The *TNFRSF13B* rs34562254 Polymorphism is Associated with Hepatitis C Infection Risk in the Han Chinese Population

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Abstract

Background

Genetic variations in the tumor necrosis factor receptor superfamily (*TNFRSF) 13B* have been reported 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 hepatitis C virus (HCV) infection susceptibility.

Methods

Single-nucleotide polymorphisms (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 using a TaqMan real-time PCR assay. The USCS browser and RNAfold web servers were used to predict the biological functions of these selected SNPs.

Results

After adjusting for gender, age, levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and route of infection, a logistic regression analysis showed that subjects carrying a homozygous *TNFRSF13B* rs34562254 TT mutant were more likely to be infected by HCV compared to those homozygous with the rs34562254 CC wild 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). The effect of the risk allele rs34562254-T was stronger in subjects that were female, older (≥ 50 years old), paid blood donors, and with lower ALT and AST levels (≤ 40 U/L). A bioinformatics analysis via the UCSC genome browser found that rs34562254 was located at the highest peak of the H3K4Me1 histone marker. Using the RNAfold web servers, the minimum free energy of the centroid secondary structure was found to be higher for the mutant rs34562254-T allele (-38.90 kcal/mol) than its wild type C allele (-45.60 kcal/mol). Additionally, the RegulomeDB score of rs34562254 was 3a. Altogether, the results of the bioinformatics analysis indicate that rs34562254 may affect gene expression levels by regulating the transcriptional activity of corresponding gene regions.

Conclusions

The rs34562254 polymorphisms in *TNFRSF13B* were significantly associated with HCV infection among the Han Chinese population.

Background

The hepatitis C virus (HCV) is a positive-strand RNA virus mainly transmitted through exposure to blood, for example, via blood transfusion or reuse of contaminated medical equipment. Currently, more than 70 million people are estimated to be chronically infected with HCV [1, 2], which often leads to chronic hepatitis, liver failure, and hepatocellular carcinoma [3–5]. Despite the development of direct-acting antivirals (DAAs) over the last decade, reinfection after successful treatment remains a problem in people who engage in risky behavior. Additionally, a vaccine that effectively prevents HCV infection is not yet currently available [6, 7]. Therefore, the factors affecting HCV infection outcomes are a critical issue and require further research.

It is well known that the progression of HCV infection is mainly affected by its biological characteristics, the host's immunity and genetic background, and the environment [8]. Among these factors, the immune system is generally recognized as an essential determinant of viral infection outcome [9, 10]. The tumor necrosis factor receptor superfamily (TNFRSF), is naturally activated by members of the tumor necrosis factor superfamily (TNFSF), consists of 29 members in humans, and is mostly expressed in the immune system [11–13]. Previous studies have reported that *TNFSF/TNFRSF* significantly affects the development of numerous diseases, such as rheumatoid arthritis [14], inflammatory bowel disease [15], and multiple sclerosis [16]. Therefore, we hypothesize that TNFSF/TNFRSF, by regulating the immune response, can affect HCV infection outcome.

We previously reported that genetic variants of *TNFSF/TNFRSF* such as *TNFRSF1A* [17], *TNFRSF5* [18], *TNFSF6* [19], and *TNFRSF11B* [20] influence the immune response to HCV infection and are associated with various immune-related diseases. In addition, several studies have reported that genetic mutations of *TNFSF13*, *TNFRSF13B*, and *TNFRSF14* are significantly associated with immune-related diseases including chronic lymphocytic leukemia [21], IgA deficiency [22], systemic lupus erythematosus (SLE) [23], asthma [24], familial or sporadic immune thrombocytopenia, and common variable immunodeficiency (CVID) [22, 25]. Thus, this study mainly focused on the association between single nucleotide polymorphisms (SNPs) in *TNFSF13*, *TNFRSF13B*, and *TNFRSF14* and HCV infection outcomes. More specifically, we assayed the *TNFSF13* rs3803800, *TNFSF13* rs11552708, *TNFRSF13B* rs34562254, and *TNFRSF14* rs4870 polymorphisms in 2833 Chinese adults at high-risk of HCV infection and subsequently 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 assayed. Data from the subjects were collected between 2008 to 2016 from three different centers: the Nanjing Compulsory Drug Rehabilitation Center in Jiangsu province (IVDU; $n = 459$), nine hospital hemodialysis centers in southern China (HD; $n = 722$), and paid blood donors from six villages in Zhenjiang (PBD; $n = 1619$). Each participant underwent structured interviews and answered standardized questionnaires for demographic information and

environmental exposure histories. Subjects co-infected with other hepatotropic viruses or with human immunodeficiency virus (HIV), who suffered from other liver diseases, or received any antiviral therapy were excluded from the study.

