RESEARCH
VOLUME 9 ISSUE 3
ISSN: 2639-5274, 2096-5168

Frequencies of IL-28B-rs12979860 and rs8099917 variants and the role of IL-28B-rs12979860 as predictors of response in HCV patients in case of direct acting antiviral therapy

Dr. Sajid Ali[1] *, Nourin Mahmood[1], Fazal Jalil[1], Naila Imtiaz [1]

[1]Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan, Khyber Pakhtunkhwa, Pakistan.

*** Corresponding author:**

Dr. Sajid Ali

Department of Biotechnology, Abdul Wali Khan University Mardan, Mardan, Khyber Pakhtunkhwa, Pakistan.

E-mail addresses:

SA: sajid@awkum.edu.pk

NM: (Nourin_jid@yahoo.com)

FJ: fjalil@awkum.edu.pk

NI : nailaimtiaz46@gmail.com

Abstract

The treatment outcomes in Hepatitis C virus (HCV) are attributed to various factors like viral load, viral genotype, ethnicity, and host genome. Therefore, this study was carried out to determine the genetic frequencies of IL-28B-rs12979860 and rs8099917 variants and to explore the role IL-28B-rs12979860 as predictors of therapeutic response in direct acting antiviral therapy. Two study subjects, chronic HCV patients (182) and control individuals (59), were recruited in this project. The patients were initially diagnosed through ELISA (Biotech-USA); however, active, and non-active infection status of the patients was determined through Real Time PCR (Amplisense-HCV FRT, Russia). Genomic DNA was extracted from whole blood using silica spin column extraction kit (MN, Germany) followed by genotyping of two SNPs IL-28B-rs12979860 C/T and IL-28B-

RESEARCH
VOLUME 9 ISSUE 3
ISSN: 2639-5274, 2096-5168

rs8099917 T/G using a real time PCR assay (Amplisense HCV FRT, Russia). The genotype frequency of IL-28B-rs12979860 was significant in co-dominant [$\chi 2$ =13.78; P=0.001], homozygous dominant (TT vs CC+CT) [OR=3.903 (0.123-0.519); P=0.0002], homozygous recessive (CC vs TT+CT) [OR=0.322 (0.160-0.625); P=0.0016], and additive C vs T) [OR=0.354 (0.22-0.572); P<0.0001] models. Similarly, a significant genotype distribution of IL-28B-rs8099917 was observed in co-dominant [χ^2 =16.53; P=0.0003], homozygous dominant (GG vs TT+GT) [OR= 2.304 (1.03- 5.11); P=0.04], homozygous recessive (TT vs GG+GT) [OR=0.222 (0.108-0.476); P=0.00], and additive (T vs G) [OR= 0.361 (0.221-0.591); P<0.0001] models. Furthermore, we found that out of the total 101 chronic HCV patients, 80 (79.2%) showed SVR while 21 (21.8%) did not achieve SVR and were called as relapsed patients' group. Thus, evaluating response of certain genotype of IL-28B-rs12979860 to direct-acting antiviral (DAA), the TT genotype was observed 33 (71.7%) in SVR and 13 (28.3%) in relapsed patients. However, CC genotype was found 23 (95.8%) and 1 (4.2%) in SVR and relapsed patients, respectively. While CT genotype was 24 (77.4%) in SVR and 7 (22.6%) in relapsed patients.

Key words: PCR, IL-28, DAA, CT, HCV, GENOTYPE

Introduction

Hepatitis C is the liver inflammation caused by the Hepatitis C Virus (HCV). HCV is the leading cause of death among all other hepatitis viruses. It is a major cause of chronic liver infection, causing almost 0.4 million deaths annually (Parsons, 2022). In 2015, the global prevalence of HCV infection was estimated to be 1% (0.5–2.3%), corresponding to 71 million chronically infected people (Liakina et al., 2017) and its highest prevalence rates are found in Egypt (6.3%), followed by Pakistan (3.8%) and Russia (3.3%). In Pakistan, its prevalence varies and is about 3-5% among the general population. It has become an endemic status in some of the areas of Pakistan especially in rural and non-developed cities (Haqqi et al., 2019).

ISSN: 2639-5274, 2096-5168

HCV has been treated with different antiviral drugs either indirect or direct in the purpose to eradicate virus from the body. Different factors related with both virus and host contribute in predicting the viral response to therapy. Among the host related factors one which is considered to being involved is interleukin-28B polymorphism (Geddawy et al., 2017).

