MICROSOMAL CYTOCHROME P450 ENZYME ACTIVITIES IN NONALCOHOIC STEATOHEPATITIS LIVERS

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INTRODUCTION

The prevalence of nonalcoholic steatohepatitis (NASH), a chronic liver disease, has increased drastically in parallel with the increased incidence of obesity in the US. This condition affects hepatic drug metabolism and has potential to impact drug-drug interactions. Our study aimed to evaluate microsomal cytochrome P450 (CYP) enzyme activities, organ fibrosis and microvesicular steatosis in NASH tissues deposited in the XenoTech Biobank, and to establish whether these tissues have application as a test system for the study of the impact of NASH on metabolism of xenobiotics. NASH-positive tissues were identified based on the presence and extent of intra-lobular inflammation, ballooning necrosis, macrovesicular fat and history of alcohol consumption. The fibrosis stage was assigned based on Brunt *et al*. (1). Four tissue microarrays (TMA) focused on different aspects of fatty liver disease were assembled. The arrays, which feature distinctive pathologies and two kinds of control samples, are a research tool for efficient evaluation of histological markers of the disease. A microsomal pool of five NASH donors and tissue micro arrays containing NASH and fatty livers from donors with and without history of alcohol consumption were prepared to assist in disease evaluation. The NASH pattern of CYP enzyme activities seen in the patients and in the microsomes prepared from non-transplantable NASH livers suggest that the pooled subcellular fraction is an appropriate test system for analysis of CYP-mediated xenobiotic metabolism associated with the disease.

MATERIALS & METHODS

Human livers harvested with an intent for transplantation were obtained from National Disease Research Initiative and International Institute for Advancement of Medicine. Identification of NASH donors and scoring of organ fibrosis followed criteria proposed by Brunt *et al*. (1). Triglycerides and total cholesterol were measured according to published methods (2, 3). Characteristics of 16 NASH tissues stored in the Biobank are presented in Table 1. A pool of NASH hepatic microsomes was prepared from five of these tissues. Fibrosis stage 0, stage 1 focally present, and stage 2 in the tissues included in the pool are illustrated in Figure 1.

Four different human liver TMA focused on progressive stages of fatty liver disease were constructed by XenoTech. The composition of the arrays and donor demographic and health data are presented in Table 2. A Masson's trichrome image of the array focused on steatosis with a history of alcohol use is presented in Figure 2. Arrays are provided unstained and the cores are not covered with paraffin. For preservation of sensitive epitopes, arrays are stored at 4 °C in atmosphere depleted of oxygen (4).

RESULTS

The XenoTech Biobank is a collection of livers from donors with steatosis, steatohepatitis and normal controls. The tissues were characterized to facilitate study of fatty liver disease. Anonymous donor data provided by the organ procurement organizations include demographics, serology, cause of death, body mass index (BMI), alcohol use and diabetes history. Macrovesicular fat, lobular inflammation, ballooning necrosis and fibrosis were evaluated based on hematoxylin and eosin (H&E) and Masson's trichrome stains. Tissues deposited in the bank are flash frozen in liquid nitrogen and stored at -80°C. Cells isolated from some tissues deposited in the bank are available as cryopreserved hepatocytes. Photomicrographs of each tissue can be viewed at www.xenotech.com. Currently the bank contains about 250 normal and fatty liver tissues.

Macrovesicular steatosis correlated positively with tissue triglyceride contents. In a cohort consisting of normal (n=10), steatosis (n=20, with and without history of alcohol consumption) and steatohepatitis tissues (n=21, with and without history of alcohol consumption) microvesicular steatosis did not correlate with the BMI (R^2=0.004, Figure 3A). In the same cohort the extent of microvesicular steatosis correlated positively with tissue triglyceride contents (R^2=0.66, Figure 3B), but not with total cholesterol levels (R^2=0.016). Microsomal protein yield was weakly negatively correlated with microvesicular fat content, but not the BMI (data not shown).

