The functional role of sodium taurocholate co-transporting polypeptide NTCP in the life cycle of hepatitis B, C and D viruses

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Abstract

Chronic hepatitis B, C and D virus (HBV, HCV and HDV) infections are a major cause of liver disease and cancer worldwide. Despite employing distinct replication strategies, the three viruses are exclusively hepatotropic and therefore depend on hepatocyte-specific host factors. The sodium taurocholate co-transporting polypeptide (NTCP), a transmembrane protein highly expressed in human hepatocytes that mediates the transport of bile acids, plays a key role in HBV and HDV entry into hepatocytes. Recently, NTCP has been shown to modulate HCV infection of hepatocytes by regulating innate antiviral immune responses in the liver. Here we review the current knowledge of the functional role and the molecular and cellular biology of NTCP in the life cycle of the three major hepatotropic viruses, highlight the impact of NTCP as an antiviral target and discuss future avenues of research.

Keywords: Liver cell biology, bile acid transport, host factor, anti-viral therapy, hepatocytes.

Introduction

Every year, viral hepatitis is estimated to cause around 1.3 million deaths worldwide, mainly through chronic liver disease and hepatocellular carcinoma (HCC). Approximately 95% of these deaths are caused by hepatitis B and C viruses (HBV, HCV) [1]. Despite the availability of an effective vaccine for HBV, 250 million people are chronically infected by the virus worldwide [2]. An estimated 5% of HBV patients are co-infected with hepatitis D virus (HDV), a satellite virus hijacking HBV envelope proteins to assemble its infectious viral particles. HDV co-infection worsens the outcome of HBV infection and treatment of HBV-HDV co-infected patients is less effective [3, 4]. Moreover, around 70 million people are living with chronic HCV infection and, despite the existence of effective curative strategies, the incidence of HCV is still increasing [3].

Remarkable progress has recently been made for treatment of HCV infection. The development and approval of direct acting antivirals (DAAs) specifically targeting viral proteins now allows for HCV cure, but these therapies remain inaccessible for the majority of HCV patients [5]. For chronic HBV infection, two therapeutic approaches are used to suppress viral replication: pegylated interferon and nucleos(t)ide analogues (NUCs). While these treatments allow control of HBV infection, viral eradication is rare and, in most cases, lifelong therapy is required [6]. For patients with chronic HBV/HDV co-infection, the current treatment options are limited to interferon-alpha (IFNα) and its pegylated derivative. Furthermore, although current antivirals decrease the risk of HCC, they are not sufficient to eliminate the risk [7, 8]. In order to effectively combat these hepatotropic viruses,

58 it is necessary to improve existing therapies and uncover new strategies for prevention and treatment of viral

59 hepatitis.

60   Alternative strategies against chronic HBV and HCV infection include host-targeting agents (HTA),

61 which target host factors required for viral replication. HTAs have been shown to be promising candidates for the

62 prevention and treatment of infections by various pathogens, including HBV and HCV [9–11]. This approach

63 requires a profound understanding of the viral life cycle and the virus-host interactions involved. Indeed, the

64 identification of the human sodium taurocholate co-transporting polypeptide (NTCP) as a functional receptor for

65 HBV/HDV infection [12, 13] opened perspectives for new antiviral strategies. Several entry inhibitors for

66 treatment of HBV infection targeting NTCP are now in development [14–19]. Furthermore, this crucial discovery

67 has allowed the development of novel infectious model systems that will enable an improved understanding of the

68 complete HBV/HDV viral life cycle [20]. However, the regulatory role of NTCP in HCV host cell infection, and

69 its potential immunomodulatory activities in hepatocytes, should not be overlooked. The aim of this review is to

70 summarize what is known about the interactions of NTCP with three major hepatitis viruses during infection, to

71 describe the molecular mechanisms, and to highlight possible applications in research and therapy.

