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Research article

Host pharmacogenetic factors that may affect liver neoplasm incidence upon using direct-acting antivirals for treating hepatitis C infection

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ABSTRACT

Introduction: Direct-acting antivirals (DAAs) represent a breakthrough in hepatitis C virus (HCV) treatment as they directly inhibit HCV nonstructural (NS) proteins (NS3/4A, NS5A, and NS5B). However, ongoing debates exist regarding their relationship with hepatocellular carcinoma (HCC) whose incidence is widely debated among investigators. This study was conducted to identify host pharmacogenetic factors that may influence HCC incidence upon using HCV DAAs.

Materials and methods: Details regarding 16 HCV DAAs were collected from literature and DrugBank database. Digital structures of these drugs were fed into the pharmacogenomics/pharmacovigilance *in-silico* pipeline (PHARMIP) to predict the genetic factors that may underpin HCC development.

Results: We identified 184 unique genes and 40 unique variants that may have key answers for the DAA/HCC paradox. These findings could be used in different methods to aid in the precise application of HCV DAAs and minimize the proposed risk for HCC. All results could be accessed at: https://doi.org/10.17632/8ws8258hn3.2.

Discussion: All the identified factors are evidence related to HCC and significantly predicted by PHARMIP as DAA targets. We discuss some examples of the methods of using these results to address the DAA/HCC controversy based on the following three primary levels: 1 - individual DAA drug, 2 - DAA subclass, and 3 - the entire DAA class. Further wet laboratory investigation is required to evaluate these results.

1. Introduction

Hepatitis C is a liver disease caused by hepatitis C virus (HCV), which can cause both acute and chronic hepatitis. Worldwide, an estimated more than 70 million people have chronic hepatitis C infections [1]. HCV complications include liver cirrhosis and hepatocellular carcinoma (HCC), of which the latter is considered as the fourth most common neoplasm and the second commonest cause of cancer-related deaths in the world [2].

Several approaches have been applied in HCV treatment. The use of pegylated interferon plus ribavirin was the traditional approach, which achieved eradication of infection in 40%–50% of cases [3, 4]. In 2011, the FDA approved two drugs (boceprevir and telaprevir) that act directly on nonstructural (NS) HCV protein 3 and 4A (NS3/4A) protease [5]. This was an impetus for a new era of interferon-free direct-acting antiviral (DAA) treatment paradigms [6]. DAAs interfere with the life cycle of the virus by directly inhibiting HCV NS proteins (NS3/4A, NS5A, and NS5B), thus providing promising cure rates of >90% [7]. However, this dramatic increase in cure rates was not at no cost.

The shift to all-oral DAA-based regimens has significantly increased the cure rates of HCV to >90% in all patient groups. However, this shift has come at the expense of increasing some serious side effects [8, 9, 10]. As a new drug class, the side effects of DAAs are widely debated. One of the most controversial issues in this aspect is the relationship between the use of HCV DAAs and the incidence of de novo occurrence and recurrence of HCC and some other liver neoplasms [11].

Regarding the risk for HCC after DAA viral treatment, there are three significant developments as follows: 1 -DAA therapy reduced the incidence of HCC development in patients with chronic HCV with preexisting cirrhosis, but it did not eradicate the risk, implying that patients need

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ongoing surveillance for HCC after viral clearance [12], 2- some reports have indicated that there is a high probability of HCC recurrence in cirrhotic patients who received DAAs [12] and HCC occurrence especially after the use of sofosbuvir without ribavirin regimens [13], and 3 – no difference exists in HCC incidence rates between different patient groups under DAA treatment [14]. Ongoing debates in favor of or against DAA links to HCC recurrence and/or de novo occurrence are frequently reported in the literature. Elucidating the entire profile of HCV DAAs/HCC relationship should consider the patient's (host's) genetic factors as approximately 80% of variability in drug efficacy and side effects are influenced by patient's pharmacogenomics [15].

Several studies have focused on host genetic factors as possible predictive markers for HCV DAA therapy response and side effects [16, 17]. For instance, variation in *IFNL4* gene could affect the outcomes of ledipasvir/sofosbuvir treatment regimen [18], whereas variations in *ITPA* gene were found to be associated with decrease in hemoglobin levels related to treatment with sofosbuvir-containing regimen [19]. Moreover, polymorphism in *IFNL3* gene (known also as *IL28B*) was found to influence the risk for hypercholesterolemia after clearance of HCV using DAA treatment [20].

