ganiere-Monteil *et al.* reported that TPMT activity was lower in children than adults, but was not likely to be clinically relevant [61]. Similarly, TPMT activities have been reported to be comparable to previously reported adult values in a population of Korean schoolchildren aged 7–9 years [62].

Thiopurine S-methyltransferase is also subject to genetic variation. Although approximately 89% of Caucasians and African-Americans have high TPMT activity, and 11% have

intermediate activity, 1 in 300 individuals inherit TPMT deficiency as an autosomal recessive trait. Three mutations have been identified in the *TPMT* gene (*2, *3A and *3C), which account for 98% of Caucasian subjects with low activity [63]. In patients with intermediate or low activity, more drug is shunted toward production of cytotoxic thioguanine nucleotides. TPMT can also methylate 6-thioinosine 5'-monophosphate to generate a methylated metabolite that is capable of inhibiting *de novo* purine synthesis. In the 0.3% of treated patients who are homozygous for variant (extremely low activity) *TPMT* alleles, severe and potentially life-threatening myelo-suppression can develop in those receiving standard doses of thiopurine. Subsequently, starting doses must be reduced to 6–10% of the normal dose. In the treatment of acute lymphoblastic leukemia, genetic variation in *TPMT* appears to be a more important determinant of 6-MP toxicity than ontogeny.

Likewise, it is probable that genetic variation is more relevant than ontogeny as a determinant of interindividual variability in TPMT, contributing to the clinical manifestations of hematopoietic toxicity in patients who utilize 6-MP and azathioprine for diseases such as inflammatory bowel disease and systemic lupus erythematosus [64,65]. Relling and Evans have recently demonstrated that genetic variation in additional enzymes in the mercaptopurine metabolic pathway, specifically inosine triphosphate pyrophosphatase, also contribute to mercaptopurine metabolism and clinical neutropenic toxicity, even when doses are adjusted based on *TPMT* genotype [65]. This illustrates that drug metabolism genotype–phenotype relationships are likely to be even more complex than currently appreciated, and that a systems-based approach will be necessary to further unravel the complexity of these relationships.

Balance between bioactivation & detoxification pathways: VPA

Valproic acid is a broad-spectrum antiepileptic drug used widely in children to treat idiopathic generalized epilepsy, absence epilepsies, primary generalized tonic-clonic seizures and juvenile myoclonic epilepsy [66,67]. More recently, its use has been expanded to include bipolar disorder and migraine prophylaxis in children and adolescents [68,69]. VPA hepatotoxicity may occur at any age, but epidemiologic studies have revealed that the risk of fatal hepatotoxicity is highest (~1:600) in children less than 2 years of age receiving concurrent anticonvulsant therapy. This is a 20-fold increase in risk relative to children of the same age on VPA monotherapy (1:13,000) [17–19]. Other studies have confirmed polytherapy as a risk factor but found little difference in risk occurring between younger (<3 years of age) and older children, 3–6 years of age [70–72].

A terminal olefin metabolite of VPA, 2-n-propyl-4-pentenoic acid (4-ene-VPA), is thought to be responsible for the observed toxicity owing to its structural similarity to known hepatotoxins, such as 4-pentenoic acid [73]. In mitochondria, 4-ene-VPA-CoA undergoes β -oxidation to (E)-2-propyl-2,4-pentadienoic acid-CoA (2,4-diene-VPA-CoA), a chemically reactive metabolite that is subject to glutathione conjugation [28,29]. Identification of various *N*-acetylcysteine conjugates in the urine of VPA-treated patients indicates that this bioactivation-detoxification pathway occurs *in vivo* in humans [28,29,74,75], but its role in the pathogenesis of VPA hepatotoxicity is unknown.

