

# Patterns of Hyaluronan Deposition in Normal and Diseased Pediatric Livers

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## INTRODUCTION

The incidence of obesity and non-alcoholic fatty liver disease (NAFLD) is growing in children worldwide. Hyaluronan (HA), an extracellular matrix (ECM) glycosaminoglycan, is increased in liver and in blood from adults with advanced liver disease. In adult chronic persistent hepatitis, as compared to normal tissue, HA is increased in fibrous areas around the portal tracts and the central vein, but it is rarely detected in the sinusoidal walls. In contrast, in chronic aggressive hepatitis, HA is present in periportal sinusoidal walls in addition to fibrous tissues of the portal tract (1). HA accumulation is also associated with inflammation and fibrosis in extrahepatic tissues. In tissue microarrays (TMAs) constructed from adult livers with macrovesicular steatosis, alcohol consumption is associated with elevated hepatic hyaluronan content by three fold (2), however HA levels have not been examined in healthy or diseased livers from pediatric patients.

Here, we sought to determine if hepatic HA accumulation was associated with donor age and/or disease in pediatric liver tissues. A pediatric liver TMA constituted the test system for this study.

## MATERIALS & METHODS

The TMA contained 25 tissues from pediatric donors (ages 4 months to 18 years and five tissues from adult donor controls (3 mm diameter cores, 4 µm-thick array, Sekisui XenoTech product TMA.PED lot #1810266). Donor demographics and health data were collected from interviews with next of kin and hospital records. Demographics and abbreviated pathologist comments are shown in Table 1. Earlier, the age-related abundance and localization of CYP1A2 protein in liver zones 3 and 2 were demonstrated in this TMA (3). To localize HA, three consecutively cut arrays were stained with hematoxylin and eosin (H&E), Gomori's trichrome (Fig 1) and biotinylated HA-binding protein (HABP). We used an avidin-biotin complex and a tyramide signal amplification method to detect HA. Images of the arrays were obtained with a Nikon HCA system and a Hamamatsu Orca Flash 4 camera (HABP) or a Nikon DS-Fi3 camera (H&E and Gomori's trichrome).

Table 1. Pediatric Tissue Microarray, Donor Information

Core	Donor	Pathology	Gender	Age	Ethnicity	BMI	Alcohol Consumption
A01	H1393	Very near normal	Male	4 months	C	18.8	None
A02	H0395	Hepatocyte degeneration 15-20%	Male	5.5 months	C	17.3	None
A03	H1383	Normal	Male	12 months	C	19.7	None
A04	H0322	Diffused feathery degeneration	Male	19 months	H	14.9	None
A05	H1351	Patchy diffused hepatic cell damage (ischemia)	Female	20 months	H	23.5	None
A06	H1334	Patchy cholestasis, feathery degeneration	Female	21 months	AA	22.8	None
B01	H1397	Feathery and ballooning degeneration	Female	21 months	H	18.9	None
B02	H0551	Near normal	Male	2 years	C	15.5	None
B03	H0852	Diffused hepatic cell death (ischemia)	Male	2 years	H	16.1	None
B04	H0872	Massive liquefaction necrosis	Male	2 years	C	19.3	None
B05	H0346	Necrotic and apoptotic cells ~5%	Male	3 years	C	15.0	None
B06	H0776	Massive necrosis, rapid ischemic event	Female	4 years	AA	18.0	None
C01	H1096	Near normal	Female	6 years	C	13.9	None
C02	H1301	Ischemic changes ~20%, steatosis ~7-10%	Female	7 years	C	14.5	None
C03	H1092	Ischemic damage, single cell necrosis	Female	9 years	A	18.8	None
C04	H0485	Diffused patchy feathery degeneration	Male	10 years	C	20.5	None
C05	H0996	NAFLD (potentially lowest grade of NASH)	Female	10 years	C	32.4	None
C06	H0377	NAFLD, steatosis ~50%	Male	11 years	C	32.5	None
D01	H0591	Feathering degeneration	Female	11 years	C	19.8	None
D02	H0072	Near normal (no inflammation)	Male	11 years	C	19.2	None
D03	H0703	Near normal, scattered microvesicular steatosis	Male	13 years	AA	12.1	None
D04	H0326	NAFLD, steatosis ~70% in zone 3	Female	14 years	C	32.2	None
D05	H0781	Focal mild lobular infiltration	Female	15 years	C	24.2	None
D06	H0707	Near normal	Male	17 years	C	25.4	None
E01	H1263	Near normal	Male	18 years	C	25.3	None
E02	H1279	Near normal - Control	Male	21 years	C	24.6	None
E03	H1357	Microvesicular steatosis ~20% - Control	Male	22 years	H	22.0	Occasional
E04	H1238	Normal - Control	Female	22 years	C	21.0	Occasional
E05	H1265	Normal - Control	Male	26 years	C	21.1	Occasional
E06	H1325	Normal - Control	Female	27 years	C	20.3	None

