Int J Clin Exp Pathol 2017;10(1):671-679
www.ijcep.com /ISSN:1936-2625/IJCEP0026597

Original Article

Association of a functional intronic polymorphism rs735396 in *HNF1A* gene with the susceptibility to hepatocellular carcinoma in Han Chinese population

Ming-Ming Jiang*, Xing Gu*, Juan Yang, Meng-Meng Wang, Hui-Ming Li, Meng Fang, Chun-Fang Gao

*Department of Laboratory Medicine, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, P. R. China. *Equal contributors.*

Received February 24, 2016; Accepted May 21, 2016; Epub January 1, 2017; Published January 15, 2017

Abstract: Background: Single nucleotide polymorphism rs735396 located in *HNF1A* gene was highly associated with the levels of glycan groups which changed during carcinogenesis and progression of HCC after HBV infection. In present study, we investigated if rs735396 are associated with the susceptibility to HBV infection and HBV-related HCC. *Methods:* Totally 568 HBV-related HCC patients, 110 HBV carriers without HCC and 380 healthy controls in Han Chinese population were recruited and genotyped using polymerase chain reaction-ligation detection reaction (PCR-LDR) method. To evaluate the effects of rs735396 polymorphisms on transcription of *HNF1A,* the luciferase activity for allelotype A or G was detected in HepG2.2.15 cells. *Results:* The genotype distribution and allelic frequencies were significantly different between HBV-related HCC patients and healthy controls ($P < 0.05$). Also, compared with genotype GG, genotypes AA+GA are connected with a higher risk of HBV-related HCC (Odds Ratio = 1.908; 95% Confidence Interval = 1.390-2.619, $P < 0.001$). Moreover, luciferase activity for allelotype A was significantly lower than that of allelotype G ($P = 0.036$). *Conclusion:* Taken together, we demonstrated that the variation of *HNF1A* rs735396 influences host susceptibility to HBV-related HCC by altering transcriptional activity of *HNF1A*.

Keywords: *HNF1A*, HBV-related HCC, single nucleotide polymorphisms, genotype, susceptibility

Introduction

Hepatocyte nuclear factor 1 alpha (HNF1A) is an indispensable transcript factor for a large number of hepato-specific and -enriched genes, such as albumin, alpha-fetoprotein, alpha-fibrinogen, beta-fibrinogen, alpha-1-antitrypsin, transthyretin, aldolase B, and the hepatitis B virus large surface protein [1]. Previous studies showed that HNF1A tightly associated with digestive diseases including diabetes, pancreatic cancer, hepatic adenoma, and HCC [2-7]. It has been report that the level of HNF1A was disordered in HCC, and might play an important part in the development and differentiation of cancer cells by acting on a set of genes or microRNAs, which involved in molecular carcinogenesis of HCC [8-12].

Single nucleotide polymorphisms (SNPs) are the most common type of genomic variation, occurred approximately every 1200 base pairs (bps). Genome-wide association studies (GWAS) have identified numerous single nucleotide polymorphisms (SNPs) that contribute to human diseases. Compared with SNPs occurred in gene coding regions lead to severe genetic disorders, vast majority of SNPs located in non-coding intergenic and intronic regulatory regions show modest effects that might modify gene function more subtly. These regulatory SNPs can regulate gene expression through several mechanisms including transcription factor binding, RNA splicing, DNA methylation and miRNA recruitment [13]. Studies have been reported influences of *HNF1A* genetic polymorphism on susceptibility to diabetes, cardiovascular diseases, pancreatic cancer, and hepatocellular adenomas [2-6]. In addition, several SNPs in *HNF1A* were tightly associated with plasma indexes of diseases, including CRP, Lipoprotein, and N-glycan [14-17]. In 2010 and 2011, two studies were conducted to screen out N-glycan associated SNPs, one of which is rs735396

located in the ninth intron of the *HNF1A* gene [16, 18]. Our studies and the others had demonstrated that aberrant glycosylation is a significant characteristic of the development of various diseases, and is tightly associated with the progression of tumor such as adhesion, invasion and metastasis [7, 15, 19-21]. Moreover, alterations in the N-glycosylation profiles of glycol-proteins occurred during the carcinogenesis and progression of HCC lesions [19, 21, 22]. These findings indicated that SNPs located in *HNF1A* might lead to the aberrant glycosylation of genes, which play an important role in HCC oncogenesis.

In this work, a case-control study was designed to assess the association of *HNF1A* genetic variant rs735396 with the susceptibility to HBV infection and HBV-related HCC in Han Chinese population using polymerase chain reaction–ligation detection reaction (PCR-LDR) method, and then we evaluated the effects of allelotype A and G of rs735396 on transcription of HNF1A using Luciferase reporter gene detection.

