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A Strategic Partnership to Advance AI Applications in Genomics and Bioinformatics for Health Innovation

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Abstract

JMIR Bioinformatics and Biotechnology announced a strategic partnership with the MidSouth Computational Biology and Bioinformatics Society (MCBIOS) in late 2025; this partnership establishes *JMIR Bioinformatics and Biotechnology* as the official journal of MCBIOS. This collaboration reflects a shared commitment to advancing computational biology, bioinformatics, and biotechnology through open science, interdisciplinary collaboration, and real-world data. By connecting MCBIOS' community-building and professional development initiatives with *JMIR Publications'* open-access publishing platform, this partnership aims to support emerging researchers, accelerate the dissemination of innovative computational methods and artificial intelligence applications, and strengthen data-driven research across medicine and biology.

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KEYWORDS

artificial intelligence; bioinformatics; genomics; AI; health innovation

In late 2025, *JMIR Bioinformatics and Biotechnology* announced a new strategic partnership with the MidSouth Computational Biology and Bioinformatics Society (MCBIOS), under which it will serve as the official journal of MCBIOS. This collaboration represents a shared commitment to advancing computational biology, bioinformatics, and biotechnology through open science, interdisciplinary collaboration, and real-world data.

MCBIOS is dedicated to advancing the fields of computational biology and bioinformatics by fostering collaboration, networking, and professional development among scientists, educators, and trainees. The society promotes interdisciplinary research, supports educational initiatives, and provides opportunities—particularly for trainees and early-career researchers to engage with peers and leaders in the field. Through annual conferences and community outreach, MCBIOS works to enhance scientific knowledge, encourage innovation, and contribute to solving complex biomedical questions [1].

The vision of *JMIR Bioinformatics and Biotechnology* is to promote high-quality, interdisciplinary research at the intersection of bioinformatics, computational biology, biotechnology and artificial intelligence (AI). The journal aims

to provide an open-access platform for innovative computational methods, data-driven biological discoveries, and translational applications that bridge algorithm development with real-world biomedical questions. Emphasizing collaboration among bioinformaticians, biologists, clinicians, and data scientists, the journal seeks to foster rigorous, reproducible research that leverages emerging technologies such as AI, machine learning, and big data analytics to accelerate scientific progress and translational impact in the life sciences and medicine.

The partnership between MCBIOS and *JMIR Bioinformatics and Biotechnology* is well-timed within today's scientific and academic landscape for several key reasons. First, computational biology and bioinformatics have entered a phase defined by large-scale data generation, AI, and translational research. Second, aligning a scientific society that fosters community, mentorship, and scholarship with a global, open-access journal amplifies both visibility and impact. Furthermore, in an era where rapid dissemination and interdisciplinary collaboration have become key components in research, this partnership creates a streamlined pathway from conference presentation and networking to peer-reviewed publication in a journal that prioritizes accessibility and methodological innovation.

Together, these organizations will support emerging scholars and strengthen open, collaborative, and AI-enabled data science.

Strengthening a Community at the Intersection of Bioinformatics, Biotechnology, and AI

MCBIOS has long served as a vibrant forum for academic researchers, industry scientists and trainees working across computational biology, bioinformatics, systems biology, machine learning, medicine and data science. This partnership offers not only a conference or a journal to showcase research, but also an integrated professional ecosystem that amplifies visibility, supports career development, and fosters innovation—making participation both professionally valuable and intellectually impactful. As part of this partnership, *JMIR Bioinformatics and Biotechnology* will work closely with MCBIOS leadership to support the society's mission through:

- **Official society journal designation:** Being the official journal of MCBIOS reinforces *JMIR Bioinformatics and Biotechnology's* credibility in the publishing landscape wherein researchers must identify trusted journals. This designation will provide quality, community oversight, and alignment with the interdisciplinary standards of computational biology and bioinformatics, giving authors confidence that their work will reach the right audience.
- **Annual conference-linked theme issues:** By publishing issues connected to MCBIOS annual meetings, *JMIR Bioinformatics and Biotechnology* will help capture emerging trends and high-impact research that might otherwise remain limited to conference presentations. This addresses the challenge of slow publication process and provides authors, especially early-career researchers, a pathway from presentation to peer-reviewed publication, increasing the visibility and impact of their work in a timely manner.
- **Publishing benefits for MCBIOS members:** Discounted article processing fees in reducing financial barriers to open access publishing, addressing a major concern for researchers.
- **Education and capacity building:** Through educational and career development webinars, and resources on peer review, publishing ethics, and research dissemination, *JMIR Bioinformatics and Biotechnology* and MCBIOS aim to address a gap in formal education on modern publishing practices.

These initiatives are designed to amplify the visibility and impact of work emerging from the MCBIOS community, while welcoming impactful contributions from the broader global research ecosystem to the *JMIR Bioinformatics and Biotechnology*.

A Shared Focus: From Computational Methods to Meaningful Clinical Impact

MCBIOS and *JMIR Bioinformatics and Biotechnology* share a vision of advancing interdisciplinary data-driven research with

clinical impact through collaboration, transparency, and open dissemination. Both partners are committed to fostering innovation at the intersection of bioinformatics, computational biology, biotechnology and AI while supporting emerging investigators and strengthening research communities. Together, MCBIOS and *JMIR Bioinformatics and Biotechnology* aim to provide a scholarly home for research that not only advances methods and technologies but also demonstrates meaningful applications across biomedical, clinical, population health, and AI contexts. For example, machine learning tools that improve early disease detection or risk stratification, computational approaches that predict drug response, or real-world data analyses that evaluate the viability of computational tools in clinical settings.

We invite submissions that address, but are not limited to:

- Novel computational methodologies, algorithms, and statistical approaches
- AI and machine learning applications in genomics, bioinformatics, biotechnology, and health
- Data integration and multimodal analytics, including genomics, transcriptomics, proteomics, imaging, and clinical data
- Demonstrated real-world applications in translational bioinformatics, including clinical, public health, and biotechnological use cases
- Scalable technologies and platforms enabling reproducible and open science

By emphasizing both methodological rigor and impactful applications, *JMIR Bioinformatics and Biotechnology* seeks to serve as a bridge between innovation and implementation of computational methods, supporting data-driven research that advances science while contributing to improved health outcomes.

Looking Ahead

This partnership between *JMIR Bioinformatics and Biotechnology* and MCBIOS is not only a recognition of shared values but also an investment in the future of bioinformatics and biotechnology research. As the pace of innovation accelerates, there is a growing need for journals that can thoughtfully integrate advances in bioinformatics, data science, AI, and biotechnology within a health-focused, open access framework. We look forward to working with the MCBIOS community to shape this next chapter through collaborative theme issues, high-quality submissions, and ongoing dialogue about the evolving role of bioinformatics and biotechnology in transforming health.

We warmly invite MCBIOS members and the wider research community to submit their work to *JMIR Bioinformatics and Biotechnology* [2] and to join us in advancing research that bridges bioinformatics, data, AI, and innovation for real-world health impact.

Conflicts of Interest

EDGU serves as the editor in chief of *JMIR Bioinformatics and Biotechnology* at the time of this publication. AT serves as the president of MCBIOS and is an associate editor for *JMIR Bioinformatics and Biotechnology* at the time of this publication.

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Abbreviations

AI: artificial intelligence

MCBIOS: MidSouth Computational Biology and Bioinformatics Society

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The AudioGene Translational Dashboard for Diagnosing Autosomal Dominant Nonsyndromic Hearing Loss: Phenotypic Data Visualization and Analysis Study

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Abstract

Background: Autosomal dominant nonsyndromic hearing loss (ADNSHL) is highly heterogeneous, with more than 64 genes implicated in its etiology. This complexity limits the diagnostic power of clinical examinations and audiometry alone, while existing computational approaches have achieved only moderate accuracy and often lack interpretability. As precision medicine increasingly emphasizes genotype-phenotype correlations, there is a recognized need for diagnostic tools that provide clinicians with transparent, interpretable outputs.

Objective: This study aimed to develop and evaluate the AudioGene Translational Dashboard, an interpretable clinical informatics tool that integrates machine learning models and interactive visualizations to enhance genotype-phenotype correlations and support diagnostic decision-making in ADNSHL.

Methods: We developed the AudioGene Translational Dashboard, integrating 2 machine learning models (AudioGene version 4 and AudioGene version 9.1) with 6 interactive visualization tools. AudioGene version 4 uses a multi-instance support vector machine classifier for patients with multiple audiograms, while AudioGene version 9.1 combines adaptive boosting, k-nearest neighbors, random forest models, and logistic regression for patients with a single audiogram. Visualizations include audiometric profile plots, audioprofile surfaces, clustering analyses, and data distribution charts designed to facilitate clinical interpretation.

Results: The AudioGene Translational Dashboard was developed to address the “70/30” phenomenon, indicating a 74% likelihood that the causative gene is among the top 3 predicted genes, thereby providing clinicians with a clear confidence indicator (“green flag”) or a caution alert (“red flag”) during diagnosis. While this level of performance is well suited for hypothesis generation, the remaining uncertainty underscores the need for interpretive context in clinical decision-making. Visualization tools enhanced clinicians’ ability to interpret and correlate phenotypic data with predicted genetic outcomes, improving diagnostic confidence and interpretability.

Conclusions: The AudioGene Translational Dashboard advances clinical informatics in genetic diagnosis of ADNSHL by integrating explainable artificial intelligence with interactive visualizations, enhancing clinical interpretability and diagnostic accuracy. This approach facilitates informed clinical decision-making, highlights the translational potential of genotype-phenotype computational models, and supports precision medicine in hearing loss diagnostics. Future enhancements will target improving class balance and incorporating additional user-customizable features to further optimize clinical applicability.

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KEYWORDS

autosomal dominant nonsyndromic hearing loss; machine learning; explainable artificial intelligence; clinical decision support systems; genotype-phenotype correlation; audiometry; genetic testing

Introduction

Background

Autosomal dominant nonsyndromic hearing loss (ADNSHL) presents a significant genetic diagnostic challenge due to its underlying heterogeneity—more than 64 genes are implicated in its etiology [1]. Because of this complex genetic landscape, computational tools designed to correlate audiogram profiles (commonly called audioprofiles) with specific genes have achieved only moderate success [2,3]. One such tool, which we developed approximately 15 years ago, is AudioGene. AudioGene uses numerous machine learning (ML) approaches to improve diagnostic precision [2,4,5]. These approaches include semisupervised support vector machines (SVMs), ensemble models, and hyper-tuning methods. However, challenges such as data imbalance and class sparsity continue to restrict the accuracy of these models.

Precision medicine harnesses information about the genome of an individual, environment, and lifestyle to guide medical care. With heterogeneous disorders such as ADNSHL, genetic variant interpretation can be challenging, complicating the diagnostic process and impacting patient care. Computational tools may improve the precision and reliability of genetic assessments by capitalizing on genotype-phenotype associations [6,7].

Current diagnostic methods for ADNSHL largely rely on clinical examination and audiometry, which do not provide sufficient resolution for the complex genetic landscape of ADNSHL [2,4]. However, with the availability of ML and artificial intelligence-driven approaches, there has been a shift toward integrating computational and visualization tools with genetic diagnostics to improve accuracy and predictive power [8,9].

To address these challenges, we have developed the AudioGene Translational Dashboard with the goal of enhancing both the accuracy and interpretability of genetic predictions. A feature of the AudioGene Translational Dashboard is the “70/30” phenomenon: by integrating the results from both models on a training dataset comprising 3189 audiograms from 1445 patients, we observed that the correct disease-causing gene was predicted within the top 3 predictions 74% of the time, with incorrect predictions accounting for the remaining 26%, hence “70/30.” This observation signals to health care providers when they can have confidence in the top predictions, serving as a “green flag” or “red flag” in the diagnostic process. Having a true positive rate of 70% is beneficial from a research perspective; however, for a diagnostic tool, the remaining 30% represents some risk that necessitates additional interpretative context. By providing this context, the AudioGene Translational Dashboard enables health care providers to weigh their confidence in the predictions, supporting more informed diagnostic decisions.

The AudioGene Translational Dashboard was introduced into the AudioGene toolset to increase transparency into the “black box” underlying the models by providing explainable artificial intelligence (XAI) to enhance model interpretability and utility in clinical settings, in line with trends in precision medicine that emphasize the importance of genotype-phenotype associations in improving diagnostic outcomes [7,10].

Related Works

Early attempts to map audiometric phenotypes to their underlying genotypes were spearheaded by AudioGene version 4 (AG4), a semisupervised multi-instance SVM that treats the collection of audiograms for a single patient as a “bag” and ranks loci according to pair-wise-coupled probability estimates [2,11]. Building on this foundation, AudioGene version 9.1 (AG9.1) introduced selective intraensemble data partitioning: training examples are first divided by gene-specific data volume, patient age, and audiogram shape, then modeled with a committee of k-nearest neighbor (KNN), adaptive boosting, and random forest subclassifiers, whose outputs are fused by logistic regression. AG9.1 offers a top-3 accuracy of 77.8%, with a precision of 0.51 and a recall of 0.56, at the cost of introducing a more complex model. We report top-3 accuracy rather than top-1 accuracy because, in the context of gene prioritization for validation sequencing, the cost of excluding the true causative gene is higher than the cost of evaluating a small number of candidate genes. In addition, the top-3 threshold represents a practical trade-off between high confidence in predictions and an acceptable loss of significance when selecting genes for sequence-based validation [4,5,12,13]. Both frameworks have improved locus-ranking accuracy for the 23 well-curated ADNSHL genes that account for roughly three-quarters of cases in populations of European ancestry [14]. Nonetheless, their predictions can still be difficult to interpret when class imbalance, sparse age coverage, or atypical audiogram morphologies are present.

Complementary to algorithmic advances, domain-specific visualization has been welcomed as a potentially beneficial tool for clinical use. Audioprofile surfaces (APS) plot 3D trajectories of frequency-specific threshold shift over time, revealing gene-characteristic progression patterns that are not obvious in 2D audiograms [15]. Circle-based genome views (eg, Circos [version 0.69-10; Krzywinski, Canada’s Michael Smith Genome Sciences Center] enable high-density comparison of structural variation or copy number events [16], while integrative genome browsers such as Integrative Genomics Viewer (version 2.19.7; UC San Diego and Broad Institute of MIT and Harvard) allow rapid inspection of read evidence at candidate loci [17]. More recent health care dashboards use fuzzy logic overlays and interactive filtering to expose outliers or low-confidence regions directly to end users [18]. Despite these advances, few systems combine genotype-prediction engines with audiogram-aware visual contexts; therefore, clinicians must cross-reference separate tools, a workflow that can erode trust in algorithmic suggestions and slow decision-making [19]. These limitations reflect shortcomings in how models communicate their reasoning and how results are presented to end users.

Accordingly, the literature reveals two unmet needs:

1. Model transparency—while ensemble and semisupervised approaches improve predictive accuracy, they do not inherently communicate *why* a particular gene is ranked highly, especially when training data are imbalanced or noisy.
2. Unified, clinician-friendly interfaces—existing genomic viewers excel at sequence-level detail but lack

phenotype-specific visualization; conversely, stand-alone audiogram tools rarely link observed hearing profiles back to the underlying variant evidence.

The AudioGene Translational Dashboard addresses these gaps by (1) merging the complementary strengths of AG4 and AG9.1 and (2) embedding 6 interactive visual modules—APS, 2D audioprofiles, uniform manifold approximation and projection (UMAP) cluster projections, gene count bar charts, region-of-origin pie charts, and age distribution plots—around the model output. This hybrid XAI-driven design supports clinicians in validating or questioning the algorithm’s “70/30” confidence observation and thus advances the state of practice in ADNSHL diagnostics.