Regarding HCC as a consequence of HCV infection, host genetics also play an essential role. For example, the single-nucleotide polymorphisms (SNPs) rs2596542, rs1012068, rs17047200, and rs2856723 of the genes *MICA*, *DEPDC5*, *TLL1*, and *HLA-DBQ1*, respectively, were found to be significantly associated with HCC development in patients with HCV [21].

On the other hand, studies focusing on the role of host genetics in the development of HCC as an adverse drug reaction (ADR) of HCV DAAs are still in their early infancy. For instance, a recent study reported no relationship between the SNPs rs12979860 in *IFNL3* and rs4986791 in *TLR4* and the development of HCC after sofosbuvir/daclatasvir combination regimen [22].

In this context, it is worth mentioning that HCV infection induces genome-wide epigenetic histone modifications that correlate with host gene expression reprogramming. This "epigenetic signature" persists after virus eradication by DAA treatment and has been associated with HCC progression [23, 24, 25, 26], which thus suggests using this epigenetic change as a biomarker for HCV infection [27]. Combing DNA methylation inhibitors (e.g. histone deacetylase inhibitors) with DAAs could be a better approach to overcome the HCC risk after DAA treatment [28, 29, 30]. Moreover, "sonoporation via the microbubble" approach could be helpful to synergize the epigenetic treatment of HCC using DAAs and histone deacetylase inhibitors [31].

The scarcity of information and studies focusing on host pharmacogenetics role in DAAs/HCC relationship highlights the importance of the present study. The current gold standard for identifying pharmacogenomic associations of a drug is the expensive and labor-intensive genome-wide association studies (GWAS) [32, 33]. In a previous research, we introduced the pharmacogenomics/pharmacovigilance *in-silico* pipeline (PHARMIP) as a method that could be used to predict candidate genetic factors that underpin a certain ADR [34].

In the present study, PHARMIP was used with 16 approved HCV DAAs to predict candidate genetic factors that may affect HCC development upon their use. The genetic factors retrieved in this study could be helpful for further in-depth investigations focusing on the HCV DAA/HCC controversial relationship.

2. Materials and methods

2.1. HCV DAA drugs

A total of 16 DAAs, covering three DAA subclasses, were selected for this study (Table 1). In more detail, 8 NS3/4A, 6 Ns5A, and 2 NS5B inhibitors were collected from literature [35] and DrugBank database [36]. Three of these DAAs (asunaprevir, boceprevir, and telaprevir) are withdrawn from the market. However, their results were retained to enrich the analyses of results. Digital structure files were retrieved from DrugBank in two primary formats, viz., the simplified molecular input line entry system (SMILES) [37] and structural data file (SDF) [38] (3D-SDF format was used when available), and used to run the PHARMIP pipeline.

2.2. Neoplastic case reports for the investigated drugs

To have a wider view on the problem addressed in our study, we retrieved neoplastic individual case study reports (ICSRs) for the 16 drugs from VigiBase [39]. On May 7, 2020, a total of 1594 neoplastic reports were retrieved for the 16 drugs, among which 972 reports (~61%) were for different liver neoplasms.

2.3. PHARMIP pipeline

Drug structure files were used as input for the PHARMIP pipeline to predict host off-label targets (OLTs) that are related to HCC. The pipeline comprises three primary steps, as simplified in Figure 1, and detailed as follows:

- Retrieving the drug in SMILES and SDF formats from DrugBank. The 3D-SDF format was used whenever possible.
- The drug in SMILES format was fed into SwissTargetPrediction [40], similarity ensemble approach server [41], and polypharmacology browser (PPB) [42] to predict possible OLTs using the similarity approach.
- The drug in SDF format was fed into PharmMapper [43] to predict possible OLTs using the pharmacophore mapping approach. The list of OLTs is obtained and filtered at a significance level of P value of <0.05 (one-tailed positive Z-score >1.645). Redundancies were removed using Excel.
- PharmMapper retrieves genes identified by their accession number in the Uniprot [44] database. We used the Uniprot retrieve/ID mapping tool to convert Uniprot accessions into gene names.
- The results obtained from similarity and pharmacophore mapping approaches were concatenated to generate the list of OLTs to be used in the next step.
- The lists of OLTs were used to feed DisGeNet [45] to retrieve genes and variants related ADRs (diseases).
- Results were downloaded in a tabulated text format (.tsv) and analyzed using Excel.
- DisGeNet results contain a column of disease "semantic type." This column was filtered by "neoplastic process." The results were further filtered by the column "disease" for diseases containing "liver" or "hep*."
- Visualization and basic analyses of gene lists were conducted using STRING [46].
- Comparisons were performed using InteractiVenn [47] to identify unique and intersected genetic factors.
- Human Genome Nomenclature Consortium (HGNC) [48] guidelines were followed for all gene symbols in this study.

