GEORGETOWN SCIENTIFIC RESEARCH JOURNAL

Volume One
Edition One
February 2021

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Georgetown Scientific Research Journal
https://doi.org/10.48091/LKML8578

Determinants of Hepatocellular Carcinoma in the United States: Differences in Risk Factor and Genetic Susceptibility by Race/Ethnicity

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Abstract

Background: Hepatocellular carcinoma (HCC) is one of the few cancers with an increasing incidence and mortality worldwide. This study aims to determine the contribution of known risk factors for HCC by race and ethnicity.

Methods: Data on race, ethnicity, age, and gender were obtained from National Health and Nutrition Examination Survey (NHANES). Population attributable fractions (PAFs) of risk factors were estimated using non-invasive scoring measures of Hepatitis B and C virus infection, excessive alcohol use, smoking, diabetes and emerging metabolic risk factors [non-alcoholic steatohepatitis advanced cirrhosis (NASH) and non-alcoholic fatty liver disease-advanced fibrosis (NAFLD-fib)] over a 10-year period, 1999-2002 and 2009-2012. Genetic analysis was performed using DisGenet platform by attaining the top enriched genes strongly related to HCC. Furthermore, cytoscape network was used to form a gene-disease network association.

Results: NASH-cirrhosis increased in the overall population and among all race and ethnic groups. Both liver fat accumulation and ALT levels vary among different populations; however, Hispanics have the highest prevalence of NAFLD and elevated ALT levels. Non-Hispanic (NH) blacks and Hispanics had a 3 to 4 times higher PAF for HCC than whites attributed due to chronic liver diseases, including NASH-cirrhosis and NAFLD-fib. Our genetic analysis demonstrated that PNPLA3 polymorphism is strongly associated with NAFLD-fib, which appears to represent susceptibility to liver disease among the Hispanic community.

Conclusion: Hispanics and NH blacks are at a disproportionately higher risk for HCC in part due to the higher prevalence of liver disease comorbidities, including NASH-cirrhosis and NAFLD-fib. Compared

https://doi.org/10.48091/LKML8578

to NH whites, Hispanics and NH blacks have a higher baseline risk for liver cancer due to non-metabolic factors, which may include a genetic susceptibility. Metabolic risk factors have increased and are now contributing to nearly half of HCC cases in the US.

Keywords: non-alcoholic fatty liver disease advanced fibrosis; hepatocellular carcinoma; non-alcoholic steatohepatitis; metabolic risk factors; non-metabolic risk factors; population attributable fraction

1. Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer related deaths worldwide.[1] In the US, HCC incidence and mortality rates are increasing at a rate of 3% per year and are distributed disproportionally among certain racial/ethnic groups. [2]

HCC most often occurs among individuals who have chronic liver diseases. Nonalcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are growing and becoming the leading risk factors for HCC. NAFLD-fib and its subtype NASH-cirrhosis affect approximately 30% and 5%, respectively, of the US population.[3] The major risk factors are hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, cigarette smoking, excessive alcohol consumption, hereditary genetic diseases, and metabolic disorders (diabetes, obesity, impaired glucose tolerance, metabolic syndrome, and non-alcoholic fatty liver disease).[4] Some risk factors of metabolic diseases have been shown to be more prominent in certain ethnic groups. Data from the United States National Center for Health Statistics (2000-2006) identified chronic liver diseases as the sixth most common cause of death in the Hispanic population.[5] Obesity and diabetes are highly prevalent among both Hispanic and non-Hispanic (NH) Blacks due to lifestyle choices, diet, or genetic polymorphism, which causes all race-related genetic differences between different groups. The proportion of incident cases of heavy drinkers in the United States between 1984 and 1992 was highest among NH blacks (51%), followed by Hispanics (43%) and whites (32%). There are several lines of evidence suggesting that NH blacks who consume alcohol have greater liver enzyme elevation than whites, which further leads to liver disease .[6,7]

The prevalence of NAFLD-fib and risk of progression is higher among Hispanics than other racial and ethnic groups.[8] The higher incidence of HCC among Hispanics is driven by higher levels of sugar, carbohydrates and intake of saturated fat as compared to whites. Obesity and insulin resistance, two important risk factors for the metabolic syndrome, have been found to have a positive correlation with NASH-cirrhosis in Hispanic persons only.[5] Hispanics and NH blacks have also been shown to have higher HCC rates than whites. Cirrhosis rates are higher for NH blacks than for whites, and the highest cirrhosis mortality rates are observed among Hispanics.[7] Mortality from chronic liver disease in Hispanic people in the United States is nearly 50% higher than in NH white persons (13.7 per 100,000 in Hispanic persons vs 9.2 in NH whites and 7.5 in African American persons).[5]

In addition to known HCC risk factors, it is likely that access to preventive health education and early treatment may be a barrier to some racial and ethnic groups. The incidence of HCC varies by race and ethnicity primarily as a result of differences in the prevalence of major risk factors and also disparities in access to high-quality healthcare.[9] Socioeconomic disadvantage, lack of health insurance, and language barriers limit access to cancer screening and treatment among NH blacks and Hispanics.[10] It has also been found that

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Hispanics and NH blacks were less likely to be diagnosed with early-stage HCC compared with whites.[10]

There is a need to understand how HCC risk factors contribute to HCC prevalence rates within racial and ethnic groups, to reduce health-care disparities. The aim of this study was to determine contribution of specific known risk factors for HCC by race and ethnicity, using a nationally representative US population. A further network study of gene specificity and HCC was conducted.