Methods

Tool Overview

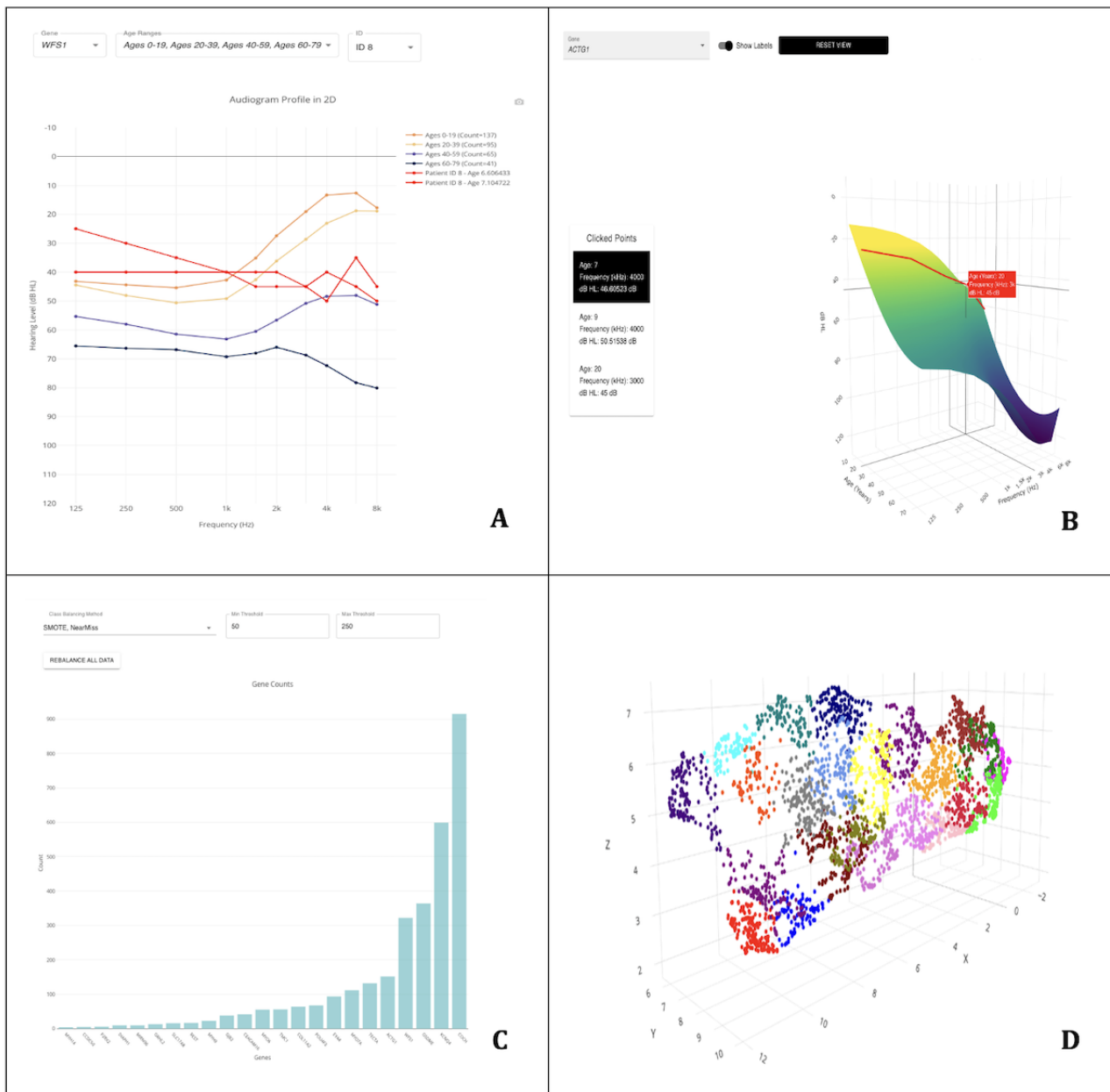
The AudioGene Translational Dashboard integrates 2 ML models to increase diagnostic accuracy for ADNSHL:

- AG4—it is a multi-instance classifier designed for patients with multiple audiograms that uses a semisupervised SVM and ranks loci based on modified SVM probability outputs [4]. It was developed using the Waikato Environment for Knowledge Analysis (version 3.7.2; WekaIO Inc) platform [11].
- AG9.1—it is a single-instance classifier for patients with only 1 audiogram, developed using the scikit-learn library

in Python. It comprises multiple submodels: 3 KNNs, 6 adaptive boosting models, 2 random forest models, and a logistic regression module for combining outputs [2,5,8].

The AudioGene Translational Dashboard interface provides six distinct visualization tools for health care providers and researchers to interactively assess genetic data and model predictions in real time: (1) audioprofile, a 2D plot displaying the average hearing loss (in dB) over 10 frequencies (125 Hz to 8000 Hz) for each age group, allowing comparison with a patient’s hearing loss over time (Figure 1A); (2) APS, a 3D surface plot depicting gene-specific hearing loss progression over time across frequencies, illustrating age-related changes in decibel loss (Figure 1B) [20]; (3) a region-of-origin pie chart, which displays the geographic origin distribution (eg, Dutch, German, and Chinese) for audiograms associated with each gene; (4) a count bar chart, which illustrates the audiogram count for each gene, highlighting class imbalance challenges (Figure 1C); (5) spatial analysis and clustering, which shows the cluster position of each gene and a 3D plot of audiograms (clusters are created using the k-means algorithm to partition data into 23 gene-specific groups [15]; the 3D plot compresses 11 features [age plus 10 frequencies] into 3 dimensions using the UMAP method (Figure 1D) [21]); and (6) an age distribution scatter plot, which shows the age distribution for each gene within the training dataset, providing context for the predictive model outputs.

Figure 1. Visualization components of the AudioGene Translational Dashboard. (A) Audioprofile for the selected gene (*WFS1*), displaying data from all age ranges along with patient data; (B) audioprofile surface view for the selected gene (*ACTG1*); (C) count bar chart showing the counts of each gene in the training data; and (D) 3D uniform manifold approximation and projection of genetic case data used in the AudioGene Translational Dashboard. (each point represents a classified genetic case, and the color coding corresponds to 1 of the 23 unique clusters identified through different genetic diagnoses).



The first 3 visualization tools were developed to compare patient-specific data to average thresholds for each of the 23 ADNSHL-associated genes. This comparison allows patient audiograms to be contextualized for each gene. The audioprofile visualization shows how a patient's audiogram compares to the expected audiograms associated with each gene.

The APS adds time as the third axis to provide a 3D rendering of gene-specific audiometric thresholds over time. Audiometric thresholds are represented as a 3D plane, depicting the expected dB loss over time (in years) at each frequency, thereby enabling comparisons between a patient's hearing thresholds and gene-specific expectations.

The spatial analysis and clustering tool uses a bar chart to show the distribution of each prediction among 23 different clusters.

These clusters are created using k-means clustering [15]. Additionally, a 3D plot visualizes the audiograms within our data that have a confirmed genetic diagnosis, compressing 11 features into 3 dimensions using UMAP [21]. This feature allows users to interact with the bar chart, highlighting corresponding clusters in the 3D plot (Figure 1). Users can compare their patient's audiogram, represented by a large red dot, with others in the cluster, facilitating the identification of similar audiograms and associated genetic diagnoses.

By using these 3 visualizations, we aim to either enhance or reduce confidence in the predictions. For example, if the model ranks the *COCH* gene second among the top 3 predictions, health care providers can analyze the APS and spatial analysis tools to determine the degree of correlation with patterns

typically associated with the *COCH* gene, potentially increasing confidence in that diagnosis.

The last 3 visualization tools provide context for the data in the AudioGene dataset. The count bar chart highlights significant class imbalance, showcasing the challenge the model encounters in predicting smaller classes due to underrepresentation. The region-of-origin pie chart and age distribution scatter plot provide additional context about data distribution, allowing health care providers to understand model limitations and adjust diagnostic strategies accordingly.

The AudioGene Translational Dashboard integrates into the workflow of a clinician as a secondary validation layer. For example, within our clinical workflow, clinicians, genetic counselors, and bioinformaticians review the results of a clinical genetic test, including patient history, family structure, audiograms, and identified variants in hearing loss genes. This team can then inspect a patient's audiometric data relative to the landscape of audiometric data across all genes and patients, considering variance within a gene, rarity or abundance of cases within a cluster, and distance to genetically validated cases.

System Design

The system was designed using the SERN (SQL, Express.js, React.js, and Node.js) stack, which uses a client-server architecture where computationally intensive tasks are performed by the server, deployed in a Docker (version 28.5.1; Docker Inc) container [19,22]. The client is supported by React (version 18.2.0; React Foundation), a JavaScript library facilitating user interactions [23]. Data preprocessing used linear interpolation and extrapolation for missing values. The same methods for handling missing values were applied in the ML models [2,5,8].

The *Pandas* library in Python was used for data manipulation and analysis [8], and visualization libraries such as Plotly were used to create interactive graphs and plots [24].

For more information, please refer to the master's thesis by DeSollar [3] and the GitLab repository.

Ethical Considerations

This study was reviewed and approved by the University of Iowa Institutional Review Board (199701065). The institutional review board granted a waiver of informed consent under US federal regulation 45 CFR 46.116(f) (also known as the

“Common Rule”), because, although audiograms were originally collected in clinical and research settings, the dataset used for this study was fully deidentified prior to analysis [25]. All procedures adhered to the ethical standards of the institutional and national research committees and to the 1964 Declaration of Helsinki and its later amendments. This paper does not contain any individual's data in any form, including individual details, images, or videos. No compensation was provided to participants, as this study involved secondary analysis of a fully deidentified existing dataset and no participants were directly recruited or enrolled.

Results

Introduction to AudioGene Translational Dashboard

The AudioGene Translational Dashboard combines advanced ML models with several visualization tools to create a platform that facilitates the prioritization of ADNSHL-associated genes in genetic testing results. Using patient data, the AudioGene Translational Dashboard generates gene rankings and enables auditory scientists and health care providers to explore these predictions interactively through various visualization tools. Gene ranking in phenotype-genotype associations can aid in the interpretation of complex genetic data, thereby providing greater context and confidence in diagnostic decisions [5,15].

The “70/30” phenomenon serves as an indicator for health care providers, providing them with the necessary context through visualizations to assess the reliability of the predictions. When the top 3 predictions include the correct gene, health care providers can have greater confidence in proceeding with targeted genetic testing.

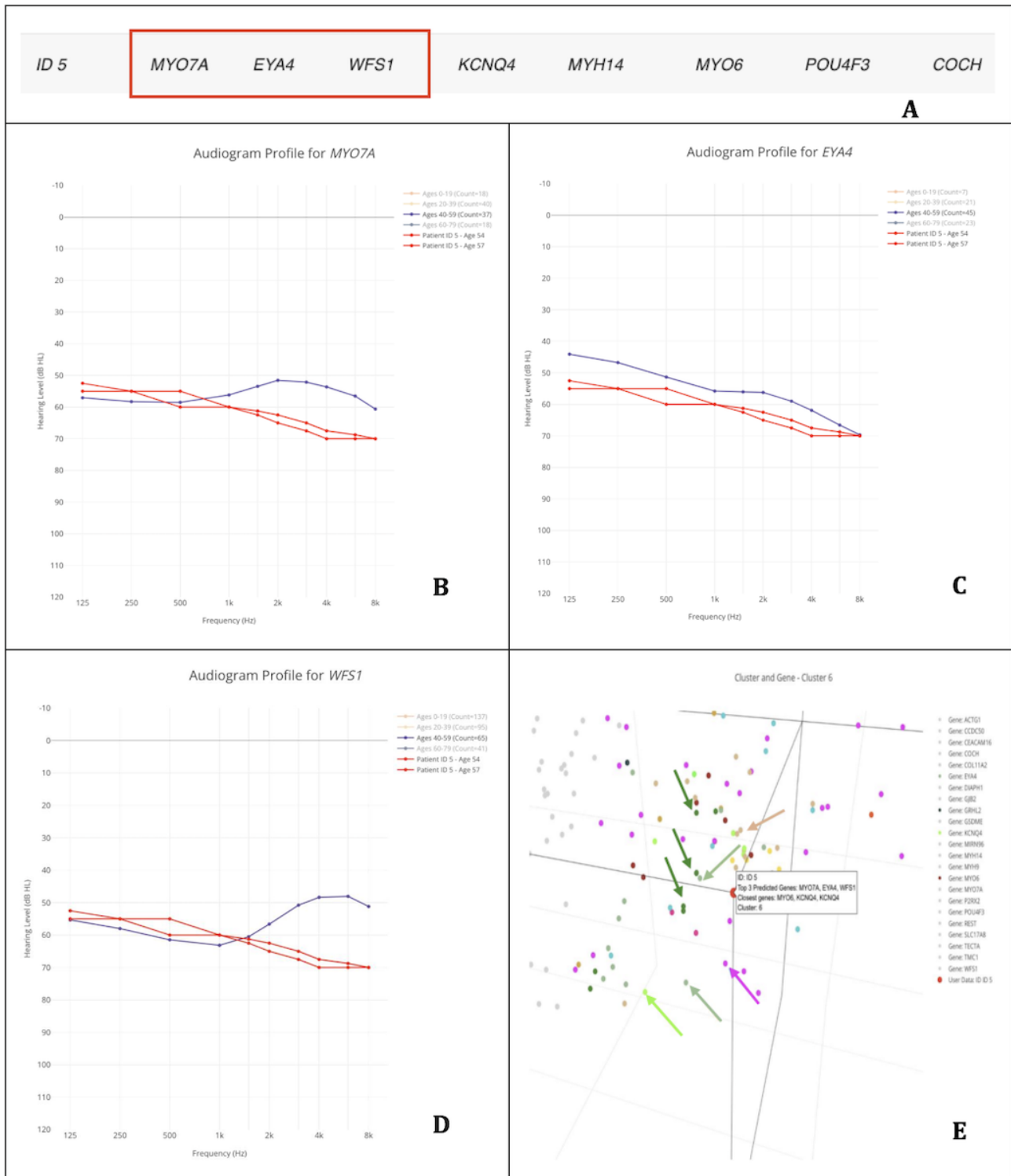
Case Studies and Clinical Implications

Several case studies have been carried out to demonstrate the effectiveness of the AudioGene Translational Dashboard in the diagnosis of specific genetic types of ADNSHL [3].

Case 1: *MYO7A* Gene—Patient 1 (ID 5)

In this case study, we analyzed the results from a patient diagnosed with *MYO7A*-related hearing loss. Our dataset included 2 audiograms, which were predicted by AG4 to be associated with *MYO7A*-related, *EYA4*-related, or *WFS1*-related hearing loss (Figure 2A).

Figure 2. Application of the AudioGene Translational Dashboard for patient-level gene prediction and visualization. (A) Predictions for patient (ID 5), highlighting the top 3 genes associated with the audiological characteristics observed; (B) audioprofile of MYO7A with the patient’s (ID 5) audiograms in red, taken at the ages of 54 and 57 years; (C) audioprofile of EYA4 with the patient’s (ID 5) audiograms in red, taken at 54 and 57 years of age; (D) audioprofile of WFS1 with the patient’s (ID 5) audiograms in red, taken at the ages of 54 and 57 years; and (E) 3D plot of audiograms in the training set reduced to 3 dimensions for visualization, with genes in cluster 6 colored (genes not in cluster are light gray; patient [ID 5] is the red dot hovered over by the displayed label; and the green arrows point to MYO7A [green dots], the pale green arrows point to EYA4 [pale green dots], the pale yellow arrow points to WFS1 [pale yellow dots], the pink arrow points to COCH [pink dots], and the light green arrow points to KCNQ4 [light green dots]).



Examining these predictions relative to the patient’s audiograms, the following observations can be made: (1) MYO7A’s audioprofile is similar in the low-to-mid frequencies but diverges in the high frequencies (Figure 2B); (2) EYA4 displays a comparable shape, but the patient’s thresholds are consistently

lower than typical values (Figure 2C); and (3) WFS1 shares some similarities in the low-to-mid frequencies but diverges in the high frequencies, as observed with MYO7A (Figure 2D).

