CRISPR and Artificial intelligence to improve precision medicine: Future Perspectives and Potential Limitations

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Abstract

The CRISPR/Cas9 genome editing system is a unique and new technology that allows genetics and medical researchers to modify or edit parts of the genome. This is done by deleting, inserting or changing parts of the DNA sequence. This method is currently the simplest, most widely used, and the most accurate method of genetic manipulation. This system has great potential to treat a wide range of genetic diseases. However, more research is needed to determine the advantages and disadvantages of the CRISPR system and establish best practices. On the other hand, individual patient treatment is one of the main goals of the medical field. Achieving this goal has been elusive due to a complex set of factors affecting disease and health. This work highlights key advances in the development of enabling technologies that advance the goal of personalized and precision medicine. Artificial intelligence is a simulation of human intelligence for computers, and is used in the interface of machines that is programmed to think and behave like a human. This review summarizes recent developments in the artificial intelligence (AI), CRISPR/Cas9, and examining clinical trials and potential based on these two in the advancement and development of precision and personalized medicine, cancer treatment and current and future challenges. Finally, the application of (AI) in the modification of the CRISPR/Cas9 system is highlighted.

Keywords: CRISPR, precision medicine, Artificial intelligence (AI), Gene therapy, Cancer, Clinical Trials.

Introduction

The CRISPR/Cas technique is a new and powerful technology that, as a precise tool without the need for complex engineering, performs gene editing to selectively modify DNA sequences at any specific location in the genome with much greater precision than other genome editing

technologies such as TALEN and ZFN [1-3]. With the discovery of this system, it has created a revolution in the development of cell-molecular biology along with genetics with profitable applications in biotechnology and medicine [4]. In recent years, CRISPR/Cas has been an attractive research area for scientists due to its potential application to discover hidden and complex science and facts and its high potential to treat diseases behind the obscure and complex processes of life [5,6]. When the CRISPR/Cas system was identified in bacteria [7], Extensive research has been conducted to identify newer and more efficient CRISPR/Cas systems, with the aim of expanding the range of applications in molecular biology and genetic engineering [8]. CRISPR systems are found in about 40 percent of bacteria, which are part of an adaptive immune system against invading genetic material such as viruses [9,10]. So far, CRISPR-Cas systems have been classified into two classes and six main types, among which the CRISPR/Cas9 system has attracted much attention [8]. The CRISPR-Cas9 genome editing system is based on the activity of the protein endonuclease, Cas9, which is guided by a guide RNA (gRNA) to the desired location in the genome [11]. Another important factor for the proper functioning of the system is another sequence called Protospacer Adjacent Motif (PAM), which is located near the target site and is identified by the CRISPR/Cas9 system and is very important for the proper functioning of Cas9 and the reduction of off-targets[12]. The Cas9 protein binds to the target site with high fidelity in the presence of gRNA and activates a double-strand break followed by repair mechanisms [13,14]. The Origins of many human diseases is related to genetic defects[15,16]; a better understanding of human genetic structure has been of interest to researchers and scientists for years [17]. But unfortunately, due to the complexity and high volume of required data, the progress in this field was stopped [18-20]. With the development and progress in the applications of AI and machine learning in biology, researchers can interpret genomic data more completely and better, and ultimately make decisions through genetic sequencing to treat patients through gene editing techniques [21,22]. One of the most interesting aspects of genetic technology is its contribution to the development of precision and personalized medicine in the treatment of diseases [23,24]. This enables the development of unique and specific medical services for a patient or a population of individuals with a similar genetic structure [25]. In the past, the limiting factors in the development of personalized medicine for patients were considered to be cost and technology [26,27]; But AI as well as machine learning methods help to overcome these obstacles [28]. It has become possible to identify patterns in genetic data sets with the help of machines, after

which AI can predict the probability of a person contracting a disease or reacting to drug interactions [29].

