Identification of *RPL5* gene variants and the risk of hepatic vein thrombosis in Saudi patients

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ABSTRACT

الأهداف: التعرف على المتغيرات الجينية لبروتين الريبوسوم L5 وخطر الإصابة بتجلط الأوردة الكبدية لدى المرضى السعوديين.

المنهجية: تم إجراء دراسة حالات تجلط الأوردة الكبدية والأشخاص السليميين لمجموعة ضابطة خلال الفترة من مايو 2018م إلى سبتمبر 2019م. حيث تم اختيار 65 مريضا، 50 فردًا سليمًا من نفس الأعمار وكلا الجنسين كمجموعة ضابطة. قمنا بتحديد النمط الجيني لبروتين الريبوسوم L5 بواسطة اختبارات التسلسل الجيني مع تطبيق تسلسل سانجر للمتغيرات التي تم فحصها وراثيًا على الجين L5.

النتائج: الأليلات A في المتغير rs182018447 و T عند المتغير rs559377519 كانت مرتبطة ارتباطًا وثيقًا مع خطر الإصابة بتجلط الأوردة الكبدية لدى المرضى السعوديين (p=0.009 وp=0.037، على التوالي). ارتبطت ترددات النمط الجيني للجين RPL5 والأنماط الجينية A/A في rs182018447 وT/T في rs559377519 ارتباطًا وثيقًا بتجلط الأوردة الكبدية (p=0.000 و p=0.004 ؛ على التوالي) والزيادة في خطر الإصابة بين هؤلاء المرضى.

الخلاصة: تشير النتائج التي توصلنا إليها إلى أن المتغيرات الجينية الخمسة الجديدة التي تم فحصها في جين RPL5 كانت مرتبطة بخطر الإصابة بتجلط الأوردة الكبدية في جميع المرضى بالسعودية. بالإضافة إلى ذلك، وجود الأنماط الجينية A/A في rs182018447 و T/T في rs559377519 لـ HVT في هؤلاء المرضى لها دور أكبر في الاصابة بتجلط الأوردة الكبدية.

Objectives: To identify ribosome protein L5 gene variants and the risk of hepatic vein thrombosis in Saudi patients.

Methods: A case-control study was conducted during the period of May 2018 to September 2019. Sixty-five patient cases of hepatic vein thrombosis (HVT) were chosen, and 50 healthy individuals of the same ages and both gender were set as a control group. The genotype of the gene *RPL5* was determined by PCR please provide abbreviation in full and capillary electrophoresis. Sanger sequencing for genetically screened variants was applied for the RPL5 gene.

Results: Alleles A at variant rs182018447 and T allele at variant rs559377519 were strongly corelated (p=0.009 and p=0.037, respectively) with the risk of HVT. The genotype frequencies of the *RPL5* gene, the A/A genotypes at rs182018447 and T/T at rs559377519 were associated with HVT (p=0.000 and p=0.004; respectively) and an increase in risk for HVT among these patients. Please rephrase the highlighted text without using the word respectively.

Conclusion: Our findings indicate that the 5 genetic novel variants examined in the *RPL5* gene were associated with a risk of HVT in all our Saudi cases. Additionally, the A/A at rs182018447 and T/T at rs559377519 genotypes were substantially susceptible to HVT in all these patients.

Keywords: *RPL5* Gene, risk, liver, hepatic vein thrombosis, Saudi Arabia

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Hepatic vein thrombosis (HVT) disorder is a rare condition attributable to hypercoagulable causes, such as pregnancy, the use of birth control pills containing hormones, and infection.[1] It is marked by blocking of the liver veins, which contain the liver's blood flow.[2] Hepatic vein thrombosis may be caused by some drugs, infections, and hereditary disorders.[2] Additionally, it has been associated with myeloproliferative disorders.[3] One study found that a significant potential risk factor for polycythemia vera (PV) was primary myeloproliferative