To identify a VPA metabolite signature predictive of increased risk of hepatotoxicity, we have applied a longitudinal phenotyping study design to characterize VPA metabolite patterns at steady state in a pediatric patient population without hepatotoxicity. Preliminary data from this study suggests that considerable variability in VPA metabolite patterns exists among VPA-treated patients [MANUSCRIPT IN PREPARATION]. However, novel relationships between metabolites reflecting bioactivation and detoxification pathways may serve as future biomarkers of susceptibility. These will provide novel phenotypes for further

investigation of pharmacogenetic determinants of susceptibility, warranting further investigation for predicting the risk of hepatotoxicity in this patient population.

Searching for appropriate outcome predictors of methotrexate in juvenile idiopathic arthritis

Methotrexate (MTX) is the most common second-line therapeutic agent used to treat juvenile idiopathic arthritis (JIA) worldwide. Interestingly, there is significant interpatient variability in the therapeutic response and toxicity observable with MTX in rheumatoid arthritis and JIA, with no clear predictors of efficacy and toxicity to the drug existing in either condition. Gastrointestinal toxicity is reported in up to 15% of JIA patients [76], and specifically liver toxicity in 4–17% [77]. Toxicity frequently results in dose adjustment, interruption and at times, termination of therapy.

Genetic variability resulting in increased or decreased function of key enzymes within the folate pathway (Figure 3) potentially plays a role in the biotransformation of MTX and therapeutic response to the drug. Although pharmacogenomic studies in adults with rheumatoid arthritis have been conducted over the last decade, results are variable and at times contradictory [78]. How genetic variation in the folate pathway alone, or by affecting intracellular concentrations of MTX or patterns of MTX polyglutamate concentrations, results in clinical efficacy or toxicity has not been adequately illustrated in JIA.

Considerable interpatient variability was observed in MTX polyglutamate concentrations (MTXglu_n) and patterns in approximately 100 JIA patients studied at our center. Concentrations of MTXglu_{1–7} were measured both individually and as a percentage of each patient's total MTX polyglutamation concentration. Among clinical variables investigated, route of administration and dose of medication per kg of bodyweight were significantly associated with variability in MTXglu_n subtypes. After correcting for dose of drug administered, higher concentrations of MTXglu₁₊₂ were observed in patients receiving oral doses of MTX, whereas higher concentrations of MTXglu_{3–5} were observed in patients receiving subcutaneous doses of MTX ($p < 0.0001$) (Figure 4). An explanation for these apparently route-specific patterns of MTXglu_n is not obvious at this point. However, saturability of oral absorption of MTX has been well reported with increasing doses of MTX [79]. If bypassing gastrointestinal absorption played the sole part in our findings, we would expect all glutamate moieties to be uniformly higher in the subcutaneous group, which was not observed. One possibility is that subcutaneous dosing may allow for higher and more consistent peak plasma concentrations after each dose, which could induce a more pronounced folate-depleted state in the cell relative to that observed following oral dosing [76]. In cultured cells, growth in folate-depleted medium results in upregulation of polyglutamation and increased cellular retention of polyglutamated folates [80]. As a folate analog, the cellular response to a MTX-mediated folate-depleted state could be to enhance polyglutamation in an attempt to retain as much folate as possible in the cell [76]. The intracellular stability of long chain MTXglu_{3–5} allows for preferential retention and therefore accumulation of the longer chain glutamates. When we evaluated the correlation between mean corpuscular volume (used as a potential surrogate of folate deficiency) and total MTX polyglutamation concentration stratified by route, an association with the subcutaneous route ($p = 0.04$), but no association in the oral group ($p = 0.44$), was observed [81]. Until the measurement of intracellular folate concentrations and polyglutamate patterns are completed, we will not have any further support for this hypothesis. If higher concentrations of long chain MTXglu_{3–5} are truly associated with improvement in disease, as some authors have suggested [82–85], these data may advocate for the use of subcutaneous dosing in more patients. However, prospective studies evaluating the effect of route of MTX administration on cellular folate status will be necessary.

