Polymorphism Rs34562254 In *TNFRSF13B* is Associated with HCV Infection Outcomes Among Chinese Han Population

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Abstract

Genetic variations in tumor necrosis factor receptor superfamily (*TNFRSF*) 13B have already proved to be associated with immune-related diseases. This study aimed to explore the relationship of tumor necrosis factor superfamily (*TNFSF*) 13, *TNFRSF13B*, and *TNFRSF14* missense mutations with the susceptibility of HCV infection. SNPs in *TNFSF13* (rs3803800, rs11552708), *TNFRSF13B* (rs34562254), and *TNFRSF14* (rs4870) were genotyped in 469 intravenous drug users, 728 hemodialysis patients and 1636 paid blood donors by TaqMan real-time PCR assay. And the USCS Brower and RNAfold web servers were used to predict the potential biological functions of the positive SNPs. After adjustment for gender, age, ALT level, AST level and route of infection, the results of logistic regression analysis showed that the subjects carried rs34562254 TT mutant homozygous type in *TNFRSF13B* were more likely to be infected by HCV compared to those with rs34562254 CC wild homozygous type (co-dominant model: OR = 1.52, 95% CI: 1.17–1.98, $P = 0.002$; recession model: OR = 1.41, 95% CI: 1.10–1.81, $P = 0.007$; additive model: OR = 1.21, 95% CI: 1.07–1.37, $P = 0.002$). And the effect of the risk allele (rs34562254-T) is more evident in female, elder (≥ 50), paid blood donors, lower ALT level (≤ 40 U/L) and lower ALT level (≤ 40 U/L) subgroup. The result of bioinformatics analysis showed that rs34562254 was located at the highest peak of the H3K4Me1 histone marker with the UCSC genome browser. And the minimum free energy (MFE) of the centroid secondary structure for the mutant T allele (-38.90 kcal/mol) of rs34562254 was higher than that of the wild C allele (-45.60 kcal/mol) with the RNAfold web servers. In addition, the RegulomeDB score of rs34562254 was 3a with the RegulomeDB web servers. All the above results of bioinformatics analysis suggested that rs34562254 may affect the gene expression level by regulating the transcriptional activity of the corresponding gene region. The rs34562254 polymorphisms in *TNFRSF13B* were significantly associated with HCV infection among Chinese Han population.

Introduction

Hepatitis C virus (HCV) is a positive-strand RNA virus and mainly transmitted through blood exposure including blood transfusion and reuse of contaminated medical equipment. Currently, it is estimated that more than 70 million people are chronically infected with HCV [1, 2], which often leads to chronic hepatitis, liver failure and hepatocellular carcinoma (HCC) [3–5]. Despite direct-acting antivirals (DAAs) have been well developed during the past decade, reinfection remains an important problem even after successful treatment in subjects with continued at risk behavior. In addition, currently no effective vaccine could be used for the prevention of HCV infection [6, 7]. Therefore, research on factors affecting the outcomes of HCV infection remains a critical issue of concern to people.

It is well known that the progression of HCV infection is mainly affected by the biological characteristics of HCV, host immunity, genetic background, and environmental factors[8]. Among of them, the immune system is generally recognized as an essential determinant of viral infection outcome [9, 10]. The tumor necrosis factor receptor superfamily (TNFRSF), naturally activated by ligands of tumor necrosis factor superfamily (TNFSF), is composed of 29 members in the human and most of which are expressed in the immune system [11–13]. Previously, a large number of studies have reported that *TNFSF/TNFRSF*

significantly affected the development of many diseases, such as rheumatoid arthritis (RA) [14], inflammatory bowel disease (IBD) [15], multiple sclerosis (MS) [16] and so on. Therefore, we speculate that the *TNFSF*/*TNFRSF* can affect the outcome of HCV infection by regulating the immune response.

Our previous researches have shown that genetic variants in *TNF* would influence the immune response of HCV infection and relevant to different immune-related diseases, such as *TNFRSF1A* [17], *TNFRSF5* [18], *TNFSF6* [19] and *TNFRSF11B* [20]. In addition, several studies have observed that genetic mutations of *TNFSF13*, *TNFRSF13B* and *TNFRSF14* were significantly associated with immune-related diseases including chronic lymphocytic leukemia (CLL) [21], IgA deficiency [22] systemic lupus erythematosus (SLE) [23], asthma symptoms [24], familial or sporadic immune thrombocytopenia and common variable immunodeficiency (CVID) [22, 25]. Thus, this study focuses mainly on association between single nucleotide polymorphisms (SNPs) in *TNFSF13*, *TNFRSF13B* and *TNFRSF14* and the outcomes of HCV infection. In the present study, we determined polymorphisms of *TNFSF13* rs3803800, *TNFSF13* rs11552708, *TNFRSF13B* rs34562254 and *TNFRSF14* rs4870 in 2833 Chinese adults at high-risk of HCV infection, and then analyzed the association between these variants and HCV infection.

