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Expression of IGF-1 Receptor and GH Receptor in Hepatic Tissue of Patients with Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis

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Abstract

Objective: The GH and IGF-1 axis is a candidate disease-modifying target in nonalcoholic fatty liver disease (NAFLD) given its lipolytic, anti-inflammatory and anti-fibrotic properties. IGF-1 receptor (IGF-1R) and GH receptor (GHR) expression in adult, human hepatic tissue is not well understood across the spectrum of NAFLD severity. Therefore, we sought to investigate hepatic IGF-1R and GHR expression in subjects with NAFLD utilizing gene expression analysis (GEA) and immunohistochemistry (IHC).

Design: GEA (n=318) and IHC (n=30) cohorts were identified from the Massachusetts General Hospital NAFLD Tissue Repository. GEA subjects were categorized based on histopathology as normal liver histology (NLH), steatosis only (Steatosis), nonalcoholic steatohepatitis (NASH) without fibrosis (NASH F0), and NASH with fibrosis (NASH F1-4) with GEA by the Nanostring nCounter assay. IHC subjects were matched for age, body mass index (BMI), sex, and diabetic status across three groups (n=10 each): NLH, Steatosis, and NASH with fibrosis (NASH F1-3).

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IHC for IGF-1R, IGF-1 and GHR was performed on formalin-fixed, paraffin-embedded hepatic tissue samples.

Results: IGF-1R gene expression did not differ across NAFLD severity while IGF-1 gene expression decreased with increasing NAFLD severity, including when controlled for BMI and age. GHR expression did not differ by severity of NAFLD based on GEA or IHC.

Conclusions: IGF-1R and GHR expression levels were not significantly different across NAFLD disease severity. However, expression of IGF-1 was lower with increasing severity of NAFLD. These findings implicate the GH/IGF-1 axis as a potential target in the treatment of NAFLD and NASH.

Keywords

Insulin-like growth factor-1 (IGF-1); IGF-1 receptor; growth hormone (GH); GH receptor; Nonalcoholic fatty liver disease (NAFLD); Nonalcoholic steatohepatitis (NASH); immunohistochemistry; gene expression analysis

Introduction

Nonalcoholic fatty liver disease (NAFLD) is present in 80% of individuals with obesity in the United States (1). Nonalcoholic steatohepatitis (NASH) is the progressive form of the disease characterized by inflammation and cell death, and NASH cirrhosis is predicted to be the most common indication for liver transplantation in the near future (2–4). While insulin resistance, oxidative stress and inflammation have all been implicated as mechanisms contributing to NAFLD and NASH, their pathogenesis is only partially understood, and no FDA-approved treatments currently exist (3).

The GH and IGF-1 axis is a candidate disease-modifying target in NAFLD and NASH because of its lipolytic, anti-inflammatory and anti-fibrotic properties (5,6). GH is secreted from the pituitary and acts through GH receptors (GHR) on hepatocytes to produce insulin-like growth factor-1 (IGF-1) (7). Obesity is a state of relative GH and IGF-1 deficiency, with GH production reduced to 25% of normal and concomitant moderate reductions in IGF-1 (5,6), although one study noted a potentially compensatory increase in hepatic GH sensitivity in this population (8). Animal models (9–15) and clinical data in patients with severe GH deficiency (16–18) are consistent with an etiopathologic role of relative GH and IGF-1 deficiency in NAFLD/NASH.

However, the mechanisms of the impact of GH and IGF-1 on hepatic steatosis, inflammation and fibrosis in NAFLD and NASH have not been fully elucidated, particularly in humans. GH has well known lipolytic actions in humans and has been shown to decrease visceral adipose tissue in pituitary patients with severe GH deficiency (19–21) and in otherwise healthy individuals with overweight/obesity (22,23). The GH receptor is expressed on normal human hepatocytes and signals through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathways. Multiple mouse models have demonstrated that hepatocyte-specific knockouts of the GH receptor or components of the JAK-STAT signaling pathway lead to the NAFLD phenotype (10,13,14).

