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 Sekisui 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-lipid 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 in a microarray 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 Sekisui XenoTech. The composition of the arrays and donor demographic and health data are presented in Table 1. 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 Sekisui XenoTech Biobank is a collection of livers donated from NASH, 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, intra-lipid inflammation, ballooning necrosis and fibrosis were evaluated based on hematoxylin and eosin (H&E) staining. In a cohort consisting of normal (n=10), steatosis (n=20, with and without history of alcohol consumption) and hepatocytes. Photomicrographs of each tissue can be viewed at www.xenotech.com. Currently the bank donor data provided by the organ procurement organizations include demographics, serology, cause of death, body mass index (BMI), alcohol use and diabetes history.

### MICROSOMAL CYTOCHROME P450 ENZYME ACTIVITIES IN NONALCOHOLIC STEATOHEPATITIS LIVERS

The prevalence of NASH 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 Sekisui 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-lipid 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.

### CONCLUSIONS

The conditions of harvesting human livers with an intent for transplantation and the transfer of the tissues to Sekisui 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.

### REFERENCES

7. Woolsey, SJ et al., CYP3A4 activity and expression in nonalcoholic fatty liver disease, Drug Metabolism and Disposition 43, 1484-1490, 2015.