International Journal of Clinical and Translational Medicine
https://www.sciltp.com/journals/ijctm

Review

CRISPR-Cas System: Novel Experimental Therapeutic and Diagnostic Approaches for Chronic Liver Diseases

Teja Naveen Sata and Senthil Kumar Venugopal *

Faculty of Life Sciences and Biotechnology, South Asian University, New Delhi 110068, India
* Correspondence: drsenthil@sau.ac.in; Tel.: +91-1135656620; Fax: +91-1124122511

How To Cite: Sata, T.N.; Venugopal, S.K. CRISPR-Cas System: Novel Experimental Therapeutic and Diagnostic Approaches for Chronic Liver Diseases. *International Journal of Clinical and Translational Medicine* **2025**, *1*(2), 3. https://doi.org/10.53941/ijctm.2025.1000010.

Received: 7 February 2025
Accepted: 26 February 2025
Published: 8 April 2025

Abstract: Chronic liver disease (CLD), a significant ailment, contributes to nearly two million deaths annually. CLD can be caused by alcohol consumption, fat, viral infections, and genetic disorders. Accurate diagnosis and application of therapeutics are crucial strategies for enhancing the management of CLD. The CRISPR-Cas system, originally a prokaryotic innate immunity mechanism, has evolved into a current-generation tool for therapeutic and diagnostic applications. The cis-cleavage feature of the CRISPR-Cas system involves crRNA-guided specific target cleavage. This mechanism is utilized for the development of therapeutics. Few CRISPR-Cas systems possess the additional feature of trans-cleavage, which is non-specific cleavage, also known as collateral cleavage. This unique feature can be exploited to generate diagnostics. In viral hepatitis, CRIPSR-Cas systems have been concurrently applied and reported for viral genome-targeted therapeutics and detection systems. Research on alcoholic and non-alcoholic fatty diseases mainly focuses on CRISPR-Cas therapeutics targeting disease progression factors. Also, CRISPR-Cas-based gene editing can be used to manage genetic disorders. In hepatocellular carcinoma, CRISPR-Cas systems are used for oncogene-targeted therapies and biomarker diagnostics. Various viral and non-viral delivery systems for CRISPR-Cas are been proposed for developing therapeutic applications. Despite limited progress, CRISPR-Cas systems have significant potential for broader application in CLD. This review describes the comprehensive use of the CRISPR-Cas system in experimental therapeutic and diagnostic approaches for CLD.

Keywords: CRISPR; Chronic liver disease; therapeutics; diagnostics; Cas9; Cas12; Cas13

1. Introduction

The liver is a crucial organ and center for numerous physiological processes. It is involved in macronutrient metabolism, lipid and cholesterol homeostasis, endocrine control of growth signaling pathways, blood volume regulation, immune system support, and the breakdown of xenobiotic compounds, including many current drugs [1]. It is affected by multiple maladies due to viral infections, fat, alcohol, and auto-immune disorders, which lead to chronic liver disease (CLD). CLD causes progressive deterioration of liver functions [2]. CLD is a significant health burden worldwide, causing more than 1.32 million deaths. Most of these deaths can be attributed to cirrhosis, i.e., advanced liver scarring, the last stage of chronic liver disease [3]. In the verge of treatment of CLD, therapeutics and diagnostics play a vital role.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a well-known current-generation genome-editing application for therapeutics and diagnostics [4]. It was first reported while exploring the mechanisms of adaptive immunity in bacteria and archaea against their respective viral pathogens [5]. It is defined

as a cluster in the genome containing unique, similar-length spacers regularly separated by short (nearly 20 to 50 bases) palindromic sequences. This cluster has a unique feature: a flanking leader sequence, which is AT-rich [6]. These CRISPR sequences coordinate with CRISPR-associated (Cas) proteins in prokaryotes forming a basis of the CRISPR-Cas adaptive immune system.

2. Mechanism of CRISPR-Cas System

This CRISPR-Cas immune system consists of three main steps: adaptation, expression, and interference [7]. The adaptation step involves spacer acquisition. When a viral pathogen invades a prokaryote possessing CRISPR-Cas system, a part of the viral genome will be acquired by resistant organisms. This genetic material, either DNA or RNA (which will be reverse-transcribed into DNA), will be incorporated as a new spacer along with the short, direct repeats (palindromic sequence) in the CRISPR arrays. The expression step is the biogenesis of CRISPR RNA (crRNA). It involves the transcription of the CRISPR locus into a single transcript, namely pre-crRNA. Respective Cas proteins or RNases will process this pre-crRNA into mature crRNA containing the spacer sequence. In some systems, pre-crRNA pairs with trans-acting small RNA (tracrRNA), which is processed further to mature crRNA [8].

Finally, in the interference step, the effector complex, either a single multidomain Cas protein or a multiprotein Cas complex, binds to crRNA. This bound crRNA guides the effector complex to identify any invading phage containing protospacer (sequence complementarity to the spacer sequence). Upon recognition, the phage will be destroyed through the nuclease activity present in the effector complex [9]. In most systems, the acquirement of the spacer sequences depends on the presence of a 2-5 nucleotide Protospacer Adjacent Motif (PAM) or Protospacer flanking sequence (PFS) found next to the protospacer sequence. This PAM-specific selection yields an advantage of the specificity of foreign DNA cleavage and also avoids self-targeting of PAM-free CRISPR cluster [10].

3. Classification

CRISPR-Cas systems, involved in the adaptive immune steps, are classified into different types and classes. The CRISPR-Cas systems are classified into six types, further grouped into two classes. The six types were classified based on signature genes: Type I, Type II, Type III, Type IV, Type V, and Type VI. The signature proteins of Type I to Type VI are Cas3, Cas9, Cas10, cas7-like gene (csf2), Cas12, and Cas13, respectively. These types are grouped into two classes based on the effector complex in the interference step. Type I, III, and IV are grouped into class 1 due to the presence of a multi-subunit effector complex. Type II, V, and VI are grouped into class 2 due to the presence of a single-subunit effector complex [11]. Most CRISPR-Cas systems in Type I–V are DNA-targeting, and in Type VI, are RNA targeting.

Among these classes, class 2 CRISPR-Cas systems are widely utilized for genome engineering. It is because of their unique feature of the presence of all components of interference effector complex in one single Cas protein. Particularly, combining the crRNA and tracrRNA into a single guide RNA (sgRNA) has allowed researchers to utilize these systems in multiple applications [12]. Among various Cas proteins, Cas9, Cas12, and Cas13 have been widely utilized for genome engineering, majorly in therapeutic and diagnostic applications. Cas9 and Cas12 were RNA-guided DNA nucleases, and Cas13 was RNA-guided RNA nuclease and hence utilized for DNA and RNA targeting, respectively [13].

4. Therapeutic and Diagnostic Approaches Using CRISPR-Cas System

Cas9, Cas12, and Cas13 nuclease generally possess two major domains, namely the recognition (REC) lobe and the nuclease (NUC) lobe. The REC lobe facilitates in the interaction with guide RNA. The NUC lobe is responsible for PAM specificity and target cleavage. The presence of the PAM Interacting domain in the NUC lobe confers this nuclease PAM sequence recognition. In the NUC lobe, RuvC (Crossover junction endodeoxyribonuclease) and HNH (presence of conserved characterized His/Asn/Asp/Glu residues) domains in Cas9, RuvC domains in Cas12 and HEPN (higher eukaryotes and prokaryotes nucleotide-binding) domains in Cas13 are present. These domains participate in nucleic acid cleavage [14–16].

When Cas9 and Cas12 nucleases are employed, they yield double-strand breaks (DSBs). However, Cas9 nuclease cleavage yields blunt ends, and Cas12 nuclease cleavage yields staggered ends [15]. In eukaryotic cells, Non-homologous end joining (NHEJ) and homology-directed repair (HDR) pathways are the two mechanisms involved in the repair of DSBs [17]. Researchers utilized these mechanisms by employing the Cas9 protein to induce DSBs, enabling the development of a tool for introducing targeted insertions and deletions into the genome, either causing knockdown or knock-in of the target gene [14]. When Cas13 nucleases were employed, they create

RNA cleavage, resulting in knockdown of the specific RNA [16]. The nucleic acid cleavage capability of these nucleases was leveraged to develop therapeutics.

