WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 4, 859-874. Review Article ISSN 2277– 7105

# A REVIEW ON GENOME EDITING (CRISPR-CAS 9) FOR CHRONIC DISEASES

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Article Received on
09 Feb. 2021,

Revised on 02 March 2021,
Accepted on 23 March 2021

DOI: 10.20959/wjpr20214-20143

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## ABSTRACT

Genome editing has entered another time with the beginning of CRISPR/Cas-9 Clustered Regulatory Interspaced Short Palindromic Repeats/CRISPR associated protein 9 innovations. This genome editing tool is no more, a quality altering apparatus is one kind of innovation that empowers geneticists and clinical researchers to alter the portion of the genome by evacuating, including, or modifying areas of the DNA (deoxyribonucleic acid). It is as of now the simplest, flexible, and precise method of genetic manipulation and is, therefore, causing a buzz in the genetic world. The purpose of this is to make a quick, successful, and adaptable genome-altering instrument to energize hereditary control. The tool made adjusted from commonly happening genes, altering framework in microorganisms. The microscopic organisms get bits of DNA from assaulting infection and use them to make DNA fragments known as CRISPR arrays, the CRISPR array consists of the number of spacers. This array permits the bacteria to remember the viruses. On the off chance that the viruses attack again, the bacteria produce (RNA) ribonucleic acid fragments from the arrays exhibits to focus on the virus DNA. The bacteria then use the protein Cas 9 or a comparable enzyme to cut the DNA apart, which disables the viruses. In this review, the article states that this framework is a noteworthy genome adjusting innovation with the possibility to make an assortment of novel therapeutics for an extent of illnesses, various of which regions of now rare immedicable.

**KEYWORDS:** CRISPR-CAS-9, genome editing tool, Cas cascade, cancer, diabetes mellitus, cardiovascular diseases.

## I. INTRODUCTION

This editing tool was first found in quite a while in prokaryotic cells and this framework is resembles a sort of immune framework in the bacterial or prokaryotic cells so it is a region on the bacterial genome. CRISPR locus is defined as an array of short direct repeats interspaced with spacer sequences and this spacer DNA are regular, between each two repeats, there is a DNA spacer. Editing tool length is somehow between 28-37 base pairs and spacer DNA length is between 32-38 base pairs. If bacteriophage (virus) infects the bacterial cell by attaching itself on the bacterial surface and infuses its genome inside the microorganism and the viral genome will drive the cell to create viral proteins and chemicals and afterward it will change the entire cell apparatus in the bacterial cell. Presently bacterial cell as a result of this framework can forestall this to happen a subsequent time by this way it is a versatile safe framework in human its a sort of memory to forestall a similar bacteriophage tainting the cell repeatedly.[1]

### Components of The Crispr Framework

The component of the CRISPR framework is a three-advance instrument and they are 1) spacer accession 2) crRNA preparing 3) Interference. Spacer accession, when an infection contaminate the cells just because the bacterial cell hack up the viral genome and take a bit of it and supplement this piece into the spacer DNA. However Spacer DNA isn't a bit of the distinctive viral genome that influence the cells already. Close to the CRISPR locus there is the Cas genes that gives Cas chemicals .Cas proteins act as a shield for prokaryotic cells and it will invade phages and plasmids by recognizing and split foreign nucleic acid sequence determined by CRISPR RNA spacers. As enzymes, in general, nearly all of them are nucleases and helicases. Nuclease can cut the DNA by cut the link in the nucleotide but Helicase can slash the hydrogen bonds between the two strands and then it can separate the two strands of DNA from each other cas1 and cas2, are the two key players in spacer acquisition.[2] The two are dimers and structure complex together so as to experience spacer acquistion.cas 1 is nuclease integrase so it will cut the viral genome and coordinates the bit of the genome in spacer DNA and Cas 2 is endoribonuclease and for the most part cut RNA, a few bacteriophages experience a RNA genome.[3] (crRNA handling CRISPR RNA preparing) in CRISPR locus we have various bits of viral DNA in spacer DNA. one of the two strands of the bacterial DNA is interpreted to messenger RNA and its corresponding to the CRISPR DNA i.e. lower strand and for this situation, we called lower strand as a coding strand expressed in (Fig:1) Three distinct kinds of crRNA preparing, In type 1 the CRISPR rehashes

