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CRISPR-Cas13d-mediated transcriptome editing and in silico off-target landscape prediction for RNA therapeutics: A review

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ABSTRACT

CRISPR-Cas13d is a kind of smaller RNA-targeting nuclease. It shows an increasing number of uses in RNA therapeutics and transcriptome engineering. However, its main advantage over DNA-editing systems is its relatively low activity towards the genome. This study therefore assessed the effectiveness of Cas13d for predicting off-target effects using modern machine learning tools to target of mammalian transcriptome. The CRISPR-Cas13d guides RNAs (crRNAs) were design for the Cas13d (RfxCas13d/CasRx) system with the intention of targeting the human transcriptome. Thereafter, high-throughput cellular assessments were done to measure the performance of the cells and their viability. The data was used to both construct and verify machine learning models, such as the CNN-based TIGER predictor and DeepCas13, a hybrid CNN–RNN framework. We gauged crucial quantitative indices, which included the AUC, sensitivity, accuracy, and specificity parameters. The result of the prediction model was crosschecked for accuracy with the experimental result, with the use of qRT-PCR technique. It was thereafter compared with nonessential gene controls to reduce the false predictions. The comparative analysis revealed a remarkable improvement in predictive power when transitioning from an empirical rule-based model to a machine learning model. It was observed that the DeepCas13 model outperformed the TIGER model. The former recorded an AUC of ~0.90 and an accuracy of ~85%, while the latter had an AUC of ~0.82 and an accuracy of ~78%. Also, it improved interpretability by combining sequence, structural, and contextual RNA characteristics than the TIGER model. The ROC and radar curve analyses consistently demonstrated that advanced deep learning frameworks reduced off-target errors while maintaining high sensitivity. We used both CRISPR-Cas13d and modern deep learning models to predict the outcomes of direct-to-edit editing in the gene sequence. Hence, modern machine learning tools, especially DeepCas13, have shown promising prospective in the development of RNA therapeutics for research and clinical applications by proffering solutions to the limitations that come with using computational approaches in CRISPR-Cas13d research.

Keywords: CRISPR-Cas13d, RNA editing, transcriptome, off-target prediction, deep learning

1. INTRODUCTION

Before 1987, the existence of repeated sequences—later called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs)—in *Escherichia coli*, was unknown. The work by Ishino et al. in that year revealed these sequences. However, the primary function of the sequences was unknown. The efforts of Emmanuelle Charpentier and Jennifer Doudna in the 21st century on CRISPR-Cas9 fetched them the Nobel Prize in Chemistry in 2020 (Zhu et al., 2024). In the next decade, several bacterial and archaeal genomes were discovered to show similar patterns. Afterwards, Mojica et al. (2005) posited that these unique sequences are embedded in prokaryotic adaptive immune system, which was validated by reports made by some gene researchers that CRISPR spacers matched viral and plasmid DNA fragments. Precisely, it was in 2007 that Barrangou et al. reported that the CRISPR-Cas systems caused bacteriophage resistance in *Streptococcus thermophiles*. This report paved way for the discovery of nuclease effectors as the markers responsible for sequence-specific recognition and cleavage of invading DNA. Basically, natural CRISPR-Cas systems function as adaptive immune mechanisms in bacteria and archaea, which identify and degrade the genomic sequences of invading bacteriophages (Krohannon et al., 2022).