disorders.[4] Ribosome protein L5 (*RPL5*) consists of a 40S small unit and a 60S large sub-unit, which relate to the cellular mechanism that translates messenger ribonucleic acid (mRNA) into proteins. Among patients with Diamond-Blackfan anemia (DBA), the *RPL5* mutation has been described. This gene was found through its fifth intron and with small nucleolar RNA gene U21. There are numerous processed pseudogenes of this type of gene scattered around the genomes, which is common for genes that encode ribosomal proteins. Diamond-Blackfan anemia patients have been identified to have impaired ribosome biogenesis and function in *RPL5*.[5,6] The mutation gene in ribosomal proteins (*RPL10*, *RPL5*, and *RPL11*) have been shown to be present in acute T-cell leukemia (T-ALL) and aggressive chronic lymphocytic leukemia (CLL), and somatic mutations involving RPS15 have also been identified.[7-11] Ribosome protein L5 and RPL22 mutated in multiform and uterine corpus endometrial carcinoma glioblastoma have been identified by cancer genome atlas (TCGA) and inactivating gastric cancer RP22 mutations have been recorded.[12-17] To date, a limited number of mutated genes have been identified in tumors, which in turn indicate the relationship between thrombosis and their function and correlation with tumors; there has also been research on genes of ribosomal protein and the HVT-hypercoagulable state.[18] The relation between ribosome alterations and HVT was confirmed by the detection in which heterozygous suppression of some of these hypercoagulable state ribosomal protein genes was heterozygous.[19] There is a lack of comprehensive studies of *RPL5* genes across HVT groups, and some defects need to be investigated in more detail. Regarding our aim of understanding ribosome defects and the higher risk of thrombosis in HVT patients, for the first screening of possible HVT-driving *RPL5* genes, it could reveal exciting novel targets to be investigated in a follow-up study. This study involved detecting gene mutations and examining novel pathways associated with HVT, and we chose the *RPL5* gene to achieve DNA Sanger sequencing; this identification of *RPL5* genetic mutations can reflect its function as a risk factor, which can help us discover novel HVT factors.

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Methods. Sixty-five cases of HVT were chosen through a convenience non-probability sample of available 100 patients diagnosed with different types of thrombosis and who were referred to the King Fahad University Hospital, AL-Khobar, Kingdom of Saudi Arabia (KSA), for follow up testing during the period of May 2018 to September 2019. The intended sample size was determined using G-power 3.1 software and thus, the relevant values: power of the test (1-β)= %, type I error (α)= 5%, sample size suggested the needed for HVT patients among thrombosis groups. Patients with other types of thrombosis were excluded from the study (n=35 cases). Fifty healthy individuals of the same ages and both gender were set as the control group. This observational case control study was approved ethically by the Deanship of the Scientific Research Committee at Imam Abdelrahman Bin Faisal University according to the Helsinki declaration (IRB number: IRB-2019-03-188). The STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement checklist for observational studies was used to evaluate the patient's selection in this study. We assessed using the following criteria: 1) research setting, 2) sample size, 3) diagnostic test, and 4) outcome data. The participants obtained written and verbal permission after a consultation with the individual regarding the role, goal, and risks of the experiment. All patients' records were collected from the center of diagnosis and submitted an interview process. Hepatic vein thrombosis cases were diagnosed and confirmed via ultrasound. Peripheral blood samples were collected in compliance with the instructions of the manufacturer (Promega, USA). The use of well-designed pairs of DNA nucleotides was applied for molecular identification of the RPL5 single nucleotide polymorphisms (SNPs). The primer reference sequences were designed for polymerase chain reaction (PCR) according to the NCBI GenBank database as follows (forward: 5' GCCTGTAATCCCAGCACTTC 3', reverse: 5' TTCTAATCTCCCCACCCCAC 3'). The genotype of the gene RPL5 was determined by PCR and capillary electrophoresis.[20] Sanger sequence for genetically screened variants was applied to the *RPL5* gene, which is listed in the NCBI public database. Our sequence results were blasted using nucleotide software from the NBCI database pairwise to the reference RPL5 sequencing references associated with the HVT patients results, and the protein coding intron 5 of the RPL5 gene was chosen as the target area; the final target area spanned 234 bp. Mutations variants from the HVT Saudi patients for the RPL5 genes encoding ribosomal proteins were annotated using genetic variants to the

