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Developmental Pharmacogenetics

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Abstract

Human development is a continuous and dynamic process but developmental changes are more important in certain periods, i.e. in neonates, young infants and near adolescence. The maturation process itself varies between individuals.
Immaturity in drug metabolism and disposition is associated with variability in pharmacokinetics and drug response.
Most cytochrome P450 activities are not detectable during fetal life, low at birth and increase after birth. CYP3A7 is the major fetal form, with a shift between CYP3A7 and CYP3A4 occurring after birth. Most conjugation reactions are immature at birth but increase in the first months after birth. The pattern of maturation of various metabolic activities is depending on the enzyme.
The physiological development and maturation of the child may interact with the expression of genotypic variation in a way that is different from the expression in adults. In children, interactions between pharmacogenetics and development have major impacts on the pharmacokinetics and response to standard dosage regimen and are central for cancer therapy, neonatal care and risks of adverse drug reactions.

Résumé

Le développement humain est un processus dynamique continu. Cependant, les modifications sont plus importantes à certaines périodes de la vie: chez le nouveau-né, le jeune enfant ou l'adolescent. De plus, la maturation est variable en fonction des enfants.
L'immaturité du métabolisme des médicaments est responsable de grandes variabilités dans la pharmacocinétique des médicaments et la réponse aux traitements.
La majorité des activités cytochromes P450 (CYP) présents chez l'adulte ne sont pas détectables pendant la vie foetale et apparaissent après la naissance. A l'inverse CYP3A7 est une forme strictement fœtale qui disparaît après la naissance et est remplacée par CYP3A4. Les réactions de conjugaison sont, elles aussi, immatures à la naissance mais augmentent dans les premiers mois de vie. Le profil de maturation des différentes voies métaboliques varie en fonction des enzymes.
Chez l'enfant, les interactions entre pharmacogénétique et développement peuvent avoir un impact important sur la pharmacocinétique et la réponse thérapeutique. Ceci explique que pour la plupart des médicaments la posologie soit adaptée à l'âge de l'enfant. Ceci est particulièrement important pour l'administration des médicaments chez le nouveau-né ainsi que dans le risquè d'effets indésirables.

Intra- and inter-individual variation in drug metabolism and disposition are central to differences in therapeutic response to a standard dosage regimen. In adults, variability is the result of complex interactions between genetic determinants and the environment. In children, the variability of drug disposition is more complex as the expression of genetic and environmental factors is modified by the impact of the physiological development / maturation[1)–3)]. Indeed, human development is a continuous and dynamic process. Developmental changes are rapid and important in certain periods, i.e in neonates, young infants and near adolescence. In addition, the maturation process itself varies between individuals and may be influenced by exogenous factors such as maternal factors during pregnancy, nursing, disease and drug administration.

Pharmacogenetics has been defined as «… a monogenic trait caused by the presence in the same population of more than one allele at the same gene locus and more than one phenotype regarding drug interaction with the organism. The genotype of lowest frequency usually represents more than 1% individuals». Such polymorphisms generally remain undetected in the absence of drug intake. They may be characterized by the genotype, referring to an individual's genetic constitution and/or the phenotype referring to the individual expression of genotype.

1. The major pharmacogenetic polymorphisms

1.1. Polymorphisms of drug metabolizing enzymes

Most drugs are lipophilic and metabolized into hydrophilic compounds which are eliminated through the kidneys. Biotransformation reactions are usually classified into phase 1 and phase 2 reactions, associated in various combinations depending on the drug concerned. Many enzymes operating in the metabolism of drugs are subject to genetic polymorphisms. For most of them, the molecular basis responsible for the allelic variants with compromised function is known, so that it is possible to determine individual genotypes. By measuring the *in vivo* metabolism of drug substrates specific to the polymorphic enzyme activity, it is possible to identify different phenotypes, e.g. fast and slow metabolizers.

Phase 1 reactions.