In other words, personalized medicine involves using technologies to acquire and critically evaluate one's own data for the sole purpose of treating one's self [30]. For example, this might involve using AI to design drug combinations based on the patient's own sample, followed by specific dosing protocols, depending on the widespread deployment of both [31].
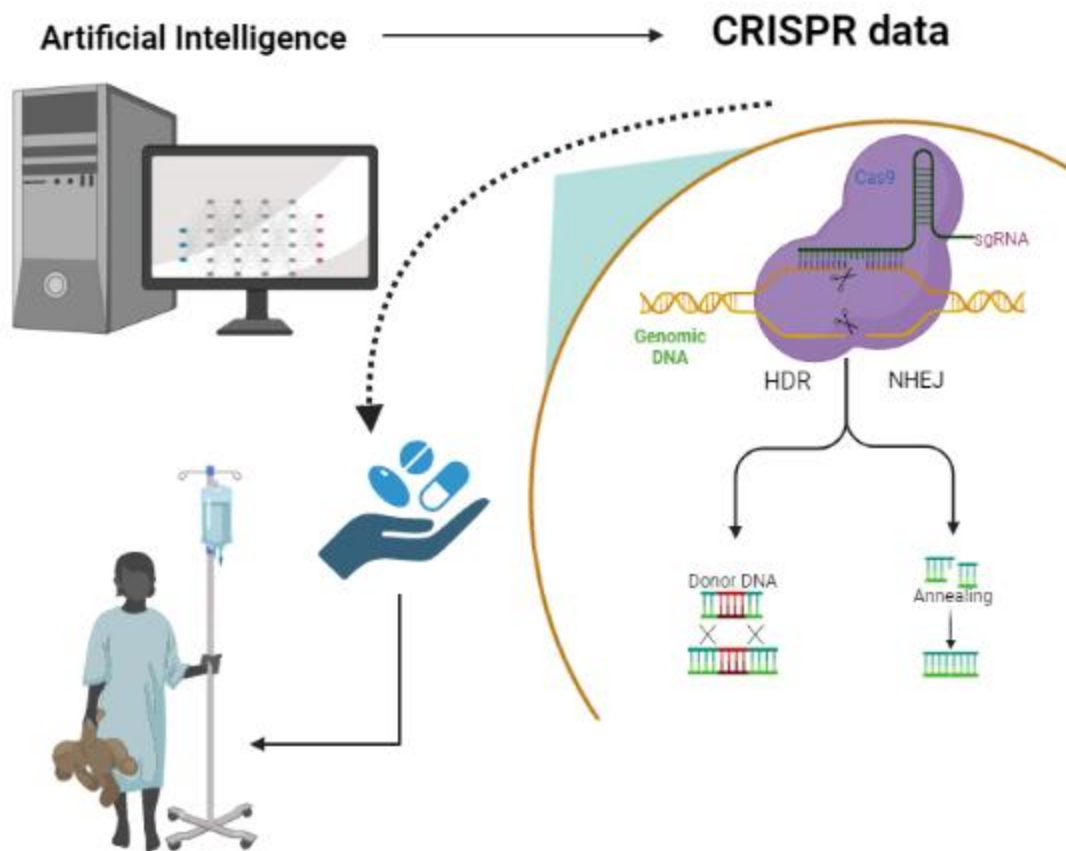

Figure 1: Schematic illustration of the power and potential of CRISPR gene editing system along with artificial intelligence for data analysis and treatment of genetic patients in the form of precise and personalized medicine.

Artificial intelligence and Precision medicine

Precision and personalized medicine, excellent and efficient and powerful technologies and smart policy choices that open a door to disease prevention, optimal targeted treatments and a fundamental change in the way doctors and patients think. As a practical example, the 2013 SPIRIT Statement (Standard Protocol Items: Recommendations for Interventional Trials) aims to improve overall clinical trial protocol reporting by providing evidence-based recommendations for a minimum set of items to be considered [32]. This guidance has been effective in promoting transparent evaluation of new interventions. Recently, it has been recognized that AI-related interventions must undergo rigorous and prospective evaluation to demonstrate their impact on health outcomes. In general, bioengineering is the foundation for the implementation of precision and personalized medicine and related enabling technologies in the clinic (Table 1), as knowledge gained from traditional biomedical fields makes its way to clinical solutions through engineering tools and approaches. Currently, 890 clinical trials have been registered on https://clinicaltrials.gov/ (some of them are in different phases in Table 1) and this number is increasing. Engineers in general will play an important role in the advancement of personalized and precision medicine, although it may not yet be entirely clear how their enabling technologies will provide solutions to the many unmet clinical needs [30].