Methods

Participants

2833 Chinese adults (18–80 years old) from three high-risk groups (Intravenous drug users, IVDU; hemodialysis patients, HD and paid blood donors, PBD) were included in this case-control study. All the subjects were collected from 2008 to 2016 in three different centers: the Nanjing compulsory drug rehabilitation center in Jiangsu province of China (459 IVDU), nine hospital hemodialysis centers in southern China (722 HD), and the crowd of paid blood donors from six villages in Zhenjiang (1619 PBD). Subjects co-infected with any other hepatotropic virus or human immunodeficiency virus (HIV), or suffered from other types of liver diseases, or received any antiviral therapy were excluded from this study.

Structured interviews and standardized questionnaires were carried out with every participant to obtain demographic and environmental exposure history information. When exposed to HCV, subjects may be infected by the virus (subjects carried seropositive anti-HCV). Therefore, only about 30% of those newly infected by HCV can effectively clear the virus (subjects carried seropositive anti-HCV and seronegative HCV RNA), while others who failed to clear HCV within 6 months will develop into chronic HCV infection (subjects carried seropositive anti-HCV and seropositive HCV RNA) [26, 27]. According to the above [27], the participants were assigned to three groups based on their sera HCV antibody and sera HCV RNA: Subjects carried seronegative anti-HCV and seronegative HCV RNA were divided into HCV uninfected control group (Group A); Subjects carried seropositive anti-HCV and seronegative HCV RNA were divided into HCV spontaneous clearance group (Group B); Subjects carried seropositive anti-HCV and seropositive HCV RNA were divided into HCV chronic infection group (Group C). Additionally, HCV spontaneous clearance group (Group B) and HCV chronic infection group (Group C) were combined into the case group (Group B + C), as compared with the HCV uninfected control group (Group A). Thus, this study compared the difference of

genotype frequencies between Group A and Group (B + C) to explore the association between the candidate SNPs or other risk factors and the susceptibility of HCV infection, and also compared the difference of genotype frequencies between Group B and Group C to explore the association between the candidate SNPs or other risk factors and chronic HCV infection.

All study procedures were approved by the medical ethics committee of Nanjing Medical University in accordance with the Declaration of Helsinki. The written informed consent was obtained from each participant.

Serological testing

10 mL of EDTA anticoagulative venous blood was taken from subjects enrolled in this study. The plasma, white blood cells and red blood cells were centrifuged and stored at -20°C for further examination, respectively. Anti-HCV, viral load and virus genotype, serological markers of HBV/HIV, and biochemical indicators of liver function (alanine aminotransferase, ALT; aspartate aminotransferase, AST) were measured using commercial reagents according to the manufactures' introduction.

SNPs selection

The genetic informations about *TNFSF13*, *TNFRSF13B* and *TNFRSF14* genes in HapMap Phase II CHB (Chinese in Beijing; up to 2 kb in the upstream and downstream) were downloaded from the 1000 Genomes database (<http://www.1000genomes.org/>). To yielded the corresponding tagSNP, we loaded the data within the Haploview 4.2 software, set the minor allele frequency (MAF) as > 0.05 and the correlation coefficient r^2 as ≥ 0.8 . Furthermore, we used HaploReg database (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>) and SNP Function Prediction (<https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html>) to select four missense mutation SNP candidates (rs3803800, rs11552708, rs34562254 and rs4870) for the present analysis.

SNPs genotyping

Genomic DNA was extracted from venous blood samples by the traditional phenol-chloroform method, and the purity and concentration of DNA were determined by ultraviolet spectrophotometry, which was uniformly standardized and stored in -20°C. Genotyping of four SNPs was performed with a TaqMan allelic discrimination assay on the LightCycler® 480 II Real-Time PCR System (Roche, Switzerland). The primers and probe sequences for candidate SNPs are shown in Table S1. The whole genotyping process was performed by experimenters without knowing the grouping of the research objects. The accordance rate of each SNP was 100% for the repeated experiments of 10% random samples. Moreover, the genotyping success rate of each polymorphism was above 95%.

In silico analysis

We used the following methods to analyze the biological functions of selected SNPs: (1) RegulomeDB online database (<http://www.regulomedb.org/>) for obtaining different RegulomeDB scores which represents different detailed information (Table S2); (2) Vienna RNA Web Servers (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>) for predicting secondary structures of

single stranded RNA sequences based on the latest ViennaRNA Package (Version 2.4.4);(3) UCSC Genome Bioinformatics website (<http://genome.ucsc.edu/>) for annotating the potential biological function. The data of H3K4Me1 histone markers of seven cell lines (GM12878, H1-hESC, HSMM, HUVEC, K562, NHEK and NHLF) was collected and analyzed.

