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Genetic and pharmacological perturbation of hepatitis-C virus entry

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Hepatitis-C virus (HCV) chronically infects 58 million individuals worldwide with variable disease outcome. While a subfraction of individuals exposed to the virus clear the infection, the majority develop chronic infection if untreated. Another subfraction of chronically ill proceeds to severe liver disease. The underlying causes of this interindividual variability include genetic polymorphisms in interferon genes. Here, we review available data on the influence of genetic or pharmacological perturbation of HCV host dependency factors on the clinically observed interindividual differences in disease outcome. We focus on host factors mediating virus entry into human liver cells. We assess available data on genetic variants of the major entry factors scavenger receptor class-B type I, CD81, claudin-1, and occludin as well as pharmacological perturbation of these entry factors. We review cell culture experimental and clinical cohort study data and conclude that entry factor perturbation may contribute to disease outcome of hepatitis C.

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Introduction

Viruses have evolved to exploit cell surface proteins as attachment and entry factors. Consequently, genetic or pharmacological perturbation of host factors may modulate virus uptake. One example is the deletion in the *chemokine receptor-5* (*CCR5*) gene protecting a small group of individuals from human immunodeficiency virus (HIV-1) infection. The *CCR5* gene encodes the co-receptor required for cell entry of macrophage tropic HIV-1 [1–3]. In the case of hepatitis-C virus (HCV), polymorphisms in the IL28B gene result in enhanced spontaneous clearance of the virus and in an increase in sustained virological response to treatment [4–6]. However, the impact of HCV entry factor variants or pharmacological perturbation of entry factors on susceptibility to HCV or treatment response remained elusive. In this review, we summarize available data on the impact of genetic and pharmacological perturbation of HCV entry and how this knowledge helps understand interindividual variability in hepatitis-C disease progression and treatment response. In fact, interindividual variance in hepatitis C occurs at multiple levels. An estimated 30% of acute infections is cleared, and of those patients developing chronic infection, yet only a subfraction of approximately 20% develop severe liver disease 15–25 years post contraction of the virus [7].

HCV entry is a multistep process requiring at least the four entry factors: scavenger receptor class-B type I (SR-BI) [8], CD81 [9], claudin-1 (CLDN1) [10], and occludin (OCLN) [11]. Instead of SR-BI, HCV can attach to low-density lipoprotein receptor (LDLR) [12–19]. The interaction of HCV with lipoprotein receptors is on the one hand mediated by the lipoprotein parts of the HCV lipoviro particles (LVPs), which are assemblies of virions, lipids, and apolipoproteins (Apo) [20,21]. On the other hand, SR-BI binds the N-terminal hypervariable region 1 of the viral glycoprotein E2 triggering a conformational change and exposing the binding domain to the receptor CD81 [22]. Binding of E2 to CD81 activates the epidermal growth factor receptor (EGFR), which is part of the CD81 protein interaction network on human hepatocytes [23,24]. EGFR signaling in turn leads to actin rearrangements and lateral translocation of the HCV–SR-BI–CD81 complex toward tight junctions [25–28]. This translocation promotes the interaction of the HCV–CD81 complex with the tight junction protein CLDN1 [10,24,29,30]. Instead of EGFR, the receptor tyrosine

kinase ephrin A2 can promote the interaction of CD81 with CLDN1 [24]. Transferrin receptor 1 (TfR1) may additionally enhance HCV entry [31]. At the tight junctions, OCLN is critically required for productive HCV entry [11,32–34]. HCV engages these entry factors and cofactors sequentially to then enter cells by clathrin-mediated endocytosis [26,35–39]. Two entry cofactors calpain-5 (CAPN5) and casitas B-lineage lymphoma b (CBLB) facilitate this endocytosis step. Both proteins form a complex with CD81, which is in line with co-endocytosis of HCV particles and CD81 [23]. During HCV–CD81 interaction, the protein network of CD81 changes and the entry cofactor serum response factor-binding protein 1 (SRFBP1) forms a complex with CD81. SRFBP1 promotes a post-binding step during HCV entry [40]. Similarly, the cholesterol-uptake receptor Niemann–Pick C1-like 1 (NPC1L1) enhances HCV entry at a post-binding step [41]. After V-type ATPase-dependent pH drop in the endosome, the viral envelope undergoes conformational changes triggering fusion with the limiting endosomal membrane [42–44]. Finally, the viral capsid disassembles, releasing the viral genome into the cytoplasm and concluding the HCV entry process.