72

73 **Sodium taurocholate co-transporting polypeptide, a bile acid transporter**

74 The circulation of bile and bile components between human intestine enterocytes and liver parenchymal cells is

75 known as the enterohepatic circulation (EHC) [21]. In the liver, bile acids are mainly involved in cholesterol

76 metabolism and elimination of toxic compounds [22]. Interestingly, bile acids have also been shown to inhibit

77 interferon (IFN) signaling pathways, resulting in reduced expression of IFN-stimulated genes (ISG) [23, 24]. In

78 hepatocytes, bile acid homeostasis is maintained by the interplay between uptake, synthesis and secretion of bile

79 acids. The major hepatic uptake transporter for conjugated bile acids in humans is sodium taurocholate

80 co-transporting polypeptide (NTCP) [25]. NTCP is predominantly expressed at the hepatic basolateral membrane

81 and is involved in the recycling of bile acids from portal blood to hepatocytes in a sodium-dependent manner [21].

82 NTCP is a member of the solute carrier family SLC10 and is encoded by *SLC10A1* [26, 27]. *SLC10A1* mRNA is

83 translated into a 349 amino acid glycosylated phosphoprotein with seven or nine transmembrane domains [21, 28–

84 31]. While the exact function of some SLC10 family members remains unknown, all of them are thought to be

85 sodium-dependent transporters [21]. Interestingly, bile acid transport through NTCP can be blocked by small

86 molecules already in clinical use, such as cyclosporine A (CsA, an immunosuppressive drug used in

87 transplantation) or ezetimibe (used for hypercholesterolemia) [16, 32].

88   Hepatic bile acid metabolism is tightly regulated, including at the transcriptional level (see Figure 1) [33].

89   Upon bile acid activation, the nuclear factor Farnesoid X Receptor (FXR) indirectly downregulates several target

90   genes through transcriptional induction of the small heterodimer partner (SHP) [34, 35], including the first and

91   rate-limiting enzyme in bile acid biosynthesis cholesterol 7α-hydroxylase (CYP7A1) [36, 37]. FXR also directly

92   activates the expression of the bile salt export pump (BSEP, ABCB11), which is expressed at the apical membrane

93   and secretes conjugated bile acids into the bile canaliculus in an ATP-dependent manner [38, 39]. FXR does not

94   directly interact with the promoter of human *SLC10A1* but induces the expression of different factors to indirectly

95   repress *slc10a1* expression in rat and mouse, although mechanisms of transcriptional regulation of human NTCP

96   remain unknown [40–42]. In hepatic inflammation, the cytokines tumor necrosis factor alpha (TNF-α), interleukin

97   (IL)-1β, and IL-6 downregulate mRNA levels of *SLC10A1* and reduce the transporter protein expression [43–45].

98   The downregulation of NTCP expression in the human liver has been implicated in several cholestasis pathologies.

99   The reduction of NTCP expression could explain impaired hepatic bile acid uptake, resulting in cholestatis and

100   jaundice. Several studies have shown a downregulation of bile salt transporters in primary biliary cirrhosis [46,

101   47]. Interestingly, a recent study showed a suppression of NTCP expression via cyclin D1 in hepatocellular

102   carcinoma (HCC) [48]. These data may explain the low expression level of NTCP in HCC-derived cell lines, such

103   as Huh7 and clones or HepG2.

104   The localization and membrane expression of NTCP is controlled by post-translational mechanisms [49].

105   For example, cyclic adenosine monophosphate (cAMP) plays a role in stimulating the dephosphorylation and

106   membrane translocation of NTCP (see Figure 1) [50–52]. Sequencing analysis of NTCP revealed the existence of

107   several ethnic-dependent single nucleotide polymorphisms (SNPs) which may alter NTCP activities [53]. For

108   example, mutation S267F, found in 7.5% of allele frequencies in Chinese Americans, is associated with an almost

109   complete loss of bile acid uptake function. However, no pathologies have been described resulting from these

110   NTCP polymorphisms and their clinical roles remain controversial [54]. Besides its major role in the bile acid

111   uptake system, Yan *et al.* described the crucial role of NTCP on HBV and HDV entry [12]. For the time being,