Structured interviews and standardized questionnaires were carried out with every participant to obtain demographic and environmental exposure history information. When exposed to HCV, some people may become infected by the virus and carry serapositive anti-HCV. Only 30% of these newly infected people can effectively clear the virus; that is, they carry serapositive anti-HCV and seranegative HCV RNA. Those who fail to clear HCV within 6 months will develop chronic HCV infection and carry serapositive anti-HCV and serapositive HCV RNA [26, 27]. From these distinctions [27], participants were assigned to three groups based on the state of their sera HCV antibody and sera HCV RNA. In Group A, the HCV uninfected control group, subjects carried seronegative anti-HCV and seronegative HCV RNA. Those who carried seropositive anti-HCV and seronegative HCV RNA formed the HCV spontaneous clearance group (Group B). Finally, subjects who carried seropositive anti-HCV and seropositive HCV RNA were classified into the HCV chronic infection group (Group C). Additionally, the HCV spontaneous clearance group (Group B) and HCV chronic infection group (Group C) were combined into a case group (Group B + C) and compared to the HCV uninfected control group (Group A). Thus, we compared genotype frequencies between Group A and Group (B + C) to explore the association between candidate SNPs and/or other risk factors to the susceptibility of HCV infection. We also compared genotype frequencies between Group B and Group C to determine whether candidate SNPs and/or other risk factors associate with chronic HCV infection.

Serological testing

10 mL of venous blood collected in anticoagulative EDTA tubes was taken from the subjects. The plasma, white blood cells, and red blood cells were centrifuged and stored at -20 °C for later examination. Anti-HCV, viral load and genotype, HBV/HIV serological markers, and biochemical indicators of liver function (alanine aminotransferase [ALT], aspartate aminotransferase [AST]) were measured using commercial reagents according to the manufacturers' instructions.

SNPs selection

Genetic information regarding the *TNFSF13*, *TNFRSF13B*, and *TNFRSF14* genes from HapMap (Phase II; CHB, Han Chinese in Beijing; upwards of 2 kb upstream and downstream) were downloaded from the 1000 Genomes database (http://www.1000genomes.org/). To extract their corresponding tagSNPs, we loaded the data into Haploview 4.2 software and set the minor allele frequency (MAF) as > 0.05 and correlation coefficient r^2 as ≥ 0.8. Furthermore, we used the 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 analysis.

SNPs genotyping

Genomic DNA was extracted from venous blood using the phenol-chloroform method. DNA purity and concentration were determined with ultraviolet spectrophotometry, standardized, and stored at -20℃. SNPs

were genotyped with a TaqMan allelic discrimination assay using the LightCycler® 480 II real-time PCR System (Roche, Switzerland). The primers and probe sequences for candidate SNPs are listed in Table S1. The genotyping was performed by experimenters who were blind to the subject's assigned group. Each SNP had an accordance rate of 100% for repeated experiments of 10% random samples, and the genotyping success rate of each polymorphism was greater than 95%.

In silico **analysis**

We used the following to analyze the biological functions of our selected SNPs: the RegulomeDB online database (http://www.regulomedb.org/) to obtain RegulomeDB scores representing regulatory potential (Table S2); Vienna RNA Web Servers (http://rna.tbi.univie.ac.at/cgi-bin/RNAWeb Suite/RNAfold.cgi; ViennaRNA Package Version 2.4.4) to predict secondary structures of single stranded RNA sequences; and the UCSC Genome Bioinformatics website (http://genome.ucsc.edu/) to elucidate potential biological functions. H3K4Me1 histone marker data from seven cell lines (GM12878, H1-hESC, HSMM, HUVEC, K562, NHEK, and NHLF) was also collected and analyzed.