Table 1: Clinical Trials Involving Artificial intelligence

Technology Platform	Status	diseases	Intervention/treatment	Clinical phase	NCT Number
Artificial intelligence	Completed Has Results	Depression Anxiety	Behavioral: IntelliCare Behavioral: Hub App with the Recommender System Behavioral: Coaching	Not Applicable	NCT02801877
Artificial intelligence	Completed Has Results	Back Pain	Behavioral: Behavioral: AI-CBT Behavioral: Behavioral: Standard Telephone CBT	Not Applicable	NCT02464449
Artificial intelligence	Completed Has Results	Venous Thrombosis Atrial Fibrillation Myocardial Infarction	Device: Genomics	Not Applicable	NCT00872079
Artificial intelligence	Completed Has Results	Major Depressive Disorder	Behavioral: IntelliCare	Not Applicable	NCT02176226

		Anxiety Disorders			
Artificial intelligence	Completed Has Results	Bone Age	Device: BoneAgeModel	Not Applicable	NCT03530098
Artificial intelligence	Completed Has Results	Colonic Polyp	Device: AI polyp detection system based on deep learning	Not Applicable	NCT04693078
Artificial intelligence	Completed Has Results	Hypertension	Device: optima4BP Medication Management	Not Applicable	NCT02988193
Artificial intelligence	Recruiting	Breast Cancer Prostate Cancer Quality of Life Survivorship Toxicity	Other: ASCAPE-based follow-up strategy	Phase 2	NCT04879563
Artificial intelligence	Recruiting	Lung Cancer, Non-small Cell	Drug: Camrelizumab + Nab-paclitaxel + Carboplatin	Phase 2	NCT04541251
Artificial intelligence	Recruiting	Cervical Cancer	Drug: Hyperpolarized 13C Pyruvate	Phase 2	NCT04951921
Artificial intelligence	Active, not recruiting	Head and Neck Squamous Cell Carcinoma	Radiation: Intensity modulated radiation therapy (IMRT)	Phase 2	NCT03953976
Artificial intelligence	Recruiting	Solid Tumor	Device: CURATE.AIDrug: CapecitabineDrug: XELOXDrug: XELIRIDrug: Ibrutinib	Phase 1/Phase 2	NCT04522284
Artificial intelligence	Not yet recruiting	Solid TumorGastrointestinal CancerBreast Cancer	Device: QPOP Device: CURATE.AI Drug: Azacitidine + docetaxel Drug: Azacitidine + paclitaxel Drug: Azacitidine + irinotecan	Phase 1/Phase 2	NCT05381038
Artificial intelligence	Recruiting	Solid Tumor	Device: CURATE.AI Drug: Nivolumab, Pembrolizumab	Phase 1/Phase 2	NCT05175235
Artificial intelligence	Unknown	Community-associated Infections Health-care	Other: antibiotic treatment of by TREAT/PCR	Phase 3	NCT01338116

		Acquired Infections Nosocomial Infections			
Artificial intelligence	Completed	Type 2 Diabetes	Drug: AI assisted insulin system Drug: Physician based insulin regime	Phase 4	NCT04053959
Artificial intelligence	Recruiting	First Episode Psychosis	Other: Smartphone App Other: Usual Care	Phase 4	NCT04046497
Artificial intelligence	Recruiting	Exudative Macular Degeneration	Drug: anti-VEGF agent	Phase 4	NCT05093374
Artificial intelligence	Not yet recruiting	Hepatocellular Carcinoma	Drug: Irinotecan Drug: Sonidegib Drug: Sorafenib	Phase 1	NCT05669339
Artificial intelligence	Recruiting	the Application of Artificial Intelligence in the Diagnosis of Prostate Cancer	Diagnostic Test: the clinical use of artificial intelligence in the diagnosis of prostate cancer	-	NCT05513638
Artificial intelligence	Recruiting	Multiple Myeloma	Drug: Bortezomib Drug: Cyclophosphamide Drug: Dexamethasone Other: CURATE.AI-Guided dosage modulation Drug: Thalidomide	Phase 2/Phase 3	NCT03759093
Artificial intelligence	Recruiting	Facial Discrimination Multitasking	Behavioral: MATB	Not Applicable	NCT03832101
Artificial intelligence	Completed	HIV Infections	Drug: Tenofovir(TDF)+Lamivudine(3TC)+Efavirenz(EFV)	Phase 4	NCT02632474
Artificial intelligence	Completed	Liver Transplant Kidney Transplant	Other: PPM-based Computation Assisted Drug Dosing Drug: Tacrolimus	Phase 2	NCT03527238

