CYP1A2 Enzyme Activity and Protein Abundance In Normal And Diseased Pediatric Livers

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INTRODUCTION

Multiple drug metabolizing enzymes are absent or expressed at a very low level during human gestation and increase substantially within the first one to two years after birth. CYPs 1A2, 2C9, 2D6, 2E1 and 3A4 exhibit this general pattern of expression (1). CYP1A2 is a drug-metabolizing enzyme whose expression begins between birth and 4 weeks of age, and gradually increases to about half of the adult levels by 6 years of age. The enzyme constitutes 4 - 16% of the hepatic CYP pool, and is a major determinant of the biotransformation of ~9% of clinically used drugs. Interestingly, CYP1A2 activity decreases in adults with non-alcoholic fatty liver disease (NAFLD). Considering the increase in the number of medications given to children, as well as the rise in childhood obesity and NAFLD, this study aimed to determine whether donor age and health status influence CYP1A2 abundance, lobular localization, and enzyme activity. Pediatric liver microsomes and a corresponding tissue microarray (TMA) were our test system.

Figure 1. Test System – Pediatric Liver Tissue Microarray

In this H&E image, macro- and microvesicular steatosis, a % of organ volume replaced by fat, is seen in NAFLD tissues boxed in red. Normal adult controls are boxed in black.