CYP2D6 polymorphism was discovered following therapeutic accidents occurring with the use of debrisoquine for hypertensive adults[4)]. More than 50 mutations and 70 different «poor metaboliser» alleles have been described, with large ethnic differences in frequency: 7 to 10% of Caucasians and 1 to 2% of Asians are poor metabolisers, while 2 to 3 % are ultrarapid metabolisers, due to gene duplications or gene multiplications. This polymorphism is inherited through an autosomal recessive gene. Homozygous individuals are characterized by negligible or no metabolism of a variety of drugs. Several β-adrenoceptor-blocking agents, antidepressants, antipsychotics and antiarrythmic drugs belong to this group. Numerous adverse drug reactions were associated with CYP2D6 poor metaboliser phenotype such as perhexiline hepatotoxicity. CYP2D6 activity was not detectable in the fœtus, increased rapidly during the postnatal period, independently of gestational age, but remained low during the first month of life (about 20%

Enzyme	Substrates
CYP1A2	Caffeine, Carbamazepine, Theophylline
CYP2A6	Acetaminophen, Nicotine
CYP2B6	Cyclophosfamide, Ifosfamide
CYP2C8	Diazepam, Diclofenac, *Tricyclic antidepressants*
CYP2C9*	Losartan NSAIDs (Celecoxib, Ibuprofen, Indomethacin, Naproxen) Phenytoin
CYP2C19*	Diazepam, Citalopram Lanzoprazole Omeprazole S-mephenytoin
CYP2D6*	*Antiarrhythmic drugs,* Codeine Dextromethorphan Ethylmorphine Odansetron Perphenazine *Serotonin reuptake inhibitors* S-Mianserin Tolterodine *Tricyclic antidepressants,* Zuclopenthixol
CYP2E1	Acetaminophen Caffeine Ethanol
CYP3A4	Cisapride Cortisol Cyclosporine, Diazepam Erythromycin Ethosuximide Midazolam Nifedipine Ritonavir Tacrolimus
CYP3A7	Dehydroepiandrosterone, Ethinylestradiol
NAT2	Caffeine Dapsone Isoniazid Sulfametoxazole
TPMT	6-Mercaptopurine 6-Thioguanine
UDPGTs	Acetaminophen Bilirubin Chloramphenicol Diclofenac Ibuprofen Ketoprofen Mycophenolic acid Morphine Naproxen

Table 1: **Major phase 1 and phase 2 enzymes with some substrates used in paediatric patients.**
(The list of substrates is not exhaustive)

of adult's levels. Phenotypic studies conducted in small populations of children, have shown that the adult phenotypic distribution pattern was attained at 3 years of age. In children, the number of clinically used drugs dependent on CYP2D6 is limited. However, codeine and tramadol are metabolized to active compounds morphine and O-desmethyl tramadol, respectively and the analgesic effectiveness of these drugs is low in patients of the slow metabolizer (developmental or pharmacogenetic) phenotype.

Additional polymorphisms were described: CYP2C19 deficient patients 5% of Caucasians and more than 20 % of Orientals are characterized by negligible or no metabolism of a variety of drugs, such as S-mephenytoin, citalopram, diazepam, omeprazol, lanzoprazol, etc. Although the polymorphism may be of clinical importance in certain situations in pediatric pharmacotherapy, very little is known about the clinical implications in children. CYP2C9 is polymorphic and responsible for the oxidative metabolism of widely used drugs such as anticoagulants, non-steroidal antiinflammatory drugs, phenytoin, etc. with clinical implications predominantly in the treatment of cardiovascular disease and epilepsy.

The CYP3A subfamily[5] is the predominant cytochrome P450 subfamily, and comprises three major isoforms: CYP3A4, CYP3A5, CYP3A7, very closely related as they share at least 85 % amino acid sequence. The CYP3A4 enzyme is the predominant form in adults important for the metabolism of a large number of commonly used drugs within the groups of antiepileptics, immunosuppressants, cytostatics, antibiotics etc. CYP3A5 is predominantly an extrahepatic form, under genetic control. CYP3A7 is the major CYP isoform detected in embryonic, fetal and newborn liver with a shift between the CYP3A7 and CYP3A4 occurring after birth. CYP3A7 is the enzyme responsible for the drug metabolising activity initially described in the human fetal liver.

Phase 2 reactions.
N-acetyltransferases (NAT1 and NAT2) are polymorphically expressed in human populations. NAT2 activity is transmitted as an autosomal recessive trait. In Caucasian populations, 50–70 % of individuals are slow acetylators, whereas the percentage is only 5% in Eskimo populations, and more than 90% in Egyptians [During fœtal life, low NAT2 activity becomes perceivable already in mid trimester After birth, all children are slow metabolizers up to about the age of 2 months, after which the proportion of fast metabolizer phenotype is successively increased. At about 3 years of age, phenotypic distribution is similar to that found in adults.