In contrast, the IGF-1R is not expressed in normal, healthy adult rat, mouse (24), or human hepatocytes (25,26). However, the IGF-1 receptor has been shown to be expressed in hepatocytes in the presence of liver disease, including in mice after hepatectomy (25) and adults with chronic hepatitis C (26). Hepatic IGF-1 receptor expression was demonstrated in children with NAFLD, and expression was increased with fibrosis progression, although predominantly in hepatic stellate cells (27). No study has examined the expression of the IGF-1 receptor or GH receptor in adult, human hepatic tissue, particularly hepatocytes, across the spectrum of NAFLD and NASH.

In this study, we sought to investigate IGF-1 receptor and GH receptor physiology in patients with NAFLD and NASH, with quantification of hepatic gene expression and localization of receptor expression by immunohistochemistry. We hypothesized that expression of hepatic IGF-1 receptor and GH receptor would be increased with worsening NAFLD disease severity given the potentially positive effects of GH and IGF-1 on hepatic steatosis, fibrosis and hepatocyte regeneration. We additionally hypothesized that IGF-1 receptor would be expressed specifically on hepatocytes of human subjects in the setting of hepatic injury due to NAFLD/NASH.

Methods

Study approval and informed consent

The present study was approved by the Partners Human Research Committee as a secondary use of the NAFLD repository. All subjects signed informed consent to participate in the NAFLD repository.

Study Cohort

Subjects were identified from the Massachusetts General Hospital (MGH) NAFLD Repository, a prospective study of consented and enrolled patients with suspected or diagnosed NAFLD from the MGH Gastroenterology Unit and MGH Weight Center. Subjects with history of other chronic liver disease or drug-induced NAFLD/NASH due to tamoxifen, oral steroids or methotrexate were excluded. Excess hepatic tissue samples were obtained during routine percutaneous liver biopsy or routine wedge liver biopsy performed during bariatric surgery. One part of the specimen was immediately flash frozen and stored at −80 degrees Celsius for future gene expression analysis. An additional section of each biopsy was formalin fixed and paraffin embedded (FFPE) for histologic evaluation and future immunohistochemistry analysis.

Liver biopsy assessment

Liver biopsies were reviewed by a single blinded hepatopathologist (RM) per the NASH Clinical Research Network (NASH CRN) criteria (28). H&E stained slides were used to assess steatosis grade (0 = <5%; 1 = 5–33%; 2 = 33–66%; 3 >66%), lobular inflammation (foci/200x; 0 = none; 1 = <2; 2 = 2–4; 3 = >4 foci), hepatocyte ballooning (0 = no ballooning; 1 = few; 2 = many), NAFLD activity score (NAS) (assigned from 0–8 as a sum of the steatosis, lobular inflammation and hepatocyte ballooning grades). NASH was defined by the predominance of zone 3 macrovesicular steatosis, hepatocyte ballooning grade ≥1,

and lobular inflammation grade ≥1 (presence of ≥1 foci per 200x field) as defined by the NASH Clinical Research Network (NASH CRN). Masson's trichrome stained slides were used to assess fibrosis, according to the modified Brunt stage 0–4, with 4 representing cirrhosis (28). Subjects for the immunohistochemistry cohort were initially selected based on a clinical pathology read which was then verified using the blinded NASH CRN read as described above.

Subject Identification and Characterization

For the gene expression cohort, 318 individuals with paired histologic analysis and available flash frozen tissue were identified as previously published (29). Subjects were categorized into the following histologically defined subgroups: normal liver histology (NLH), Steatosis, NASH without fibrosis (NASH F0) and NASH with fibrosis (NASH F1–4).

For the immunohistochemistry cohort, 30 adult subjects who had adequate archived FFPE hepatic tissue samples available were identified. Subjects were selected from three distinct histological groups: normal liver histology (NLH), steatosis only without inflammation, hepatocyte ballooning or fibrosis (Steatosis) and NASH with fibrosis stage 2–3 (NASH F2–3). Subjects were matched across groups of three for BMI (± 6 kg/m^2), age (± 6 years), sex and menopausal status, so that each matched triplet contained a subject with NLH, Steatosis and NASH F2–3.