1. Methods

1.1 Study Population

The National Health and Nutrition Examination Survey (NHANES) is a biennial cross-sectional survey representative of the US civilian, non-institutionalized population. Details of the NHANES methods and sampling strategy have been described by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC).[11] Briefly, subjects were recruited though a multistage probability sampling design, which was used to select participants representative of the civilian, non-institutionalized US population, with a sample weight assigned to each person.[12] Next, each subject was interviewed and underwent a physical examination, including a blood draw. General demographic characteristics, including age, sex, race/ethnicity (non-Hispanic white, non-Hispanic Black, Mexican American, other Hispanic, other race including Asian descent and multiracial (other/mixed)), and smoking behavior were collected during the Mobile Examination Center interview stage. During the examination, body measurements, including height, weight, and waist circumference (cm) were also collected. Serum samples were obtained and analyzed for albumin (g/dL), alanine aminotransferase (ALT, U/L), aspartate (aminotransferase (AST, U/L), alkaline phosphatase (U/L), fasting glucose (mg/dL), fasting insulin (uU/mL), gamma-glutamyl transpeptidase (GGT, U/L), platelet count (1000 cells/μL), total bilirubin (mg/dL), hemoglobin A_{1C} (%), total cholesterol (mg/dL), high density lipoprotein (HDL), low density lipoprotein (LDL) cholesterol (mg/dL), and triglycerides (mg/dL). All participants provided informed consent. NHANES is approved by the Institutional Review Board of the CDC. Subjects who were less than 18 years old or pregnant were excluded from this analysis (**Figure 1**).

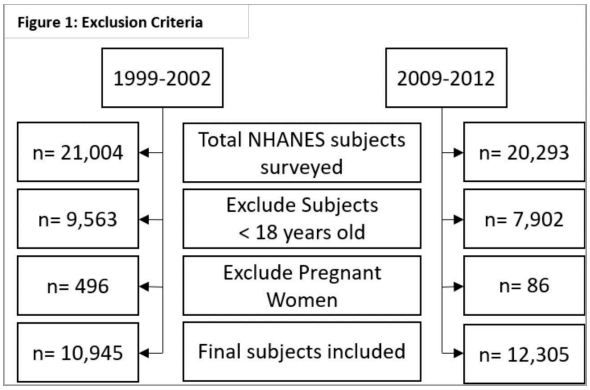

Figure 1. Exclusion Criteria

The prevalence of HCC risk factors was determined using interview, physical exam, and/or laboratory NHANES data. Hepatitis C virus was defined as having a positive hepatitis C virus antibody (anti-HCV) in laboratory testing. Hepatitis B viral infection was defined as having a positive surface antigen (HBsAg) on laboratory testing. Persons were identified as smokers when they reported current smoking on the NHANES questionnaire. Men who reported consuming more than 14 drinks per week and women who reported more than 7 drinks per week were defined as excessive drinkers.[13]

Of the metabolic risk factors, obesity was defined by BMI greater than or equal to 30 from body measurements taken on physical exam. Metabolic syndrome was defined using the

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International Diabetes Federation definition.[14] Persons with any three of the following five criteria were defined as having metabolic syndrome: 1) Elevated waist circumference (men >102cm, women >88cm) which was measured during physical exam; 2) Elevated triglycerides (>= 150 mg/dL or currently taking prescription to lower lipids); 3) Reduced high-density lipoprotein (<40mg/dL for males or <50mg/dl for females); 4) Hypertension (blood pressure measurements greater than 140mg/dL for systolic blood pressure or greater than 90 for diastolic); or 5) Elevated fasting glucose (≥100mg/dL). Diabetes was defined as having answered yes to the questionnaire question of "Have you ever been diagnosed by a physician as having diabetes?" or "Are you currently taking a blood glucose lowering medication?", and/or having a fasting glucose level greater than 126 mg/dL, or having a hemoglobin A_{1C} level greater than 6.5%.