Materials and methods

Study population

In this study, a hospital-based case-control study was performed in 568 HBV-related HCC patients, 110 HBV carriers without HCC and 380 healthy controls. All recruited subjects are exclusively Han Chinese without any restrictions on gender and age. All subjects were recruited between January 2011 and June 2012 from the Eastern Hepatobiliary Surgery Hospital and Changzheng Hospital of Second Military Medical University. All the HBV carriers and HCC patients were infected with HBV, and were further confirmed by HBsAg (hepatitis B virus surface antigen) positive, HBcAb (hepatitis B virus core antibody) positive and HBeAg (hepatitis B virus e antigen)or HBeAb (hepatitis B virus e antibody) positive for at least 6 months. The presence of HCC was excluded in non-HCC patients and healthy controls by histology, computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography and laboratory tests. Moreover, patients with positive laboratory tests for HIV, HCV (anti-HCV and/or HCV-RNA), alcoholic liver disease, suspected autoimmune diseases with antinuclear antibody titer greater than 1:160 were excluded from this study. The following laboratory parameters were tested in almost 5 ml of venous whole-blood collected from each participant: serum albumin level (ALB), total bilirubin level (T-Bil), alanine aminotransferase (ALT) level, alpha fetoprotein (AFP) level, hepatitis B virus serum markers (HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb) and platelet count (PLT). This study was approved by the Committee on Ethics of Biomedicine Research of Second Military Medical University.

DNA extraction and genotype detection

Genomic DNA extraction was performed with the QIAamp DNA Blood MINI Kit (Qiagen, Hilden, Germany). *HNF1A* genetic variant rs735396 was genotyped using PCR-LDR method. The specific primers synthesized by Invitrogen (Carlsbad, USA) were: 5'-TGAGTACCCCTAGGGACAG-3' (forward) and 5'-ACACTGCAGAGGCAAACAAG-3' (reverse). The PCRs were carried out on the Gene Amp PCR system 9600 (Applied Biosystems, Foster city, USA) in a total volume of 20 μl including 5 ng genomic DNA, 1 × PCR Buffer, 0.1 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 μM each primer, and 0.2 U hot-start Taq DNA polymerase. Cycling parameters were as follows: 95°C for 2 min; 40 cycles at 94°C for 90 s, 56°C for 90 s, 65°C for 30 s; A final extension step at 94°C for 10 min. The probes for LDR were: common probe, 5'-P-AGAGTTTTAGGATTGTGGAGGGGGAttt-FAM-3'; C-specific probe, 5'-ttttAGTGCTGGCCCCTCATCTGTTTTGC-3'; G-specific probe, 5'-ttttttttAGTGCTGGCCCCTCATCTGTTTTGG-3'. The common probe was labeled at the 3'-end with 6-carboxyXuorescein (FAM) and was phosphorylated at the 5'-end. The ligation reaction for each PCR product was carried out with a final volume of 10 μl, containing 1 μl 10 × ligation buffer, 3 μl of PCR product, 1 pmol of each discriminating probe, 5 U Taq DNA ligase (Takara Bio, Dalian, China). The LDR parameters were as follows: 40 cycles at 94°C for 30 s and 60°C for 3 min. Following the LDR reaction, 1 μl LDR reaction product was mixed with 2 μl loading Dye (with Marker). The mixture was denatured at 95°C for 3 min, and then ice-cooled immediately. At last the samples were analyzed by the ABI 3730 DNA Sequencer (Applied Biosystems, Foster city, USA). In addition, the representative PCR products were subjected to direct DNA sequencing to confirm the accuracy of this method.

Table 1. Demographic and laboratory parameters of the subjects included in the study

Groups	Controls (n = 375)	HCC (n = 568)	HBV (n = 110)
Demographic parameters			
Gender (M/F)	309/66	473/95	82/28
Age (Y) (mean ± SD)	47.1±11.8	50.6±10.3	38.1±15.8
Laboratory parameters [median (range)]			
AFP (ng/mL)	3.3 (0.7-18)	454.6 (0.61-1210)†	2.4 (0.6-11.6)‡
T-Bil (μmol/L)	13.5 (4-42)	14.7 (3.8-156)	13.9 (5-47)‡
ALB (g/L)	48.7 (40-56)	41.5 (25.7-51.8)†	48.5 (43-57)‡
ALT (U/L)	25.8 (6-193)	45.7 (8-1259)†	23 (7-160)‡
PLT (10^9/L)	223.5 (95-400)	160.6 (5-431)†	192.5 (72-311)‡

Controls, Healthy controls; HCC, HBV-related HCC patients; HBV, HBV Carriers without HCC. AFP, serum alpha fetoprotein level; T-Bil, serum total bilirubin level; ALB, serum albumin level; ALT, serum alanine transaminase level; PLT blood platelet count. †Indicates a significant difference between healthy controls and HBV-related HCC patients. ‡Indicates a significant difference between healthy controls and HBV carriers without HCC.

