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**Title:** **Pediatric development of glucuronidation: the ontogeny of hepatic UGT1A4.**

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**Running Title:** **Ontogeny of UGT 1A4**

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**Non-standard Abbreviations:**
λ = wavelength, 4MU: 4-methylumbelliferone, 4MUG: 4-methyl umbelliferone glucuronide, ANOVA: analysis of variance, Cl: clearance, $Cl_{int}$: intrinsic clearance, CV: coefficient of variation, CYP450: cytochromes P450, Fu: fraction unbound, FU: fluorescence units, Q = [blood] flow, TFP: Trifluoperazine, UDPGA: Uridine diphosphate glucuronic acid, UGT: Uridine diphosphate glucuronosyl transferase, Wi: individual weight, $W_{std}$: standard average weight

**Abstract**

This paper reports on the development of Uridine diphosphate glucuronosyltransferase (UGT) enzyme activity in pediatric livers. The substrates 4-methylumbelliferone (4MU) and trifluoperazine (TFP) were used as probes for general glucuronidation and specific UGT1A4 activity respectively. The activity of hepatic β-glucuronidase enzymes was also determined so as to investigate the balance between glucuronide clearance and systemic re-circulation. UGT activity towards 4MU reached maximum levels by 20 months of age, while the activity of β-glucuronidase was highest in the neonatal liver and decreased to steady-state adult levels by 4 months. The average Vmax and Km for UGT1A4 in pediatric samples were 151.9 ± 63.5 pmol/min/mg protein and 14.4 ± 9.6 μM respectively. Average Vmax was understandably low due to developmental dynamics, but Km was similar to those reported elsewhere. When a constant rate of enzyme development is assumed, maximum activity of UGT1A4 occurs at 1.4 years of age. When the intrinsic hepatic clearance of TFP was scaled with an allometric model, hepatic clearance of TFP by UGT1A4 did not reach maximum levels until 18.9 years of age and scaled results underestimated reported *in vivo* clearances in adult males. No significant differences in UGT activities or hepatic clearances were observed with gender or ethnicity. The developmental dynamics of most drug metabolizing enzymes are unknown and this paper contains the first description of the development of a single UGT isoform in childhood. Ultimately work such as this is important for predicting drug responses and for developing and evaluating new medications in children.

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**Introduction:**

One of the main causes of adverse drug reactions in children is believed to be a lack of substantial drug metabolism due to immaturity of drug metabolizing enzymes (Benedetti et al., 2005). Although the vast majority of drug metabolism is performed by enzymes in the human liver, developmental profiles for most drug metabolizing systems in the liver (and other organs) are not defined.

Within the many families of metabolizing enzymes, the UDP-glucuronosyltransferase (UGT) super family is critical for the metabolic clearance of most biological substances including drugs, dietary, environmental and endogenous compounds (Radominska-Pandya et al., 1999; Williams et al., 2004). In the liver, the UGT super family is divided into the UGT1A and UGT2B subfamilies that contain nine and seven isoforms respectively (Radominska-Pandya et al., 1999; Levesque et al., 2001). They are high capacity, low affinity enzymes that demonstrate considerable overlapping affinity for multiple substrates; however, some substrate specificities do occur. Of the known UGT isoforms, twelve are expressed in the adult human liver and three have no known physiological or xenobiotic substrates (Radominska-Pandya et al., 1999; Levesque et al., 2001). Thus, biologically relevant drug and hormone metabolism is mediated by the remaining nine isoforms.

Within the UGT1A subfamily, UGT1A4 is one of the lesser-known isoforms. It was first cloned by Ritter *et al.* in 1991, shows high sequence homology with UGT1A3 and is expressed in the gastrointestinal system (Ritter et al., 1991; Radominska-Pandya et al., 1999). The isoform is active towards primary, secondary, and tertiary amines, aromatic amines, androgens, progestins and plant steroids (Mori et al., 2005). In terms of clinically used drugs, UGT1A4 metabolizes the antidepressants amitryptiline and imipramine, the antipsychotic clozapine, quinine antimalarials and tamoxifen

(Radominska-Pandya et al., 1999; Ogura et al., 2006; Uchaipichat et al., 2006a).