Cirrhosis was defined as having an AST-to-platelet ratio index (APRI) >2 and any one of the following abnormal liver function tests: 1) elevated ALT levels (>40 U/L for men or >30 U/L for women); 2) Elevated alkaline phosphatase (>113 U/L); or 3) elevated total bilirubin (>1.3 mg/dL). NAFLD-advanced fibrosis (NAFLD-fib) was defined using three different noninvasive formulas: hepatic steatosis index (HSI), the FIB-4 index (FIB4), and the NAFLD fibrosis score (NFS).[15,16] Persons who had fatty liver based on the HSI and had fibrosis based on the FIB4 and/or the NFS were defined as having NAFLD-advanced fibrosis.

$$\boldsymbol{HSI} = 8 \times \left(\frac{ALT}{AST}\right) + BMI \ [+2 \ if \ Diabetes, +2 \ if \ Female]$$

$$\boldsymbol{FIB4} = \frac{Age_{\ Years} \times AST \ (\frac{U}{L})}{Platelet \ Count \ \left(\frac{10^9}{L}\right) \times \sqrt{ALT \ (\frac{U}{L})}}$$

$$\boldsymbol{NAFLD\ Fibrosis\ Score} = \frac{-1.675 + 0.037 \ (Age_{Year}) + 0.094 \ (BMI) + 1.13(Fasting \ Glucose)}{(Diabetes \ (yes = 1, no = 0)) + 0.99 \left(\frac{AST}{ALT}\right) - 0.013 \left(Platelet \ Count(\frac{10^9}{L}\right) - 0.66(Albumin \left(\frac{g}{L}\right))}$$

NHANES does not include genetic disorders which are risk factors for HCC or impaired glucose tolerance. Thus, these risk factors could not be included in our analysis.

1.2 Statistical Analysis.

This analysis used the required weighting procedures to account for the survey design of NHANES. Descriptive analyses were done to compare the NHANES population in 1999-2002 to the NHANES population in 2009-2012. Categorical variables were compared using χ^2 tests. Continuous variables were compared using the Students t-test after confirming all data were normally distributed. Age-adjusted prevalence rates for HCV infection, HBV infection, smoking, excessive alcohol use, obesity, diabetes, NASH-cirrhosis, and NAFLD-fib for the two four-year time periods were calculated using the projected population of the United States for the year 2000.[17]

To determine the predicted contribution of each risk factor towards the development of HCC, the population attributable risk was calculated. A medical literature review was done to find the relative risk (RR) of each risk factor (HCV, HBV, etc.) towards the development of HCC. The literature review was done using PubMed and with the term for each risk factor, risk, and hepatocellular carcinoma.

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Results were sorted by year of publication with US populations, and recent meta or pooled analyses preferred. The population attributable fraction (PAF) for each risk factor was then calculated using the formula developed by Levin for each sex and race group.[18]

$$Population\ Attributable\ Fraction = \frac{Risk\ Prevalence\ (RR_{Exposure} - 1)}{Risk\ Prevalence\ (RR_{Exposure} - 1) + 1}$$

In determining the PAF, the risk factors were analyzed independently, without accounting for interaction between their effects.

The combined effect of all risk factors and risk factors by type (metabolic vs. non-metabolic) were calculated using the formula below:

$$Total\ Attributable\ Fraction = 1 - \prod_{i=1}^{n}(1 - PAF_i)$$

All statistical analyses were conducted using SAS 9.4 (Cary, NC) with p <0.05 considered significant. Figures were developed using GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA.

The top enriched pathogenic genes associated with liver diseases were analyzed using the DisGenet platform (Table 1).[19] The DisGeNet database uses information of human gene-disease association (GDAs) and variant-disease association (VDAs) from expert curated repositories. The GDA score was calculated using the formula developed by DisGeNet. Scoring (gda) was used to rank the gene-disease according to their level of evidence.[19]

The DisGeNET Score (S) for GDAs is computed according to:

$$S = C + M + I + L$$

$$C = \begin{cases} 0.6 & if\ N_{sources_i} > 2 \\ 0.5 & if\ N_{sources_i} = 2 \\ 0.3 & if\ N_{sources_i} = 1 \\ 0 & otherwise \end{cases}$$

where:

$N_{sourcesi}$ is the number of CURATED sources supporting a GDA

i ∈ CGI, CLINGEN, GENOMICS ENGLAND, CTD, PSYGENET, ORPHANET, UNIPROT

$$M = \begin{cases} 0.2 & if\ N_{sources_j} > 0 \\ 0 & otherwise \end{cases}$$

where:

j ∈ Rat, Mouse from RGD, MGD, and CTD

$$I = \begin{cases} 0.1 & if\ N_{sources_k} > 0 \\ 0 & otherwise \end{cases}$$

where:

k ∈ HPO, CLINVAR, GWASCAT, GWASDB

$$L = \begin{cases} 0.1 & if\ N_{pubs} > 9 \\ N_{pubs} * 0.01 & if\ N_{pubs} < 9 \end{cases}$$

where:

N_{pubs} is the number of publications supporting a GDA in the sources LHGDN and BEFREE

DisGeNET uses two other metrics to facilitate the ranking of the genes associated with hepatocellular carcinoma. The Disease Specificity Index (DSI) was used, which is inversely proportional to the number of diseases associated to gene. A gene associated with multiple diseases

https://doi.org/10.48091/LKML8578

gets a score close to zero, and a gene associated with only one disease has DSI of 1.[18] It is computed according to :

$$DSI = \frac{log_2(\frac{N_d}{N_T})}{log_2(\frac{1}{N_T})}$$

where:

N_d Is the number of diseases associated to the gene/variant

N_T is the total number of diseases in DisGeNET

The Disease Pleiotropy Index (DPI) was the second metric used to rank the genes. It ranges from 0 to 1 and is proportional to the number of different (MeSH) disease classes a gene is associated with. The DPI is computed according to :

$$DPI = (\frac{N_{dc}}{N_{TC}}) * 100$$

where:

N_{dc} is the number of the different MeSH disease classes of the diseases associated to the gene/variant

N_{TC} is the total number of MeSH diseases classes in DisGeNET.