are structure circles and the messenger RNA will be cut utilizing cas6e or cas6f compounds and end up with little bits of RNA each piece contains CRISPR grouping which structures circle and bit of the viral genome. These little bits of RNA considered as crRNAs was shown in (Fig:2). In type 2 key player of crRNA preparing is tracer RNA and this will bound to the CRISPR groupings of the mRNA and the mRNA is hacked up by Cas 9 and RNAase 3 and end up with little bits of RNA each piece contains CRISPR arrangement and bit of viral genome and tracrRNA(tracer CRISPR RNA)bound in the CRISPR succession. These little bits of RNA considered as crRNAs was shown in (Fig:3). In type 3 cas6 homolog is going to hack the mRNA legitimately and end with crRNAs carrying CRISPR repeats and the bit of the viral genome (Fig: 4).[4]

**Protospacer Adjacent Motif (Pam) And Types of Interfernce**

When the microorganism slashes the bit of the viral genome it doesn't take any piece of the viral genome it takes the viral genome which is adjoining the PAM arrangement so bacterial cell perceive the PAM grouping and afterward it takes the nearby succession so as to include into the spacer DNA and so as to frame RNA complex.Cas protein will perceive PAM succession it will build the explicitness of acknowledgment.[5] Interference, as a rule, crRNAs coordinated with Cas protein and structure an unpredictable, obstruction is a hereditary irritation strategy that allows for grouping explicit constraint of quality articulation. There are three kinds of the CRISPR framework, contingent upon the sort of bacterial cells crRNAs, Cas proteins, CRISPR grouping will vary. Type 1 contains crRNAs (each piece contains CRISPR arrangement which structures circle and bit of viral genome) viral genome (lower strand) will tie to the corresponding strand of bacterial RNA when this coupling happens it enact cas cascade course catalysts and these Cas course will create Cas 3 to cleave up viral genome, so in type 1 cas3 assists with hacking up the viral genome to wind up with the corrupted viral genome and this infection can't attack the cell any longer(fig 5). In type 2 the most significant and the principle player is cas9, PAM grouping is additionally significant in type 2, in light of the fact that for acknowledgment and furthermore, RNA arrangement will perceive DNA succession and afterward they will tie together by RNA is corresponding to bring down strand and they will tie together and Cas chemical itself experiences twofold strand break in the viral genome and it implies it breaks the two strands precisely at a similar spot Cas compound has two spaces and the catalyst used to do the twofold strand break are HNH, RuvC, RNase2H-like endonuclease domains(fig 6). In type 3 is basic and furthermore there is Cas protein and there is no PAM in type 3 and RNA succession perceive the

correlative in the viral genome and predicament together and there will likewise a Cas cascade course like sort 1 and viral genome will be cleaved up(fig7).[6]