Gene expression analysis

RNA extraction was performed as previously described using the following kits: miRNeasy Mini Kit (Qiagen) for flash-frozen samples or RNA-later liver or High Pure FFPET RNA Isolation Kit (Roche Life Science) for Formalin-Fixed Paraffin-Embedded (FFPE) Tissue. An Agilent 2010 bioanalyzer (Santa Clara, CA) was used to assess RNA quality and quantity with RIN numbers from 1.5–9 appropriate for the nCounter platform (29). We quantified gene expression in liver samples using a NanoString probeset as previously published that included GH receptor, IGF-1 receptor and IGF-1. GH expression was not available in this NanoString probeset.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on FFPE hepatic tissue samples for expression of IGF-1 receptor and GH receptor. Anti-IGF-1 receptor antibodies that would cross-react with the insulin receptor, which has a similar homology, were avoided. Three IGF-1 receptor antibodies underwent attempted validation using human breast cancer tissue samples (D4O6W, Cell Signaling, Danvers, MA, USA; Ab131476, Abcam, Cambridge, MA, USA and LS-B2905, LSBio, Seattle, WA, USA). One antibody was successfully validated (D4O6W, Cell Signaling) and utilized to stain all subject slides [dilution 1:50, antigen heat mediated retrieval (Biocare Medical), manual staining]. Two additional anti-IGF-1 receptor antibodies failed validation (Ab131476 [Abcam] for absent staining on breast cancer control samples and LS-B2905 [LSBio] for nonspecific staining on breast cancer control samples). One anti-GH receptor antibody was successfully validated using normal hepatic tissue (HPA045339, Atlas, Sweden) and was then utilized to stain all subject slides (dilution 1:25, antigen heat mediated retrieval [Biocare Medical], manual staining). Results were

systematically reviewed by a single pathologist (RM) with blinded assessment of all samples for staining intensity and zonal distribution. Staining intensity was categorized as either weak, moderate or strong and zonality was categorized as centrilobular (zone 3>1), absent or peri-portal (zone 1>3).

Statistical analysis

Continuous variables are reported as mean ± SD and categorical variables as n (%), unless specified otherwise. JMP Pro Statistical Database Software (Version 12; SAS Institute, Cary, NC) was used to perform all IHC analyses and R was used to perform Nanostring data analysis. Gene expression data were not normally distributed in all groups, and therefore, a generalized linear model (GLM) approach for non-normal data was utilized to determine differences in gene expression (30). This approach has been utilized in order to address biologic variability in RNAseq analyses (31). Specifically, the GLM function in R was utilized with a binomial family parameter for binary logistic regression of non-normally distributed data, and these models were adjusted for age and BMI.

Results

Gene expression cohort clinical characteristics

Clinical characteristics of the gene expression cohort were previously published and are presented in Table 1 (29). In brief, the cohort (n=318) was 76.4% (n=243) female with a mean BMI of 47±7 kg/m^2 and mean age of 44±12 years. The subjects were divided into four histologic groups, including NLH (n=76), Steatosis (n=88), NASH F0 (n=72) and NASH F1–4 (n=82). Mean age and BMI did not differ across groups.

Gene expression results

Gene expression analysis demonstrated that there was no difference in IGF-1 receptor (Fig. 1A) or GH receptor gene expression (Fig. 1B) between NLH, Steatosis, NASH F0 and NASH F1–4 in multivariable models controlled for BMI and age. However, consistent with prior literature (32), IGF-1 gene expression decreased across disease stages (Fig. 1C). A sensitivity analysis was performed excluding subjects with cirrhosis (NASH F4, n=3), and the results of these analyses were unchanged.

Gene expression by sex and presumed menopausal status

GH receptor gene expression was significantly higher in women versus men across all disease severity groups (Fig. 2B). When broken down by age as a proxy of menopausal status, women <50 years (presumed premenopausal) had significantly higher expression of GH receptor compared to men only but not compared to women ≥50 years (presumed postmenopausal) (Fig. 2E); there was no difference in GH receptor expression between presumed postmenopausal women and men (Fig. 2E).

In contrast, there were no significant differences in IGF-1 receptor (Fig. 2A and 2D) or IGF-1 expression (Figs. 2C and 2F) between men and women, including when classified by presumed menopausal status, with the exception of higher IGF-1 gene expression in men versus all women in the Steatosis group only (Fig. 2C).