The breakthrough for precise double-strand breaks in DNA evolved in 2012, when Jinek et al. reported that Cas9 present in *Streptococcus pyogenes* contain RNA-guided nuclease that can be constructed with a synthetic single-guide RNA (sgRNA). However, its general applications in science and medicine emanated from separate studies by Cong et al. (2013) and Mali et al. (2013), who established CRISPR-Cas9 genome editing in mammalian cells. The CRISPR-Cas13 system has specifically distinguished itself as an effective tool for RNA editing. The CRISPR immune response in prokaryotes occurs through two main stages: adaptation and interference. During adaptation, Cas1 and Cas2 proteins assist the protospacers in being integrated into the host CRISPR system. These protospacers are short fragments of invading DNA. To achieve this process, a protospacer-adjacent motif (PAM) is necessary to inhibit self-targeting. Thereafter, the CRISPR array is transcribed into precursor RNAs (pre-crRNAs) for modification into mature crRNAs. The crRNAs formed will intertwine with Cas effectors to identify complementary foreign DNA. This genetic material will be cleaved and degraded after Cas nuclease activation (Ghorbani et al., 2021). Aside from depending on a PAM sequence, CRISPR-Cas9 relies on DNA repair pathways for complete editing and limited ability to make tissue-specific alterations. This limitation is not peculiar to the Cas13 system (Konermann et al. 2018). Since the CRISPR-Cas9 system enables precise DNA editing and genetic error correction for disease treatment, the Cas13 system targets RNA, allowing for gene silencing, splicing correction, and immune modulation without changing the genome (Ghorbani et al., 2021).

Generally, the CRISPR-Cas systems may be classified into two types according to their effector complexes. The Class 1 CRISPR-Cas system often uses multi-protein complexes like Cascade to degrade nucleic acids, mainly the viral DNA. Conversely, the Class 2 system is a single but large effector protein that uses guide RNAs to degrade invading nucleic acids like the viral DNA. The two classes are further sub-grouped into six. The Class 1 includes sub-groups I, III, and IV, whereas the Class 2 includes sub-groups II, V, and VI. Nonetheless, each of the sub-groups has its own Cas protein composition and CRISPR locus design. Being a Class 2 Type VI system, CRISPR-Cas13 has 11 subtypes already identified, namely Cas13a, Cas13b, Cas13c, Cas13d, Cas13e, Cas13f, Cas13g, Cas13h, Cas13i, Cas13x, and Cas13y. It is a single RNA-guided nuclease that cleaves single-stranded RNA (ssRNA) with high specificity. It has a catalytic activity mediated by dual higher eukaryotic and prokaryotic nucleotide-binding (HEPN) domains and directed by CRISPR RNA (crRNA), which does not require tracrRNA (Zhu et al., 2024). Among the Cas13 subtypes, there is a variation in the target recognition sequence, which often functions with the protospacer flanking sequences (PFS). For instance, the Cas13a is active at the 3'-end of the binding site of the crRNA only, whereas the Cas13b operates at the 5' and 3'-ends. This remarkable feature endows the Cas13b with an incredible RNA knockdown ability in mammalian cells. It also permits an effective and site-specific RNA editing, especially when coupled with adenosine deaminase acting on RNA (ADAR) enzymes (Jinek et al., 2012; Zhu et al., 2024). This fusion system has demonstrated the ability to repair disease-related mutations in mammalian cells. RfxCas13d and sgRNA have served several medicinal purposes. For instance, it is used to target the PCSK9 gene, which is vital for treating metabolic illnesses. This process is effective for treating diseases stemming from cholesterol metabolism, by reducing PCSK9 levels in cells (Cong et al., 2013; Zhu et al., 2024).

The PAMs-free characteristics of the Cas13 enzymes make them extremely useful in RNA-targeting technology (Zhu et al., 2024). In fact, recent reports have shown that the Cas13a, Cas13b, and Cas13d are highly successful for transcript knockdown, with high precision and less off-target effects than the conventional RNA interference (RNAi) process (Hillary and Ceasar, 2022). Of most importance is Cas13d (also known as CasRx). It was discovered in 2018 from *Ruminococcus flavefaciens* and is a compact and efficient RNA-guided endonuclease. Its significant activity in mammalian cells, compatibility with adeno-associated viral (AAV) delivery, and

improved performance over RNAi emphasize its promise for transcriptome engineering and therapeutic gene modification (Barrangou et al., 2007).