NASH-positive tissues had decreased CYP enzyme activities. Microsomal CYP activities in the Biobank NASH donors were compared to the general population of liver donors (Table 3). The results demonstrated that the majority of the microsomal CYP activities in NASH livers were lower than in the general population of liver donors. The microsomal activities of CYP2C19 and CYP3A4 were the most affected with decreases of 34% and 54% compared to the microsomal activities of their respective controls. In contrast, NASH did not change the average CYP2E1 microsomal activity as compared to the general population of liver donors. These observations were in agreement with published clinical data (5-7).

Table 1. NASH tissues in the XenoTech Biobank

Donor	Gender/age/race	Macrovesicular steatosis (%)	BMI (kg/m²)	Diabetes	Fibrosis stage
H0448	F 58 Caucasian	60	32.3	No	1 – 2, focal
H0499	F 55 Caucasian	40	41.5	No	0[1]
H0501	F 58 Caucasian	30	30.2	No	1
H0522	F 56 Caucasian	3	34.2	Yes, type unknown	1 – 2, focal
H0554	F 43 Caucasian	25	50.4	No	3
H0571	F 45 Caucasian	20?	23.9	IDDM[2]	0
H0602	F 83 Caucasian	20	21.9	No	1
H0613	M 65 Caucasian	50	32.9	No	3
H0656	F 56 Hispanic	70	34.3	No	3
H0847	F 55 Caucasian	50 - 60	36.9	NIDDM	0
H0958[3]	M 47 Caucasian	90	40.7	NIDDM	2
H1027	F 63 Caucasian	50	43.0	NIDDM	0
H1028	F 51 African American	70	40.0	Yes, type unknown	0
H1060	M 49 Hispanic	50	44.9	Yes, type unknown	0
H1069	M 39 Caucasian	25	62.7	No	1, focal
H1097	M 36 Caucasian	65	37.0	IDDM	0

[1] 0 = no fibrosis;
[2] IDDM – insulin dependent diabetes mellitus, NIDDM – non-insulin dependent diabetes mellitus;
[3] Shaded donors indicate those in the pool of NASH microsomes

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Figure 1. Different stages of liver fibrosis in tissues present in the pool of hepatic NASH microsomes

