Mohsen *et al.*
Egyptian Journal of Medical Human Genetics (2022) 23:110
https://doi.org/10.1186/s43042-022-00322-9

Egyptian Journal of Medical Human Genetics

CORRESPONDENCE Open Access

A single-nucleotide polymorphism of *IL12A* gene (rs582537 A/C/G) and susceptibility to chronic hepatitis B virus infection among Iraqi patients

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Abstract

A case–control study (80 patients with chronic hepatitis B virus [HBV] infection and 96 controls) was performed to evaluate the association of an *IL12A* gene variant (rs582537 A/C/G) with HBV infection. Allele *G* showed a significantly lower frequency in patients compared to controls (31.2 *vs*. 46.9%; probability [*p*] = 0.009; corrected *p* [*pc*] = 0.027) and was associated with a lower risk of HBV infection (odds ratio [OR] = 0.49; 95% confidence interval [CI] = 0.29–0.83). A similar lower risk was associated with genotypes CG (17.5 *vs*. 29.2; OR = 0.25; 95% CI = 0.08–0.81; *p* = 0.02) and GG (10.0 *vs*. 16.7; OR = 0.25; 95% CI = 0.07–0.91; *p* = 0.036), but the *pc* value was not significant (0.12 and 0.126, respectively). Serum IL-35 levels showed significant differences between individuals of different genotypes (*p* = 0.007). The highest median was associated with CA genotype (286.5 pg/mL), followed by genotypes CG (227.0 pg/mL), GG (206.5 pg/mL), CC (169.0 pg/mL), AA (137.5 pg/mL) and finally AG (125.0 pg/mL). In conclusion, rs582537 appears to be an important genetic variant that may influence not only susceptibility to HBV infection but IL-35 levels.

Keywords: Hepatitis B virus, Interleukin-35, *IL12A*, Single-nucleotide polymorphism, rs582537

Introduction

We recently demonstrated that interleukin (IL)-35 showed significantly lower levels in serum of patients infected with hepatitis B virus (HBV) compared to a healthy control group [1]. IL-35 is heterodimeric cytokine consisting of two subunits: IL-12α chain p35 (IL-12p35) and IL-27β chain Epstein–Barr virus-induced 3 (EBI3). IL-12p35 subunit is encoded by *IL12A* gene, which is located in the long arm of human chromosome 3 (3q25.33) [2]. Two single-nucleotide polymorphisms (SNPs) located in intron 2 of the *IL12A* gene (rs582054 and rs583911) were also studied by our group to assess their association with HBV infection. rs583911 showed no association, while *A* allele and AT genotype of rs582054 were significantly associated with the risk of HBV infection [3]. rs582537 is a SNP located between the SNPs rs582054 and rs583911, and studies have associated this SNP with susceptibility to primary biliary cholangitis (PBC) [4–6]. PBC, formerly known as primary biliary cirrhosis, is a chronic autoimmune disorder that results in progressive destruction of intrahepatic bile ducts resulting in cholestasis, which in turn leads to cirrhosis [7]. It has been reported that previous infection with HBV may exacerbate the severity of PBC and may lead to poorer outcomes [8]. Therefore, we hypothesized that SNP rs582537 may also be associated with the risk of HBV infection. To the best knowledge of investigators, this SNP has not been studied in HBV infection.

A case–control study (80 patients with chronic HBV infection and 96 controls) was performed during

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January−July 2020 to evaluate the association between rs582537 and susceptibility to HBV infection. Information for patients and controls was previously detailed [1, 3]. An allele-specific polymerase chain reaction (PCR) assay was used to amplify a 251-bp DNA region comprising rs582537 A/C/G (ancestral allele: C) using three forward primers (FA: 5′-TTTGGGCA**A**TTGTCTGTC TCA-3′, FC: 5′-TTTGGGCA**C**TTGTCTGTCTCA-3′ and FG: 5′- TTTGGGCA**G**TTGTCTGTCTCA-3′) and one reverse primer (5′-TTGCAGTGCACAGACGC-3′). Agarose gel electrophoresis was performed to detect genotypes of PCR products. These methods were previously detailed [3].

Genotype frequencies were tested for Hardy–Weinberg equilibrium (HWE) using Pearson's chi-square goodness-of-fit test. Two-tailed Fisher's exact test was used to assess significant differences between allele and genotype frequencies. Age- and gender-adjusted multinomial logistic regression was performed to calculate odds ratio (OR) and 95% confidence interval (CI). Serum levels of IL-35 were given as median and interquartile range (IQR). Significant differences between medians were assessed with Kruskal–Wallis test. A probability (p) value ≤ was considered significant. Bonferroni correction was applied to correct p value (pc) due to multiple comparisons. GraphPad Prism version 8.0.0 (San Diego, California, USA) and IBM SPSS Statistics 25.0 (Armonk, NY: IBM Corp.) were used to perform statistical analysis.

rs582537 was recognized by six genotypes (CC, CA, CG, AA, AG and GG) corresponding to three alleles (*C*, *A* and *G*). Genotype frequencies of rs582537 were in good agreement with HWE in HBV patients and controls ($p=0.529$ and 0.127, respectively). Allele *G* showed a significantly decreased frequency in patients compared to controls (31.2 *vs*. 46.9%; $p=0.009$; $pc=0.027$) and was associated with a lower risk of HBV infection (OR = 0.49; 95% CI = 0.29–0.83). A similar lower risk was associated with genotypes CG (17.5 *vs*. 29.2; OR = 0.25; 95% CI = 0.08–0.81; $p=0.02$) and GG (10.0 *vs*. 16.7; OR = 0.25; 95% CI = 0.07–0.91; $p=0.036$), but the pc value was not significant (0.12 and 0.126, respectively) (Table 1).