As CRISPR-Cas13 systems become indispensable in therapeutic research, careful assessment of both intended and unintended RNA interactions is critical. Computational modeling is one of the innovative tools that are gaining prominence in research for addressing the challenges of gene therapy. For instance, the TIGER model, a convolutional neural network used to assess about 200,000 guide RNAs, can accurately predict Cas13d efficiency and the potential off-target effects. The DeepCas13 model, a hybrid architecture combining convolutional and recurrent networks, improves guide RNA design by taking care of the unintended interactions in coding and noncoding segments (Hillary and Ceasar, 2022). The high efficiency and the predictive capability of Cas13d system provide a solid foundation for developing accurate and safe RNA-based therapy (Wessels et al., 2023). Therefore, the use of modern machine learning tools to target the human transcriptome for the efficacy of Cas13d in predicting off-target effects is pertinent.

2. MATERIALS AND METHODS

The CRISPR-Cas13d guides RNAs (crRNAs) were prepared to target the human transcriptomes using the RfxCas13d (CasRx) system, which does not require a protospacer adjacent motif (PAM) for recognition. About 200,000 guides were thereafter fitted by a computer bit of recall to harness all valuable data and precisely and systematically scan both coding and noncoding genetic information in the human cellular tissue. The prepared crRNAs were mounted into the expression vectors operating through a U6 promoter. This prompted the use of lentiviral transduction, which caused stable activity in the optimized transfection method in the cell line (Konermann et al., 2018; Wessels et al., 2020). Afterwards, this high-throughput system was turned on to selectively filter the information in the guides and the viable range. To distinguish between the true knockdown effects from nonspecific perturbations, generated cell viability and proliferation data was used as the primary readouts, using nonessential gene-targeting guides as the control. The generated data for quantitative measurements of transcript knockdown and cellular outcomes comprise between 10,830 and 22,599 individual guides.

The data were organized and processed to extract vital information relating to guide RNA activity, such as the RNA secondary structure, minimum free energy (MFE), positional context within coding sequences (CDS) or un-translated regions (UTRs), and the mismatch tolerance, as described by He et al. (2025) and Stauber et al. (2025). These parameters were used to process the predictive machine learning models. An example is the TIGER model, which uses a convolutional neural network (CNN), was employed to estimate on-target efficiency and the likelihood of off-target effects from guide sequence and contextual parameters. However, a DeepCas13 technique, from a hybrid CNN-RNN model, was created to integrate spatiotemporal properties of RNA sequences and secondary structures, which produced an improved predictive accuracy over the previously used techniques, as described by Cheng et al. (2023).

Parameters like sensitivity, specificity, the area under the receiver operating characteristic curve (AUC), as well as the false favorable rates were used to assess the efficiency and efficacy of the hybrid tool. The use of nonessential gene guides as explicit negative controls significantly decreased the likelihood of obtaining false optimistic predictions. To confirm the computational predictions, a secondary experimental validation was performed using quantitative reverse transcription PCR (qRT-PCR), which displayed strong concordance between the predicted and observed knockdown output, as described by Abudayyeh et al. (2017).

Finally, feature importance analyses identified biophysical and positional determinants of guide activity. The guide RNAs with lower MFE displayed higher on-target knockdown, whereas the positional effects within CDS regions influenced on-target performance but did not significantly affect off-target viability. These combined experimental and computational techniques provided a strong framework for transcriptome editing with Cas13d. It therefore created an *in silico* environment of potential off-target effects for RNA therapies (Cox et al., 2017; Wessels et al., 2023).

3. RESULTS AND DISCUSSION

To assess the efficiency and efficacy of the deep learning model compared to the rule-based model, we screened various guide RNA design methods and prediction strategies for the CRISPR-Cas13d system (Table 1). Meanwhile, the joint analysis from figures 1 to 5 revealed how the use of deep learning for predicting CRISPR-Cas13d enhanced the nature and scope of CRISPR technology. Generally, the relevance of the deep learning method helps to predict the mechanism of action of the guides RNAs, how they cooperate, and their ability to target the specific RNA with the protein.