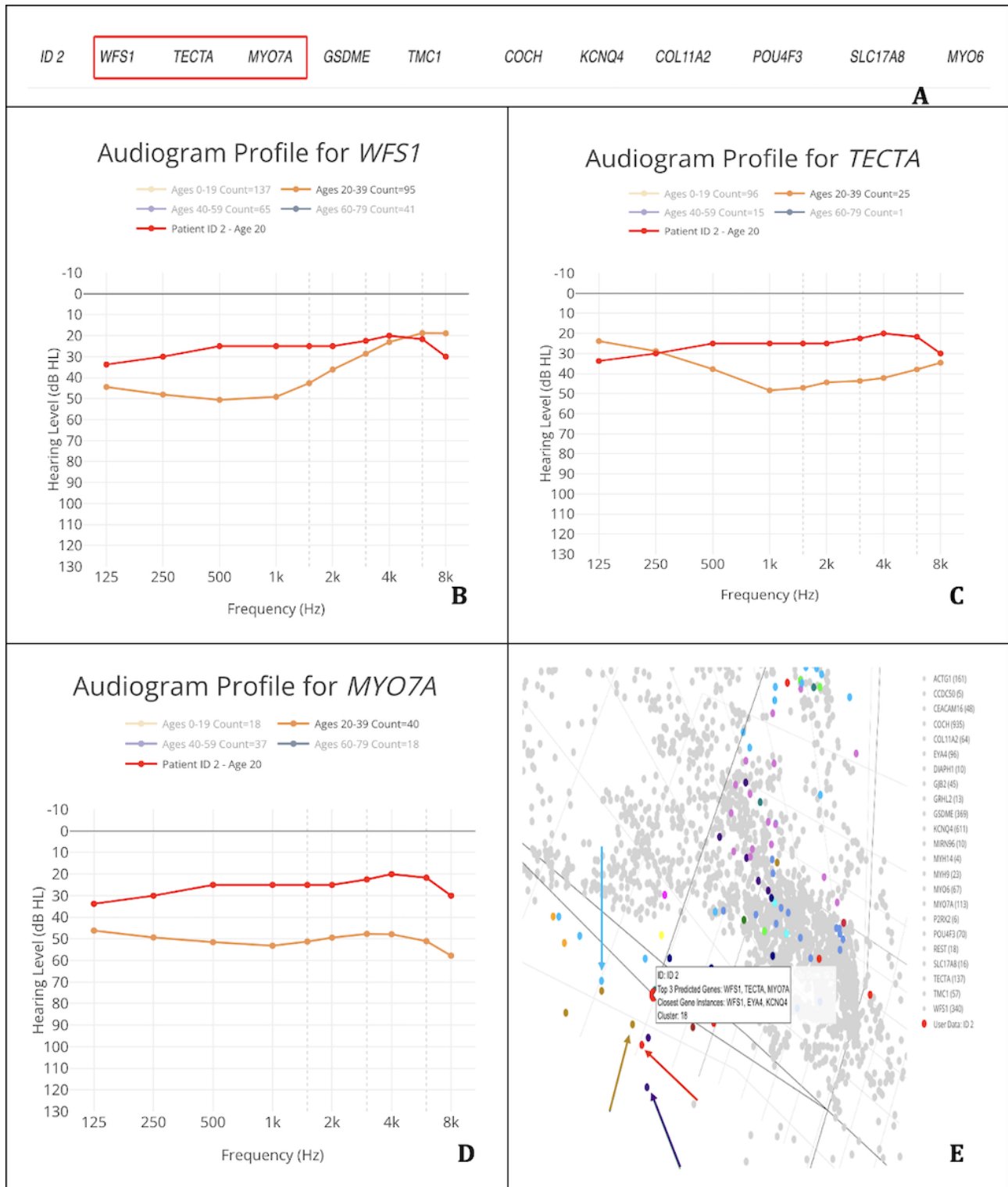
The audioprofiles for the 3 genes all show moderate to moderately severe hearing loss thresholds, with either close

similarity (<5 dB) at several frequencies or similar shapes. Therefore, we can conclude that the correct gene is likely captured within the top 3 predictions.

Case 2: MYO6 Gene—Patient 2 (ID 2)

In our second case, we explored the results from a patient (ID 2) previously diagnosed with *MYO6*-related hearing loss. Our dataset contains only 1 audiogram, with gene predictions shown in [Figure 3A](#) by AG9.1.

Figure 3. Application of the AudioGene Translational Dashboard for patient-level gene prediction, audioprofile comparison, and cluster-based visualization. (A) Predictions for the patient (ID 2), with the top 3 genes being *WFS1*, *TECTA*, and *MYO7A*; (B) audioprofile of *WFS1* with the patient’s (ID 2) audiograms in red, taken at 20 years of age; (C) audioprofile of *TECTA* with the patient’s (ID 2) audiograms in red, taken at the age of 20 years; (D) audioprofile of *MYO7A* with the patient’s (ID 2) audiograms in red, taken at the age of 20 years; and (E) 3D plot of audiograms in the training set reduced to 3 dimensions for visualization, with genes in cluster 18 colored (genes not in the cluster are light gray; patient [ID 2] is the red dot hovered over by the displayed label; and the light blue arrow points to *KCNQ4* [light blue dots], the red arrow points to *WFS1* [red dots], the purple arrow points to *POU4F3* [purple dots], and the brown arrow points to *GSDME* [brown dots]).



None of the top 3 candidate genes (*WFS1*, *TECTA*, or *MYO7A*) display an audioprofile that closely aligns with the patient’s thresholds (Figure 3B–D). This mismatch strongly suggests that the true causative gene (*MYO6*) does not appear among the model’s top 3 predictions for this patient.

From the clustering interface, we observe that the genes closest to the patient’s audiogram by the KNN metric (*WFS1*, *EYA4*, and *KCNQ4*) also fail to match the patient’s observed audioprofile in any convincing way. Furthermore, *KCNQ4*, *GSDME*, and *POU4F3*, which are noted to have multiple data

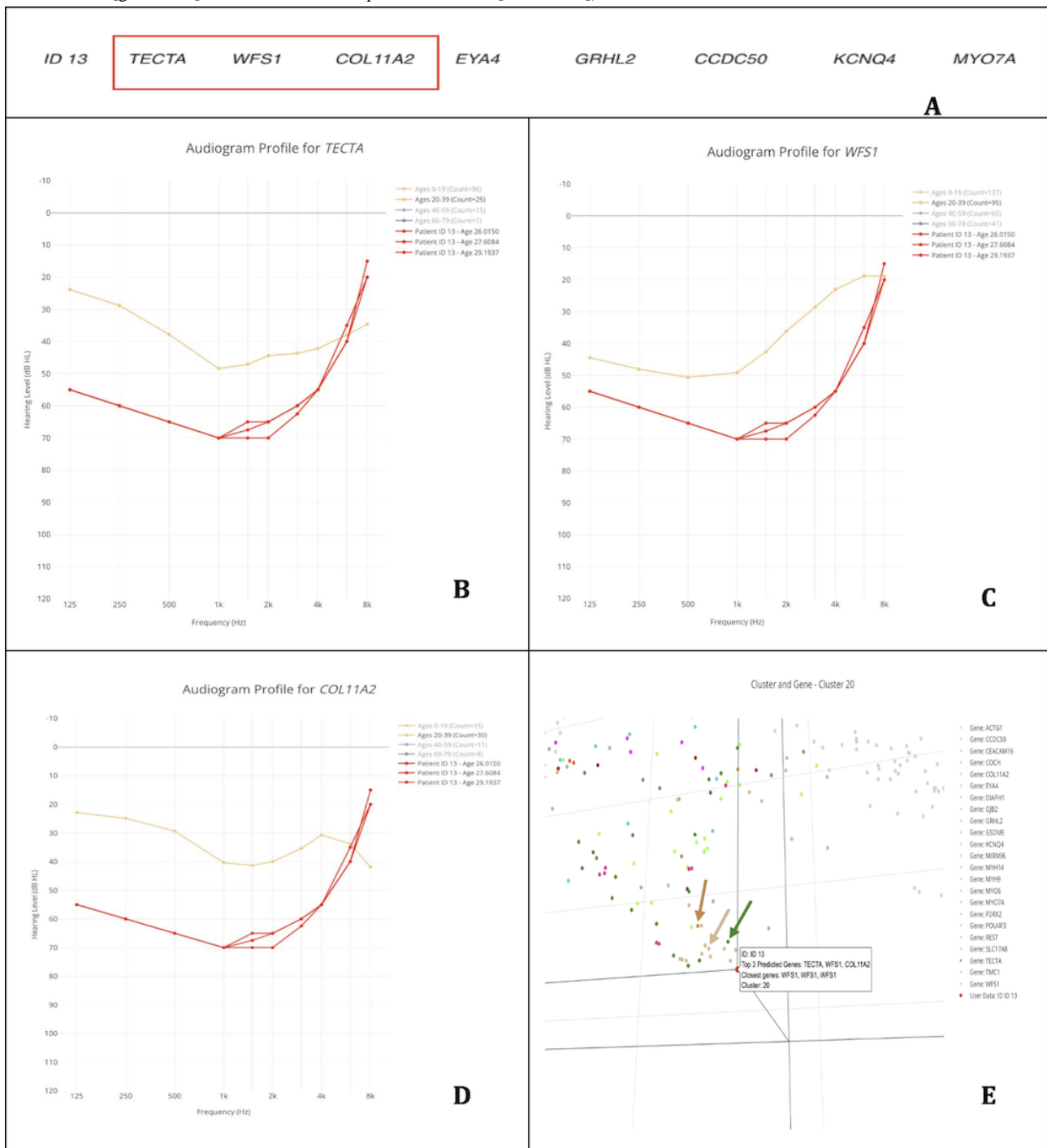
points near the patient’s cluster, likewise show audioprofiles inconsistent with the patient’s hearing loss. These factors combine to produce inconclusive predictions in this case. When we then integrated these data with the genetic data—which identified no genetic variants in *WFS1*, *TECTA*, or *MYO7A* and confirmed a known variant in *MYO6*—we verified that the correct gene was not found among the model’s top 3 predictions. This outcome highlights how conflicting audioprofiles and

clustering results can indicate that a prediction should be viewed with caution.

Case 3: *WFS1* Gene—Patient 3 (ID 13)

In our third case, the patient (ID 13) was diagnosed with *WFS1*-related hearing loss and, based on 3 audiograms, was predicted by AG4 to have *TECTA*-related, *WFS1*-related, or *COL11A2*-related hearing loss (Figure 4A).

Figure 4. Application of the AudioGene Translational Dashboard for patient-level gene prediction, audiometric profile comparison, and cluster-based visualization. (A) Predictions for the patient (ID 13), with the top 3 genes being *TECTA*, *WFS1*, and *COL11A2*; (B) audioprofile of *TECTA* with the patient’s (ID 13) audiograms in red, taken at the ages of 26, 27, and 29 years; (C) audioprofile of *WFS1* with the patient’s (ID 13) audiograms in red, taken at the ages of 26, 27, and 29 years; (D) audioprofile of *COL11A2* with the patient’s (ID 13) audiograms in red, taken at the ages of 26, 27, and 29 years; and (E) 3D plot of audiograms in the training set converted into 3 dimensions, with genes in cluster 20 colored (genes not in cluster are light gray; patient [ID 13] is the red dot hovered over the displayed label; and the light brown arrow points to *WFS1* [light brown dots], the green arrow points to *MYO7A* [green dots], and the brown arrow points to *TECTA* [brown dots]).



When examining these predictions in relation to the patient's audiograms, the following conclusions emerge regarding why *WFS1* is likely the correct gene and is captured within the top 3 predictions. First, the audioprofile for *TECTA* (Figure 4B) shows some similarities; however, it does not fully capture the nuanced relationship between low-frequency and high-frequency thresholds observed in the patient's data. Second, *COL11A2* (Figure 4D) also exhibits differences that diverge from the patient's pattern. Finally, *WFS1* (Figure 4C) demonstrates an especially close match to the patient's audiometric profile, particularly in the way it mirrors better hearing at the high frequencies relative to the low frequencies. Although one might contend that *TECTA* or *COL11A2* could also be considered candidates based on partial pattern matches, the overall evidence—supported by the 3D clustering in Figure 4E—reinforces that *WFS1* provides the best fit.

Thus, whether one emphasizes the possibility of *TECTA* or *COL11A2* as contenders, the integrated data confirm that the correct gene, *WFS1*, is indeed within the top 3 predictions. This close alignment between the patient's audiometric data and the *WFS1* reference profile, combined with supporting clustering analysis, enhances confidence in the diagnostic utility of the AudioGene Translational Dashboard.

Discussion

Principal Findings

These studies demonstrate how the model can raise or lower confidence in variant interpretation based on whether the correct genetic cause of ADNSHL appears among the top 3 predicted genes. Cases 1 and 3 illustrate scenarios in which the model successfully includes the causative gene in its top predictions and closely matches the patient's audiometric data, thereby justifying a higher level of trust in the result. In contrast, case 2 underscores how mismatched audioprofiles and inconclusive clustering can reveal when the actual gene of interest is likely

missing from the top 3 predictions. The interactive visualizations of the AudioGene Translational Dashboard, such as the APS and spatial analysis tools, remain valuable in identifying gene-specific patterns that align with clinical observations [20].

However, there are important limitations of the AudioGene Translational Dashboard, especially concerning smaller gene classes. The sparsity of data and the lower accuracy of models in these categories can make the AudioGene Translational Dashboard and phenotypic predictions less reliable. However, by presenting visualizations of the data distribution and class imbalance, these limitations become more apparent, allowing data interpretation to be adjusted accordingly [2,4].

Conclusions

The AudioGene Translational Dashboard represents an advancement in the field of genetic diagnostics for ADNSHL. By integrating advanced ML algorithms with interactive visualization tools, the AudioGene Translational Dashboard enhances health care providers' ability to interpret genetic data and make more informed diagnostic decisions.

A central feature of the AudioGene Translational Dashboard is the "70/30" phenomenon, which provides health care providers with critical context for confidence in genetic predictions. When the top 3 predictions are likely to contain the correct gene, the tool serves as a "green flag" for health care providers, increasing diagnostic confidence. Conversely, it alerts health care providers when predictions may be less reliable, serving as a "red flag" and prompting further investigation.

The AudioGene Translational Dashboard is an example of XAI in clinical settings, offering a context-driven method with increased transparency for the diagnosis of ADNSHL. Future developments will focus on incorporating custom model building, enhancing class imbalance functionality, and implementing user suggestions. The AudioGene Translational Dashboard not only advances genetic diagnostics for hearing loss but also serves as an example of a hybrid ML system.

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Data Availability

The datasets generated or analyzed during this study are not publicly available but are available from the corresponding author on reasonable request. The source code for the AudioGene Translational Dashboard is publicly available [26] under the GNU General Public License version 3.0 or later.

Authors' Contributions

BD led the design and development of the AudioGene Translational Dashboard, implemented the machine learning models, and drafted the manuscript. NS contributed to the integration of visualization tools and assisted with manuscript preparation. DW and AMO were responsible for data acquisition and preprocessing and contributed to tool validation. KTAB and HA provided expertise in genetic diagnostics and guided the tool's clinical relevance. MS supported statistical analysis and interpretation of the results. RJHS and TB provided project oversight, secured funding, and critically revised the manuscript. TC supervised the software engineering components and contributed to system architecture design. All authors reviewed and approved the final manuscript.

Conflicts of Interest

None declared.

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Abbreviations

ADNSHL: autosomal dominant nonsyndromic hearing loss

AG4: AudioGene version 4
AG9.1: AudioGene version 9.1
APS: audioprofile surface
KNN: k-nearest neighbor
ML: machine learning
SERN: SQL, Express.js, React.js, and Node.js
SVM: support vector machine
UMAP: uniform manifold approximation and projection
XAI: explainable artificial intelligence

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Systematic Mining of Bioactive Compounds for Wound Healing From *Cayratia japonica* Exosome-Like Nanovesicles: A Workflow Combining LC-MS and DeepSeek Models

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Abstract

Background: Plant-derived exosome-like nanovesicles (P-ELNs) effectively deliver bioactive compounds due to their high biocompatibility and low immunogenicity. While liquid chromatography-mass spectrometry (LC-MS) profiles compounds in complex samples, its analysis of large datasets remains limited by traditional methods. Recent advances in large language models (LLMs) and domain-specific systems have enhanced Chinese biomedical data processing and cross-modal pharmaceutical research.