Statistical analysis

For basic genetic information, Hardy-Weinberg equilibrium (HWE) was estimated for each SNP among the controls with a goodness-of-fit χ^2 test. To compare the demographic characteristics difference among the three groups, we used one-way analysis of variance (ANOVA), chi-square (χ^2) test, or Kruskal-Wallis test where appropriate. The associations between selected SNPs with the susceptibility of HCV infection or the outcomes of HCV infection were estimated by constructing logistic regression models including age, gender, route of infection and each of the SNPs, and were explained by odds ratios (ORs), 95% confidence intervals (CIs) using co-dominant model, dominant model, recessive model and additive model. The statistical analysis was performed using STATA14.0 software. P -value < 0.05 was considered as significant in all analysis except multiple comparisons for SNPs, in which P -values < 0.0125 (0.05/4) were considered as significant after Bonferroni correction.

Results

Participant characteristics

According to the characteristics of anti-HCV and HCV RNA, the subjects were divided into three groups, including 1543 cases of HCV uninfected control group (Group A), 522 cases of HCV spontaneous clearance group (Group B) and 768 cases of HCV chronic infection group (Group C). The demographic and clinical information about the three groups were shown in Table 1. No significant difference in the distribution of age and gender was observed among three groups (both $P > 0.05$). However, there were significant differences in the abnormal rates of ALT, AST, the types of infection routes, and the distribution of HCV genotypes among three groups (both $P < 0.05$).

Association of candidate SNPs with HCV infection outcomes

Table S1 showed that MAFs of selected SNPs were greater than 20%, and all of them were consistent with HWE in the HCV uninfected group (Group A). As the results shown in Table 2, after adjusting for age, gender, and other potential confounding factors, logistic regression analyses suggested that the participants carried *TNFRSF13B* rs34562254-T had an increased risk of HCV infection (co-dominant model: OR=1.52, 95%CI: 1.17-1.98, $P=0.002$; additive model: OR=1.21, 95%CI: 1.07-1.37, $P=0.002$; recessive model: OR=1.41 95%CI=1.10-1.81, $P=0.007$). However, our present results did not show any significant association between selected SNPs and HCV chronicity (all $P > 0.05$).

Stratification analysis of TNFRSF13B-rs34562254

Furthermore, we explored the rs34562254 loci by stratified analysis to exclude the influence of confounding factors based on the additive model. As the results shown in Table S3, the rs34562254 genotype remained significantly related to the susceptibility risk of HCV infection in age and gender subgroup (both $P < 0.0125$). In addition, other variables including ALT ≤ 40 U/L (OR=1.22, 95%CI=1.07-1.39, $P=0.003$), AST ≤ 40 U/L (OR=1.23, 95% CI=1.08-1.40, $P=0.002$) and PBD (OR=1.29, 95%CI=1.09-1.53, $P=0.003$), were also statistically correlated with HCV susceptibility. Based on the results of the stratified analysis, we further tested the heterogeneity of each stratified variable and found that there was no heterogeneity between the sub-layers (all $P > 0.05$).

In silico analysis

As the results shown in Fig.1, the RegulomeDB score of rs34562254 was 3a, suggesting that rs34562254 might be located at the site of TF binding, motif, and DNase peak. In addition, RNAfoldweb servers was used to predict *TNFRSF13B* RNA secondary structure. The MAF of the centroid secondary structure for the mutant T allele (-38.90 kcal/mol) of rs34562254 was higher than that of the wild C allele (-45.60 kcal/mol), indicating that the polymorphism of rs34562254 in *TNFRSF13B* could affect the transcription of related RNA (Figure 1). Moreover, we explored the potential biological functions of rs34562254 in the Encyclopedia of DNA Elements (ENCODE) project using the UCSC genome browser. As the results shown in Figure 2, rs34562254 was located on the highest peak of the H3K4Me1 histone marker in seven cell lines. H3K4me1 is the predominant histone modification involved in nucleosomes around enhancer elements, and linked to transcriptional regulation of genes.

Discussion

This study found that *TNFRSF13B* rs34562254 was associated with the susceptibility of HCV infection. Compared to subjects with rs34562254-CC (the wild type), the subjects with CT/TT have an increased risk of HCV infection. Moreover, the effect of the T allele was more evident in elder (≥ 50 years old), female, paid blood donors, lower AST level (≤ 40 U/L) and lower ALT level (≤ 40 U/L) subgroups. In addition, bioinformatics analysis implied that rs34562254 was located at the highest peak of the H3K4Me1 histone marker and might regulate the mRNA transcription by affecting the binding of the transcription factors.