Artificial intelligence and CRISPR/Cas9

Many properties or modulators of gRNA have been identified that affect the efficiency of cleavage of the resulting gRNA and its off-targets, and many of them are still unknown to scientists. In addition to properties of gRNA in unwanted and off-target effects, gRNA sequence, use of site-specific nucleotides in gRNA design, specific nucleotide composition in flanking positions, protospacer proximity (PAMs), secondary structure of gRNA and TracRNA, features Epigenetics, extracellular and intracellular immune barriers and many other factors that have not yet been discovered but are influential[33,34]. Considering gRNA design in silico as a critical parameter in successful gene editing by CRISPR-Cas9 genome editing system, scientists are currently focused on modifying gRNA design with high efficiency and efficiency and minimal off-target effects [35]. Later investigate has portrayed different computations that offer assistance anticipate the different on- and off-target impacts of CRISPR gRNAs, hence progressing the action and the individual specificity of the CRISPR-Cas9 framework [36]. These prescient apparatuses have essentially made strides program advance and the victory rate of the CRISPR. Since it is possible, the efficiency of gRNA is highly dependent on various factors, investigation of cellular environment, gRNA and target action pathway, experimental conditions, which challenges can be overcome by machine learning (ML) based computation [37,38]. ML models are prepared utilizing existing datasets and can be utilized to foresee on/off target impacts of test datasets. Progressed ML has empowered the sending of DL strategies such as manufactured neural systems (ANN) for the CRISPR-Cas9 system [39,40], which empowers high-precision target expectation. In the CRISPR/Cas9 system, DL models are composed of several layers of interconnected computing units [39].

Strategies based on artificial intelligence and deep learning have now become extremely important in gRNA and CRISPR applications[41]. This method uses algorithms based on a growing gene editing data set reported globally and helps to predict activity and score gRNA features for best CRISPR performance [42]. In addition, compared to experimental detection tools such as IDLV, GUIDE-seq or HTGTS, MDL-based methods are more cost-effective, efficient and faster. Some of the MDL-based computational algorithms developed to predict CRISPR sufficiency and effectiveness on target are: , WU-CRISPR , CRISPRScan , CRISPRater, DeepCRISPR ,

Azimuth 2.0 , DeepCas9, SgRNAScorer, DeepHF, CRISPRpred , TUSCAN, C-RNNCrispr and CNN-SVR [43 39,44-50 41].

The effectiveness of MDL-based prediction tools in different types of cells is not yet fully known [51]. Development of species-specific software such as CRISPRscan for zebrafish [50] and fryCRISPR for Drosophila [52] is under consideration due to species diversity. Algorithms including dsNickFury, FlashFry, CNN_std, Elevation and DeepCRISPR have been described to help predict important off-target effects of the CRISPR-Cas9 system [41]. The results of the studies have shown that the off-target effects of CRISPR, which may not always be random, may be prevented by the proper design of gRNA sequences [53]. Furthermore, studies have shown that gRNA truncation, especially the 5′ end, as well as the addition of some chemicals may reduce the possible off-target abundance [54,55].

Hence, the calculation of gRNA design serves as a fundamental advance that may reveal the correct application and productivity improvement of CRISPR. In any case, the current algorithms and models suffer from disadvantages such as the lack of training datasets, heterogeneity and imbalance in data, inapplicability between species, which must be corrected before being fully used in the treatment of diseases in medical centers. It is anticipated that possibly with the development of more advanced and updated algorithms, the CRISPR/Cas9 system will advance its compensatory movement with negligible off-target effects, which could be an essential prerequisite in its clinical and regenerative applications.

Challenges and suggestions

Along with the CRISPR editing system, artificial intelligence is facing many challenges in all fields, especially the treatment of diseases. The integration of these two may help solve each other's challenges and cause diseases to be treated with less error and faster. To achieve these goals, some researchers are trying to expand the potential of basic editors.

In this part, some challenges related to Artificial intelligence and CRISPR technique will be discussed, and solutions will be provided to improve and develop them and solve the challenges.