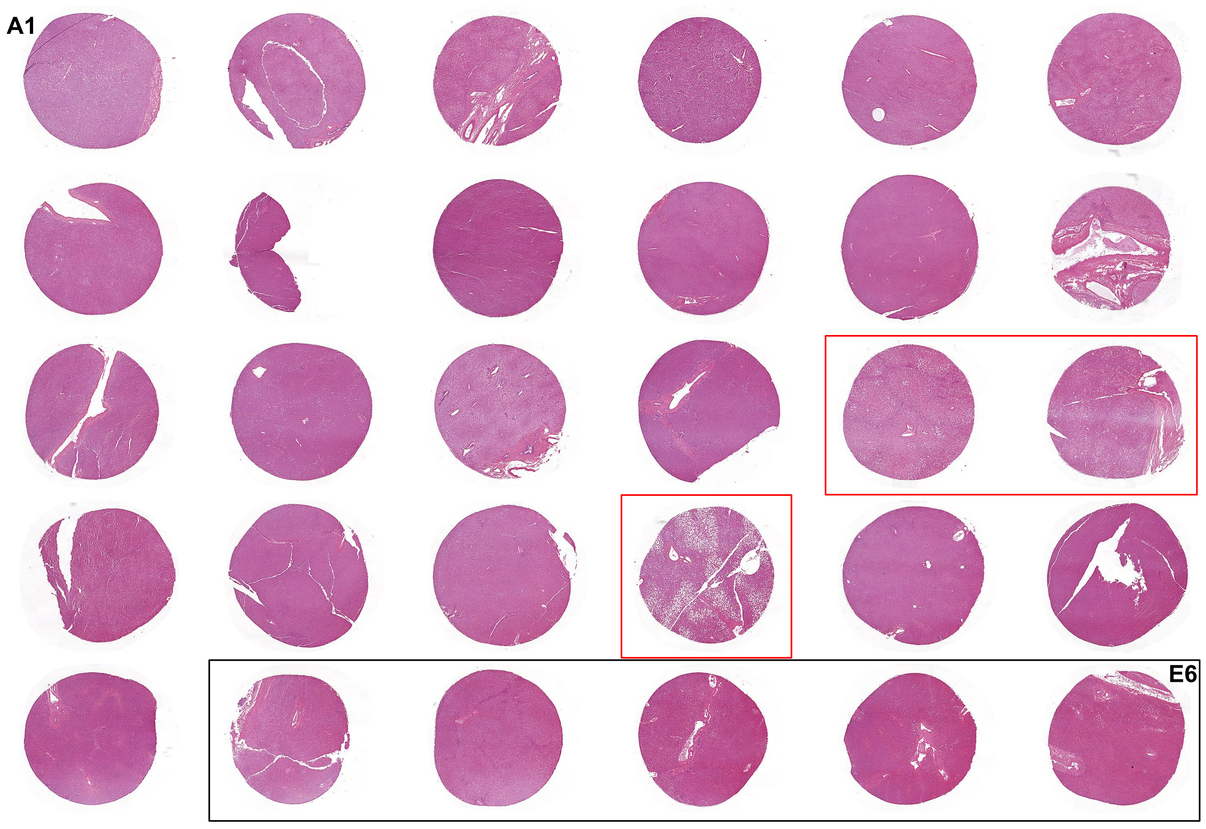


Figure 2A. Donor age and CYP1A2 abundance in pediatric tissue microarray

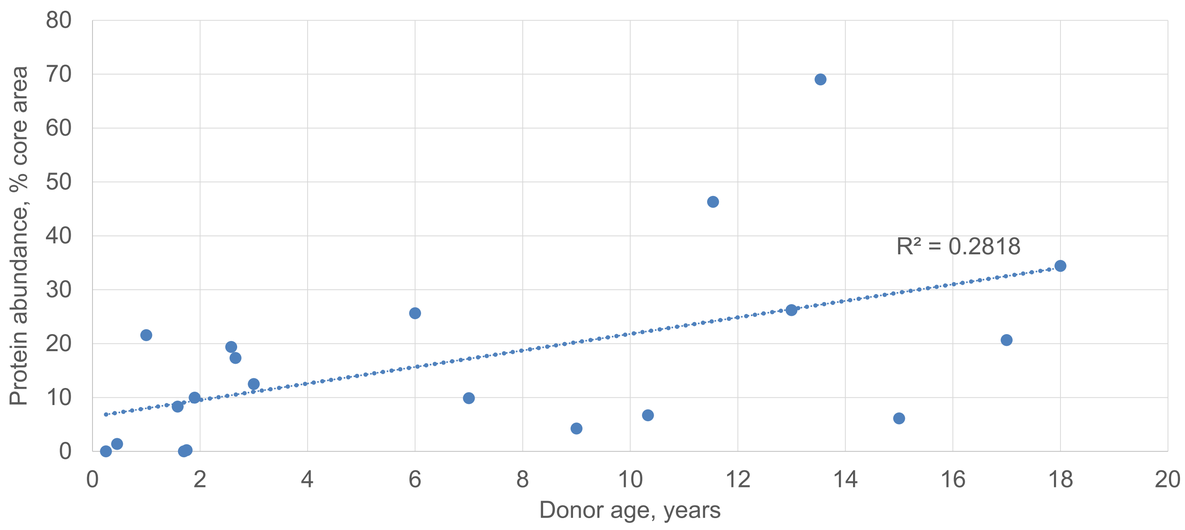
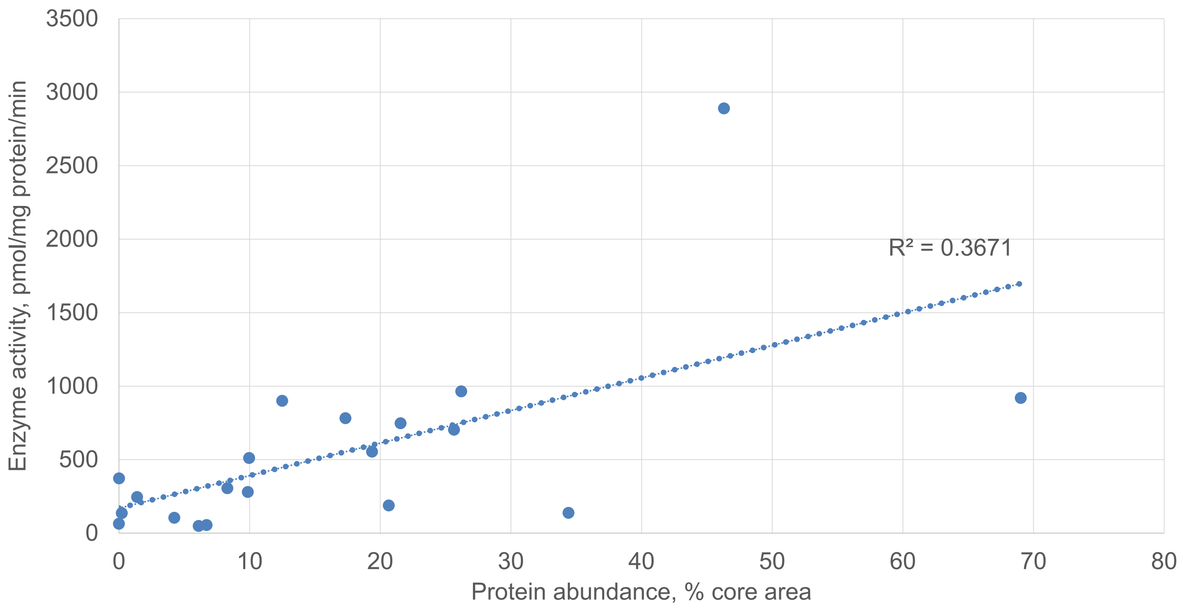