Furthermore, cytoscape network was used to form a gene-disease association to visualize interaction among different genes (Figure 2). Cytoscape is an open-source platform for visualizing molecular interactions.[20]

1. Results

There were 10,945 individuals in the 1999-2002 sample and 12,305 in the 2009-2012 sample (**Figure 1**). The groups did not differ among the distribution of sex, age, or race/ethnicity. The mean age at screening was 45.4 (1999-2002) and 46.4 (2009-2012) and 50.7% of the participants were female (**Table 2**).

Figure 2: *HCC hub gene*: Gene network representing the interaction of most significant genes involved in HCC

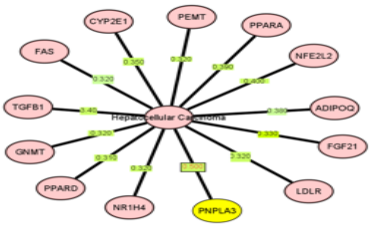

Figure 2: Each gene is associated with a score generated by DisgGeNET platform. Green score (0.500)represents how strongly associated each gene is with HCC. Based on the scoring and our genetic analysis demonstrates PNPLA3 gene represents the strongest gene associated with HCC. Each gene node in the figure is representing association of the gene in the process of tumorigenesis and have an important influence on the occurrence of HCC.

Table 1. Genes associated with hepatocellular carcinoma from the DisGeNET database

Gene	DSI	DPI	GDA score
PNPLA3	0.556	0.692	0.500
NFE2L2	0.357	0.885	0.400
PPARA	0.432	0.885	0.390
ADIPOQ	0.376	0.885	0.380
CYP2E1	0.459	0.692	0.350
TGFB1	0.287	0.962	0.340
FGF21	0.485	0.769	0.330
LDLR	0.449	0.885	0.320
FAS	0.372	0.923	0.320
PEMT	0.653	0.538	0.320
NR1H4	0.513	0.808	0.320
GNMT	0.626	0.462	0.320
PPARD	0.513	0.846	0.310

DSI = disease specificity index

DPI = disease pleiotropy index

Score $_{GDA}$ = ranking of the gene-disease association

In the overall NHANES population, obesity, excessive alcohol consumption, and smoking were the most prevalent HCC risk factors in both 1999-2002 and 2009-2012. The prevalence of HBV, HCV, or excessive alcohol consumption did not change over the ten-year period. All metabolic risk factors (obesity, diabetes, NAFLD-fib, NASH-cirrhosis) increased over the ten years of study in the overall population (**Table 2**). Concurrently, BMI, waist circumference, fasting glucose levels, and triglyceride levels all increased between the two time periods (all $p < 0.001$, **Supplemental Table 1**).

Table 2. Participant Characteristics

Characteristics		Survey Period		
		1999-2002	2009-2012	p-value
Demographics	Gender			0.15
N %	Male	5395 (49.3)	6072 (49.4)	
	Female	5550 (50.7)	6233(50.7)	
	Age at Screening (years) *	45.4(0.33)	46.4(0.51)	0.22
	Race/Ethnicity			0.19
	Non-Hispanic White	5132(46.9)	5158(47.9)	
	Non-Hispanic Black	2163(19.8)	2734(22.2)	
	Hispanic	3273(29.9)	3065(24.9)	
	Mixed/Other	377(3.44)	1348(10.9)	
	Smoking Status			<0.001
	Current Smoker	2125(24.5)	2441(20.3)	
	Former Smoker	2625(26.8)	2768(23.7)	-
Disease Prevalence	Hepatitis C Virus	2.0(54)	1.6(48)	0.37
% Prevalence (Population Attributable Fraction)	Hepatitis B Virus	0.2 (5)	0.4(7)	0.67
	Excessive Alcohol	23.8 (12)	21.6(12)	0.83
	Current Smokers	24.5 (12)	20.3(10)	<0/001
	Diabetes	9.8(9)	11.8(11)	<0.001
	Obesity	29.9(25)	35.2(28)	<0.001
	NASH-Cirrhosis	0.07(3)	0.2(6)	0.02
	NAFLD-fib	1.5(1.4)	4.0(3.7)	<0.001

NASH = non-alcoholic steatohepatitis; NAFLD-fib = non=alcoholic fatty liver disease advanced fibrosis

