Literature Review: Role of Pharmacogenetics in the Treatment of HBV

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Abstract

Background: Hepatitis B virus (HBV) infection is a serious and in some cases life threatening infection accounting for numerous deaths annually. The virus can be transmitted through sexual contact or exchange of bodily fluids. Progression to a chronic infection is age dependent with 90% of newborns of HBeAg-positive mothers developing a chronic infection. The risk is lowest with adults, with only <5% of adults progressing into a chronic infection. The goal of treatment is to prevent disease progression and further chronic HBV related complications like cirrhosis, hepatic failure, and cancer. The 2018 AASLD practice guidelines recommends the following preferred drugs: Pegylated interferon alpha 2a, Entecavir, Tenofovir dipivoxil fumarate, Tenofovir alafenamide in treatment of chronic Hepatitis B (CHB). There are several other non-preferred drugs used to treat CHB infection. The aim of this study was to review the current published literature recommending use based on genetic test results. Ongoing research has shown that pharmacogenomics can play a pivotal role in treatment efficacy and safety of HBV medications.

Keywords: Pharmacogenetics, Chronic infection, Adults, Hepatic failure, drugs

1. Introduction

Hepatitis B is an important topic of discussion with more than 296 million people affected and contributes to about 820,000 deaths annually [01, 80]. The Hepatitis B virus (HBV) can be transmitted through sexual contact or exchange of bodily fluids either by perinatal, percutaneous or by close person-to-person contact (through cuts and sores, particularly among children in high risk areas). Perinatal transmission remains the main cause of chronic infection in most countries [02-05]. The presence of HBsAg is the cornerstone of hepatitis B diagnosis [05]. Hepatitis B manifests as either an acute or a chronic infection. A chronic infection is when the HBsAg is present for at least 6 months [05]. Chronic infections require treatment due to the risks of serious health problems, such as liver damage, cirrhosis, cancer, and death. Age plays a pivotal role in the progression of the disease, with younger patients being at a higher risk of developing a chronic infection [02].

The risk of progressing into a chronic infection after acute exposure to HBV varies depending on age. The risk is highest in newborns of HBeAg-positive mothers with 90% of them developing chronic infection, and it drastically declines to 25-30% in infants and children under the age of 5 [05]. The risk is lowest in adults, with only <5% of adults experiencing chronic infection [05]. The goal for the treatment of chronic hepatitis B (CHB) is preventing disease progression and further complications like cirrhosis, hepatic failure, and cancer [04].

HBV Virus

HBV is a double-stranded DNA virus from the family Hepadnaviridae [01]. The core particle contains the viral genome, nucleocapsid protein and polymerase, as well as an envelope composed of viral antigens. The virion invades cells by entering the hepatocytes and binding to the cellular sodium taurocholate cotransporting polypeptide via the pre-S1 region on the virion envelope [01]. Once the virion is bound to the target cell, the virion is uncoated, and the core particle contents are transported into the nucleus [01]. Inside the nucleus, covalently closed circular DNA (cccDNA) is formed through covalent ligation and the viral genome is replicated through reverse transcription by reverse transcriptase. Understanding the viral life cycle is important for guideline-directed treatment regimen for patients with hepatitis B infection.

Pharmacogenomics

Many drugs are prescribed on the basis of “one size fits all.” Cli-
Pharmacogenomics is the relationship between genetic variation and drug response [07]. These genetic variations can dictate the efficacy and safety profile of the study drug in individual patients typically through alterations in pharmacokinetics (ADME) or pharmacodynamics, by modifying the pathway(s) the drug exerts its effects upon [08]. Understanding the role our genes play in response to therapy is an active area of research. Many studies have made advances in pharmacogenomics by identifying associated genes and/or drugs that are clinically relevant. Upon the discovery and validation of a pharmacogenomic relationship, the challenge remains in its implementation and translation into clinical practice [08].

Once the genotypes are determined, they are converted into phenotypes which have clinical relevance. These phenotypes are referenced as one of the 4 different categories of metabolizers as follows: poor metabolizer (PM) having two non functional alleles resulting in no activity, intermediate metabolizer (IM) having one nonfunctional allele and one reduced function variant resulting in reduced activity, extensive metabolizer with two alleles that have full or reduced function; 1 full plus 1 nonfunctional or reduced function allele resulting in normal activity, and ultra rapid metabolizers (UM) with more than 2 copies of full functional alleles resulting in increased activity. Each phenotype correlates with an activity score. PM have an activity score of 0, IM metabolizers will have an activity score of 0.5. EMs activity score can be 1-2, and UMs have an activity score greater than 2 [9-11].

Barrier for Clinical Implementation of Pharmacogenomic Findings: There are multiple factors that impede the implementation and translation of pharmacogenomics into clinical practice. One such barrier is the lack of incentive for healthcare providers and systems to conduct pharmacogenetic testing to reduce adverse events and maximize efficacy [08]. There has been a limited amount of research that focuses on the cost-effectiveness of pharmacogenetic testing and health economists believe that if time and cost burden of disease monitoring is uncomplicated and affordable, then genomic testing is not cost effective [09]. They believe that the most cost-effective genomic tests are those done for rather complicated conditions where monitoring is expensive and challenging [09]. Furthermore, the majority of healthcare systems do not offer financial reimbursement for screening services and as such create a barrier to conducting pharmacogenetic testing [08].