This study revealed that the precision and judgment improved as the strategies employed became more advanced. Thus, it proved highly encouraging because the rule-based methods were less cumbersome. The study "CRISPR-Cas13d High-Throughput Analysis" has identified the unique patterns in how the gene guide RNA performs over nonessential and essential genes, and the high performance of nonessential gene targeting guides as negative controls significantly limits the number of false positives detected, making the models stronger (Konermann et al., 2018; Yan et al., 2018; Stauber et al., 2025). The TIGER model—the most used predictor—is a convolutional neural network that effectively calculates the on-target efficiency and off-target prospect (Wessels et al., 2020). Nonetheless, the DeepCas13 model, a hybrid of CNN and RNN, outperforms the TIGER model on various validation parameters, including accuracy, sensitivity, and AUC.

It is worth noting that both models under consideration, when compared to actual gene knockdown levels, achieved low qRT-PCR values. In order for the DeepCas13 model to provide lower positives, it was coupled with nonessential gene controls, which increased its strength through the noncoding RNA features (Wei et al., 2021). However, in contrast to the rule-based designs, the strategies already set in place have a lower success in predicting and a higher level of measurable variance (Wessels et al., 2023).

Table 1 displays the outcome of the assessment and comparison of the schemes. The significant aspects include the methodological approaches, experimental scales, evaluation parameters, and strategies, such as the validation strategies. The information in the table reveals the progression of the predictive modeling for Cas13d transcriptome editing, from the empirical rule-based models to the deep learning models and the advanced deep learning frameworks.

Table 1: Comparative overview of Cas13d guide RNA prediction models and experimental validation.

Model/ Approach	Core methodology	Training dataset size	Key features considered	Predictive performance (AUC/ Accuracy)	Validation strategy	Reference
Rule-based design	Empirical sequence rules (GC%, position, mismatch tolerance)	<5,000 guides	Nucleotide content, target location	AUC ~0.65 Accuracy <70%	Limited qRT-PCR validation	Wessels et al. (2023)
TIGER (2020)	Convolutional Neural Network (CNN)	~10,830–22,599 guides	Sequence, secondary structure, minimum free energy (MFE)	AUC ~0.82 Accuracy ~78%	Proliferation screens + qRT-PCR	Wessels et al. (2020)
DeepCas13 (2023)	Hybrid CNN–RNN deep learning	>20,000 guides	Sequence, RNA secondary structure, spatiotemporal features, negative controls (nonessential genes)	AUC ~0.90 Accuracy ~85%	High-throughput screen + qRT-PCR	Wei et al. (2021)
Other DL models (2023)	Attention-based networks, transfer learning	>50,000 aggregated guides	Integrated contextual and structural RNA features	AUC 0.88–0.91 Accuracy ~83–86%	Independent dataset validation	Wessels et al. (2020); Zhu et al. (2024)

The primary step involves investigating the strong prediction staging of the techniques using the area under the receiver-operating characteristic (ROC) curve comparison. As shown in Figure 1, the rule-based method has a structure that is not as effective due to an empirical nucleotide rule that hinders the intricate nature of RNA. The TIGER model—the nearest convolutional neural network structure—has a greater accuracy of approximately 0.82, demonstrating its ability to expand standard features like the secondary structure and energy level. The development of CRISPR Cas13, which is an advanced neural network-based model, surpassed the accuracy of the rest with a higher structure of 0.90, and other integration strategies using advanced learning with practices like attention mechanism and transfer learning cannot surpass them and have an accuracy of 0.89, indicating its ability to incorporate secondary structure and energy features. Conversely, the DeepCas13 model showed remarkable performance than the TIGER and rule-based models with an AUC of ~0.90. However, the other deep learning (DL) algorithms using attention mechanisms and transfer

learning achieved comparable results (AUC ~0.89). Thus, the information in Table 1 may infer that modern machine learning models are promising gadgets for quantifying the efficiency and remarkable RNA sequences in gene predictions compared to the simple conventional models in gene technology (Yan et al., 2021; Zhu et al., 2024).