Luciferase reporter gene detection

DNA fragment carrying allelotype A of rs735396 was amplified from genomic DNA of HepG2.2.15 cells (HCC cell line). The primers for PCR were: 5'-GGGGTACCTACCTACCTCGGCAT-3' (forward) and 5'-GAAGATCTATCACCCCCCAAGCAG-3' (reverse). The PCR products (439 bp) were cloned in the pGL3-Promoter vector (pGL3) as pGL3-A. The pGL3-G vector (allelotype G of rs735396) were generated from pGL3-A using the Quickchange Lightning Site-Directed Mutagenesis Kit (Agilent Technologies, La Jolla, USA). HepG2.2.15 cells were co-transfected with the pGL3-A or with the pGL3-G (1 μg) along with the pRL-TK renilla luciferase plasmid (Promega, Madison, USA) (200 ng) using Lipofectamine™ 2000 (Invitrogen, Carlsbad, USA) according to the manufacturer's recommendations. After 48 hours of transfection, luciferase activity was measured using the Dual-Luciferase® Reporter Assay kit (Promega, Madison, USA) according to manufacturer' s instructions. The data were showed as fold activity (firefly/renilla) and reported as a mean (duplicate transfections of three experiments) ± standard deviation (SD). A Student' s *t* test (paired, two-tail) was performed to find the differences in fold activity. A value of $P < 0.05$ was considered statistically significant.

Statistical analysis

Mann-Whitney U test were performed to determine associations of laboratory parameters with the presence of HBV infection and HBV-related HCC. We assessed the association of genotypes or alleles with presence of HBV-related HCC and HBV infection using a standard chi-squared test. We also estimated the relative risk of *HNF1A* gene polymorphism rs735396 with HCC and HBV infection using Binary logistic regression, adjusted for age and gender. Odds ratio (OR) and their 95% confidence interval (CI) were calculated to assess the association of rs735396 with HCC and HBV infection. Stratification analysis was performed by gender, tumor grades, Child-Pugh grades, TNM stages and the presence of liver cirrhosis in HBV-related HCC group. The two-sided *P* value < 0.05 was defined as statistically significance. All statistical analyses were performed with the software of SPSS 17.0 version (SPSS Inc., Chicago, USA).

Results

Population characteristics

As shown in **Table 1**, in HBV-related HCC patients, median values of AFP and ALT were significantly higher than reference ranges, while median values of ALB, PLT and T-Bil were normal. The levels of AFP, ALB, ALT, and PLT showed statistical differences between HCC patients and controls ($P < 0.05$). There were also significant differences in the level of AFP, T-Bil, ALB, ALT, and PLT between HBV carriers and controls ($P < 0.05$).

Allele and genotype distribution of rs735396 in cases and controls

Several samples could not be genotyped in each group because we failed to extract the DNA or detect genotype from these samples.

Table 2. Genotype and allele distribution of rs735396 in cases and healthy controls

Groups	Controls (n = 375, %)	HCC (n = 545, %)	HBV (n = 109, %)
Genotype			
AA	101 (27%)	137 (25%)	29 (27%)
GA	169 (45%)	315 (58%)	53 (49%)
GG	105 (28%)	93 (17%)	27 (24%)
Allele			
A	371 (49%)	589 (54%)	111 (51%)
G	379 (51%)	501 (46%)	107 (49%)

Controls, Healthy controls; HCC, HBV-related HCC patients; HBV, HBV Carriers without HCC.

As shown in **Table 2**, the genotype and allele distributions of rs735396 in healthy controls were AA: 27%, GA: 45% and GG: 28%, and A allele: 49% and G allele: 51%, which conform to Hardy-Weinberg equilibrium.