TNFRSF13B, a lymphocyte-specific tumor necrosis factor receptor that interacts with the NF- κ B pathway and regulates B-cell development [28, 29], has been reported to be associated with a variety of human immune-related diseases such as CVID [22], asthma symptoms[24] and SLE[23]. Furthermore, Rand. K, et al have found that polymorphism rs34562254 in *TNFRSF13B* was correlated with multiple myeloma and coronary artery lesion [30]. In current study, based on the analyses of four genetic models, we also found that the subjects with rs34562254-T were linked to the risk of HCV infection.

From NCBI dbSNP (<https://www.ncbi.nlm.nih.gov/snp/>) browser, we found that rs34562254 was a missense mutation located in the exon region of *TNFRSF13B*. According to RegulomeDB, we found the RegulomeDB score for rs34562254 was scored as 3a (<https://www.regulomedb.org/regulome-search/?regions=rs34562254&genome=GRCh37>). RegulomeDB is a database that annotates SNPs with known

and predicted regulatory elements in the intergenic regions of the *H. sapiens* genome, and the RegulomeDB score represents a model integrating functional genomics features along with continuous values such as eQTL, TF binding, TF motif, ChIP-seq signal, DNase Footprint and DNase Footprint[31]. Therefore, we speculated that rs34562254 SNP might have potential transcriptional regulatory functions including TF binding and DNase peak. Through RNAfold web server, we found wild C allele of the centroid secondary structure had a lower MAF than mutant T allele (-45.60 vs. -38.90 kcal/mol); Through ENCODE project and the UCSC genome browser, we found rs34562254 was located on the highest peak of the H3K4Me1 histone marker, which was the predominant histone modification involved in nucleosomes around enhancer elements. When the enhancer region is enriched with only H3K4me1 modification, the enhancer is in a state of equilibrium, and when the enhancer region is enriched with both H3K4me1 and H3K27ac modifications, the enhancer is in an activated state to promote gene expression[32]. As a result, we speculated genetic variants in rs34562254 might affect *TNFRSF13B* expression by influencing the binding of transcription factors.

The results of stratified analysis showed that the rs34562254-T risk allele was significantly associated with HCV susceptibility in the female, and the subjects over 50 years old, PBDs, subjects with lower ALT level (≤ 40 U/L) and lower ALT level (≤ 40 U/L). In general, females usually develop more intense innate, humoral and cellular immune responses to viral infections and to vaccination compared to male subjects [33], which meant that the risk effect of allele is more significant in female subgroup. For AST and ALT, AST and ALT level are a key indicator for the degree of liver injury. When hepatitis C occurs, AST and ALT level are abnormal (> 40 U/L), which may confuse the effects of genes [19]. Given that HCV is a common infection among older adults in certain areas of China [34]. Due to older people have worse health and require exposure to potentially contaminated needles or blood products than younger people[35], the result showed that the risk effect of allele is more significant in age up 50 years subgroup. For PBDs, they may rely on blood donation as a source of income. As blood-borne pathogens, both HCV and HIV were frequently detected among PBDs in China during the early 1980s [36], so they had a very high prevalence of HCV infection [37].

However, there are several limitations in this study. First, we did not test more SNPs in *TNF/ TNFR* that might associate with HCV infection. Therefore, studies with extensive genome coverage are required in the future to increase understanding of the role of *TNFRSF13B* polymorphisms in process of HCV infection. Second, in the multivariate regression analysis of *TNFRSF* SNP distribution and HCV infection outcome, HCV genotype was not included due to the lack of data. But we used the existing data of HCV genotype to perform a logistic regression on rs34562254 and found that the *P* value did not change much. We'll proceed to the further study and collect as much baseline data as possible to improve the reliability of our study. Third, potential biological functions of *TNFRSF13B* rs34562254 were only speculated *in silico* analysis. The exact mechanisms of rs34562254 in *TNFRSF13B* in HCV infection should be further investigated.

Conclusion

In conclusion, our present study firstly reported that *TNFRSF13B* missense mutation (rs34562254) was significantly associated with HCV susceptibility among Chinese Han population. Moreover, the effect of risk mutant genotype was more evident in female, elder (≥ 50), paid blood donors, lower ALT level (≤ 40 U/L) and lower ALT level (≤ 40 U/L) subgroup. These findings can provide valuable genetic information to deepen understanding of the susceptibility to HCV infection and offer a reference for developing a prevention and control strategy among high risk population of HCV repeated infection.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Nanjing Medical University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

All authors made substantial contributions to editing and drafting of the manuscript. H.Z.F, Z.Q.F. and M.Y. designed and organized the study and supervised the whole project. H.Z.F, Z.Q.F., Z.J.G., C.H.W., J.L. and M.Y. contributed to field survey, data collection, laboratory detection and quality control. H.Z.F, Z.Q.F., D.C., C.S. and R.B.Y. performed data cleansing and statistical analysis. H.Z.F, Z.Q.F and Y.Z. provided analysis tools and performed data interpretation. H.Z.F, Z.Q.F., C.D. and M.Y. wrote and critical revised the manuscript. All authors read and approved the final manuscript.