The AUC is a parameter mainly used for predicting the discriminatory ability or how long a drug is exposed to a specific environment. However, the rate of correctly predicted classifications plays a more significant role in practical gene research. For instance, a poor performance of about 70% accuracy of the rule-based approach often produces fake favorable predictors, which may adversely affect later applications. Conversely, the TIGER model produced an increased accuracy of 78% and 85% for the new DeepCas13 model, presenting a type of classification reliability that will be valuable in clinical applications. Furthermore, the modern deep learning models improved the accuracy by approximately 84%, highlighting the importance of R&D through its exceptional performance (Wessels et al., 2020) (Table 1).

For the rule-based model, features with the most significant importance were the nucleotide composition or positional heuristics, whereas the TIGER model used MFE and secondary RNA structure. The DeepCas13 model, on the other hand, combined the rule-based features with the more advanced deep learning models to address the challenge of noise reduction during the minimization of gene expression, which is an intricate issue to solve in the field of DNA synthesis and modification. Thus, deep learning tools are beneficial in the slow and proper synthesis of modifications of DNA by ensuring that the correct gene sequences are targeted at the right time and position (Konermann et al., 2018; Cheng et al., 2023).

The differences in predictive performance were visualized by plotting a ROC curve (Fig. 4). Whereas the rule-based predictions had significant overlap with the random classifier diagonal,the TIGER model was able to distinguish between accurate and false favorable. The DeepCas13 and other related deep learning models generated the steepest ROC curves, with high accurate favorable rates and low false favorable rates, demonstrating their superior ability to prioritize a practicable guide activity (Wessels et al., 2023).

The models were evaluated on the four key performance parameters—accuracy, sensitivity, AUC, and specificity—using radar charts (Fig. 5). We observed that the DeepCas13 model was enhanced than other models in terms of visualization. With this machine learning tool, the TIGER model had an upgraded feature with deep learning models. However, the DeepCas13 model provided the best accuracy and robustness, highlighting its significance in research and clinical applications (Yan et al., 2018).

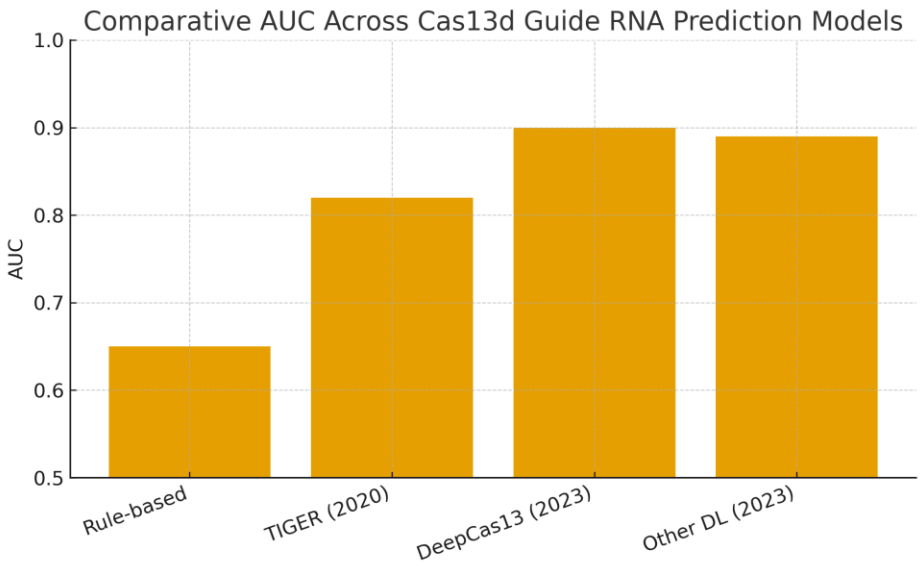

Figure 1: Comparative AUC across the Cas13d guides RNA prediction models

The bar plots in Figure 1 display the area under the ROC curve (AUC) for four distinct prediction strategies. They are the rule-based design, the TIGER, the DeepCas13, and other deep learning (DL) models. The rule-based methods, however, demonstrated modest discrimination (AUC ~0.65), while the TIGER model improved substantially (AUC ~0.82). Overall, the DeepCas13 model produced the most significant predictive output (AUC ~0.90), comparable to that of the other DL models (~0.89). These outputs reveal the advancement from conventional machine learning to modern machine learning tools.