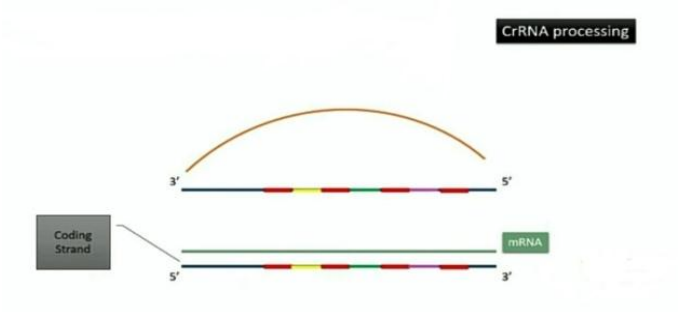


**Figure 1: the bacterial DNA is deciphered to messenger RNA**

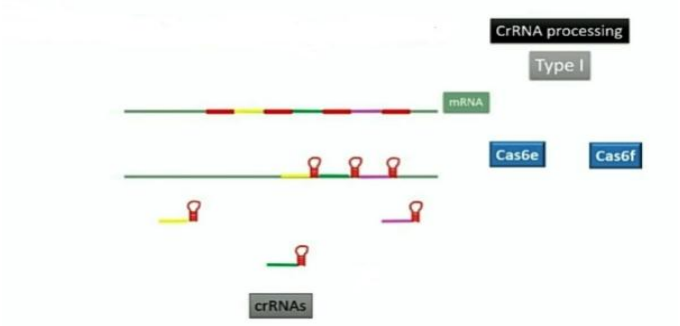


**Figure 2: little bits of RNA in type I mechanis.**

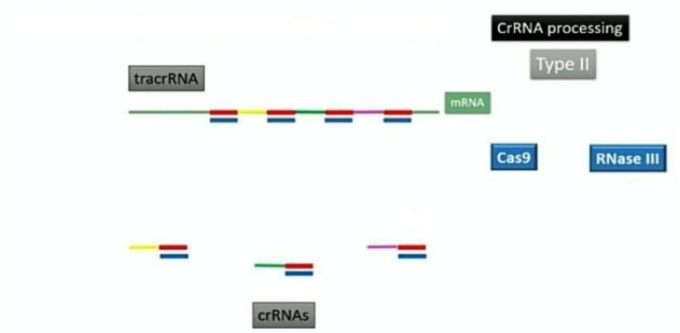


**Figure 3: little bits of RNA intype ii mechanism.**

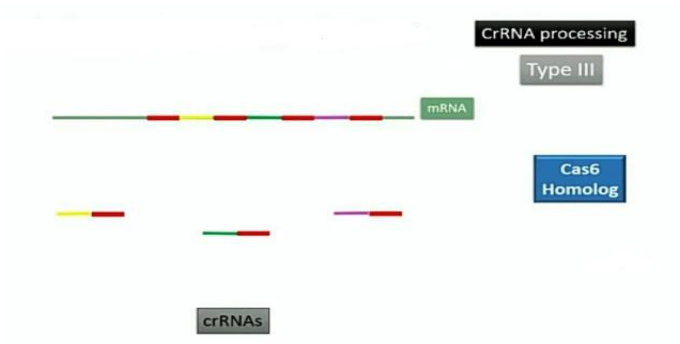


**Figure 4: little bits of RNA in type iii mechanism.**

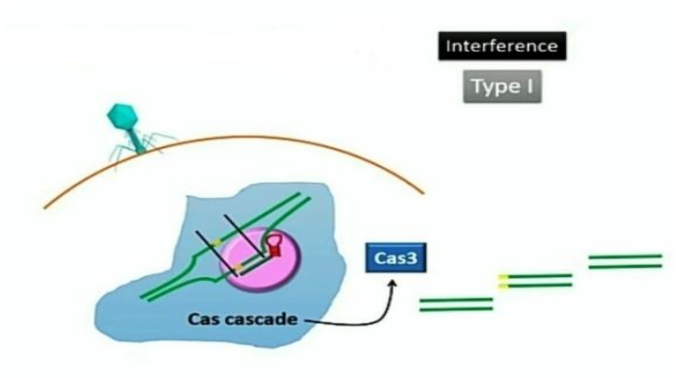


**Figure 5: cleave up viral genome using cas 3 enzyme.**

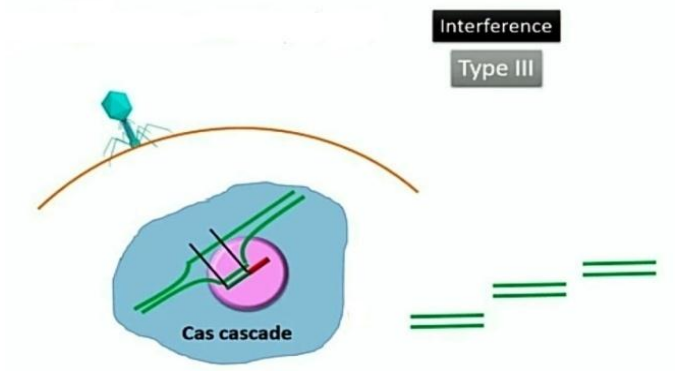


**Figure 6: double strand break.**

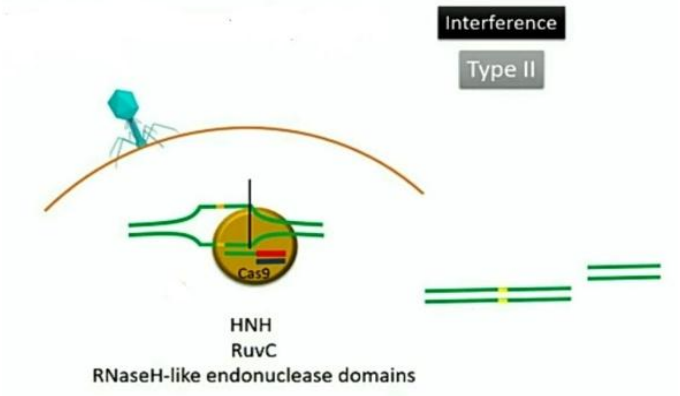


**Figure 7: genome will be cleaved up using casprotein.**

### Genome Editing Tool As Therapeutic Tool In Cancer

By the utilization of the gene editing tool instrument in the therapy of malignant growth became aggressive later for manage and initiate tumor generating transformation genome editing tool may be used. The advancement started with the research, that one concentrated high-chance of HR-HPV that was demonstrated to be a significant ingenious specialist related to human cervix malignant growth. Human papilloma virus contamination apparently causes

changes related to E6 and E7 qualities which assume crucial jobs in keeping up the harmful idea of disease. It was uncovered that utilizing an altered CRISPR framework editing tool human papilloma viruse type 16 E7 protien, guide RNA direct CRISPR/Cas structure), whichever upsets DNA tool of human papilloma viruse type 16 E7 protien at unequivocal regions, would realize programmed cell death and improvement limitation of HPV-positive SiHa and Caski cells, anyhow not in tool human papilloma viruse -negative C-33-A and HEK-293 cells. Moreover it notice that the aggravation of protien E7 DNA direct provoked that cell decreases the quantity of cellular component of E7 and upregulate of cancer silencer enzyme protein Rb. Additionally headways happened accompanied by the usage of the CRISPR in balanced structures to incite cancerogenesis in imitation animalia in significantly less mind boggling and invaluable