Challenges of Artificial intelligence

The application of artificial intelligence in many advanced countries in all fields, especially the treatment of diseases, faces many challenges [56]. The main challenge that they deal with in the application of artificial intelligence is related to people and manpower, data and information required or preferences and trade balances and legal issues [57].

Artificial intelligence (AI) algorithms, taking advantage of high-performance computing capabilities, can now achieve reasonable success in predicting risk in some cancers and cardiovascular diseases from multidimensional clinical and biological data [58,59]. In contrast, less progress has been made in neurodevelopmental disorders, which include intellectual disability (ID), autism spectrum disorder (ASD), epilepsy, and broader neurodevelopmental disorders [60,61]. Great hopes have been placed on the opportunity to quantify risk arising from patterns of genomic variation, including functional characterization of genes and variants, but this ambition is confounded by phenotypic and causal heterogeneity, along with the rare and variable penetrant nature of underlying risk variants. So far, structural and functional brain imaging and neuropsychological and neurophysiological markers may provide additional dimensions, but often require further development to achieve sensitivity for diagnosis. In general lies a puzzle in precision medicine: Can artificial intelligence make advances in risk prediction and prognostication of disorders and diseases? In this review, we explore these complexities and consider some strategies that AI might overcome.

Challenges of CRISPR

We concur that CRISPR/Cas9 may be a broadly utilized quality altering development, but it has several problems due to off-target effects, efficacy, and safety challenges. Another major challenge in applying CRISPR-Cas technology to living organisms is the lack of an efficient delivery system. Encapsulating CRISPR components in a vehicle is challenging due to the larger size of Cas proteins in this tool. As a suggestion for researchers, we can use artificial intelligence and computational tools to design compact size Cas proteins to be more efficient than existing Cas proteins. A peak transfection method for CRISPR/cas9 should have high transfection efficiency, significant capacity concentration, and ease of mass production. However, current strategies are still far from achieving these goals. Consideration of the PAM sequence is critical for sgRNA design and is limiting, although essential for CRISPR/Cas9. Creating designable PAMs with the help of artificial intelligence is very important to expand the application of

CRISPR/Cas9. Antibodies against Cas9 that have been widely detected in the human body represent a potential risk for the CRISPR/Cas9 technique to enter therapy. Also, paying attention to the immunity created by carriers, especially viruses, is another challenge because the patient may have already been involved with that type of virus. An important limitation and risk in gene therapy using the CRISPR/Cas9 technique is the off-target effect. The design and selection of meaningful gRNA features to predict the efficiency of a process requires a good knowledge of the mechanism of CRISPR gene editing, which still exists and is not fully resolved. Furthermore, feature identification can be extended to hypothesis generation and CRISPR design and deep learning experiments. Although several computer programs have optimized the design of sgRNA, its specificity cannot be fully guaranteed, and perhaps artificial intelligence can help researchers better predict effects. For example, the DeepCRISPR evaluation showed that insufficient data renders predictive models ineffective, even when data augmentation techniques are used. However, different methods of combining data sets can lead to different results.

Future perspective

CRISPR/Cas9 techniques as a powerful gene editing technology due to its accuracy and efficiency can provide an incredible opportunity to treat several gene-related diseases by deleting, inserting, regulating and blocking different genes. The CRISPR genome editing technique offers an advanced approach to cancer screening and treatment, which is done by activating tumor suppressor genes and removing oncogenes alone or in combination, which is a smart approach to cancer treatment. One of the applications of this The strategy of producing CAR-T cells by the Cas9 system, which has successfully reached the stages of clinical trials. Of course, a number of problems mentioned in this technique remain unsolved, but in this case, CRISPR/Cas9 is an efficient tool for editing. It is a gene, most scientists and researchers came to the same conclusion, but it is still not an ideal treatment approach.

What exactly will happen in the future of artificial intelligence is still unclear. There is no definitive consensus on the definitions of what artificial intelligence is. In fact, AI means that humans are constantly trying to make machines behave smarter and use it to help humanity. It should be mentioned that the main and important effects of artificial intelligence have not yet been observed and it is the duty of all researchers to play their role because artificial intelligence will play a role in the future.

Until a few years ago, the future of artificial intelligence was chatbots and image generators such as ChatGPT and Midjourney, which have been available to the public for some time and are expected to achieve significant improvements in the next few years. For example, the company OpenAI is working on the fourth version of the GPT big language model, which Silicon Valley people claim is going to do wonders in the world of chatbots. At one time, the idea that two people with two different languages could talk to each other and understand each other at the same time was only possible in science fiction stories and Mass Effect games; But it is not unlikely that artificial intelligence will turn such an idea into reality.