Figure 2B. CYP1A2 abundance and microsomal enzyme activity in pediatric livers



MATERIALS & METHODS

The TMA used for evaluation of abundance and lobular localization of CYP1A2 protein contained 25 tissues from pediatric donors (4 months to 18 years old) and five tissues from adult donor controls (3 mm diameter cores, 4 µm-thick array, XenoTech product TMA.PED lot #1810266). Donor demographics and health data collected from interviews with next of kin and hospital records are shown alongside abbreviated pathologist comments (Table 1). Earlier, lobular distribution of hyaluronan was demonstrated in this TMA (2). To localize CYP1A2, three consecutively cut arrays were stained with hematoxylin and eosin (H&E, Fig. 1), Gomori's trichrome for visualization of fibrosis, and anti-CYP1A2 antibody (MyBioSource, cat# MBS395073). We used an avidin-biotin complex and a tyramide signal amplification method to detect CYP1A2. Images of the arrays were obtained with a Nikon HCA system and a Hamamatsu Orca Flash 4 camera (CYP1A2) or a Nikon DS-Fi3 camera (H&E and Gomori's trichrome). O-dealkylation of CYP1A2 marker substrate phenacetin [80 µM], was measured in liver microsomes.

Table 1. Pediatric Liver Tissue Microarray, Donor Information

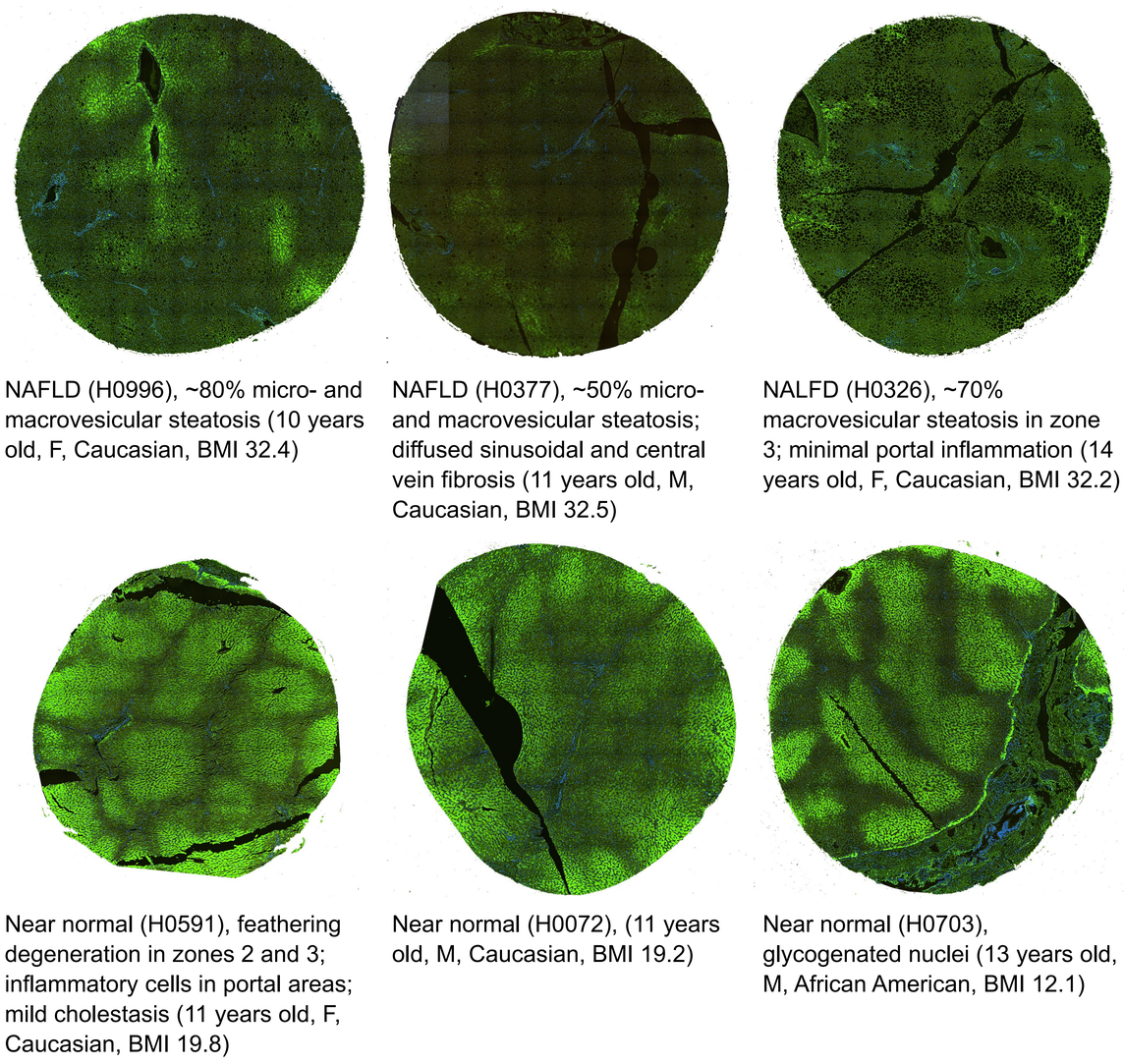
Core	Donor	Pathology	Gender	Age	Ethnicity	BMI	Alcohol Consumption
A1	H1393	Very near normal	Male	4 months	C	18.8	None
A2	H0395	Hepatocyte degeneration 15-20%	Male	5.5 months	C	17.3	None
A3	H1383	Normal	Male	12 months	C	19.7	None
A4	H0322	Diffused feathery degeneration	Male	19 months	H	14.9	None
A5	H1351	Patchy diffused	Female	20 months	H	23.5	None
A6	H1334	Patchy cholestasis, feathery degeneration	Female	21 months	AA	22.8	None
B1	H1397	Feathery and ballooning degeneration	Female	21 months	H	18.9	None
B2	H0551	Near normal	Male	2 years	C	15.5	None
B3	H0852	Diffused hepatic cell death (ischemia)	Male	2 years	H	16.1	None
B4	H0872	Massive liquefaction necrosis	Male	2 years	C	19.3	None
B5	H0346	Necrotic and apoptotic cells ~5%	Male	3 years	C	15.0	None
B6	H0776	Massive necrosis, rapid ischemic event	Female	4 years	AA	18.0	None
C1	H1096	Near normal	Female	6 years	C	13.9	None
C2	H1301	Ischemic changes ~20%, steatosis ~7-10%	Female	7 years	C	14.5	None
C3	H1092	Ischemic damage, single cell necrosis	Female	9 years	A	18.8	None
C4	H0485	Diffused patchy feathery degeneration	Male	10 years	C	20.5	None
C5	H0996	NAFLD (potentially lowest grade of NASH)	Female	10 years	C	32.4	None
C6	H0377	NAFLD, steatosis ~50%	Male	11 years	C	32.5	None
D1	H0591	Feathering degeneration	Female	11 years	C	19.8	None
D2	H0072	Near normal (no inflammation)	Male	11 years	C	19.2	None
D3	H0703	Near normal, scattered microvesicular steatosis	Male	13 years	AA	12.1	None
D4	H0326	NAFLD, steatosis ~70% in zone 3	Female	14 years	C	32.2	None
D5	H0781	Focal mild lobular infiltration	Female	15 years	C	24.2	None
D6	H0707	Near normal	Male	17 years	C	25.4	None
E1	H1263	Near normal	Male	18 years	C	25.3	None
E2	H1279	Near normal - Control	Male	21 years	C	24.6	None
E3	H1357	Microvesicular steatosis ~20% - Control	Male	22 years	H	22.0	Occasional
E4	H1238	Normal – Control	Female	22 years	C	21.0	Occasional
E5	H1265	Normal – Control	Male	26 years	C	21.1	Occasional
E6	H1325	Normal – Control	Female	27 years	C	20.3	None