Another barrier is the lack of clear clinical guidelines for translating pharmacogenetic findings into actionable recommendations and often professional societies or those responsible for making guidelines are in disagreement on whether to proceed with the pharmacogenetic testing [08]. Nowadays, with the advances in pharmacogenetic testing and the popularity of the inexpensive multi-gene tests, many professional societies are less concerned with whether a genetic test should be ordered and more concerned with how they should use the “already” generated genetic results to guide their drug regimen decision [08]. Publicly available resources like the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Pharmacogenetics Working Group within the Royal Dutch Association for the Advancement of Pharmacy are working on creating guidelines that focus on the implementation of genetic testing into practice guidelines [10].

There are a number of barriers currently interfering with the use of pharmacogenetic test results in various clinical settings where patients seek care. Networks for organizing health care records and genomic variation databases lack continuity, and standard ontology for interpreting genomic testing remains in its infancy, preventing the exchange of information between the two domains [12]. Efforts are being made to create a homogenous set of terms to ameliorate this deficit in interoperability. The Institute of Medicine Roundtable on Translating Genomic-Based Research for Health and CPIC are beginning to generate the conventional terminology for pharmacogenomic tests necessary for upload into existing electronic healthcare record databases, which will contribute to clinical decision support (CDS) geared toward personalized medicine [12]. Progress is slow; however, new terminology useful for CDS will greatly facilitate the implementation of pharmacogenetic information longitudinally [12].

Additional barriers include the lack of education amongst clinicians, the scarcity of evidence-based implementation systems, the concern about true findings versus incidental findings, and the resistance to change in the healthcare systems. These are not solely barriers that impede pharmacogenomic advances but rather barriers to moving medicine forward in general. In order to implement pharmacogenomics into clinical practice, we must overcome some if not all of these barriers. As the general public is becoming more aware of the importance of genetic testing, clinicians and policy makers will be more encouraged to include pharmacogenomics as part of their routine testing.

Pharmacogenomics and HBV: The 2018 AASLD practice guidelines have recommended several drugs to treat hepatitis B infections, and pharmacogenomics can play an important role for some of these medications. Research is ongoing regarding the efficacy of HBV medications, their safety and tolerability, patient’s quality of life and the long-term outcomes. Although there are guidelines determining preferred therapies, the relationship of pharmacogenomics has not been discussed.
We will be covering the relationship between pharmacogenetics and the therapies that can be used to treat HBV in order to gain a better understanding of their efficacy. In this review, we will be discussing Tenofovir, Entecavir, Pegylated Interferon, Lamivudine, Adefovir, Pradefovir and Telbivudine. Drug development in the field of HBV infection is resurfacing and there are currently several novel drugs that are under investigation either phase III or phase IV clinical trials and the future holds high hopes for individuals affected by these viral infections.

Figure 1: Timeline of current therapies used in the treatment of CHB. The drugs denoted by (*) are only approved in South Korea.

**Tenofovir Disoproxil Fumarate and Tenofovir Alafenamide**

Tenofovir disoproxil fumarate (TDF) is a prodrug that undergoes diester hydrolysis to tenofovir (TFV), which is a nucleotide reverse transcriptase inhibitor commonly prescribed for the treatment and prevention of hepatitis B virus as well as HIV [13-16]. It is the most used antiretroviral worldwide and as such it is the first line and salvage therapy recommended by most guidelines [16]. It has many beneficial aspects, including once-daily dosing (300 mg daily), high efficacy, relatively favorable safety profile and lack of interaction with the cytochrome P450 enzyme family [17, 18]. Although literature suggests low overall toxicity profile for TDF and only a modest effect on eGFR, there have been case reports that link TDF renal tubular dysfunction and Long-term kidney toxicity [18-20]. One clinic observed that Tenofovir-associated nephropathy was the most common reason for an HIV-related referral to nephrologists, accounting for over 20% of the referrals. Among the side effects associated with Tenofovir are nephropathy, Fanconi syndrome, osteomalacia, and lactic acidosis [05].

Mechanisms involved in kidney tubular dysfunction are not yet well understood and research in this field is still ongoing; however, mitochondrial toxicity and/or interference with normal tubular cell function has been proposed [18]. There are certain risk factors that have been associated with tubular dysfunction including comorbid conditions (such as hepatitis C and/or diabetes), low body weight, older age, and concomitant use of nephrotoxic drugs, low CD4+ cell count, and length of therapy [18].

Another risk factor that is commonly associated with the development of kidney damage is high concentrations of Tenofovir in the plasma, which is likely related to transporters involved in uptake and efflux of Tenofovir [18]. The loss of hepatitis B surface antigen is rarely seen and most patients with CHB are on chronic long-term therapy in order to control their disease progression and symptoms [19-23]. It has been reported that TDF has high potency against HBV and no reported resistance in CHB patients; however, long-term use of the drug is associated with renal toxicity and reduction in bone mineral density [22]. These adverse effects are mainly due to the systemic exposure of TDF to tenofovir.