112   NTCP remains the only described HBV and HDV entry receptor.

113

114

NTCP is a host factor for HBV/HDV infection

Hepatitis B virus is the prototypic member of the *Hepadnaviridae* family of small enveloped hepatotropic DNA viruses. Its envelope consists of three different forms of the HBV surface protein (HBsAg) – the small (S), middle (M) and large (L) proteins. Importantly, the preS1-domain of L envelope protein is known to bind the hepatocyte cell surface and is required for HBV and HDV entry [55]. The HBV capsid is comprised of HBV core protein (HBcAg) and encapsidates a partially double-stranded relaxed circular DNA (rcDNA) genome of 3.2 kilobases. Upon infection of hepatocytes, genomic rcDNA is converted into covalently closed circular DNA (cccDNA), a minichromosome-like structure that persists in the nucleus as a central transcription template for all viral RNAs [56]. The presence of cccDNA in the nucleus is thought to be responsible for viral rebound after withdrawal of NUC therapy that targets reverse transcription, a late step in the HBV life cycle. Therefore, removal of cccDNA from HBV-infected hepatocytes will be essential to achieve the goal of HBV cure [57].

HDV is a defective hepatotropic virus which depends on HBV surface proteins for assembly of infectious virions and viral entry [58]. The HDV genome is a negative single-stranded circular RNA of nearly 1700 nucleotides containing one functional open reading frame, which encodes the hepatitis delta protein (HDAg) expressed in small and large form. Replication of HDV RNA and transcription of HDAg mRNA in the nucleus depends on host cell polymerases, including DNA-dependent RNA polymerase II. Both forms of the delta protein are then produced and reimported in the nucleus where they bind to genomic RNA to form the ribonucleoprotein (RNP), which is then exported into the cytoplasm and is associated with HBV envelope proteins to form a mature HDV virion [59]. Thus, HDV enters hepatocytes using the same pathways as HBV, and depends on the same host factors for host cell binding and entry. HDV is therefore a useful surrogate model for HBV entry.

The first step of viral infection is virion binding to attachment factors and receptors at the host cell surface. This specific interaction between viral surface proteins and host entry receptors often determines the tissue tropism and host range of the virus [60]. HBV and its satellite virus HDV share HBV envelope proteins and are known to exclusively infect human, chimpanzee and tree shrew (*Tupaia belangerii*) hepatocytes, suggesting the involvement of species- and liver-specific cell surface factors in the common entry process of these viruses [20]. Two elements of the HBV envelope proteins are necessary for interaction with these factors. One determinant of infectivity resides in the surface-exposed cysteine-rich antigenic loop (AGL), a polypeptide located in the S domain of all three envelope proteins [61, 62]. The second known infectivity determinant is a receptor binding site in the N-terminal pre-S1 domain of the L-HBsAg [55]. This domain is post-translationally modified by addition of myristic

144   acid [63], and this myristoylation is essential for virion infectivity [64, 65]. A synthetic myristoylated peptide

145   comprising the N-terminal amino acids 2 to 78 of the pre-S1 domain prevents HBV infection [66].

146   As for many viruses [67, 68], HBV/HDV infection requires the initial attachment to the

147   glycosaminoglycan (GAG) side chains of heparan sulfate proteoglycans (HSPGs) [69]. Both the antigenic loop of

148   all HBV envelope proteins and the preS1-region of HBsAg-L are involved in this interaction [69, 70]. Indeed,

149   glypican-5 (GPC5), a member of the glypican family of HSPGs, acts as an entry factor for HBV and HDV (see

150   Figure 2) [71]. After this initial step of HBV/HDV attachment to HSPGs, the virions bind to a high-affinity receptor

151   via the preS1-domain [72], allowing uptake into hepatocytes. Despite the discovery of several preS1-interacting

152   proteins that did not affect HBV infectivity [73–78], the identity of the HBV/HDV entry receptor remained unclear

153   until 2012, when Yan *et al.* identified NTCP as a functional receptor for HBV and HDV infection. Using a labeled

154   preS1 peptide as a bait in *Tupaia* hepatocytes, a mass spectrometry purification of preS1-bound proteins, and

155   validation in human hepatocytes, it was shown that NTCP specifically interacts with the HBV receptor-binding

156   domain preS1, allowing viral entry [12]. Zhong *et al.* showed that *Tupaia* NTCP mediates entry of woolly monkey