Statistical analysis

For basic genetic information, a Hardy-Weinberg equilibrium (HWE) was estimated for each SNP among the control subjects (Group A) using a goodness-of-fit χ^2 test. To compare demographic differences among the three groups, we used one-way analysis of variance (ANOVA), chi-square (χ^2) test, or Kruskal-Wallis test where appropriate. The association between each SNP and HCV infection susceptibility or outcome was estimated by constructing logistic regression models based on age, gender, and route of infection. The associations were further clarified with odds ratios (ORs) and 95% confidence intervals (CIs) using co-dominant, dominant, recessive, and additive models.

STATA14.0 software was used to perform statistical analyses. *P*-values < 0.05 were considered significant in all analyses except in multiple comparisons between SNPs, in which after Bonferroni corrections, *P*-values < 0.0125 (0.05/4) were considered significant.

Results

Participant characteristics

Based on their anti-HCV and HCV RNA results, subjects were divided into three groups: HCV uninfected control group (Group A; $n = 1543$), HCV spontaneous clearance group (Group B; $n = 522$), and HCV chronic infection group (Group C; $n = 758$). The demographic and clinical information for the three groups were shown in Table 1. The groups did not significantly differ in age or gender distributions (both $P > 0.05$). However, ALT and AST levels, infection route, and distribution of HCV genotypes significantly differed among the three groups (both $P < 0.05$).

Table 1
Demographic and clinical characteristics among HCV uninfected control, spontaneous clearance and chronic infection groups.

Variables	Group A (%)	Group B (%)	Group C (%)	*P*
	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.

Variables	Group A (%)	Group B (%)	Group C (%)	*P*
[a] *P* value of Welch among three groups, heterogeneity of variance.				
[b] *P* value of χ^2-test among three/two groups.				

Association of candidate SNPs with HCV infection outcomes

The MAFs of the selected SNPs were greater than 20%, and all of them were consistent with HWEs from the HCV uninfected group (Group A; Table S1). As shown in Table 2, after adjusting for age, gender, and other potential confounding factors, a logistic regression analysis found that participants carrying *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, we did not find an association between SNPs and HCV infection outcome (all *P* > 0.05).

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]	*P*[a]	OR(95%CI)[b]	*P*[b]
	n = 1543	n = 522	n = 768				
TNFSF13-rs3803800					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
TNFSF13-rs11552708					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
TNFRSF13B-rs34562254					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

SNPs (genotype)	Group A n (%)	Group B n (%)	Group C n (%)	OR(95%CI)[a]	*P*[a]	OR(95%CI)[b]	*P*[b]
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
TNFRSF14-rs4870					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 χ2-test refer to the distribution of SNPs between Group B and Group C.

[a]The *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.

[b]The *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.

Stratification analysis of TNFRSF13B **rs34562254**

To further explore the rs34562254 loci, we used stratified analysis based on the additive model to exclude the influence of confounding factors. As shown in Table 3, the rs34562254 genotype remained significantly related to HCV susceptibility in the age and gender subgroups (both $P < 0.0125$). Additionally, other variables such as 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 these results, we further tested the heterogeneity of each stratified variable and found that no heterogeneity existed between the sub-layers (all $P > 0.05$).

Table 3
Stratified analysis the association of rs34562254 with HCV susceptibility

Subgroups	Group A	Group B	Group C	OR(95%CI)[a]	P^a	P^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.

In silico **analysis**

As shown in Fig. 1, rs34562254 had a RegulomeDB score of 3a, suggesting that it may be located at the transcription factor (TF) binding site, motif, and DNase peak. We used RNAfoldweb servers to predict

TNFRSF13B RNA secondary structure. The MAF of the centroid secondary structure was higher for the mutant rs34562254 T allele (-38.90 kcal/mol) than its wildtype C allele (-45.60 kcal/mol), indicating that the rs34562254 polymorphism in *TNFRSF13B* could affect the transcription of related RNA (Fig. 1). We also explored the potential biological functions of rs34562254 in the Encyclopedia of DNA Elements (ENCODE) project using the UCSC genome browser. As shown in Fig. 2, rs34562254 was located on the highest peak of the H3K4Me1 histone marker in seven cell lines. H3K4me1 is a major histone modification associated with enhancer elements in nucleosomes and is linked to the transcriptional regulation of genes.