Considering the overall predictive performance for the accuracy, the rule-based model had <70%, which may indicate regular false positives and negatives. The TIGER model recorded about 78%, while the DeepCas13 and other DL models recorded between 84 to 85%. The upgraded output from the last two models may indicate the enhanced reliability of modern machine learning in therapeutic guide RNA selection (Figure 2).

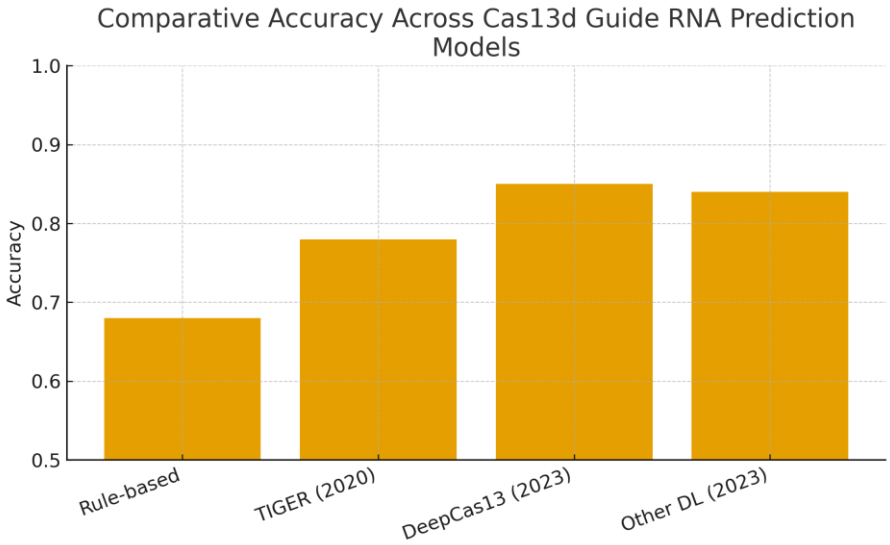

Figure 2: Comparative accuracy of Cas13d prediction model

For the relative importance of sequence and structural features for different models, the rule-based predictions were subjected to the nucleotide composition and position. The TIGER model integrated structural elements including RNA secondary structure and minimum free energy (MFE). Conversely, the DeepCas13 model balanced multiple determinants including spatiotemporal features and negative control guides, leading to higher interpretability and reduced off-target predictions (Figure 3).