Association analysis of rs735396 with the risk of HCC and HBV infection

In present study, the most common genotype GG in healthy controls was considered as the reference. As shown in **Table 3**, the frequency of genotype AA and GA was significantly different between HCC patients and controls ($P = 0.024$ and $P < 0.001$), and odds ratio (OR) for AA and GA was 1.546 (95% CI: 1.058-2.258) and 2.124 (95% CI: 1.519-2.971). Individuals with genotypes (AA+GA) had an OR of 1.908 (95% CI: 1.390-2.619; $P < 0.001$) for HCC, compared with individuals with reference genotype GG. The minor allele (A) showed a much higher frequency in the HCC patients than in the controls ($P = 0.047$), and OR for the allelotype A was 1.21. The significant differences of rs735396 genotype and allele frequencies between HCC and controls remained steady after adjusting for age and gender. No significant differences were found between HBV carriers and controls. In conclusion, these results suggested that *HNF1A* gene polymorphism rs735396 was significantly associated with the susceptibility to HBV-related HCC, but not HBV infection.

Effects of rs735396 G > A on HNF1A transcriptional activity

The pGL3-Promoter vector contains an SV40 promoter upstream of the luciferase gene and can be used for detecting the activity of putative enhancer elements. In this study, DNA fragments located in intron region of *HNF1A* gene were inserted into the multiple cloning site of pGL3-Promoter vector (pGL3). *HNF1A* luciferase reporter gene vector varied at the rs735396 position (pGL3-A and pGL3-G) were created to examine the effects of rs735396 polymorphism on *HNF1A* transcription. There are significant differences in luciferase activity between pGL3-Promoter (no insert) and pGL3-A or pGL3-G in HepG2.2.15 cells ($P < 0.001$). Since pGL3-Promoter vector is used to detect the activity of enhancer inserted in multiple clone sites, these results suggested that the inserted fragments might possess enhancer elements. Also, luciferase activity for pGL3-A was significantly lower in comparison to that of pGL3-G ($P = 0.036$) (**Figure 1**). These data indicated that allelotype A of rs735396 might reduce the activity of this putative enhancer in *HFN1A* gene.

Stratification analysis in HBV-related HCC patients

To found the association of rs735396 with clinical characteristics in HBV-related HCC patients, we performed a Stratification analysis according to serum AFP level, TNM stages, Child-Pugh grades, tumor grades and the presence of liver cirrhosis. Patients were divided into two subgroups depending on TNM (I vs II-III = 252 vs 279), Child-Pugh (A vs B+C = 519 vs 24), tumor grades (I-II vs III-IV = 105 vs 420), cirrhosis vs non-cirrhosis = 373 vs 150) and the level of AFP (< 20 ng/ml vs ≥ 20 ng/ml = 179 vs 353). The data shown in **Table 4** revealed that no statistical differences were found in genotype and allele frequencies between subgroups. These data indicated that rs735396 might have no effect on these clinical parameters in HCC patients.

Discussion

Numerous studies have well characterized the dysregulation of HNF1A in the development of HCC. In the tissue samples from HCC patients, HNF1A protein level was higher in well-differentiated carcinoma than that in the adjacent non-HCC tissues, whereas HNF1A protein level was lower in moderately and poorly differentiated carcinoma [4, 23]. Consistent results were found at the mRNA level of HNF1A [24]. Zeng et

Table 3. Association analysis of rs735396 with the risk of HBV-related HCC and HBV infection

Groups	Genotype and Allele			
	AA vs GG	GA vs GG	AA+GA vs GG	A vs G
Controls vs HCC				
OR (95% CI)	1.546 (1.058-2.258)	2.124 (1.519-2.971)	1.908 (1.390-2.619)	1.207 (1.002-1.454)
P	0.024*	< 0.001*	< 0.001*	0.047*
OR[II] (95% CI)	1.583 (1.073-2.336)	2.042 (1.450-2.876)	1.876 (1.390-2.619)	1.221 (1.010-1.477)
P[II]	0.021*	< 0.001*	< 0.001*	0.039*
Controls vs HBV				
OR (95% CI)	1.117 (0.618-2.017)	1.220 (0.723-2.059)	1.167 (0.714-1.905)	1.060 (0.784-1.433)
P	0.764	0.512	0.624	0.758
OR[II] (95% CI)	1.203 (0.698-2.105)	1.305 (0.797-2.135)	1.253 (0.806-2.117)	1.137 (0.864-1.513)
P[II]	0.701	0.491	0.602	0.734

Controls, Healthy controls; HCC, HBV-related HCC patients; HBV, HBV Carriers without HCC. [II]Genotype and allele frequencies between HBV carriers and controls after adjusting for age and gender using Binary logistic regression. *Indicates a statistically significant difference.