With four critical entry factors and a multitude of cofactors, HCV cell entry is one of the best-studied virus uptake processes, and the complexity of the entry process likely reflects the strong adaptation of the virus to the human liver. Although the human liver consists of several cell types such as hepatocytes, Kupffer cells, stellate cells, cholangiocytes, liver sinusoidal endothelial cells, macrovascular endothelial cells, and immune cells, HCV targets mainly hepatocytes. Recent studies using single-cell RNAseq from nine independent donors show that the expression of some entry factors indeed differs between liver cell types. For instance, SR-BI is expressed mostly in hepatocytes and to a lower extent in Kupffer cells, endothelial cells, and cholangiocytes [45]. According to the human liver cell atlas, CLDN1 and OCLN similarly to SR-BI are more specifically expressed in albumin-positive hepatocytes, while CD81 is expressed in all liver-resident cells (Figure 1) [45].

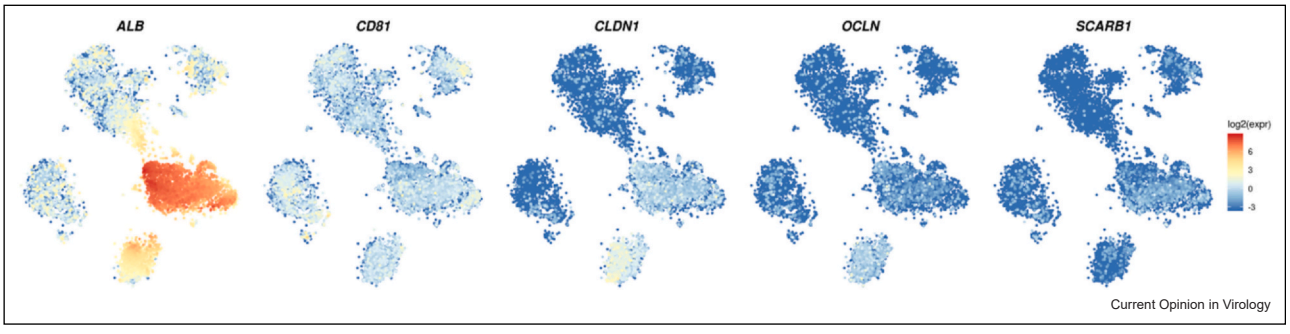
In the liver, hepatocytes are grouped into different zones depending on proximity to the portal vein. These zones differ in metabolic activity and oxygen availability [46]. With regard to entry factor expression, CLDN1 localizes to apical–canalicular tight junction regions and at basolateral–sinusoidal regions of liver lobes. At both sites, CLDN1 colocalizes with CD81, but only at the basolateral–sinusoidal side with SR-BI [47]. Despite this heterogeneity, only limited intrahepatic compartmentalization occurs in HCV infection and 1–50% of all hepatocytes become infected with a large interindividual variability [48].

Genetic variants for several entry factors occur in the human population and several clinically used drugs interfere with entry factors. Thus, it is conceivable that genetic and pharmacological perturbation of entry factors contributes to the interindividual variability in hepatitis-C progression and antiviral drug response.

Genetic polymorphisms and genetic variants of hepatitis-C virus entry factors

Genetic polymorphisms in virus entry factors can render individuals resistant or differentially susceptible to infection. The best-studied example is the gene encoding for the HIV co-receptor CCR5 [1–3]. A defective *CCR5* allele harboring a 32-base-pair deletion results in a truncated protein, which protects homozygous individuals from C5-tropic HIV infection [49]. For HCV, genome-wide association studies (GWAS) identified three single-nucleotide polymorphisms (SNPs) (rs12979860, rs368234815, and rs8099917), which are in strong linkage disequilibrium and associated with HCV clearance and sustained virological response to PEG–IFN-alpha/ribavirin treatment [4–6]. These SNPs are located in the same region as the *interferon-λ 3* and *4* (*IFNL3* and *IFNL4*) genes. The variant rs368234815 (TT/ΔG) causes a frame shift upstream of the *IFNL3* gene, leading to generation of a new IFNL4 protein. Clinically, the described SNPs are more commonly found in individuals of African descent and thus the GWAS results contributed to the understanding of

Figure 1



Expression level of *SCARB1, CD81, CLDN1,* and *OCLN* transcripts in human liver according to the human liver cell atlas.