3. Results

A total of 184 unique genes and 40 unique variants were obtained by the application of PHARMIP to 16 HCV DAAs. A general view and some basic analyses for this set of genes could be performed using the following STRING link: =https://version-11-0.string-db.org/cgi/network.pl?networkId=ICEMmXPPw86b.

According to our study, these results resemble candidate host pharmacogenetic factors that may influence the incidence of HCC upon using HCV DAAs. Results could be viewed and analyzed in different methods according to the researcher's interest in an individual DAA, in a DAA subclass, or in the entire DAA class. All the data obtained in this study can be found at: https://doi.org/10.17632/8ws8258hn3.2.

Table 1. Names, DrugBank accession numbers, and VigiBase liver neoplastic ICSRs of the 16 investigated DAA drugs.

Class of DAA	Target Protein/Enzyme	Drug (DrugBank #)	Liver neoplastic ICSRs
NS3/4A protease inhibitors	NS3/4A membrane-targeted serine protease.	Asunaprevir (DB11586) Withdrawn	• Hepatic cancer (21) • Hepatocellular carcinoma (8) • Total neoplastic reports (66)
		Boceprevir (DB08873) Withdrawn	• Hepatic cancer (20) • Hepatocellular carcinoma (11) • Hepatic cancer metastatic (1) • Total neoplastic reports (94)
		Glecaprevir (DB13879)	• No reports • Total neoplastic reports (0)
		Grazoprevir (DB11575)	• Hepatic cancer (1) • Total neoplastic reports (11)
		Paritaprevir (DB09297)	• No reports • Total neoplastic reports (2)
		Simeprevir (DB06290)	• Hepatocellular carcinoma (58) • Hepatic cancer (9) • Hepatic cancer metastatic (1) • Metastases to liver (1) • Total neoplastic reports (111)
		Telaprevir (DB05521) Withdrawn	• Hepatic cancer (16) • Hepatocellular carcinoma (15) • Hepatic cancer recurrent (3) • Hepatic cancer metastatic (2) • Total neoplastic reports (159)
		Voxilaprevir (DB12026)	• Hepatocellular carcinoma (1) Total neoplastic reports (1)
NS5A inhibitors	NS5A zinc-binding and proline-rich hydrophilic phosphoprotein required for HCV viral reproduction.	Daclatasvir (DB09102)	• Hepatocellular carcinoma (128) • Hepatic cancer (36) • Hepatic cancer metastatic (1) • Total neoplastic reports (267)
		Elbasvir (DB11574)	• Hepatic cancer (1) • Total neoplastic reports (11)
		Ledipasvir (DB09027)	• Hepatocellular carcinoma (10) • Total neoplastic reports (10)
		Ombitasvir (DB09296)	• Hepatocellular carcinoma (2) • Total neoplastic reports (5)
		Pibrentasvir (DB13878)	• No reports • Total neoplastic reports (0)
		Velpatasvir (DB11613)	• Hepatocellular carcinoma (4) • Total neoplastic reports (4)
NS5B polymerase inhibitors	NS5B protein that act as RNA dependent RNA polymerase.	Dasabuvir (DB09183)	• Hepatocellular carcinoma (51) • Hepatic cancer (11) • Total neoplastic reports (102)
		Sofosbuvir (DB08934)	• Hepatocellular carcinoma (410) • Hepatic cancer (65) • Hepatic cancer recurrent (23) • Hepatic cancer metastatic (6) • Total neoplastic reports (751)
3 classes	3 targets	16 drugs	1594 reports (972 for liver neoplasms)

3.1. Results for individual drugs

As shown in Table 2, the highest number of retrieved genes was 53 for daclatasvir, and the lowest number was 25 for boceprevir. At the level of variants, the highest number was 14 for grazoprevir, and the lowest number was 2 for glecaprevir. Table 3 shows sample results for glecaprevir variants retrieved during the study.

