Original Article

Polymorphisms of AZIN1 rs2679757 and TRPM5 rs886277 are Associated with Cirrhosis Risk in Chinese Patients with Chronic Hepatitis B

Li-jun Peng¹, Jin-sheng Guo¹, Zhe Zhang¹, Li-li Liu¹, Yi-rong Cao¹, Hong Shi¹, Jian Wang¹, Scott L. Friedman², John J. Sninsky³ and Ji-yao Wang¹

Objective Genome-wide association studies (GWAS) have linked many single nucleotide polymorphisms (SNPs) to the outcomes of a variety of liver diseases. The aim of the present study was to evaluate the association of several candidate SNPs with the risk and severity of cirrhosis due to chronic hepatitis B in a Chinese population.

Methods A total of 714 Chinese participants with persistent HBV infection were studied. Patients were divided into cirrhotic (n = 429) and non-cirrhotic (n = 285) groups based on clinical and pathological evidence. The progression rate and severity of liver cirrhosis were evaluated with an arbitrary *t*-score system. Genotypes of six SNPs in five candidate genes were detected with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The genotypic distributions of the SNPs were compared between the age-matched cirrhotic and non-cirrhotic subjects. The association between the risk of SNPs and the severity and progression rate of cirrhosis was further analyzed.

Results Rs2679757 polymorphism of the antizyme inhibitor 1 (AZIN1) gene and Rs886277 in the transient receptor potential cation channel subfamily M, member 5 gene (TRPM5) were found to be associated with cirrhosis risk in CHB. They were also correlated with the overall severity and progression rate of cirrhosis. Genotype frequencies of other SNPs were not different between the cirrhosis and non-cirrhosis groups.

Conclusions AZIN1 rs2679757 and TRPM5 rs886277 are associated with the risk and the progression rate of HBV-related liver fibrosis in Chinese patients. The emerging SNPs associated with cirrhosis prognosis warrant further clinical validation in other CHB cohorts or ethnic groups, and merit mechanistic studies to reveal their roles in fibrosis progression.

Key words: Hepatitis B; Cirrhosis; Single nucleotide polymorphism; Antizyme inhibitor 1; Transient receptor potential cation channel subfamily M, member 5

Based on data from Poynard et al, patients with chronic hepatitis C have highly variable rates of disease progression; some progress rapidly, others are slow, and on average the progression to cirrhosis requires approximately 30 years.¹ Host genetic factors may contribute significantly to the variable risk of fibrosis progression to cirrhosis. Single nucleotide polymorphisms (SNPs) are the stable replacement of a single nucleotides in a human gene. A SNP is the most common genetic variant in humans, appearing in more than 1% of the population. SNPs may affect biological processes and disease development if they impact on the function of relevant genes.^{2,3} In recent

decades, genome-wide association studies (GWAS) and candidate gene association studies have been widely performed to delineate the genetic impact of SNPs on human liver diseases.⁴ Notably, Huang et al identified 9 SNPs residing in 9 genes that are associated with liver fibrosis progression in Caucasian patients infected with hepatitis C virus (HCV). Cirrhosis risk scores (CRS) built on 7 of these SNPs were predictive for fibrosis progression in Caucasian chronic hepatitis C patients in longitudinal studies.⁵⁻⁸ However, few studies have investigated the roles of host gene polymorphisms on the progression of HBV-related liver cirrhosis, which, like hepatitis C, is a public health threat worldwide and is especially endemic in China.⁹⁻¹¹ As the biology of chronic hepatitis B fibrogenesis is similar to that of hepatitis C or other etiologies of liver diseases, these different diseases may share common genetic

¹Division of Digestive Diseases, Department of Internal Medicine, Zhongshan Hospital, Shanghai Medical College, Fudan University, No.180 Feng Lin Road, Shanghai 200032, China; ²Division of Liver Diseases, Mount Sinai School of Medicine, No.1425 Madison Ave, New York 10029, USA; ³Celera Corporation, Alameda, CA, USA

Correspondence: Jin-sheng Guo, E-mail: guo.jinsheng@zs-hospital.sh.cn

risk factors for fibrosis progression. Therefore, the present study was designed to evaluate the association of specific cirrhosis risk SNPs reported in the recent studies in Caucasian hepatitis C patients with the risk of cirrhosis among Chinese with chronic HBV infection.