Despite playing a role in placental dynamics during gestation, UGTs are largely absent from the fetal liver (Coughtrie et al., 1988; Collier et al., 2002a; Collier et al., 2002b). Their subsequently low activity in the neonate and the child's inability to excrete bilirubin is the major cause of jaundice in newborns (Onishi et al., 1979; Kawade and Onishi, 1981; Strassburg et al., 2002). Despite this serious effect, the developmental dynamics of UGTs in neonates have not been defined. Kawade and Onishi showed that premature and term neonates develop UGT activity at the same rate from the neonatal period up to 6 months of age, implying a level of environmental as well as genetic control (Onishi et al., 1979; Kawade and Onishi, 1981). More recently, Strassburg *et. al.* have demonstrated that while mRNAs for all hepatic UGTs are expressed within 6 months, even at the age of two, UGT activities are up to 40-fold lower than adults' (Strassburg et al., 2002). After the age of 2, further development of UGT enzymes in children has not been previously reported.

Although it is well recognized that children have altered drug disposition, a comprehensive picture of their pharmacokinetics is lacking (Benedetti et al., 2005). Because children are commonly switched from pediatric to adult dosing schedules at or around 12-14 years old without noticeable adverse effect, it has been assumed that drug metabolism likely reaches full adult activity sometime during early childhood. However, recent reports, such as those highlighting adverse reactions of teenagers and adolescents to anti-depressant drugs, seem to contradict this assumption (Duff, 2003).

Thus, if the nominal age of adulthood is at or around 20 years, we supposed that UGT enzymes, being a major path of drug metabolism, must develop before this time. To test our hypothesis, we determined

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total UGT activity in subcellular fractions (microsomes) from 27 normal pediatric liver samples (0-20 years of age) and compared these to UGT activity in pooled adult liver fractions. We used one compound, 4-methylumbelliferone (4MU); that is metabolized by all the hepatic UGT isoforms except UGT1A4 (Uchaipichat et al., 2004) and another, the antipsychotic drug trifluoperazine (TFP), that is a specific substrate for UGT1A4 alone (Uchaipichat et al., 2006a). We also determined the activity of hepatic β-glucuronidase enzymes to assess the relative balance between conjugation/excretion and hydrolysis/enterohepatic re-circulation of drugs in children and neonates.



**Materials and Methods**

4-methylumbelliferone sodium salt (4MU), 4-methylumbelliferone glucuronide (4MUG), alamethicin (from *trichoderma viridae*), β-glucuronidase (from *E. coli*), $MgCl_2$, D-saccharic acid 1,4-lactone (saccharolactone), trifluoperazine hydrochloride (10-[3-(4-Methylpiperazin-1-yl)propyl]-2-(trifluoromethyl)-10H-phenothiazine dihydrochloride, TFP), Tris-HCl and uridine diphosphoglucuronic acid (UDPGA) were purchased from Sigma Chemical Company (St Louis, MO). Hecogenin was from Sciencelab.com Inc. (Houston, TX).

**Pediatric and Adult Liver Samples and Demographics**

Pediatric and adult liver microsomes were purchased from Xenotech LLC (Lenexa, KS) and were derived from post-mortem donors with healthy livers. Pediatric microsomes (27) were derived from single livers and our study included Asian (4%), Hispanic (11%), African American (19%) and Caucasian (67%) descent, with 10 female and 17 male donors. Pooled adult liver microsomes were derived from 50 donors and contained Asian (4%), Hispanic (6%), African American (6%) and Caucasian (84%) ethnicities with equal numbers of men and women (25 each). The average age was 50

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with a range of 17-78.

**Total UGT activity with 4MU**

The assay for UGT activity with 4MU was carried out as previously described (Collier et al., 2000). Three reactions were performed per sample and samples were assessed on 3 separate days. Initial reaction velocities were calculated by least squares linear regression with substrate depletion as a marker of product formation. Fluorescence units (FU) were converted to concentrations by comparison to a standard curve of 4MU. The average $r^2$ and slopes of the standard curves were 0.98 ± 0.01 and 550.5 ± 12.9 FU/μM respectively (n = 6). The intra- and inter assay CVs for the slopes of the standard curves were 3 and 8 % respectively and for pooled adult human liver microsomes (positive control) 14.8 and 16.1% respectively.

**De-conjugation (β-glucuronidase) activity**

The assay for β-glucuronidase activity was performed with 4-methylumbelliferone glucuronide essentially according to the method of Trubetskoy and Shaw (Trubetskoy and Shaw, 1999). Briefly, microsomes (0.1 mg) and Tris-HCl containing 5 mM $MgCl_2$ were placed in a microplate and pre-warmed to 37 °C for 2 minutes. The reaction was started by addition of 4-methylumbelliferone glucuronide (4MUG) to a final concentration of 100 μM. Reactions were monitored continuously at 37 °C for 20 minutes at λ = 355 nm excitation and λ = 460 nm emission (Gemini XS, Molecular Devices, Sunnyvale CA). Three reactions were performed per sample on 3 separate days. Initial reaction velocities were calculated by least squares linear regression using the appearance of fluorescence. FU were converted to concentrations by comparison to a standard curve of 4MU. Each day a positive control (0.2 mg β-glucuronidase from *E. coli*), negative control (containing 5 mM sacchrolactone) and

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reference comparison (pooled adult human liver microsomes of n = 50 donors) were performed. Negative controls averaged 0.07 ± 0.3 pmol/min/mg activity – essentially zero. Positive controls averaged 0.5 ± 0.24 pmol/min/mg protein and pooled adult human liver microsomes 1.7 ± 0.9 pmol/min/mg protein. The accuracy and precision of the standard curves was the same as reported above for 4MU activity.