Objective: This study aimed to create a multimodal framework of LC-MS combined with DeepSeek models for data mining of compounds with wound-healing properties from exosome-like nanovesicles derived from *Cayratia japonica* (CJ-ELNs).

Methods: LC-MS identified compounds enriched in CJ (n=3) and CJ-ELNs (n=3), and then compounds specifically enriched in CJ-ELNs were filtered via a four-step filtering workflow. The CJ-ELNs-specific compounds were processed by DeepSeek models for screening naturally active compounds with targeted functions of antioxidation, anti-inflammation, anticellular damage, antiapoptosis, wound healing and tissue regeneration, and cell proliferation.

Results: A multimodal framework of LC-MS combined with the DeepSeek-DF model was created. With the assistance of artificial intelligence (AI), a total of 46 naturally active compounds derived from CJ-ELNs with targeted functions were identified.

Conclusions: A self-designed multimodal framework of LC-MS, combined with DeepSeek models, rapidly and accurately identifies naturally active compounds from CJ-ELNs. This AI-powered system innovatively integrates the traditional analytical technique with modern LLMs, thus greatly favoring data mining of active ingredients in traditional Chinese medicine herbs.

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KEYWORDS

DeepSeek; liquid chromatography-mass spectrometry; LC-MS; *Cayratia japonica* exosome-like nanovesicles; CJ-ELNs; artificial intelligence; AI-powered multimodal framework; wound healing and tissue regeneration

Introduction

Plant-derived exosome-like nanovesicles (P-ELNs) contain abundant bioactive molecules, serving as novel carriers of natural products to mediate intercellular communication and mediate physiological processes [1,2]. P-ELNs are superior to conventional mammalian-derived exosomes, possessing unique

advantages such as high biocompatibility, high skin permeability, low cytotoxicity and low immunogenicity [3,4]. Multiple *in vitro* and *in vivo* studies indicate that these P-ELNs possess intrinsic therapeutic activity, offering promise for disease treatment and enhancing human health [5,6]. *Cayratia japonica*, a traditional Chinese medicinal herb, is widely used for the treatment of traumatic injuries such as contusions and lacerations [7]. Recent clinical studies have confirmed that

topical application of CJ ointment effectively alleviates local inflammation and promotes the repair and regeneration of damaged tissue, demonstrating favorable therapeutic outcomes in the management of postoperative infectious wounds around the anus [8]. However, research and application of exosome-like nanovesicles (ELNs) derived from CJ remain incomplete. Our research team successfully extracted and characterized a novel type of P-ELNs from the traditional Chinese medicinal herb *Cayratia japonica*, namely *Cayratia japonica* exosome-like nanovesicles (CJ-ELNs). They possess efficient delivery of bioactive compounds to wound sites, thus favoring tissue regeneration from infectious wound-related disorders. Bioactive constituents encapsulated within CJ-ELNs are dominant in wound healing. Consequently, the identification and characterization of bioactive compounds responsible for wound healing are of paramount significance.

Great strides have been made in the screening of active ingredients from natural products via omics techniques [9]. Liquid chromatography–mass spectrometry (LC-MS) has emerged as a powerful tool for profiling trace-level compounds in complex samples, although its performance in processing massive data is limited by traditional manual or rule-based analytical approaches [10,11]. In recent years, large-scale pretrained language models (LLMs), such as ChatGPT, GPT-4, and domain-specific systems like DeepSeek, have significantly transformed the landscape of biomedical data analysis and knowledge discovery [11,12]. These models exhibit powerful capabilities in natural language understanding, semantic reasoning, and prompt-based knowledge retrieval [13–15]. They are promising tools to assist omics analysis. In particular, DeepSeek models have been widely adopted for optimizing Chinese-language biomedical contexts, and supporting cross-modal tasks in pharmaceutical research, such as entity recognition, document summarization, and semantic ranking [16,17].

In this study, we innovatively created a multimodal framework of LC-MS combined with DeepSeek models for data mining of compounds with wound-healing properties from CJ-ELNs. This work illustrates the potential of artificial intelligence (AI) as a computational engine in natural compound discovery and offers a scalable solution for mining multimodal biochemical data.

Methods

Preprocessing of LC-MS Data

Untargeted metabolomic profiling of CJ and CJ-ELNs was performed by LC-MS. A total of 6 samples (including 3 CJ samples and 3 CJ-ELNs samples) were analyzed using an ultra-high-performance liquid chromatography (UHPLC) system coupled to a Q Exactive HF-X mass spectrometer (Thermo Scientific). Chromatographic separation was performed on an HSS T3 column (maintained at 40°C) with a 12-minute linear gradient from 2% to 98% mobile phase B at a flow rate of 0.3 mL/min. Mass spectrometry (MS) data were acquired in both positive and negative electrospray ionization (ESI) mode (\pm ESI) using a data-dependent acquisition strategy (top 10 most intense ions). Raw data were first converted to the mzML format using ProteoWizard, followed by processing, using Compound Discoverer 3.3 (Thermo Fisher Scientific) for peak alignment (with maximum retention time shift of 0.5 min and mass tolerance of 10 ppm) and normalization (using the median of maximum peak areas). Compound identification was achieved by matching MS/MS spectra against the following databases: mzCloud, LipidMaps, KEGG, HMDB, and MassBank. The matching criteria were set to a mass tolerance of 10 ppm and a minimum match factor threshold of 10. A four-step filtering workflow was designed to quantitatively identify target compounds as follows (Figure 1).

1. Filtering of match confidence: compounds with spectral match scores ≥ 80 were retained [18];
2. Filtering of unique compounds of CJ-ELNs: compounds identified in CJ and CJ-ELNs were compared with isolated compounds unique to CJ-ELNs;
3. Filtering of biological relevance: candidate compounds were screened for associations with wound healing-related signaling pathways using the DeepSeek-Bio model;
4. Semantic recognition and prompt engineering: final candidate molecules were refined through semantic analysis and prompt-based selection.

Common and unique compounds derived from CJ and CJ-ELNs were visualized in a Venn diagram, and a word cloud analysis was conducted via Python. Functions and tools, and databases of key terms used in this study are listed in Table 1.

Figure 1. A four-step filtering workflow. CJ-ELNs: *Cayratia japonica* exosome-like nanovesicle; LC-MS: liquid chromatography-mass spectrometry.

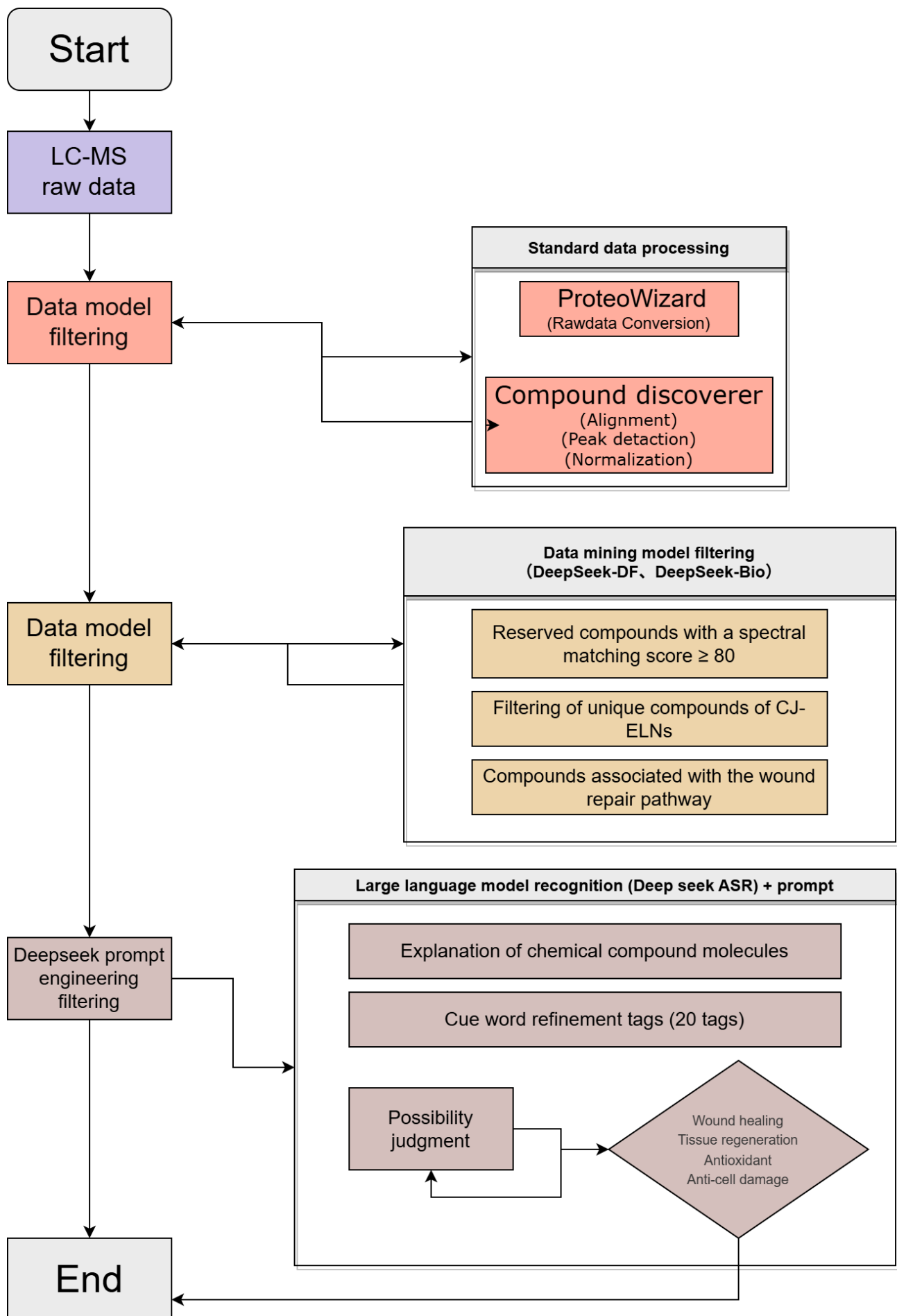


Table . Key terms, functions, tools and databases used in this study.

Key terms	Functions	Tools/databases
mzML	Standardized data storage	ProteoWizard
DeepSeek-Bio	Biological pathway association analysis	Deepseek 671B Model Network Edition KEGG database
Morgan	Digital characterization of molecular structures	Chemoinformatics software packages
PubMedBERT	Literature feature extraction	PubMed.pro
Grad-CAM	Visualization of model decisions	Deep learning frameworks (eg, PyTorch)
ASR	automatic semantic recognition	The Great Prophecy Model of Human-Computer Interaction

Construction of a Multimodal Framework of LC-MS Combined With DeepSeek Models

A multimodal framework of LC-MS combined with the DeepSeek-DF model was created, consisting of two major components of the input and output layers. The input layer integrated structural features of compounds (Morgan

fingerprints), quantitative features (z score normalization), and literature-derived features (PubMedBERT embeddings). The core architecture was listed in [Figure 2](#). Additionally, the output layer used multitask learning to simultaneously predict wound-healing activity via Sigmoid output and mechanism category via Softmax output.

Figure 2. The core architecture of the input layer.

```
class DualAttentionNN(nn.Module):
    def __init__(self):
        super().__init__()
        self.struct_net = GATv2Conv(
            in_channels=2048,
            hidden_channels=512
        )
        self.quant_net = TransformerEncoder(
            layers=4,
            d_model=256
        )
        self.fusion = DeepSeekCrossAttention(
            embed_dim=768
        )
```

Interpretability-Based Filtering

The Automated Semantic Recognition (ASR) module and prompt engineering techniques of DeepSeek-R1 32B, as well as web searching were used to interpret the potential biological functions of the screened candidate compound with an annotation of functional labels. A plausibility assessment was then performed based on predefined criteria, including antioxidation, anti-inflammation, anticellular damage, antiapoptosis, wound healing and tissue regeneration, and cell proliferation. Each compound was evaluated and categorized

using the following scoring scheme: \checkmark (confirmed), \times (not supported), and $?$ (uncertain). Taking the metabolite (-)-Epicatchin 3-O-gallate as an example, its function, category and possibility in the involvement of wound healing, tissue regeneration, antioxidant, and anticellular damage were predicted via the multimodal framework ([Table 2](#)). Following this preliminary filtering, manual curation was conducted to eliminate compounds of nonplant origin and those with low abundances. Ultimately, a refined set of characteristic natural products from CJ-ELNs with potential wound-healing properties was selected.

Table . Functions, categories and possibility in the involvement of biological processes of representative metabolites.

Compound	Functions	Categories	Possibility
(-)-Epicatechin 3-O-gallate	Antioxidant, anti-inflammatory, anti-cancer, cardiovascular protection, glucose and lipid metabolism regulation.	Organic compound, antioxidant factor, anti-inflammatory factor, energy metabolism, phenolic factor	Wound healing: ×, tissue regeneration: ×, antioxidant: √, anti-cellular damage: ?
Rutin	Antioxidant and anti-inflammatory, maintaining vascular resilience, reducing vascular permeability and fragility, exhibiting certain antiviral and anticancer effects.	Flavonoids, antioxidant, anti-inflammatory	Wound healing: ×, tissue regeneration: ×, antioxidant: √, anti-cellular damage: ?
Caffeine	Central nervous system stimulants, enhance mental alertness, alleviate fatigue.	Organic compounds, alkaloids, energy metabolism	Wound healing: ×, tissue regeneration: ×, antioxidant: ×, anti-cellular damage: ×

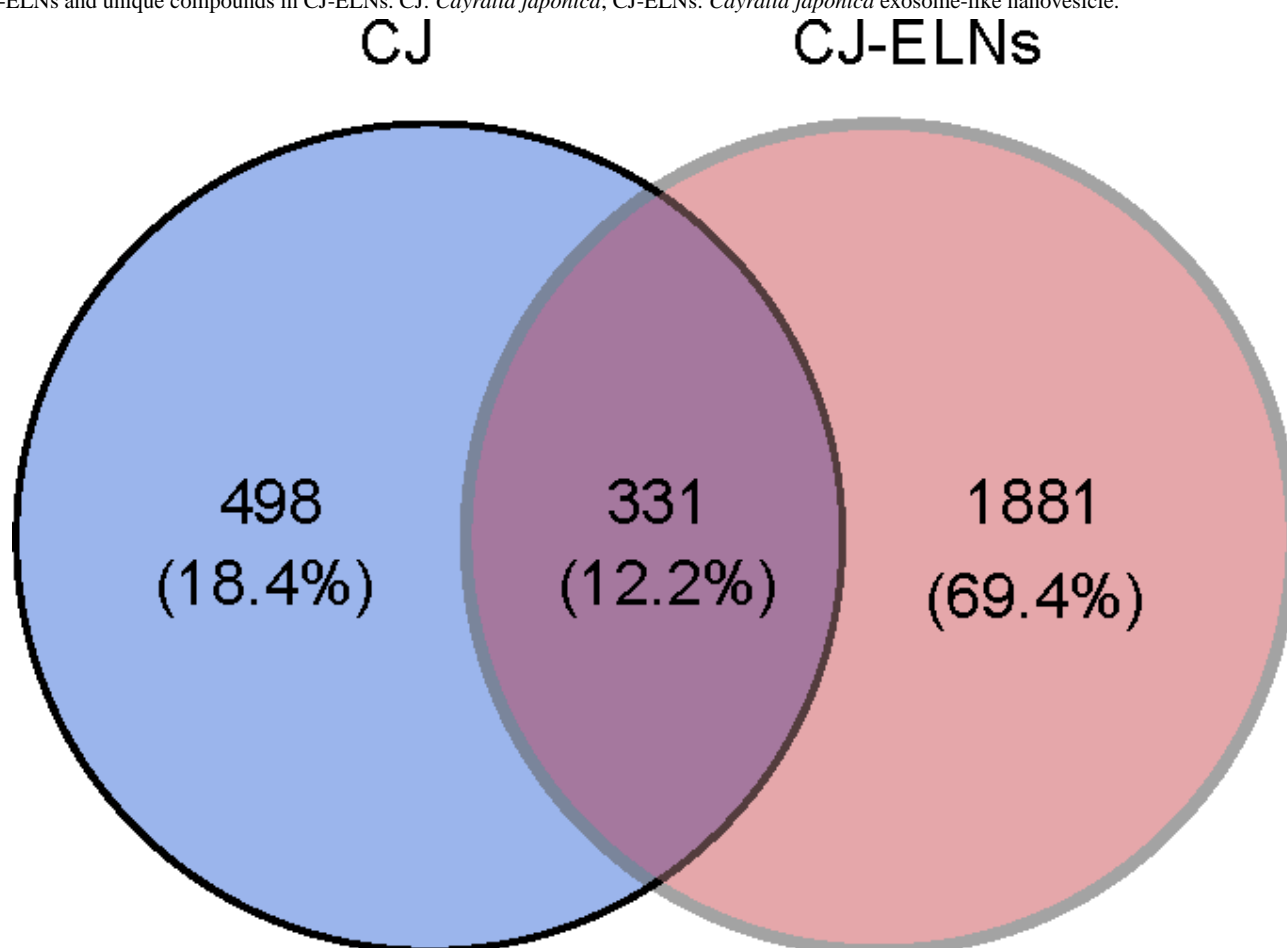
Results

Acidic Compounds Are Enriched in CJ-ELNs

After conversion and normalization of the raw LC-MS data, a total of 829 and 2212 compounds were identified from CJ and

CJ-ELNs. A Venn diagram visualized 1881 specific compounds in CJ-ELNs (Figure 3). “Acid,” as the most frequent term across all entries of metabolite names, was detected by a word cloud analysis (Multimedia Appendix 1). It suggested that acidic compounds were highly enriched in CJ-ELNs.