Tenofovir alafenamide (TAF) is a novel prodrug of Tenofovir and a nucleotide reverse transcriptase inhibitor [19, 23]. TAF has a greater plasma stability than TDF and delivers the active drug more efficiently to the hepatocytes, resulting in 90% less systemic tenofovir exposures [19, 23]. In a recent randomized, double-blind, phase 3, non-inferiority trial, 426 HBeAg-negative CHB patients were randomly, to receive once daily oral dose of TAF 25 mg or TDF 300 mg in 105 centers in 17 countries [13, 14, 24]. It was shown that CHB patients with HBeAg-negative receiving TAF had similar efficacy and improved bone and renal effects compared to patients receiving TDF, suggesting that TAF therapy may have lower incidence of renal and bone adverse effects [24].

Another group of researchers studied non-cirrhotic treatment-naïve patients with CHB and randomized them (1:1:1:1:1) to receive TAF 8 mg, 25 mg, 40 mg, or 120 mg, or TDF 300 mg for 28 days. Study groups were then assessed for safety, treatment response, and pharmacokinetic parameters [25]. TAF was shown to be similarly efficacious as TDF at all the evaluated doses and when given at doses 25 mg or lower, it had significantly reduced TFV exposures when compared to TDF [25].

**Pharmacokinetics and Metabolism:** The recommended oral consumption of TDF is 300 mg once a day with food, as the oral bioavailability increases with increased stomach acid [17]. Following oral administration, TDF is converted to TFV. TDF require initial diester hydrolysis for conversion to tenofovir. It is phosphorylated twice to the diphosphate form (TFV-DP), which is the active form of the antiviral drug [21]. The phosphorylation steps are carried out by adenylyl kinases (AK2 and AK4) and nucleotide diphosphate kinases (NME1 and NME2). TFV has low bioavailability (25%), low protein binding (<0.7%), and long plasma and intracellular half-life (12-18 hours and 69-139 hours, respectively) [16]. The drug is not a substrate for cytochrome P450 enzyme and...
is not metabolized by the family [21]. It is negatively charged at physiological pH and it is mainly excreted through the kidneys by both glomerular filtration and active tubular secretion [16]. Clearance is dependent on membrane transport proteins.

Organic Anion Transport 1 (OAT1, encoded by SLC22A6 gene) and organic anion transporter 3 (OAT3, encoded by SLC22A8) on the basolateral membrane of the proximal tubule and multidrug resistance associated protein-4 (MRP-4, encoded by ABC4 gene) on the apical side are the major transporters involved [16, 21]. While SLC22A6 and SLC22A8 are involved in the uptake of the drug into the proximal tubule, the ABC4 gene controls its efflux across the apical membrane [21]. The multidrug resistance protein-2 (MRP-2, encoded by ABCC2 gene) is also potentially involved with the efflux process [16, 21]. Genetic variation in SLC22A6, SLC22A8 and ABCC4 genes may influence exposure of the kidney to tenofovir and play a major role in renal toxicity associated with Tenofovir [21].

TAF is the phosphonate produg of TFV. TAF is hydrolyzed to TFV by intracellular enzymes like the carboxylesterase 1 (CES1), which is mainly expressed in hepatocytes of HBV-infected patients, as well as cathepsin A in lymphoid cells of HIV-infected individuals the improved systemic stability of TAF allows for a greater concentration of TFV-DP, the active form of TFV, when given at lower doses than TDF 300 mg [25].

**Polymorphism:** There have been many proposed mechanisms for TFV kidney toxicity including drug TFV interaction with transporters in the kidney tubules [26]. Efflux transporters ABCC4 (MRP4) and ABCC 2 (MRP2) have been associated with the elimination of TFV. Previously polymorphism in these two transporters have been associated with kidney tubular disease in addition to older age and lower body weight, which are known risk factors of kidney tubular disease [26]. Another transporter that was recently shown to transport anticancer agents was studied by Pushpakom et. al (2011) [26]. The researchers investigated whether TFV was a substrate for ABCC10; furthermore, in a cohort of TFV-treated HIV positive patients ABCC10 genetic variation was studied to see if there’s an association between genetic polymorphism and kidney tubular disease susceptibility [26]. Fourteen SNPs in ABCC10 were genotyped in HIV–positive patients with kidney tubular disease (population = 519) or without, which were the controls (population = 596) [26]. They found that two ABCC10 SNPs (rs9349256 and rs2125739) were significantly associated with kidney tubular disease. They concluded that TFV is indeed a substrate for ABCC10 and genetic polymorphism in this gene may be associated with kidney toxicity [26]. Research is still needed to explore the relationship between genetic polymorphism and Tenofovir toxicity and efficacy in patients with CHB.