**Measurement of UGT1A4 activity**

UGT 1A4 activity was measured using the assay conditions described by Uchaipichat et. al. (2006b) with detection of glucuronidation performed by monitoring the fall in fluorescence in solution (substrate depeletion) at wavelengths described by Rele et. al. (2004). Briefly, reactions were carried out in 0.1 ml containing 0.2 mg/ml microsomal protein, 2 mM UDPGA, 10 mM $MgCl_2$, 0.025 mg/ml alamethicin and 0-200 uM TFP in 50 mm Tris (pH 7.5). Reactions were incubated at 37 °C for 20 minutes in the dark and detection of activity was performed fluorimetrically in a Gemini XS microplate spectrophotometer (Molecular Devices, Sunnyvale, CA) at $\lambda$ = 310 nm excitation and $\lambda$ = 475 nm emission (Rele et al., 2004). Each sample was assessed in triplicate. Substrate depletion was used to measure UGT1A4 activity as fluorescence became masked by glucuronidation. Quantitation was achieved by comparison to standard curves that were prepared fresh each day in shielded tubes. The average $r^2$ for standard curves was 0.985 ± 0.010 with intra- and inter-day CV of 3.5 and 3.6% respectively (n = 6, in triplicate).

To confirm that the loss of fluorescence was specifically due to glucuronidation of TFP, we performed triplicate incubations as described above except that incubations proceeded for 1 hour at 37 °C. One set of incubations contained microsomes and 100 µM TFP with no UDPGA and the second contained microsomes and 100 µM TFP with UDPGA. A third incubation proceeded for 1 hr after which we added

1000 Units of β-glucuronidase enzyme and continued the incubation for 30 minutes. Subsequently the emission spectra (excitation λ = 310 nm) for each set of incubations were scanned from 350 – 500 nm (Gemini XS, Molecular devices, Sunnyvale CA).

The specificity of glucuronidation was confirmed with pooled adult human liver microsomes using the incubation conditions described above with 100 μM TFP substrate and the specific UGT1A4 inhibitor hecogenin (200 μM in ethanol, (Uchaipichat et al., 2006a). Total solvent amounts did not exceed 1% of the individual incubations.

**Pharmacokinetics, Scaling and Statistical Analyses**

Data for 4MU and 4MUG were performed at a single concentration of substrate (100 μM) and velocities were plotted directly. Experimental data for TFP were derived from 11 concentrations of TFP (and a blank) per sample and were performed at least twice, in triplicate. They were subsequently fit to the Michaelis-Menten equation using Prism 3.0 (GraphPad, San Diego, USA). There has been some discussion of the correct interpretation of UGT1A4 kinetics based on whether the enzyme exhibits substrate inhibition by TFP at higher concentrations (Uchaipichat et al., 2006a). We fit our data to both Michaelis-Menten kinetics and the equation for substrate kinetic inhibition defined by Houston and Kenworthy and compared these with an F test for suitability (Houston and Kenworthy, 2000). Of the 65 fits performed, only 20 (31%) were better fit to the substrate inhibition equation. Subsequently all TFP data were fit to the Michaelis-Menten models

Because concentration-dependent non specific binding of TFP is known to occur under the conditions used, kinetic data were analyzed with respect to the unbound fraction of drug only ($F_u$) using published

data for protein binding (Uchaipichat et al., 2006b).

To evaluate hepatic drug clearance, we scaled our enzyme kinetic results using both the well-stirred and parallel tube models (Equations 1 and 2).

$$Cl_{hepatic} = \frac{Q_{hepatic} \bullet F_u \bullet Cl_{\text{int}}}{Q_{hepatic} + F_u \bullet Cl_{\text{int}}}$$

….. Equation 1 Well Stirred Model

$$Cl_{hepatic} = Q_{hepatic}\left(1 - e^{\frac{-Cl_{\text{int}} \bullet F_u}{Q_{hepatic}}}\right)$$