RESULTS & CONCLUSION

We detected CYP1A2 protein in all normal pediatric livers and those having minor pathological findings, except in one 4-month-old donor. As anticipated, in tissues excluding those with significant necrosis or NAFLD, abundance of immunoreactive CYP1A2 protein correlated with donor age (R^2 = 0.28; Fig. 2A) and with CYP1A2 microsomal phenacetin O-dealkylase activity (R^2 = 0.37; Fig. 2B). CYP1A2 was localized in zone 3 and 2 (Fig. 3).

A diffuse localization, associated with reduced CYP1A2 level, was seen in tissues affected by necrosis and ischemia. In pediatric NAFLD donors (n = 3, average 11.7 years old, BMI 32.6, Fig. 3) CYP1A2 abundance was 63% of age-matched control (n = 10, average 11.7 years old, BMI 19.4). CYP1A2 microsomal phenacetin O-dealkylase activity in the pediatric NAFLD livers was reduced to 45% of control in agreement with observations made in adult tissues (3). As expected, sever ischemic changes, seen in two samples, were associated with dramatic reduction of CYP1A2 protein abundance and activity, as well as diffused localization of the remaining immunoreactive protein. These data indicated that the TMA is a suitable test system for analysis of biomarkers of pediatric liver disease.

Figure 3. Diminished abundance of CYP1A2 in pediatric NAFLD



NAFLD (H0996), ~80% micro- and macrovesicular steatosis (10 years old, F, Caucasian, BMI 32.4)

NAFLD (H0377), ~50% micro- and macrovesicular steatosis; diffused sinusoidal and central vein fibrosis (11 years old, M, Caucasian, BMI 32.5)

NALFD (H0326), ~70% macrovesicular steatosis in zone 3; minimal portal inflammation (14 years old, F, Caucasian, BMI 32.2)

Near normal (H0591), feathering degeneration in zones 2 and 3; inflammatory cells in portal areas; mild cholestasis (11 years old, F, Caucasian, BMI 19.8)

Near normal (H0072), (11 years old, M, Caucasian, BMI 19.2)

Near normal (H0703), glycogenated nuclei (13 years old, M, African American, BMI 12.1)

REFERENCES

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