Once researchers can sequence and interpret DNA, AI systems will do it faster, cheaper and more reliably, gaining insight into the essential genetic code that organizes the behaviors of all organisms. With all this information, the researcher will be able to make informed choices about where an organism may be vulnerable in the future, what kinds of changes lead to multiple diseases, and how to plan to deal with them in the future. . Given that individual conditions achieved throughout life are largely determined by unique genetics, further understanding of human genetic makeup has been of interest over the years—the complexity and value of the activities to be assessed hindered our progress. Thanks to advances in machine learning and artificial intelligence, scientists can make advanced assessments and interpret genomic data by decoding and modifying DNA. The landscape of artificial intelligence and genetic engineering is predicted to include precision medicine, pharmacogenomics, genetic screening tools for newborns, advances in agriculture, and more. While the future remains unpredictable, we are convinced that machine learning and artificial intelligence will accelerate our understanding of the genetic makeup of humans and other living organisms.

References

1.   Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal transduction and targeted therapy*. 2020;5(1):1.
2.   Hwang S, Maxwell KL. Diverse mechanisms of CRISPR-Cas9 inhibition by type II anti-CRISPR proteins. *Journal of Molecular Biology*. 2023:168041.
3.   Khoshandam M, Soltaninejad H, Mousazadeh M, Hamidieh AA, Hosseinkhani S. Clinical applications of the CRISPR/Cas9 genome-editing system: Delivery options and challenges in precision medicine. *Genes & Diseases*. 2023;

4.   Zhang F. Development of CRISPR-Cas systems for genome editing and beyond. *Quarterly Reviews of Biophysics*. 2019;52:e6.

5.   McGuire AL, Gabriel S, Tishkoff SA, et al. The road ahead in genetics and genomics. *Nature Reviews Genetics*. 2020;21(10):581-596.

6.   Foulkes AL, Soda T, Farrell M, Giusti-Rodríguez P, Lázaro-Muñoz G. Legal and ethical implications of crispr applications in psychiatry. *North Carolina law review*. 2019;97(5):1359.

7.   Wang Y, Liu KI, Sutrisnoh N-AB, et al. Systematic evaluation of CRISPR-Cas systems reveals design principles for genome editing in human cells. *Genome biology*. 2018;19:1-16.

8.   Bhatia S, Yadav SK. CRISPR-Cas for genome editing: Classification, mechanism, designing and applications. *International Journal of Biological Macromolecules*. 2023:124054.

9.   Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. *Science*. 2010;327(5962):167-170.

10.   Marino ND. Phage against the machine: discovery and mechanism of type V anti-CRISPRs. *Journal of Molecular Biology*. 2023:168054.

11.   Didovyk A, Borek B, Tsimring L, Hasty J. Transcriptional regulation with CRISPR-Cas9: principles, advances, and applications. *Current opinion in biotechnology*. 2016;40:177-184.

12.   Zhao C, Zheng X, Qu W, et al. CRISPR-offinder: a CRISPR guide RNA design and off-target searching tool for user-defined protospacer adjacent motif. *International journal of biological sciences*. 2017;13(12):1470.

13.   Ray U, Raghavan SC. Modulation of DNA double-strand break repair as a strategy to improve precise genome editing. *Oncogene*. 2020;39(41):6393-6405.

14.   Pickar-Oliver A, Gersbach CA. The next generation of CRISPR–Cas technologies and applications. *Nature reviews Molecular cell biology*. 2019;20(8):490-507.

15.   Passarge E. Origins of human genetics. A personal perspective. *European Journal of Human Genetics*. 2021;29(7):1038-1044.

16.   Oyouni AAA. Biological and genetic basis of various human genetic disorders and the application of biological and genetic markers. *Journal of King Saud University-Science*. 2022:101961.

17.   Moraes F, Góes A. A decade of human genome project conclusion: Scientific diffusion about our genome knowledge. *Biochemistry and Molecular Biology Education*. 2016;44(3):215-223.

18.   Campbell EG, Clarridge BR, Gokhale M, et al. Data withholding in academic genetics: evidence from a national survey. *jama*. 2002;287(4):473-480.