path contemplating the ability to all the more promptly inspect the sickness and make dangerous development models. Direct change of harm delivering characteristics was finished. Hepatic cells of mice concentrating on Pten and p53 characteristics. As soon as, changes were affected simultaneously, shows that hepatic cancerous be made mirroring realized by Cre-loxP-interceded of Pten and p53 eradication. In the model that the Pten quality was changed on one's own, raised Akt phosphorylation and lipid storing up in hepatocytes were recognized; an end result of genocopying is the effects of abrogation of the quality by the use CreloxP development. The innovation has been stretched out and created to complete something other than producing creature models in relationship with disease contemplates. In myeloid malignancies, substantial changes of the epigenetic modifier and tumor silencer ASXL1 are normal. In an assessment did by scientists did an assessment were changes be modified in a myelogenousleukemia cell line (KBM5), that is accompanied by the guide of heterograft mice. Cell lines showed in a general sense longer continuance comparing to those organ graft with not unchecked cell lines. At this moment, the CRISPR/Cas9 gadget were utilized to change the ASLX1 purebred (homozygous) foolishness change accord in KBM5 cell lines. The amendment reestablished ordinary cell work and down regulated polycomb oppressive complex 2 (PCR2) target qualities.[9] In like manner, the CRISPR/Cas9 used effectively and components was seen in proficiently quieting the CDK 11. Cyclin dependent kinase is a quality imperative in the multiplication of osteogenic sarcoma cells. Monitoring by cyclin dependent kinase 11 is seen as fundamental in cell development and expansion, and quieting of the CDK11 quality apparently was related to diminished cell reasonability and multiplication and moreover saw to incite programmed cell death in KHOS and U 2O cell lines. A manner saw to be identified with diminished interruption and development of cells was the knockout quality. Further assessments finished