**Entecavir**

Entecavir (ETV) is a cyclopentyl guanosine analogue that is one of the preferred antivirals used against HBV [27, 28]. ETV is a potent inhibitor of HBV DNA polymerase, a key enzyme in HBV replication, and as such induces a rapid virologic and biochemical cascade in CHB patients [4, 28]. According to the AASLD 2018 Hepatitis B guidance, Entecavir is one of the approved antivirals used in the treatment of CHB in children and adults [27]. The recommended daily dose for adults is 0.5 mg daily and for children ≥2 years of age is weight based if they’re between 10-30 Kg and 0.5 mg daily if they are above 30 Kg. ETV 1 mg daily is given to Lamivudine refractory CHB patients [27]. The once daily dosing is because of its bi-exponential elimination half-life suggesting an accumulation half-life of roughly 24 hours [28-32]. ETV improves liver fibrosis and slows down the progression of CHB and it is generally well tolerated but can have serious liver side effects like hepatomegaly, hepatotoxicity, and steatosis as well as lactic acidosis [31, 32].

In nucleoside analogue-naïve patients, ETV has shown a superior virological, histological, and biochemical efficacy after 48 weeks of treatment when compared to lamivudine or adefovir [29, 32]. Furthermore, it has also shown relatively low genotypic resistance in this patient population through 5 years of continuous therapy [30]. The efficacy of ETV is significantly reduced in LAM-refractory CHB patients with ETV resistance and patients with a virologic breakthrough [30]. Currently, ETV is approved for use in NA-naïve patients, LAM-resistant patients who not only have compensated liver disease but also have an active viral replication, as well as patients who are suffering from decompensated liver disease [31, 32].

**Pharmacokinetics and Metabolism:** ETV is a deoxy guanosine nucleoside analog. To exert its antiviral effects, it is phosphorylated to the active triphosphate form and competes with the endogenous ligand deoxy guanosine triphosphate in order to suppress HBV replication, thereby suppressing all activities of HBV polymerase including base priming, synthesis of the positive strand and reverse transcription of the negative strand from the pregenomic messenger RNA [32].

Following oral administration, ETV peak plasma concentrations and steady state occurs in 30 minutes to 1.5 hours and 6 to 19 days respectively [31, 32]. ETV should be taken either 2 hours before or 2 hours after a meal to maximize absorption, Cmax, and AUC [31, 32]. In healthy patients, ETV has a 100% bioavailability in the tablet formulation relative to the oral solution, indicating they may be used interchangeably [31]. ETV is extensively distributed into the tissues and shows a relative protein binding of 13% in vitro [31]. Following administration of ETV in human and rodent subjects, it was determined that there are no oxidative or acetylated metabolites and only minor amounts of phase II metabolites such as glucuronide and sulfate conjugates were observed [31]. It was also demonstrated that ETV does not in any way interact with any CYP450 enzymes. The drug is mostly eliminated through the kidneys via filtration and secretion with 62%-73% of the administered dose recovered in urine unchanged [32].
Polymorphism: The progression of HBV infection is associated with the interaction of the virus and the host immune system. The immune system defends the host from a myriad of viral and bacterial infections and if successful it can manage to suppress the infection and diminish its progression. On the other hand, invaders have evolved immune evasion strategies to allow them to escape the immune cells and infect the host. Natural Killer (NK) cells are cytotoxic lymphocytes that play a pivotal role as part of the innate immunity in the control of both tumors and virally-infected cells [33, 34]. Activation of NK cells are important in the control of viral hepatitis and in control of the pathogenesis of liver injury and inflammation [33]. Furthermore, NK cells secrete cytokines that modulate the immune response against the viral infection by activating the adaptive immune cells like the T lymphocytes [34].

Killer Cell Immunoglobulin-Like Receptor (KIR) Gene Polymorphism

Glycoproteins on the surface of NK cells are involved in the interaction between NK cells and the Major Histocompatibility Complex (MHC) class I [34]. KIR receptors are one group of glycoproteins that modulate the inhibition or activation of such interactions. KIR receptor nomenclature depends on the number of extracellular immunoglobulin domains it possesses (2D or 3D) and the length of its cytoplasmic tail (L for long and S for short) [34]. The length of the cytoplasmic tail dictates the activity of the KIR receptor. A long cytoplasmic tail with two Immunoreceptor Tyrosine-based Inhibition Motif (ITIM) dictates inhibitory activity to inhibitory KIRs (2DL, 3DL) and a short cytoplasmic tail means activating activity for KIR activators (2DS, 3DS) [34]. KIR genes are either A haplotype or B haplotype depending on the presence of specific genes [34]. The human KIR gene locus, on chromosome 19q13.4, encodes roughly 15 KIR genes and two pseudo genes (2DP1, 3DP1). There are 8 inhibitor genes (KIR3DL1-3, KIR2DL1-3, KIR2DL5B, KIR2DL5A), and 7 activator genes (KIR2DS1-4, KIR3DS1, KIR2DS5A, KIR2DS5B) [33, 34]. KIR2DL4 gene has been shown to have both inhibitory and activating characteristics [33, 34].