Predetermined IL-35 levels [1] were examined in all participants (patients and controls) after stratification by rs582537 genotypes. Median IL-35 levels showed significant differences between individuals of different genotypes ($p=0.007$). The highest median was associated with CA genotype (286.5 [IQR 169.0–523.0] pg/mL), followed by genotypes CG (227.0 [IQR 161.0–430.0] pg/mL), GG (206.5 [IQR 106.5–336.5] pg/mL), CC (169.0 [IQR 144.0–282.0] pg/mL), AA (137.5 [IQR 118.0–335.0] pg/mL) and finally AG (125.0 [IQR 67.0–210.0] pg/mL) (Fig. 1).

These data indicate that *G* allele may have protective effects against the development of HBV infection. Besides, IL-35 serum levels were influenced by rs582537 genotypes. Interestingly, genotypes comprising *G* allele ranked second and third among the highest level order of IL-35. Thus, the down-regulated levels of IL-35 in HBV patients [1] might be causally related to the observed lower frequency of *G* allele and GG genotype. Similar to our study, a strong association between rs582537 and PBC was reported and the SNP was considered a risk variant involved in the pathogenesis of disease [4–6].

In conclusion, rs582537 appears to be an important genetic variant that may influence not only susceptibility to HBV infection but IL-35 levels. As this study was the first, further studies in genetically different population

Table 1 Multinomial logistic regression analysis of SNP rs582537 A/C/G (Ancestral: C) in hepatitis B virus infection patients *versus* controls

Allele/Genotype	Patients ($N=80$)		Controls ($N=96$)		OR	95% CI	p value (pc)
	N	%	N	%			
C	54	33.8	48	25.0	Reference		
A	56	35.0	54	28.1	0.92	0.54–1.58	0.785 (1.0)
G	50	31.2	90	46.9	0.49	0.29–0.83	**0.009 (0.027)**
CC	12	15.0	6	6.3	Reference		
CA	16	20.0	8	8.3	1.00	0.27–3.66	1.0 (1.0)
CG	14	17.5	28	29.2	0.25	0.08–0.81	**0.02** (0.12)
AA	10	12.5	8	8.3	0.63	0.162–2.41	0.495 (1.0)
AG	20	25.0	30	31.3	0.33	0.11–1.03	0.057 (0.45)
GG	8	10.0	16	16.7	0.25	0.07–0.91	**0.036** (0.126)
HWE-*p* value	0.529		0.127				

HWE Hardy–Weinberg equilibrium, *OR* Odds ratio, *CI* Confidence interval, *p* Two-tailed Fisher's exact probability, *pc* Bonferroni correction probability.

Significant *p* value is indicated in bold

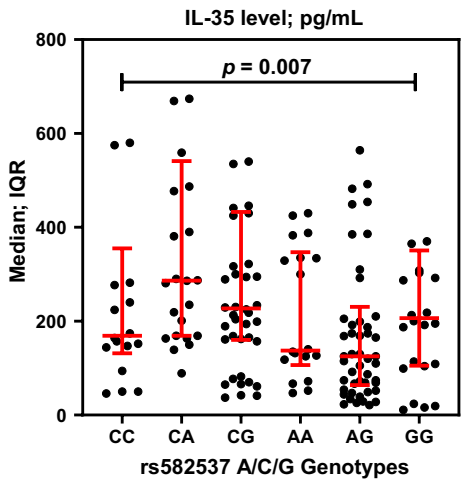

Fig. 1 Scatter dot plot of IL-35 levels in all participants (hepatitis B virus infection patients and controls) stratified according to rs582537 SNP genotypes. Horizontal red lines indicate medians, while vertical red lines indicate interquartile range (IQR). Significant differences were assessed with Kruskal–Wallis test

groups [9, 10] are warranted to understand the role of rs582537 in the pathogenesis of HBV infection.

Abbreviations

CI: Confidence interval; EBI3: IL-27β chain Epstein–Barr virus-induced 3; HBV: Hepatitis B virus; HWE: Hardy–Weinberg equilibrium; IL: Interleukin; IL-12p35: IL-12α chain p35; IQR: Interquartile range; OR: Odds ratio; *p*: Probability; PBC: Primary biliary cholangitis; *pc*: Corrected probability; SNP: Single-nucleotide polymorphism.

Acknowledgements

We appreciate the kind help and cooperation of the medical staff at the Specialized Center for Gastroenterology and Hepatology and Central Blood Bank in Baghdad.

Author contributions

The three authors (RTM, RHA and AHA) contributed equally to data management, statistical analyzes and manuscript writing and reviewing. All authors read and approved the final manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Availability of data and materials

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

Declarations

Ethics approval and consent to participate

The Ethical Approval Committee at the University of Anbar approved the study (Reference: 23).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 22 February 2022 Accepted: 5 July 2022
Published online: 14 July 2022

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