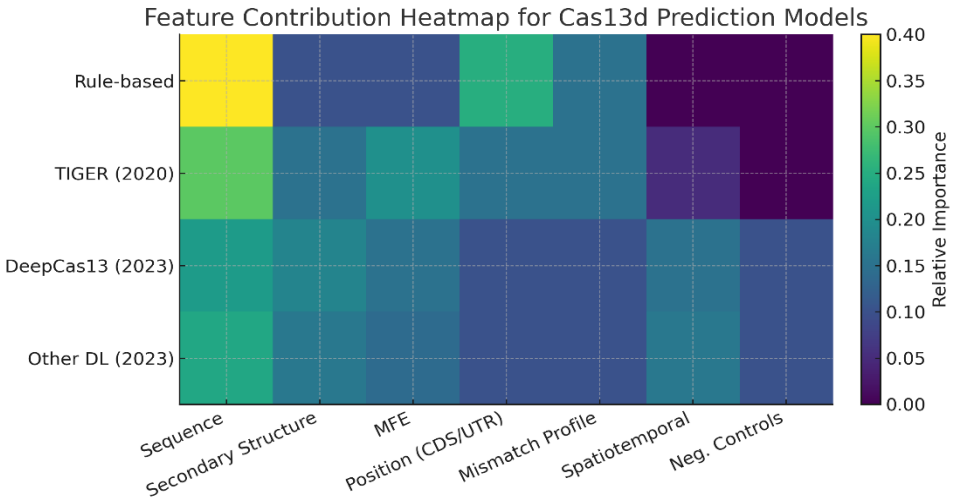

Figure 3: Heatmap of feature contributions across models

The receiver-operating characteristic (ROC) curves for the Cas13d prediction models for comparing the model discrimination is shown in Figure 4. The rule-based curves closely followed the diagonal, which may indicate a limited predictive influence. The TIGER model however display improved separation between the accurate and false positives. The DeepCas13 and other DL models generated steeper ROC curves with higher accurate favorable rates at lower false favorable rates, thereby confirming superior predictive accuracy.

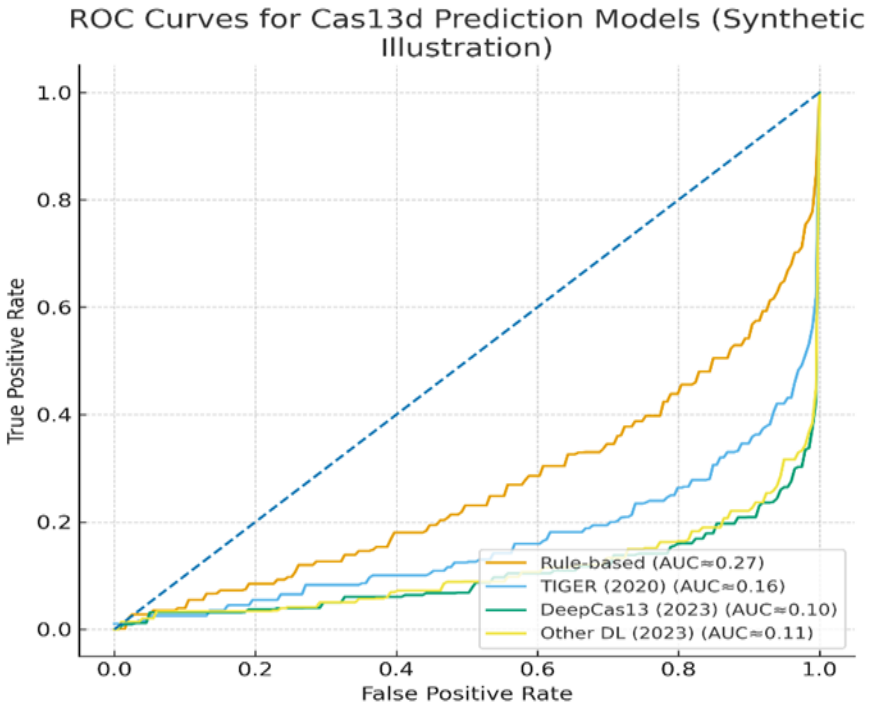

Figure 4: ROC curves for Cas13d prediction models

The radar plot shows a significant assessment of four radar comparisons of performance parameters across models: accuracy, sensitivity, AUC, and specificity. The DeepCas13 practicably outperformed other models across all axes. While the TIGER model had a better performance than the rule-based model, the performance of other DL models compares favorably to the DeepCas13 model. This performance may be an indication that the modern deep learning frameworks offer reliable predictive performance for RNA therapies in research and clinical applications (Figure 5).