RefSeq database, 1000 Genomes pilot release (March 2010) using Annovar software and the Statistical Package for Social Sciences (SPSS) version 21 (IBM Corp., Armonk, NY, USA) was used to analyze the collected data.[21,22] The frequency and percentages for categorical variables was represented as variations of the gene by an unadjusted Odd ratio. To compare the mean values of age and HVT in relation to demographic variables, the t-test and Fisher's test were used. The statistical significance and validity of the findings were reported using a *p*-value <0.05 and 95% confidence intervals (CI).

Results. This research was performed at Imam Abdulrahman Bin Faisal University, Dammam, KSA, as a case-control hospital study and used as a systematic procedure to recruit cases of HVT that were previously confirmed in King Fahad University Hospital. In addition, controls were selected from co-patients of the hospital who were matched by ethnicity, gender, and age group. After exclusion of patients who were diagnosed with other types of thrombosis and those who has no permission, the total cases were 65 HVT patients (46 males and 19 females); of these, 19 patients (of both gender) with HVT were further selected for the Sanger sequencing besides the controls. The medical and diagnostic characteristics in this study would be a significant rise in mean age of HVT patients when compared to controls (p=0.0001) despite matching both groups (>40 years). Approximately 20% of patients had a family history of HVT, while the other 80% had no family history of the condition (Table 1). Table 2 shows the type of genome variations, and 5 novel SNVs in the target area were identified. The characteristics were coding nonsynonymous SNV intron that was not involved in the db SNP b153 v2 and a 1000 Genomes allele frequency. Five variants were discovered in HVT cases, indicating that these nonsynonymous single nucleotide variants may have a risk impact on these patients.

The impact of polymorphisms detected in the RPL5 gene and the risk of developing HVT in our study and alleles of the novel 5 variants (rs138079590, rs558220259, rs576892621, rs182018447, and rs559377519) were compared with control frequencies; the alleles T for variant rs138079590, A for variant rs182018447 and T allele for variant rs559377519 were significantly (p=0.006, p=0.009 and p=0.032, respectively) associated with risk of HVT (OR=2.00; 95% CI: [1.23-3.32] and OR=1.73; 95% CI: [1.02-2.92], respectively), as detailed in Table 3.

Table 1 - Characteristics of the hepatic vein thrombosis (HVT) Saudi patients included in this study.

Characteristic	HVT cases (n=65)	Controls (n=50)	*P*-value*
Age (years) (mean±SD)	47.2±3.1	43.5±2.6	0.0001*
Gender (%)			
Male	46 (70.7)	32 (64.0)	0.5463†
Female	19 (29.3)	18 (36.0)	
Family history of HVT (%)			
Yes	13 (20.0)		
No	52 (80.0)		

*t-test compares the means of 2 groups, †Chi-square to comparing small groups frequency

The genotype frequencies of the RPL5 gene are summarized in Table 4, the A/A genotypes at rs182018447 and T/T at rs559377519 were statistically significantly associated with HVT (p=0.000; OR=2.00; 95% CI: [1.39-2.86] and p=0.004; OR=1.69; 95% CI: [1.17-2.45], respectively) and an increased risk factor of HVT among these patients.