Immunohistochemistry cohort clinical characteristics

The mean age for the immunohistochemistry cohort was 57±8 years and mean BMI was 44±6 kg/m^2. Cohort characteristics and liver histology by group are reported in Table 2. Mean (±SD) BMI, age, sex and diabetes status did not differ across the NLH, steatosis and NASH F2–3 groups, per study design. Of note, one subject assigned to the NLH group based on clinical pathologic read was found to have mild liver fat based on subsequent blinded pathological review but was included in the NLH group for analysis based on the *a priori* intention to analyze matching categorization.

Mean NAFLD Activity Score (NAS) was higher in the NASH versus Steatosis and NLH groups, per study design. Mean NAS score for the NASH group was 5.9±1.0 (range 0–8), consistent with steatohepatitis.

IGF-1 receptor staining in NAFLD/NASH

Despite adequate validation of the Cell Signaling anti-IGF-1 receptor antibody with expected staining of breast cancer control tissue, there was no staining of the hepatic tissue across any subject group. Confirmatory testing was attempted with two additional anti-IGF-1 receptor antibodies that both failed validation as described in the methods.

GH receptor staining in NAFLD/NASH

GH receptor staining was localized to hepatocytes based on morphology. There was no significant difference between GH receptor staining intensity or zonal distribution between the three groups (Table 2).

Discussion

We hypothesized that the degree of expression of both IGF-1 receptor and GH receptor would directly correlate with the severity of NAFLD/NASH. Contrary to our hypothesis, IGF-1 receptor and GH receptor expression by gene expression analysis remained unchanged across the spectrum of disease severity in NAFLD/NASH. GH receptor expression also appeared to be estrogen dependent when analyzed by sex and menopausal status, with higher GH receptor expression in presumed premenopausal women. These data suggest that IGF-1 receptors and GH receptors remain a potentially actionable target for novel therapeutics in this disease state, and that the interaction between estrogen and GH may play an important role in mediating sex differences in NAFLD and NASH.

We additionally hypothesized that IGF-1R would be pathologically expressed specifically on hepatocytes of patients with liver damage due to NAFLD/NASH. We were unable to demonstrate IGF-1R expression in hepatocytes or hepatic tissue of individuals with NAFLD and NASH by immunohistochemistry, findings that were likely limited by technical factors, as gene expression analysis did demonstrate consistent IGF-1 receptor expression across all severity of NAFLD. In contrast, immunohistochemistry methods were able to successfully demonstrate robust GH receptor staining localized to human hepatocytes, with intensity and zonality that was not affected by the severity of NAFLD or NASH.

Sex differences in GH receptor gene expression are of particular interest, as GH receptor expression was higher in premenopausal women (presumed by age) with high estrogen exposure compared to men. However, no differences were seen in IGF-1 gene expression by sex or menopausal status. These data are consistent with animal models demonstrating higher levels of hepatic GH receptor binding sites in females versus males as well as increased expression of hepatic GH receptor in rats with estrogen exposure (33–35). Estrogen is known to induce hepatic GH resistance by inhibiting GH receptor signaling via the JAK/STAT pathway (36), although IGF-1 levels and age-adjusted normal ranges do not differ between men and women (37). Therefore, these data may suggest that GH receptor is potentially upregulated in the presence of estrogen in order to maintain similar serum IGF-1 levels in women as in men. This is of particular interest given that premenopausal women are relatively protected from the development of NAFLD (38), particularly until menopause (39), and that postmenopausal women in particular have a higher risk of NASH and advanced fibrosis as compared to men (38,40,41). It is possible that GH regulation of lipolysis and IGF-1 production may be somewhat uncoupled, with endogenous estrogen inducing GH resistance with respect to IGF-1 production but not lipolysis (42). Further investigation of the interaction between estrogen and the GH axis as a contributor to sex differences in the development of NAFLD and progression to NASH is needed to define this complex pathophysiology.

This study also demonstrated that hepatic IGF-1 gene expression significantly decreased with increasing histologic severity of NAFLD, including when controlled for age and BMI. While we were unable to obtain serum IGF-1 levels to correlate with hepatic IGF-1 gene expression, data from the current study are concordant with our findings from a prior cohort that hepatocyte ballooning, lobular inflammation, NASH and fibrosis were associated with lower serum IGF-1 levels (32).