Cis-cleavage refers to the cleavage of the bound nucleic acid target under the catalysis of Cas nucleases. However, some types of Cas proteins, along with cis-cleavage, exhibit cleavage of unbound nucleic acid called trans-cleavage. Generally, cis-cleavage is specific depending on the guide RNA spacer sequence, and trans-cleavage is non-specific. Most Cas12 and Cas13 proteins are reported to exhibit this cis-cleavage, and parallel trans-cleavage is termed collateral cleavage (Figure 1) [18]. Interestingly, this collateral cleavage has been detected in prokaryotic hosts and not in eukaryotes [19]. Researchers have taken advantage of this collateral cleavage feature by applying detectable reporters with those Cas proteins to measure collateral cleavage in a diagnostic approach [20]. Moreover, the advent of isothermal amplification techniques has simplified diagnostic approaches, eliminating the need for costly equipment and making it feasible to implement in rural areas [21].

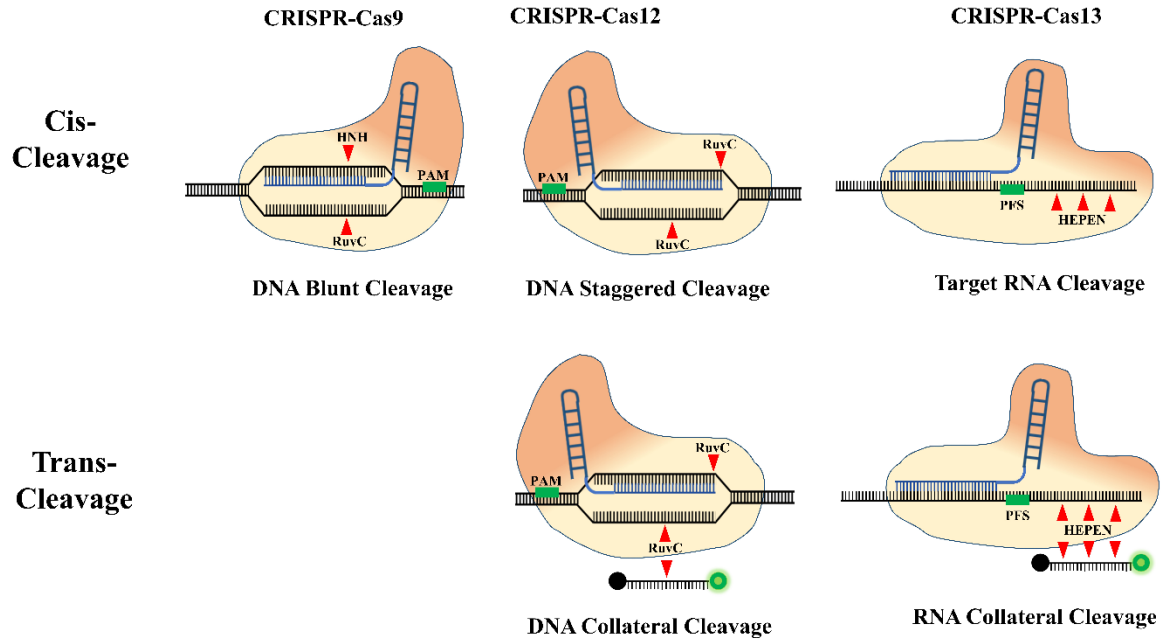

Figure 1. Use of CRISPR-Cas technology as therapeutic and diagnostic modalities. All Cas nucleases perform cis-cleavage, which involves cleaving the bound target nucleic acid, which is the basis for therapeutic approach. Some special Cas nucleases perform trans-cleavage, which involves cleaving the unbound target nucleic acid. This parallel trans-cleavage is non-specifical, hence termed collateral cleavage, which is used for diagnostic approach.

5. Viral Hepatitis

Viral Hepatitis is a virus-induced inflammatory liver disease. It was known to be caused by multiple viruses, namely, hepatitis virus A (HAV), B (HBV), C (HCV), D (HDV), E (HEV), and G (HGV). These viral infections under acute conditions may aggravate fatal acute liver failure. Under chronic conditions, it may lead to cirrhosis and further hepatocellular carcinoma (HCC) [22]. Except for the HBV having DNA, the remaining hepatitis virus has genomic material as RNA. Various CRISPR-based diagnostic platforms have been reported in regular screening of this virus. Furthermore, various CRISPR-based therapeutics are currently under development to tackle these infections [23].

5.1. Hepatitis B Virus

Hepatic B virus (HBV), being a DNA virus, is always the prime target for developing CRISPR-Cas9-based therapeutics. The first study of CRISPR-Cas9 targeting performed against HBPol and HBX region of the HBV genome. It disrupted the HBV-expressing templates in HBV-expression vectors in Huh7 cells and in vivo in a HBV hydrodynamics-mouse model [24]. Later, CRISPR-Cas9 system targeted against ENII/CP (Enhancer II/Core Promoter) region and the PC (Pre-Core) regions on the HBV genome. This lead to specifically target covalently closed DNA (cccDNA), which is derived from HBV relaxed circular DNA (rcDNA) [25]. Further, the combination of CRISPR-Cas9 against HBV with antivirals has been shown to inhibit HBV infection more effectively [26]. Similarly, additional studies enhanced the usage of CRISPR-Cas9 systems by adopting strategies like multiplexed

all-in-one CRISPR-Cas9 vector [27,28], targeting multiple components of the HBV genome with minimal off-target effects [29], and removal of integrated HBV DNA fragments [30], to effectively prevent viral infection [31,32]. Moreover, nickase-Cas9 with a pair of sgRNA, nuclease dead-Cas9 [33], and CRISPR-Cas-mediated "base editors" (BEs) [34] were also employed to suppress HBV replication. In a novel approach, CRISPR-Cas13b was specifically utilized to target the HBV pregenomic RNA and viral mRNAs to reduce HBV replication [35]. Though several in vitro and in vivo studies targeted HBV, no studies have shown to eradicate the virus from hosts. Future studies could be aimed at eradicating the virus from the host cells.

The combination of isothermal amplification techniques with Cas12 and Cas13 has been employed to formulate diagnostic approaches for HBV in clinical samples. Cas13a coupled with polymerase chain reaction (PCR), has been reported to specifically detect HBV DNA and drug resistance mutations [36], and rolling circle amplification (RCA) is also employed to detect cccDNA [37]. Further, an isothermal amplification reaction, Recombinase-aided amplification (RAA) [38], and a one-pot detection platform [39] were employed along with Cas13a to detect HBV.

Likewise, Cas12a integrated with loop-mediated isothermal amplification (LAMP) [40], strand displacement amplification (SDA) [41], and recombinase polymerase amplification (RPA) [42] were designed to detect HBV detection. In addition, PCR-assisted Cas12a detection with DNA cubes is utilized for HBV detection [43]. Further, Recombinase amplified CRISPR enhanced chain reaction (RACECAR) was developed with Cas12a. It employs RPA and a cascade detection reaction, Hybridization chain reaction (HCR). It detects as little as 40 copies of the HBV genome [44]. Also, an amplification-free biosensor was developed using CRISPR-Cas12a associated with Surface-Enhanced Raman Spectroscopy-Active Nanoarray to detect hepatitis B virus (HBV). It has an extremely low detection limit and a broad detection range from 1 aM to 100 pM [45].

Similarly, Cas12b assisted with LAMP utilized to detect HBV in a one-pot reaction [46]. Cas12b, along with multiple cross displacement amplification (MCDA), is also utilized to identify and distinguish HBV genotypes B and C [47]. In addition, the SDA assisted CRISPR-Cas12a method [48] and Mxene-probe DNA-Ag/Pt nanohybrids [49] were employed to detect HBV viral nucleic acid for the colorimetric analysis. These studies have successfully shown that HBV can be detected in patients as low as single copies.

5.2. Hepatitis C Virus

Being an RNA virus, direct targeting of HCV by CRISPR-Cas9 was challenging. Thus, researchers have utilized this machinery to target pro-viral factors that facilitate viral progression. Ribosomal protein eS25 (RPS25) knockdown by CRISPR-Cas9 helped to know that RPS25 performs 40S subunit recruitment to IRES of HCV for translation [50]. Further knockout of claudin-1 (CLDN1) and claudin-6 (CLDN6) helped to study HCV entry [51].