Discussion. In this study, we evaluated the genetic polymorphism of RPL5 effects on the development of HVT in the Saudi population. Our observations showed that RPL5 polymorphisms are associated with HVT risk. Our research has many advantages, such high involvement of suitable participants from equally indigenous relatively homogenous Saudi population along with all experimental normal controls with Hardy-Weinberg equilibrium. Furthermore, most of our HVTs have been pathologically registered. Gene variants were discovered in a study conducted throughout the current study among the phenotypes studied. The findings showed a correlation of greater HVT exposure in alleles A (rs182018447) and T allele (rs559377519). Yoshihama et al,[23] emphasized the impact of being able to examine all identified ribosomal variants. A combined analysis with all ribosomal protein genomes in all affected people was suitable for this study of various groups of factors that influence HVT therapeutically, which showed disorder in the protein translation system. In addition to the detection of single variations that may display a statistical relationship with risk, this technique can discover new disease-predisposing processes. The Sangar sequencing in this study properly utilized the sequence data of patients. In 19 individuals, testing of the RPL5 gene revealed 5 new genetic variants of various functional types and frequencies. The annotations of the variants describe reported variety-related diseases, a subset of which may have been documented with HVT. Many novel variants

Table 2 - Nonsynonymous single nucleotide variants in the *RPL5* genes.

Variants	Substitution	Transcript ID	db SNP (b153 v2)	Variation type	1000 genomes allele frequency	Position
rs138979590	G>C G>T	NG_011779.2	Novel	SNV, intron	not present	chr1:92833927
rs558220259	A>G	NG_011779.2	Novel	SNV, intron	not present	chr1:92833948
rs576892621	A>G	NG_011779.2	Novel	SNV, intron	not present	chr1:92833969
rs182018447	G>A	NG_011779.2	Novel	SNV, intron	not present	chr1:92834024
rs559377519	A>T	NG_011779.2	Novel	SNV, intron	not present	chr1:92834047

Ch1: chromosome 1, SNV: single nucleotide variant, SNP: single nucleotide polymorphism.

Table 3 - Allele frequency of the *RPL5* genetic polymorphisms in hepatic vein thrombosis (HVT) patients compared with the control subjects.

db SNP b153 v 2	Alleles	Patients	Control	*P*-value*	Unadjusted OR [95% CI]
rs138979590	G	224 (87.3)	198 (87.6)		Reference
	C	26 (9.1)	16 (7.1)	0.350	1.44 [0.75-2.76]
	T	36(12.6)	12(5.3)	0.006*	2.65 [1.34-5.24]
rs558220259	A	221 (77.3)	183 (80.9)	0.362	Reference
	G	65 (22.7)	43 (19.1)		1.25 [0.81-1.93]
rs576892621	A	233 (81.5)	189 (83.6)	0.603	Reference
	G	53 (18.5)	37 (16.3)		1.16 [0.73-1.84]
rs182018447	G	229 (80.1)	201 (88.9)	0.009*	Reference
	A	57 (19.9)	25 (11.1)		2.00 [1.23-3.32]
rs559377519	A	238 (83.2)	202 (89.4)	0.032*	Reference
	T	48 (16.8)	24 (10.6)		1.73 [1.02-2.92]

Values are presented as number and percentage (%).
*P-values were calculated by chi-square analyses. differences were statistically significant at $p<0.05$.
OR: odds ratio, CI: confidence interval. Single-variant association analyses was performed.

have been identified to have a harmful health impact on the role of the main ribosomal proteins. These findings are consistent with the hepatocellular carcinoma (HCC) study by Pengbo et al,[24] which proposed accelerated ribosome pathogenesis, facilitated ribosomal RNA development, and altered *RPL5* and *RPL11* localization, leading to enhanced p53 proteolysis. In many other reports, it has been shown that a raised risk of cancer is related to RPL5 polymorphism.[25,26] Additionally, in response to our findings, recent research, particularly numerous cohort studies, has indicated that there is an association between *RPL5* genotype polymorphism (A/A at rs182018447 and T/T at rs559377519) and an increased HVT hazard in Saudi populations.[27] Although some studies have shown indications of variant genotypes within RPL5 genes leading to reduced cell translation of proteins, some results have not been confirmed in other studies.[28]