Association between KIR Genes and Efficacy of Treatment of HBeAg-Positive Chronic Hepatitis B Patients with Entecavir

KIR gene polymorphisms have been associated with either susceptibility to or protections from infectious diseases like hepatitis C virus, Treponema pallidum, HIV, and Ebola virus [34-36]. Zhuang et al. (2018) investigated whether KIR genes are associated with HBV DNA suppression and seroconversion in ETV-treated HBeAg-positive CHB patients [33]. They analyzed the frequency of KIR genes in the Chinese Han population among 198 patients with HBeAg-positive CHB who were on 0.5 mg daily ETV for 48 weeks and 200 healthy blood donors using polymerase chain reaction with sequence-specific primers (PCR-SSP). After therapy, patients with negative HBeAg and HBV DNA copies <300 were grouped as complete response group (CRG) (59 patients were in this group), those with positive HBeAg and HBV DNA copies >300 were the null response group (NRG) and lastly those with positive HBeAg and HBV DNA copies <300 were the null response group (NRG) and lastly those with positive HBeAg and HBV DNA copies <300 were in the partial response group (PRG). Together, the null or partial response group (NPRG) consisted of 139 patients [33].

The researchers found that in HBeAg-positive CHB patients when compared to healthy controls the frequencies of KIR2DS2 and KIR2DS3 genes were significantly higher (P=0.030, 95% CI=2.36-1.05 and P=0.018 and 95% CI=2.77-1.13 respectively) while the frequencies of KIR2DL3, KIR2DS1 and KIR3DS1 were significantly lower (P=0.038, 95%CI=0.96-0.29, and P=0.031, 95%CI =0.95-0.43, and P=0.035, 95%CI =0.96-0.43, respectively) [33]. KIR2DS3 gene frequency was significantly higher in the NPRG than in the CRG (P=0.018, 95%CI=0.83-0.20) while the frequencies of KIR2DL3 and KIR3DS1 genes were significantly higher in CRG than those in NPRG (P=0.019, 95%CI=10.83-1.21 and P=0.041, 95%CI=3.65-1.04, respectively). These results suggest that patients with the KIR2DS3 gene are more susceptible to negative responses to ETV therapy. Furthermore, it is possible that patients with KIR2DL3 and KIR3DS1 genes might have an advantage over other gene polymorphisms in ETV therapy [33].

Role of KIR Genes and Genotypes in Susceptibility to or Protection Against HBV Infection in a Turkish Cohort

Another study by a group of researchers in Turkey investigated the role of KIR genes in susceptibility to or protection against CHB infection or spontaneous remission of the infection [37]. The study looked at 37 patients with CHB infection, 36 patients in spontaneous remission of HBV infection, and 85 healthy patients from the Cukurova region in Turkey. DNA was extracted from each subject’s blood sample and was used in genotyping of KIR genes. A locus-specific oligonucleotide probes analysis was performed to investigate 16 KIR genes [37].

The rate of inhibitory KIR2DL3 (P=0.0) and KIR3DS1 (P=0.0) were higher in healthy controls than the group with CHB infection and spontaneous remission [37]. These results are like those found by Zhuang et. al (2018). They also looked at the haplotype frequencies and found that there were no statistically significant differences between the rate of AA and Bx genotypes of CHB infection patients and those with spontaneous remission and the healthy controls (P>0.05) [37]. These results suggest that KIR2DL3 and KIR3DL3 genes could potentially be protector genes for HBV infection and may be good markers in determining antiviral immunity [37].

Insights into the Interplay between KIR Gene Frequencies and Chronic HBV Infection in Burkina Faso

Another research group in Ghana, investigated the interplay between KIR gene frequencies and the risk of developing CHB [34]. The researchers recruited a total of 244 subjects from the Burkina Faso region in their study, 110 of the subjects had CHB (HBsAg positive for more than 6 months) and 134 were controls. Their results suggest that inhibitory genes KIR2DL2 (P<0.001), KIR2DL3
with decreased levels of demyelination in the CNS. Therefore, of virus-specific IFN-γ-secreting CD8+ cells in the spleen along chemokines monokine induced by IFN-γ and IFN-inducible T cell it was found that with reduced levels of IFN-γ, IFN-γ-induced into sites where inflammation is occurring. In a study with mice, lipopolysaccharide. This protein is possibly a recruiter of T-cells protein is secreted from cells stimulated with type I and II IFNs and IFN inducible protein 10 (IP-10) is a Inducible Protein 10: Polymorphisms

Pharmacokinetics and Metabolism

Pegylated Interferon is cleared through the liver and kidney, with about 30% of Pegylated Interferon alpha 2b being cleared through the kidney [21]. The remaining is cleared through the liver or degraded after interactions occur between the drug and cellular IFN receptors [21].

Polymorphisms

Inducible Protein 10: IFN-γ inducible protein 10 (IP-10) is a chemokine that is a chemoattractant for activated T cells. This protein is secreted from cells stimulated with type I and II IFNs and lipopolysaccharide. This protein is possibly a recruiter of T-cells into sites where inflammation is occurring. In a study with mice, it was found that with reduced levels of IFN-γ, IFN-γ-induced chemokines monokine induced by IFN-γ and IFN-inducible T cell alpha chemoattractant in the brain, there was decreased numbers of virus-specific IFN-γ-secreting CD8+ cells in the spleen along with decreased levels of demyelination in the CNS. Therefore, data suggests that impaired IP-10 may affect a person’s ability to recover from an infection [39].