Supplemental Table 1: Metabolic Measures

Metabolic Measures	1999-2002		2009-2012		
	mean	s.e.m.	mean	s.e.m	p-value
BMI (kg/m^2)	27.9	0.13	28.6	0.49	<0.001
Waist Circumference (cm)	95.3	0.16	98.1	0.16	<0.001
Mean Systolic Pressure (mmHg)	123.2	0.42	121.1	0.41	<0.001
Fasting Glucose (mg/dL)	104.3	0.55	107.1	0.45	<0.001
Fasting Insulin (uU.mL)	13.0	0.34	13.8	0.26	0.17
Cholesterol, total (mg/Dl)	197.9	0.78	193.1	0.73	<0.001
HDL-cholesterol (mg/Dl)	51.2	0.36	52.9	0.59	<0.001
LDL-cholesterol (mg/Dl)	122.4	0.96	115.0	0.71	<0.001
Triglycerides	144.6	2.62	150.4	2,24	>0.001
Fasting Glucose > 100 (%)	16.0	0.56	19.5	0.63	<0.001
Albumin(g/dL)	4.37	0.01	4.29	0.01	<0.001
ALT (U/L)	25.9	0.42	25.4	0.23	0.44
AST (U/L)	24.4	0.22	25.9	0.16	<0.001
GGT (U/L)	84.9	0.49	69.0	0.24	<0.001
Platelet count (SI)	268.5	1.39	239.0	1.13	<0.001
Total Bilirubin (mg/dL)	0.58	0.01	0.74	0.01	<0.001
GGT (U/L)	30.3	0.46	26.6	0.50	<0.001
Iron (ug/dL)	87.2	0.39	84.2	0.34	<0.001

s.e.m. = standard error of the mean; ALT= Alanine aminotransferase; AST=Aspartate aminotransferase; GGT= Gamma-Glutymyl Transpetidase;

Both NASH-cirrhosis and NAFLD-fib increased in prevalence between 1999-2002 and 2009-2012. The prevalence rates of NAFLD-fib increased from 1999-2002 (1.53%) to 2009-2012 (4.0%) (p<0.001), while NASH-cirrhosis

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increased from 0.07% to 0.20%, p=0.03). This represents a 164% increase for NAFLD-fib and 186% increase for NASH-cirrhosis overall. The largest increase of NAFLD-fib occurred among the Hispanic population (a 296% increase, p<0.001) (**Table 3**).

Table 3A and B: Hepatocellular Carcinoma Risk Factor Prevalence and PAF by Sex and Race/Ethnicity

Table3a: Hepatocellular Carcinoma Risk Factor Prevalence and PAF Stratified by Sex and Race/Ethnicity Subgroup (1999-2002)

		HCV		HBV		Excessive alcohol		Current smoker		Diabetes		Obesity		NASH cirrhosis		NAFLD-fib	
		Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev (%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF (%)
Overall	Total	**2.0**	**54**	**0.2**	**5**	**23.8**	**12**	**24.5**	**12**	**9.8**	**9**	**29.9**	**25**	**0.07**	**3**	**1.5**	**1**
	M	**2.6**	**52**	**0.3**	**7**	**22.3**	**11**	**26.9**	**13**	**11.2**	**10**	**26.7**	**23**	**0.05**	**2**	**1.5**	**1**
	F	**1.4**	**53**	**0.2**	**2**	**26.9**	**13**	**22.1**	**11**	**8.5**	**8**	**32.8**	**27**	**0.09**	**4**	**1.6**	**1**
NH White N=5132	**Total**	1.9	53	0.1	1	24.5	12	25.0	12	8.4	8	28.7	25	0.08	4	1.3	1
	M	2.4	60	0.0	0	24.1	12	26.1	13	10.7	10	27.2	24	0.04	2	1.1	1
	F	1.3	34	0.1	0	25.3	13	24.0	12	6.1	6	30.1	25	0.13	6	1.4	1
NH Black N=2163	**Total**	4.1	70	0.8	19	22.9	11	26.5	13	14.2	13	38.9	31	0	0	1.9	1
	M	6.0	78	0.8	10	18.8	9	33.9	16	10.6	10	26.8	23	0	0	1.2	1
	F	2.6	62	0.7	3	30.9	14	20.5	10	16.8	15	48.7	35	0	0	2.5	1
Hispanic N=3273	**Total**	2.0	44	0.0	0	22.3	10	22.9	11	14.1	12	30.1	25	0.10	4	1.3	1
	M	2.0	53	0.1	2	22.1	10	27.2	13	13.5	12	23.1	21	0.23	9	1.2	1
	F	1.8	52	0.0	0	24.1	9	18.9	9	13.9	12	36.5	29	0	0	1.3	1
Other/Mixed N=377	**Total**	1.1	40	2.3	8	28.4	13	22.3	11	17.9	15	20.3	19	0	0	0.8	1
	M	1.4	35	3.6	5	27.6	12	31.4	15	18.3	16	22.3	20	0	0	0.7	1
	F	1.0	36	0.9	21	28.2	16	14.3	7	17.5	15	19.2	18	0	0	0.9	1

Table 3B. Hepatocellular Carcinoma Risk Factors Prevalence and PAF Stratified by Sex and Race/Ethnicity Subgroup (2009-2012)