….. Equation 2 Parallel Tube Model

Here, $Q_{hepatic}$ is hepatic blood flow, $Cl_{int}$ is intrinsic clearance and $F_u$ is the unbound fraction of drug. Intrinsic clearances were generated by using experimental intrinsic clearances (Vmax/Km), assuming liver size of 1500 g, a hepatic flow rate of 1.5 L/min, protein content of 45 mg/g and plasma unbound fraction for TFP of 0.05.(Midha et al., 1983; Verbeeck et al., 1983; Houston, 1994). Subsequently an allometric model was needed to scale clearance (Equation 3) to children's weight:

$$Cl_{pediatric} = Cl_{hepatic\ calculated} \bullet \left(\frac{W_i}{W_{std}}\right)^{\frac{3}{4}}$$

….. Equation 3 allometric model

Where $W_i$ id the weight of the individual and $W_{std}$ was taken from the adult (20 yrs) weights of men and women from the same charts. Age and gender were known for each donor; thus, average weights for age and gender were taken from the National Center for Health Statistics growth charts (Statistics., 2000) and substituted for the variable $W_i$ to scale hepatic clearance for individual donors.

To define adult levels of enzyme activity in the population, one-phase exponential association (4MU,

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TFP, Equation 4) or one-phase exponential decay (4MUG, Equation 5) non-linear least squares regression equations were used. These models assume a constant rate of change (K) in the development of the enzyme/s that fits a curve ending in a plateau rate or action. A table of XY co-ordinates defining the curve was generated and the "age of adult activity" was defined as the youngest age at which plateau levels were reached.

$$Y = Y\ \max \bullet (1 - e^{-KX}) \qquad \textbf{… Equation 4}$$

$$Y = Span \bullet e^{-KX} + plateau \qquad \textbf{… Equation 5}$$

Goodness of fit was assessed with F tests, $R^2$ values, absolute sums of squares and sy.x.

## Results

### Balance between Glucuronidation and Hydrolysis in the Pediatric Liver

The balance of metabolism in neonatal life shifts from metabolite cleavage and systemic recirculation in the neonate to detoxification in the child. Assuming a constant rate of increase in UGT activity, the combined activity of UGT isoforms towards 4MU increased to maximum adult levels of 1.53 nmol/min/mg protein by 20 months of age (Figure 1A). When compared to the activity measured in pooled adult human liver microsomes (2.35 ± 0.38 nmol/min/mg protein) the maximum adult UGT activity from the model showed reasonable agreement with measured UGT activity. No significant differences between genders and ethnicities were reported (P = 0.3655, t-test and P = 0.5678 ANOVA respectively).

The activity of β-glucuronidase was highest in the neonatal liver and decreased to steady-state adult levels of 1.61 pmol/min/mg protein by 4 months of age (Figure 1B). The measured activity in pooled adult liver microsomes of 1.7 ± 0.9 pmol/min/mg protein showed excellent agreement with the adult activities derived by the model. No significant differences between genders or ethnicities were observed for either enzyme (P = 0.2258 t-test and P = 0.3715, ANOVA respectivey).

**Pediatric Development of UGT1A4**

After adjustment for non-specific binding to microsomal protein, the derived Vmax and Km in pediatric samples averaged 151.9 ± 63.5 pmol/min/mg protein (range 61.6 – 276.8) and 14.4 ± 9.6 μM (range 5.4 – 42.3 μM) respectively. This compares with the kinetic parameters measured from pooled human adult liver microsomes of 348.2 ± 14.93 pmol/min/mg protein and 15.86 ± 1.98 μM for Vmax and Km respectively. A typical kinetic plot for one pediatric liver sample is presented in Figure 2A.

Hecogenin suppressed UGT1A4 activity in pooled adult human liver microsomes. The activity of TFP alone in pooled human liver microsomes (50 donors) was normalized to 100% and when hecogenin was included in incubations, activity was 31.6 ± 9.0 % of control levels (n = 3, in triplicate Figure 2B).

Assuming a constant rate of development, UGT1A4 activity reached maximum (adult) levels of 113.1 ± 10.17 pmol/min/mg protein at 1.4 years of age (Figure 3A) This modeled maximum activity was somewhat lower than the measured Vmax of UGT1A4 in pooled adult microsomes of 230.5 ± 15.9 pmol/min/mg protein. TFP glucuronidation did not differ significantly with gender or ethnicity (P = 0.7758, t-test and P = 0.2258, ANOVA respectively).

The well-stirred and Parallel Tube models returned the same maximum clearances and times to adult activities. When data were scaled to pediatric hepatic clearance using the allometric model, apparent adult clearance was reached at 18.9 years with a modeled maximum hepatic clearance of 0.357 ± 0.037 L/hr (range 0.05 – 1.2 L/hr, Figure 3B). This shows moderate correlation with the adult clearance experimentally gained from liver microsomes of 0.80 L/hr .