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References

1. WHO.: **Global hepatitis report. Geneva World Health Organization.**2017
<http://apps.who.int/iris/bitstream/10665/255016/1/9789241565455-eng.pdf?ua=1>.
2. Agner KJ. Principles for automation of quantitative chemical analysis by a discontinuous flow system and their application. *Scand J Clin Lab Invest Suppl.* 1967;100:139.
3. Chinese Society of Hepatology and Chinese Society of Infectious Diseases CMA. Guidelines for the prevention and treatment of hepatitis C (2019 version). *J Clin Hepatol.* 2019;35(12):1–17.
4. Cacoub P, Comarmond C, Domont F, Savey L, Desbois AC, Saadoun D. Extrahepatic manifestations of chronic hepatitis C virus infection. *Therapeutic advances in infectious disease.* 2016;3(1):3–14.
5. Allison RD, Tong X, Moorman AC, Ly KN, Rupp L, Xu F, Gordon SC, Holmberg SD. **Increased incidence of cancer and cancer-related mortality among persons with chronic hepatitis C infection, 2006–2010.** *J Hepatol* 2015, **63**(4):822–828.
6. Honegger JR, Zhou Y, Walker CM. Will there be a vaccine to prevent HCV infection? *Semin Liver Dis.* 2014;34(1):79–88.
7. WHO.: **GUIDELINES FOR THE CARE AND TREATMENT OF PERSONS DIAGNOSED WITH CHRONIC HEPATITIS C VIRUS INFECTION.** 2018.
8. Grebely J, Dore GJ, Kim AY, Lloyd A, Shoukry NH, Prins M, Page K. Genetics of spontaneous clearance of hepatitis C virus infection: a complex topic with much to learn. *Hepatology.* 2014;60(6):2127–8.
9. Larrubia JR, Moreno-Cubero E, Lokhande MU, Garcia-Garzon S, Lazaro A, Miquel J, Perna C, Sanz-de-Villalobos E. Adaptive immune response during hepatitis C virus infection. *World journal of gastroenterology.* 2014;20(13):3418–30.
10. Piconese S, Cammarata I, Barnaba V. Viral hepatitis, inflammation, and cancer: A lesson for autoimmunity. *J Autoimmun.* 2018;95:58–68.
11. Fletcher NF, Sutaria R, Jo J, Barnes A, Blahova M, Meredith LW, Cosset FL, Curbishley SM, Adams DH, Bertoletti A, et al. Activated macrophages promote hepatitis C virus entry in a tumor necrosis factor-dependent manner. *Hepatology.* 2014;59(4):1320–30.
12. Fletcher NF, Clark AR, Balfe P, McKeating JA. TNF superfamily members promote hepatitis C virus entry via an NF-kappaB and myosin light chain kinase dependent pathway. *J Gen Virol.* 2017;98(3):405–12.
13. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nature reviews Immunology.* 2003;3(9):745–56.
14. Durie FH, Fava RA, Foy TM, Aruffo A, Ledbetter JA, Noelle RJ: **Prevention of collagen-induced arthritis with an antibody to gp39, the ligand for CD40.** *Science (New York, NY)* 1993, **261**(5126):1328–1330.
15. Hikosaka Y, Nitta T, Ohigashi I, Yano K, Ishimaru N, Hayashi Y, Matsumoto M, Matsuo K, Penninger JM, Takayanagi H, et al. The cytokine RANKL produced by positively selected thymocytes fosters