IL-28B gene is found on the 19th chromosome at 19q13 and consisted of 6 exons (Witte et al., 2010b). Interleukin is a sort of form of IFN. IFNs are the key cytokines in establishing a multifaceted antiviral response. There are 3 types of IFN, based on their biological activities, structural features, and receptor usage. (Type I, II, and III) (Donnelly et al., 2010). Type-III IFN was discovered (1993) in humans. This group contain 3 strongly associated IFN gamma proteins, IFN gamma 1, gamma 2, and gamma 3, which are also known as IL-29, IL-28A, and IL-28B, respectively (Witte et al., 2010a). IL28B/IFN-λ3 shows effective action against HCV, whereas IFN-λ induces innate antiviral activity against many viruses such as encephalo myocarditis virus, vesicular stomatitis virus and HBV, both in cell culture as well as in animals (Witte et al., 2010a, Donnelly and Kotenko, 2010). IFN-λ3 also shows polymorphisms in gene that includes rs12980275A/G, rs8099917T/G, rs28416813C/G, and rs12979860C/T. Alleles such as rs12979860 C and rs8099917 T are actually the much valuable gene of IFN gamma 3 that shows impulsive approval. IFN-λ induces an amazing antiviral protection in large numbers of the cells, particularly when collaborating with type I IFN (Donnelly et al., 2010). It shows a critical antiviral action in HCV infection because near the 3kb upstream of the gene, polymorphism emerge to be associated with the result of infection although the mode of action is not known yet (Langhans et al., 2011). According to a report, the outcome of hepatitis C infection is affected by numbers of factors such as age, gender, route of transmission, jaundice, co-infection and viral genotype (Fedorchenko et al., 2010). However, recently few reports have established the important role of heritability and ethnicity in the immune response of host to the infection caused by HCV (Thomas et al., 2009b). The impulsive viral clearance following acute infection varies among the patients of different races. According to the recent reports, the genetic variation has also an important role in IFN gamma 3 gene region, located on chromosome 19, which can response to PEG-IFN in combination with ribavirin therapy and can also fight against the HCV infection (Thomas et al., 2009a). This finding has been confirmed in other independent cohorts as well (Tillmann et al.,

ISSN: 2639-5274, 2096-5168

2010a). Genetic variation in the IL28B/IFN-λ3 gene, the rs12979860 C and rs8099917 T allele are believed to be strongly associated with viral clearance (Tillmann et al., 2010b). HLA class-I and II (Kuniholm et al., 2013), IL-10 (Mangia et al., 2004), IL-4 (Ramos et al., 2012), IFN-γ are reported to be associated with the natural clearance of HCV infection (Ramos et al., 2012), and programmed cell death protein-1 (PD-1) (Ramos et al., 2012). Some studies were also conducted on polymorphisms on natural clearance of HCV infection in Iran. Accordingly, in those studies the genotypes and allele frequency of IFN gamma 3 at rs12979860C/T and the rs8099917T/G SNPs in those patients suffering from HCV were explored in one of the provinces of south Iran named Fars. It was examined that whether their allele and genotype relate to HCV infection resulted in spontaneous clearance versus chronic infection.

This study was aimed to explore the IL-28B polymorphism roles as predictor of response in case of direct-acting antiviral (DAA) therapy as earlier studies have shown its correlation with indirect therapies but no one or least has shown its correlation with DAA.

Methodology

Recruitment of the study subjects

A total of 241 study subjects (including 182 chronic HCV patients and 59 healthy controls) were enrolled in this study. All the subjects were screened for coinfection of HBV and HIV. The ICT positive patients were initially screened through ELISA (Biotech, USA). For further active infection confirmation RNA was extracted followed by real time PCR (Amplisense HCV FRT, Russia) as per manufacturer guidelines. The sensitivity for real time PCR was less than 10 IU/ml. An informed consent form was signed from each participant in the study.

DNA extraction and genotyping

Genomic DNA was extracted from whole blood using silica spin column extraction kit (MN, Germany). Two SNPs rs12979860 C/T and rs8099917 T/G from IL-28B gene were genotyped using a real time PCR assay (Amplisense HCV FRT, Russia). Two tubes were prepared for each sample, labelled as Tube 1 (rs12979860 + internal control) and Tube 2 (rs8099917 + internal control) to determine the homologous and heterozygous status of each sample.