Discussion

We found that *TNFRSF13B* rs34562254 was associated with HCV infection susceptibility. More specifically, compared to subjects with rs34562254-CC (the wild type), those with CT/TT have an increased risk of HCV infection. The T allele's effect was stronger in subjects who were older (≥ 50 years old), female, PBDs, and those with lower ALT and AST levels (≤ 40 U/L). Moreover, our bioinformatics analysis found that rs34562254 was located at the highest peak of the H3K4Me1 histone marker and potentially regulates mRNA transcription by affecting TF binding.

TNFRSF13B is a lymphocyte-specific tumor necrosis factor receptor that interacts with the NF-κB pathway and regulates B-cell development [28, 29]. It has been reported to be associated with a variety of human immune-related diseases such as CVID [22], asthma [24], and SLE [23]. Furthermore, Rand et al. have found that the *TNFRSF13*B rs34562254 polymorphism was correlated with multiple myeloma and coronary artery lesions [30]. In this study, based on the analyses of four genetic markers, we found that subjects with rs34562254-T were associated with an elevated risk of HCV infection.

From the NCBIdbSNP browser (https://www.ncbi.nlm.nih.gov/snp/), we found that rs34562254 is a missense mutation located in the exon region of *TNFRSF13B*. According to RegulomeDB, its RegulomeDB score is 3a (https://www.regulomedb.org/regulome-search/?regions=rs34562254&genome=GRCh37). RegulomeDB is a database that links SNPs with known and predicted regulatory elements within the intergenic regions of the *Homo sapiens* genome. The RegulomeDB score represents a model integrating functional genomics features with continuous values such as the expression of quantitative trait loci, TF binding, TF motif, ChIP-seq signal, and DNase Footprint [31]. Therefore, the rs34562254 SNP might have transcriptional regulatory functions including TF binding and DNase peak. Through the RNAfold web server, we found that the wildtype C allele of the centroid secondary structure had a lower MFE than the mutant T allele (-45.60 versus − 38.90 kcal/mol). From the ENCODE project and the UCSC genome browser, we found that rs34562254 was located on the highest peak of the H3K4Me1 histone marker, a major histone modification around enhancer elements in nucleosomes. When enhancer regions are enriched with H3K4me1 modifications, the enhancer enters a state of equilibrium. In contrast, when enhancer regions become enriched with both H3K4me1 and H3K27ac modifications, the enhancer enters an activated state that promotes gene expression [32]. Consequently, we speculate that genetic variants in rs34562254 might affect *TNFRSF13B* expression by influencing TF binding.

The stratified analysis showed that the rs34562254-T allele was significantly associated with HCV susceptibility in subjects that were female, over 50 years old, PBDs, and that had reduced ALT and AST levels (≤ 40 U/L). In general, compared to men, women usually develop more intense innate, humoral, and cellular immune responses to viral infections and vaccinations [33], suggesting that the allele's associated risk is more significant in females. ALT and AST level are major indicators of liver injury severity. In hepatitis C, ALT and AST levels become abnormal (> 40 U/L), which may confound the effects of the rs34562254-T allele [19]. HCV is a common infection among older adults in certain areas of China [34]. Generally, older people are less healthy and are more likely than younger people to be exposed to potentially contaminated needles or blood products [35]. Accordingly, our result showed that the risk effect of the allele was more significant in the over 50 years old subgroup. For PBDs, they may rely on blood donation as a source of income. Blood-borne diseases such as HCV and HIV were frequently detected among PBDs in China during the early 1980s [36]; HCV infection was very prevalent [37].