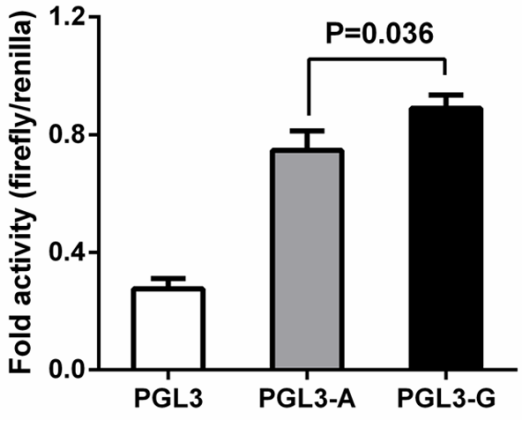

Figure 1. Effects of rs735396 G > A on HNF1A enhancer activity. Nonspecific luciferase activity was measure using pGL3 (pGL3-Promoter vector, no insert). Data were normalized by co-expression of pRL-TK renilla luciferase. Luciferase activity was detected using the Dual-Luciferase® Reporter Assay kit. The data are showed as fold activity (firefly/renilla) and reported as a mean (duplicate transfections of three experiments) ± SD.

al. also reported that in 20 paired tissues samples from HCC patients, compared with their adjacent tissues, HNF1A protein was reduced in 52.94% samples, whereas HNF1A protein expression remained unchanged in 23.53% and elevated in 23.53% of the samples [25].

Previous studies also showed that HNF1A played key role in the carcinogenesis of HCC *in vitro* and *in vivo*. HNF1A was found to be down-regulated in HCC cell lines and animal models, and function as a critical regulator on a series of genes and microRNAs which were decreased in HCC, such as Hepassocin, miR-122, miR-192, and miR-194 [8, 9, 12]. These researches suggested that the down-regulation of HNF1A might be conducive to process of carcinogenesis in HCC. However, the contrary results showed that knocking-down of HNF1A reduced cell proliferation and the Wnt pathway activity in MHCC97L cells, and elevated levels of HNF1A expression were closely associated with a higher expression of CDH17, which was highly expressed in liver cancer and associated with poor clinical outcomings [11]. Taken together, the foregoing studies indicated that the level of HNF1A varied at differentiation stages in HCC, and HNF1A could be either tumor promoter or suppressor in the carcinogenesis of HCC though different mechanisms.

As is known, SNP was considered to be an important genetic factors, which might be involved in carcinogenesis and post-translational modification [16, 18, 22, 26-28]. It has been reported that rs735396 in *HNF1A* gene showed significant associations with glycosylation (one type of post-translational modification), which might involve in development of HCC and HBV immune escape [22, 29, 30]. In this case-control study, association analysis showed that rs735396 was tightly associated with the susceptibility to HCC, but not with HBV infection. Also, our results indicated that the G > A variant might involve in carcinogenesis of HCC. As rs735396 is located in the intron of *HNF1A* gene, we hypothesized that rs735396-locating region might interact with some DNA-binding factors and then regulate *HNF1A* gene

Table 4. Stratification analysis of HBV-related HCC patients

Groups	AFP < 20 vs AFP ≥ 20 ng/ml		TNM I vs TNM II-III		Child-Pugh A vs Child-Pugh B+C		tumor grades I-II vs tumor grades III-IV		cirrhosis vs non-cirrhosis	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Genotype										
AA vs GG	0.876 (0.468-1.571)	0.658	0.915 (0.534-1.569)	0.747	1.963 (0.429-8.982)	0.377	1.036 (0.507-2.119)	0.922	0.885 (0.492-1.594)	0.684
GA vs GG	1.283 (0.776-2.122)	0.330	1.020 (0.635-1.639)	0.935	0.772 (0.253-2.354)	0.648	1.364 (0.735-2.531)	0.323	0.893 (0.532-1.499)	0.669
AA+GA vs GG	1.149 (0.706-1.871)	0.576	0.987 (0.624-1.561)	0.956	0.951 (0.317-2.849)	0.928	1.262 (0.691-2.305)	0.448	0.891 (0.541-1.467)	0.649
Allele										
A vs G	0.922 (0.714-1.190)	0.558	0.928 (0.752-1.220)	0.758	1.281 (0.718-2.287)	0.46	0.986 (0.728-1.335)	0.938	0.954 (0.729-1.249)	0.784

AFP, serum alpha fetoprotein level; TNM, tumor node metastasis stages; Child-Pugh, Child-Pugh class.

expression. In order to confirm this inference, fragments with allelotype A or G of rs735396 were inserted into pGL3-Promoter vector to detect the effects of polymorphism on luciferase activity. The results indicated that the gene region in which rs735396 site located might possess enhancer elements, and the allelotype A might reduce the expression of HFN1A by altering activity of enhancer.