157   HBV, indicating that NTCP orthologs act as a common cellular receptor for known primate hepadnaviruses [79].

158   Differential gene expression patterns between non-susceptible undifferentiated and susceptible differentiated

159   HepaRG cells validated the role of NTCP as a specific receptor for HBV and HDV [13]. Moreover, silencing of

160   NTCP in primary *Tupaia* hepatocytes (PTH) or differentiated HepaRG cells inhibits HBV and HDV infection [12,

161   13]. Exogenous expression of NTCP directly renders non-susceptible hepatoma cell lines susceptible to HBV and

162   HDV infection, while entry inhibitors derived from the preS1 peptide efficiently inhibit this infection [12]. In

163   addition, the S267F mutant of NTCP, conferring a loss of bile acid uptake function, is significantly associated with

164   resistance to chronic hepatitis B and decreased risk of cirrhosis and liver cancer development, supporting the role

165   of NTCP as cellular receptor for HBV in human infection [80–82]. However, S267F homozygote patients can still

166   be infected by HBV, suggesting the existence of alternative receptors allowing viral entry in the absence of

167   functional NTCP [83].

168   Interestingly, expression of human (but not mouse) NTCP in non-susceptible hepatocarcinoma cells

169   confers limited susceptibility to infection. For robust infection, addition of dimethyl sulfoxide (DMSO) to culture

170   medium is essential [13]. The fact that human hepatoma cell lines HepG2 and Huh7 are not susceptible to HBV

171   and HDV infection without exogenous expression of NTCP is consistent with reports that NTCP expression is

172   reduced in human hepatocellular carcinoma cells [48, 84]. NTCP expression rapidly decreases over time following

173   isolation of cultured PTHs, which supports observations that primary human hepatocytes (PHH) remain

174 susceptible to HBV infection *in vitro* only for a few days after isolation [12, 85]. Considering the predominant

175 expression of NTCP in the liver, this receptor is likely to contribute to the hepatotropism of both viruses [12]. In

176 addition, NTCP protein sequences vary among mammalian species, which might contribute to the narrow species

177 tropism of HBV and HDV infection. For example, monkey NTCP does not support HBV and HDV infection

178 despite a high protein sequence homology to human NTCP. Replacing amino acids 157–165 of nonfunctional

179 monkey NTCP with the human counterpart conferred susceptibility to both HDV and HBV infection [12]. The

180 fact that hepatocytes from cynomolgus and rhesus macaques and pigs become fully susceptible to HBV upon

181 hNTCP expression indicates that NTCP is the key host factor limiting HBV infection in these species [86].

182   As a key host factor enabling HBV and HDV infection *in vitro*, the discovery of NTCP has been crucial

183 for the development of novel animal models supporting virus infection. Indeed, only chimpanzees and *Tupaia* can

184 experimentally support HBV and HDV infections [87]. The state-of-the-art mouse model for the study of

185 HBV/HDV consists of liver-engrafted humanized chimeric uPa/SCID or FRG mice, which support virus entry and

186 replication, but lack an efficient immune system, limiting the study of virus-host interactions [87]. The recent

187 development of human NTCP-expressing transgenic mice opened perspectives for the development of novel

188 immune-competent animal models for the investigation of HDV infection and HDV-induced pathogenesis *in vivo*

189 [88]. As HBV infection is limited in mouse cells expressing hNTCP, probably due to the lack of a key host factor

190 [89], it should be noted that hNTCP-transgenic mice are not susceptible to HBV infection. Recently, an elegant

191 study demonstrated that vector-mediated expression of hNTCP in the hepatocytes of rhesus macaques conferred

192 susceptibility to HBV infection, providing a robust and relevant model for the study of HBV infection, including

193 its interaction with adaptive immunity and the understanding of viral clearance [90].

194   Overall, NTCP was identified as the long-sought preS1-specific HBV receptor contributing to HBV liver

195 tropism and species specificity [13]. Targeting the interactions between the HBV preS1-domain and its receptor

196 NTCP required for HBV/HDV entry is a promising strategy to block viral entry for both viruses.

197

198 **NTCP as a therapeutic target for HBV/HDV infection**

199 Even before the identification of NTCP as HBV/HDV receptor, entry inhibitors derived from the HBV preS1 were

200 shown to efficiently inhibit HBV infection *in vitro* and *in vivo* [91, 92]. One of these compounds, the myristoylated

201 preS1-derived peptide (also called Myrcludex B or MyrB), efficiently prevents HBV dissemination *in vivo* and