Inducible Protein 10 Polymorphisms

A cohort of 121 chronic hepatitis B (CHB) patients were randomly assigned to complete a full course of Pegylated Interferon for 9 months at King Chulalongkorn Hospital in Bangkok. All patients had been seropositive for HBsAg for a minimum of 6 months before therapy, had elevated serum alanine aminotransferase levels and had serum HBV DNA levels >20,000 IU/mL. Exclusion criteria included patients coinfected with HCV and/or HIV. The study was conducted to evaluate whether or not inducible protein 10 (IP-10) polymorphisms have an effect on treating patients with Pegylated Interferon for the primary endpoint of virological response (defined as HBeAg clearance plus HBV DNA level <2000 IU/mL and assessed at 24 weeks post treatment). IP-10 is a chemokine that binds to the CXCR3 receptor and mediated T-cell trafficking and function. Studies have shown that pre-treatment serum IP-10 levels have a correlation to IFNL3 polymorphisms and treatment response to Pegylated Interferon therapy in patients with chronic HCV infection. This study examined the effects of G-201A and rs12979860 and rs3077 genotypes, on the treatment response to Pegylated Interferon on Thai patients with chronic HBV [40].

The results of the treatment showed different rates of viral clearance in HBeAg-positive chronic HBV patients. After reviewing treatment responses, patients with GG genotype achieved significantly higher rates of viral response (48.8% vs 19.2% p=0.011) and HBsAg declined <100 units/mL (25.6% vs 4% p=0.019) than those with a non-GG-genotype. Although viral response was significantly different, HBsAg clearance rate showed no difference between GG and non-GG genotypes [40].

Vitamin D Receptor Gene

New research has shown that Vitamin D plays a role in immune response to viral infections. The active form of Vitamin D (1,25-DihydroxyvitaminD [1,25(OH)2D]) is primarily generated in the kidneys by a 1-a-hydroxylase, CYP27B1. When the body responds to an injury, CYP27B1 is up-regulated in keratinocytes, as well as toll-like receptor (TLR) activation [41-43]. TLRs are able to recognize viral proteins and nucleic acids [44]. When activated by viral proteins or nucleic acids, TLRs release cytokines, inducing antimicrobial peptides and reactive oxygen species expression. Although few studies have shown an effect of Vitamin D on HBV, a study of 2015 Gambian tuberculosis patients identified a single nucleotide polymorphism in codon 352 of the VDR was associated with significantly lower rates of HBV infection [45]. The SNP affects vitamin D levels, VDR mRNA stability, and VDR mRNA levels [46-49]. Similar effects were also seen in a study on dengue fever [41]. In addition, VDR SNPs showed an association that patients with lower vitamin D levels failed to clear hepatitis C virus [41].
Vitamin D Receptor Gene Polymorphisms

The purpose of this study was to observe the effect of vitamin D receptor (VDR) gene polymorphisms FokI T>C (rs10735810), BsmI A>G (rs1544410), Apal (rs7975253), and TaqI C>T (rs731236) in response to PEG-IFN treatment in Egyptian HBV patients. The control ratio was 2:1. Inclusion criteria included: male/females 18-60 years old, HBsAg positive for more than 6 months with either normal or abnormal ALT levels. Exclusion criteria included: co-infections with HCV, HDV positive and bilharzia liver disease. In this study, patients with FOKI Ff or ff alleles had an increased response to PEG-IFN than those with FF allele. This was shown through significantly lower HBV viral load determined from PCR [42-50].

The most common SNPs of VDR that are genotyped include, FokI C>T (rs2228570), exon 2), BsmI G>A (rs1544410, intron 8), Apal C>A (rs7975232, intron 8), and TaqI T>C (rs731236, exon 9). In this study, patients with the bb allele in the VDR BSMI SNP responded more to PEG-IFN treatment compared to those with the Bb allele which was determined by viral DNA at the end of treatment. In the TaqI SNP, patients with TT allele showed significantly less HBV compared to patients with Tt or tt alleles. No significant differences were found in Apal patients. More research is required to determine the significance [50].

Lamivudine

According to the AASLD 2018 guidelines Lamivudine is not a preferred drug for HBV therapy due to the high resistance rates [50]. Lamivudine is a potent reverse transcriptase inhibitor [50]. Lamivudine is generally well tolerated but it can cause headache, nausea, malaise, fatigue, nasal signs and symptoms, diarrhea and cough. More severe effects can occur such as lactic acidosis and pancreatitis [51]. The common dose is 3 mg/kg with a maximum daily dose of 100 mg for at least 2 years [53].

Pharmacokinetics and Metabolism: Lamivudine (2’-deoxy-3’-thiacytidine, 3TC) is a pyrimidine analog reverse transcriptase enzyme inhibitor [21]. It has one known metabolite, trans-sulfoxide which is formed into its active form from multiple kinases [21]. The metabolite competes with deoxycytidine triphosphate for binding to reverse transcriptase, which then adds 3TC-TP into the virus DNA resulting in chain termination. Lamivudine enters the cell by either passive diffusion or it is actively transported into the cell by uptake transporters (SLC22A1, SLC22A2, and SLC22A3). The rate limiting step of the mechanism of lamivudine is the phosphorylation of 3TC-DP to 3TC-TP [21]. Lamivudine is not significantly metabolized by cytochrome P450 enzymes and is 70% eliminated unchanged in urine [51]. Lamivudine is actively transported out of the cell by efflux transporters (ABCB1, ABCC2, ABCC3, ABCC4 and ABCG2). Unless lamivudine is in its monophosphate form, it will be effluxed out of cell by ABCC4 [21].