Enhancers, one of transcriptional regulatory elements, can be pivotal for temporal regulation of gene transcription in the form of interacting with some *trans*-acting factors [31]. Since no significant differences of stratification analysis on clinical materials of HCC patients was shown, these results suggested that rs735396 which is located in an enhancer might not be involved in the progression of HCC. We supposed that the temporal interaction between different *trans*-acting factors and *cis*-regulatory elements of HNF1A might affect the gene expression in various stages of carcinogenesis of HCC. However, besides transcription regulatory, anther hypothesis is that HNF1A might have different biological function in late progression of HCC from its initiation period.

HNF1B, another member of the HNF-1 family, and HNF1A have been shown to act on the promoters or enhancers of many hepato-specific and -enriched genes. These two transcriptional factors share 47% sequence identity in DNA-binding POU domain that regulates the expression of hepato-specific genes including AFP [32]. However, in HepaG2 cells, HNF1B was found to be more potent than HNF1A in activating the AFP promoter [33]. In our study, rs735396 was not associated with AFP levels, which also indicated that HNF1A might play only a minor role in regulating AFP gene expression.

In conclusion, we report for the first time that *HNF1A* gene polymorphism rs735396 was correlated with increased HBV-related HCC risk in Han Chinese population. Our findings suggested that rs735396 might be an available marker to identify the people with high risk of HCC after HBV infection, and may provide the possibilities to modify the design of HCC surveillance programs for patients with chronic HBV infection. However, we only recruited Han Chinese population in our study, and thus further studies based on different ethnicity may be needed. Since our results indicated rs735396 was irrelevant to HBV infection, we should also explore the relationship between rs735396 and HCC with different etiology to investigate the underlying mechanism of its influence on HCC susceptibility, such as HCV infection, chronic alcohol consumption, and etc. In addition, the levels of HNF1A in liver tissues should be detected to verify the effects of rs735396 polymorphism on *HNF1A* gene expression. Screening of more polymorphisms and functional studies would be useful to found the precise molecular mechanisms of HNF1A involved in carcinogenesis of HCC.

Acknowledgements

This work was funded by the China National Key Project for Infectious Diseases (2012zx10002016001003) and National Natural Science Foundation of China (NSFC No 81271925).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chun-Fang Gao, Department of Laboratory Medicine, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai, P. R. China. E-mail: gaocf1115@163.com

References

[1] Hayashi Y, Wang W, Ninomiya T, Nagano H, Ohta K and Itoh H. Liver enriched transcription factors and differentiation of hepatocellular carcinoma. Mol Pathol 1999; 52: 19-24.

[2] Pierce BL and Ahsan H. Genome-wide "pleiotropy scan" identifies HNF1A region as a novel pancreatic cancer susceptibility locus. Cancer Res 2011; 71: 4352-4358.

[3] Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M and Bell GI. Mutations in the hepatocyte nuclear factor-4alpha gene in maturity-onset diabetes of the young (MODY1). Nature 1996; 384: 458-460.

[4] Wang W, Hayashi Y, Ninomiya T, Ohta K, Nakabayashi H, Tamaoki T and Itoh H. Expression of HNF-1 alpha and HNF-1 beta in various histological differentiations of hepatocellular carcinoma. J Pathol 1998; 184: 272-278.

[5] Kishnani PS, Chuang TP, Bali D, Koeberl D, Austin S, Weinstein DA, Murphy E, Chen YT, Boyette K, Liu CH and Li LH. Chromosomal and

genetic alterations in human hepatocellular adenomas associated with type Ia glycogen storage disease. Hum Mol Genet 2009; 18: 4781-4790.

[6] Estrada K, Aukrust I, Bjorkhaug L, Burtt NP, Mercader JM, Garcia-Ortiz H, Huerta-Chagoya A, Moreno-Macias H, Walford G, Flannick J, Williams AL, Gomez-Vazquez MJ, Fernandez-Lopez JC, Martinez-Hernandez A, Centeno-Cruz F, Mendoza-Caamal E, Revilla-Monsalve C, Islas-Andrade S, Cordova EJ, Soberon X, Gonzalez-Villalpando ME, Henderson E, Wilkens LR, Le Marchand L, Arellano-Campos O, Ordonez-Sanchez ML, Rodriguez-Torres M, Rodriguez-Guillen R, Riba L, Najmi LA, Jacobs SB, Fennell T, Gabriel S, Fontanillas P, Hanis CL, Lehman DM, Jenkinson CP, Abboud HE, Bell GI, Cortes ML, Boehnke M, Gonzalez-Villalpando C, Orozco L, Haiman CA, Tusie-Luna T, Aguilar-Salinas CA, Altshuler D, Njolstad PR, Florez JC, MacArthur DG and Jimenez-Morales S. Association of a low-frequency variant in HNF1A with type 2 diabetes in a Latino population. JAMA 2014; 311: 2305-2314.