202 hinders amplification of the cccDNA pool in infected human hepatocytes [14]. MyrB is the first HBV/HDV entry

203 inhibitor targeting NTCP to reach clinical trials [93], where it was shown to have a good safety profile with a mild

204 and reversible elevation of serum bile acid salts [93, 94]. Phase IIa clinical studies revealed a marked antiviral

205 effect of MyrB, as measured by HDV RNA, HBV DNA and improvement of biochemical disease activity (ALT),

206 when used in combination with IFN therapy, although there was no significant decrease in HBsAg levels. In

207 monotherapy, however, MyrB did not show significant antiviral activity [94]. Further studies are necessary to

208 confirm these results obtained in small patient cohorts [95].

209 Importantly, the identification of NTCP as the first HBV/HDV entry receptor has accelerated the

210 discovery and development of several new potential entry inhibitors. Binding of myristoylated preS1-derived

211 peptide to NTCP was shown to interfere with the physiological bile acid transport function of NTCP, indicating

212 that NTCP-inhibiting drugs might be able to block HBV infection [96]. In a study evaluating FDA approved

213 therapeutics with documented inhibitory effect on NTCP cellular function against HDV entry, three of these

214 molecules (irbesartan, ezetimibe, and ritonavir) inhibited HDV infection *in vitro* [97]. The inhibitory effect of

215 ezetimibe on HBV infection had already been described previously without understanding its interactions with

216 NTCP [98]. In 2014, Watashi *et al.* evaluated the effect of compounds on the early phase of the HBV life cycle to

217 identify cyclosporine A as an HBV entry inhibitor targeting NTCP [15]. In the same year, Nkongolo *et al.*

218 characterized the effect of cyclosporine A, a cholestasis-inducing drug inhibiting NTCP bile acid transport [32, 97,

219 98], against HBV/HDV infection and found that inhibition of entry resulted from interference with the NTCP

220 receptor [16]. The screening of FDA/EMA-approved drugs or small molecules for interaction with NTCP allowed

221 the identification of several additional potential HBV/HDV entry inhibitors targeting NTCP [18, 19]. All of these

222 NTCP-targeting HBV/HDV entry inhibitors concomitantly inhibit the transporter function of NTCP and impair

223 bile acid uptake into hepatocytes, increasing the risk of adverse effects. NTCP-deficient mice and a patient with

224 NTCP deficiency were shown to exhibit an elevated level of serum bile acids and to develop related pathologies

225 including growth retardation and hypercholanemia [101, 102].

226 Two different strategies to selectively inhibit HBV entry without impairing bile acid uptake have been

227 suggested recently. Shimura *et al.* showed that cyclosporine A derivatives SCY450 and SCY995 inhibit

228 HBV/HDV entry without interfering with the NTCP transporter activity (see Figure 2) [17]. Tsukuda *et al.*

229 identified an oligomeric flavonoid, proanthocyanidin (PAC) and its analogs, as a new class of entry inhibitors,

230 which directly target the preS1-domain of the HBV large envelope protein and thereby prevent its attachment to

231 NTCP. By directly targeting HBV particles, PAC impairs HBV infectivity without affecting the NTCP-mediated

232 bile acid transport activity [103]. Further studies are required to determine if these novel inhibitory strategies will

233 show efficacy *in vivo* and in clinical studies in co-treatment with NUC therapy.

234

NTCP is a host factor for HCV infection

Hepatitis C virus is an enveloped single-stranded positive-sense RNA virus in the *Flaviviridae* family. The host-cell derived lipid envelope contains the two viral envelope glycoproteins, E1 and E2 [104]. Within the envelope, an icosahedral capsid contains the RNA genome of 9.6 kilobases. Like HBV and HDV, attachment of HCV to hepatocytes is mediated by HPSGs on the host cell surface [105–107]. Following attachment, the envelope glycoprotein E2 mediates interactions with a series of specific cellular entry factors, including CD81 and claudin-1 (see Figure 2) [108–111]. HCV is internalized via endocytosis in a clathrin- and dynamin-dependent process [112]. Following fusion with early endosomal membranes, the HCV genome is released into the cytosol, where it is translated into a polyprotein cleaved by viral and host proteases. The HCV genome is replicated directly into RNA without passing through a DNA intermediate [113]. Therefore, HCV entry and replication steps are very distinct from those described for HBV/HDV. Nonetheless, the mutual hepatotropism of these three viruses mediated by tissue specific factors suggests a possible overlap in usage of common hepatocyte specific host factors like NTCP.