First Cas9 mediated direct target of HCV was performed using CRISPR-*Francisella novicida* Cas9 (FnCas9). It can carry out endonucleolytic cleavage of the endogenous bacterial transcript. Here, the RNA-inactivating effect is mediated by a different guide RNA, which is termed small CRISPR-Cas-associated RNA (scaRNA). scaRNA interacts with the tracrRNA to form the targeting complex with FnCas9. This feature was utilized to reprogram FnCas9 with an RNA-targeting guided RNA (rgRNA) to target the HCV genome [52]. Later, RNA targeting Cas13a was utilized to knock down HCV by targeting highly conserved regions of the HCV internal ribosomal entry site (IRES). This inhibited viral infection and did not affect the host cells [53]. However, few studies have successfully removed HCV from the host cells.

The first detection was reported with CRISPR-Cas12a coupled with reverse transcription-loop-mediated isothermal amplification (RT-LAMP) to detect HCV [54]. Later, a Visual One-Pot RT-RAA-Cas12a was designed for HCV for in-field detection [55]. Further, a highly thermostable Cas12b coupled with RT-LAMP reported the detection of HCV in one pot [56]. Later, a biosensor-based Cas13a/crRNA-Mediated CRISPR-FET (field-effect transistor) was developed to detect HCV without any pre-amplification step [57]. A single-pot dual detection of HBV and HCV by CRISPR-Cas12 and CRISPR-Cas13 systems by lateral flow was reported [58]. More studies are required to establish the detection of HCV at low levels using human samples.

5.3. Other Hepatitis Viruses

Very few studies have focused on other hepatitis viruses. For example, to detect the HDV, an RNA virus, the RNA-targeting Cas13a system was integrated with either RAA or RT-PCR for accurate identification [59]. Similarly, Hepatitis E virus being an RNA virus, Cas13a coupled with RAA was utilized to detect HEV [60]. Recently, a one-pot reaction was designed to detect HEV with Cas13a coupled with RAA or RT-PCR [61]. Since these viruses are not known to cause chronic liver diseases, little research has been directed at uncovering the diagnostics or therapeutic potential of the causative viruses.

6. Alcoholic Liver Disease (ALD)

Etiologically, ALD can be caused by chronic consumption of alcohol with chronic consumption of 12–24 g of alcohol per day [62]. Clinically, ALD is a spectrum comprising of alcoholic fatty liver (AFL) and alcoholic steatohepatitis (ASH), which can potentially cause fibrosis and cirrhosis, and, in some cases, HCC [63]. Various approaches have been utilized to tackle ALD. With the advent of the CRISPR-Cas system, this system was further utilized to target the proteins involved in disease progression as a therapeutic approach.

CRISPR-Cas9-mediated knockdown of Nuclear Transcription Factor Y Subunit Alpha (NFYA) in ALD attenuated Fatty Acid Synthase (FASN) and Sterol Regulatory Element Binding Transcription Factor 1 (SREBP1) expression, which are lipogenic markers [64]. Also, knockout of 3-hydroxy-3-methylglutaryl-coenzyme A synthase 2 (HMGCS2) with CRISPR-Cas9 attenuated SLC25A5 lysine β-hydroxybutyrylation (Kbhb). This showed reduced lipid accumulation in ALD [65]. Point gene mutation with CRISPR-Cas9 towards Methionine adenosyltransferase alpha1 (MATα1) K48 in mice was reported to show protective role from ethanol-induced fat accumulation and liver injury [66].

7. Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD is characterized by the accumulation of fat in >5% of hepatocytes. This accumulation should not be due to secondary causes, such as alcohol consumption or the use of drugs that can cause steatosis. NAFLD is strongly associated with features of metabolic syndrome, including obesity, insulin resistance (IR) or type2 diabetes mellitus (T2DM), hyperglycemia, and dyslipidemia [67]. Similar to ALD, NAFLD also exists as a spectrum of diseases, ranging from simple steatosis or non-alcoholic fatty liver (NAFL), non-Alcoholic Steatohepatitis (NASH), which may further lead to fibrosis, cirrhosis, and even HCC as an end-stage disease [68]. The CRISPR-Cas9 system was utilized to produce NAFLD models for therapeutic approach and disease investigation.

The initial application of Cas9 was documented in the establishment of a rat model for investigating NAFLD. It was reported that hydrodynamic injection of CRISPR-Cas9 targeting Phosphatase And Tensin Homolog (PTEN) plasmid efficiently knocked down the expression of PTEN in rat liver, resulting in lipid deposition and NAFLD [69,70]. Similarly, later, many studies utilized Cas9 to study NAFLD [71].

Multiple studies have shown that CRISPR-Cas9 knockout of NAFLD progression factors leads to the suppression of NAFLD pathology. CRISPR-Cas9-mediated Factor D knockout mice showed downregulated expression of genes related to fatty acid uptake and *de novo* lipogenesis in the liver [72]. Blocking liver mortality factor 4-like protein 1 (MORF4L1) via CRISPR technology significantly attenuated liver steatosis and improved hepatic lipid metabolism in animals subjected to a high-fat diet [73]. Microsomal triglyceride transfer protein (MTTP) forms as a heterodimer with protein disulfide isomerase (PDI), catalyses lipidation and assembly of apolipoprotein B (ApoB)-containing lipoproteins for secretion by hepatocytes. MTTP variants have been linked with susceptibility to NAFLD. A rare genetic variant in the gene MTTP, p.I564T, has been identified as responsible for the development of NAFLD. CRISPR-Cas9 was used to correct the I564T mutation in the MTTP [74]. Phosphoglycerate mutase 5 (PGAM5), a phosphatase involved in mitochondrial homeostasis, was knocked out using Cas9. This PGAM5-KO relieved hepatic steatosis and inflammation in high-fat high fructose (HFHF)-fed mice [75]. Depleting Insulin-like growth factor binding protein 7 (IGFBP7) effectively using CRISPR Cas9 alleviated zebrafish NAFLD progression by inhibiting hepatic ferroptosis [76]. Silencing Suppressor Of Cytokine Signaling 3 (SOCS3), Salt Inducible Kinase 1 (SIK1), and Dual Specificity Phosphatase 1 (DUSP1) expression using Cas9 in human adipose-derived mesenchymal stem cells (hADMSCs)-derived adipocytes reduces hepatocyte lipid storage in vitro [77]. When fed with a western high-fat diet, CRISPR-Cas9 knockout G protein-coupled receptor 75 (GPR75) mice displayed higher energy expenditure to remain in energy balance, thereby preventing NAFLD [78]. Also, CRISPR-Cas9 mediated knockdown of pyruvate kinase M2 (PKM2) decreases inflammation and increases autophagy in NAFLD [79]. Hepatic Mitochondrial Calcium uniporter (MCU) knockdown by Cas9 has been shown to protect against diet-induced MASH and fibrosis in mice [80].

8. Genetic Disorders of the Liver

In addition to the conditions mentioned above, the liver, as a primary site for secretory proteins production, is implicated in various genetic disorders that have been reported [81]. Notable genetic disorders of liver are Acute Hepatic Porphyria, Alagille Syndrome, Alpha-1 Antitrypsin Deficiency, Crigler-Najjar Syndrome, Dubin-Johnson Syndrome, Hereditary Tyrosinemia Type I (HT1), Gilberts Syndrome, Galactosemia, Haemochromatosis, Lysosomal acid lipase deficiency, Reye Syndrome, Wilson's Disease. These are reported to have mutations in Hydroxymethylbilane Synthase (HMBS), Hydroxymethylbilane Synthase (JAG1), Serpin Family A Member

1(SERPINA1), UDP Glucuronosyltransferase Family 1 Member A1 (UGT1A1), ATP Binding Cassette Subfamily C Member 2 (ABCC2), Fumarylacetoacetate Hydrolase (FAH), promoter of UGT1A1 gene, UDP-Galactose-4-Epimerase (GALE), Galactokinase 1 (GALK1), & Galactose-1-Phosphate Uridylyltransferase (GALT), Homeostatic Iron Regulator (HFE), Lipase A, Lysosomal Acid Type (LIPA), Acyl-CoA Dehydrogenase Medium Chain (ACADM) and ATPase Copper Transporting Beta (ATP7B) gene respectively. Genetic engineering tools were a major priority in the treatment of these disorders.