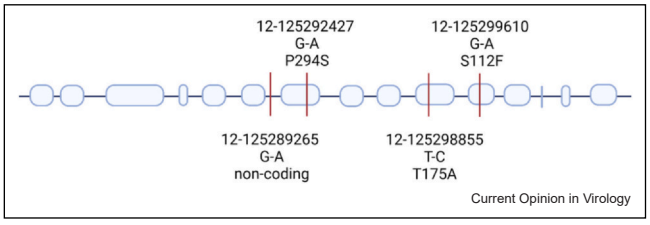
differential single-nucleotide variants (SNVs) in different patient groups in the era preceding direct-acting antivirals (DAAs).

With regard to HCV entry factors, SNPs or SNVs could modulate susceptibility by at least four different mechanisms. First, genetic variants could modulate virus–host interaction, for example, CD81–E2 affinity. Second, variants could affect host protein–protein interactions during HCV entry, for example, CD81–SR-BI binding. Both of these mechanisms would be caused by nonsynonymous coding variants that alter protein function and affinities. A third option is variants outside the ORFs, for example, in regulatory or promoter regions, which would alter expression levels of HCV entry factors and thereby modulate entry efficiency. Finally, and in analogy to the Δ32 CCR5 polymorphisms in HIV, indel variants could cause premature termination of translation and thus expression of truncated entry factors, which would render individuals refractory or less susceptible to HCV infection.

For the four major HCV entry factors (SR-BI, CD81, CLDN1, and OCLN), rare coding and noncoding variants occur in the human population and were analyzed in the context of *in vitro* HCV infection assays or retrospective patient cohort studies (Table 1).

The *SCARB1* gene encodes the SR-BI receptor and contains several coding and noncoding variants. Two coding mutations are associated with increased levels of high-density lipoprotein (HDL) in patients [58]. These mutations result in amino acid substitutions S112F and T175A (highly conserved residues among species) causing deficient HDL binding [59]. Both variants show reduced affinity to HCV E2 glycoprotein and cells carrying these SR-BI variants are poorly susceptible to HCV [53]. As both variants are rare in the human population, no cohort data on their role in clinical course of disease are available. In contrast, the noncoding SNP rs3782287 (MAF = 0.34) in intron 7 of the *SCARB1* gene causes reduced SR-BI expression levels in the liver and a concomitant slight, but observable decrease in viral load in chronic hepatitis-C patients [53] (Figure 2).

Figure 2



Overview of the *SCARB1* gene. Exons are shown as blue boxes and position of SNVs reported to affect HCV susceptibility *in vitro* or *in vivo* is marked with a red line. Data were obtained from the gnomAD database.

CD81 is a tetraspanin present on the cell surface in tetraspanin-enriched microdomains and consisting of four transmembrane domains, two extracellular loops (large and small), one intracellular loop, and two intracellular tails (N-terminal and C-terminal). The protein coordinates a cholesterol between the transmembrane helices. The CD81 large extracellular loop (LEL) harbors the residues responsible for E2 binding [60], including amino acids F186 [61], I182, and N184 [62]. In E2, the motif