Figure 3. Enrichment of acidic compounds in CJ-ELNs. (A) A Venn diagram visualizing an intersection of compounds identified from both CJ and CJ-ELNs and unique compounds in CJ-ELNs. CJ: *Cayratia japonica*; CJ-ELNs: *Cayratia japonica* exosome-like nanovesicle.



Rapid and Accurate Data Mining of Compounds in CJ-ELNs With Functional Properties

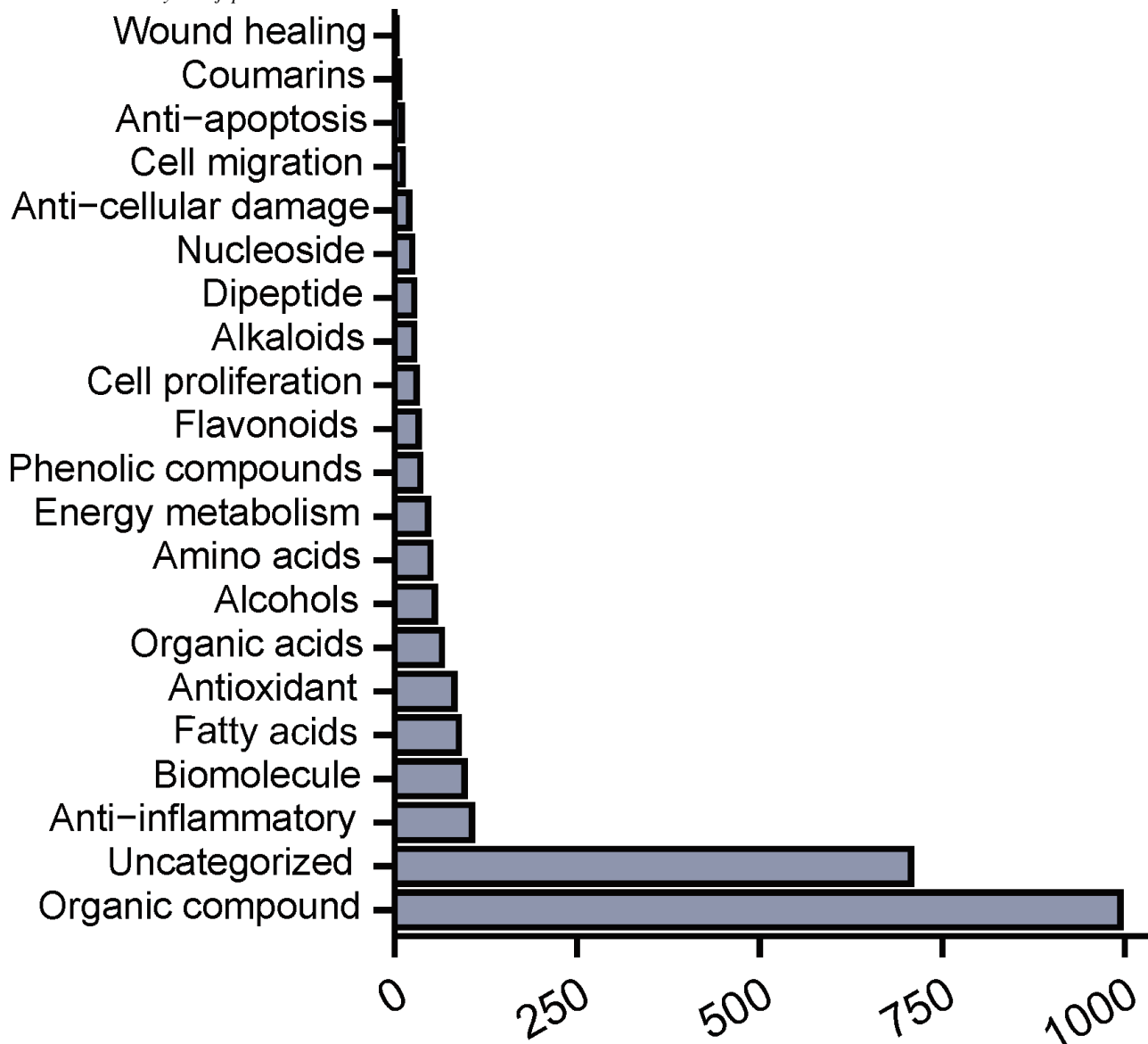
A total of 1881 candidate compounds enriched in CJ-ELNs were functionally annotated and classified using the self-designed multimodal framework of LC-MS combined with

DeepSeek models. They were categorized into 20 distinct classes, including organic compounds, alkaloids, amino acids, biomolecules, organic acids, antioxidants, anti-inflammatory agents, energy metabolism-related molecules, phenolics, cytoprotective agents, alcohols, and others. Organic compounds were the leading category of compounds enriched in CJ-ELNs

(Figure 4, Multimedia Appendix 2). Functionally, 43.33% (n=39) of compounds enriched in CJ-ELNs possessed the antioxidant property. With the assistance of DeepSeek, we specifically screened compounds enriched in CJ-ELNs with

targeted functions of antioxidation, anti-inflammation, anticellular damage, antiapoptosis, wound healing and tissue regeneration, and cell proliferation.

Figure 4. Rapid and accurate data mining of compounds in CJ-ELNs with functional properties. Top 20 classifications of compounds enriched in CJ-ELNs. CJ-ELN: *Cayratia japonica* exosome-like nanovesicle.



Bioactive Compounds of CJ-ELNs Responsible for Wound Healing and Tissue Regeneration

We estimated the overall expression levels of compounds across the six target functions derived from the DeepSeek model within this multimodal framework, visualizing the results in radar chart format after log₂-transformation. (Figure 5). Notably, compounds with the antioxidant function possessed the highest expression levels, proving the antioxidant mechanism of CJ-ELNs in wound repair. Finally, a secondary filtering of

compounds with targeted functions was conducted. We manually excluded nonplant-derived compounds, including those of animal origin, synthetic chemicals, and other nonbotanical sources. In addition, compounds with low expression levels in CJ-ELNs were also removed. As a result, a total of 46 naturally active compounds derived from CJ-ELNs with targeted functions were identified (Figure 6 and Multimedia Appendix 3). Citric acid was the most abundant compound with the targeted functions, which was consistent with the finding from the word cloud analysis.

Figure 5. Radar plots visualizing bioactive compounds of *Cayratia japonica* exosome-like nanovesicles with targeted functions.

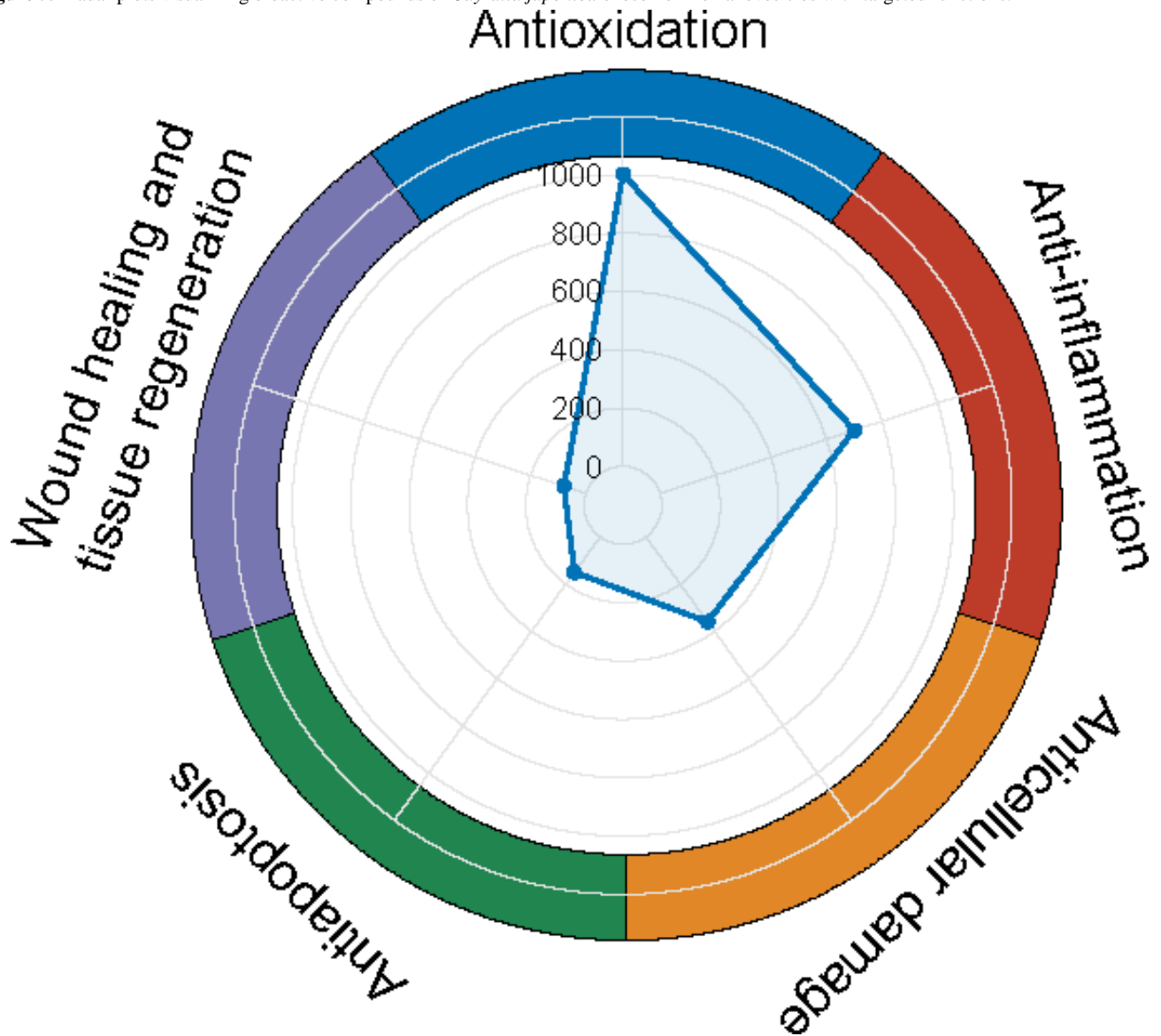
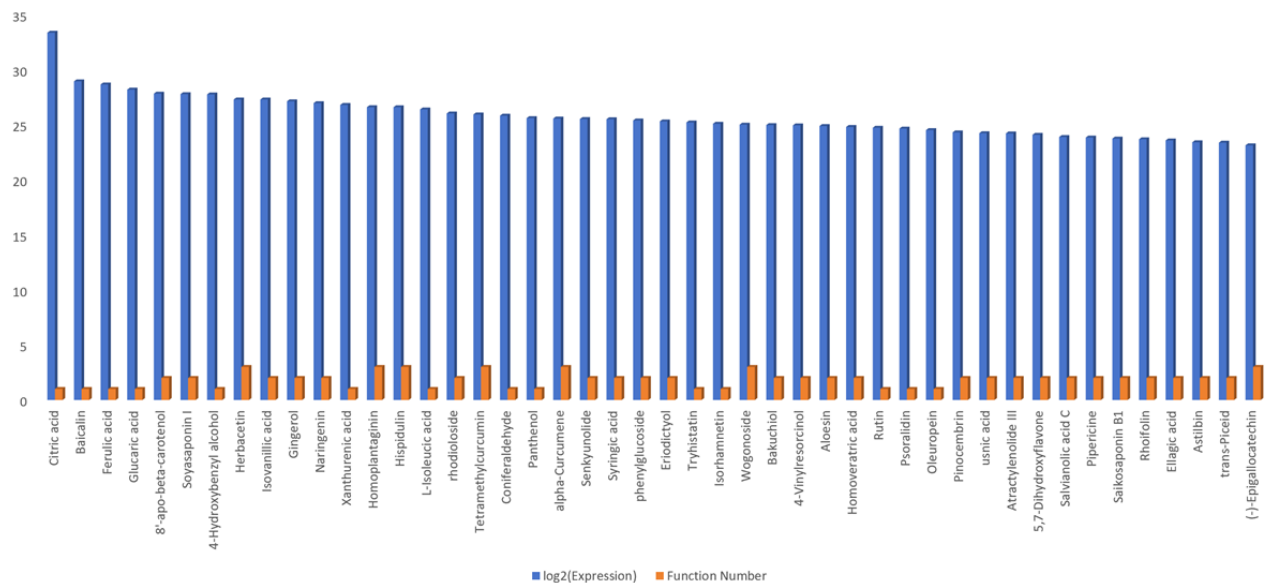


Figure 6. Expression levels (log₂-transformed) of naturally active compounds derived from *Cayratia japonica* exosome-like nanovesicle identified by an integration of liquid chromatography-mass spectrometry and DeepSeek models.



Discussion

Principal Findings

This study innovatively integrated DeepSeek models with LC-MS to successfully predict the major natural products of CJ-ELNs responsible for wound healing. DeepSeek's ASR semantic recognition and prompt engineering worked together to generate initial classification labels. Moreover, an automatic assessment effectively, rapidly, and accurately achieved the goal of data mining of specific compounds for targeted functions.

AI techniques, particularly LLMs, have become an unstoppable force for reshaping medical research [19,20]. Traditionally, LC-MS is a powerful analytical technique to identify and quantify active ingredients in traditional Chinese medicine (TCM) herbs. However, a rapid and accurate recognition of compounds with targeted functions, and a quantitative analysis of trace concentrations in complicated samples can be challenging [21]. We expected that an integration of LC-MS and LLMs would benefit TCM research, including the acceleration of active ingredient screen, precise targeting of interested compounds for certain diseases, and anchoring the promising candidates for developing new drugs. DeepSeek is an intelligent system based on a large-scale pre-trained language model, exhibiting strong capabilities in text understanding, knowledge reasoning, and cross-modal collaborative analysis, particularly excelling in processing information within Chinese-language contexts [22,23]. It enables rapid processing and analyzing massive volumes of both unstructured and structured data, thus digging biological insights out of complex omics datasets [24,25].