METHODS

Study population

Total of 988 HBV-infected subjects were collected from Zhongshan Hospital, Fudan University between March 2009 and January 2011 and a total of 714 Chinese subjects with persistent HBV infection were studied. They were all Chinese Han subjects from an epidemic region of HBV, eastern coastal region of China. The study was approved by the ethical committee of the hospital and was registered on the Chinese Clinical Trials Register (<http://www.chictr.org>) numbered ChiCTR-ONC-00000439. The protocol conformed to the provisions of the Declaration of Helsinki (as revised in Tokyo, 2004). Informed consent was obtained from all subjects.

Clinical information and grouping of the patients

Information including hepatitis B history, alcohol consumption, serum HBV markers, liver function test, prothrombin time, platelets count, liver imaging (computed tomography or magnetic resonance imaging) and liver histology if available, was collected to establish the diagnosis and judge the severity of HBV-related liver disease. The status of their chronic HBV infection is indicated by HBV surface antigen positive with or without e antigen, and high or low HBV DNA load. The 714 chronic HBV infected subjects were divided into cirrhotic and the non-cirrhotic groups. The cirrhotic patients were defined by evident liver cirrhosis clinically or histologically, the presence of decompensated cirrhosis based on presence of ascites, variceal bleeding, encephalopathy,

spontaneous bacterial peritonitis, or Child-Pugh grade of B or C. The non-cirrhotic patients were defined as inactive HBV carriers, without cirrhosis-related clinical abnormalities or exam findings. Patients with evidence of chronic hepatitis C, autoimmune liver diseases, nonalcoholic steatohepatitis, drug-induced liver disease, Wilson's disease and serious clinical condition of other organs were excluded. Patients with alcohol consumption of > 40 g/day in males and > 20 g/day in females for at least 5 years were deemed alcoholics and excluded from the study. Patients with long-term anti-HBV therapy were also excluded.

The overall severity of liver cirrhosis was arbitrarily scored with a *t*-score system containing items included in Child-Pugh grade, platelet count, and the occurrence of decompensate complications of liver cirrhosis, with a range from 10 to 27 (Table 1). The progression rate of liver cirrhosis was expressed as a divided *t*-score by the duration of infection (Table 1).

Six SNPs in five genes reported by previous studies that were associated with cirrhosis risk in hepatitis C patients in Caucasian were selected (Table 2). Among them, there are SNPs within aquaporin 2 (AQP2), antizyme inhibitor 1 (AZIN1), degenerative spermatocyte homolog 1, lipid desaturase (DEGS1), toll-like receptor 4 (TLR4), transient receptor potential cation channel subfamily M, member 5 (TRPM5). Details of the SNP location (missense, synonymous, promoter, intronic or other changes), known function or potential biological impact of the SNPs are also listed in Table 2.¹⁴⁻²²

Preparation of DNA samples

From each subject, 2 ml blood was collected in an EDTA-containing tube. Genomic DNA was extracted with QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA, USA). DNA samples were analyzed by nucleic acid spectrophotometer and gel electrophoresis to

Table 1. The arbitrary *t*-score system for the evaluation of overall severity of liver cirrhosis (score 10-27)