Improved understanding of the mechanisms and impact of GH and IGF-1 on hepatic steatosis, inflammation and fibrosis could lead to additional insights into the pathophysiology of this multifactorial disease process. However, teasing apart the specific effects of GH and IGF-1 is challenging, as interruption of GH signaling leads to a concurrent decline in IGF-1 levels and reconstitution of functional GH signaling leads to a concurrent restoration of IGF-1. GH has known lipolytic and anti-inflammatory effects, and disruption of GH signaling pathways in preclinical models lead to hepatic steatosis (9–15) as well as inflammation (9,43) and fibrosis (9). While IGF-1 is not lipolytic, it improves insulin sensitivity, and there are preclinical and clinical data that implicate IGF-1 in hepatic regeneration and fibrosis reduction. However, it is not known whether these regenerative effects are mediated through hepatocytes, hepatic stellate cells or both. Overexpression of IGF-1 receptor in hepatic stellate cells has been shown to enhance hepatic regeneration in cirrhotic rats by reducing stellate cell activation and decreasing fibrogenesis (44). The IGF-1 receptor is not expressed in normal hepatocytes in adult mice (44) or in humans (25,45). However, hepatocyte IGF-1 receptor expression has been demonstrated in the presence of liver damage, suggestive of a compensatory regenerative response. IGF-1 receptor expression has been demonstrated in mouse hepatocytes post injury by hepatectomy (25), and a hepatocyte specific IGF-1 knockout mouse demonstrated reduced regeneration with corresponding reduction in the IGF-1 receptor, IRS-1/ERK signaling pathway (45).

Adults with chronic hepatitis C have increased hepatic and hepatocyte expression of IGF-1 receptor mRNA that normalizes after virologic response to treatment (26). Finally, Alisi *et al.* were able to demonstrate higher hepatic IGF-1R expression with increasing levels of fibrosis in a pediatric cohort, although this may have predominantly been due to an increased expression of IGF-1 receptor specifically in hepatic stellate cells (27). We were unable to make conclusions about IGF-1R expression in hepatocytes in the setting of NAFLD and NASH from this investigation due to technical factors, as no staining was seen in any hepatic tissue even when utilizing an anti-IGF-1R antibody that had been successfully validated in breast cancer tissue.

Finally, it is important to acknowledge the newly proposed nomenclature of Metabolic Associated Fatty Liver Disease (MAFLD), which is defined as fatty liver with associated with one of the following: 1) overweight/obesity, 2) diabetes or 3) evidence of metabolic dysfunction (46,47). This MAFLD nomenclature seeks to provide an alternative to the term nonalcoholic fatty liver disease, which is a diagnosis of exclusion that also carries the stigmata of being defined by extent of alcohol use. The MAFLD definition sets up a scaffolding by which patients with fatty liver can be further characterized and studied, both in clinical care and research. In fact, all subjects in this study meet criteria of hepatic steatosis with overweight/obesity, and thus could be characterized as having MAFLD. Perhaps in the future, characterization of the GH/IGF-1 axis could be utilized as a biomarker to identify a novel subtype of MAFLD associated with this relative deficiency of the endogenous hormone axis.

This study was limited by a small sample size in the immunohistochemistry cohort, which was limited by our goal of strict matching on age, sex, BMI and diabetes status. However, the sample size was robust in the gene expression analysis cohort. Additionally, we were unable to localize IGF-1 receptor expression, despite attempting to utilize three different anti-IGF-1 antibody receptors. In the gene expression analysis cohort, we were unable to quantify GH gene expression, which was not included in our Nanostring nCounter gene panel. We additionally did not have estradiol levels and/or specific information regarding menopausal status, thus used aged as a proxy for this analysis.

In conclusion, our study is the first to demonstrate preserved expression of the IGF-1R and GHR despite worsening severity of NAFLD and NASH in adults. Additionally, this is the first study in humans to demonstrate that women, and particularly premenopausal women, have higher levels of hepatic GHR expression as compared to men, which possibly underlies sex differences in NAFLD and NASH. Additional research is needed regarding the contribution of the GH/IGF-1 axis to the pathophysiology of NAFLD and NASH as well as the potential impact of GH administration on hepatic endpoints in this highly prevalent and morbid disease.