19.   Bellazzi R, Zupan B. Predictive data mining in clinical medicine: current issues and guidelines. *International journal of medical informatics*. 2008;77(2):81-97.

20.   Butler JM. *Forensic DNA typing: biology, technology, and genetics of STR markers*. Elsevier; 2005.

21.   Dias R, Torkamani A. Artificial intelligence in clinical and genomic diagnostics. *Genome medicine*. 2019;11(1):1-12.

22.   MacEachern SJ, Forkert ND. Machine learning for precision medicine. *Genome*. 2021;64(4):416-425.

23.   Pokorska-Bocci A, Stewart A, Sagoo GS, Hall A, Kroese M, Burton H. 'Personalized medicine': what's in a name? *Personalized Medicine*. 2014;11(2):197-210.

24.   Chen R, Snyder M. Promise of personalized omics to precision medicine. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*. 2013;5(1):73-82.

25.   Ginsburg GS, Willard HF. Genomic and personalized medicine: foundations and applications. *Translational research*. 2009;154(6):277-287.

26.   Lightbody G, Haberland V, Browne F, et al. Review of applications of high-throughput sequencing in personalized medicine: barriers and facilitators of future progress in research and clinical application. *Briefings in bioinformatics*. 2019;20(5):1795-1811.

27.   Nicholls SG, Wilson BJ, Castle D, Etchegary H, Carroll JC. Personalized medicine and genome-based treatments: why personalized medicine≠ individualized treatments. *Clinical Ethics*. 2014;9(4):135-144.

28.   Barberis E, Khoso S, Sica A, et al. Precision Medicine Approaches with Metabolomics and Artificial Intelligence. *International Journal of Molecular Sciences*. 2022;23(19):11269.

29.   Zitnik M, Nguyen F, Wang B, Leskovec J, Goldenberg A, Hoffman MM. Machine learning for integrating data in biology and medicine: Principles, practice, and opportunities. *Information Fusion*. 2019;50:71-91.

30.   Jain KK. Personalized medicine. *Current opinion in molecular therapeutics*. 2002;4(6):548-558.

31.   Ho D, Quake SR, McCabe ER, et al. Enabling technologies for personalized and precision medicine. *Trends in biotechnology*. 2020;38(5):497-518.

32.   Rivera SC, Liu X, Chan A-W, et al. Guidelines for clinical trial protocols for interventions involving artificial intelligence: the SPIRIT-AI extension. *The Lancet Digital Health*. 2020;2(10):e549-e560.

33.   Hiranniramol K, Chen Y, Wang X. CRISPR/Cas9 guide RNA design rules for predicting activity. *RNA Interference and CRISPR Technologies: Technical Advances and New Therapeutic Opportunities*. 2020:351-364.

34.   Manghwar H, Li B, Ding X, et al. CRISPR/Cas systems in genome editing: methodologies and tools for sgRNA design, off-target evaluation, and strategies to mitigate off-target effects. *Advanced science*. 2020;7(6):1902312.

35.   Konstantakos V, Nentidis A, Krithara A, Paliouras G. CRISPR–Cas9 gRNA efficiency prediction: an overview of predictive tools and the role of deep learning. *Nucleic Acids Research*. 2022;50(7):3616-3637.

36.   Wilson LO, O'Brien AR, Bauer DC. The current state and future of CRISPR-Cas9 gRNA design tools. *Frontiers in pharmacology*. 2018;9:749.

37.   Teng SY, Yew GY, Sukačová K, Show PL, Máša V, Chang J-S. Microalgae with artificial intelligence: A digitalized perspective on genetics, systems and products. *Biotechnology advances*. 2020;44:107631.

38.   Dimauro G, Barletta VS, Catacchio CR, Colizzi L, Maglietta R, Ventura M. A systematic mapping study on machine learning techniques for the prediction of CRISPR/Cas9 sgRNA target cleavage. *Computational and Structural Biotechnology Journal*. 2022;

39.   Zhang G, Dai Z, Dai X. C-RNNCrispr: Prediction of CRISPR/Cas9 sgRNA activity using convolutional and recurrent neural networks. *Computational and structural biotechnology journal*. 2020;18:344-354.