Alpha-1 Antitrypsin (AAT) is a circulating serine protease inhibitor secreted from the liver. In humans, the SERPINA1 gene encodes the AAT protein. In Alpha-1 Antitrypsin Deficiency, a point mutation (commonly referred to as PiZ (Glu342Lys) and the PiS (Glu264Val)) occurs. This causes aggregation of the miss-folded protein in hepatocytes, resulting in subsequent liver damage. CRISPR-Cas9, designed against hSERPINA1, has been shown to completely revert the phenotype associated with the PiZ mutation, human AAT protein levels, liver fibrosis, and protein aggregation [82]. Similar studies also reported utilizing the CRISPR-Cas9 system to correct mutation by knock-in and a preference for therapeutic [83–86].

Crigler–Najjar syndrome is a recessively inherited disorder caused by variation in the UGT1A1 gene. It encodes an enzyme that conjugates bilirubin in the liver [87]. CRISPR-*Staphylococcus aureus* Cas9 (*Sa*Cas9) was recently used to edit the UGT1A1 locus, resulting in decreased plasma bilirubin to safe levels and partially rescuing neonatal lethality by correcting the mutant UGT1A1 gene [88].

Hereditary Tyrosinemia Type I (HT1) is an inborn autosomal recessive disorder caused by single base mutations in the FAH gene. This encodes the last enzyme of the tyrosine catabolic pathway [89]. This loss of function leads to hepatic failure due to the accumulation of toxic metabolites from impaired hepatocyte metabolism. Using the CRISPR-Cas9 system, a knock-in therapeutic was developed through NHEJ and HDR pathways, which showed better efficiencies. CRISPR-Cas9 and the donor template were delivered using an adeno-associated virus to a newborn HT1 phenotype rabbit. The rescue of lethal phenotypes was observed, and the liver displayed normal phenotype and function [90].

Wilson's disease is an autosomal recessive disorder caused by loss-of-function mutations in ATP7B gene. This gene encodes a copper-transporting protein crucial for copper metabolism. A combination of the CRISPR-Cas9 system combined with single-strand DNA oligonucleotides (ssODNs) was utilized to produce an in vivo model of rabbits for the study of Wilson's disease. This is achieved by producing ATP7B site-directed point mutation p.Arg778Leu, a significant mutation type in Asians [91]. The ATP7B point mutation in the human cell lines was corrected using a combination of CRISPR-Cas9 gene editing and ssODNs [92]. A similar approach was adopted in hepatocytes derived from induced pluripotent stem cells originating from a patient with Wilson's disease [93].

Till now, CRISPR-Cas systems have been utilized to develop multiple therapeutic agents for the treatment of Alpha-1 Antitrypsin Deficiency, Crigler-Najjar Syndrome, Hereditary Tyrosinemia Type I (HT1), and Wilson disease. There is a vast opportunity in the CRISPR-Cas toolbox to develop therapeutics for Acute Hepatic Porphyria, Alagille Syndrome, Alpha-, Dubin-Johnson Syndrome, Gilberts Syndrome, Galactosemia, Haemochromatosis, Lysosomal acid lipase deficiency (LAL-D) and Reye Syndrome.

9. Hepatocellular Carcinoma (HCC)

Most of the CLD, except genetic disorders, can progress toward HCC in the final stages. CRISPR-Cas system has been widely applied to develop therapeutic methods for treating HCC. Knockout of oncogenic factors has been a significant approach. Initially, the CRISPR-Cas9 system mediated knockdown of hypoxia-inducible factor-1α (HIF-1α) and nuclear receptor binding SET domain-containing protein 1 (NSD1) shown regression of HCC [94]. Alongside the development of therapeutics, models for the study of HCC were also developed using CRISPR-Cas systems by targeting PTEN and p53. Further, multiple oncogenic factors, including coding and non-coding genes knockdown by CRISPR-Cas9, showed a decrease in the proliferation and metastasis of HCC [95,96].

Recent studies performed CRISPR-Cas9-based library analysis and identified Solute Carrier Family 7 Member 5 (SLC7A5) [97], Splicing factor proline and glutamine-rich (SFPQ) [98], Vasorin (VASN) [99], scavenger receptor class B member 2 (SCARB2) [100], long intergenic non-protein coding RNA 1607 (LINC01607) [101], TAR (HIV-1) RNA Binding Protein 1 (TARBP1) [102] and other players, involved in the progression of HCC.

Recently, diagnostic platforms have also been developed to detect HCC markers efficiently. Glypican-3 (GPC3) was not found in healthy adult liver but is overexpressed in HCC. The protein marker GPC3 on extracellular vesicles (GPC3+ EVs) is a helpful marker for HCC detection. An extraction-free one-pot immuno-RPA-CRISPR was developed for the direct and extremely sensitive detection of EV proteins like GPC3 [103].

Moreover, a CRISPR-Cas12a amplification strategy was developed to detect HCC-related microRNAs. This approach utilizes nanozymes as signal reporters, enabling binary visual and colorimetric detection for enhanced diagnostic precision developed [104].

10. Delivery Methods

As discussed above, CRISPR-Cas systems are mainly developed as a therapeutic approaches for CLD. The delivery of these therapeutics also underwent various advancements. These delivery systems can be divided into two categories: viral and non-viral delivery systems [105]. The commonly used viral delivery system in liver therapeutics include primarily adeno-associated viruses (AAV) [106], Murine leukemia virus (MLV) [107], Engineered virus-like particles (eVLPs) [108]. Non-viral delivery systems employed mostly are lipid nanoparticles (LNPs). Viral vectors have the advantages of better transfection efficiency and longer period persistence. Still, they also have the risk of triggering the innate immune system, which leads to the production of neutralizing antibodies. Viral vectors have specific limitations including, limited cloning capacity and the risk of mutagenesis. Non-viral vectors have the advantage of simple production, better stability, high encapsulation efficiency, efficient cellular internalization, endosomal escape capabilities, and low immunogenicity. Still, they also have the risk of cytotoxic effects, lack inherent targeting capabilities, and short half-life [109]. Currently, several LNP-based therapeutics are under clinical trials. These include the inactivation of Transthyretin (TTR), Kallikrein B1 (KLKB1), and Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) [105].

11. Future Perspectives and Conclusion

With the evolution of the CRISPR-Cas system toolkit, its applications are increasingly being reported. Linking the CRISPR-Cas nickase system with reverse transcriptase and base editors leads to hybrid technologies, namely prime-editing and base editing, respectively. Likewise, integrases, recombinases, and transposases are utilized in the hybridization with the CRISPR-Cas system for various applications. These technologies are emerging as key players in precise gene editing therapeutics. To date, most of the applications of CRISPR-Cas systems have been directed towards therapeutics and diagnostics towards CLD. However, these applications have yet to be thoroughly scrutinized and made robust for better management of the treatment of CLD. In summary, this review demonstrates that the CRISPR-Cas system has not been fully exploited in CLD and we believe that further research is advocated to expand the application scope of the CRISPR-Cas system. Future studies should focus on developing diagnostics and management strategies for chronic liver disease by leveraging the CRISPR-Cas technology.

Author Contributions

T.N.S. wrote the original manuscript. S.K.V. (correspondence) conceptualized, reviewed, and edited the manuscript. Both authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable

Data Availability Statement

Not applicable.

Acknowledgments

We thank Department of Biotechnology, Ministry of Science and Technology, Government of India, for providing “DBT-Junior Research Fellowship” (DBT-JRF) to Teja Naveen Sata.

Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

No AI tools were utilized for this paper.