Items/scores	1	2	3
Encephalopathy	no	1-2	3-2
Ascited	no	Mild	Moderate -severe
Child-Pugh grade	Serum bilirubin (μmol/L)	< 34.2	34.2-51.3
	albumin (g/L)	> 35.0	28.0-35.0
	Prothrombin time elongation (sec)	< 4.0	4.0-6.0
Platelet count (×10 ⁹ /L)	> 100.0	50.0-100.0	< 50.0
Gastroesophagous varices/bleeding	Without evidence	With evidence but no-bleeding	With bleeding
Spontaneous bacterial peritonitis	no	Yes	
Hepatorenal syndrome	no	Yes	
Hepatocellular carcinoma	no	Yes	

Table 2. General information on the 6 candidate SNPs in the study

Rs Number	Genes	Chromosome	SNP locations	Reported clinical association	Biological function of gene	Functional linkage with fibrogenesis
rs2878771	AQP2	12	UTR	HCV-related liver fibrosis ⁶	Water reabsorption, vasopressin regulation ¹⁴	unknown
rs2679757	AZIN1	8	Intron region	HCV-related liver fibrosis ⁶	Polyamine biosynthesis, cell proliferation ¹⁵	unknown
rs4290029	DEGS1	1	Intergenic	HCV-related liver fibrosis ⁶	Lipid metabolism and transport, cell growth ^{16,17}	unknown
rs960312	TLR4	9	Intergenic	HCV-related liver fibrosis ^{6,18}	LPS-stimulated inflammatory responses, profibrogenic signals ¹⁹⁻²¹	unknown
rs1927911	TLR4	9	Intron region	HCV-related liver fibrosis ^{6,18}	LPS-stimulated inflammatory responses, profibrogenic signals ¹⁹⁻²¹	unknown
rs886277	TRPM5	11	Missense	HCV-related liver fibrosis ⁶	Taste responses ²²	unknown

Notes: SNP, single nucleotide polymorphism; MAF, minor allele frequency; UTR, un-translated region; HCV, hepatitis C virus; AQP2, aquaporin 2; AZIN1, antizyme inhibitor 1; DEGS1, degenerative spermatocyte homolog 1, lipid desaturase; HSC, hepatic stellate cell; TLR4, toll-like receptor 4; LPS, lipopolysaccharides; TRPM5, transient receptor potential cation channel subfamily M, member 5

Table 3. Characteristics of the study populations

Total (n =714)	Cirrhotic group	Non-cirrhotic group	P value
N	429	285	-
Age (years)	49.3 ± 11.5	47.5 ± 13.5	0.10
Male, n (%)	334 (80.5)	205 (67.8)	< 0.01
Platelet (10 ⁹ /L)	96.1 ± 59.6	195.3 ± 62.1	< 0.01
Total bilirubin (μmol/L)	33.1 ± 64.2	12.9 ± 11.0	< 0.01
ALT (U/L)	77.4 ± 169.0	40.1 ± 25.4	0.04
Albumin (g/L)	35.1 ± 6.8	41.1 ± 4.2	< 0.01
PT (second)	14.0 ± 3.4	11.5 ± 0.9	< 0.01
Child-Pugh grades, n	A: 235, B: 145, C: 49	A: 285, B: 0, C: 0	< 0.01

Notes: ALT, alanine aminotransferase; PT, prothrombin time, normal numeral ranges for platelet, total bilirubin, ALT, albumin, and PT were 101 to 320 (×10⁹/L), 3.4 to 20.4 (μmol/L), < 75 (U/L), 35 to 52 (g/L), and 10 to 13 (sec), respectively. Data were expressed as mean ± standard deviation. 20.4 (μmol/L), < 75 (U/L), 35 to 52 (g/L), and 10 to 13 (sec), respectively. Data were expressed as mean ± standard deviation.

ensure the purity and for quantification.

SNPs detection

SNPs were genotyped using matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), which determines the allele-specific primer extension products with Sequenom's MassARRAY system and iPLEX technology (Sequenom, San Diego, CA, USA; www.sequenom.com).^{23,24} After laser desorption/ionization, automated spectral acquisition analysis was performed and the spectral data was saved to the MassARRAY database.