in this effect have uncovered knowledge into a gigantic proportion of information, especially as for the usage of viral transmitter passing on tranquilize inductive guide RNAs to changes the nature of interest via motivate Cas-9- Research undergo exhibited the sweeping substantiality about already stated structure in taking out changes similarly on the point of their selection easily. For example, myeloid leukemia cell partition protein, for the proceeded with continuance of human hodgkin's lymphoma (BL) cells, (myeloid cell leukemia-1) an antiapoptotic enzymes, is fundamental. By using a lentiviral CRISPR-Cas9 stage, research undergo exhibited the limit of controlling myeloid cell leukemia -1 in human BL cells which realized the programmed cell death of BL cells at a high repeat. In addition, in human BL heterograft replica in vivo, electrifying cancer backslide, else hindered improvement by the redo selection of guide RNAs was viewed. CRISPR-Cas9 arbitrate knockdown of explicit characteristics, for instance, SHCBP1 (SH2-region limiting protein 1) in chest harmful development cells in the test-tube and (kelch area containing 4) KLHDC4 innasopharynx cancer cells, both within and outside the living organism seemed to create good conclusion, accompanied by the two knockdown related to decreased increase of cells similarly as prevented cell movement and assault in the last referenced. To sum up the implementation of the CRISPR as editing tool was givenin(Table1).

**Table 1: Genome Editing Tool As Therapeutic Tool In Cancer.**

| Controlled gene | Target | Cell type | Species | Invivo/invitro | Delivery | End result | Ref |
|---|---|---|---|---|---|---|---|
| human papillomavirus type16 genome | coding DNA 7 | human papillomavirus negative C33 A HEK 293 : human papillomavirus positive SiHa and Caski cells | Human | Invitro | phosphoresence correspondent pSSA Rep3-1 plasmid | programmed cell death and development restraint of cells. No hindrance and programmed cell death. Downregulate of E7 enzyme and upright of cancer concealment enzyme pRb | [7] |
| phosphate and tensin homolog quality and p53 quality all the while | - | phosphate and tensin homolog quality and p53 quality all the while | Musmusculus | Invivo | Hydrokinetics infusion via convey of CRISPR associated protein9 and guide RNAs | Longer cell endurance was seen when contrasted with cells that were not amended. Typical cell work amended and downregulated polycomb oppressive complex 2 qualities (pten quality only). Liver tumors like those brought about by CreloxP interceded erasure of p53.By using Cre-loxp, deletion of Akt phosphorylation and lipid amassing phenocopying quality happen by cre-loxp | [8] |
| putative polycomb group protein ASXL1 homozygous | Genomic area covering AsLX1 transformations saw in KBM5 cells | KBM5 cell line | Musmusculus | Invivo | pSpCas9(BB)-2A-GFP transfection | Longer endurance in mice with Xenograft of remedied cell lines instead of this Xenografted with undiscplined cell lines. | [9] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| transformation | | | | | | Rectification of driver changes in leukemia cells builds endurance within a living mice by rectifying the changes in operator | |
| Cyclin dependent kinase 11 | fourth coding exon of Cyclin dependent kinase 11 | type of bone cancer that begin in the cells that form bones, KHOS and U 205 | Human | Invitro | U6g ribonucleic acid-crispr associated protein 9-2A-Green fluorescent protein | KHOS and U20 cell lines leads to programmed cell death. Relocation of cells, Diminished attack. | [10] |
| MCL 1 | Burkitt'stumour cells present in lymphatic system in human .Burkitt's lymphoma heterograft models. | - | Mouse | Invivo | . Dual lentiviral transmitter framework | programmed cell death of lymphoma cells at high recurrence. Tumor relapse or impeded development | [11] |
| SGCBP1 | estronol receptive MCH-7 and estronol insensitive MDA-MB-231 human breast cancer cell lines | - | Human | Invitro | LentiCRISPR/CAS9 trnasmitter | The inhibited proliferation of bosom malignant growth cells. | [12] |
| KLHDC4 | Targeting exon 5 of kelch domain containing 4 gene | Nasopharynx cancer cells | Musmusculus | In vivo | pX330 transfectable transmitter | Inhibited development, relocation, cell multiplication, movement of cells, and expanded apoptosis | [13] |