Polymorphism

Deoxycytidine Kinase: Lamivudine can have mutations and polymorphisms in the pathway that are gene specific and could alter the pharmacokinetics. However, few studies have reported variability in population pharmacokinetics. Of the findings, there has been an association with deoxycytidine kinase (dCK) polymorphisms on the effect of treatment response. Function of dCK is critical because it phosphorylates lamivudine (LAM) to its active triphosphates which then allows it to terminate the viral replication [52].

Single Nucleotide Polymorphisms in Deoxycytidine Kinase

A clinical trial with 127 patients ages 18-75 years old were enrolled and followed for at least 24 months. Patients were treated with LAM monotherapy 100 mg for at least 12 months. Six different dCK SNPs were found: -2052C/A, IVS3-46G/DEL, IVS4+40G/T, IVS4+39T/C, IVS5-72A/T, and 966-975T10/T11. Two promoter SNPs were found to be more prevalent in Korean patients. In addition, there is a SNP in exon 3 that was found to be prevalent in Caucasians. HBV sero-clearance rates increased with long follow-up periods in patients carrying the -260GG/-201CT or -360/-201T genotypes. The study found significant difference in the allele frequency of -360G/-201T between HBsAg sero-clearance group and the HBsAg non-sero-clearance group (P=0.045). These results infer that having the -360/-201T haplotype may increase viral clearance when patients are given lamivudine treatment [52].

OCT1 and OCT2 Polymorphisms Decreasing Lamivudine Uptake

As stated previously, Lamivudine is a substrate for OCT1 and OCT2. The exact details of the polymorphism have not been noted, but OCT1 variants are found in the loops of the protein between transmembrane helices (TMH) 6 and 7, which interacts with TMH4 in the secondary conformation [53-55]. The regions where the variants occur in the OCT1 protein are important for substrate recognition or as functional domains [56].

Reduced uptake of lamivudine into transporters OCT1 and OCT2 could reduce the hepatic and renal elimination of lamivudine and may be responsible for genetic variability as well as variability in pharmacokinetics and drug response. Genetic variants in this study were shown to affect the transport function in a substrate-dependent manner. Overall, this study found that oocytes expressing OCT1-283L and -P341L variants had decreased OCT2 uptake of lamivudine compared to OCT-WT. Similarly, OCT2-T1991, -T201M and -A270S variants had decreased OCT2 uptake of lamivudine compared to OCT-WT in oocytes. This data was measured in terms of clearance and all P values were less than 0.5. Although these genetic variants can affect therapeutic treatment, the study claims that further research is needed to clarify the results [56].

Adefovir

Adefovir, 9-(2-phosphonylmethoxyethyl) adenine (PMEA), is an acyclic nucleotide analog of adenosine monophosphate that exhibits antiretroviral activity targeting the human hepatitis B reverse transcriptase [57, 58]. It is one of the non-preferred antiretroviral therapy drugs used to inhibit reverse transcriptase [58]. The drug...
has not been shown to cause liver damage. However, rapid initiation and withdrawal of the drug may cause an impermanent spike in HBV symptoms [58]. Adefovir dipivoxil (ADV) and pradefovir, which are converted to PMEA in the body, can also be administered [59, 60]. Adefovir use may cause asthenia or Hepatitis B exacerbation. The common adult dosage is 10mg by mouth daily.

**Pharmacokinetics and Metabolism:** ADV, like PMEA, is a nucleoside reverse transcriptase inhibitor analogue [61]. The drug is not metabolized by any of the cytochrome P450 enzymes. It requires an initial diester hydrolysis for conversion into PMEA, its active form, in addition to being phosphorylated twice by kinases into the diphosphate form. ADV demonstrates little binding to human plasma and serum proteins at less than 10% [57].

The major pathway of excretion for ADV is via glomerular filtration in the kidneys as well as tubular secretion. Organic ion transporter 1 (OAT1) (SLC22A6 gene), organic ion transporter 3 (OAT3) (SLC22A8 gene), and multidrug resistant protein 4 (ABCC4) are the three main transporters involved in the clearance of ADV [57]. SLC22A6 and SLC22A8 lie on the basolateral membrane of the proximal convoluted tubule and control the uptake into the tubule while ABCC4 lies on the apical side of the tubule and controls the active passage of the drug across the apical membrane [57]. As a result, single nucleotide polymorphisms associated with SLC22A6, SLC22A8, ABCC4, and ABCC2 may play a role in the renal toxicity that has been observed with the use of ADV [57]. There is a need for more credible research regarding the specific effects of the polymorphisms listed above.