In DisGeNet results, some genes were associated to synonymous diseases with different GDA scores. In such cases, we retained only the highest score and removed all other redundancies. For example, the gene *F2* is associated to "adult hepatocellular carcinoma" with GDA = 0.01 and to "liver carcinoma" with GDA = 0.4. In this case, we retained the 0.4-GDA result and removed the others. It is worth mentioning that targets with low scores were retained as they could have synergetic effects with other high-score targets [49].

3.2. Results for drug subclasses

For investigators who may be interested in a certain DAA subclass rather than a certain drug, the results could be analyzed at the level of DAA subclasses. Figure 2 shows an example of the possible intersections between resulting genes of the six NS5A drugs included in this study (daclatasvir, elbasvir, ledipasvir, ombitasvir, pibrentasvir, and velpatasvir). Three genes (*MAPK14*, *AKR1B1*, and *PTPN1*) were found to be commonly predicted for these six drugs.

3.3. Results for the entire DAA class

We collected nonredundant hits for each subclass and obtained the intersection between the three subclasses. A total of 23 genes and 7 variants were found to be common between the three subclasses. Figure 3

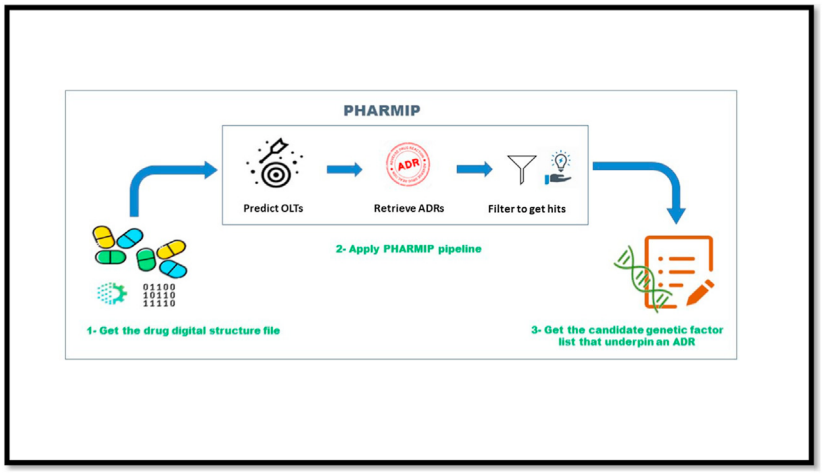

Figure 1. Study design showing the three primary steps of the used pipeline (PHARMIP) to answer the proposed question of what pharmacogenetic factors could be involved in the DAA/HCC relationship.

Table 2. Number of genes and variants retrieved from the application of PHARMIP to the 16 investigated DAAs.

No.	Drug	No. of genes	No. of variants
1.	Asunaprevir	44	8
2.	Boceprevir	25	7
3.	Glecaprevir	29	2
4.	Grazoprevir	37	14
5.	Paritaprevir	31	5
6.	Simeprevir	37	7
7.	Telaprevir	31	9
8.	Voxilaprevir	29	3
9.	Daclatasvir	53	3
10.	Elbasvir	47	12
11.	Ledipasvir	26	4
12.	Ombitasvir	50	8
13.	Pibrentasvir	39	8
14.	Velpatasvir	42	8
15.	Dasabuvir	45	5
16.	Sofosbuvir	51	13

summarizes the intersection results of genes between subclasses, and the intersections results of variants are depicted in Figure 4. The genetic map of these common 23 genes is shown in Figure 5. Additional analyses of these 23 genes could be performed by STRING using the following link: =https://version-11-0.string-db.org/cgi/network.pl?networkId=kgoIUsJ8Egh1.

4. Discussion

4.1. Defining candidate pharmacogenetic factors

The revolution in biological (especially genetic) data generation and analysis has significantly influenced healthcare practice. Implementation of the precision medicine model began in the past few years, resulting in massive changes in the traditional methods of practicing medicine [50]. Decreasing the occurrence of ADRs, and hence the cost of healthcare services, is one of the most important opportunities and challenges of precision medicine. Developing tools that help in achieving such goals and rationalize pharmacogenomic study designs is extremely important to address this challenge [51]. A potential strategy to define candidate genes that modulate a phenotype (e.g., developing an ADR) is to predict drug targets, disease-related genes, and genetic pathways that underlie this phenotype [52]; this is the strategy used in PHARMIP.