Statistical analysis

The parametric Student *t*-test and the non-parametric Wilcoxon rank-sum test, Pearson χ^2 test, or Fisher's test were utilized to assess differences in the mean data between groups for the characteristics and liver function profiles of the studied populations. The χ^2 test was also used in the evaluation of Hardy–Weinberg equilibrium. Binary logistic regression analysis was performed to determine the adjusted significance of the genotypic distribution of each SNP between the

cirrhotic and non-cirrhotic groups, where age and sex were covariables. Each statistically significant SNP in univariate analysis was further assessed with logistic regression analysis using the dominant, recessive and additive genetic models, and the odds ratio (OR) and 95% confidence interval (CI) were calculated. Furthermore, the association of the significant SNPs with the severity and the progression rate of cirrhosis were also analyzed with χ^2 test and logistic regression analysis. Statistics was performed with SPSS version 15.0 software (SPSS, Chicago, IL, USA). *P* value < 0.05 was considered to be statistically significant.

RESULTS

Population characteristics

The characteristics of the 714 subjects in the study were shown in Table 3. There was no difference between the cirrhotic and non-cirrhotic groups in mean age and age stratification. There is a higher ratio of male gender in the cirrhotic group when compared with the non-cirrhotic group. The blood tests and Child-Pugh grades suggested apparent liver decompensation in the

Table 4. Minor allele frequency of genotyped SNPs

SNP	allele	Detected MAF in cirrhotic group	Detected MAF in non-cirrhotic group	Reported MAF in Caucasian
AQP2 rs2878771	G/C	C: 41.78%	C: 40.32%	C: 18.6%
AZIN1 rs2679757	A/G	G: 25.76%	G: 20.07%	G: 40.8%
DEGS1 rs4290029	G/C	C: 38.88%	C: 38.95%	C: 22.4%
TLR4 rs960312	A/G	G: 27.34%	G: 29.05%	G: 15.8%
TLR4 rs1927911	C/T	T: 40.21%	T: 42.55%	T: 26.5%
TRPM5 rs886277	C/T	T: 31.31%	T: 38.20%	C: 34.8%

Notes: MAF: minor allele frequency; Data of reported MAF in Caucasian is from HapMap.

Table 5. Genotypic frequency for each SNP with genotype diversity in the cirrhotic and non-cirrhotic groups

Gene and SNP	Genotype	cirrhotic group, n (%)	Non-cirrhotic group, n (%)	<i>P</i> value	HWP
AQP2 rs2878771	GG	143 (33.6)	100 (35.2)	0.86	0.77
	GC	210 (49.3)	139 (48.9)		
	CC	73 (17.1)	45 (15.8)		
AZIN1 rs2679757	AA	231 (54.1)	180 (63.4)	0.03	0.59
	AG	172 (40.3)	94 (33.1)		
	GG	24 (5.6)	10 (3.5)		
DEGS1 rs4290029	CC	62 (14.5)	45 (15.8)	0.80	0.66
	CG	208 (48.7)	132 (46.3)		
	GG	157 (36.8)	108 (37.9)		
TLR4 rs960312	AA	232 (54.2)	147 (51.8)	0.79	0.25
	AG	158 (36.9)	109 (38.4)		
	GG	38 (8.9)	28 (9.9)		
TLR4 rs1927911	TT	70 (16.3)	55 (19.5)	0.55	0.34
	TC	205 (47.8)	130 (46.1)		
	CC	154 (35.9)	97 (34.4)		
TRPM5 rs886277	CC	206 (48.1)	104 (36.6)	0.01	0.26
	CT	176 (41.1)	143 (50.4)		
	TT	46 (10.7)	37 (13.0)		

Notes: SNP, single nucleotide polymorphism; HWP, *P* value for Hardy-Weinberg equilibrium test; AQP2, aquaporin 2; AZIN1, antizyme inhibitor 1; DEGS1, degenerative spermatocyte homolog 1, lipid desaturase; TLR4, toll-like receptor 4; TRPM5, transient receptor potential cation channel subfamily M, member 5

cirrhotic group (Table 3).