**Discussion**

The balance of glucuronide metabolism shifts very early in the neonate from cleavage and re-circulation to conjugation and clearance. The activity of β-glucuronidase is initially high, but decreases from birth to reach apparent adult levels by 4 months of age while general UGT activity is initially low and rises to adult levels at or around 20 months of age. These data parallel early work performed in animals where the livers of fetal, neonatal and juvenile rabbits and guinea pigs showed the same profile (Lucier et al., 1977).

Our report that total UGT activity may develop by 20 months of age (1.7 years) agrees with previously published data for drugs and compounds metabolized by multiple UGT isoforms (Strassburg et. al., 2002). The early development of apparent “maximum” levels of activity may mask the contribution of iso-enzymes that have lower rates of activity and/or develop later. This is because low rates of metabolism performed by one isoform (such as in the picomolar range) can be drowned out by the development of high-capacity isoforms that work, for example; in the micromolar range. For the data presented, this is almost certainly true because although the substrate 4MU is considered non-specific for UGT activity, it is metabolized primarily by the UGT1A family (Burchell et al., 1995). Some members of the UGT2B family including UGT2B4 (Jin et al., 1993), UGT2B15 (Green et al., 1994) and

UGT2B7 (Ritter et al., 1990; Jin et al., 1993) have also been reported to metabolise 4MU *in vitro* although at rates up to 10-fold lower than the UGT1A isoforms.

Despite this, the age at which enzymes reach full adult activities may not be universally indicative of *in vivo* drug clearance. For example, using allometric scaling intrinsic hepatic clearance of TFP did not appear to reach adult levels until 18.9 years of age, despite UGT being maximally active well before this. In addition, the range of intrinsic clearances calculated was far lower than clinical pharmacokinetic studies with TFP that show clearances of around 500 L/hr (Midha et al., 1988). There is still much discussion over the application and accuracy of the three quarter allometric model, particularly at the individual organ level (Wang et al., 2001). When added to the fact that the parameter for liver blood flow (Q) certainly differs between neonates, children and adults, some uncertainty exists in our model. The underestimation of drug clearance using scaled *in vitro* UGT data is also an acknowledged problem with this family of enzymes – in contrast to that of the cytochromes P450 (Lin and Wong, 2002).

Our derived Vmax and Km values for UGT 1A4 towards Trifluoperazine are in good agreement with recently published values which is interesting due to the difference in models (Michaelis-Menten *vs.* substrate inhibition) used (Uchaipichat et al., 2006a). Inter-laboratory variation, the greater number of human livers assessed by us (27 individual plus a pool of 50 in the current study *versus* 4 individual livers in the previous study) and differences in sensitivity between our fluorometric assay and HPLC detection employed by Uchaipichat *et. al.* (2006) may also be involved.

Aside from scaling issues, it is not unreasonable for modeled intrinsic clearances to reach apparent adult maxima after full enzyme activity. This may be related to the relative contribution of different metabolic

pathways for TFP. As the contribution of UGT1A4 to total metabolism of TFP is unknown, it is likely that other enzymes metabolize TFP to a greater extent than UGT1A4. Phenothiazine drugs are largely metabolized by CYP2D6 (Llerena et al., 2000) with subsequent phase II metabolism. The major phase II metabolites commonly reported are sulfate-conjugates (Hartigan-Go et al., 1996) but glucuronide conjugates have also been reported as the major urinary metabolite of phenothiazine drugs (Pieniaszek et al., 1999). The precise metabolic profile for Trifluoperazine is unknown but the contribution of UGT1A4 may not be the deciding factor in total hepatic clearance. Redundancy in metabolic pathways is extremely useful in humans for avoiding toxic consequences - where one pathway may be blocked another simply takes over. However, this also means that study of a single path may not give an accurate picture of whole-body metabolism.

A functional single nucleotide polymorphism in the UGT1A4 isoform recently identified with incidences as high as 9% (Ehmer et al., 2004; Mori et al., 2005). This polymorphism has functional effects towards the anti-cancer drug tamoxifen (Sun et al., 2006) and the atypical anti-psychotic clozapine (Mori et al., 2005). Both of these studies reported apparently higher intrinsic clearances (Vmax/Km ratios) for the polymorphism due to lower Km values being reported. Although the presence of polymorphisms in UGT1A4 have not been assessed in this study, using the statistical incidence of the polymorphism we would expect that no more that 3 of our samples would contain the polymorphism (corresponding to the highest reported 9% incidence in the German population (Ehmer et al., 2004)).