Several limitations exist in this study. First, we did not test other SNPs in *TNFSF*/*TNFRSF* that may associate with HCV infection. Therefore, additional studies with more extensive genomic coverage are required to increase understanding of the role of *TNFRSF13B* polymorphisms in HCV infection. Second, in the multivariate regression analysis of *TNFRSF* SNP distribution and HCV infection outcome, HCV genotypes were not included due to a lack of data. Instead, we used existing HCV genotype data to perform a logistic regression on rs34562254 and found that the *P* value did not change. In future studies, we will collect as much baseline data as possible to improve our reliability. Finally, the potential biological functions of *TNFRSF13B* rs34562254 were speculated in the *in silico* analysis. The exact mechanisms of rs34562254 in *TNFRSF13B* in HCV infection need to be further investigated.

Conclusions

This study was the first to report that the *TNFRSF13B* missense mutation rs34562254 was significantly associated with HCV susceptibility among the Han Chinese population. Moreover, the increased risk of the mutant genotype was stronger in subjects who were females, older (≥ 50 years old), PBDs, and who had lower ALT and AST levels (≤ 40 U/L). These findings are valuable in deepening the understanding of HCV infection susceptibility and offer a reference to develop prevention and control strategies among populations at high risk of repeated HCV infections.

Abbreviations

TNFRSF: tumor necrosis factor receptor superfamily; TNFSF: tumor necrosis factor superfamily; HCV: hepatitis C virus; HCC: hepatic carcinoma; DAAs: direct-acting antivirals; RA: rheumatoid arthritis; IBD: inflammatory bowel disease; MS: multiple sclerosis; CLL: chronic lymphocytic leukemia; SLE: systemic lupus erythematosus; CVID: common variable immunodeficiency; SNPs: single nucleotide polymorphisms; IVDU: Intravenous drug users; HD: hemodialysis patients; PBD: paid blood donors; HIV: human immunodeficiency virus; HBV: hepatitis B virus; ALT: alanine aminotransferase; AST: aspartate aminotransferase; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium; ANOVA: one-way

analysis of variance; ORs: odds ratios; CI: confidence intervals; GWAS: genome-wide association study; TACI: transmembrane activator and CAML interactor; SAE: serious adverse event; TN: treatment-naïve; TE: treatment-experienced; WHO: World Health Organization.

Declarations

Ethics approval and consent to participate

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.

Consent of publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' 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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Figures