Association between individual SNPs and liver cirrhosis

Also a highly variable rate of cirrhosis progression was observed in the group of hepatitis B patients (Figure 1). Minor allele frequency (MAF) of each SNP was shown in Table 4. The genotypic distributions for the polymorphic sites were in Hardy–Weinberg equilibrium in this population ($P > 0.05$). Two loci, rs4986791 in TLR4 gene and rs62522600 in AZIN1 gene had no polymorphisms in the Chinese population. Rs17740066 in STXBP5L gene had an MAF < 5%. Some of the other alleles also showed differences from those reported in the NCBI SNP database (<http://www.ncbi.nlm.nih.gov/projects/SNP/>) in Caucasian (Table 4).

There was no significant association between these SNPs and liver cirrhosis except AZIN1 rs2679757 and TRPM5 rs886277 (Table 5). Regression analysis with adjustment for age and sex revealed that rs2679757

genotypes were associated with a significant risk of liver cirrhosis with the use of either a dominant or an additive genetic model for the G risk allele. Similarly, rs886277 genotypes in the TRPM5 gene were associated with liver cirrhosis risk in the recessive model for the risk C allele (Table 6).

Correlation of AZIN1 and TRPM5 SNPs with the progression rate and severity of liver cirrhosis

AZIN1 rs2679757 and TRPM5 rs886277 were also correlated well with *t*-score and associated the severity of liver cirrhosis (Figure 2). Patients with the risk of SNPs also had a higher progression rate to liver cirrhosis as expressed by progression rate (*t*-score/duration of infection) (Figure 3).

In a further analysis for the association of rs2679757 and rs886277 polymorphisms with the severity of cirrhosis judged by the Child-Pugh classification, the GG homozygote and AG heterozygote (GG + AG) of AZIN1 rs2679757 as a risk of SNP were found to have

Figure 1. Variable rate of disease progression in hepatitis B patients.

AZIN1 rs267957, $P < 0.05$

AZIN1 rs267957, $P < 0.01$

TRPM5 rs886277, $P < 0.01$

TRPM5 rs886277, $P < 0.05$

Figure 2. Association of AZIN1 rs267957 and TRPM5 rs886277 with the severity of liver cirrhosis.

Figure 3. Genotypic diversity for AZIN1 rs267957 and TRPM5 rs886277 with the progression rate of liver cirrhosis.

Figure 4. Association of AZIN1 rs2679757 or TRPM5 rs886277 with the occurrence of decompensated cirrhosis.

Notes: Decompensation events: ascites, gastroesophageal varices bleeding, encephalopathy, spontaneous bacterial peritonitis, hepatocellular carcinoma, Child-Pugh score > 8

a higher frequency in Child-Pugh B plus C subgroup ($n = 194$) than in Child-Pugh A subgroup ($n = 235$) ($\chi^2 = 8.54$, $P = 0.003$). The analysis of CC homozygote of TRPM5 rs886277 as another risk SNP also achieved a similar result ($\chi^2 = 8.4$, $P = 0.004$).

The risk allele of AZIN1 rs2679757 and TRPM5 rs886277 also had higher frequencies among patients with clinical events of decompensated cirrhosis, such as ascites, gastroesophageal varices bleeding, encephalopathy, spontaneous bacterial peritonitis, hepatocellular carcinoma, and Child-Pugh score > 8 (Figure 4).