After amplification the data was analyzed through real time PCR software (Mygo, USA) by detecting florescence signals. To measure florescence signals, three fluorophores channels were

used i.e., FAM, JOE and ROX. The data was recorded in terms of threshold cycle (Ct) value for each sample. FAM and JOE were used to detect nucleotide type in rs8099917 G/T and rs12979860 C/T. While ROX was used as an internal control (Table 1). Ct values detected from FAM and ROX shows that individual is homozygous for one allele, while Ct values from JOE and ROX shows homozygosity for second allele. However, Ct values detected from all three channels shows that the patient is heterogeneous at the given variant.

The genetic data was statistically analyzed through GraphPad Prism software using Chi Square and Fisher´s Exact test at 95% CI (confidence interval). The p value of <0.05 was considered a significant range.

RESULTS

The frequency distribution of IL-28B-rs12979860 and rs8099917 SNPs in chronic HCV patients and controls is given in Table 1. The genotype frequency of IL-28B-rs12979860 was significant in co-dominant [$\chi 2$ =13.78; P=0.001], homozygous dominant (TT vs CC+CT) [OR=3.903 (0.123-0.519); P=0.0002], homozygous recessive (CC vs TT+CT) [OR=0.322 (0.160-0.625); P=0.0016], and additive C vs T) [OR=0.354 (0.22-0.572); P<0.0001] models.

Similarly, a significant genotype distribution of IL-28B-rs8099917 was observed in co dominant [χ^2 =**16.53**; P=0.0003], homozygous dominant (GG vs TT+GT) [OR= 2.304 (1.03- 5.11); P=0.04], homozygous recessive (TT vs GG+GT) [OR=0.222 (0.108-0.476); P=0.00], and additive (T vs G) [OR= 0.361 (0.221-0.591); P<0.0001] models (Table 1).

Furthermore, for IL-28 B rs12979860 response of certain genotype to DAA was assessed. We found that out of the total 101 chronic HCV patients, 80 (79.2%) showed SVR while 21 (20.8%) did not achieve SVR and were called as relapsed patients' group. The TT genotype was observed 33 (71.7%) in SVR and 13 (28.3%) in relapsed patients. However, CC genotype was found 23 (95.8%) and 1 (4.2%) in SVR and relapsed patients, respectively. While CT genotype was 24 (77.4%) in SVR and 7 (22.6%) in relapsed patients.

Table 1: Frequency distribution of IL-28 B rs12979860 and rs8099917 SNPs

RESEARCH
VOLUME 9 ISSUE 3
ISSN: 2639-5274, 2096-5168

Genes	***Models***	***Genotypes***	***Cases (101)***	***Controls (59)***	***Odd Ratio***	χ^2	*P value*
rs12979860	*C0-dominant*	*CC* *CT* *TT*	*24 (23.8%)* *31 (30.7%)* *46 (45.5%)*	*29 (49.1%)* *18 (30.5%)* *12 (20.4%)*	*--*	***13.78***	*0.001*
	Dominant	*TT* *CC+CT*	*46 (45.5%)* *55 (54.5%)*	*12 (20.4%)* *47 (79.6%)*	*3.903* *0.123-0.519*	*-*	*0.0002*
	Recessive	*CC* *TT+CT*	*24 (23.8%)* *77 (76.2%)*	*29 (49.1%)* *30 (50.9%)*	*0.322* *0.160-0.625*	*-*	*0.0016*
	Heterozygous	*CT* *CC+TT*	*31 (30.7%)* *80 (69.3%)*	*18 (30.5%)* *41 (69.5%)*	*0.882* *0.452-1.735*	*-*	*0.72*
	Additive	*C* *T*	*79 (39.1%)* *123 (60.9%)*	*76 (64.4%)* *42 (35.6%)*	*0.354* *0.22-0.572*	*-*	*<0.0001*
rs8099917	***Models***	***Genotypes***	*Cases (81)*	*Controls (59)*	***Odd Ratio***	X^2	***P value***
	C0-dominant	*TT* *GT* *GG*	*16 (19.8%)* *35 (43.2%)* *30 (37%)*	*31 (52.6%)* *16 (27.1%)* *12 (20.4%)*	-	***16.53***	*0.0003*
	Dominant	*GG* *TT+GT*	*30 (37%)* *51 (53%)*	*12 (20.4%)* *47 (79.6%)*	*2.304* *1.03- 5.11*	*-*	*0.04*
	Recessive	*TT* *GG+GT*	*16 (19.8%)* *65 (80.2%)*	*31 (52.6%)* *28 (47.4%)*	*0.222* *0.108-0.476*	*-*	*<0.0001*
	Heterozygous	*GT* *GG+TT*	*35 (43.2%)* *46 (56.8%)*	*16 (27.1%)* *43 (72.9%)*	*2.045* *0.999-4.246*		*0.07*
	Additive	*T* *G*	*67 (41.3%)* *95 (58.7%)*	*78 (66.1%)* *40 (33.9%)*	*0.361* *0.221-0.591*	*-*	*<0.0001*