Table 1

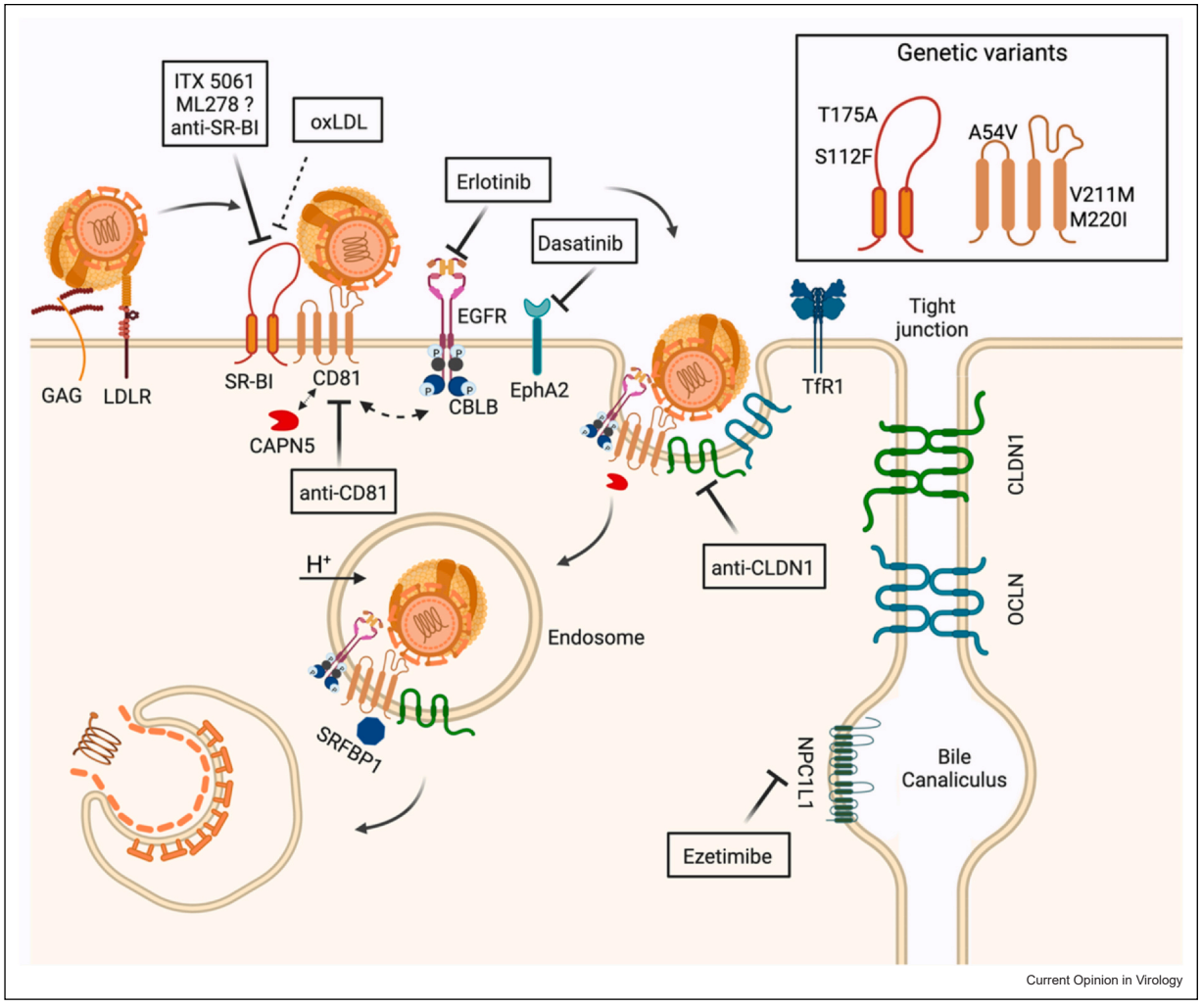
Overview of selected HCV entry factor SNVs and their impact on HCV infection.

Gene	GnomAD ID/rsID	Amino acid substitution	Impact on HCV infection	Reference	Protein domain
CD81	11-2395464-G-A	V135M	-	[50]	LEL
	11-2395927-A-G	N173S	-	[50]	LEL
	11-2395926-A-G	N173D	-	[50]	LEL
	11-2396650-A-G	D195G	-	[50]	LEL
	11-2396649-G-A	D195N	-	[50]	LEL
	11-2396697-G-A	V211M	Decreased susceptibility	[51]	TM4
	11-2396703-G-A	A213T	-	[51]	TM4
	11-2396815-G-A	M220I	Decreased susceptibility	[51]	TM4
	11-2390506-C-T	A54V	Decreased susceptibility	[51]	TM4
	11-2390452-G-A	R36L	-	[51]	SEL
SCARB1	rs397514572	S112F	Reduced receptor function	[52]	LEL
	12-125298855-T-C	T175A	Reduced receptor function	[53]	LEL
	12-125289265-G-A	Noncoding variant	Decreased viral load	[53]	Intron
	12-125292427-G-A	P297S	-	[54]	LEL
OCLN	rs28562785	A151V	-	[55]	TM2
	rs17852716	L233S	-	[55]	EL2
	rs28418826	V255E	-	[55]	TM4
CLDN1	rs9880018	Noncoding variant	Reduced susceptibility to infection	[56]	Promoter region
	rs9865082	Noncoding variant	Reduced susceptibility to infection	[56]	Promoter region
	rs10212165	Noncoding variant	Reduced susceptibility to infection	[56]	Promoter region
EGFR	rs11506105	Noncoding variant	HCV clearance	[57]	Intron