Following establishment of the pivotal role of NTCP for HBV and HDV entry into hepatocytes, a recent study identified a role for NTCP in HCV infection (see Figure 2). Exogenous overexpression or silencing of NTCP increased or decreased HCV infection *in vitro*, respectively [114]. Unlike HBV, however, no direct interaction between HCV envelope proteins and NTCP was identified. Instead, the bile acid transporter function of NTCP was found to be important for HCV entry [114]. Bile acids are known to modulate cellular antiviral responses by inhibiting interferon (IFN) type I signaling and thereby decreasing the expression of IFN-stimulated genes (ISGs) [23, 24]. NTCP was shown to regulate HCV infection by inducing the bile acid-mediated repression of ISG expression in hepatocytes, including IFITM1, IFITM2 and IFITM3 [114]. These transmembrane proteins are known to restrict the entry of several viruses, including HCV [115]. IFITM1 blocks the interaction between HCV and its receptors [116], whereas IFITM2 and IFITM3 inhibit entry at a post-endocytosis step by blocking the release of virions into the cytoplasm [117]. NTCP facilitates HCV infection by modulating innate antiviral responses via its bile acid transport function. As bile acids have been shown to enhance HCV replication [118], it is likely that NTCP expression and activity modulates HCV infection through a multimodal mechanism of action. Interestingly, MyrB-mediated inhibition of NTCP blocks the import of bile acids, which in turn stimulates the expression of ISGs, inhibiting HCV entry and infection [114]. However, it still needs to be determined whether the inhibition of NTCP-mediated bile acid entry affects the HBV life cycle through similar mechanisms as

264 described for HCV. The potential of NTCP-targeting antivirals to enhance innate antiviral responses and to engage

265 the host immune system to clear infection may be a useful property for the treatment of all hepatotropic viruses,

266 including HBV, HCV and HDV.

267

Conclusions

269 The discovery of NTCP as the first HBV/HDV receptor was a milestone in the study of the life cycle of these

270 viruses. This landmark discovery enabled significant progress in understanding HBV/HDV entry and virus-host

271 interactions. Moreover, based on this discovery, novel infectious model systems based on transduced cell lines

272 stably expressing NTCP have been developed which allow detailed study of the early steps of the viral life cycle.

273 By allowing the study of authentic infection in cell lines, these model systems will help to understand the formation

274 and degradation of HBV cccDNA, which is a key target to achieve the ultimate goal of HBV cure. Robust human

275 NTCP-expressing animal model systems will enable the *in vivo* validation of virus-host interactions and antiviral

276 therapies. Moreover, NTCP has been established as an antiviral target, and several molecules targeting NTCP are

277 in clinical development with the goal to improve current therapies in the future. The recent discovery of NTCP as

278 a host-dependency factor in HCV infection underscores its essential role in virus-hepatocyte interactions.