Table 2: IL-28B genotypes association with Response

ISSN: 2639-5274, 2096-5168

Genotypes	SVR (n=80)	Relapse (n=21)	
CC (n=24)	23 (95.8%)	1 (4.7%)	
CT (n=31)	24 (77.4%)	7 (22.6%)	
TT (n=46)	33 (71.7%)	13 (28.3%)	

Discussion

HCV infection has created alarming situation in some of the areas of Pakistan and even in KPK. To treat HCV infection different parameters are kept in mind to have a good treatment response (Al Kanaani et al., 2018). Different predisposing factors are contributing to HCV treatment outcome. Among those, some are viral related like, genotypes and viral load whereas some are host related like, gender, ethnicity, and host genome (Manns et al., 2017). Among the host genome, the most important are IL-28 B genetic polymorphism. This association has been explored in many of the studies, using drugs like IFN and Ribavirin and very few studies have been done to explore its association with DAA drugs that is sofosbuvir and ribavirin in combination.

IL-28 polymorphism at rs60 and 17 has been explored in Chronic hepatitis C (CHC) and individuals having non-active hepatitis C infection, which revealed that among different genotypes, the most associated genotypes with CHC patients were CT and TT and comparatively low prevalence was that of CC (Table 2). Genome wide association study has been done, revealing that genetic variation near the region of IL-28 B gene are connected with absence of HCV RNA in anti-HCV antibody positive patients, presumed to have HCV spontaneous clearance (Mazzaro et al., 2011). According to Mazzaro et al., 2011 individuals having C/C genotype at rs12979860, are most capable of resolution of HCV infection in different population of subjects like European

ISSN: 2639-5274, 2096-5168

and African. It is further stated that those individuals who have C/C genotypes are more associated with HC resolution compared to individuals with TT (Mazzaro et al., 2011).

Our results showed that most of the CHC patients are associated with TT genotypes (Table 2). This is consistent with the study done by (He et al., 2011) in which the genotype TT was closely linked with chronic hepatitis C infection in Italian population. Similarly in another study conducted by Prokunina-Olsson et al it was revealed that TT genotype at rs 60 in CHC patients were more prone to cirrhosis (Prokunina-Olsson et al., 2013).

Besides this, persons having C allele at rs 60 tend to have constant higher serum level of IL-28 and IL-29 than those having T/T allele (Casato et al., 1991), indicating favorable response to antiviral therapy. At this stage, it would be analyzed that CC genotype at rs 60 might be associated more with spontaneous cleared patients than CHC patients, revealing IFN–λ level, and this high level in turn is responsible for HCV clearance. Similarly many studies have been done to show association of IL-28 b polymorphism with response to antiviral therapy (Zhang et al., 2016). Our results showed that CC genotypes were associated more with sustained virological response (SVR) as compared to T and G allele (Table 2). This is in agreement with a study conducted in Iran by (Cocquerel et al., 1998) in which individuals with C/C and C/T genotype at rs 60 showed higher SVR rate than those having TT genotype. Similarly, our study results are consistent with the study conducted by (Lavie et al., 2006), that in Caucasians, IL-28 B polymorphism CC genotypes at rs 60 was associated with more viral kinetics and likely improved SVR than TT genotypes. Another study also has shown the same relationship of CC genotypes with viral kinetics and SVR and also with HCV clearance (Rauch et al., 2010). This association has also been shown in uremic patients (Penin et al., 2001).

The presence of TT genotypes has great influence over the HCV clearance and SVR in some regions of the world like USA, Brazil, Europe and Morocco (Rauch et al., 2010), while in some regions like china, Iran and also even in Pakistan, TT genotypes have no such relationship. Although in some studies association of rs 17 TT genotypes have been shown with HCV clearance and outcome (Zhang et al., 2016). Although haplotype analysis is most important in predisposing genetic factors in some complex diseases like this study has shown the importance of Il-28 B host

genetic polymorphism, but this study had some limitation that is, small size of cleared HCV infected individuals and some uneven amount of female to male those might be considered in next future study.