Thiopurine methyltransferase (TPMT)[6)-7)]. TPMT is a cytosolic enzyme under pharmacogenetic control, metabolizing thiopurine drugs, 6-mercaptopurine, azathioprine and thioguanine, widely used in pediatric patients. 6-MP is prescribed during maintenance therapy for childhood acute lymphoblastic leukemia, azathioprine is used in the management of inflammatory bowel disease. The genetic polymorphism of TPMT activity is of major clinical importance and TPMT genotype should be determined in all patients prior to treatment with thiopurines as patients deficient in TPMT activity are at very high risk of developing myelosuppression when treated with standard doses of 6-mercaptopurine. In addition, in children with acute lymphoblastic leukemia having a detectable TPMT activity, 6-TGN concentrations were negatively correlated with TPMT activity and were predictive of better outcome.

Additional polymorphisms were described for 5'-Uridine-diphosphate -glucuronosyl transferases (UDPGTs), gluthathione S-tranferases (GST)… Indeed, the glucuronidation of acetaminophen (a substrate of UDPGT1A6 and 1A9) is absent in human fetal liver and replaced by sulphate conjugaison. The glucuronidation of morphine (a substrate of UDPGT2B7), is negligeable in human fetal liver, immature at birth and rapidly increasing thereafter. In infants, morphine clearance increases with age reaching 80% of adult values by 6 months[8].

Therapeutic consequences. The therapeutic consequences depend on the importance of the deficient enzyme for the overall metabolism of the drug and/or the influence on the metabolism pattern. In slow metabolizers, the accumulation of the drug in a poor metabolizer may be associated with excessive therapeutic or toxic effects. However, metabolic deviation leading to metabolites not formed in extensive metabolizers is also possible. In fast metabolizers, excessive metabolism is a result causing risk of undertreatment and insufficient clinical efficacy. Disequilibrium between phase I and phase II reactions may account for a high risk of adverse consequences including induction of cancer, or immunotoxicity.

1.2. Polymorphism of ABC transporters

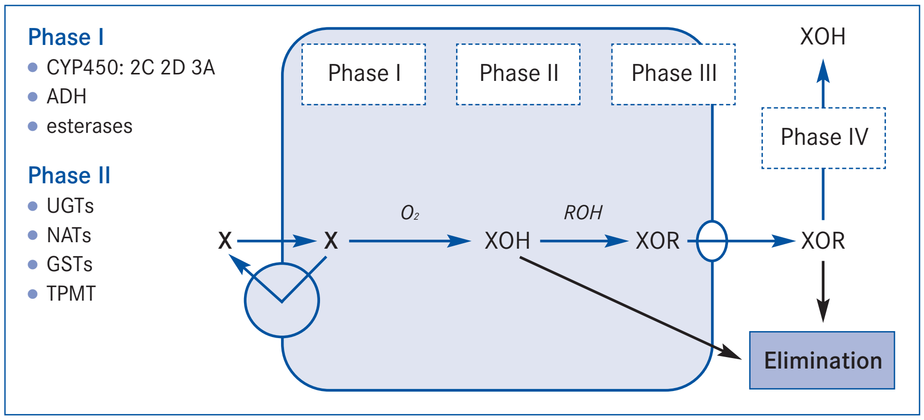
Data on the development aspects of transporters or drug targets are still limited, although this may have a major impact on the pharmacological response in children.

The ATP binding cassette (ABC) family comprises at least eight multi-drug resistance-associated proteins with a central role in the absorption, distribution and elimination of many drugs. P-glycoprotein (P-gp) is a transmembrane ATP-dependent efflux pump [9]. It is encoded by the *MDR1* (ABCB1) gene. It is located in many tissues: in the gastrointestinal tract its location in the brush border of the apical surface of mature enterocytes in the small intestine contributes to the chemical protection of the organism. Its expression is genetically controlled and MDR1 function is correlated with a mutation in exon 26 (C3435T) of the MDR1 gene. Substrates of P-glycoprotein include glucocorticoids, anticancer drugs, immunossuppressants, HIV1 protease inhibitors and many other drugs. As an example, digoxin pharmacokinetics is affected by the C3435T polymorphism in exon 26.