		HCV		HBV		Excessive Alcohol		Current Smokers		Diabetes		Obesity		NASH cirrhosis		NAFLD-fib		
		Prev (%)	PAF (%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev(%)	PAF(%)	Prev (%)	PAF(%)	Prev(%)	PAF(%)	Prev (%)	PAF(%)	
Overall	**Total**	**1.6**	**48**	**0.4**	**7**	**21.6**	**12**	**20.3**	**10**	**11.8**	**11**	**35.2**	**28**	**0.20**	**8**	**4.0**	**4**	
	M	**2.2**	**47**	**0.4**	**10**	**27.4**	**11**	**22.7**	**11**	**13.7**	**12**	**34.3**	**28**	**0.37**	**14**	**4.1**	**4**	
	F	**1.1**	**46**	**0.3**	**4**	**23.7**	**13**	**18.0**	**9**	**10.1**	**9**	**36.0**	**29**	**0.06**	**3**	**4.0**	**4**	
NH White N=5158	**Total**	1.6	49	0.1	2	21.3	12	21.1	10	9.3	9	33.2	27	0.19	8	3.5	3	
	M	2.1	56	0.2	1	27.1	11	21.9	11	11.5	10	34.0	28	0.43	16	3.6	3	
	F	1.2	31	0.0	0	26.6	13	20.3	10	7.4	7	32.4	27	0	0	3.4	3	
NH Black N=2734	**Total**	2.5	59	0.9	21	23.1	12	24.4	10	18.1	15	48.8	36	0.21	8	4.8	4	
	M	3.3	66	1.2	14	31.8	11	30.0	11	18.3	16	38.2	30	0.00	0	4.5	4	
	F	2.0	55	0.7	2	23.7	14	20.1	9	18.0	15	57.2	39	0.41	15	5.1	5	
Hispanic N=3065	**Total**	1.4	35	0.1	0	23.1	11	17.0	10	18.9	16	40.8	32	0.23	9	4.0	4	
	M	2.1	55	0.1	3	25.3	11	23.1	11	20.4	17	38.4	30	0.29	11	3.4	3	
	F	0.6	27	0.2	2	22.1	12	10.7	10	17.4	15	43.2	33	0.17	7	4.5	4	
Other/Mixed N=1348	**Total**	0.8	34	2.4	9	20.3	10	17.1	12	17.2	15	19.8	18	0.10	4	0.9	1	
	M	1.8	41	2.5	4	19.4	9	22.3	14	19.8	17	21.1	19	0.23	9	0.9	1	
	F	0.0	0	2.3	41	19.2	10	12.5	10	15.2	13	18.5	17	0	0	0.9	1	

Prev=prevalence (%); PAF= Population Attributable Fraction (%); M=males; F=Females; SEM=Standard error or the mean; HCV= Hepatitis C Viral infection; HBV= Hepatitis B viral Infection; NASH=Non-alcoholic steatohepatitis; NAFLD-fib=Non-alcoholic Fatty liver disease advanced fibrosis

The risk factors to decrease over the ten-year period were the number of current smokers (prevalence 24.5% to 20.3%, p=<0.001) and access alcohol. There were no temporal changes in the prevalence of excessive drinking behaviors overall, which remained between 23.8% and 21.6% (p = 0.83, **Table 2**).

1.1 Population Attributable Fractions

Overall, 77.9% of HCC cases from 1999-2002 and 76.2% of HCC cases from 2009-2012 could be attributed to the risk factors analyzed in this study. In the overall population, metabolic risk factors (diabetes, obesity, NAFLD-fib, NASH-cirrhosis) composed 35% of HCC cases in 1999-

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2002 and increased to accounted for 42% of HCC cases in 2009-2012. Synchronously, non-metabolic risk factors (HBV, HCV, excessive alcohol use, current smokers) decreased from a PAF of 66% in 1999-2002 to 59% in 2009-2012 (**Table 4**). Other/mixed category was removed from the study because it was not statistically significant enough to make conclusions about the PAF due to low sample size. Among both metabolic and non-metabolic risk factors, HCV was the single highest attributable cause of HCC in both time periods; 54.1% of HCC cases were estimated to be attributable to HCV in 1999-2002 and 48.4% in 2009-2012.

Table 4. PAF by Risk Category Stratified by Sex and Race/Ethnicity

Table 4A: PAF by Risk Category Stratified by Sex and Race/Ethnicity 1999-2002

		Metabolic	Non-Metabolic	Unknown	Total
Overall	**Total**	**34.9**	**66.0**	**22.1**	**77.9**
	M	**33.4**	**65.4**	**23.1**	**76.9**
	F	**36.4**	**64.6**	**22.5**	**77.5**
NH White	Total	13.1	63.7	31.5	68.5
	M	32.8	69.3	20.6	79.4
	F	34.5	49.0	33.4	66.6
NH Black	Total	40.4	81.1	11.3	88.7
	M	31.5	85.0	10.3	89.7
	F	46.1	71.3	15.5	84.5
Hispanics	Total	38.2	54.7	28.0	72.0
	M	37.3	64.1	22.5	77.5
	F	38.7	60.1	24.5	75.5