[7] Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI and Young RA. Control of pancreas and liver gene expression by HNF transcription factors. Science 2004; 303: 1378-1381.

[8] Krutzfeldt J, Rosch N, Hausser J, Manoharan M, Zavolan M and Stoffel M. MicroRNA-194 is a target of transcription factor 1 (Tcf1, HNF1alpha) in adult liver and controls expression of frizzled-6. Hepatology 2012; 55: 98-107.

[9] Xu H, He JH, Xiao ZD, Zhang QQ, Chen YQ, Zhou H and Qu LH. Liver-enriched transcription factors regulate microRNA-122 that targets CUTL1 during liver development. Hepatology 2010; 52: 1431-1442.

[10] Ladeiro Y, Couchy G, Balabaud C, Bioulac-Sage P, Pelletier L, Rebouissou S and Zucman-Rossi J. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. Hepatology 2008; 47: 1955-1963.

[11] Zhu R, Wong KF, Lee NP, Lee KF and Luk JM. HNF1alpha and CDX2 transcriptional factors bind to cadherin-17 (CDH17) gene promoter and modulate its expression in hepatocellular carcinoma. J Cell Biochem 2010; 111: 618-626.

[12] Yu HT, Yu M, Li CY, Zhan YQ, Xu WX, Li YH, Li W, Wang ZD, Ge CH and Yang XM. Specific expression and regulation of hepassocin in the liver and down-regulation of the correlation of HNF1alpha with decreased levels of hepassocin in human hepatocellular carcinoma. J Biol Chem 2009; 284: 13335-13347.

[13] Huang Q. Genetic study of complex diseases in the post-GWAS era. J Genet Genomics 2015; 42: 87-98.

[14] Reiner AP, Gross MD, Carlson CS, Bielinski SJ, Lange LA, Fornage M, Jenny NS, Walston J, Tracy RP, Williams OD, Jacobs DR Jr and Nickerson DA. Common coding variants of the HNF1A gene are associated with multiple cardiovascular risk phenotypes in community-based samples of younger and older European-American adults: the Coronary Artery Risk Development in Young Adults Study and The Cardiovascular Health Study. Circ Cardiovasc Genet 2009; 2: 244-254.

[15] Hegele RA, Cao H, Harris SB, Hanley AJ, Zinman B and Connelly PW. The private hepatocyte nuclear factor-1alpha G319S variant is associated with plasma lipoprotein variation in Canadian Oji-Cree. Arterioscler Thromb Vasc Biol 2000; 20: 217-222.

[16] Lauc G, Essafi A, Huffman JE, Hayward C, Knezevic A, Kattla JJ, Polasek O, Gornik O, Vitart V, Abrahams JL, Pucic M, Novokmet M, Redzic I, Campbell S, Wild SH, Borovecki F, Wang W, Kolcic I, Zgaga L, Gyllensten U, Wilson JF, Wright AF, Hastie ND, Campbell H, Rudd PM and Rudan I. Genomics meets glycomics-the first GWAS study of human N-Glycome identifies HNF1alpha as a master regulator of plasma protein fucosylation. PLoS Genet 2010; 6: e1001256.

[17] Thanabalasingham G, Huffman JE, Kattla JJ, Novokmet M, Rudan I, Gloyn AL, Hayward C, Adamczyk B, Reynolds RM, Muzinic A, Hassanali N, Pucic M, Bennett AJ, Essafi A, Polasek O, Mughal SA, Redzic I, Primorac D, Zgaga L, Kolcic I, Hansen T, Gasperikova D, Tjora E, Strachan MW, Nielsen T, Stanik J, Klimes I, Pedersen OB, Njolstad PR, Wild SH, Gyllensten U, Gornik O, Wilson JF, Hastie ND, Campbell H, McCarthy MI, Rudd PM, Owen KR, Lauc G and Wright AF. Mutations in HNF1A result in marked alterations of plasma glycan profile. Diabetes 2013; 62: 1329-1337.

[18] Huffman JE, Knezevic A, Vitart V, Kattla J, Adamczyk B, Novokmet M, Igl W, Pucic M, Zgaga L, Johannson A, Redzic I, Gornik O, Zemunik T, Polasek O, Kolcic I, Pehlic M, Koeleman CA, Campbell S, Wild SH, Hastie ND, Campbell H, Gyllensten U, Wuhrer M, Wilson JF, Hayward C, Rudan I, Rudd PM, Wright AF and Lauc G. Polymorphisms in B3GAT1, SLC9A9 and MGAT5 are associated with variation within the human plasma N-glycome of 3533 European adults. Hum Mol Genet 2011; 20: 5000-5011.