References

1. Kalra, A.; Yetiskul, E.; Wehrle, C.J.; et al. Physiology, Liver. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2025.
2. Sharma, A.; Nagalli, S. Chronic Liver Disease. In *StatPearls*; StatPearls Publishing: Treasure Island, FL, USA, 2025.
3. Younossi, Z.M.; Koenig, A.B.; Abdelatif, D.; et al. Global Epidemiology of Nonalcoholic Fatty Liver Disease-Meta-Analytic Assessment of Prevalence, Incidence, and Outcomes. *Hepatology* **2016**, *64*, 73–84. https://doi.org/10.1002/hep.28431.
4. Pickar-Oliver, A.; Gersbach, C.A. The next Generation of CRISPR-Cas Technologies and Applications. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 490–507. https://doi.org/10.1038/s41580-019-0131-5.
5. Labrie, S.J.; Samson, J.E.; Moineau, S. Bacteriophage Resistance Mechanisms. *Nat. Rev. Microbiol.* **2010**, *8*, 317–327. https://doi.org/10.1038/nrmicro2315.
6. Jansen, R.; van Embden, J.D.A.; Gaastra, W.; et al. Identification of Genes That Are Associated with DNA Repeats in Prokaryotes. *Mol. Microbiol.* **2002**, *43*, 1565–1575. https://doi.org/10.1046/j.1365-2958.2002.02839.x.
7. Makarova, K.S.; Wolf, Y.I.; Iranzo, J.; et al. Evolutionary Classification of CRISPR–Cas Systems: A Burst of Class 2 and Derived Variants. *Nat. Rev. Microbiol.* **2020**, *18*, 67–83. https://doi.org/10.1038/s41579-019-0299-x.
8. Charpentier, E.; Richter, H.; van der Oost, J.; et al. Biogenesis Pathways of RNA Guides in Archaeal and Bacterial CRISPR-Cas Adaptive Immunity. *FEMS Microbiol. Rev.* **2015**, *39*, 428–441. https://doi.org/10.1093/femsre/fuv023.
9. van der Oost, J.; Jore, M.M.; Westra, E.R.; et al. CRISPR-Based Adaptive and Heritable Immunity in Prokaryotes. *Trends Biochem. Sci.* **2009**, *34*, 401–407. https://doi.org/10.1016/j.tibs.2009.05.002.
10. Mojica, F.J.M.; Díez-Villaseñor, C.; García-Martínez, J.; et al. Short Motif Sequences Determine the Targets of the Prokaryotic CRISPR Defence System. *Microbiology* **2009**, *155*, 733–740. https://doi.org/10.1099/mic.0.023960-0.
11. Wright, A.V.; Nuñez, J.K.; Doudna, J.A. Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering. *Cell* **2016**, *164*, 29–44. https://doi.org/10.1016/j.cell.2015.12.035.
12. Jiang, W.; Marraffini, L.A. CRISPR-Cas: New Tools for Genetic Manipulations from Bacterial Immunity Systems. *Annu. Rev. Microbiol.* **2015**, *69*, 209–228. https://doi.org/10.1146/annurev-micro-091014-104441.
13. Tao, J.; Bauer, D.E.; Chiarle, R. Assessing and Advancing the Safety of CRISPR-Cas Tools: From DNA to RNA Editing. *Nat. Commun.* **2023**, *14*, 212. https://doi.org/10.1038/s41467-023-35886-6.
14. Asmamaw, M.; Zawdie, B. Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. *Biologics* **2021**, *15*, 353–361. https://doi.org/10.2147/BTT.S326422.
15. Paul, B.; Montoya, G. CRISPR-Cas12a: Functional Overview and Applications. *Biomed. J.* **2020**, *43*, 8–17. https://doi.org/10.1016/j.bj.2019.10.005.
16. Liu, L.; Li, X.; Ma, J.; et al. The Molecular Architecture for RNA-Guided RNA Cleavage by Cas13a. *Cell* **2017**, *170*, 714–726.e10. https://doi.org/10.1016/j.cell.2017.06.050.
17. Cannan, W.J.; Pederson, D.S. Mechanisms and Consequences of Double-Strand DNA Break Formation in Chromatin. *J. Cell Physiol.* **2016**, *231*, 3–14. https://doi.org/10.1002/jcp.25048.
18. Feng, W.; Zhang, H.; Le, X.C. Signal Amplification by the Trans-Cleavage Activity of CRISPR-Cas Systems: Kinetics and Performance. *Anal. Chem.* **2023**, *95*, 206–217. https://doi.org/10.1021/acs.analchem.2c04555.
19. Abudayyeh, O.O.; Gootenberg, J.S.; Essletzbichler, P.; et al. RNA Targeting with CRISPR–Cas13. *Nature* **2017**, *550*, 280–284. https://doi.org/10.1038/nature24049.
20. Weng, Z.; You, Z.; Yang, J.; et al. CRISPR-Cas Biochemistry and CRISPR-Based Molecular Diagnostics. *Angew. Chem. Int. Ed.* **2023**, *62*, e202214987. https://doi.org/10.1002/anie.202214987.
21. Glökler, J.; Lim, T.S.; Ida, J.; et al. Isothermal Amplifications—A Comprehensive Review on Current Methods. *Crit. Rev. Biochem. Mol. Biol.* **2021**, *56*, 543–586. https://doi.org/10.1080/10409238.2021.1937927.
22. Castaneda, D.; Gonzalez, A.J.; Alomari, M.; et al. From Hepatitis A to E: A Critical Review of Viral Hepatitis. *World J. Gastroenterol.* **2021**, *27*, 1691–1715. https://doi.org/10.3748/wjg.v27.i16.1691.
23. Bartosh, U.I.; Dome, A.S.; Zhukova, N.V.; et al. CRISPR/Cas9 as a New Antiviral Strategy for Treating Hepatitis Viral Infections. *Int. J. Mol. Sci.* **2023**, *25*, 334. https://doi.org/10.3390/ijms25010334.
24. Lin, S.-R.; Yang, H.-C.; Kuo, Y.-T.; et al. The CRISPR/Cas9 System Facilitates Clearance of the Intrahepatic HBV Templates In Vivo. *Mol. Ther. Nucleic Acids* **2014**, *3*, e186. https://doi.org/10.1038/mtna.2014.38.