By exploring the pharmacogenomics knowledge base (PharmGKB) [53] and the electronic pharmacogenomics assistant (ePGA) [54] for the genetic annotations of the 16 DAAs, only limited, if any, information was obtained regarding the possible explanation of the pharmacogenomics for their HCC relationship paradox. Most of the retrieved information was related to the role of interferon lambda 3 and 4 (*IFNL3* and *IFNL4*) genetic polymorphism in the efficacy and safety of treatment using different DAAs. Using PHARMIP, 184 unique genes and 40 unique variants were successfully predicted as pharmacogenetic factors that may modulate HCC development in patients treated with HCV DAAs. These results could be analyzed in different manners based on the interests of researchers. As a discussion on all the results and possible analyses of this study is not feasible, we represent some of these possible analyses of the results and how they may help in resolving the DAA/HCC paradox.

4.2. Analysis of gene results

Considering GDA score, the MET proto-oncogene, a receptor tyrosine kinase (*MET*) gene, appeared as a very interesting hit. This gene was significantly predicted as an OLT for five DAAs (elbasvir, ombitasvir, pibrenatasvir, simeprevir, and velpatasvir). As a well-investigated gene for its overexpression relationship with HCC [55, 56], it may be worth exploring its effect as a mediator for HCC development in DAA-treated patients. The same is applicable to HRas proto-oncogene, GTPase (*HRAS*), androgen receptor (*AR*), coagulation factor II, thrombin (*F2*), and peroxisome proliferator-activated receptor γ (*PPARG*). These candidate genes were significantly retrieved as DAA OLTs and related to HCC development with relatively high GDA scores. *PPARG* is an interesting hit from another point of view. Its prediction as a DAA OLT and its relationship with lipid metabolism [57] support our results and may explain the role of DAAs in lipid metabolism.

Considering these genes in a pharmacogenomic study design could disclose some key relationships of DAAs/HCC. However, genes with lower GDA scores could not be excluded as they may exert synergistic effects with genes with higher GDA scores.

From another point of view, the gene results could be analyzed depending on their repetition rather than their scores. For instance, protein tyrosine phosphatase nonreceptor type 1 (*PTPN1*) is significantly predicted as an OLT for all DAAs (except boceprevir). *PTPN1* is upregulated in HCC, and its knockdown therapies are sex-linked (more effective in men than in women) [58, 59]. The mechanism of DAA interaction with this gene may deserve further investigation under wet laboratory settings, especially when patient sex is considered.

At the subclass level, 23 genes were found to appear at least once in each DAA subclass. Gene enrichment analysis of this gene set using

Table 3. Example of variant results showing two reference SNPs that may influence HCC development upon using glecaprevir.

Variant	Gene	Gene_id	Chr	Consequence	Alleles	Class	Disease	Disease_id	Score_vda
rs1057519958	RXRA	6256	9	missense variant	C/A,T	snp	Liver carcinoma	C2239176	0.7
rs31223	ITK	3702	5	intron variant	T/A,C	snp	Liver carcinoma	C2239176	0.01