Table 4B. PAF by Risk Category Stratified by Sex and Race/Ethnicity

Table 4B. PAF by Risk Category Stratified by Sex and Race/Ethnicity 2009-2012

		Metabolic	Non-Metabolic	Unknown	Total
Overall	**Total**	**42.0**	**59.0**	**23.8**	**76.2**
	M	**46.3**	**60.9**	**21.0**	**79.0**
	F	**37.8**	**61.7**	**23.8**	**76.2**
NH White	Total	39.5	61.0	23.6	76.4
	M	45.1	65.7	18.8	81.2
	F	34.0	46.7	35.2	64.8
NH Black	Total	47.9	67.6	16.9	83.1
	M	43.5	78.9	11.9	88.1
	F	51.0	69.6	14.9	85.1
Hispanics	Total	47.6	49.7	26.4	73.6
	M	49.5	64.0	18.2	81.8
	F	45.1	43.8	30.8	69.2

M=males; F=Females; Metabolic Diseases = Diabetes, Obesity, Nonalcoholic steatohepatitis, Non-alcoholic fatty liver disease-advanced fibrosis; Non-Metabolic Diseases = Hepatitis B, Hepatitis C, Excessive alcohol use, Current smokers

Differences in PAF magnitudes were observed among race/ethnic subgroups. The highest HCV PAF was observed among non-Hispanic Blacks (59% in 2009-2012), while the lowest was among other/mixed (34% in 2009-2012). These values did not significantly decrease over the ten-year period. Non-Hispanic Blacks had the highest obesity PAF (31% in 2009-2012 compared to 25% in the overall population), which decreased from 36% in 1999-2002.

NASH-cirrhosis or NAFLD-fib could be attributed to, at most, 8% of HCC cases; these liver diseases had the largest fold change of any risk factor over the study period. Specifically, NAFLD-fib had the greatest fold increase of any risk factor studied, increasing 2.6-fold. NASH-cirrhosis had the second greatest PAF, increasing to a similar degree of 2.4-fold. NASH-cirrhosis

https://doi.org/10.48091/LKML8578

increased from 3.8% of cases to 5.8% and NAFLD-fib increased from 1.4% to 3.7% (p=0.022 and p<0.001, respectively, **Figure 3**).

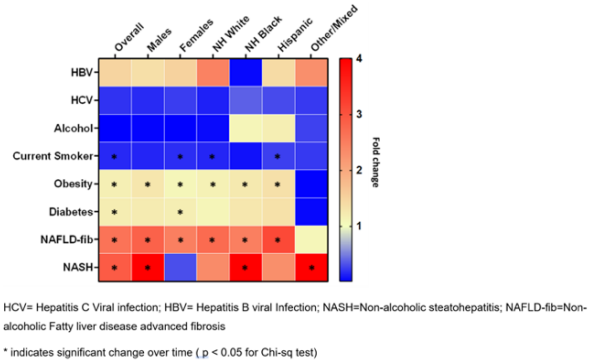

Figure 3: Fold Change in Hepatocellular Carcinoma Risk Factor Population Attributablr Fractions 1999-2002 and 2009-2012

Stratification by race/ethnicity showed that obesity and NAFLD-fib increased across all groups. The largest increase was seen among Hispanics with a 208% increase in NAFLD-fib. NASH-cirrhosis was more common among men than women in 2009-2012 (relative risk = 6.2). The largest increase in NASH-cirrhosis was among non-Hispanic Blacks, increasing from PAF of 0% in 1999-2001 to 8% in 2009-2012. NASH-cirrhosis increased significantly, accounting for 6.3% of HCC cases in 2009-2012 (**Table 3, Figure 3**).

1.1.1 Genetic Analysis.

There are differences in HCC outcomes between different ethnic groups. The gene *PNPLA3* was shown to play a major role in the development of liver disease such as NAFLD-fib and NASH-cirrhosis. *PNPLA3 represents* a GDA score of 5.00(figure 2). The specificity for HCC was 0.556, and the association of the PNPLA3 with HCC specifically is 0.692. This is based on an evidence score of 0.500, which was calculated from DisGenet . *PNPLA3* gene represents a cytosine to guanine substitution, resulting in an isoleucine to methionine switch at codon 148 and individuals with the G allele have a higher hepatic triglyceride level and elevated serum of ALT.[21] Our study found a total of 13 pathogenic genes from DisGeNET platfrom with a DSI , DPI and a GDA score based on DisGeNet ranking system. Our analysis represents high frequency of *PNPLA3* gene among Hispanic groups. Furthermore, gene-diet interaction plays a vital role in the pathogenesis of liver cancer in Hispanics.

Figure 2 represents the 13 gene strongly associated with HCC. Based on the GDA score from DisGeNET, *PNPLA3* (0.500) holds a strong association with increased risk of HCC. The GDA score of *PNPLA3* (0.500) is the highest as compared to other genes in list. The lowest GDA score based on DisGeNET ranking is *PPARD*. Genes *LDLR, FAS, PEMT, NR1H4, GNMT* represents the same GDA score of 0.320 which means they all are equally associated in the development of HCC.