In conclusion, this study has found greater association of CC genotypes and C allele at rs 60 but not with TT genotypes at rs 17, with HCV clearance and final HCV outcome. Therefore, it can be suggested that IL-28 host genetic factor can influence HCV infection outcome in Pakistani population, and it can be put in guidelines in some serious complicated cases of HCV infection.

References

AL KANAANI, Z., MAHMUD, S., KOUYOUMJIAN, S. P. & ABU-RADDAD, L. J. J. R. S. O. S. 2018. The epidemiology of hepatitis C virus in Pakistan: systematic review and meta-analyses. 5**,** 180257.

CASATO, M., LAGANA, B., ANTONELLI, G., DIANZANI, F. & BONOMO, L. 1991. Long-term results of therapy with interferon-alpha for type II essential mixed cryoglobulinemia. *Blood*, 78**,** 3142-3147.

COCQUEREL, L., MEUNIER, J.-C., PILLEZ, A., WYCHOWSKI, C. & DUBUISSON, J. 1998. A retention signal necessary and sufficient for endoplasmic reticulum localization maps to the transmembrane domain of hepatitis C virus glycoprotein E2. *Journal of virology*, 72**,** 2183-2191.

DONNELLY, R. P. & KOTENKO, S. V. 2010. Interferon-lambda: a new addition to an old family. *Journal of Interferon & Cytokine Research*, 30**,** 555-564.

DONNELLY, R. P., KOTENKO, S. V. J. J. O. I. & RESEARCH, C. 2010. Interferon-lambda: a new addition to an old family. 30**,** 555-564.

FEDORCHENKO, S. V. E., MARTYNOVICH, T., LYASHOK, O., KARYUK, Z. A., YANCHENKO, V., FEDORCHENKO, S., FEDORCHENKO, S., MARTYNOVICH, T., LYASHOK, O. & YANCHENKO, V. J. T. A. 2010. Spontaneous HCV clearance: an association with gender, age, viral genotypes, infection transmission routes, and markers of HBV and HIV. 82**,** 52-56.

GEDDAWY, A., IBRAHIM, Y. F., ELBAHIE, N. M. & IBRAHIM, M. A. J. J. O. T. I. M. 2017. Direct acting anti-hepatitis C virus drugs: clinical pharmacology and future direction. 5**,** 8-17.

HAQQI, A., MUNIR, R., KHALID, M., KHURRAM, M., ZAID, M., ALI, M., SHAH, Z. H., AHMED, H. & AFZAL, M. S. J. V. I. 2019. Prevalence of hepatitis C virus genotypes in Pakistan: current scenario and review of literature. 32**,** 402-413.

HE, L., CHEN, Z., CHEN, Y., XU, H., TANG, H., LEI, B. & LEI, X. 2011. Association between the influential factors and the effectiveness of pegylated interferon alpha-2a plus ribavirin as a combination treatment for chronic hepatitis C patients. *Zhonghua gan zang bing za zhi= Zhonghua ganzangbing zazhi= Chinese journal of hepatology,* 19**,** 34-37.

KUNIHOLM, M. H., ANASTOS, K., KOVACS, A., GAO, X., MARTI, D., SETTE, A., GREENBLATT, R. M., PETERS, M., COHEN, M. H. & MINKOFF, H. 2013. Relation of HLA class I and II supertypes with spontaneous clearance of hepatitis C virus. *Genes and immunity*, 14**,** 330.

LANGHANS, B., KUPFER, B., BRAUNSCHWEIGER, I., ARNDT, S., SCHULTE, W., NISCHALKE, H. D., NATTERMANN, J., OLDENBURG, J., SAUERBRUCH, T. & SPENGLER, U. 2011. Interferon-lambda serum levels in hepatitis C. *Journal of hepatology*, 54**,** 859-865.

LAVIE, M., GOFFARD, A. & DUBUISSON, J. 2006. HCV glycoproteins: assembly of a functional E1-E2 heterodimer. *Hepatitis C viruses: Genomes and molecular biology***,** 1-30.

LIAKINA, V., SPEIČIENĖ, D. M. & VALANTINAS, J. 2017. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study.