**Genome Editing Tool As Therapeutic Tool In Diabetes**

Fruitful period of insulin-insufficient porker by the interference of the INS quality by methods for CRISPS/Cas9 was practiced by a social affair of specialists possibly preparing for progressions inside the usage of porker models in considers.[14] Synchronous examinations manifest the unforeseen growth of rat models where co-microinjection of guide RNAs and and CRISPR associated protein messengerRNA assist in the period of Letine quality and LEP receptor quality knockdown murine. The previously mentioned murine seemed vague genotypic results in murine models including the usage of powerful and diabetiesmurine.[15] To make animal model in a reasonably worthwhile manner utilization of CRISPR has been accepted. By use of CRISPR Cas 9 animals used as models was widened to human juvenile microorganism. By the usage of the CRISPR/Cas9 contraption pluripotential cells of the human that are fit for isolating a cell and are self-restoring will change. At this moment changes started in the protein called (HNF) hepatocyte nuclear factor 1b quality that is well known to rise off diabetes mellitus by methods for underdevelopment of pancreatic parenchyma and beta-cell dysfunction. To summarize the use of CRISPR as a medicinal tool for diabetes mellitus appeared in (Table 2).

**Table 2: Genome Editing Tool As Therapeutic Tool In Diabetes.**

| Controlled gene | Target | Cell type | Species | Invivo/invitro | Delivery | End result | Ref |
|---|---|---|---|---|---|---|---|
| NS quality | coding DNA 2 and coding DNA 3 | Piggish essential mechanocyte cells | Piglet | In vivo | px458 vector | Successful models created for research | [14] |
| Letine or leptine receptor qualities | coding DNA 2 | C57BL/6J incipient organism (mice) | Mouse | Invivo | via genetic engineering of Cas9 messenger RNA and guide RNAs | genotypically indistinguishable from murine models including the utilization of large and diabetic murine | [15] |
| Hepatocyte atomic factor 1B | | Human iPSCs | Human | In vitro | Plasmid vectors | It gives broad knowledge about the impact and can see the improvement of diabetes and the sub-atomic instruments engaged with pancreatic turn of events by changes in HNF1B knockout | [16] |

**Genome Editing Tool As Therapeutic Tool In Cardiovascular Diseases**

The utilization of the CRISPR/Cas9 component has been appeared to be successful in the age of mouse models to better comprehend disease etiologies related to CVD. Later work distributed consolidates a broad overview that joins various pieces of the utilization of the CRISPR/Cas enzyme genetic makeup mechanical assembly in vascular system investigation close by its requirements. Research was expanded to consolidate model animal as zebra fish moreover. Analysts adequately used the CRISPR/Cas9 framework to alter zebra fish lacking living beings with a knockdown of a lamin A/C quality that is a quality similar to the human quality lamin A/C. The latter is viewed as obligated for starting stage cardiovascular conduction passing. Research are in the manner to finish the attempt revision and adjustment of changes identified with a high pace of the CVD. In one mice extended degree of PCSK9 should present in blood for that PCSK gene is highly responsible. which therefore causes an ascent of (LDL-C) low-thickness lipoprotein cholesterol by performing vice versa on low density lipoprotein structure effectively and at maximum level change of shapes or types can seen. In the examination end results captivating that greater than 50% of the mucrine exhibiting a maximum limit in the PCSK9 quality, by this way diminished degrees of LDL is seen. It was found that 30-45% of plasma glucose levels diminished. What is phenomenally significant was that the degree of to one side changes was in every way that really matters missing in the 10 picked regions. An all the more late assessment in February 2018 used an adjusted of the clustered interspaced short pallindromic repeats instrument known as (BE3) Base Editorial director 3. Base supervisor can convey transformation in the base of the genome. In assessment the particular structure will help in the needed areas by familiarizing changes in the thymine and cyosine. The genetic makeup changing mechanical assembly enlighten to make lost limit (ANGPTL3) which is identified with lessened risk of chronic heart disease and diminished blood lipids level and low density lipoprotein. Correspondingly, the essential for pig is a model in the examination of CVD, which are uncommonly searched for after in the medical speciality field, assessment owing to wide resemblances with individuals, was associated with the development of six biallelic (relating to two alleles of a solitary quality gene) knockdown swine. (ApoE)and (LDLR) are the characteristics be at the same time accompanied by the use of CRISPR/Cas9 structure, in a singular development knockdown models were created, subsequently making a perfect replica for the examination of Cardiovascular disease. To recapitulate the application of CRISPR as a remedial instrument for cardiovascular disease shown in (Table 3).