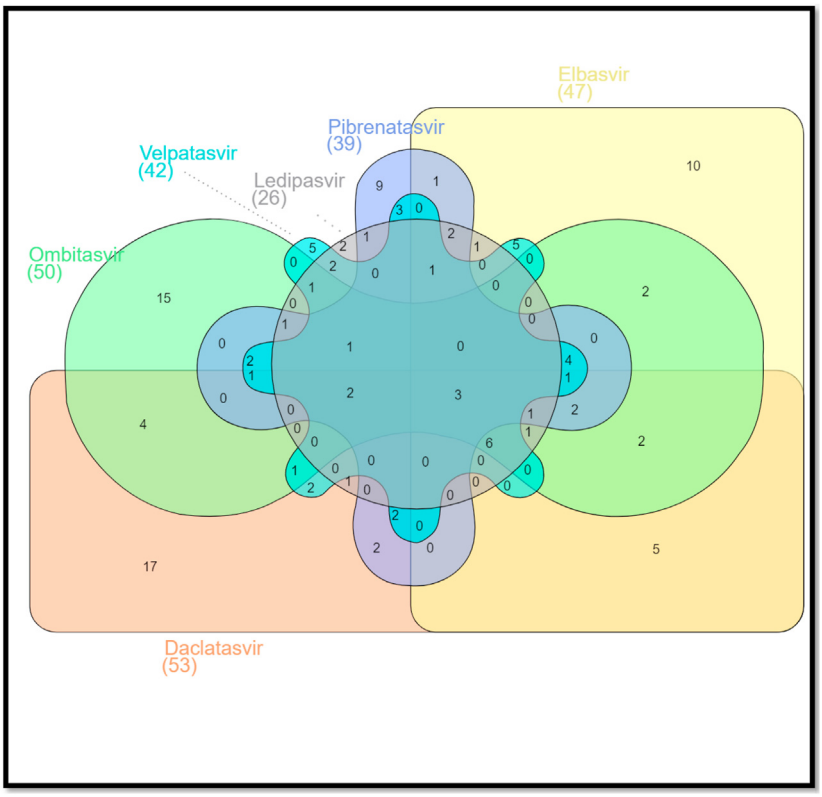

Figure 2. Intersection results of genes between the six NS5A drugs included in the study.

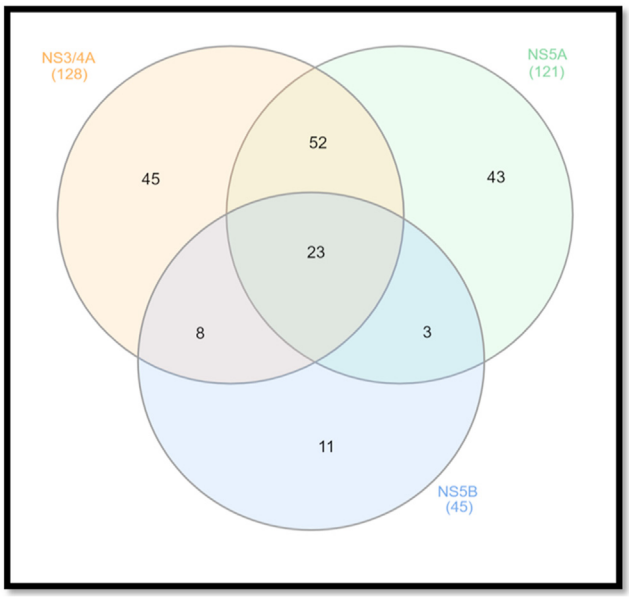

Figure 3. Gene intersections between the three DAA subclasses.

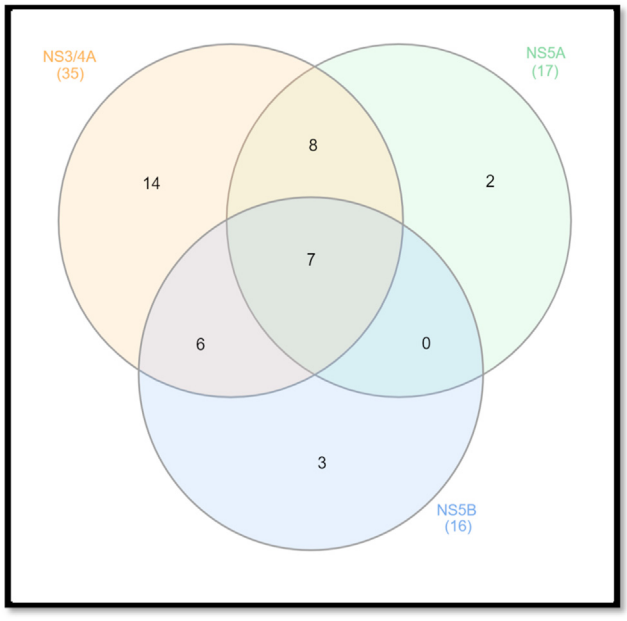

Figure 4. Variant intersections between the three subclasses.