G^{436}WLAGLFY and the conserved residues W420, Y527, W529, G530, and D535 are critical for binding to CD81 [63,64]. Additionally, four E2 amino acid residues (A524, P525, Y527, and W529) determine binding of E2 to the monoclonal antibody (1H8) capable of neutralizing HCV *in vitro* [65]. A recent study on the protein crystallographic model of E2 in complex with the LEL of CD81 highlights the relevance of these E2 residues [44]. Genetic variants in the CD81 LEL are rare (MAF < 0.001) and do not impair the HCV entry factor function [50] to our current knowledge.

Apart from the E2-binding domains of CD81, other domains are critical for HCV entry. This was demonstrated by testing CD81 chimeras [66] with a LEL of human CD81 and the remaining domains of CD81 orthologs from other species. Chimeras containing non-LEL domains from distantly related species lose entry factor function. In particular, loss of the amino acid E219 in the fourth transmembrane domain of CD81 reduces HCV susceptibility of cells. Glutamate-219 is critical for cholesterol binding [66], which is in line with cholesterol dependency of HCV entry [67]. *CD81* non-synonymous genetic variants resulting in amino acid changes in proximity to the CD81 cholesterol-binding pocket reduce cell susceptibility to HCV in cell culture, highlighting the relevance of CD81 domains outside the LEL 51 (Figure 3).

Figure 3



Schematic representation of the HCV entry pathway including entry inhibitors. Entry of HCV into hepatocytes is a multistep process involving several host factors. Initial attachment is of low specificity and mediated by glycosaminoglycans (GAG) and LDLR. Afterward, HCV binds to SR-BI and subsequently to CD81. This activates the EGFR and the virus-receptor complex translocates to the tight junctions. Here, HCV interacts with CLDN1 and OCLN following virus internalization by clathrin-mediated endocytosis. Acidification of endosomes initiates fusion of the viral envelope with the endosomal membrane and delivery of the viral genome into the cytoplasm. In addition, other host factors facilitate HCV entry such as CAPN5, CBLB, ephrin receptor A2 (EphA2), TfR1, NPC1L1, and SRFBP1. Reported HCV entry inhibitors are highlighted as well as blocking antibodies against entry factors. In addition, genetic variants of SR-BI and CD81, which reduce HCV susceptibility, are shown in the upper-right box. Illustration created with BioRender.com.

A follow-up study confirmed that amino acid changes in the cholesterol-binding pocket of CD81 alter HCV receptor function. This is likely due to an allosteric mechanism in which cholesterol binding to transmembrane domains induces a closed conformation of the CD81 ectodomain, which allows E2 binding. In contrast, in the absence of cholesterol, an open ectodomain conformation may decrease HCV receptor activity [68]. Hence, for both early HCV host factors, namely SR-BI and CD81, variants, which reduce lipid binding, seem to decrease HCV E2 binding as well.

For *CLDN1*, four genetic variants in promoter or intron regions of the gene are linked to a reduced HCV infection risk as assessed in injection drug user patient cohorts [56].

Together with CD81, OCLN is one of the entry factors mediating HCV species specificity. Murine orthologs of CD81 and OCLN fail to support HCV entry [11,69–73] and this species comparison led to the identification of critical amino acid residues in both proteins. Two alanine residues at positions 223 and 224 in the second extracellular loop of human OCLN mediate OCLN entry factor function [55]. Nonsynonymous SNPs in the *OCLN* ORF coding for amino acids in the transmembrane domains (A151V, V255E) or the second extracellular loop (L233S) did not impair HCV entry factor function *in vitro*.

For the entry cofactor EGFR, the variant rs11506105 has been associated with HCV clearance and PEG–IFN-alpha treatment response, but it remains to be studied whether this polymorphism affects EGFR function directly or the interaction of EGFR with CD81 [57].

Taken together, genetic variants in HCV entry factors occur with very low frequency in the population, and to our current knowledge, in patients, only variants in the noncoding regions of *SCARB1*, *CLDN1*, and *EGFR* cause interindividual differences in disease progression and susceptibility.