Figure 1. Test System – Pediatric Liver Tissue Microarray

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

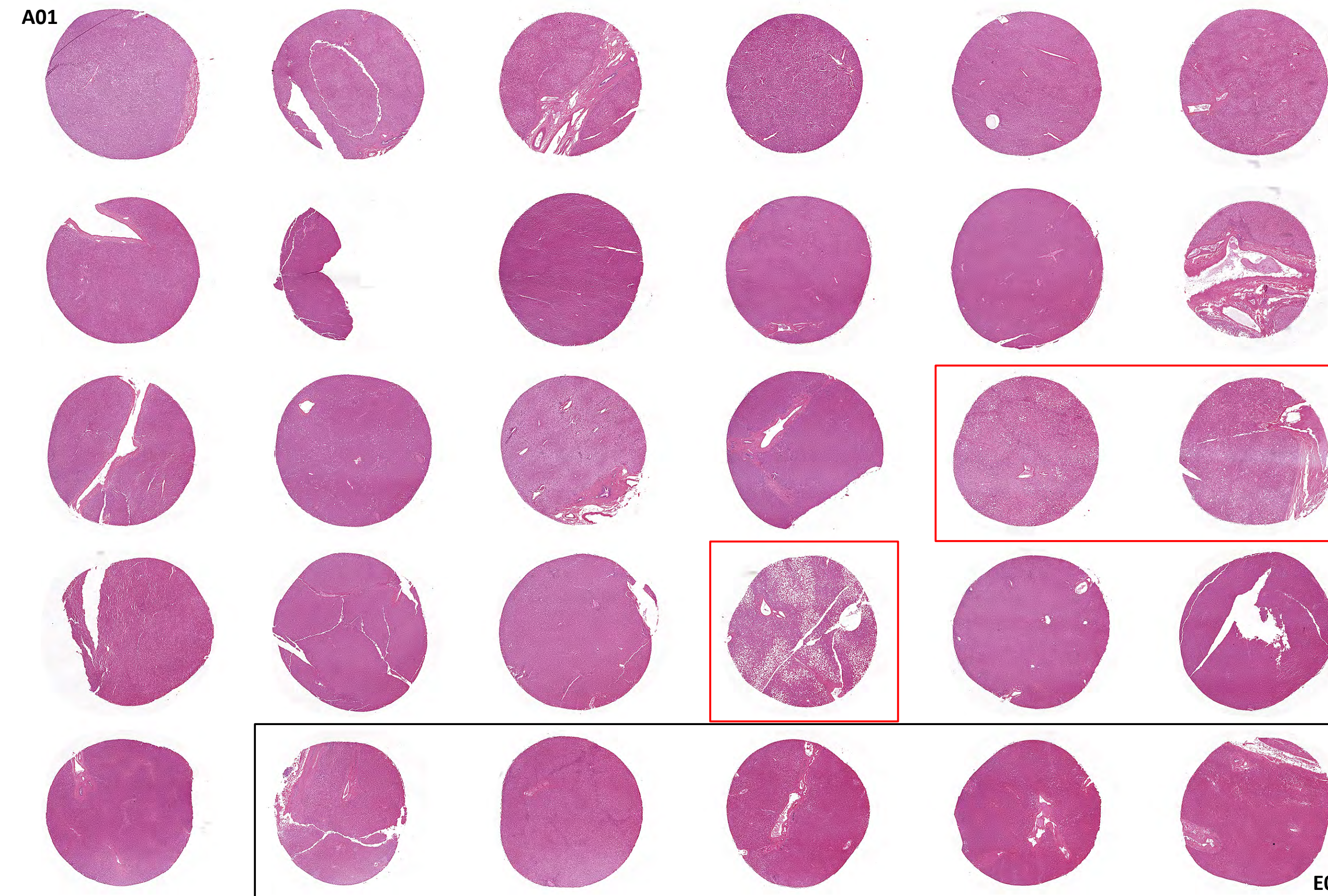


Figure 2.

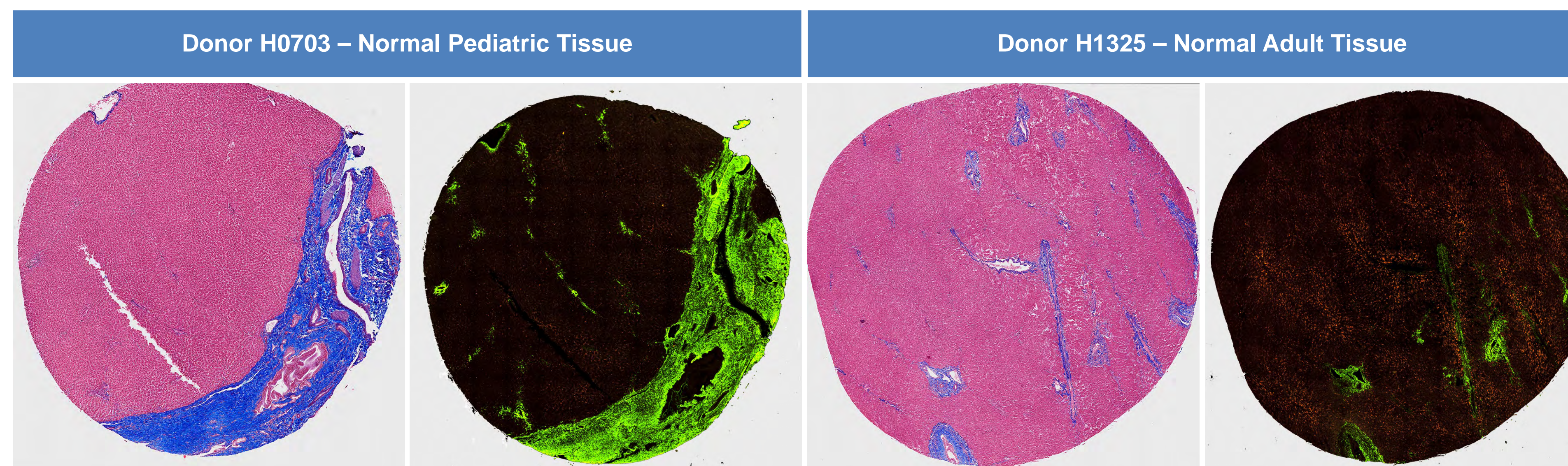
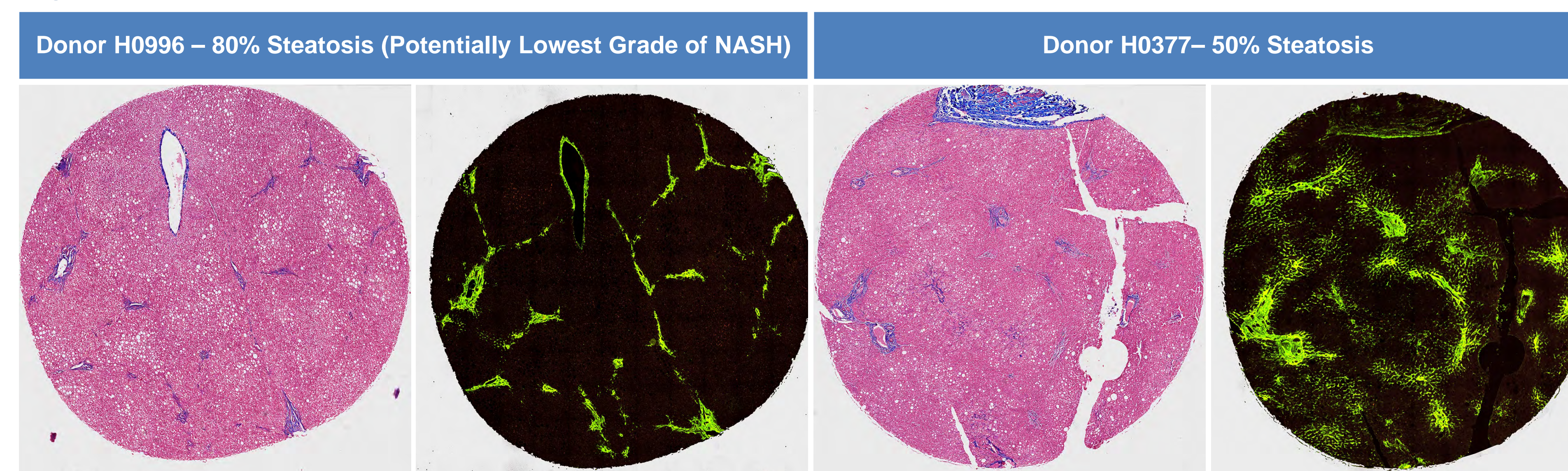