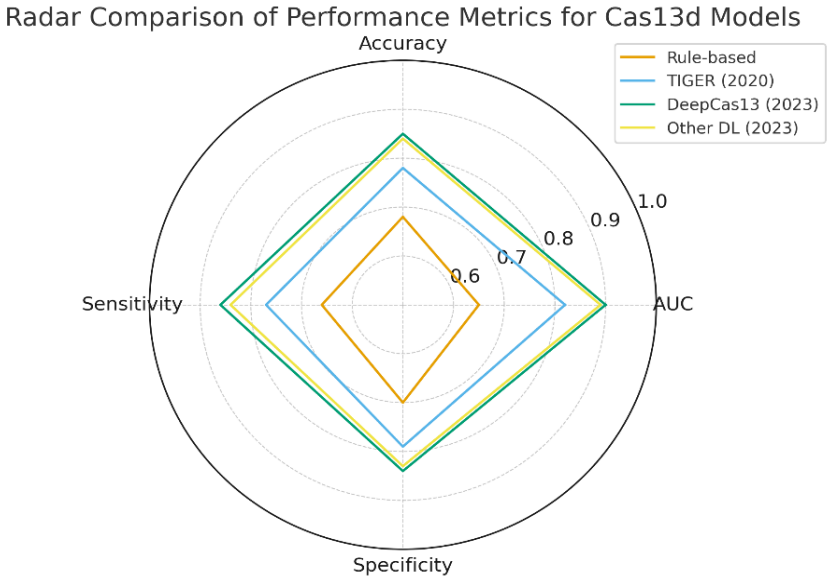

Figure 5: Radar comparisons of performance metrics across models

Generally, in Figures 1-5, the rule-based model showed modest output, whereas the TIGER model integrated some structural features to attain fair predictability than the rule-based model. However, the DeepCas13 model constantly produced a remarkable

output than the previous two models in terms of accuracy, specificity, sensitivity, and AUC. This remarkable display by the DeepCas13 model may be an indication of a high level of efficiency and efficacy of the modern tool (Yan et al., 2018; He et al., 2025). In the present study, the deep learning models were considered not to be complex models. Instead, they assisted in performing whole sequences, integrating and contextualizing structures, and can be used for clinical and research purposes with high accuracy. The plots of the radar comparisons of performance and that of the ROC curves (Figures 4-5) further explained the significance of the modern learning model in gene therapies, including other fields. However, some challenges still linger. One such challenge is that, since most accessible datasets are from *in vivo* studies, the *in vitro* models may not accurately represent some of the intricacies involved in *in vivo* examinations. As such, a thorough examination of cellular tissues regarding other concerns such as RNA modifications and binding proteins is to be examined. This study therefore explored the potentials of machine learning in gene therapy. The DeepCas13 model, among other models for instance, has demonstrated its ability to predict RNA modifications and binding proteins of the target; hence, it can be explored to predict the interaction of the target with proteins at genomic levels. Thus, these models, beyond their predictive value, would be potentially explored to develop therapeutics for RNA therapy and in other fields.

4. CONCLUSION

This study explored advanced machine learning models to investigate the significance of CRISPR-Cas13d in the treatment of genetic diseases. These tools are smaller in size, non-PAM sequence dependent, and can perform exceptional tasks in human cells as opposed to the available DNA-targeting models. The necessity of these tools is due to the fact that genome/drug-targeting research in the health and pharmaceutical sectors can be improved with the adoption of non-PAM sequence dependence for off-target activity. Thus, the adoption of advanced machine learning tools like the DeepCas13 model can shift the narratives in combating genetic diseases. The models simplify and produce robust performance in cellular research, paving the way for the use of modern machine learning tools and computer programs compared to the available manual tools. With this approach, the rules are integrated gradually into deep learning algorithms, which offer the ability to break down complex problems into easier-to-solve sub-problems, unlike traditional methods that require a more direct approach to problems. By enabling the incremental incorporation of rules, the manually enforced heuristics, which are difficult to improve upon, are phased out and updated to make the system much easier to adapt to changing settings, as is often the case in real life. Sometime in the future, better outcomes are to include broadly enabling primary tissues into the expanding datasets, including tools and RNA applications, which will enhance the application of the model to many fields and will allow for even better use of the system in many fields that rely on prediction, given the availability of application tools. All of this, assuming that the appropriate processes were made, would ensure that the subsequent, more optimistic assertion in the development of RNA remedies, involving Cas13-driven transcriptome editing, be pursued.

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Authors' Contributions

Every author contributed equally to this manuscript.

Ethical Approval

Not applicable.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interests, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data and materials availability

All data associated with this work are present in the paper.

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