Pharmacological perturbation of hepatitis-C virus host factors

Direct antiviral agents (DAAs) against HCV infection are currently available and show > 90% cure rates. In total, seven oral DAAs are approved by the Food and Drug Administration to treat chronic hepatitis caused by HCV infection [74]. These drugs target different stages of the HCV replication cycle and result in rapid decrease in viral load, especially when two or more DAAs are combined. However, some challenges to HCV treatment remain, such as the rise of resistance-associated substitutions [75] and reinfection of cured patients in high-exposure risk groups such as injection drug users [76].

Before the advent of highly efficacious DAAs, host-targeting agents with a high barrier of resistance development were considered as alternative therapeutics [77,78]. Several HCV host factor antagonists can inhibit HCV infection in cell culture or small-animal models. The SR-BI antagonist ITX 5061 was tested up to clinical phase I. ITX 5061 reduces hepatic HDL uptake by inhibition of SR-BI activity [79]. Consequently, treatment with ITX 5061 inhibits HCV infection in primary human hepatocytes and human hepatoma cell lines [80]. The possibility of virus resistance emergence after ITX 5061 treatment has been evaluated by *in vitro* selection. A single amino acid exchange in HCV (N415D) arose and conferred high-level resistance to ITX 5061. On the other hand, no cross-resistance between ITX 5061 and HCV protease inhibitors occurred, which indicates the safety of ITX 5061 when used in combination with other antivirals [81]. Despite these promising aspects, results from a clinical phase-1b trial are discouraging [82] as only in one out of seven subjects, virus titers dropped. Most likely, ITX 5061 monotherapy is insufficient and a combination with other antivirals would improve the outcome. Moreover, in chronic HCV infection, inhibition of only SR-BI might not be a sufficient strategy to combat HCV, as SR-BI-independent cell-to-cell spread may be an important mode of virus maintenance [82].

Alternatively, treatment with another class of SR-BI modulators such as ML278 [83] could block HCV entry. ML278 belongs to a group of indolinyl–thiazole-based inhibitors of SR-BI that show promising characteristics such as reversibility and lack of cytotoxicity [83]. However, the impact of this class of SR-BI inhibitors on HCV entry is currently unknown. Another strategy to block SR-BI is treatment with monoclonal antibodies, which was proven in humanized mouse models of HCV infection [84].

Monoclonal antibodies targeting CD81 are putative therapeutics for hepatitis C both *in vitro* and *in vivo*. Prophylactic treatment using anti-CD81 antibodies protected humanized mice during a subsequent exposure to HCV [85]. *In vitro*, a CD81 antibody not only impaired cell-free viral spread but also direct cell-to-cell viral spread [86].

Another druggable HCV host entry factor is EGFR [87]. EGFR plays a dual role in chronic hepatitis C, first by promoting HCV entry as described above and second by activating the STAT3 and mitogen-activated protein kinase pathways [88]. The latter two pathways are involved in the development of liver fibrosis and hepatocellular carcinoma [88]. Erlotinib is an EGFR tyrosine kinase inhibitor and was tested up to clinical phase 1b in HCV patients infected with genotype 1 [87]. In this study, erlotinib proved to be safe and well-tolerated by the patients at all doses tested. Erlotinib treatment for

Table 2

Host-targeting agents and lipid homeostasis modulators with inhibitory effects on HCV entry.

Class	Host factor	Inhibitor	Reported *in vitro* mutations	Stage	References
Host-targeting agents	SR-BI	ITX 5061	N415D (E2)	Clinical phase I	[79]
		Anti-SR-BI antibody	-	Preclinical phase	[84]
	CD81	Anti-CD81 antibody	-	Preclinical phase	[85]
	OCLN	Anti-OCLN antibody	-	Preclinical phase	[92]
	CLDN	Anti-CLDN1 antibody	-	Preclinical phase	[90]
	TfR1	Anti-TfR1	-	Preclinical phase	[31]
	EGFR	Erlotinib	-	Preclinical phase	[87]
		Anti-EGFR antibody	-	Preclinical phase	[24]
	Ephrin A2	Dasatinib	-	Preclinical phase	[89]
	NPC1L1	Ezetimibe	-	Clinical phase 2	[93]
LLDs	HMG-CoA reductase	Fluvastatin	-	*In vitro* results	[94]
					[95]
		Simvastatin			[96]
		Rosuvastatin			
		Atorvastatin			
	SR-BI	oxLDL	-	*In vitro* results	[97]

14 days did not yield an immediate antiviral effect but reduced viral loads in the follow-up period. Similarly, the ephrin A2 kinase inhibitor dasatinib presents a putative HCV drug and treatment with dasatinib reduced HCV entry *in vitro* [89].