Figure 3.



## RESULTS & CONCLUSION

HA was detected in 24 pediatric tissues and 5 adult control tissues. Tissue from the 20 month old donor (H1351), characterized by patchy diffuse hepatic cell damage indicative of an acute ischemic event, was the single sample negative for HA in the study. The donor died of anoxia, secondary to drowning. The effect of anoxia on HA is unknown. The CYP1A2 protein was very low in this donor (3).

Multiple pathological changes are recognized in the pediatric tissues contained in the TMA (Table 1). Some of these pathological changes may be expected since donated healthy tissues are directed toward pediatric transplantation.

Tissue donors represented in the TMA differed in factors (such as health conditions, age, course of hospitalization and the cause of death) that may affect HA. Differences in the area of the tissue cores occupied by tracts of connective tissue and large blood vessels were confounding technical factors that limited the utility of determining the HA-positive fraction of the area of each tissue core. These observations lead us to focus on the patterns of HA localization rather than the overall abundance of the glycosaminoglycan.

Figure 2. Normal Pediatric and Adult Tissue

Typically, HA in normal pediatric (H0703) and adult (H1325) tissues was observed predominantly in the ECM-rich portal tracts while thin but distinct rims of HA were observed surrounding central veins. Discrete HA staining was also seen on the perimeter of nerves in a fibrous region of the liver from donor H0703. A minimal amount of HA was detected in the periportal regions (zone 1) of normal tissues. Normal histological appearance of the tissues can be appreciated in the trichrome images.

Figure 3. NAFLD Pediatric Tissue

In the pediatric NAFLD tissues, HA was localized in the portal tracts that were typically expanded, as compared to the normal tissues. In addition, the glycosaminoglycan was found surrounding steatotic hepatocytes outside of portal tracts. In these areas, HA was co-localized with periportal fibrosis; minimal in tissue H0996 and moderate in tissue H0377. There was variability in this staining pattern as this assessment was complicated by both micro- and macrovesicular steatosis ranging from 50 – 80%

in the NAFLD patients. In tissues deemed normal or near normal based on microscopic examination and medical history, the HA staining was less intense than in diseased tissues – independent of donor age.

We concluded that the TMA allowed demonstration of normal and disease-associated patterns of HA localization and may therefore be a suitable tool for evaluation of disease biomarkers in pediatric livers.

## REFERENCES

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3. Ogilvie BW, et al. CYP1A2 Enzyme Localization in Normal and Diseased Pediatric Livers. Platform presentation at this meeting, March 13.