25. Seeger, C.; Sohn, J.A. Targeting Hepatitis B Virus With CRISPR/Cas9. *Mol. Ther. Nucleic Acids* **2014**, *3*, e216. https://doi.org/10.1038/mtna.2014.68.
26. Kennedy, E.M.; Bassit, L.C.; Mueller, H.; et al. Suppression of Hepatitis B Virus DNA Accumulation in Chronically Infected Cells Using a Bacterial CRISPR/Cas RNA-Guided DNA Endonuclease. *Virology* **2015**, *476*, 196–205. https://doi.org/10.1016/j.virol.2014.12.001.
27. Sakuma, T.; Masaki, K.; Abe-Chayama, H.; et al. Highly Multiplexed CRISPR-Cas9-Nuclease and Cas9-Nickase Vectors for Inactivation of Hepatitis B Virus. *Genes. Cells* **2016**, *21*, 1253–1262. https://doi.org/10.1111/gtc.12437.
28. Schiwon, M.; Ehrke-Schulz, E.; Oswald, A.; et al. One-Vector System for Multiplexed CRISPR/Cas9 against Hepatitis B Virus cccDNA Utilizing High-Capacity Adenoviral Vectors. *Mol. Ther. Nucleic Acids* **2018**, *12*, 242–253. https://doi.org/10.1016/j.omtn.2018.05.006.
29. Kennedy, E.M.; Kornepati, A.V.R.; Cullen, B.R. Targeting Hepatitis B Virus cccDNA Using CRISPR/Cas9. *Antivir. Res.* **2015**, *123*, 188–192. https://doi.org/10.1016/j.antiviral.2015.10.004.
30. Li, H.; Sheng, C.; Wang, S.; et al. Removal of Integrated Hepatitis B Virus DNA Using CRISPR-Cas9. *Front. Cell Infect. Microbiol.* **2017**, *7*, 91. https://doi.org/10.3389/fcimb.2017.00091.
31. Ely, A.; Moyo, B.; Arbuthnot, P. Progress with Developing Use of Gene Editing to Cure Chronic Infection with Hepatitis B Virus. *Mol. Ther.* **2016**, *24*, 671–677. https://doi.org/10.1038/mt.2016.43.
32. Yang, H.-C.; Chen, P.-J. The Potential and Challenges of CRISPR-Cas in Eradication of Hepatitis B Virus Covalently Closed Circular DNA. *Virus Res.* **2018**, *244*, 304–310. https://doi.org/10.1016/j.virusres.2017.06.010.
33. Kurihara, T.; Fukuhara, T.; Ono, C.; et al. Suppression of HBV Replication by the Expression of Nickase- and Nuclease Dead-Cas9. *Sci. Rep.* **2017**, *7*, 6122. https://doi.org/10.1038/s41598-017-05905-w.
34. Yang, Y.-C.; Chen, Y.-H.; Kao, J.-H.; et al. Permanent Inactivation of HBV Genomes by CRISPR/Cas9-Mediated Non-Cleavage Base Editing. *Mol. Ther. Nucleic Acids* **2020**, *20*, 480–490. https://doi.org/10.1016/j.omtn.2020.03.005.
35. McCoullough, L.C.; Fareh, M.; Hu, W.; et al. CRISPR-Cas13b-Mediated Suppression of HBV Replication and Protein Expression. *J. Hepatol.* **2024**, *81*, 794–805. https://doi.org/10.1016/j.jhep.2024.05.025.
36. Wang, S.; Li, H.; Kou, Z.; et al. Highly Sensitive and Specific Detection of Hepatitis B Virus DNA and Drug Resistance Mutations Utilizing the PCR-Based CRISPR-Cas13a System. *Clin. Microbiol. Infect.* **2021**, *27*, 443–450. https://doi.org/10.1016/j.cmi.2020.04.018.
37. Zhang, X.; Tian, Y.; Xu, L.; et al. CRISPR/Cas13-Assisted Hepatitis B Virus Covalently Closed Circular DNA Detection. *Hepatol. Int.* **2022**, *16*, 306–315. https://doi.org/10.1007/s12072-022-10311-0.
38. Tian, Y.; Fan, Z.; Xu, L.; et al. CRISPR/Cas13a-Assisted Rapid and Portable HBV DNA Detection for Low-Level Viremia Patients. *Emerg. Microbes Infect.* **2023**, *12*, e2177088. https://doi.org/10.1080/22221751.2023.2177088.
39. Wang, T.; Bai, L.; Wang, G.; et al. SATCAS: A CRISPR/Cas13a-Based Simultaneous Amplification and Testing Platform for One-Pot RNA Detection and SNPs Distinguish in Clinical Diagnosis. *Biosens. Bioelectron.* **2024**, *263*, 116636. https://doi.org/10.1016/j.bios.2024.116636.
40. Ding, R.; Long, J.; Yuan, M.; et al. CRISPR/Cas12-Based Ultra-Sensitive and Specific Point-of-Care Detection of HBV. *Int. J. Mol. Sci.* **2021**, *22*, 4842. https://doi.org/10.3390/ijms22094842.
41. Li, T.; Wang, J.; Fang, J.; et al. A Universal Nucleic Acid Detection Platform Combing CRISPR/Cas12a and Strand Displacement Amplification with Multiple Signal Readout. *Talanta* **2024**, *273*, 125922. https://doi.org/10.1016/j.talanta.2024.125922.
42. Zhao, C.; Du, L.; Hu, J.; Hou, X. Recombinase Polymerase Amplification and Target-Triggered CRISPR/Cas12a Assay for Sensitive and Selective Hepatitis B Virus DNA Analysis Based on Lanthanide Tagging and Inductively Coupled Plasma Mass Spectrometric Detection. *Anal. Chem.* **2024**, *96*, 15059–15065. https://doi.org/10.1021/acs.analchem.4c03715.
43. Wang, J.; Luo, L.; Li, Y.; et al. A Signal On-off Strategy Based on the Digestion of DNA Cubes Assisted by the CRISPR-Cas12a System for Ultrasensitive HBV Detection in Solid-State Nanopores. *Analyst* **2022**, *147*, 5623–5632. https://doi.org/10.1039/d2an01402e.
44. Kachwala, M.J.; Smith, C.W.; Nandu, N.; et al. Recombinase Amplified CRISPR Enhanced Chain Reaction (RACECAR) for Viral Genome Detection. *Nanoscale* **2022**, *14*, 13500–13504. https://doi.org/10.1039/d2nr03590a.
45. Choi, J.-H.; Shin, M.; Yang, L.; et al. Clustered Regularly Interspaced Short Palindromic Repeats-Mediated Amplification-Free Detection of Viral DNAs Using Surface-Enhanced Raman Spectroscopy-Active Nanoarray. *ACS Nano* **2021**, *15*, 13475–13485. https://doi.org/10.1021/acsnano.1c03975.
46. Xu, H.; Lin, G.; Chen, R.; et al. CRISPR/Cas12b Assisted Loop-Mediated Isothermal Amplification for Easy, Rapid and Sensitive Quantification of Chronic HBV DNA in One-Pot. *Anal. Chim. Acta* **2024**, *1310*, 342702. https://doi.org/10.1016/j.aca.2024.342702.

47. Chen, X.; Tan, Y.; Wang, S.; et al. A CRISPR-Cas12b-Based Platform for Ultrasensitive, Rapid, and Highly Specific Detection of Hepatitis B Virus Genotypes B and C in Clinical Application. *Front. Bioeng. Biotechnol.* **2021**, *9*, 743322. https://doi.org/10.3389/fbioe.2021.743322.
48. Gong, S.; Zhang, S.; Wang, X.; et al. Strand Displacement Amplification Assisted CRISPR-Cas12a Strategy for Colorimetric Analysis of Viral Nucleic Acid. *Anal. Chem.* **2021**, *93*, 15216–15223. https://doi.org/10.1021/acs.analchem.1c04133.
49. Tao, Y.; Yi, K.; Wang, H.; et al. CRISPR-Cas12a-Regulated DNA Adsorption and Metallization on MXenes as Enhanced Enzyme Mimics for Sensitive Colorimetric Detection of Hepatitis B Virus DNA. *J. Colloid. Interface Sci.* **2022**, *613*, 406–414. https://doi.org/10.1016/j.jcis.2022.01.038.
50. Fuchs, G.; Petrov, A.N.; Marceau, C.D.; et al. Kinetic Pathway of 40S Ribosomal Subunit Recruitment to Hepatitis C Virus Internal Ribosome Entry Site. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 319–325. https://doi.org/10.1073/pnas.1421328111.
51. Hopcraft, S.E.; Evans, M.J. Selection of a Hepatitis C Virus with Altered Entry Factor Requirements Reveals a Genetic Interaction between the E1 Glycoprotein and Claudins. *Hepatology* **2015**, *62*, 1059–1069. https://doi.org/10.1002/hep.27815.
52. Price, A.A.; Sampson, T.R.; Ratner, H.K.; et al. Cas9-Mediated Targeting of Viral RNA in Eukaryotic Cells. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 6164–6169. https://doi.org/10.1073/pnas.1422340112.
53. Ashraf, M.U.; Salman, H.M.; Khalid, M.F.; et al. CRISPR-Cas13a Mediated Targeting of Hepatitis C Virus Internal-Ribosomal Entry Site (IRES) as an Effective Antiviral Strategy. *Biomed. Pharmacother.* **2021**, *136*, 111239. https://doi.org/10.1016/j.biopha.2021.111239.
54. Kham-Kjing, N.; Ngo-Giang-Huong, N.; Tragoolpua, K.; et al. Highly Specific and Rapid Detection of Hepatitis C Virus Using RT-LAMP-Coupled CRISPR-Cas12 Assay. *Diagn.* **2022**, *12*, 1524. https://doi.org/10.3390/diagnostics12071524.
55. Wei, X.; Shen, Y.; Yuan, M.; et al. Visualizing In-Field Detection of HCV Using a One-Pot RT-RAA-CRISPR/Cas12a Platform. *Anal. Methods* **2024**, *16*, 7484–7493. https://doi.org/10.1039/d4ay01253d.
56. Nguyen, L.T.; Rananaware, S.R.; Yang, L.G.; et al. Engineering Highly Thermostable Cas12b via de Novo Structural Analyses for One-Pot Detection of Nucleic Acids. *Cell Rep. Med.* **2023**, *4*, 101037. https://doi.org/10.1016/j.xcrm.2023.101037.
57. Li, J.; Tang, L.; Li, T.; et al. Tandem Cas13a/crRNA-Mediated CRISPR-FET Biosensor: A One-for-All Check Station for Virus without Amplification. *ACS Sens.* **2022**, *7*, 2680–2690. https://doi.org/10.1021/acssensors.2c01200.
58. Shahni, S.N.; Albogami, S.; Azmi, I.; et al. Dual Detection of Hepatitis B and C Viruses Using CRISPR-Cas Systems and Lateral Flow Assay. *J. Nucleic Acids* **2024**, *2024*, 8819834. https://doi.org/10.1155/2024/8819834.
59. Tian, Y.; Fan, Z.; Zhang, X.; et al. CRISPR/Cas13a-Assisted Accurate and Portable Hepatitis D Virus RNA Detection. *Emerg. Microbes Infect.* **2023**, *12*, 2276337. https://doi.org/10.1080/22221751.2023.2276337.
60. Li, M.; He, Q.; Li, T.; et al. Development and Evaluation of a CRISPR-Cas13a System-Based Diagnostic for Hepatitis E Virus. *Clin. Chem. Lab. Med.* **2024**, *62*, 1237–1247. https://doi.org/10.1515/cclm-2023-1007.
61. Fan, Z.; Xu, L.; Cao, Y.; et al. One-Pot Assay Based on CRISPR/Cas13a Technology for HEV RNA Point-of-Care Testing. *J. Med. Virol.* **2024**, *96*, e70115. https://doi.org/10.1002/jmv.70115.
62. Rehm, J.; Taylor, B.; Mohapatra, S.; et al. Alcohol as a Risk Factor for Liver Cirrhosis: A Systematic Review and Meta-Analysis. *Drug Alcohol. Rev.* **2010**, *29*, 437–445. https://doi.org/10.1111/j.1465-3362.2009.00153.x.
63. Seitz, H.K.; Bataller, R.; Cortez-Pinto, H.; et al. Alcoholic Liver Disease. *Nat. Rev. Dis. Primers* **2018**, *4*, 16. https://doi.org/10.1038/s41572-018-0014-7.
64. Zhang, Y.; Sun, Y.; Zhang, Y.; et al. Nuclear Factor Y Participates in Alcoholic Liver Disease by Activating SREBP1 Expression in Mice. *Biochem. Biophys. Res. Commun.* **2021**, *541*, 90–94. https://doi.org/10.1016/j.bbrc.2021.01.011.
65. Chen, N.; Luo, J.; Zhou, T.; et al. Lysine β-Hydroxybutyrylation Promotes Lipid Accumulation in Alcoholic Liver Disease. *Biochem. Pharmacol.* **2024**, *228*, 115936. https://doi.org/10.1016/j.bcp.2023.115936.
66. Floris, A.; Chandla, S.; Lim, Y.; et al. Sumoylation of Methionine Adenosyltransferase Alpha 1 Promotes Mitochondrial Dysfunction in Alcohol-Associated Liver Disease. *Hepatology* **2024**, *80*, 102–118. https://doi.org/10.1097/HEP.0000000000000717.
67. de Alwis, N.M.W.; Day, C.P. Non-Alcoholic Fatty Liver Disease: The Mist Gradually Clears. *J. Hepatol.* **2008**, *48*, S104–S112. https://doi.org/10.1016/j.jhep.2008.01.009.
68. Hardy, T.; Oakley, F.; Anstee, Q.M.; et al. Nonalcoholic Fatty Liver Disease: Pathogenesis and Disease Spectrum. *Annu. Rev. Pathol.* **2016**, *11*, 451–496. https://doi.org/10.1146/annurev-pathol-012615-044224.
69. Yu, Q.; Tan, R.-Z.; Gan, Q.; et al. A Novel Rat Model of Nonalcoholic Fatty Liver Disease Constructed Through CRISPR/Cas-Based Hydrodynamic Injection. *Mol. Biotechnol.* **2017**, *59*, 365–373. https://doi.org/10.1007/s12033-017-0025-8.