One of the relative strengths of this study is that our microsomes derive from normal pediatric livers. The only other study to present data on pediatric UGT activity used tissue from patients 2 years of age or less undergoing liver resection for extrahepatic biliary atresia (Strassburg et al., 2002). While most

markers appeared normal, the authors could not rule out confounding of their results secondary to liver pathology because it has been demonstrated that UGT mRNA levels can be induced under conditions of acute liver inflammation (Congiu et al., 2002). The main caveat to our pediatric liver tissue is that the posst-mortem time of liver collection is unknown. Thus the possibility exists that enzyme activity in our samples had declined from that in live, healthy livers. Despite this, our study presents a valuable addition through our novel UGT1A4 data as well as through being strongly supportive of the earlier paper.

By indicating that drug metabolizing enzyme activity is particularly lacking in infants under 2 years old, our study matches the reported incidences of pediatric adverse drug reactions startlingly well. The rates of adverse drug reaction reported in children show that these are almost 5 times more likely to occur in children under one and 3.5 times more likely in children over one but under four when compared with older children (Menniti-Ippolito et al., 2000). We suggest, based on data presented, that in some cases this is related to a lack of UGT-mediated drug clearance causing systemic accumulation of parent drug and/or reactive metabolites.

Understanding the disposition of drugs in the human body is one of the most fundamental pillars of medicine. Until the development of metabolizing enzymes in childhood is better understood, prescribing for children will remain problematic. This paper contains the first description of the development of the UGT1A4 isoform in pediatric liver. Detailed study of the development of enzyme activity is particularly important for preventing adverse drug reactions as well as for evaluating and developing new medications for children – a desperately under-served population in modern medicine.

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**Footnotes:**

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**Figure Legends:**

**Figure 1: The balance of total glucuronidation and deconjugation in the pediatric liver with age. 1A: UGT activity towards the pan-specific substrate 4MU (100 µM)**. Plateau is reached at 1.7 years (20 months) and apparent mean adult activity is 1.53 nmol/min/mg protein. Bars are means of n = 3, in triplicate ± SD. **1B: β-glucuronidase activity towards 4MUG.** Plateau is reached at 4 months of age and adult activity of the enzyme is 1.61 pmol/min/mg protein. Points are means of n = 3, in triplicate ± SD.

**Figure 2: UGT1A4 activities in pediatric liver. 2A: Typical kinetic graph of UGT1A4 in one human liver sample.** In this sample, Vmax = 212.7 ± 12.4 pmol/min/mg protein, Km = 12.28 ± 1.96 µM. Points are means of triplicate determinations ± SD. **2B: Emission spectra for Trifluoperazine with and without microsomal incubations (excitation at 310 nm).** Spectra were generated from 1 hr incubations (in triplicate) performed as detailed in materials and methods. The three spectra are derived from incubations containing microsomes and TFP only (solid line ——) and Microsomes, TFP and UDPGA (dotted line ·····). To prove the loss of fluorescence was due to glucuronide conjugation, after 1 hour 1000 Units of β-glucuronidase were added to the third incubation and further incubated for 30 minutes (dashed line — – —). Recovery of fluorescence can be observed **2C: Inhibition of UGT1A4 activity in pooled adult human liver microsomes by hecogenin (100 µM)**. Average activity was 31.6 ± 9.0 % of control levels. Bars are means ± SD of 3 experiments, each performed in triplicate.

**Figure 3: The development of UGT1A4 activity in the pediatric liver. 3A: The development of UGT1A4 activity in the pediatric liver (Vmax)**. Apparent maximum adult activities are reached at 1.4 years with apparent mean adult activity at 113.1 ± 10.17 pmol/min/mg protein (range 62.7 – 268.9

pmol/min/mg protein). The model for UGT1A4 development is weighted by $1/Y^2$ and constrained by strict convergence criteria requiring five consecutive iterations of the fit to change the sum-of-squares by less than 0.000001%. **3B: The scaled hepatic clearance of trifluoperazine in neonates and children aged 13 days to 20 years using an allometric model.** Plateau is reached at 18.9 years with an apparent mean adult clearance of 0.357 L/hr (range 0.05 – 1.3 L/hr). The model is unweighted and standard criteria for convergence are applied.