Finally, monoclonal antibodies targeting CLDN1 have been tested and shown to decrease HCV infection with different genotypes and patient isolates (HCV quasispecies) in primary human hepatocytes *ex vivo* [90]. Despite efforts to develop drugs targeting HCV entry factors, this approach has caveats such as difficulties to block HCV cell-to-cell spread. This direct spread not only prevents neutralization by antibodies but also bypasses SR-BI [91]. Today, the advent of highly efficacious combinatorial DAAs with advantageous side effect profiles makes host-targeting agent development superfluous. The above-described small-molecular inhibitors and antibodies targeting HCV entry factor are however essential research tools to probe for the mode of action of host proteins during HCV infection (Table 2).

Lipid-lowering drugs and their effect on hepatitis-C virus entry

A unique characteristic of HCV, compared with other enveloped RNA viruses, is its tight association with host-derived lipoproteins such as Apo B and E. The resulting particles are therefore designated as LVPs that have a low buoyant density [21,98,99]. Lipoprotein receptors such as SR-BI or LDLR promote HCV entry [8,13,18]. Furthermore, replication as well as assembly and release are tightly associated with the host lipid machinery [100,101]. Thus, interference of the host lipid metabolism may influence HCV infection. Lipid-lowering drugs (LLDs) such as statins (HMG-CoA reductase inhibitors) are used to treat patients with hypercholesterolemia in order to decrease blood low-density lipoprotein (LDL) levels [102]. LLDs are among the most frequently prescribed drugs in Europe and North America. Several studies have examined the effect of LLDs on HCV infection. For instance, Wuestenberg et al. showed that statins decrease HCV replication *in vitro* by induction of the heme oxygenase-1 and interferon response [94]. Another LLD, lovastatin, was reported to reduce HCV replication by inhibition of host protein geranylgeranylation [95]. Another statin, pravastatin, has no reported antiviral effect. This indicates that inhibition of the HMG-CoA reductase alone is insufficient to inhibit HCV infection [94–96]. In line with this, we could show that fluvastatin reduces SARS-CoV-2 infection *in vitro* independent of cholesterol synthesis inhibition. Proteomic analysis revealed that fluvastatin in the context of SARS-CoV-2 infection downregulates host proteins involved in viral replication, protein translation, and DNA damage response, thereby indirectly dampening virus replication [103]. Furthermore, Blanchet and colleagues demonstrated a concentration-dependent, dual function of statins on HCV infection. At low concentrations, statins had a proviral effect through upregulation of LDLR, while at higher concentrations, they exerted their antiviral function by downregulation of the HCV entry factor CLDN1 [96]. Finally, in one of our studies, we did not detect a reduction of HCV infection upon statin treatment in hepatoma cells and primary human hepatocytes. However, in cells lacking either SR-BI or LDLR or both lipoprotein receptors simultaneously, simvastatin treatment reduced HCV infection [19]. This suggests that under conditions of low lipoprotein receptor expression, LLDs may reduce HCV infection.

In addition to the described *in vitro* studies, retrospective studies have analyzed the effect of statin treatment in hepatitis-C patient cohorts. One study of chronically infected HCV patients observed no correlation between statin treatment and patient viral loads

Box 1 Definition of terms used in virus entry, host genetics, and antiviral research

- ✓ Receptor: Host factor able to bind to virus particles and necessary for productive entry of the virus into cells.
- ✓ Attachment factor: Host factor that concentrates viral particles at the plasma membrane, thereby facilitating entry.
- ✓ Entry factor: Host factor necessary for virus entry into cells; in the HCV field, a collective term for receptors and entry factors, for which virus particle-binding information is lacking.
- ✓ Entry cofactor: Host factor supporting virus entry, typically forming a complex with entry factors, functioning in post-attachment steps.
- ✓ Genetic variant: Equivalent to mutation; change in DNA sequence that makes up a gene; usually defined by comparison to the reference genome of a species.
- ✓ Single-nucleotide variant (SNV): A single-nucleotide exchange in the genome defined by comparison of the reference genome of a species.
- ✓ Single-nucleotide polymorphism (SNP): Genetic variant present at a single position in the genome, which occurs with a frequency > 1% in a given population.
- ✓ Direct-acting antiviral (DAA): Inhibitors of the HCV protease NS3/4A, NS5A, and viral RNA-dependent RNA polymerase NS5B.
- ✓ Host-targeting agent (HTA): Drugs blocking virus production by inhibiting a critical host factor.