70. Wang, D.; Mou, H.; Li, S.; et al. Adenovirus-Mediated Somatic Genome Editing of Pten by CRISPR/Cas9 in Mouse Liver in Spite of Cas9-Specific Immune Responses. *Hum. Gene Ther.* **2015**, *26*, 432–442. https://doi.org/10.1089/hum.2015.087.
71. Hayat, U.; Siddiqui, A.A.; Farhan, M.L.; et al. Genome Editing and Fatty Liver. *Adv. Exp. Med. Biol.* **2023**, *1396*, 191–206. https://doi.org/10.1007/978-981-19-5642-3_13.
72. Tsuru, H.; Osaka, M.; Hiraoka, Y.; et al. HFD-Induced Hepatic Lipid Accumulation and Inflammation Are Decreased in Factor D Deficient Mouse. *Sci. Rep.* **2020**, *10*, 17593. https://doi.org/10.1038/s41598-020-74617-5.
73. Wei, Y.; Tian, C.; Zhao, Y.; et al. MRG15 Orchestrates Rhythmic Epigenomic Remodelling and Controls Hepatic Lipid Metabolism. *Nat. Metab.* **2020**, *2*, 447–460. https://doi.org/10.1038/s42255-020-0203-z.
74. Grove, J.I.; Lo, P.C.K.; Shrine, N.; et al. Identification and Characterisation of a Rare MTTP Variant Underlying Hereditary Non-Alcoholic Fatty Liver Disease. *JHEP Rep.* **2023**, *5*, 100764. https://doi.org/10.1016/j.jhepr.2023.100764.
75. Li, L.; Guo, C.; Yu, Y.; et al. Differential Effects of PGAM5 Knockout on High Fat High Fructose Diet and Methionine Choline-Deficient Diet Induced Non-Alcoholic Steatohepatitis (NASH) in Mice. *Cell Biosci.* **2023**, *13*, 154. https://doi.org/10.1186/s13578-023-01095-3.
76. Wang, Y.; Bo, J.; Zhao, Z.; et al. Depletion of Igfbp7 Alleviates Zebrafish NAFLD Progression through Inhibiting Hepatic Ferroptosis. *Life Sci.* **2023**, *332*, 122086. https://doi.org/10.1016/j.lfs.2023.122086.
77. Lopez-Yus, M.; Frendo-Cumbo, S.; Del Moral-Bergos, R.; et al. CRISPR/Cas9-Mediated Deletion of Adipocyte Genes Associated with NAFLD Alters Adipocyte Lipid Handling and Reduces Steatosis in Hepatocytes in Vitro. *Am. J. Physiol. Cell Physiol.* **2023**, *325*, C1178–C1189. https://doi.org/10.1152/ajpcell.00291.2023.
78. Leeson-Payne, A.; Iyinikkel, J.; Malcolm, C.; et al. Loss of GPR75 Protects against Non-Alcoholic Fatty Liver Disease and Body Fat Accumulation. *Cell Metab.* **2024**, *36*, 1076–1087.e4. https://doi.org/10.1016/j.cmet.2024.03.016.
79. Hossain, M.M.; Mishra, A.K.; Yadav, A.K.; et al. MicroRNA-122 Regulates Inflammatory and Autophagic Proteins by Downregulating Pyruvate Kinase M2 in Non-Alcoholic Fatty Liver Disease. *Mol. Cell Biochem.* **2024**. https://doi.org/10.1007/s11010-024-05174-y.
80. Li, S.; Chen, F.; Liu, M.; et al. Knockdown of Hepatic Mitochondrial Calcium Uniporter Mitigates MASH and Fibrosis in Mice. *Cell Biosci.* **2024**, *14*, 135. https://doi.org/10.1186/s13578-024-01315-4.
81. Konkwo, C.; Chowdhury, S.; Vilarinho, S. Genetics of Liver Disease in Adults. *Hepatol. Commun.* **2024**, *8*, e0408. https://doi.org/10.1097/HC9.0000000000000408.
82. Bjursell, M.; Porritt, M.J.; Ericson, E.; et al. Therapeutic Genome Editing With CRISPR/Cas9 in a Humanized Mouse Model Ameliorates A1-Antitrypsin Deficiency Phenotype. *EBioMedicine* **2018**, *29*, 104–111. https://doi.org/10.1016/j.ebiom.2018.02.015.
83. Shen, S.; Sanchez, M.E.; Blomenkamp, K.; et al. Amelioration of Alpha-1 Antitrypsin Deficiency Diseases with Genome Editing in Transgenic Mice. *Hum. Gene Ther.* **2018**, *29*, 861–873. https://doi.org/10.1089/hum.2017.227.
84. Ji, Q.; Guo, C.; Xie, C.; et al. Genetically Engineered Cell Lines for A1-Antitrypsin Expression. *Biotechnol. Lett.* **2017**, *39*, 1471–1476. https://doi.org/10.1007/s10529-017-2391-5.
85. Stephens, C.J.; Kashentseva, E.; Everett, W.; et al. Targeted in Vivo Knock-in of Human Alpha-1-Antitrypsin cDNA Using Adenoviral Delivery of CRISPR/Cas9. *Gene Ther.* **2018**, *25*, 139–156. https://doi.org/10.1038/s41434-018-0003-1.
86. Song, C.-Q.; Wang, D.; Jiang, T.; et al. In Vivo Genome Editing Partially Restores Alpha1-Antitrypsin in a Murine Model of AAT Deficiency. *Hum. Gene Ther.* **2018**, *29*, 853–860. https://doi.org/10.1089/hum.2017.225.
87. D'Antiga, L.; Beuers, U.; Ronzitti, G.; et al. Gene Therapy in Patients with the Crigler–Najjar Syndrome. *N. Engl. J. Med.* **2023**, *389*, 620–631. https://doi.org/10.1056/NEJMoa2214084.
88. Bortolussi, G.; Iaconcig, A.; Canarutto, G.; et al. CRISPR-Cas9-Mediated Somatic Correction of a One-Base Deletion in the Ugt1a Gene Ameliorates Hyperbilirubinemia in Crigler-Najjar Syndrome Mice. *Mol. Ther. Methods Clin. Dev.* **2023**, *31*, 101161. https://doi.org/10.1016/j.omtm.2023.101161.
89. St-Louis, M.; Tanguay, R.M. Mutations in the Fumarylacetoacetate Hydrolase Gene Causing Hereditary Tyrosinemia Type I: Overview. *Hum. Mutat.* **1997**, *9*, 291–299. https://doi.org/10.1002/(SICI)1098-1004(1997)9:4<291::AID-HUMU1>3.0.CO;2-9.
90. Li, N.; Gou, S.; Wang, J.; et al. CRISPR/Cas9-Mediated Gene Correction in Newborn Rabbits with Hereditary Tyrosinemia Type I. *Mol. Ther.* **2021**, *29*, 1001–1015. https://doi.org/10.1016/j.ymthe.2020.11.023.
91. Jiang, W.; Liu, L.; Chang, Q.; et al. Production of Wilson Disease Model Rabbits with Homology-Directed Precision Point Mutations in the ATP7B Gene Using the CRISPR/Cas9 System. *Sci. Rep.* **2018**, *8*, 1332. https://doi.org/10.1038/s41598-018-19774-4.
92. Pöhler, M.; Guttmann, S.; Nadzemova, O.; et al. CRISPR/Cas9-Mediated Correction of Mutated Copper Transporter ATP7B. *PLoS ONE* **2020**, *15*, e0239411. https://doi.org/10.1371/journal.pone.0239411.