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Conflict of Interest Statement:

KEC serves on the scientific advisory board for Novo Nordisk, Theratechnologies and Bristol Myers Squibb (BMS) and has received grant funding from Boehringer-Ingelheim, BMS and Novartis. LED has received drug donation from Pfizer by investigator-initiated request. DWG is a consultant to Boston Scientific, Ethicon J&J Medical Devices and Medtronic and serves on the medical advisory board for Boston Scientific, and New View Surgical. All other authors have no conflicts of interest to declare.

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HIGHLIGHTS

- GH and IGF-1 have been implicated in the pathogenesis of NAFLD
- Lower IGF-1 expression is associated with more severe NAFLD
- Expression of IGF-1 and GH receptors is unchanged with increasing severity of NAFLD
- The GH/IGF-1 axis is a potential therapeutic target in NAFLD

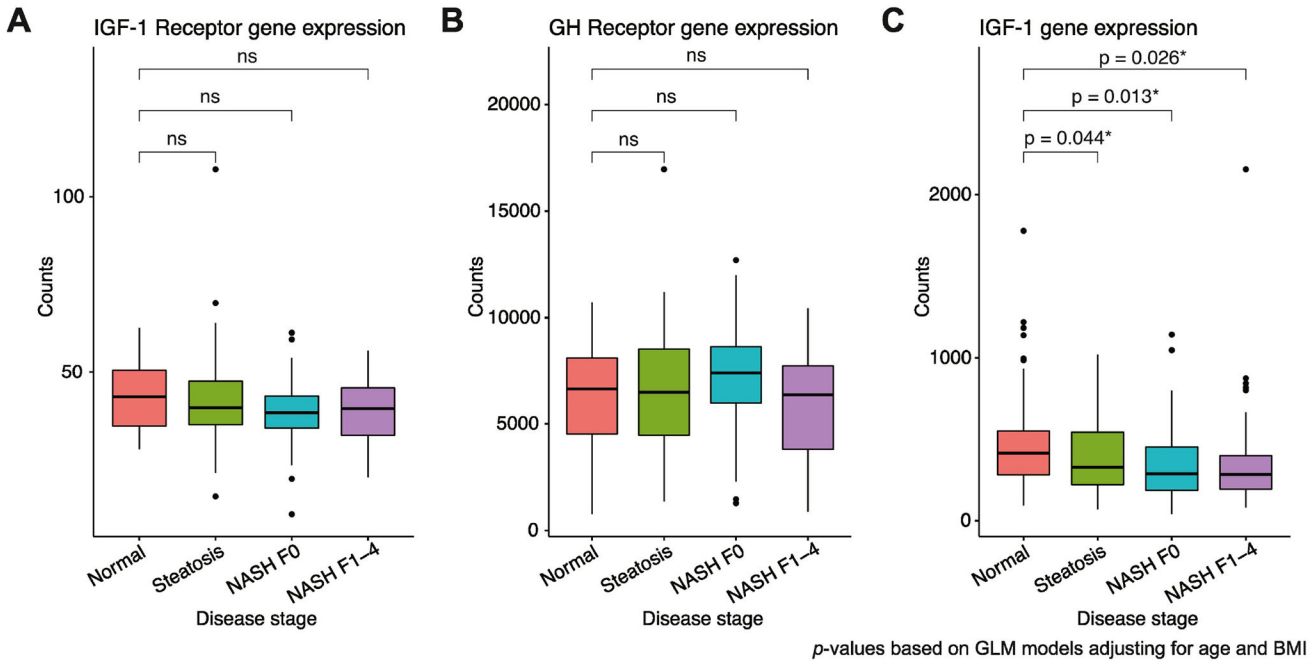

Figure 1:

Gene expression analysis for (A) IGF-1 receptor, (B) GH receptor and (C) IGF-1 across worsening stages of NAFLD/NASH. Raw data are plotted. P-values based on generalized linear models adjusted for age and BMI. Box plot lower and upper hinges correspond to the 25th and 75th percentiles, respectively. Lower whisker extends to the smallest value at most 1.5 times the interquartile range and upper whisker extends to the highest value no further than 1.5 times the interquartile range. Data points beyond the whiskers are plotted as individual outliers.