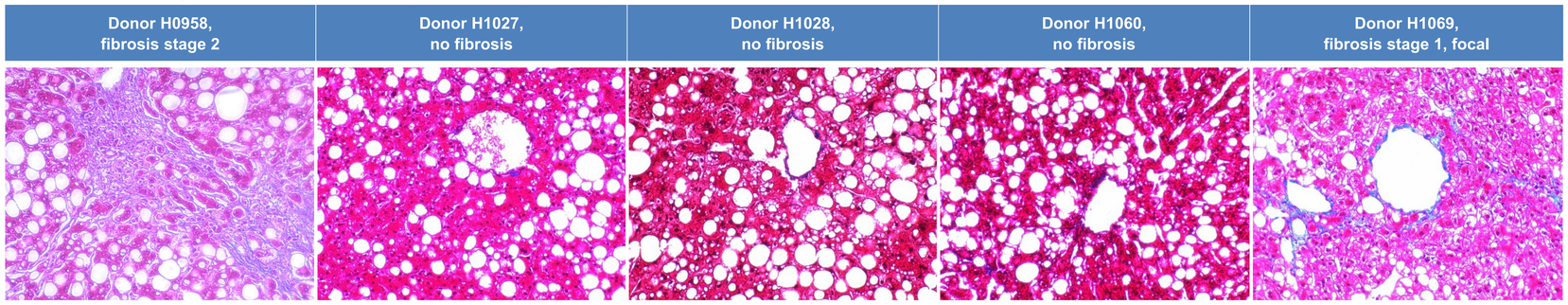


Table 2. Demographic and disease characteristics of the arrayed tissues

Focus of the tissue micro array (donors)	Male/ Female (n)	Age (avg. years)	Macrovesicular steatosis (average %)	BMI (avg. kg/m²)	Diabetes (n)
NASH (12) control 1: steatohepatitis, history of alcohol use (5) control 2: normal (5)[1]	4/8 2/3 3/2	51.8 53.6 47.0	52.3 52.5 0	41.2 26.1 27.0	7 1 0
Steatohepatitis, history of alcohol use (9) control 1: normal, history of alcohol use (5) control 2: normal (5)	4/5 3/2 3/2	53.8 45.8 47.0	41.9 0 0	25.6 24.8 27.0	2 0 0
Steatosis, no history of alcohol use (20) control 1: steatosis, history of alcohol use (5) control 2: normal (5)	13/7 3/2 3/2	50.9 46.2 47.0	37.4 54.5 0	41.4 32.4 27.0	8 0 0
Steatosis, history of alcohol use (19) control 1: steatosis, no history of alcohol use (5) control 2: normal (5)	14/5 3/2 3/2	48.4 50.4 47.0	43.7 40.4 0	33.4 41.5 27.0	0 0 0

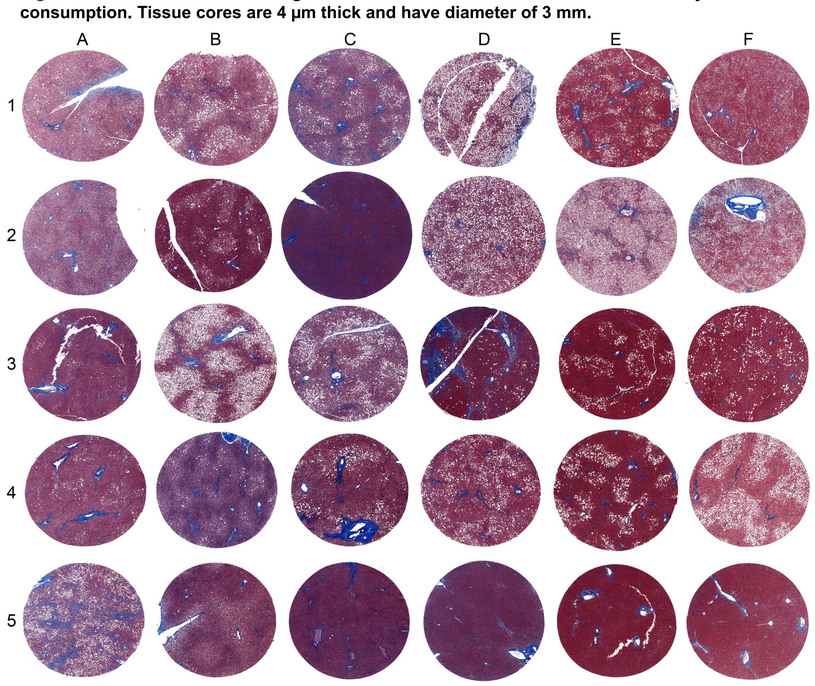
[1] The same normal controls are present on all arrays

Table 3. CYP enzyme activities in NASH liver microsomes

Enzyme	Marker Substrate Reaction ([S] µM)	Rate (n=14) (pmol/mg protein/min)	Change from control (%)[1]
CYP1A2	Phenacetin *O*-dealkylation (80)	338 ± 153	65%
CYP2A6	Coumarin 7-hydroxylation (50)	992 ± 939	93%
CYP2B6	Bupropion hydroxylation (500)	611 ± 1020	71%
CYP2C8	Amodiaquine *N*-dealkylation (20)	2480 ± 1730	80%
CYP2C9	Diclofenac 4'-hydroxylation (100)	2990 ± 1260	83%
CYP2C19	*S*-Mephenytoin 4'-hydroxylation (400)	28.7 ± 20.7	35%
CYP2D6	Dextromethorphan *O*-demethylation (80)	209 ± 170	66%
CYP2E1	Chlorzoxazone 6-hydroxylation (500)	1690 ± 502	104%
CYP3A4/5	Testosterone 6β-hydroxylation (250)	3440 ± 3040	73%
CYP3A4/5	Midazolam 1'-hydroxylation (30)	989 ± 877	54%
CYP4A11	Lauric acid 12-hydroxylation (100)	1790 ± 594	94%

[1] Control are data from a general population of liver donors (n=191)

Figure 2. Masson's trichrome image of TMA focused on liver steatosis with a history of alcohol consumption. Tissue cores are 4 µm thick and have diameter of 3 mm.