93. Wei, R.; Yang, J.; Cheng, C.-W.; et al. CRISPR-Targeted Genome Editing of Human Induced Pluripotent Stem Cell-Derived Hepatocytes for the Treatment of Wilson's Disease. *JHEP Rep.* **2022**, *4*, 100389. https://doi.org/10.1016/j.jhepr.2021.100389.
94. Wu, X.; Ma, W.; Mei, C.; et al. Description of CRISPR/Cas9 Development and Its Prospect in Hepatocellular Carcinoma Treatment. *J. Exp. Clin. Cancer Res.* **2020**, *39*, 97. https://doi.org/10.1186/s13046-020-01603-0.
95. Yu, S.; Zhao, R.; Zhang, B.; et al. Research Progress and Application of the CRISPR/Cas9 Gene-Editing Technology Based on Hepatocellular Carcinoma. *Asian J. Pharm. Sci.* **2023**, *18*, 100828. https://doi.org/10.1016/j.ajps.2023.100828.
96. Amjad, E.; Pezzani, R.; Sokouti, B. A Review of the Literature on the Use of CRISPR/Cas9 Gene Therapy to Treat Hepatocellular Carcinoma. *Oncol. Res.* **2024**, *32*, 439–461. https://doi.org/10.32604/or.2023.044473.
97. Kim, S.-Y.; Ong, Q.; Liao, Y.; et al. Genetic Ablation of LAT1 Inhibits Growth of Liver Cancer Cells and Downregulates mTORC1 Signaling. *Int. J. Mol. Sci.* **2023**, *24*, 9171. https://doi.org/10.3390/ijms24119171.
98. Lin, Y.; Zhong, W.; Lin, Q.; et al. SFPQ Promotes the Proliferation, Migration and Invasion of Hepatocellular Carcinoma Cells and Is Associated with Poor Prognosis. *Am. J. Cancer Res.* **2023**, *13*, 2269–2284.
99. Wan, F.; Li, H.; Huang, S.; et al. Vasorin Promotes Proliferation and Migration via STAT3 Signaling and Acts as a Promising Therapeutic Target of Hepatocellular Carcinoma. *Cell Signal* **2023**, *110*, 110809. https://doi.org/10.1016/j.cellsig.2023.110809.
100. Wang, F.; Gao, Y.; Xue, S.; et al. SCARB2 Drives Hepatocellular Carcinoma Tumor Initiating Cells via Enhanced MYC Transcriptional Activity. *Nat. Commun.* **2023**, *14*, 5917. https://doi.org/10.1038/s41467-023-41593-z.
101. Zhang, Y.; Zhang, Y.; Tao, H.; et al. Targeting LINC01607 Sensitizes Hepatocellular Carcinoma to Lenvatinib via Suppressing Mitophagy. *Cancer Lett.* **2023**, *576*, 216405. https://doi.org/10.1016/j.canlet.2023.216405.
102. Shi, X.; Zhang, Y.; Wang, Y.; et al. The tRNA Gm18 Methyltransferase TARBP1 Promotes Hepatocellular Carcinoma Progression via Metabolic Reprogramming of Glutamine. *Cell Death Differ.* **2024**, *31*, 1219–1234. https://doi.org/10.1038/s41418-024-01323-4.
103. Xiao, X.; Lin, X.; Ting, C.L.; et al. Extraction-Free, Immuno-RPA-CRISPR/Cas13a-Based One-Pot Detection of Glypican-3 Directly from Extracellular Vesicles. *Anal. Chim. Acta* **2024**, *1297*, 342385. https://doi.org/10.1016/j.aca.2024.342385.
104. Luo, B.; Zhou, J.; Zhan, X.; et al. Visual and Colorimetric Detection of microRNA in Clinical Samples Based on Strand Displacement Amplification and Nanozyme-Mediated CRISPR-Cas12a System. *Talanta* **2024**, *277*, 126310. https://doi.org/10.1016/j.talanta.2024.126310.
105. Huang, K.; Zapata, D.; Tang, Y.; et al. In Vivo Delivery of CRISPR-Cas9 Genome Editing Components for Therapeutic Applications. *Biomaterials* **2022**, *291*, 121876. https://doi.org/10.1016/j.biomaterials.2022.121876.
106. Chadwick, A.C.; Wang, X.; Musunuru, K. In Vivo Base Editing of PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) as a Therapeutic Alternative to Genome Editing. *Arterioscler. Thromb. Vasc. Biol.* **2017**, *37*, 1741–1747. https://doi.org/10.1161/ATVBAHA.117.309881.
107. Mangeot, P.E.; Risson, V.; Fusil, F.; et al. Genome Editing in Primary Cells and in Vivo Using Viral-Derived Nanoblades Loaded with Cas9-sgRNA Ribonucleoproteins. *Nat. Commun.* **2019**, *10*, 45. https://doi.org/10.1038/s41467-018-07845-z.
108. Banskota, S.; Raguram, A.; Suh, S.; et al. Engineered Virus-like Particles for Efficient in Vivo Delivery of Therapeutic Proteins. *Cell* **2022**, *185*, 250–265.e16. https://doi.org/10.1016/j.cell.2021.12.021.
109. Haghighi, E.; Abolmaali, S.S.; Dehshahri, A.; et al. Navigating the Intricate In-Vivo Journey of Lipid Nanoparticles Tailored for the Targeted Delivery of RNA Therapeutics: A Quality-by-Design Approach. *J. Nanobiotechnol.* **2024**, *22*, 710. https://doi.org/10.1186/s12951-024-02972-w.