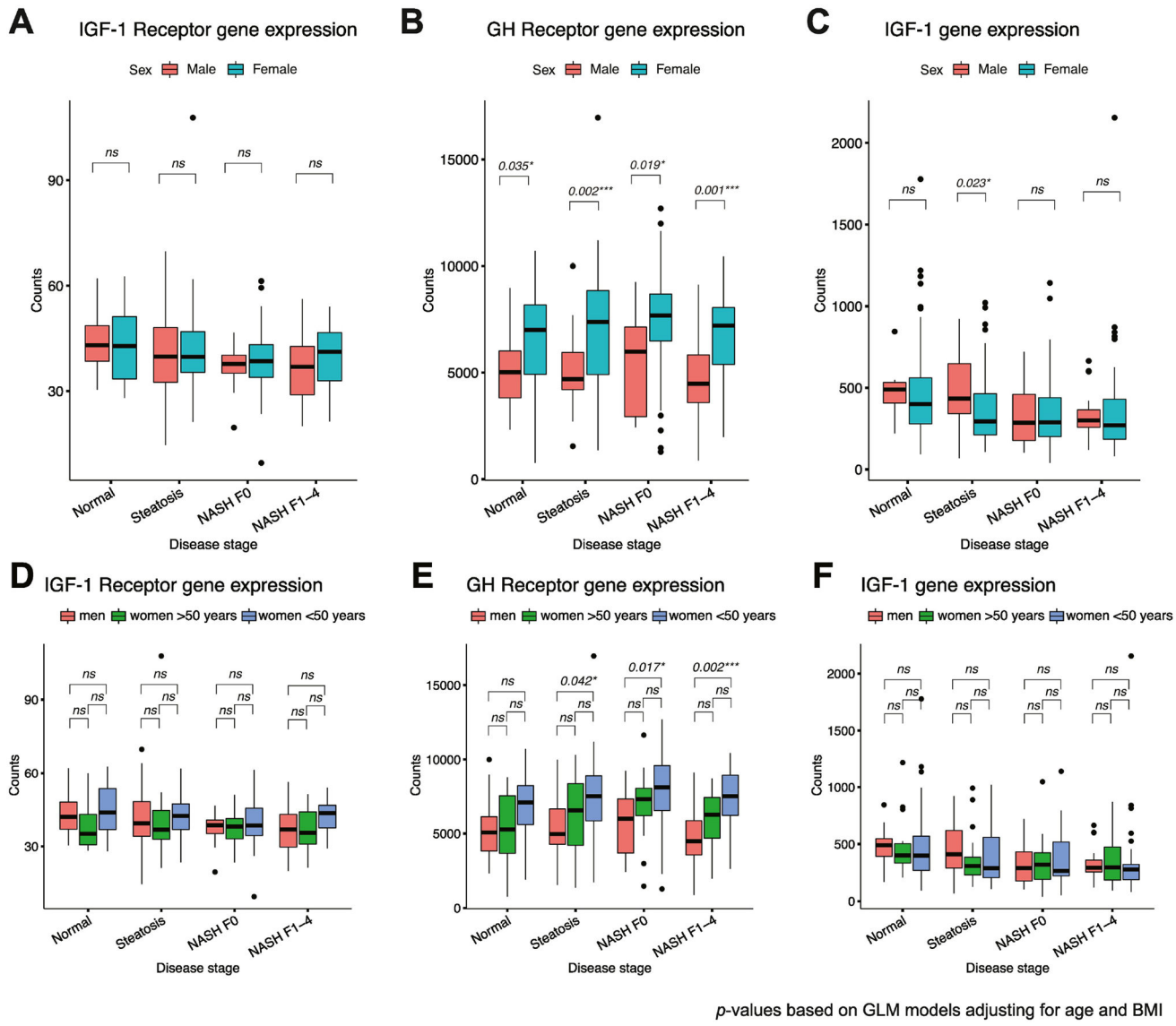

Figure 2:

(A) IGF-1 receptor, (B) GH receptor and (C) IGF-1 gene expression by sex and presumed menopausal status (premenopausal <50 years and postmenopausal ≥50 years). Box plot lower and upper hinges correspond to the 25th and 75th percentiles, respectively. Lower whisker extends to the smallest value at most 1.5 times the interquartile range and upper whisker extends to the highest value no further than 1.5 times the interquartile range. Data points beyond the whiskers are plotted as individual outliers.