[19] Ji J, Gu X, Fang M, Zhao Y, Yi C, Wang A and Gao C. Expression of alpha 1,6-fucosyltransferase 8 in hepatitis B virus-related hepatocellu-

lar carcinoma influences tumour progression. Dig Liver Dis 2013; 45: 414-421.
[20] Zhao YP, Ruan CP, Wang H, Hu ZQ, Fang M, Gu X, Ji J, Zhao JY and Gao CF. Identification and assessment of new biomarkers for colorectal cancer with serum N-glycan profiling. Cancer 2012; 118: 639-650.
[21] Fang M, Zhao YP, Zhou FG, Lu LG, Qi P, Wang H, Zhou K, Sun SH, Chen CY and Gao CF. N-glycan based models improve diagnostic efficacies in hepatitis B virus-related hepatocellular carcinoma. Int J Cancer 2010; 127: 148-159.
[22] Kamiyama T, Yokoo H, Furukawa J, Kurogochi M, Togashi T, Miura N, Nakanishi K, Kamachi H, Kakisaka T, Tsuruga Y, Fujiyoshi M, Taketomi A, Nishimura S and Todo S. Identification of novel serum biomarkers of hepatocellular carcinoma using glycomic analysis. Hepatology 2013; 57: 2314-2325.
[23] Thony B, Neuheiser F, Hauer CR and Heizmann CW. Molecular cloning and recombinant expression of the human liver phenylalanine hydroxylase stimulating factor revealed structural and functional identity to the dimerization cofactor for the nuclear transcription factor HNF-1 alpha. Adv Exp Med Biol 1993; 338: 103-106.
[24] Svensson EC, Conley PB and Paulson JC. Regulated expression of alpha 2,6-sialyltransferase by the liver-enriched transcription factors HNF-1, DBP, and LAP. J Biol Chem 1992; 267: 3466-3472.
[25] Zeng X, Lin Y, Yin C, Zhang X, Ning BF, Zhang Q, Zhang JP, Qiu L, Qin XR, Chen YX and Xie WF. Recombinant adenovirus carrying the hepatocyte nuclear factor-1alpha gene inhibits hepatocellular carcinoma xenograft growth in mice. Hepatology 2011; 54: 2036-2047.
[26] Liu N, Meng Z, Lou G, Zhou W, Wang X, Zhang Y, Zhang L, Liu X, Yen Y, Lai L, Forman BM, Xu Z, Xu R and Huang W. Hepatocarcinogenesis in FXR-/- mice mimics human HCC progression that operates through HNF1alpha regulation of FXR expression. Mol Endocrinol 2012; 26: 775-785.
[27] Serfas MS and Tyner AL. HNF-1 alpha and HNF-1 beta expression in mouse intestinal crypts. Am J Physiol 1993; 265: G506-513.
[28] Ishiyama T, Kano J, Minami Y, Iijima T, Morishita Y and Noguchi M. Expression of HNFs and C/EBP alpha is correlated with immunocytochemical differentiation of cell lines derived from human hepatocellular carcinomas, hepatoblastomas and immortalized hepatocytes. Cancer Sci 2003; 94: 757-763.
[29] Schrottner P, Wollner S, Catusse J and Burger M. Detection of elevated serum levels of the chemokine CCL18 in B-cell chronic lymphocytic leukaemia: identification of a novel biomarker. Acta Haematol 2010; 124: 110-114.
[30] Yu DM, Li XH, Mom V, Lu ZH, Liao XW, Han Y, Pichoud C, Gong QM, Zhang DH, Zhang Y, Deny P, Zoulim F and Zhang XX. N-glycosylation mutations within hepatitis B virus surface major hydrophilic region contribute mostly to immune escape. J Hepatol 2014; 60: 515-522.
[31] Miguel-Escalada I, Pasquali L and Ferrer J. Transcriptional enhancers: functional insights and role in human disease. Curr Opin Genet Dev 2015; 33: 71-76.
[32] Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C and Yaniv M. Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell 1996; 84: 575-585.
[33] Bois-Joyeux B and Danan JL. Members of the CAAT/enhancer-binding protein, hepatocyte nuclear factor-1 and nuclear factor-1 families can differentially modulate the activities of the rat alpha-fetoprotein promoter and enhancer. Biochem J 1994; 301: 49-55.