A1 – B4: Steatosis, history of alcohol use
C4 – A5: Steatosis, no history of alcohol use
B5 – F5: Normal control; C2: control

Figure 3A. Macrovesicular steatosis is independent of liver donors' BMI

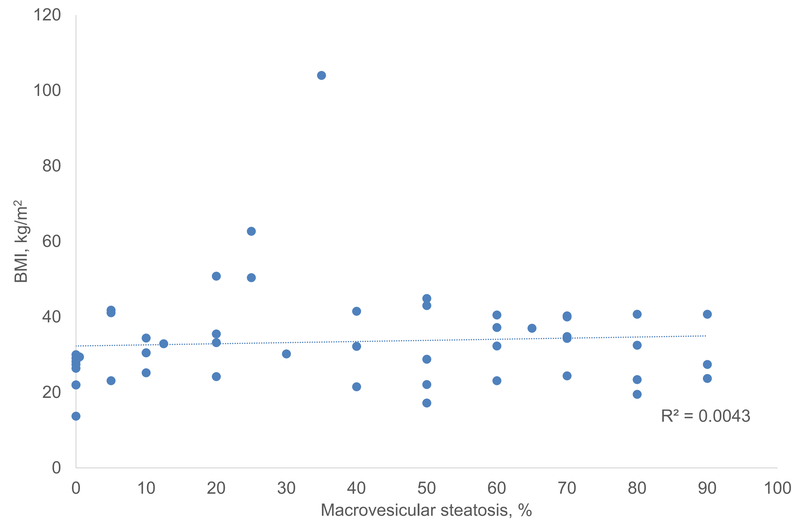
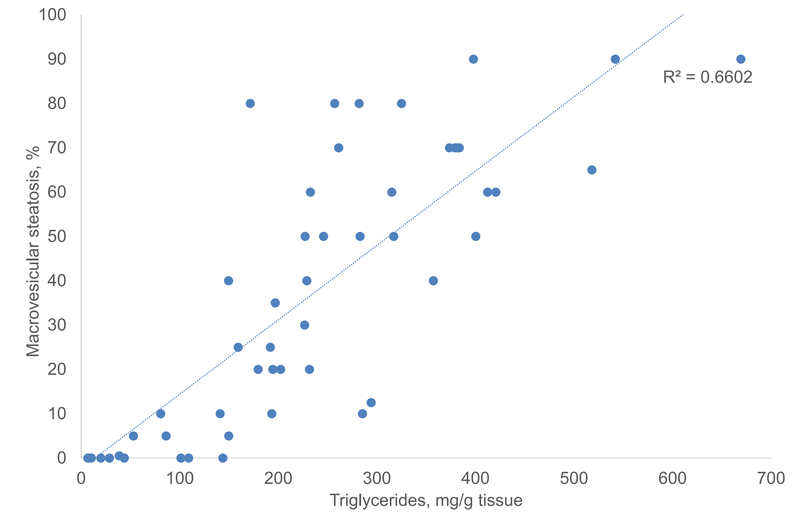


Figure 3B. Macrovesicular steatosis correlates with tissue triglyceride content



CONCLUSIONS

The conditions of harvesting human livers with an intent for transplantation and the transfer of the tissues to XenoTech followed by storage and preparation of the microsomes preserved a NASH-specific pattern of CYP expression, namely reduction in CYP3A4 and unchanged CYP2E1 enzyme activities.

The preservation of a NASH-specific pattern of CYP expression in the tissues deposited in the Biobank suggest that tissue micro arrays prepared from the same organs are suitable tools for investigating histological features of fatty liver disease.

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