In the present study, we first created a four-step filtering workflow and quantitatively identified target compounds from CJ-ELNs by LC-MS. The cloud word analysis emphasized the term of acid among screened compounds enriched in CJ-ELNs. Acidic compounds derived from traditional Chinese herbals are established for the role of clearing heat and detoxifying [26]. Numerous studies have reported that acidic compounds in plants exert antioxidant, antibacterial, and anti-inflammatory effects through mechanisms such as scavenging free radicals, alleviating oxidative stress, modulating inflammatory factors, stimulating fibroblast proliferation, promoting collagen deposition,

enhancing epithelialization, and inducing angiogenesis [27,28]. To achieve a precise data mining of compounds with relevant functions, DeepSeek models lent a hand that specifically screened compounds in CJ-ELNs with targeted functions of antioxidation, anti-inflammation, anticellular damage, antiapoptosis, wound healing and tissue regeneration, and cell proliferation. Finally, naturally active compounds in CJ-ELNs were resurfaced for their promising potentials in wound repair. For example, studies have shown that baicalin accelerates the wound healing process by downregulating the expression of pro-inflammatory cytokines (IL-6 and IL-1 β) while upregulating the anti-inflammatory factor IL-10, and by promoting the secretion of various growth factors (VEGF, FGF-2, PDGF- β , and CTGF) [29]. The combination of LC-MS with DeepSeek paves the way to further analyses of therapeutic targets from traditional Chinese herbs for wound healing and tissue regeneration [30,31].

Limitations in this study should be noted. Firstly, bioactive compounds derived from CJ-ELNs were mined via LC-MS and a single LLM, namely, DeepSeek-R1. Other cutting-edge LLMs such as Claude, GPT-4 and Llama [32] can be further analyzed for the assistance of LC-MS in identifying interested compounds. Secondly, the 46 naturally active compounds derived from CJ-ELNs with targeted functions should be validated in *in vivo* and *in vitro* experiments. Lastly, the workflow we have established requires further validation on independent datasets. We shall address the aforementioned issues in subsequent work, including evaluating the efficacy of compounds through cell migration and transdermal tissue compatibility assays, verifying their efficacy via macroscopic imaging and H&E staining following animal wound modelling interventions, and validating potential pathways involved through Western blot and immunohistochemical analysis.

Conclusion

We innovatively designed a multimodal framework of LC-MS combined with DeepSeek models that rapidly and accurately identify naturally active compounds from CJ-ELNs. This AI-powered system innovatively integrates the traditional analytical technique with modern large language models, showing a huge potential in modern medicine and TCM research.

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Data Availability

The original data used for the current study are available upon reasonable request from the corresponding authors.

Authors' Contributions

Conceptualization: MXS, QF, QJY

Data curation: YPF, WJ

Formal analysis: YPF, WJ

Funding acquisition: MXS, QF, QJY

Investigation: WJ, YPF

Methodology: WJ, YPF

Project administration: MXS, QHY

Resources: MXS, QJY

Supervision: MXS, QF, QJY

Writing-original draft: WJ, YPF

Writing-review & editing: JX, JY, MXS, QF, QJY

Conflicts of Interest

None declared.

Multimedia Appendix 1

A word cloud of common compounds identified by liquid chromatography-mass spectrometry.

[[PNG File, 283 KB - bioinform_v7i1e80539_app1.png](#)]

Multimedia Appendix 2

Distribution of the classifications of compounds enriched in CJ-ELNs, distribution of functional compounds enriched in CJ-ELNs with targeted functions of wound healing and tissue regeneration, and distribution of compounds enriched in CJ-ELNs with all functional categories.

[[TIF File, 1337 KB - bioinform_v7i1e80539_app2.tif](#)]

Multimedia Appendix 3

Function of 46 compounds.

[[XLSX File, 17 KB - bioinform_v7i1e80539_app3.xlsx](#)]

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Abbreviations

AI: artificial intelligence

ASR: Automated Semantic Recognition

CJ-ELN: *Cayratia japonica* exosome-like nanovesicle

ELN: exosome-like nanovesicles

ESI: electrospray ionization
LC-MS: liquid chromatography-mass spectrometry
LLM: large language model
MS: mass spectrometry
P-ELN: Plant-derived exosome-like nanovesicle
TCM: traditional Chinese medicine
UHPLC: ultra-high-performance liquid chromatography

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Prevalence and Associated Risk Factors of Bovine Fasciolosis in Bahir Dar, Ethiopia: Cross-Sectional Study

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Abstract

Background: Cattle are among the most important livestock resources in Ethiopia, contributing significantly to the agricultural economy and rural livelihoods. They provide meat, milk, hides, draft power for crop production, and serve as a major source of income for farmers. Despite their vital role, cattle productivity is often constrained by various diseases, particularly parasitic diseases. One of the most significant of these is bovine fasciolosis, a condition caused by ingestion of metacercariae of liver flukes belonging to the genus *Fasciola*.

Objective: This study aimed to assess the prevalence and associated risk factors of bovine fasciolosis in Bahir Dar, Ethiopia.

Methods: A cross-sectional study was conducted from November 2021 to April 2022. A total of 384 cattle were randomly selected from different locations within the study area. Animals of all age groups and both sexes were included. Fecal samples were collected directly from the rectum of each animal using clean, labeled containers. The samples were examined using standard coprological techniques, specifically the sedimentation method, to detect liver fluke eggs. All findings were recorded, and the data were analyzed using descriptive statistical methods.

Results: The overall prevalence of fasciolosis was 49.21% (n=189). Based on origin, Sebatamit had the most incidence at 61.84% (n=47), followed by Kebele 11 at 59.37% (n=57), Tikurit at 50% (n=59), and Latamma at 27.65% (n=26). Statistical analysis revealed significant disparities in occurrence among areas. Cattle in poor condition had the largest prevalence (n=80, 64%), followed by medium condition (n=85, 50%) and fat cattle (n=24, 26.96%). This variation was statistically significant. Age-group analysis revealed comparable prevalence rates, with young cattle at 50.38% (n=65), adults at 47.33% (n=71), and elderly cattle at 50.47% (n=53), with no significant differences found. There were no significant sex-related variations in prevalence, with males exhibiting a prevalence of 49.73% (n=93) and females 48.73% (n=96). Local cattle had a slightly higher prevalence (n=111, 51.62%) than crossbreeds (n=78, 46.15%), although the difference was not statistically significant ($P=0.29$).

Conclusions: These findings underscore the need for targeted, location-specific control strategies and highlight the importance of improved nutritional and health management practices to reduce the burden of fasciolosis in cattle populations.

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KEYWORDS

Bahir Dar; bovine fasciolosis; fecal examination; prevalence; risk factors

Introduction

Ethiopia hosts one of the largest livestock populations in Africa, with an estimated 55.03 million cattle, 27.32 million sheep, and 28.16 million goats as of 2019. Cattle, in particular, play a central role in the country's agricultural economy, with the dairy sector contributing over 81% of total milk production. Despite this abundance, livestock productivity remains low due to constraints such as poor nutrition, inadequate management, and widespread infectious diseases. Among these, fasciolosis is one of the most impactful parasitic diseases, causing substantial

economic losses through reduced growth, impaired fertility, decreased milk yield, and increased mortality [1].

Fasciolosis is caused by trematode parasites of the genus *Fasciola*, commonly known as liver flukes. Infection occurs when animals ingest metacercariae, the infective stage of the parasite, from contaminated pasture, water, or feed. Two species are primarily responsible: *Fasciola hepatica*, which predominates in temperate regions, and *Fasciola gigantica*, more common in tropical climates, including much of Africa and Ethiopia [2-4]. Transmission relies on the presence of aquatic snails, such as *Lymnaea truncatula* and *Lymnaea*

natalensis, which serve as intermediate hosts under suitable conditions like stagnant water and moist environments [5].

Infected cattle experience liver tissue damage due to migrating immature flukes, resulting in inflammation, bile duct obstruction, hepatocellular necrosis, and fibrosis. Clinical signs often include weight loss, jaundice, poor body condition, and reduced productivity. Severe infections compromise liver function, predispose animals to secondary infections, and may lead to significant morbidity and mortality [6]. Economically, fasciolosis causes extensive liver condemnation at slaughterhouses, higher veterinary costs, and financial losses for farmers and the meat industry [7].

Epidemiology of fasciolosis is influenced by host and environmental factors, including age, sex, breed, management practices, and ecological conditions. Older animals are often more affected due to cumulative exposure, while some breeds show variable susceptibility. Differences in grazing behavior and reproductive cycles may contribute to higher prevalence in females in some cases [8-10].

Additionally, pasture type, access to contaminated water, and use of anthelmintics play critical roles in transmission. Although previous studies have provided insights into fasciolosis in Ethiopia, many were localized, leaving gaps in regional

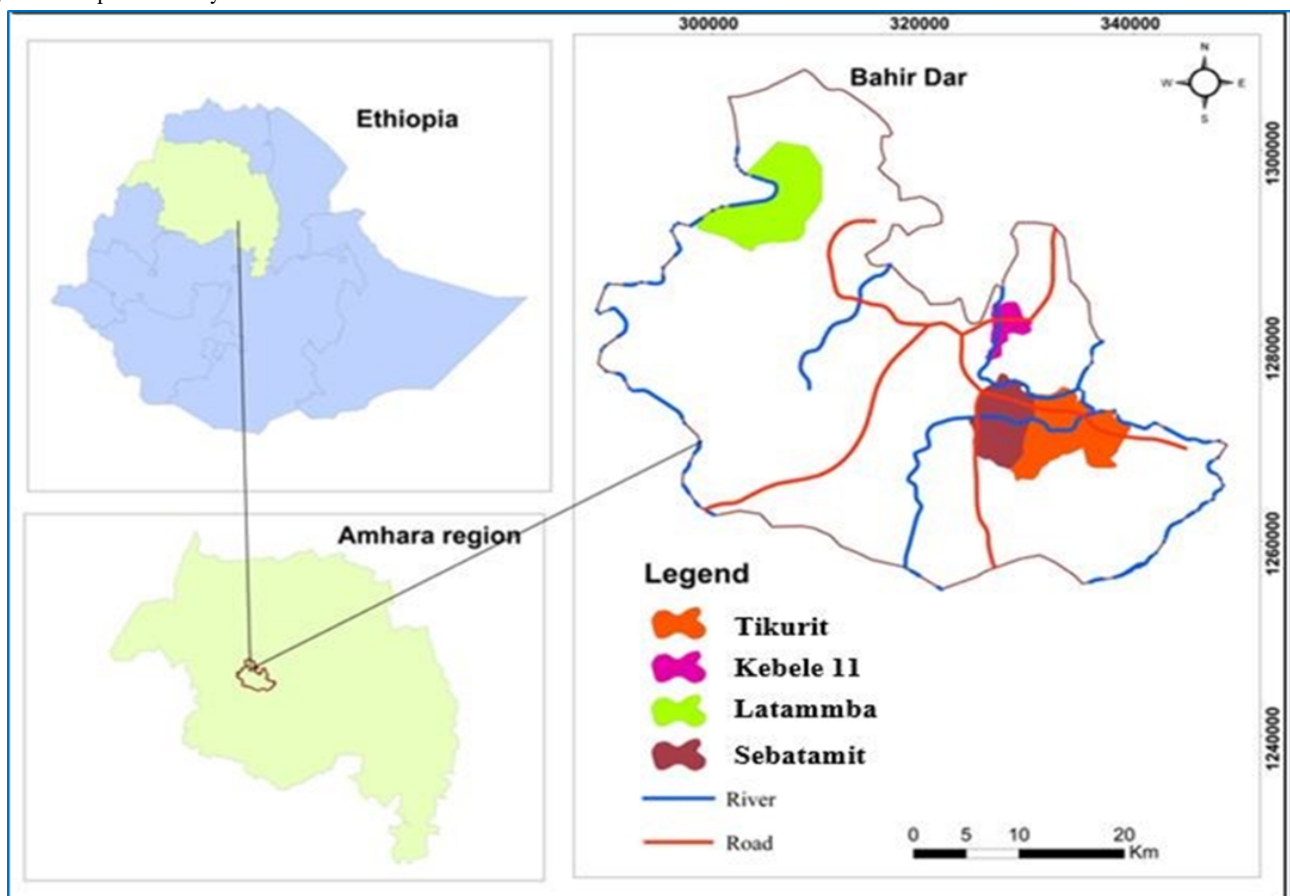
prevalence and risk factors. Given the ecological and management diversity across the country, region-specific studies are essential to inform targeted control strategies. In particular, the central and northern highlands, where cattle production is economically significant, may present unique environmental conditions that affect parasite dynamics [11-14]. Therefore, this study aimed to determine the prevalence and associated risk factors of bovine fasciolosis in Bahir Dar, Ethiopia.

Methods

Study Area

The study was conducted in Bahir Dar, Ethiopia, from November 2021 to April 2022 (Figure 1). Bahir Dar is located approximately 575 km northwest of Addis Ababa, at an elevation of 1500 - 2600 meters above sea level, with geographic coordinates of 12°29' N latitude and 37°29' E longitude. The area receives an average annual rainfall of 1200 - 1600 mm, and temperatures range from 8 °C to 31 °C. The landscape is predominantly plain plateaus, covering roughly 70% of the region, and the vegetation includes shrub formations, low woodlands, evergreen areas, and semi-humid highland vegetation. Agriculture is a key livelihood, with major crops including teff (*Eragrostis tef*), wheat (*Triticum aestivum*), maize (*Zea mays*), and various pulses [1].

Figure 1. Map of the study area



Study Animal and Sampling Method

The study animals consisted of cattle from four selected sites in Bahir Dar: Kebele 11, Sebatamit, Tikurit, and Latamma.

Both indigenous and crossbred Holstein Friesian cattle reared under local management conditions were included. A total of 384 cattle were randomly selected, representing both sexes and multiple age groups. Animal age was assessed based on dentition

and categorized as young or adult [15]. Body condition score was also evaluated for each animal following established guidelines to estimate nutritional and health status [16].

Study Design and Sample Size

A cross-sectional study was carried out in Bahir Dar, Ethiopia, from November 2021 to April 2022 to determine the prevalence and associated risk factors of bovine fasciolosis in Bahir Dar, Ethiopia. The risk factors considered included origin (location), breed, age, sex, and body condition of the cattle. The required sample size for fecal sample collection was calculated using a 95% confidence level, 5% absolute precision, and an expected prevalence of 50% in the absence of prior data for the study area [17].

$$n = Z^2 \times P_{exp} \times (1 - P_{exp}) / d^2$$

where: n =required sample size; P_{exp} =expected prevalence (0.5); d =desired absolute precision (0.05); and Z = Z -value for confidence level (1.96). Based on this formula, a total of 384 cattle were included in the study.

Fecal Examination and Identification of Fasciola Eggs

Fresh fecal samples were collected directly from the rectum of each animal using disposable gloves and placed into universal bottles containing 10% formalin as a preservative. Samples were transported under controlled conditions to the Bahir Dar Regional Veterinary Laboratory for parasitological examination. The sedimentation technique was used to detect *Fasciola* eggs, following standard procedures [18,19]. To differentiate *Fasciola* eggs from those of other trematodes, such as *Paramphistomum* species, the sediment was stained with methylene blue. *Fasciola* eggs are typically yellowish, large, and operculated, whereas *Paramphistomum* eggs stain blue [20,21].

Data Management and Analysis

The raw data collected from the study were organized and entered into a Microsoft Excel spreadsheet for initial management. Subsequently, the data were exported to STATA

(version 16.0; StataCorp) for statistical analysis. A χ^2 test was used to evaluate the correlation between infection rates and risk factors such as age, sex, breed, and location. The test evaluated infection rates based on these parameters, with a significance level of $P < .05$.

Ethical Considerations

Ethical clearance was obtained from the Ethics Research Review Committee of the University of Gondar, College of Veterinary Medicine and Animal Sciences (Ref. No. CVMASc/UoG/RERC/10/11/2021; November 7, 2021). Cattle were handled according to animal welfare guidelines, and owner consent was obtained prior to data collection.