Table 1:

Gene Expression Analysis Cohort Clinical Characteristics

	Normal Liver Histology n=76	Steatosis n=88	NASH F0 n=72	NASH F1–F4 n=82	Overall n=318	P-value
Age, years	41.6±11.5	45.2±11.7	43.9±12.2	45.1±12.9	44.0±12.1	0.146
BMI (kg/m^2)	45.7±6.6	46.1±7.2	47.4±7.6	47.9±7.3	46.8±7.2	0.169
Female, n (%)	65 (85.5)	65 (73.9)	59 (81.9)	54 (65.9)	243 (76.4)	0.018
Race, n (%)						
Black or African American	36 (47.4)	27 (30.7)	22 (30.6)	13 (15.9)	98 (30.8)	0.014
White	36 (47.4)	58 (65.9)	49 (68.1)	68 (82.9)	211 (66.4)	
Other	1 (13)	1 (11)	0 (0.0)	1 (12)	3 (0.9)	
Unknown	3 (3.9)	2 (2.2)	1 (14)	0 (0.0)	6 (1.9)	
Ethnicity, n (%)						
Hispanic	4 (5.3)	6 (6.8)	3 (4.2)	6 (7.3)	19 (6.0)	0.011
Diabetes, n (%)	6 (7.9)	20 (22.7)	21 (29.2)	46 (56.1)	93 (29.2)	<0.001
Dyslipidemia, n (%)	17 (22.4)	27 (31.0)	27 (37.5)	44 (53.7)	115 (36.3)	<0.001
ALT (U/L)	25±2	29±2	32±2	42±2	32±2	<0.001
AST (U/L)	18±2	20±2	19±2	28±2	21±2	<0.001
Creatinine (mg/dL)	0.86±0.48	0.73±0.17	0.78±0.15	0.83±0.22	0.80±0.29	0.428

Values for categorical variables are expressed as n (percentage) and continuous variables are presented in the form of mean±SD. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; NASH, nonalcoholic steatohepatitis. Data adapted from Subudhi *et al.*, Hepatology Communications, 2021.

Table 2:

Immunohistochemistry Cohort Demographics and GH Receptor Staining

	NLH n=10	Steatosis n=10	NASH n=10	P-value
Cohort Characteristics				
Age, years (mean±SD)	59±5	56±9	55±8	NS
BMI (kg/m^2)	43±7	46±5	45±7	NS
Male, n (%)	5 (50)	5 (50)	5 (50)	NS
Race				
White, n (%)	10 (100)	8 (80)	9 (90)	NS
Other, n (%)	0 (0)	2 (20)	1 (10)	
Hispanic Ethnicity, n (%)	0 (0)	0 (0)	1 (10)	NS
Diabetes, n (%)	10 (100)	10 (100)	10 (100)	NS
NAFLD Activity Score (NAS)[#]	0.1±0.3[a]	1.1±0.3[b]	5.9±1.0[c]	<0.0001
Fibrosis stage	0±0[a]	0±0[a]	2.4±0.5[b]	<0.0001
Immunohistochemistry Results				
GH Receptor Staining Intensity				
Low (weak)	4	7	4	NS
High (moderate-strong)	6	3	6	NS
GH Receptor Zonality				
Centrilobular (Z3>1)	7	4	7	NS
Absent	2	6	3	NS
Peri-portal (Z1>3)	1	0	0	NS

[#]NLH NAFLD Activity Score (NAS) was >0 due to one NLH subject being classified as having mild liver fat based on blinded assessment post group assignment. Superscript letters indicate statistical difference across different letters. Abbreviations: NLH, normal liver histology and BMI, body mass index.