279

Author contributions

281 CFE, LH, CCC, ERV, CS, TFB wrote the manuscript.

282

Conflicts of interest

284 The authors have no conflicting interests to disclose.

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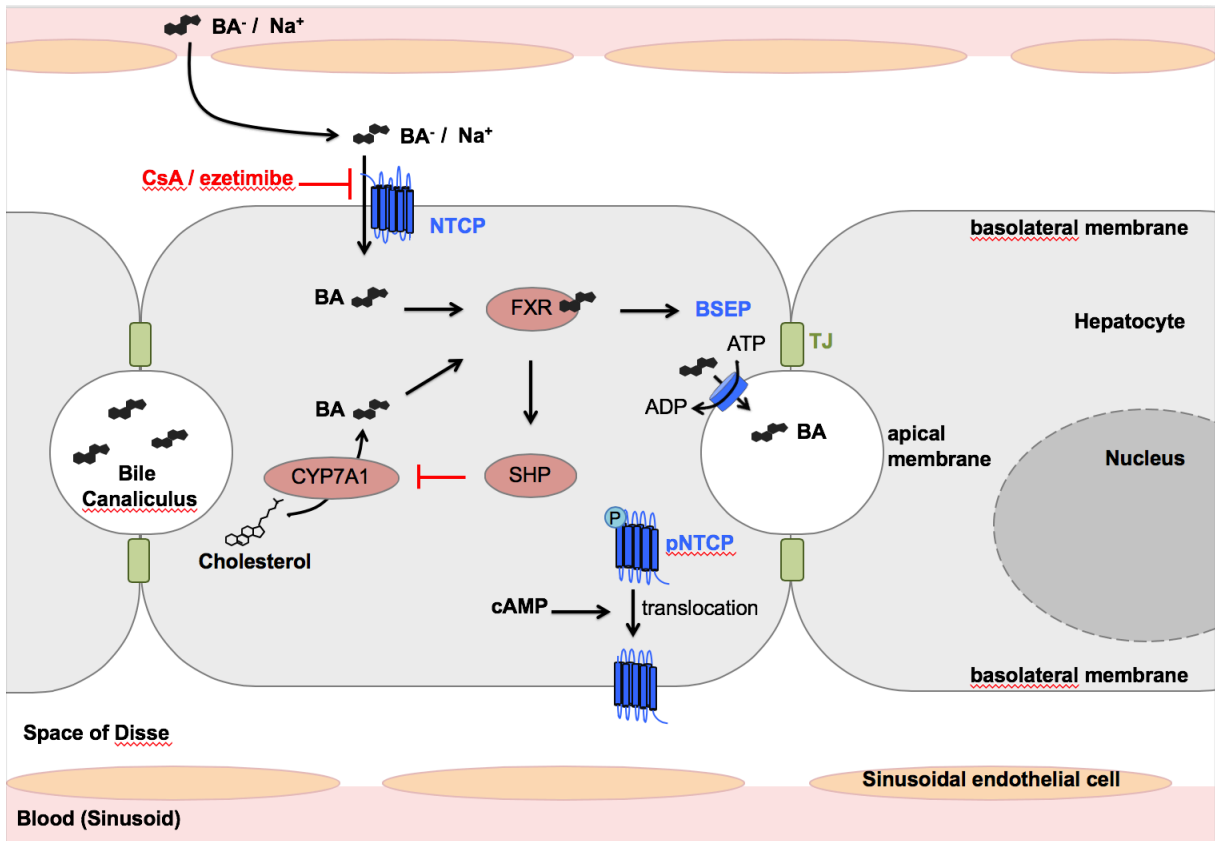**Figure legends**

Fig. 1 Model of the functional role of NTCP in hepatic bile acid transport and metabolism. Transport of bile

acids from portal blood into hepatocytes *via* NTCP depends on a sodium gradient and is inhibited by CsA or

ezetimibe. Secretion into the bile canaliculus *via* bile salt export pump (BSEP) in an ATP-dependent manner and

synthesis from cholesterol are regulated by bile acid-mediated activation of FXR. cAMP mediates

dephosphorylation and membrane translocation of NTCP. NTCP: Sodium taurocholate co-transporting

polypeptide; BSEP: bile salt export pump; FXR: Farnesoid X Receptor; SHP: small heterodimer partner; CYP7A1:

cholesterol 7α-hydroxylase; BA: bile acid; TJ: tight junction; CsA: cyclosporin A; cAMP: cyclic adenosine

monophosphate

Fig. 2 Model of interactions between NTCP and the entry of HBV, HDV, and HCV in hepatocytes. After

initial attachment to HSPG including GPC5, HBV and HDV virions bind to the receptor NTCP through the preS1-

domain of the large envelope protein. NTCP inhibitors CsA and ezetimibe block viral entry like preS1-derived

MyrB and CsA-derived SCY995. NTCP modulates HCV infection by interfering with innate immune responses.