- medullary thymic epithelial cells that express autoimmune regulator. *Immunity*. 2008;29(3):438–50.
16. Volpe E, Sambucci M, Battistini L, Borsellino G. Fas-Fas Ligand: Checkpoint of T Cell Functions in Multiple Sclerosis. *Frontiers in immunology*. 2016;7:382.
 17. Yue M, Huang P, Wang C, Fan H, Tian T, Wu J, Luo F, Fu Z, Xia X, Zhu P, et al: **Genetic Variation on TNF/LTA and TNFRSF1A Genes is Associated with Outcomes of Hepatitis C Virus Infection.** *Immunological investigations* 2020:1–11.
 18. Tian T, Huang P, Wu J, Wang C, Fan H, Zhang Y, Yu R, Wu C, Xia X, Fu Z, et al. CD40 polymorphisms were associated with HCV infection susceptibility among Chinese population. *BMC Infect Dis*. 2019;19(1):840.
 19. Huang P, Wang CH, Zhuo LY, Xia XS, Yang S, Zhang JW, Fan HZ, Wu JJ, Yu R, Yue M, et al. Polymorphisms rs763110 in FASL is linked to hepatitis C virus infection among high-risk populations. *Br J Biomed Sci*. 2020;77(3):112–7.
 20. Wu JJ, Huang P, Yue M, Wang CH, Wu C, Shao JG, Xue H, Fu ZQ, Zhuo LY, Yu RB, et al: **Association between TNFRSF11A and TNFRSF11B gene polymorphisms and the outcome of hepatitis C virus infection.** *Zhonghua liu xing bing xue za zhi = Zhonghua liuxingbingxue zazhi* 2019, 40(10):1291–1295.
 21. Enjuanes A, Benavente Y, Bosch F, Martin-Guerrero I, Colomer D, Perez-Alvarez S, Reina O, Ardanaz MT, Jares P, Garcia-Orad A, et al. Genetic variants in apoptosis and immunoregulation-related genes are associated with risk of chronic lymphocytic leukemia. *Cancer research*. 2008;68(24):10178–86.
 22. Karaca NE, Severcan EU, Guven B, Azarsiz E, Aksu G, Kutukculer N. TNFRSF13B/TACI Alterations in Turkish Patients with Common Variable Immunodeficiency and IgA Deficiency. *Avicenna journal of medical biotechnology*. 2018;10(3):192–5.
 23. Wen L, Zhu C, Zhu Z, Yang C, Zheng X, Liu L, Zuo X, Sheng Y, Tang H, Liang B, et al: **Exome-wide association study identifies four novel loci for systemic lupus erythematosus in Han Chinese population.** 2018, 77(3):417.
 24. Janzi M, Melén E, Kull I, Wickman M, Hammarström L. Rare mutations in TNFRSF13B increase the risk of asthma symptoms in Swedish children. *Genes Immun*. 2012;13(1):59–65.
 25. Freiburger T, Ravcukova B, Grodecka L, Pikulova Z, Stikarovska D, Pesak S, Kuklinek P, Jarkovsky J, Salzer U, Litzman J. Sequence variants of the TNFRSF13B gene in Czech CVID and IgAD patients in the context of other populations. *Hum Immunol*. 2012;73(11):1147–54.
 26. Spearman CW, Dusheiko GM, Hellard M, Sonderup M. Hepatitis C. *Lancet*. 2019;394(10207):1451–66.
 27. Chinese Society of Hepatology and Chinese Society of Infectious Disease CMA. Guidelines for the prevention and treatment of hepatitis C (2019 version). *Journal of Clinical Hepatology*. 2019;35(12):2670–86.
 28. Xia XZ, Treanor J, Senaldi G, Khare SD, Boone T, Kelley M, Theill LE, Colombero A, Solovyev I, Lee F, et al. TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. *The Journal of experimental medicine*. 2000;192(1):137–43.

29. Zhang X, Park CS, Yoon SO, Li L, Hsu YM, Ambrose C, Choi YS. BAFF supports human B cell differentiation in the lymphoid follicles through distinct receptors. *Int Immunol*. 2005;17(6):779–88.
30. Rand KA, Song C, Dean E, Serie DJ, Curtin K, Sheng X, Hu D, Huff CA, Bernal-Mizrachi L, Tomasson MH, et al. **A Meta-analysis of Multiple Myeloma Risk Regions in African and European Ancestry Populations Identifies Putatively Functional Loci.** *Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research*. cosponsored by the American Society of Preventive Oncology. 2016;25(12):1609–18.
31. Dong S, Boyle AP. Predicting functional variants in enhancer and promoter elements using RegulomeDB. *Hum Mutat*. 2019;40(9):1292–8.
32. Tian Y, Jia Z, Wang J, Huang Z, Tang J, Zheng Y, Tang Y, Wang Q, Tian Z, Yang D, et al. Global mapping of H3K4me1 and H3K4me3 reveals the chromatin state-based cell type-specific gene regulation in human Treg cells. *PloS one*. 2011;6(11):e27770.
33. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nature reviews Immunology*. 2008;8(9):737–44.
34. Zhang M, Sun XD, Mark SD, Chen W, Wong L, Dawsey SM, Qiao YL, Fraumeni JF Jr. Taylor PR, O'Brien TR: **Hepatitis C virus infection, Linxian, China.** *Emerg Infect Dis*. 2005;11(1):17–21.
35. He J, Lin J. HCV prevalence during the age range of peak sexual activity. *The Lancet Infectious diseases*. 2017;17(2):131–2.
36. Shan H, Wang JX, Ren FR, Zhang YZ, Zhao HY, Gao GJ, Ji Y, Ness PM. Blood banking in China. *Lancet*. 2002;360(9347):1770–5.
37. Rao HY, Sun DG, Yang RF, Liu F, Wang J, Feng B, Wu N, Fang JL, Song GJ, Ma H, et al. Outcome of hepatitis C virus infection in Chinese paid plasma donors: a 12-19-year cohort study. *Journal of gastroenterology hepatology*. 2012;27(3):526–32.