1.3. Polymorphism of target proteins

Target proteins can be a receptor, an enzyme, or another type of protein. Genetic polymorphisms affecting these drug targets can contribute to the pathogenesis of the disease and modify the pharmacological response in children[10].

Polymorphisms of the β2-adrenergic receptor (ADRB2) have been implicated in the varying response to β-agonists in patients with asthma. Polymorphisms of the promoter region (variable number of tandem repeats) affecting *ALOX5 gene* expression has been associated with the response to inhibitors of ALOX5. A number of additional polymorphisms of potential clinical importance were described in adults: polymorphisms of the angiotensin converting enzyme (ACE) affecting the sensitivity to ACE inhibitors, of the 5-hydroxytryptamine receptor affecting the response to neuroleptics, and of dopamine D3 receptor associated with drug induced tardive dyskinesia. However, studies should be conducted in this field as there is almost no data on the ontogeny of the different drug targets.



Pharmacogenetics of drug metabolising enzymes
CYP450: cytochrome P450; **ADH:** alcohol dehydrogenase; **UGTs:** uridine 5'-triphosphate glucuronosyltransferase; **NAT:** N-acetyl transferase; **GST:** glutathion S-transferase; **TPMT:** thiopurine methyltransferase

2. Methods in pharmacogenetics

At the gene level, pharmacogenetic alterations leading to allelic variants may include:
1) partial or total gene deletion, insertion or duplication,
2) SNPs (Single Nucleotide Polymorphisms) affecting the coding region, the perigenic region, or the non-coding region. The larger the gene, the larger the number of expected SNPs. On average about four SNPs of functional importance are expected per gene. Such SNPs are generally non-synonymous, i.e located in the coding region and associated with an amino acid change.

The majority of the SNPs will not have any clinical importance, depending primarily on the role of the polymorphic trait and the therapeutic index of the drug. Indeed, the functional significance of all the genetic variations identified through rapid-throughput sequencing will require demonstration of a functional correlate. Sometimes, powerful statistical approaches are required for this purpose.

3. Implications of developmental pharmacogenetics in therapeutics

3.1. Impact on pharmacokinetics

Delayed maturation and pharmacogenetic polymorphisms of drug metabolizing enzymes have an important impact on the pharmacokinetics of many drugs, resulting in a lower clearance and prolonged elimination half-life. Examples include drugs highly metabolized such as carbamazepine, morphine, phenytoin, theophyllin, or drugs primarily cleared by renal elimination such as gentamicin or digoxin. As the age related changes in pharmacokinetics are non monotonic, age-appropriate doses should be given in neonates, infants and children in order to optimize therapy.
Paediatric pharmacokinetic models, are now developed, based on genetic, physiological, demographic and clinical attributes of the patient population, in order to predict drug disposition in paediatric patients and to estimate average dose requirements according to age.

3.2. Pharmacogenetics and adverse drug reactions

The possible implications of pharmacogenetics for the risk of ADRs was underlined by Philips *et al*[11] who reported recently that 59% of drugs in the ADR studies were metabolized by at least one polymorphic enzyme in comparison to 22% of randomly selected drugs. The pharmacogenetic mechanisms may involve accumulation of the parent drug in slow metabolisers, formation of toxic metabolites with disequilibrium between the bioactivation and detoxification processes, immune-mediated ADR, most often associated with the HLA (human leukocyte antigen).
Adverse drug reactions are a significant cause of morbidity and mortality in children[12]. This is the case in neonates having immature glucuronidation, who developed a grey baby syndrome from treatment with chloramphenicol in the 1960s. The example of cisapride is more recent: the CYP3A4 meta-

bolism of cisapride is immature in neonates resulting in drug accumulation that might explain QTc prolongation[13]. In older infants, aged two to six years, some metabolic activities appear to exceed the corresponding adult activities. This may result in an inbalance between bio activation and detoxification reactions, leading to high concentrations of toxic reactive metabolites.

4. Conclusions

The relationship between phenotype and genotype may be different from what it is seen in adults, due to the physiological development and maturation of the child. As a result, variability in pharmacokinetics and response to standard dosage regimen is greater in children than in adults. Therefore, pharmacological studies as a function of age are very important for the definition of dosage regimens suitable for children and for limiting the risk of side effects and toxicity.

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