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Figure 1 DMD 15214

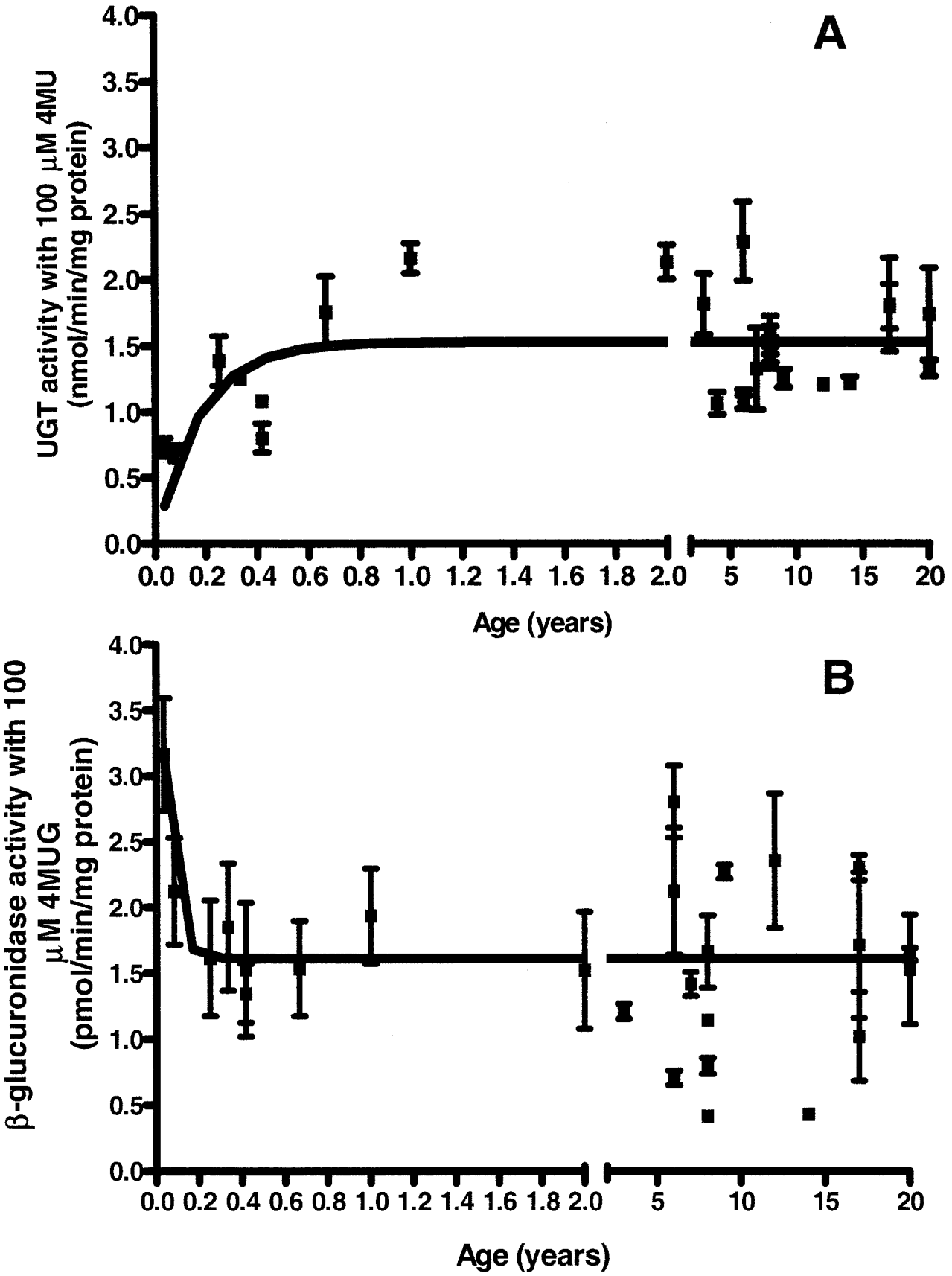

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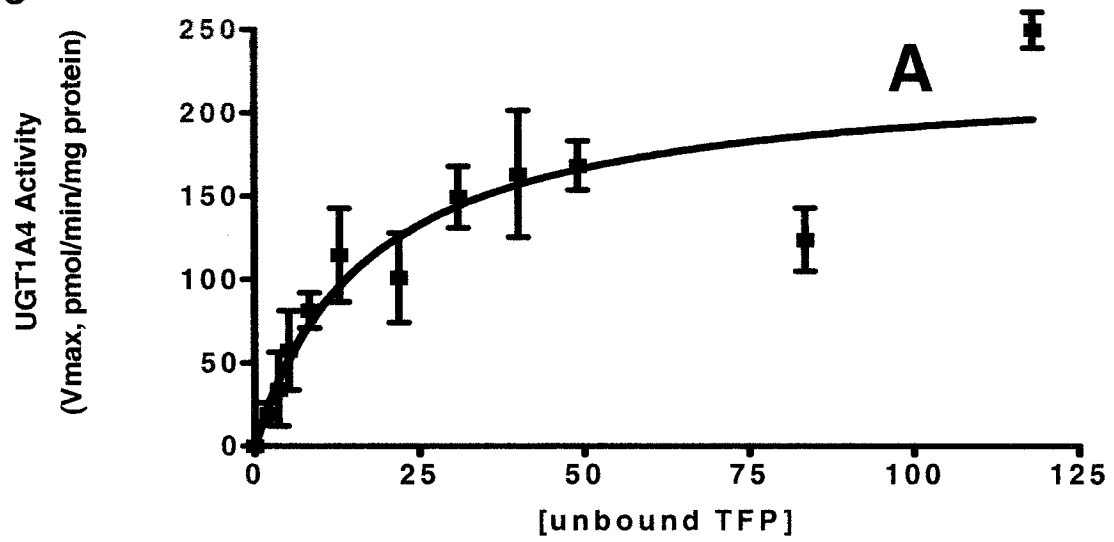