MANGIA, A., SANTORO, R., PIATTELLI, M., PAZIENZA, V., GRIFA, G., IACOBELLIS, A. & ANDRIULLI, A. 2004. IL-10 haplotypes as possible predictors of spontaneous clearance of HCV infection. *Cytokine*, 25**,** 103-109.

MANNS, M. P., BUTI, M., GANE, E., PAWLOTSKY, J.-M., RAZAVI, H., TERRAULT, N. & YOUNOSSI, Z. J. N. R. D. P. 2017. Hepatitis C virus infection. 3**,** 1-19.

MAZZARO, C., MONTI, G., SACCARDO, F., ZIGNEGO, A. L., FERRI, C., DE VITA, S., GABRIELLI, A., LENZI, M., DONADA, C. & GALLI, M. 2011. Efficacy and safety of peginterferon alfa-2b plus ribavirin for HCV-positive mixed cryoglobulinemia: a

multicentre open-label study. *Clinical and Experimental Rheumatology-Incl Supplements*, 29**,** 933.

PARSONS, G. J. P. 2022. Hepatitis C: epidemiology, transmission and presentation. 33**,** 20-23.

PENIN, F., COMBET, C., GERMANIDIS, G., FRAINAIS, P.-O., DELÉAGE, G. & PAWLOTSKY, J.-M. 2001. Conservation of the conformation and positive charges of hepatitis C virus E2 envelope glycoprotein hypervariable region 1 points to a role in cell attachment. *Journal of virology,* 75**,** 5703-5710.

PROKUNINA-OLSSON, L., MUCHMORE, B., TANG, W., PFEIFFER, R. M., PARK, H., DICKENSHEETS, H., HERGOTT, D., PORTER-GILL, P., MUMY, A. & KOHAAR, I. 2013. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nature genetics,* 45**,** 164.

RAMOS, J. A., SILVA, R., HOFFMANN, L., RAMOS, A. L. A., CABELLO, P. H., ÜRMÉNYI, T. P., VILLELLA-NOGUEIRA, C. A., LEWIS-XIMENEZ, L. & RONDINELLI, E. 2012. Association of IL-10, IL-4, and IL-28B gene polymorphisms with spontaneous clearance of hepatitis C virus in a population from Rio de Janeiro. *BMC research notes*, 5**,** 508.

RAUCH, A., KUTALIK, Z., DESCOMBES, P., CAI, T., DI IULIO, J., MUELLER, T., BOCHUD, M., BATTEGAY, M., BERNASCONI, E. & BOROVICKA, J. 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology*, 138**,** 1338-1345. e7.

THOMAS, D. L., THIO, C. L., MARTIN, M. P., QI, Y., GE, D., O'HUIGIN, C., KIDD, J., KIDD, K., KHAKOO, S. I. & ALEXANDER, G. 2009a. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature,* 461**,** 798.

THOMAS, D. L., THIO, C. L., MARTIN, M. P., QI, Y., GE, D., O'HUIGIN, C., KIDD, J., KIDD, K., KHAKOO, S. I. & ALEXANDER, G. J. N. 2009b. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. 461**,** 798-801.

TILLMANN, H. L., THOMPSON, A. J., PATEL, K., WIESE, M., TENCKHOFF, H., NISCHALKE, H. D., LOKHNYGINA, Y., KULLIG, U., GÖBEL, U. & CAPKA, E. 2010a. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. *Gastroenterology*, 139**,** 1586-1592. e1.

ISSN: 2639-5274, 2096-5168

TILLMANN, H. L., THOMPSON, A. J., PATEL, K., WIESE, M., TENCKHOFF, H., NISCHALKE, H. D., LOKHNYGINA, Y., KULLIG, U., GÖBEL, U. & CAPKA, E. J. G. 2010b. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. 139**,** 1586-1592. e1.

WITTE, K., WITTE, E., SABAT, R. & WOLK, K. 2010a. IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. *Cytokine & growth factor reviews*, 21**,** 237-251.

WITTE, K., WITTE, E., SABAT, R., WOLK, K. J. C. & REVIEWS, G. F. 2010b. IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. 21**,** 237-251.

ZHANG, J., NGUYEN, D. & HU, K.-Q. 2016. Chronic hepatitis C virus infection: a review of current direct-acting antiviral treatment strategies. *North American journal of medicine & science*, 9**,** 47.