1. Discussion

This study attributed nearly 80% of HCC cases in 1999-2001 and 2009-2012 to eight known risk factors in a large nationally representative sample of the U.S. population. Metabolic risk factors are now contributing to nearly half of HCC cases in the US. Metabolic diseases (diabetes, obesity, NASH-cirrhosis, and NAFLD-advanced fibrosis) increased from contributing an estimated 35% of HCC burden in 1999-2002 to 42% in 2009-2012. Concordantly, non-metabolic risk factors decreased from 66% to 59% of total HCC burden over the same period. Stratification by race/ethnicity showed a similar shift across all groups. The HCC risk factor prevalence rates of obesity and NAFLD-fib increased for all groups and were particularly high among Hispanics and non-Hispanic Blacks. A genetic variant in *PNPLA3* was identified as strongly associated with HCC.

https://doi.org/10.48091/LKML8578

These findings highlight vulnerabilities within certain racial and ethnic groups within one country's population of HCC, which is increasing in the US as well as worldwide. [25-28] As the obesity epidemic had increased from 1999 until 2012, metabolic risk factors (NAFLD-fib and NASH-cirrhosis) became contributers to HCC development in the US more so than non-metabolic risk factors (HBV, HCV), excessive alcohol use, and smoking. [22,23]

It is likely that there is an interplay between lack of access to health care, racial disparities, and genetics leading the whole pathogenesis and playing a major role in the development of liver disease that eventually forms into HCC. Specific genetic contributions may help explain differences observed in PAFs for HCC between race/ethnic groups.[29] While insulin resistance likely plays a role in its pathogenesis, oxidative injury and inflammatory reactions could be influenced by genetics. A study performed in the US among Hispanic, NH black and NH white individuals identified the variant, rs73809, (148M) in patatin-like phospholipase domain-containing protein 3 (*PNPLA3)* as a predictor for hepatic fat content.[30] The study confirmed that patients with NAFLD-fib who carry an allele of the gene (rs73809) *PNPLA3* have an increased risk of developing advanced diseases, including NASH-cirrhosis. Risk allele (rs73809) was the main common genetic determinant of hepatic fat content and of progressive NAFLD-fib, and this allele was mostly observed among Hispanic groups. It is not clear as to why this allele is increased among Hispanics. The variant has been reported to manifest in early life among Hispanic adolescents, [31] as well was having a prevalence of 80% in a single center study in Mexico.[32] This is consistent with the current study's findings showing higher burdens of NAFLD-fib among Hispanics. Overall, evolving knowledge in genetics along with epidemiological studies focused on race/ethnic backgrounds may help identify patients at higher risks for HCC.[33] This study demonstrates that PNPLA3 influences liver fat accumulation early in life in Hispanic children and adults. [34] This analysis also represents, individuals carrying the GG genotype of the *PNPLA3* gene are susceptible to increased hepatic fat when dietary sugar intake is high. The role of *PNPLA* gene may have an association in the development of NAFLD-fib and NASH-cirrhosis in the Hispanics. Other studies have also confirmed that this gene predispose obese children and adolescents to exhibit hepatic damage.[35]

Among non-metabolic risk factors, HCV has long been recognized as a major predictor of HCC risk.[36] This study is also consistent with previous studies indicating a largest risk of HCV in the non-Hispanic Black population. [36-37] A cross-sectional study utilizing Medicare databases have shown that the proportion of HCC cases attributable to HCV and HBV have doubled over approximately the same study period.[37,38] The differing estimations in previous studies compared to the current is likely due to the broader age of subjects surveyed presently in NHANES compared to SEER-Medicare databases. The known birth cohort effect of those born between 1945-1965 reaching the age of peak HCC risk had been previously reported, [37,39] and the greater proportion of HCV contribution was towards HCC among the older US generation.

The current study does have certain limitations. While this study has assumed independent causation of HCC for each risk factor, the course of disease from obesity, diabetes, NAFLD-fib, and NASH-cirrhosis is not a mutually exclusive path toward malignancy. [25,26,40-43] Patients can often present with multiple risk factors, raising the question of how to accurately weigh the contribution of each risk and handle overlapping interactions with other, possibly concurrent, risk factors. While this study served to focus on contributions of each specific risk factor,

https://doi.org/10.48091/LKML8578

future studies are needed to disentangle these interactions using real-world data .[44] Importantly, the average age of HCC onset is 65 while the study's cohort had an average age of 46. While this study did adjust for age, the burden of each metabolic risk factor may have been an underestimation.[16] This study also assumes that people do not change their lifestyle habits as they grow older, thereby potentially decreasing their HCC risk.[29] Thus, these results should be interpreted as epidemiological evidence for prevention strategies and public health education on risk factors.

In conclusion, these results display the changing contributions as well as the proportions of known HCC risk factors among specific racial and ethnic groups in a representative sample of the US population. The results of this study show that the increasing HCC rates are due to modifiable causes; this can be used to inform prevention and education programs with awareness as to racial and ethnic genetic and lifestyle differences.

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