**Table 3: Genome Editing Tool As Therapeutic Tool In Cardiovascular Diseases.**

| Controlled gene | Target | Cell type | Species | Invivo/invitro | Delivery | End result | Ref |
|---|---|---|---|---|---|---|---|
| LMNA | - | 1-cell stage Daniorerio incipient organism | Daniorerio | Invivo | injection performed under microscope | representation for the investigation of beginning stage CCD. | [17] |
| PCSK9 quality | coding DNA 1 and coding DNA 2 of the PCSK9 quality | liver cells | Mouse | Invivo | Adenoviral conveyance | 50% of mucrine indicated misplaying of volume and decrease of LDL cholestrol levels | [18] |
| ANGPTL3 | - | Human iPSCs | Mouse | Invivo | Adenoviral transmiter | Reduced danger of CHD, diminished blood triglycerides, and LDL | [19] |
| ApoE and LDLR quality | coding DNA 2 of apo lipoprotein-E and LDLR quality | - | Swine | In vivo | pGL3-U6-gRNA-PGK-puromycin and CRISPR associated protein 9 communicating inclusion | fruitful age of swine models. | [20] |

## DISSCUSSION

Both of the basic perspectives via the medical specialty field to that the CRISPR/Cas9 enzyme mechanism compartment turned out to be familiar with the examination and analysis of hereditarily associated afflictions.[21] The grade of progress of the CRISPR/Cas gadget usage was wide. An immense measure of examination has been completed as for this, and the possibility to reach out past creature models that are presently being utilized have been extensively featured.[22] Besides, the utilization of the genome-altering device expands past genome altering itself. Be that as it may, this audit mostly centresaround the utilization of CRISPR innovation as a remedial device for some maladies. Malignancy includes numerous hereditary modifications. The beginning of malignancy commonly includes a progression of changes in genetic makeup bringing about cancer, absence of programmed cell death, and adjustments in epigenetic guidelines.[23] The CRISPR/Cas9 framework can alter various

qualities in equal and straightforwardly focus on the reasons for disease, and researchers have promiscuity numerous records Diabetes is a run of the mill multifactorial sickness that appears in individuals everything being equivalent and before long is a huge gust on overall human prosperity. Causes of diabetes are outstandingly factor. Type 1 and type 2 are the types of diabetes survived.[24] The past juvenile diabetes normally suggested as adolescent diabetes clearly connected via an addition or reducing in the repeat of unequivocal tissue-compatibility antigens operating the 6th RNA and with islet cell immunoglobulin, despite the fact that all the additionally affectionately alluded to as adolescent diabetes mellitus, this isn't, seen as definite as it would happen at all-time.[25] The WHO in 2017 set forward figures asserting these moving toward sentiments of fear. It onetime exhibited that the amount of individuals encountering diabetes has created from 108 million out of 1980 to 422 million by 2014 with an overall power of the infirmity among young adults past 18 years of age climbing to 8.5%.[26] It is too foreseen that diabetes will be the seventh driving purpose behind demise by 2030.[27] Cardiovascular disease (CVD) remains to exist the best explanation behind bleakness and mortality around the globe. In all around Late bits of knowledge show that Cardiovascular disease is liable for 31%[28] In the United States, alone 800,000 passings are realized by CVD in 2017; to place it in setting, it speaks to 1 in each 3 passings. Organizing the heredity medicate over weapons store for the challenge in case of Cardiovascular disease is essential.[29] By utilization of the CRISPR/cas9 framework and Inherited adjusting modifying instrument have mulled over a gigantic proportion of progression. In such a way, the illness revised by methods for genomic treatment, and the meaning of animal models expect a key activity. You can’t stop science from progressing Science is what it is (Martin Jinek, 2015).

## CONCLUSION

CRISPR is set to get one of the most principal essential science research apparatuses with wide application in science and medication. Further exploration and open commitment are expected to completely make an interpretation of CRISPR’s huge potential to human health.

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