DISCUSSION

HBV infection is a public health threat worldwide, with up to two billion people harboring serological evidence of past or present infection, and 360 million with chronic infection.⁹ HBV infection is endemic highly in China with an HBV surface antigen sero-positive

rate as high as 5%-12%.¹⁰ The natural history of HBV infection varies from spontaneous recovery post infection, chronic asymptomatic carrier, to persistent infection with progression of fibrosis to decompensated cirrhosis and hepatocellular carcinoma, which confer a high mortality rate and heavy economic burden.¹¹⁻¹³ Besides factors such as alcohol abuse, gender, age, and viral genotypes contributing to this high variability, host genetic factors could be an important determinant predicting progression to cirrhosis in hepatitis B. A single nucleotide polymorphism (SNP) is the most common genetic mutation in humans and may change the amino acid sequence and protein function if present within the coding region of genes. Alternatively, SNPs may alter the gene's expression at the level of RNA transcription, splicing or protein translation if situated in the intronic or intergenic regulatory region. The identification of disease-related gene determinants may assist in the prediction of individual risk of disease progression, help to understand the pathophysiologic mechanisms of the diseases, and provide insight into the establishment of a personalized treatment strategy.

The present study provides the first evidence that the rs2679757 polymorphism in AZIN1 gene and rs886277 in TRPM5 gene were associated with the development and severity of cirrhosis in hepatitis B patients. Functionally, ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the biosynthesis of polyamines. AZIN1 titrates intracellular polyamine levels by antagonizing Az function, allowing ODC to escape Az mediated degradation and stimulate cellular polyamine uptake.¹⁵ In a recent paper,²⁵ the minor allelic SNP variant rs62522600 in the twelfth exon of AZIN1 favors the expression of a novel splice form, AZIN1 SV2, that inhibits the expression of fibrogenic genes in hepatic stellate cells via a novel, polyamine independent pathway.²⁵ However, this locus was not identified among diverse genotypes in Chinese (data not shown). The polymorphic nature of the AZIN1 gene may modulate individual responses to liver damage and serve as a common functional SNP in HBV- and HCV-induced hepatic fibrogenesis. The presence of this SNP in the intronic region of AZIN1 gene may alter the gene expression and function by creating an alternative splice site that affects post-transcriptional regulation of the gene. TRPM5 is one member of the melastatin subfamily of transient receptor potential ion channels that are widely distributed in mammalian tissues.²² It is a calcium-activated cation channel that functions in taste signal transduction.²⁶ The specific physiological function of TRPM5 in liver is unknown. The variant rs886277 leading to a missense change in amino acid sequence may influence the biological function of the TRPM5 protein.

In this study, a reliable medium-throughout genotyping platform using the Sequenom Mass ARRAY system was established for testing gene variants. This methodology allows simultaneous determination of multiple SNPs in one set with hundreds of samples, and has the potential to generate convenient polygenic tests useful in the clinical setting, and for testing the potential CRS signature built on risk of SNPs.

In conclusion, two polymorphisms among 6 candidate SNPs, rs2679757 in *AZIN1* gene and rs886277 in *TRPM5* gene, were associated with liver cirrhosis risk in Chinese hepatitis B patients. These SNPs related to cirrhosis warrant further basic investigation and clinical validation in hepatitis B. Larger efforts including research by using GWAS are needed to generate and validate CRS signatures specifically for Chinese hepatitis B patients.

Acknowledgements

This work was supported by Shanghai Pujiang Talent Program 2009 (09PJ1402600) to Jin-sheng Guo, and Wang Bao-En Liver Fibrosis Research Foundation (20090001) to Ji-yao Wang.