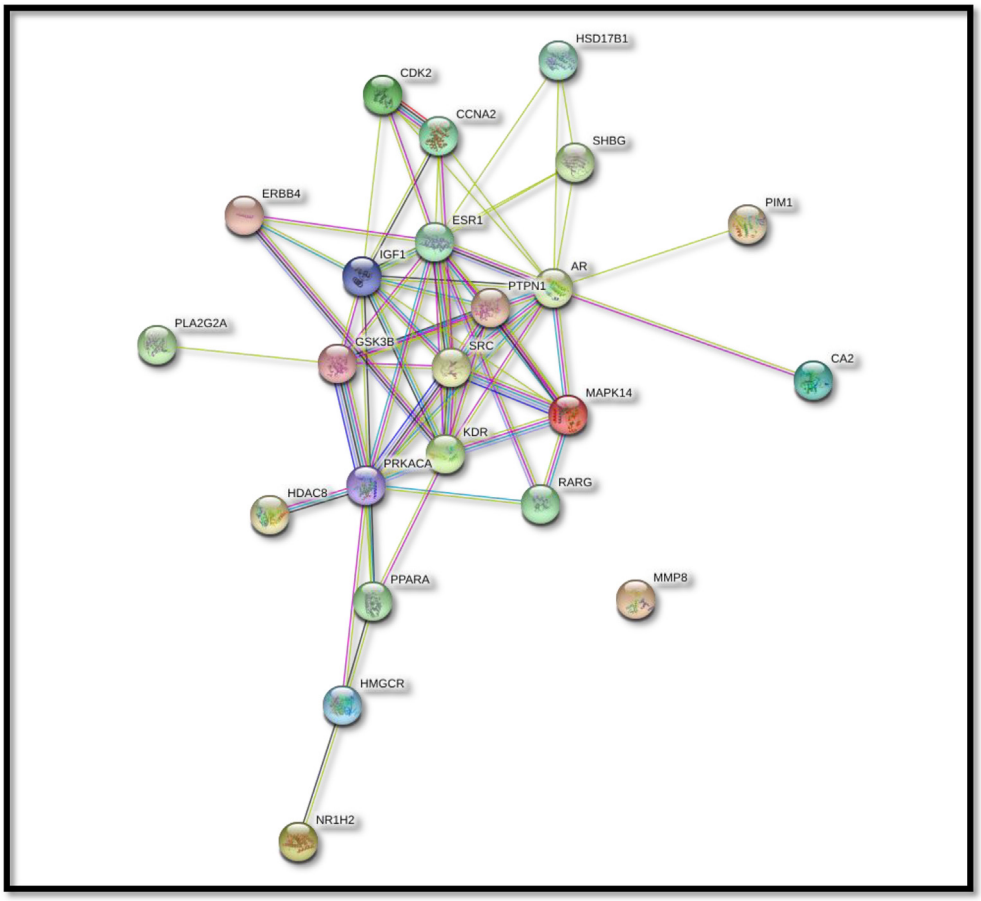

Figure 5. Genetic map of the 23 common genes repeated at least once in each of the three DAA subclasses.

Enrichr [60] showed the best KEGG [61] pathways that may be enriched by these genes. As shown in Figure 6, "proteoglycans in cancer," "progesterone-mediated oocyte maturation," and "prolactin signaling" pathways have the best predicted P values. These pathways are evidence related to HCC [62, 63, 64, 65]. Modulation of these pathways by HCV DAAs could disclose some answers regarding their HCC mysterious relationship.

Alternatively, hits in a certain DAA subclass could be beneficial in other manners. For instance, *F2* was retrieved for all the eight NS3/4A inhibitor DAAs included in this study. The functions of *F2* polymorphism in HCC development have been extensively investigated throughout literature [66, 67, 68]. The effect of DAAs on the blood coagulation system has been debated [69, 70], which coincides with our results that some DAAs may target *F2*. A further in-depth investigation of DAA relationship with *F2* may clarify their debated relationship with HCC development and coagulation system.

4.3. Variant results

Considering VDA scores, some variations are worth further investigation in wet laboratory settings. For example, the SNPs rs104894226, rs104894228, rs104894229, rs104894230, rs121913233, and