In this analysis, there was possibly no correlation between rs138079590, rs558220259, and rs576892621 variants due to the inability to recognize a thorough gene linked to thrombosis, which might support the argument that genome-based HVT susceptibility is likely to be affected by reasonable configurations of single-risk variants and some stimulatory gene-gene interactions. Although rs182018447 and T/T at rs559377519 were shown in this study to be a major risk factor for HVT and although the *RPL5* gene investigated here has been involved in the possible associations, few other studies on *RPL5* genetic variants have accessed the risk due to the challenges of processing the data. Consequently, the impact of such phenotypes on diseases related to various cancers has been studied in several studies.[29-31] In this study, the possible weakness was linked to quality control implemented by genotyping and using hospital-based studies. For all patients requiring hospitalization, the hospitals through which the cases were selected were information centers. In an experimental evaluation, our sample size was not sufficient to find definitive outcomes. Another potential limitation is that other RPL5 genetic variants could not be presented in the previous analysis. Therefore, with more participants and effective genotyping procedures, a future goal should be to confirm these results.

Table 4 - Analysis of frequency of RPL5 genotype polymorphisms and HVT risk.

db SNP b153 v2	Genotype	Patients	Control	*P*-value*	Unadjusted OR [95% CI]
		n (%)			
rs138979590	G/C	250 (24.5)	214 (25.2)		Reference
	G/T	260 (25.5)	210 (24.8)	0.708	1.06 [0.81- 1.37]
	G/C+G/T	510 (50.0)	424 (50.5)	0.842	1.03 [0.82-1.29]
rs558220259	A/A	442 (61.7)	366 (64.8)		Reference
	A/G	144 (20.1)	113 (20.0)	0.764	1.06 [0.79-1.39]
	G/G	130 (18.2)	86 (15.2)	0.173	1.25 [0.92-1.7]
rs576892621	A/A	466 (65.2)	378 (67.0)		Reference
	A/G	143 (20.0)	112 (19.9)	0.862	1.04 [0.78-1.37]
	G/G	106 (14.8)	74 (13.1)	0.413	1.16 [0.84-1.61]
rs182018447	G/G	458 (64.1)	402 (71.2)		Reference
	G/A	142 (19.9)	113 (20.0)	0.543	1.10 [0.83-1.46]
	A/A	114 (16.0)	50 (8.8)	0.000*	2.00 [1.39-2.86]
rs559377519	A/A	486 (63.7)	404 (71.5)		Reference
	A/T	148 (19.4)	113 (20.0)	0.549	1.08 [0.82-1.43]
	T/T	98 (12.9)	48 (8.5)	0.004*	1.69 [1.17-2.45]

**P*-values were calculated by chi-square analyses; differences were statistically significant at $p<0.05$. OR: odds ratio, CI: confidence interval, HVT: hepatic vein thrombosis. Single-variant association analyses was performed.

Study limitations. The main objective of this study is to recognize RPL5 gene variants as well as the risk for HVT in patients by testing the association between RPL5 genetic variations and HVT. Moreover, our analysis has included a singular community of HVT patients from a single center that provides patients with cardiovascular care. These could make sure that our findings of the study are much more relevant on a greater scale. This research, on the other hand, has a few limitations. First, research uses a case-control design, that observes correlations, but it does not draw a decision about causal relationships. Second, the questionnaire was answered in the existence of the research teams; while the researchers had been wary to also provide explanations and guidelines, the effect of their supervision on the respondents' achievement cannot be ruled out. Eventually, while the RPL5 gene was intended to measure patients' existing HVT, the test's effectiveness may be influenced by the patients' information and understanding the aim of this study.

In conclusion, our results indicate that the 5 genetic novel variants examined in the RPL5 gene are associated with a risk of HVT in our Saudi group cases. Although the A/A at rs182018447 and T/T at rs559377519 genotypes were substantially susceptible to HVT in all those patients, the negative findings in this study of other RPL5 genetic variants suggest that these associations may not have a connection with the formation of HVT. To determine the true function of genetic susceptibility in Saudi HVT patients, more studies about correlation of these 5 genetic novel variants of RPL5 gene with HVT in Saudi patients are required. Further investigation of those variants might indeed demonstrate the differences of phenotypes reported in individuals with almost the same medical assessment, as well as lead to the identification of the hereditary major risk factor, with one which the data set was insufficient.

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