Tables

Table1. Demographic and clinical characteristics among HCV uninfected control, spontaneous clearance and chronic infection groups.

Variables	Group A (%)	Group B (%)	Group C (%)	<i>P</i>
	n=1543	n=522	n=768	
Age (years)	53.2±13.7	50.3±14.1	51.9±12.4	<0.001 ^a
				0.067 ^b
<50	561(36.36)	212 (40.61)	312(40.63)	
≥50	982(63.64)	310(59.39)	456(59.38)	
Gender				0.117 ^b
Male	617(39.99)	199(38.12)	273(35.55)	
Female	926(60.01)	323(61.88)	495(64.45)	
ALT (U/L)				<0.001 ^b
≤40	1450(94.96)	416(79.85)	453(59.14)	
>40	77(5.04)	105(20.15)	313(40.86)	
AST (U/L)				
≤40	1456(95.48)	424(82.65)	467(61.69)	<0.001 ^b
>40	69(4.52)	89(17.35)	290(38.31)	
Route of infection				<0.001 ^b
HD	561 (36.36)	91 (17.43)	76 (9.90)	
IVDU	181 (11.73)	148 (28.35)	140 (18.23)	
PBD	801 (51.91)	283 (54.21)	552 (71.88)	
HCV genotype				<0.001 ^b
1b	–	42(26.25)	223(46.07)	
Non-1b	–	118(73.75)	261(53.93)	

Group A: uninfected controls; Group B: spontaneous clearance subjects; Group C: chronic infection patients.

Non-1b means viral strains other than 1b, including genotype 1a, 2, and 3 (either solely or mixed infection).

Abbreviations: HCV, hepatitis C virus; SD, standard deviation; ALT, alanine transaminase; AST, aspartate transaminase; HD, hemodialysis patients; IVDU, Intravenous drug user; PBD, paid blood donors.

^a*P* value of Welch among three groups, heterogeneity of variance.

^b*P* value of χ^2 -test among three/two groups.

Table 2. Genotypes distributions of *TNFRSF* genes among HCV uninfected control, spontaneous clearance and chronic infection groups.

SNPs (genotype)	Group A n (%)	Group B n (%)	Group C n (%)	OR(95%CI) ^a	<i>P</i> ^a	OR(95%CI) ^b	<i>P</i> ^b
	n=1543	n=522	n=768				
<i>TNFSF13- rs3803800</i>					0.834 [*]		0.593 ^{**}
GG	645 (44.00)	221 (43.50)	342 (46.22)	1.00	–	1.00	–
GA	669 (45.63)	231 (45.47)	325 (43.92)	1.01 (0.85- 1.21)	0.908	0.91 (0.70- 1.17)	0.443
AA	152 (10.37)	56 (11.02)	73 (9.86)	1.00 (0.74- 1.33)	0.977	0.85 (0.56- 1.27)	0.421
Dominant model				1.01 (0.85- 1.19)	0.93	0.89 (0.70- 1.14)	0.361
Recessive model				1.00(0.75- 1.31)	0.946	0.89 (0.60- 1.31)	0.550
Additive model				1.00 (0.88- 1.14)	0.971	0.91 (0.76- 1.10)	0.334
<i>TNFSF13- rs11552708</i>					0.024 [*]		0.588 ^{**}
GG	573 (38.00)	183 (35.47)	249 (32.98)	1.00	–	1.00	–
GA	684 (45.36)	252 (48.84)	390 (51.66)	1.18 (0.98- 1.41)	0.080	1.06 (0.814- 1.370)	0.680
AA	251 (16.64)	81 (15.7)	116 (15.36)	0.91 (0.711.72)	0.468	1.00(0.698- 1.440)	0.991
Dominant model				1.11 (0.93- 1.31)	0.257	1.04 (0.81- 1.34)	0.736
Recessive model				0.83 (0.66- 1.04)	0.112	0.97 (0.70- 1.35)	0.856
Additive model				1.03(0.92- 1.15)	0.615	1.05(0.89- 1.24)	0.576
<i>TNFRSF13B- rs34562254</i>					0.009 [*]		0.251 ^{**}
CC	661 (43.95)	190 (36.54)	312 (41.16)	1.00	–	1.00	–
CT	673 (44.75)	251 (48.27)	340 (44.85)	1.15 (0.96- 1.38)	0.119	0.81 (0.63- 1.05)	0.112
TT	170(11.30)	79 (15.19)	106 (13.98)	1.52 (1.17- 1.98)	0.002	0.86(0.60- 1.22)	0.401