Results

Prevalence of Bovine Fasciolosis

A total of 384 cattle were examined in this study, and the overall prevalence of bovine fasciolosis in the study area was 49.21% ($n=189$). Analysis by origin showed the highest prevalence in Sebatamit ($n=47$, 61.84%), followed by Kebele 11 ($n=57$, 59.37%), Tikurit (50%), and Latamma ($n=26$, 27.65%), with a statistically significant difference among sites ($\chi^2=26.31$, $P < .001$). Prevalence also varied according to body condition, with poor-conditioned cattle exhibiting the highest prevalence ($n=80$, 64%), followed by medium condition ($n=85$, 50%) and fat cattle ($n=24$, 26.96%), showing a significant difference ($\chi^2=28.6$, $P < .001$). When grouped by age, the prevalence was 50.38% ($n=65$) in young cattle, 47.33% ($n=71$) in adults, and 50.47% ($n=53$) in older animals; however, differences were not statistically significant ($\chi^2=0.84$, $P=.35$). Similarly, sex did not significantly influence prevalence: males had 49.73% ($n=93$), and females had 48.73% ($n=96$, $\chi^2=0.844$; $P=.35$). Finally, breed showed no significant effect on infection rates, although local cattle had a slightly higher prevalence ($n=111$, 51.62%) compared to crossbreeds ($n=78$, 46.15%) ($\chi^2=0.287$, $P=.29$) (Table 1).

Table . Prevalence of bovine fasciolosis based on risk factors.

Risk factor and category	Examined (n=384)	Positive (n=189), %	Chi-square (<i>df</i>)	P value
Origin			26.31 (3)	<.001
Tikurit	118	59 (50)		
Sebatamit	76	47 (61.84)		
Latamma	94	26 (27.65)		
Kebele 11	96	57 (59.37)		
Body condition			28.60 (2)	<.001
Poor	125	80 (64)		
Medium	170	85 (50)		
Fat	89	24 (26.96)		
Age			0.839 (2)	.35
Young	129	65 (50.38)		
Adult	150	71 (47.33)		
Old	105	53 (50.47)		
Sex			0.844 (1)	.35
Male	187	93 (49.73)		
Female	197	96 (48.73)		
Breed			1.134 (1)	.29
Local	215	111 (51.62)		
Crossbreed	169	78 (46.15)		

Identification of Fasciola Eggs

The Fasciola eggs detected in cattle fecal samples are shown in [Figure 2](#). Identification was based on morphological

characteristics [20,21]. The eggs are ovoid, possess a thick yellowish-brown shell, and typically have an operculum at one end.

Figure 2. Egg of *Fasciola* species: yellowish egg (arrow).



Discussion

Principal Findings

Bovine fasciolosis remains one of the most significant parasitic diseases affecting cattle in Ethiopia, causing substantial economic losses through reduced growth, poor milk production, liver condemnation, and increased susceptibility to secondary infections. The present study recorded an overall prevalence of bovine fasciolosis of 49.21% in the study area, which aligns with previous reports from different regions of Ethiopia. For instance, prevalence rates of 41.41% and 54.5% have been reported in Woreta [22] and Jimma [23], respectively. Similar rates were observed in North-East Amhara (47.10%) [13], and Eastern Shoa, Kuyu District (54.2%) [19] likely associated with common agro-ecological factors such as the presence of *Lymnaea* snails and practices like communal water use and irrigation that facilitate pasture contamination [24]. Conversely, lower prevalence rates have been reported in some regions. Investigations in Soddo (4.9%) [25], Nekemte (15.9%) [7], and Southern Ethiopia (15.9%) [8] demonstrated lower frequencies, while Zenzelma, Bahir Dar (26%) [1], and Bahir Dar (32.3%) [26] reported slightly lower prevalence. These variations are likely influenced by differences in altitude, geography, climate, snail host abundance, management practices, and anthelmintic use [27].

Geographical and Management Factors

Prevalence varied significantly among study sites in the current work, with Sebatamit recording the highest prevalence (61.84%), followed by Kebele 11 (59.37%), Tikurit (50%), and Latamma (27.65%). This observation is consistent with previous studies indicating substantial geographical variation in fasciolosis prevalence [28-31], which may be influenced by climatic conditions, animal management practices, and access to veterinary services [32].

Influence of Animal Factors

Cattle in poor body condition exhibited the highest prevalence (64%), compared to medium (50%) and fat cattle (26.96%). This pattern corresponds with several Ethiopian reports that found significantly higher infection rates in animals with poor body condition, likely reflecting increased susceptibility due to malnutrition, compromised immunity, and concurrent infections that weaken host defenses [33-36]. However, some studies reported no significant differences in prevalence among body condition categories, indicating that body condition may not always predict *Fasciola* infection [23,37].

In this study, no significant differences were observed across age groups, with young cattle (50.38%), adults (47.33%), and older cattle (50.47%) showing similar prevalence. Several studies in Ethiopia have likewise reported no significant association between age and *Fasciola* infection [38,39], although

other reports documented age-related variation, likely reflecting differences in exposure or management practices [40,41].

Similarly, sex did not significantly influence prevalence, with males at 49.73% and females at 48.73%. This finding aligns with previous studies reporting no significant differences between male and female cattle [35], although one study did observe sex-related variation, possibly due to differences in management practices or environmental exposure [25].

Finally, breed had no significant effect on infection rates, with local cattle and crossbreeds showing prevalence of 51.62% and 46.15%, respectively. This finding agrees with earlier research [24,34], suggesting similar susceptibility between breeds, although a few studies reported breed-related differences, potentially attributable to genetic factors or breed-specific traits.

Limitations

The cross-sectional design and use of a single diagnostic method provide an accurate snapshot of prevalence and associated risk factors within the study population. While longitudinal or

multimethod studies may provide additional detail, the current methodology is sufficient to address the study objectives and offers valuable insights for local disease control strategies.

Conclusion

This study revealed a high overall prevalence of bovine fasciolosis (49.21%) in the Bahir Dar area, confirming its significance as a major parasitic disease affecting cattle in the region. Prevalence varied notably across localities, with Sebatamit exhibiting the highest rate (61.84%) and Latamma the lowest (27.65%). Analysis of risk factors indicated a significant association between body condition and fasciolosis, with poorly conditioned cattle being more susceptible to infection. In contrast, no statistically significant differences were observed based on age, sex, or breed, suggesting that cattle across all demographic groups are at risk. These findings underscore the need for targeted, location-specific control strategies and highlight the importance of improved nutritional and health management practices to reduce the burden of fasciolosis in cattle populations.

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Data Availability

All data generated or analyzed during this study are included in this published article.

Authors' Contributions

TM: writing – review & editing, writing – original draft, resources, formal analysis, investigation, conceptualization, methodology, validation. TS: writing – review & editing, resources, validation, methodology, visualization. ABT: writing the original draft, review editing, funding acquisition, resources, methodology, conceptualization, formal analysis, supervision, and data curation

Conflicts of Interest

The authors declare that they have no competing interests.

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Development and Validation of a Generative Artificial Intelligence-Based Pipeline for Automated Clinical Data Extraction From Electronic Health Records: Technical Implementation Study

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Abstract

Background: The manual abstraction of unstructured clinical data is often necessary for granular clinical outcomes research but is time consuming and can be of variable quality. Large language models (LLMs) show promise in medical data extraction yet integrating them into research workflows remains challenging and poorly described.

Objective: This study aimed to develop and integrate an LLM-based system for automated data extraction from unstructured electronic health record (EHR) text reports within an established clinical outcomes database.

Methods: We implemented a generative artificial intelligence pipeline (UODBLLM) utilizing a flexible language model interface that supports various LLM implementations, including Health Insurance Portability and Accountability Act-compliant cloud services and local open-source models. We used extensible markup language (XML)-structured prompts and integrated using an open database connectivity interface to generate structured data from clinical documentation in the EHR. We evaluated the UODBLLM's performance on the completion rate, processing time, and extraction capabilities across multiple clinical data elements, including quantitative measurements, categorical assessments, and anatomical descriptions, using sample magnetic resonance imaging (MRI) reports as test cases. System reliability was tested across multiple batches to assess scalability and consistency.

Results: Piloted against MRI reports, UODBLLM processed 1800 clinical documents with a 100% completion rate and an average processing time of 8.90 seconds per report. The token utilization averaged 2692 tokens per report, with an input-to-output ratio of approximately 13:2, resulting in a processing cost of US \$0.009 per report. UODBLLM had consistent performance across 18 batches of 100 reports each and completed all processing in 4.45 hours. From each report, UODBLLM extracted 16 structured clinical elements, including prostate volume, prostate-specific antigen values, Prostate Imaging Reporting and Data System scores, clinical staging, and anatomical assessments. All extracted data were automatically validated against predefined schemas and stored in standardized JSON format.

Conclusions: We demonstrated the successful integration of an LLM-based extraction system within an existing clinical outcomes database, achieving rapid, comprehensive data extraction at minimal cost. UODBLLM provides a scalable, efficient solution for automating clinical data extraction while maintaining protected health information security. This approach could significantly accelerate research timelines and expand feasible clinical studies, particularly for large-scale database projects.

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KEYWORDS

generative artificial intelligence; artificial intelligence large language model; GPT-4; chatbot; pattern analysis; prostate cancer; kidney cancer

Introduction

Background

Electronic health record (EHR) systems contain extensive health data, but much of it is in unstructured notes such as radiology and pathology reports, making it hard to access for large-scale research. Granular clinical outcomes research often requires laborious manual chart review. The automation of this process requires significant investment, and algorithm performance varies with report parameters and automation type [1,2]. Previous attempts to automate this process have tried natural language processing on prostate cancer pathology reports, reporting a weighted F_1 score and accuracy as high as 0.97% and 93%, respectively [3].

Large language models (LLMs) represent a new opportunity for addressing this problem. LLMs are generative artificial intelligence programs capable of drafting human-like responses to specific queries. In oncological contexts, LLM applications can create medical notes, aggregate imaging findings, extract operative note data, and identify presenting symptoms [4-7]. Previous studies analyzing the overall data extraction capabilities have found accuracies ranging from 63.9% to 100% in retrieving data elements [5,8-13]. Specifically, several LLM models have also been developed to extract medical information from text, including early-stage LLM trained on medical encyclopedias and radiology datasets to read annotated radiology reports (71.6% accuracy) and inferring cancer disease response based on computed tomography reports (89% accuracy) [14,15]. Some of these groups also implemented or hypothesized implementing their systems into medical research pipelines for expediting data extraction [3,8]. Another group applied a customized, open-source LLM trained on medical data to read magnetic resonance imaging (MRI) reports with a sensitivity of 96% and specificity of 99%. In terms of data extraction, generative pre-trained transformer (GPT)-4 has been shown to extract hepatocellular carcinoma data from MRI reports with an overall accuracy of 93.4% [16]. LLMs have also proven to be flexible and frequently outperform traditional automated models, suggesting that powerful LLMs might be ready to support research endeavors via the extraction of unstructured data [5,8,17]. Implementing LLMs into practical, applicable tools remains challenging, and some private organizations have attempted to improve clinical data extraction through EHR integration [18]. Despite this, most efforts, such as the American Urological Association Quality Registry, remain dependent on manual data management, partially due to difficulty integrating new tools into existing workflows. While some larger institutions have begun implementing automated data extraction pipelines, traditional methods of data extraction require considerable technical expertise and resources to initiate, making these methods inaccessible for most institutions.

The University of California, San Francisco (UCSF) Department of Urology maintains the Urologic Outcomes Database (UODB) for prostate, bladder, and renal cancers [19]. The UODB is an SQL-based clinical data research database that holds structured manually abstracted clinical data for patients treated at the UCSF, including 7000 patients with prostate cancer over 20

years. Due to limited manual abstraction capacity and increasing patient volume, clinical events and data entry often lag. Previous in-house attempts to automate this process using traditional natural language processing solutions proved to be time-consuming to develop and maintain [1-3,20]. The aim of this study was to demonstrate a practical use of LLMs in academic clinical research by describing the successful implementation of a secure, baseline, institutional version of GPT-4 within the UODB to quickly and easily extract unstructured data and effectively reduce manual labor in gathering data from medical reports.

Related Work

Previous studies by our group have utilized UCSF's Versa, an internal, secure, Health Insurance Portability and Accountability Act (HIPAA)-compliant deployment of OpenAI's GPT models (OpenAI Inc.) that includes an application programming interface (API) for query automation [17,21]. We demonstrated that systems based on the Versa GPT-4 API can accurately extract structured data from real-world clinical reports. In one study involving 424 prostate MRI reports, our pipeline, using zero-shot prompting, achieved an overall median field-level accuracy of 98.1% (IQR 96.3% - 99.2%), with key elements such as prostate-specific antigen density (98.3%), extracapsular extension (97.4%), and TNM staging (98.1%) [21]. In a separate effort with 228 prostate MRI reports, the approach achieved similarly high concordance (over 95%) when compared with manual abstraction [17].

These validation efforts serve to confirm the accuracy of the underlying extraction prompts and Versa GPT-4 API performance. The focus of the current work, therefore, is not on additional accuracy testing; rather, we build upon this foundation to present a modular, scalable implementation pipeline that operationalizes LLM-driven extraction at scale, within a secure, clinical-grade environment.

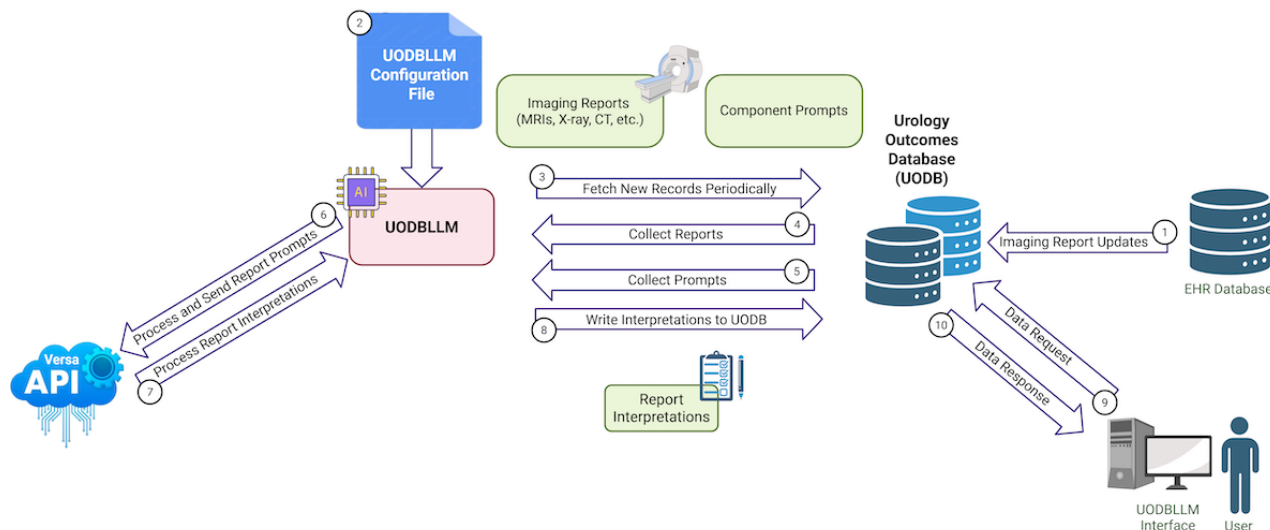
Methods

Overall Design

This study presents the implementation and performance evaluation of UODBLLM, a modular LLM-based pipeline designed for structured data extraction from a wide range of unstructured clinical reports. For this technical implementation, the system was evaluated using free-text prostate MRI radiology reports as the primary use case (Figure 1). The system was deployed within a secure, HIPAA-compliant clinical environment using the internal UCSF Versa GPT-4 API, ensuring that protected health information (PHI) remained confined to institutional systems. UODBLLM was designed with a flexible architecture to support multiple language models and API endpoints, enabling adaptability across varied clinical settings.

Prompts are stored as configurable components in dedicated database tables, allowing users to dynamically pair extraction templates with report sets without modifying the underlying code. This design supports rapid iteration, version control, and seamless adaptation to evolving information extraction needs.