Evaluation of the genetic contribution to observed variability in MTXglu_n concentrations and patterns of polyglutamation, and the association of MTXglu_n and genetic variation in folate pathway genes with outcomes in JIA, are currently being investigated. At this point, it appears that several SNP combinations in the folate pathway, including the *de novo* purine synthesis pathway as well as the mitochondrial folate pathway, may contribute to polyglutamate patterns and outcomes in JIA [86]. Further prospective analysis incorporating genotype, intra-cellular folate status and MTXglu patterns with clinical response, may be useful in better predicting how an individual in a current folate status may respond to perturbation with antifolate compounds such as MTX.

Conclusion

By 5 years of age, 95% of children have been prescribed a medication, with an average of 8.5 prescriptions and 5.5 different medications per child [85]. Despite the paucity of data investigating the safety and efficacy of drugs in children, the risks for ADRs are real and may pose significant risks for morbidity and mortality. Now, more than ever, in the era of genomics and personalized medicine, we can strive to better comprehend drug biotransformation and therapeutic response in the pediatric population. An essential first step is the recognition and characterization of the inherent differences between children and adults as manifested by differences in disease expression, drug biotransformation and utilization, and specific drug responses that may be unique to pediatric patients. In conjunction with the improved reporting of ADRs in children and, potentially, a comprehensive active surveillance system, we can systematically approach personalized therapeutics in children.

Future perspective

Serious ADRs occur in children, and yet represent an understudied area of investigation. The purpose of this article was to present a systematic approach to assess the relative contribution of ontogeny and genetic variability as predisposing factors to ADRs in children. However, future investigation of ADRs in children will require improved surveillance tools that can be used to capture unexpected drug outcomes in children. Multicenter active surveillance networks, such as the Canadian Pharmacogenomics Network for Drug Safety [87], need to be established. These networks are well-equipped to identify ADRs in children, and also to capture the clinical and genomic data that can then be used to investigate the etiology of these drug responses in children. The value of comprehensive, multicenter networks exemplified by the Canadian Pharmacogenomics Network for Drug Safety is best illustrated by the recent publication describing the association between cisplatin-induced hearing loss in children and genetic variation in the *TPMT* and *COMT* genes [88]. The passive surveillance systems currently in place are unlikely to provide a full understanding of the frequency and severity of ADRs in children, and will certainly not allow us to fully clinically characterize the reactions, nor obtain important genomic data, that ultimately may contribute to improved risk assessment.

During this era of genomic medicine, we have established methods capable of determining genotype and genetic variation in the patients that we treat. However, comprehending how genotype contributes to risk of disease or differences in drug response will be the challenge in the years ahead. For investigators, this requires a systematic approach for future research studies, including an assessment of the ontogeny of gene expression and drug metabolism when approaching these issues in the pediatric population. For clinicians, this requires an understanding of how to incorporate this new data into everyday practice when treating individual patients. Together, with the common goal of better understanding the variability in drug response in children, clinicians and researchers need first to recognize what makes

children different from adults, and then prioritize and plan future studies to specifically include the pediatric population at every stage of drug development.

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Figure 1. The number of prescriptions dispensed by US retail pharmacies and crude counts of US Adverse Event Reporting System reports for pediatric (<18 year) and adult (18 year and older) subjects, by year

AERS: Adverse Event Reporting System.

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Figure 2. Variability in CYP2D6 activity over the first year of life

(A) Population variability in the DM:DX urinary metabolite ratio. Considerable interindividual variability in phenotype is seen at both time points. (B) Genotype–phenotype relationships are concordant as early as 2 weeks postnatal, and genotype is a more important determinant of CYP2D6 activity.

DM: Dextromethorphan; DX: Dextrorphan.

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Figure 4. Comparison of MTXglu subgroups between per os and subcutaneous routes (A) Short-chain polyglutamates (MTXglu₁₊₂) were in higher concentrations in orally dosed patients and (B) long-chain polyglutamates (MTXglu₃₋₅) were in higher concentrations in patients dosed SC. Concentrations were corrected for dose in mg/kg. Box and whisker plots are superimposed on data from individual patients. Boxes include the median and interquartile range and whiskers indicate the 10th and 90th percentiles. MTXglu_n: MTX polyglutamate concentrations; PO: Per os; SC: Subcutaneous. Reproduced with permission from [81].