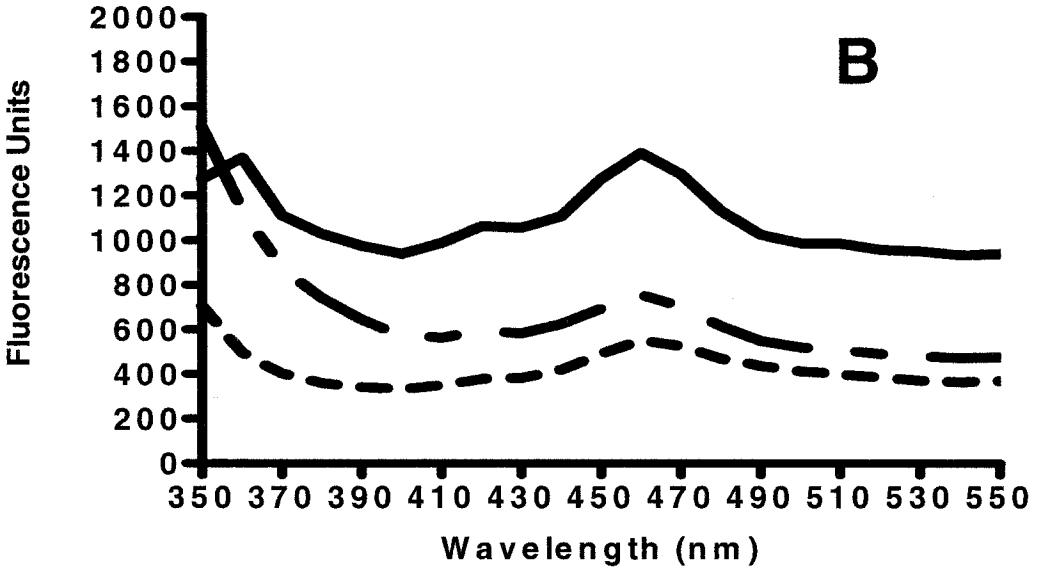
Figure 2 DMD 15214



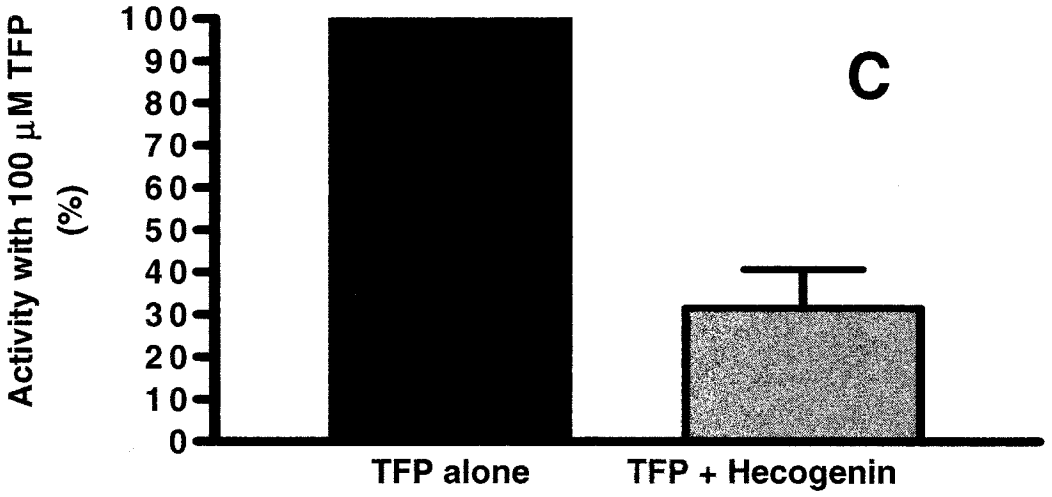



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Figure 3 DMD 15214