REFERENCES

- Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997;349:825-832.
- Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, et al. Host genetic factors influence disease progression in chronic hepatitis C. *Hepatology* 2000;31:828-833.
- Day CP. Genetic studies to identify hepatic fibrosis genes and SNPs in human populations. *Methods Mol Med* 2005;117:315-331.
- Karlsen TH, Melum E, Franke A. The utility of genome-wide association studies in hepatology. *Hepatology* 2010;51:1833-1842.
- Huang H, Shiffman ML, Cheung RC, Layden TJ, Friedman S, Abar OT, et al. Identification of two gene variants associated with risk of advanced fibrosis in patients with chronic hepatitis C. *Gastroenterology* 2006;130:1679-1687.
- Huang H, Shiffman ML, Friedman S, Venkatesh R, Bzowej N, Abar OT, et al. A 7 gene signature identifies the risk of developing cirrhosis in patients with chronic hepatitis C. *Hepatology* 2007;46:297-306.
- Marcolongo M, Young B, Dal Pero F, Fattovich G, Peraro L, Guido M, et al. A seven-gene signature (cirrhosis risk score) predicts liver fibrosis progression in patients with initially mild chronic hepatitis C. *Hepatology* 2009;50:1038-1044.
- Curto TM, Lagier RJ, Lok AS, Everhart JE, Rowland CM, Sninsky JJ, et al. Predicting cirrhosis and clinical outcomes in patients with advanced chronic hepatitis C with a panel of genetic markers (CRS7). *Pharmacogenet Genomics* 2011;21:851-860.
- Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev* 2006;28:112-125.
- Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. *J Gastroenterol Hepatol* 2000;15 Suppl:e3-e6.
- Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004;11:97-107.
- Yuen MF, Sablon E, Yuan HJ, Wong DK, Hui CK, Wong BC, et al. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. *Hepatology* 2003;37:562-567.
- Yin JH, Zhao J, Zhang HW, Xie JX, Li WP, Xu GZ, et al. HBV genotype C is independently associated with cirrhosis in community-based population. *World J Gastroenterol* 2010;16:379-383.
- Takata K. Aquaporin-2 (AQP2): its intracellular compartment and trafficking. *Cell Mol Biol* 2006;52:34-39.
- Mangold U. Antizyme inhibitor: mysterious modulator of cell proliferation. *Cell Mol Life Sci* 2006;63:2095-2101.
- Ternes P, Franke S, Zahringer U, Sperling P, Heinz E. Identification and characterization of a sphingolipid delta 4-desaturase family. *J Biol Chem* 2002;277:25512-25518.
- Kraveka JM, Li L, Szulc ZM, Bielawski J, Ogretmen B, Hannun YA, et al. Involvement of dihydroceramide desaturase in cell cycle progression in human neuroblastoma cells. *J Biol Chem* 2007;282:16718-16728.
- Li Y, Chang M, Abar O, Garcia V, Rowland C, Catanese J, et al. Multiple variants in toll-like receptor 4 gene modulate risk of liver fibrosis in Caucasians with chronic hepatitis C infection. *J Hepatol* 2009;51:750-757.
- Beutler B. Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* 2004;430:257-263.
- Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007;13:1324-1332.
- Guo J, Loke J, Zheng F, Hong F, Yea S, Fukata M, et al. Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of Toll-like receptor 4 to hepatic stellate cell responses. *Hepatology* 2009;49:960-968.
- Kaske S, Krasteva G, König P, Kummer W, Hofmann T, Gudermann T, et al. TRPM5, a taste-signaling transient receptor potential ion-channel, is a ubiquitous signaling component in chemosensory cells. *BMC Neurosci* 2007; 8:49.
- Jurinke C, Oeth P, van den Boom D. MALDI-TOF Mass Spectrometry. *Mol Biotech*, 2004;26:147-163.
- Ragoussis J, Elvidge GP, Kaur K, Colella S. Matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry in genomics research. *PLoS Genet* 2006;2:e100.
- Paris AJ, Snapir Z, Christopherson CD, Kwok SY, Lee UE, Ghiassi-Nejad Z, et al. A polymorphism that delays fibrosis in hepatitis C promotes alternative splicing of *AZIN1*, reducing fibrogenesis. *Hepatology* 2011;54: 2198-2207.
- Prawitt D, Monteilh-Zoller MK, Brixel L, Spangenberg C, Zabel B, Fleig A, et al. TRPM5 is a transient Ca²⁺-activated cation channel responding to rapid changes in [Ca²⁺]_i. *Proc Natl Acad Sci USA* 2003;100:15166-15171.