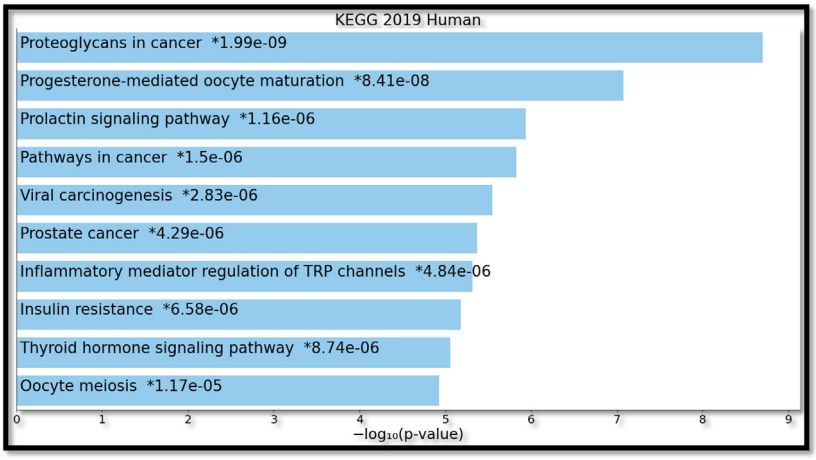

Figure 6. Gene enrichment analysis for 23 intersecting genes between DAA subclasses showing enriched KEGG pathways. Studying the modulation of these pathways by DAAs could disclose some answers about the DAA/HCC relationship.

rs28933406 in HRas proto-oncogene, GTPase (minus strand), *HRAS*, and leucine-rich repeat containing 56 (plus strand) *LRRC56* are good candidates for further investigation on their possible role in DAA/HCC relationship. These genes are significantly predicted as DAA OLTs, and at the same time, their variants are evidence related to HCC [71].

Intersecting variants of the three subclasses returned seven variants that appeared at least once in each subclass. These variants (namely rs12338, rs13332, rs1870377, rs1979277, rs2071559, rs6147150, and rs8898) belong to four genes (*CSTB*, *SHMT1*, *KDR*, and *ERBB4*). Variants (rs12338, rs8898, and rs13332) of *CTSB* are associated with high risk and tumor size in HCC [72]. The variant rs1979277 of *SHMT1* is linked to the risk for liver and colon cancer according to global DNA methylation [73]. Variants (rs2071559 and rs1870377) of *KDR* are related to overall survival and tumor resectability of patients with HCC [74]. The variant rs6147150 of *ERBB4* is a polymorphism associated with high risk for HCC [75]. Considering these variants in pharmacogenetic studies could elucidate their possible role (risk or protective) in developing HCC in DAA-treated patients. This could help in the efforts to define DAA pharmacogenetic labels and in developing clinical pharmacogenetic guidelines for the precise use of DAAs and according to the patient's genetic profile.

4.4. Future research

The rapid change in healthcare practice driven by high-throughput data technologies urges a rapid response in tools that aid implementing this change. Developing tools to help implement the patient's genetic profile in healthcare decision is becoming more interest, and there are research efforts across the world. In a previous study, we introduced PHARMIP as a tool to address some challenges in this area. In a future insight, we intend to develop a platform to automate PHARMIP to ease the process for nonspecialists. Furthermore, we intend to develop a scoring system for candidate genetic factors that take into consideration the possible synergism between them.

In a more optimistic insight, we intend to use machine-learning techniques to help in detecting the action of drugs on candidate OLTs. Supervised binary classification algorithms could help predicting the drug effect (activator/inhibitor) on its predicted OLT, which based on its role will elucidate the role of the drug (risk/protection) in developing an ADR. Embedding this feature in PHARMIP will improve its use in daily activities related to precision medicine.

We also have some goals to use our technique earlier in a “pharmacogenetic-guided-drug-design” role rather than in an “ADR-explanation” role. For instance, three genes (*MAPK14*, *AKR1B1*, and *PTPN1*) appeared as OLTs for all the six NS5A inhibitors included in this study. Such results could be helpful in pharmacogenetic-guided NS5A development and precise design of new subclass members to minimize/maximize the risk/protective effect related to HCC development.

5. Conclusion

Our results demonstrated high prediction scores with several OLTs and variants that were markedly linked to HCC development. The predicted interactions may explain the unprecedented results with HCV treatment by DAAs. It could be concluded that in addition to the direct inhibition of HCV targets by DAAs, they synergistically interact with OLTs in the infected hepatocytes to influence HCC development. Further experimental investigation on these OLTs is strongly recommended aimed at defining the role(s) of host genetics in the HCV DAA/HCC relationship controversy. Additional wet laboratory analyses are required to identify whether the genetic factor indicates HCC risk or a protective factor. We anticipate that the schema of this study would help in the ongoing revolution of personalized medicine by identifying host genetic factors underlying unexplained ADRs.

Declarations

Author contribution statement

Ahmad M. Zidan, Eman A. Saad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nasser E. Ibrahim, Amal Mahmoud: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Alaa A. Hemeida, Medhat H. Hashem: Conceived and designed the experiments; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at Mendeley (https://doi.org/10.17632/8ws8258hn3.2).

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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