Dominant model				1.22 (1.03-1.45)	0.019	0.82 (0.65-1.05)	0.114
Recessive model				1.41 (1.10-1.81)	0.007	0.96(0.69-1.34)	0.805
Additive model				1.21 (1.07-1.37)	0.002	0.90 (0.76-1.06)	0.213
<i>TNFRSF14-rs4870</i>					0.031*		0.472**
AA	455(29.88)	177 (34.57)	241 (31.84)	1.00	–	1.00	–
AG	782 (51.35)	236(46.09)	352 (46.50)	0.77 (0.64-0.93)	0.043	1.06 (0.81-1.38)	0.684
GG	286 (18.78)	98 (19.34)	164 (21.66)	0.96(0.75-1.21)	0.708	1.25 (0.90-1.74)	0.186
Dominant model				0.82 (0.69-0.98)	0.028	1.11 (0.87-1.43)	0.396
Recessive model				1.12 (0.91-1.38)	0.290	1.21 (0.904-1.63)	0.201
Additive model				0.95 (0.84-1.07)	0.391	1.11(0.94-1.31)	0.206

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio; SNP, single nucleotide polymorphism.

Group A: uninfected controls; Group B: spontaneous clearance subjects; Group C: chronic infection patients.

*The P value of χ^2 -test refer to the distribution of SNPs between Group A and Group (B+C).

**The P value of χ^2 -test refer to the distribution of SNPs between Group B and Group C.

^aThe P value, OR and 95% CIs of Group (B+C) versus Group A were calculated on the basis of the logistic regression model, adjusted by gender, age, ALT level, AST level and route of infection.

^bThe P value, OR and 95% CIs of Group C versus Group B were calculated on the basis of the logistic regression model, adjusted by gender, age, ALT level, AST level and route of infection.

Bonferroni correction was applied and the P value was adjusted to 0.0125 (0.05/4).

Bold type indicates statistically significant results.

Table 3. Stratified analysis the association of rs34562254 with HCV susceptibility

Subgroups	Group A	Group B	Group C	OR(95%CI) ^a	<i>P</i> ^a	<i>P</i> ^b
	n (CC/CT/TT)	n (CC/CT/TT)	n (CC/CT/TT)			
Age						0.632
<50	221/232/78	78/99/35	131/122/56	1.17(0.97-1.41)	0.100	
≥50	440/441/92	112/152/44	181/218/50	1.26(1.07-1.66)	0.005	
Gender						0.103
Male	234/290/73	63/103/33	122/108/42	1.07(0.89-1.31)	0.439	
Female	427/383/97	127/148/46	190/232/64	1.32(1.12-1.55)	0.001	
ALT (U/L)						0.759
≤40	622/635/162	155/203/57	181/194/71	1.20(1.06-1.37)	0.004	
>40	33/36/8	35/48/21	130/145/35	1.13(0.78-1.62)	0.505	
AST (U/L)						0.355
≤40	624/636/160	160/201/58	174/202/71	1.22(1.07-1.39)	0.002	
>40	30/35/10	26/47/19	131/136/33	1.01(0.68-1.51)	0.950	
Route of infection						0.706
HD	229/231/75	29/47/15	41/23/12	1.14(0.87-1.49)	0.357	
IVDU	77/78/22	53/72/23	57/59/24	1.18(0.89-1.55)	0.250	
PBD	355/364/73	108/132/41	214/258/70	1.29(1.09-1.53)	0.003	

Abbreviations: CI, confidence interval; HCV, hepatitis C virus; OR, odds ratio; HD, hemodialysis patients; IVDU, Intravenous drug user; PBD, paid blood donors.

Group A: uninfected controls; Group B: spontaneous clearance subjects; Group C: chronic infection patients. Group (B+C): Infected individuals (spontaneous clearance subjects + chronic infection patients).

^a The *P* value, OR and 95% CIs of Group (B+C) versus Group A were calculated on the basis of the additive model, adjusted by gender, age, ALT level, AST level and route of infection.

^b *P*-value for the heterogeneity test.

Supplementary

Supplementary Tables S2 and S3 are not available with this version

Figures

Figure 1

The influence of rs34562254 variants on mRNA centroid secondary structures of TNFRSF13B.

Figure 2

Functional annotation for SNP rs34562254 using ENCODE data from UCSC genome browser.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalTable1.docx](#)