[104]. Similarly, in our HCV patient cohort, statin treatment did not alter HCV RNA levels before and during DAA treatment. However, statin treatment before DAA therapy led to lower levels of liver damage markers (ALT and AST), indicating a beneficial effect of statins on liver integrity [19]. Another study showed that a combination of fluvastatin and interferon treatment decreased the viral relapse rate of chronically infected HCV patients [105]. This is in line with two *in vitro* studies, showing that statins in combination with interferon treatment or HCV DAAs have a strong antiviral activity and may prevent the emergence of resistance mutations [106,107]. However, due to the controversial effect, further clinical data are needed to study the role of statins in HCV infection.

Oxidized low-density lipoprotein (oxLDL), which is a SR-BI ligand, may present an alternative approach to alter the lipid metabolism. Von Hahn and colleagues showed that oxLDL but not LDL effectively reduced cell entry of HCV pseudoparticles and cell culture-derived virus [108]. The observed inhibitory effect was not caused by receptor antagonism as oxLDL did not affect binding of soluble E2 to SR-BI. The authors suggest that a ternary interaction of oxLDL, SR-BI, and HCV is involved in the mechanism. Strikingly, treatment with oxLDL reduced infection with all seven HCV genotypes with only moderate variation between genotypes and with genotype 4 being the most sensitive [97]. In contrast, data from patient cohorts show conflicting results. A study by Westhaus et al. demonstrated no correlation between endogenous oxLDL and viral load in chronic hepatitis-C patients [97], while Nakhjavani and colleagues showed that oxLDL levels were significantly higher in HCV patients compared with healthy individuals [109]. Interestingly, the second cohort included some outliers with very high oxLDL levels and viral loads.

Taken together, HCV is strongly associated with the host lipid metabolism and alteration of the latter by LLD or oxLDL treatment reduces HCV infection *in vitro*. So far, retrospective cohorts demonstrate some conflicting results, thus, further studies are needed to conclude on the role of LLDs and oxLDL in patients.

Perspective

In summary, the development of DAAs has led to high HCV cure rates and in fact makes hepatitis C the first curable chronic virus infection [110,111]. However, there are also interindividual differences in treatment success, which may in part be caused by genetic and pharmacological heterogeneity of patients [75].

In the past, studies have largely relied on available cell culture models of HCV infection. Hence, initially many studies were performed with hepatoma cell clones and genotype-2-derived infectious clones of HCV, both of which do not mimic the clinical situation well. For instance, susceptible hepatoma cells lack the RNA sensor RIG-I and are not polarized [112]. Efforts on using polarized cells, immune-competent primary hepatocytes, stem cell-derived hepatocytes, multilineage liver organoids with vasculature, and humanized mouse models have been made, some of which confirmed the role of the major HCV entry factors [113,114]. Similarly, efforts have been made to generate models for the clinically relevant HCV genotypes. These include intergenotypic chimeras and engineered cell lines for testing of pangenotype patient isolates [115,116]. A limitation of entry studies that has not been completely resolved yet is the limited availability of structural information for the LVP and the E1E2 complex. In fact, the fusion mechanism of HCV is still not discovered. Future entry studies should thus focus on solving the remaining structural riddles and on using now-available elaborate and more authentic infection models.

In these models, the effect of genetic and pharmacological perturbations could be addressed with a presumably higher predictive value. Alternatively, and in the light of recent developments of fast, comparably cheap and deep sequencing techniques, and an increasing availability of genetic patient cohort information, the influence of genetic variants with low mean allele frequency could today be addressed. Existing and

new cohorts could be analyzed with respect to those SNVs, shown to have an influence on HCV susceptibility *in vitro*. Alternatively, given that patient consent exists, completely novel genetic associations could be revealed by whole-genome sequencing of available cohorts (Box 1).

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: Gisa Gerold reports financial support was provided by German Research Foundation.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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