Pradefovir is a produg of adefovir, studied in an effort to improve the therapeutic index of adefovir. Pradefovir has increased liver perfusion compared to adefovir having higher kidney perfusion [58-79]. Currently, Pradefovir is not an FDA approved drug. Pradefovir is a cyclohydroxy cyclic nucleotide antiviral prodrug with activity against HBV. Unlike ADV, pradefovir is metabolized by the cytochrome P450 enzymes, specifically by CYP3A4, to PMEA [60]. CYP3A4 is a liver specific enzyme. Thus, pradefovir is not converted into the active form of PMEA until it reaches the liver, decreasing renal toxicity as well as systemic toxicity [62]. A study conducted in 2006 on rodents showed that CYP3A4 was the only CYP isozyme responsible for conversion of pradefovir into its active form [63]. Once pradefovir has been converted into PMEA, it follows the same metabolic pathway as ADV by first being phosphorylated by kinases to adefovir diphosphate [60]. The diphosphate form is able to effectively compete with 2'-deoxyadenosine triphosphates, allowing it to become part of the viral DNA. The resultant addition of adefovir diphosphate to the growing DNA strand inhibits HBV DNA polymerase to cause premature termination of HBV replication, preventing the virus from duplicating [60].

**Polymorphism:** A study conducted on fifty healthy Chinese subjects in 2017 looked to evaluate the adverse effects associated with pradefovir and identified three SNPs associated with the metabolism of the drug [62]. Subjects were separated into five groups and randomly received a single dose of pradefovir of either 10, 30, 60, 90, or 120 mg and 10 mg of ADV or placebo. Results of the study show that a single dose of pradefovir of 10-120 mg was tolerated overall with no renal impairments. The three metabolic SNPs identified included cytochrome P450 oxidoreductase (rs6965343), aryl amine N-acetyltansf erase (rs4986993), and CYP2F1 (rs305968). One distribution-related SNPs was also identified, orosomucoid 2 (rs12685968) [62]. Additional research is needed to understand the effect these SNPs may have on adverse effects seen with pradefovir.

**Telbivudine**
Telbivudine triphosphate (telbivudine 5'-triphosphate) is a synthetic nucleoside analog of thymidine that functions as a nucleoside reverse transcriptase inhibitor of hepatitis B [64]. It is a non-preferred antiretroviral therapy drug used to inhibit reverse transcriptase [65]. Telbivudine competes with thymidine during HBV DNA replication in its dideoxy form. Reverse transcriptase functions as the HBV DNA polymerase during viral replication. Once dideoxy telbivudine has been incorporated into the growing viral DNA strand, HBV DNA replication is terminated prematurely, thereby inhibiting the function of reverse transcriptase [64]. Telbivudine used to be a treatment option for pregnant women due to its ability to prevent vertical transmission of HBV to the fetus without harming it, but it has since been discontinued [66, 67].

**Pharmacokinetics and Metabolism:** Telbivudine is not an inhibitor of the cytochrome P450 enzymes, and it is not metabolized by the enzyme system. To become an active metabolite, telbivudine is phosphorylated three times by kinase enzymes [64].

**HBV Prevention**
According to the ACIP, HBV vaccination is recommended in all populations. Importantly, HBV vaccine is safe and effective in pregnant woman [68]. Ideally, vaccination should occur within 24 hours of birth for healthy infants weighing more than 2kg. Special precautions should be taken when a mother that is HBsAg+ gives birth. For HBsAg+ women, it may be necessary to test for HBV DNA. In addition, after birth an infant should receive post-vaccination serologic testing for an infant born to a mother of unknown HBV status. Preventing transmission to a child is important because of the risk in children to develop a chronic infection. In addition, the 2018 AASLD guidelines recommend any person with a chronic liver disease to be vaccinated for HBV [68].

**HBV/HIV Co-infection**
The hepatitis B virus (HBV) and human immunodeficiency virus (HIV) utilize the same routes of transmission. As a result, co-infection is very common in areas where both viruses are prevalent with six to ten percent of patients infected with HIV having HBV co-in-
Combinations of antiretroviral therapies (ART) are used to achieve this goal. Lamivudine (3TC), emtricitabine (FTC), and tenofovir disoproxil fumarate (TDF) are types of ART with activity against both HIV and HBV. Current treatment recommendations for co-infected HIV/HBV patients involve the use of 3TC and TDF or FTC and TDF. In cases where TDF cannot be used, entecavir is recommended in its place [75]. Despite the use of combination ART, some co-infected patients still fail to achieve viremia suppression while taking 3TC and TDF or FTC and TDF. Although viremia is still detected following TDF based combination therapy, TDF resistance is not a factor [76, 77]. HBV treatment should never be stopped, but rather tailored to the treatment of both HBV and HIV.

2. Conclusion
Pharmacogenetics has made progress in the past decade, but further research will be needed to confirm pharmacogenetic results that may affect clinical practice. Currently 2018 AASLD Hepatitis B guidance states preferred therapies are tenofovir alafenamide, entecavir, tenofovir disoproxil fumarate, and peginterferon. Other drugs may not be preferential due to increasing resistance or side effects.

Acknowledgements
We would like to thank Taimour Langaee MSPH, Ph.D. for his helpful advice on constructing this manuscript.

Disclosure
The authors report no conflicts of interest.

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