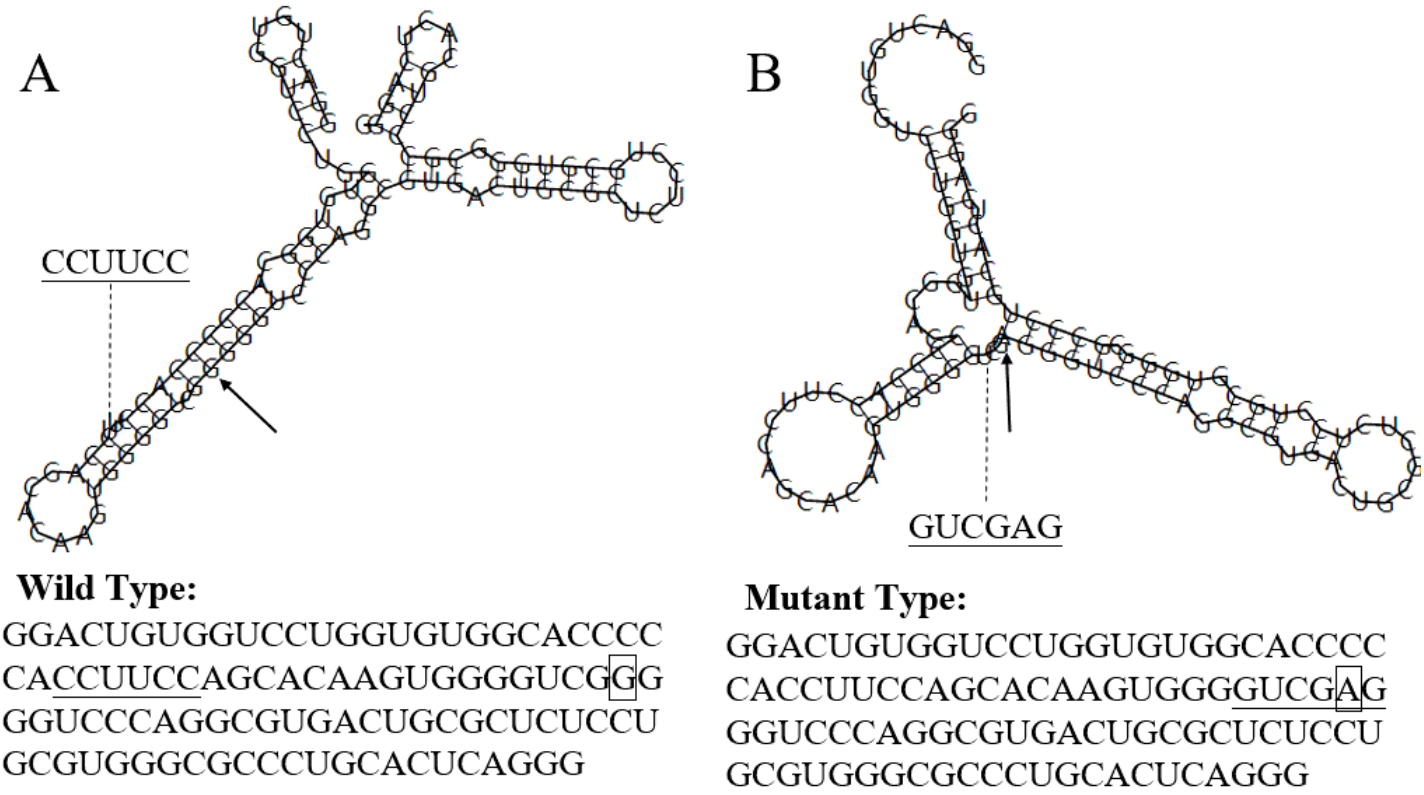

Figure 1

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

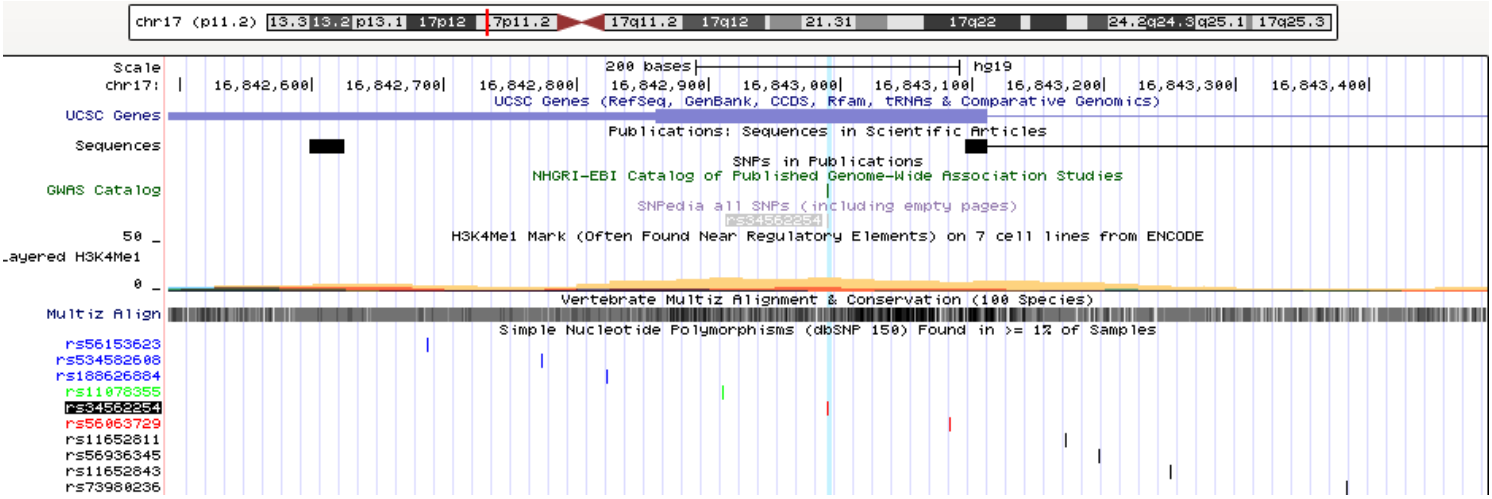

Figure 2

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

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