Bile acids interfere with the IFN signaling pathway and thereby favor HCV entry. Inhibition of NTCP-mediated

bile acid import into hepatocytes promotes inhibition of HCV entry through the upregulation of ISGs including

*IFITM*s. HBV: hepatitis B virus; HCV: hepatitis C virus; HDV: hepatitis D virus; HSPG: heparan sulfate

proteoglycan; GPC5: glypican-5; NTCP: Sodium taurocholate co-transporting polypeptide; MyrB: myrcludex B;

CsA: cyclosporin A; SCY995: synthesized CsA derivative 995; IFN: interferon; IFNAR: IFN-α/β receptor; JAK:

Janus kinase; STAT: signal transducer and activator of transcription; IRF9: Interferon regulatory factor 9; ISRE:

IFN-sensitive response element; ISG: IFN-stimulated gene; IFITM: IFN-induced transmembrane protein; CLDN1:

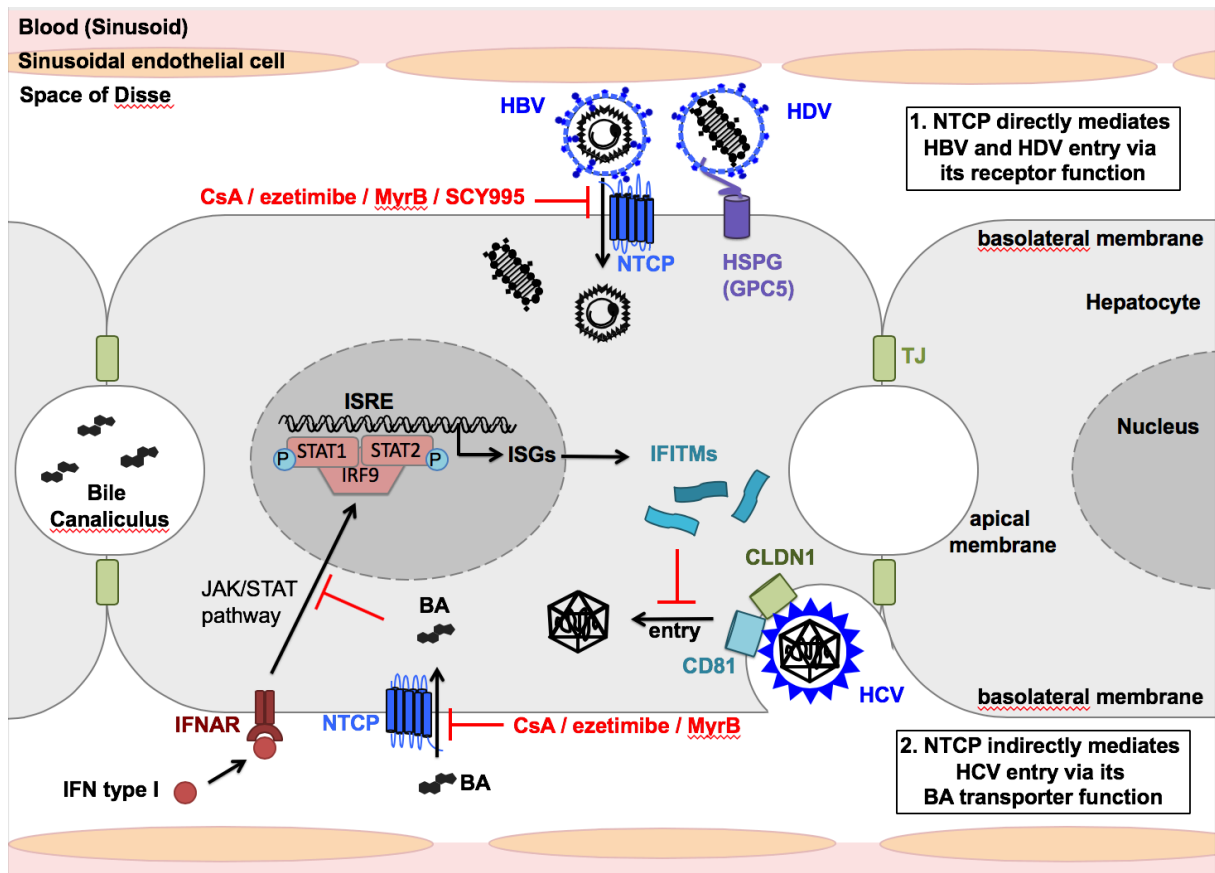Claudin 1; CD81: cluster of differentiation 81; BA: bile acid; TJ: tight junction

652   **Figure 1**

653

654

Figure 2

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