40.   Zhang G, Zeng T, Dai Z, Dai X. Prediction of CRISPR/Cas9 single guide RNA cleavage efficiency and specificity by attention-based convolutional neural networks. *Computational and structural biotechnology journal*. 2021;19:1445-1457.

41.   Bhat AA, Nisar S, Mukherjee S, et al. Integration of CRISPR/Cas9 with artificial intelligence for improved cancer therapeutics. *Journal of Translational Medicine*. 2022;20(1):534.

42.   Wang J, Zhang X, Cheng L, Luo Y. An overview and metanalysis of machine and deep learning-based CRISPR gRNA design tools. *RNA biology*. 2020;17(1):13-22.

43.   Gratz SJ, Ukken FP, Rubinstein CD, et al. Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in Drosophila. *Genetics*. 2014;196(4):961-971.

44.   Zhang G, Dai Z, Dai X. A novel hybrid CNN-SVR for CRISPR/Cas9 guide RNA activity prediction. *Frontiers in genetics*. 2020;10:1303.

45.   Wang D, Zhang C, Wang B, et al. Optimized CRISPR guide RNA design for two high-fidelity Cas9 variants by deep learning. *Nature communications*. 2019;10(1):4284.

46.   Rahman MK, Rahman MS. CRISPRpred: a flexible and efficient tool for sgRNAs on-target activity prediction in CRISPR/Cas9 systems. *PloS one*. 2017;12(8):e0181943.

47.   Wong N, Liu W, Wang X. WU-CRISPR: characteristics of functional guide RNAs for the CRISPR/Cas9 system. *Genome biology*. 2015;16(1):1-8.

48.   Xue L, Tang B, Chen W, Luo J. Prediction of CRISPR sgRNA activity using a deep convolutional neural network. *Journal of chemical information and modeling*. 2018;59(1):615-624.
49.   Chuai G, Ma H, Yan J, et al. DeepCRISPR: optimized CRISPR guide RNA design by deep learning. *Genome biology*. 2018;19:1-18.
50.   Wilson LO, Reti D, O'Brien AR, Dunne RA, Bauer DC. High activity target-site identification using phenotypic independent CRISPR-Cas9 core functionality. *The CRISPR Journal*. 2018;1(2):182-190.
51.   Chuai G-h, Wang Q-L, Liu Q. In silico meets in vivo: towards computational CRISPR-based sgRNA design. *Trends in biotechnology*. 2017;35(1):12-21.
52.   Listgarten J, Weinstein M, Kleinstiver BP, et al. Prediction of off-target activities for the end-to-end design of CRISPR guide RNAs. *Nature biomedical engineering*. 2018;2(1):38-47.
53.   Naeem M, Majeed S, Hoque MZ, Ahmad I. Latest developed strategies to minimize the off-target effects in CRISPR-Cas-mediated genome editing. *Cells*. 2020;9(7):1608.
54.   Han HA, Pang JKS, Soh B-S. Mitigating off-target effects in CRISPR/Cas9-mediated in vivo gene editing. *Journal of Molecular Medicine*. 2020;98(5):615-632.
55.   Bendixen L, Jensen TI, Bak RO. CRISPR/Cas-mediated transcriptional modulation: The therapeutic promises of CRISPRa and CRISPRi. *Molecular Therapy*. 2023;
56.   Cui M, Zhang DY. Artificial intelligence and computational pathology. *Laboratory Investigation*. 2021;101(4):412-422.
57.   Tambe P, Cappelli P, Yakubovich V. Artificial intelligence in human resources management: Challenges and a path forward. *California Management Review*. 2019;61(4):15-42.
58.   Shameer K, Johnson KW, Glicksberg BS, Dudley JT, Sengupta PP. Machine learning in cardiovascular medicine: are we there yet? *Heart*. 2018;104(14):1156-1164.
59.   Bhinder B, Gilvary C, Madhukar NS, Elemento O. Artificial intelligence in cancer research and precision medicine. *Cancer discovery*. 2021;11(4):900-915.
60.   Gupta C, Chandrashekar P, Jin T, et al. Bringing machine learning to research on intellectual and developmental disabilities: Taking inspiration from neurological diseases. *Journal of Neurodevelopmental Disorders*. 2022;14(1):28.
61.   Uddin M, Wang Y, Woodbury-Smith M. Artificial intelligence for precision medicine in neurodevelopmental disorders. *NPJ digital medicine*. 2019;2(1):112.