Figure 1. System design and data flow of the UODBLLM application. The process begins with an initial connection between the electronic health record (EHR) and the Urologic Outcomes Database (UODB) for imaging report updates (1). The UODBLLM application is governed by a configuration file defining its core parameters (2). The application periodically fetches new records from the UODB (3), collects the relevant reports (4) and component prompts (5), and sends these to the Versa application programming interface (API) for processing (6). The API returns structured interpretations of the reports (7), which are then written back into the UODB (8). A user, via the UODBLLM interface, can send a data request to the UODB (9) and receive a data response for review and analysis (10).



Study Population

The study dataset comprised 1800 prostate MRI radiology reports retrieved from the institutional EHR system. Reports were selected based on procedural coding and metadata filters to ensure relevance to downstream urologic data extraction.

Intervention

UODBLLM is a Python-based (version 3.9.6, Python Software Foundation, worldwide) application designed to extract structured information from clinical reports using a modular, API-driven architecture. Source text is retrieved from the UODB using a parameterized SQL query passed via a secure Open Database Connectivity connection. Text blocks are staged and dispatched in configurable batches, controlled by a modifiable parameter specified in a configuration file or modifiable via command-line flag.

The pipeline retrieves a version-controlled extensible markup language (XML)-based prompt template at runtime using a parameterized SQL query from the UODB. This template specifies the role, task, JSON response schema, and a structured sub-prompt with 16 XML elements that each represent a clinical field of interest (eg, prostate volume, prostate-specific antigen density, and overall Prostate Imaging Reporting and Data System score), each with plain-language extraction instructions (Figure S1 in [Multimedia Appendix 1](#)). For every report, the program inserts the full free-text report into the template's designated placeholder, producing a complete prompt that is then submitted to the Versa GPT-4 model. Embedding the report within a constant, schema-constrained envelope ensures that returned JSON follows a predictable structure, enabling reliable downstream parsing and storage.

Each batch is passed to a thin wrapper around the Versa GPT-4 API. Requests are streamed to the API endpoint; results are captured, parsed, and validated against the predefined JSON schema. Error handling includes up to 5 retry attempts per request with exponential back-off (2^n seconds, capped at 30 seconds). Failed requests are logged, and the affected reports are re-queued for later processing. Element-level completeness is defined as the proportion of reports for which the pipeline returned a non-null value.

Extracted fields are transmitted back to the database using a set of parameterized SQL UPDATE statements mapped to internal column identifiers. A custom statistics tracking module records token usage, response latency, and processing cost per report by counting model-specific numerical tokens generated from text via Byte Pair Encoding. System-wide throughput and error frequency are also recorded. The pipeline was executed on a 2019 MacBook Pro (Intel Core i9, 2.4 GHz, 64 GB RAM, macOS Ventura 13.2.1). The system's computational workload is lightweight and not hardware dependent, making it executable on a standard consumer laptop. The source code will be made available to investigators for non-commercial purposes upon request.

Ethical Considerations

The study was approved by the University of California, San Francisco Institutional Review Board (IRB #11-05329), and the requirement for informed consent was waived. The system was deployed within a secure, HIPAA-compliant clinical environment using the internal UCSF Versa GPT-4 API, ensuring that PHI remained confined to institutional systems. All reports were de-identified prior to processing.

Results

Processing Performance and Resource Utilization

The analysis of system logs demonstrated consistent performance metrics, with an average processing speed of 8.90 seconds per report across 1800 reports. UODBLLM maintained 100% completion rates across all test runs, with batch sizes of 100 reports. Token utilization, representing the count of model-specific numerical tokens generated from the input and output text via Byte Pair Encoding (calculated using the tiktoken library), averaged 2692 tokens per report. Given the model's context window capacity relative to typical report lengths, specific token optimization techniques like input text chunking were not required for this implementation. This resulted in an input-to-output ratio of approximately 13:2 (4,196,697 input tokens, 648,723 output tokens), resulting in an average processing cost of US \$0.009 per report. The total processing run successfully analyzed all 1800 test reports in 4.45 hours, showing sustained performance at scale.

Prior Validation

Although the present study did not re-evaluate extraction accuracy on this corpus, the underlying extraction logic and prompt structure have been previously validated in two independent studies by our group. In one effort involving 424 prostate MRI reports, the system achieved a median field-level accuracy of 98.1% (IQR 96.3% - 99.2%) for key clinical variables [21]. A subsequent study with 228 MRI reports demonstrated similarly high extraction fidelity, with all structured elements exceeding 95% accuracy [17]. These findings confirm the robustness of the prompt design and model configuration across settings, supporting their reliability in the context of the current implementation.

Experience

Researchers interact with UODBLLM by selecting the clinical report category (eg, MRI reports or pathology reports) through a secure web-based application that integrates with the UODB and is accessible only through local institutional network connections. UODBLLM displays quantitative processing metrics for the selected report type, including extraction completion timestamps, LLM prompts, and performance statistics from previous analyses. This longitudinal view enables investigators to evaluate existing structured data's temporal relevance and completeness before proceeding with additional processing.

Researchers can use previously extracted structured data or initiate a new extraction cycle with refined extraction parameters. When opting for new extraction, investigators can specify temporal bounds for report inclusion and modify extraction prompts stored in the database tables. This parameterization enables the analysis of specific clinical cohorts while ensuring consistent extraction methodology across research protocols.

Upon initiating the UODBLLM process, the system executes batch processing of identified reports, with real-time logging providing visibility into extraction progress. Researchers can monitor the system performance through logs that track

processing times, success rates, and any encountered exceptions. The structured JSON output is automatically integrated into the UODB, enabling immediate access for researchers.

Quality assurance is implemented through a review interface where researchers can perform comparative analysis of extracted data elements against source reports and any pre-existing manually abstracted data with the opportunity to iteratively refine prompts. Successfully processed reports are flagged in the database, preventing duplicate processing while maintaining a comprehensive audit trail of all data extraction operations.

Discussion

Principal Findings and Comparison With Previous Works

In this study, we developed and validated an automated LLM-based integration for UODB management that achieved a 100% completion rate across 1800 clinical documents, with an average processing time of 8.90 seconds per report. The UODBLLM demonstrates an implementation of a PHI-secure, LLM-agnostic system for automated data extraction from urological outcomes documentation. By leveraging institutional cloud infrastructure and established database architecture, we created a scalable solution that significantly reduces the manual effort traditionally required for data extraction while maintaining high accuracy rates [19]. This advancement represents a crucial step toward efficient, accurate, and comprehensive research database management [18].

The integration of generative artificial intelligence in clinical data management has seen rapid evolution, with several institutions developing specialized approaches for extracting structured data from clinical documentation [1,2]. While the validation of a local GPT model showed promising accuracy in the low 90th percentile for biomedical data collection, their focus on chromatin expression in cell lines addressed a more constrained data domain [20]. UODBLLM demonstrates comparable accuracy rates with the ability for researcher customization. Recent oncology initiatives using LLMs for clinical note evaluation have shown potential, but our approach differs by providing a complete pipeline that not only extracts data but also integrates directly with existing database infrastructure [5,6]. The problem of integration from clinical care to research database is common in clinical trials, clinical record management, and safety reports, encouraging other groups to design automated data capture and transfer pipelines. These pipelines have historically been evaluated as successful by the variables they extract, efficiency gained, and interoperability they provide, aligning with our key performance indicators [22,23]. The pipeline here described and designed has been estimated to improve data extraction manual time efficiency by as much as 90% if pulling multiple variables from hundreds of reports, although this enhancement varies based on report type, variable, and iterations of prompt refinement.

The technical robustness of our approach is supported by key design decisions and validated through comprehensive testing. Our choice to leverage a PHI-secure institutional version of GPT-4 addresses performance and privacy requirements, crucial

considerations for clinical data management [5]. The system's integration within the UODB piggybacks off a validated foundation for data structure and management [19]. Our validation protocol included processing reports across various batch sizes, achieving consistent performance and reliable operation at scale. The ability of the UODBLLM to efficiently process clinical documentation while maintaining high accuracy suggests the potential for significant resource optimization in research operations [6]. These efficiency gains could dramatically accelerate research timelines and expand the scope of feasible clinical studies.

Although this study did not re-assess extraction accuracy, this was a deliberate design choice. The extraction framework employed here has already undergone validation in prior work, with element-level accuracies exceeding 95% across multiple prostate MRI cohorts [17,21]. In contrast, our current objective was to evaluate the system-level performance of a scalable, generalizable implementation pipeline deployed within a secure clinical environment. Notably, the architecture is model-agnostic and allows for future integration of various LLMs or prompt schemas. This decoupling of model validation from pipeline implementation facilitates adaptability while building on established, validated components.

The limitations of our approach warrant careful consideration. While UODBLLM performs robustly for current use cases, the accuracy of LLM-based data extraction still requires human validation for critical data points, a challenge noted across multiple studies [4,5,8]. The evolving nature of clinical research

means that prompt engineering must continually adapt to new data types and research questions. Additionally, while our pipeline is LLM-agnostic, our specific performance results were achieved using a PHI-secure version of GPT-4, and performance may vary with different models or implementations. While this implementation focused on prostate MRI reports, the UODBLLM pipeline was designed for broad applicability across diverse clinical documents. This generalizability is enabled by its modular, model-agnostic architecture and a flexible prompting system where extraction templates are stored as configurable components in the database. The design allows the pipeline to be readily adapted for other unstructured texts, such as pathology results or operative notes, which aligns with plans to expand its use to other urologic cancers.

Conclusions

Our study demonstrates the feasibility and effectiveness of integrating LLM-based automation into UODB management. Our system's perfect completion rate, rapid processing speed, and cost-effective operation provides a robust framework for modernizing clinical research data management. Looking ahead, we aim to develop protocols for using LLMs to validate existing data entries and expanding to renal and bladder cancer radiology and pathology texts. The potential benefits of increased research efficiency and data quality suggest that LLM-based approaches will play an increasingly important role in clinical research infrastructure [4]. These advances may ultimately accelerate the pace of discovery in clinical oncology and serve as a model for other medical specialties.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Example of the UODBLLM Data Extraction Workflow. (A) The original unstructured text from a sample magnetic resonance imaging report. (B) The corresponding extensible markup language-structured prompt containing instructions and specific data extraction queries sent to the large language model (LLM). (C) The structured JSON data returned by the LLM based on the prompt and report.

[[DOCX File, 9 KB - bioinform_v7i1e70708_app1.docx](#)]

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Abbreviations

- API:** application programming interface
- EHR:** electronic health record
- GPT:** generative pre-trained transformer
- HIPAA:** Health Insurance Portability and Accountability Act
- LLM:** large language model
- MRI:** magnetic resonance imaging
- UCSF:** University of California, San Francisco
- UODB:** Urologic Outcomes Database
- XML:** extensible markup language

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Unpacking Genomic Biomarkers for Programmed Cell Death Receptor-1 Immunotherapy Success in Non–Small Cell Lung Cancer Using Deep Neural Networks: Quantitative Study

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Abstract

Background: Non–small cell lung cancer (NSCLC) is one of the leading causes of cancer-related mortality. Programmed cell death receptor-1 (PD-1) immunotherapy has shown results in the treatment of NSCLC; however, not all patients respond effectively to it. Identifying predictive biomarkers for PD-1 therapy response is critical to improving patient outcomes and treatment strategies. Traditional methods of biomarker discovery often fall short in terms of accuracy and comprehensiveness. Recent advancements in deep learning provide a powerful approach to analyze complex genomic data to resolve this issue.

Objective: This study aims to leverage deep neural networks (DNNs) to identify genomic biomarkers predictive of patient responses to PD-1 immunotherapy in NSCLC. DeepImmunoGene is a model designed using a reduced feature set to identify the most critical biomarkers. We use feature selection to reduce the space and apply deep learning to identify the highly predictive gene subset.

Methods: Differentially expressed genes were identified in RNA-seq data from 355 patients with NSCLC using the LIMMA package in R, followed by preprocessing with log₂ transformation, removing outliers, and detecting easily identified genes. Machine learning models, including support vector machines, extreme gradient boosting (XGBoost), and DNNs, were applied to gene expression data to predict patient responses to immunotherapy. Key predictive genes were identified through model interpretation techniques, and differences in model performance were assessed for statistical significance. Primarily, the metric used identifies which genes serve as key biomarkers in regard to immunotherapy detection.

Results: Initially, we identified 1093 differentially expressed genes from RNA-seq data of 355 patients. We then trained models using SVM, XGBoost, and DNN to predict immunotherapy response. The DNN model outperformed both SVM and XGBoost with an accuracy of 82%, an area under the curve of 90%, and recall of 85%. To identify key biomarkers, we performed a permutation importance analysis, narrowing down the gene set to 98 genes. DeepImmunoGene, trained on these 98 genes, showed superior results, with an accuracy of 87% and an area under the curve of 95%. The top 36 upregulated genes in responders and 62 upregulated genes in nonresponders were identified, which could serve as potential biomarkers for predicting response to PD-1 inhibitors. These findings suggest that DeepImmunoGene can reliably forecast immunotherapy outcomes and aid in biomarker discovery, supporting the development of more personalized treatment strategies in NSCLC.

Conclusions: The DeepImmunoGene predictive model identified 36 upregulated genes that may represent candidate genomic biomarkers associated with response to PD-1 immunotherapy in patients with NSCLC. Notably, the 10 most significant genes offer valuable insights into the underlying mechanisms of treatment responses. These biomarkers may not only aid in predicting which patients are more likely to respond to PD-1 immunotherapy but also offer insights into the molecular differences associated with nonresponse.

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KEYWORDS

lung cancer; machine learning; deep neural network; DeepImmunoGene; biomarkers; RNA-seq analysis; differential gene expression; programmed cell death receptor-1; immunotherapy

Introduction

Lung cancer is a leading cause of cancer-related deaths globally, with approximately 238,340 new cases and 127,070 deaths annually in the United States [1,2] and 2.5 million new cases and 1.8 million deaths worldwide [3]. Smoking accounts for approximately 90% of lung cancer cases [4], whereas the remaining cases in nonsmokers are due to other factors, including environmental exposure to asbestos, arsenic, nickel, pesticides, other toxic chemicals, and air pollution [5,6]. Lung cancer is classified into 2 main groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [4]. SCLC is a rare, fast-growing form of lung cancer that primarily develops in individuals with a long history of tobacco smoking, whereas NSCLC is more common, accounting for 85% of lung cancer cases compared to 15% for SCLC [5]. Although tobacco smoking is a major risk factor for NSCLC, it can also develop in nonsmokers. NSCLC is divided into 3 main types: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [5,6]. Among these, adenocarcinoma is the most prevalent type, typically developing in the outer parts of the lung and being more common in individuals aged <45 years [5,6]. In contrast, squamous cell carcinoma originates from the epithelial cells of the central airways and is strongly associated with smoking [7,8].

Over the last 10 years, lung cancer treatment has undergone significant changes, with advancements in understanding its biology leading to the development of immunotherapy, which has emerged as a promising therapeutic option [9,10]. Immunotherapy works by enhancing the immune system through the use of drugs that block inhibitory signaling pathways, allowing it to better recognize and eliminate cancer cells [9,10]. Cancer can evade immunosurveillance by expressing ligands for inhibitory checkpoint molecules, such as programmed cell death receptor-1 (PD-1) and cytotoxic T-lymphocyte-associated protein-4, which prevent T cells from recognizing and destroying cancer cells [11]. Thus, immune checkpoint inhibitors (ICIs) have become an effective cancer therapy [12]. In recent years, ICIs have been used as the first line of treatment for metastatic NSCLC as well as consolidation therapy after surgical removal and chemotherapy [10]. PD-1 is a surface receptor found on T cells in lung cancer that acts as a negative regulator of immune responses [13-15]. Recent studies have shown that inhibiting PD-1 or programmed cell death-ligand 1 (PD-L1) restores T cell function, enabling the immune system to recognize and destroy cancer cells, suggesting their potential as promising therapeutic targets for NSCLC treatment [15-17]. However, only a fraction of patients respond to this immunotherapy. Therefore, we aimed to investigate genomic features that may help distinguish responders from nonresponders to PD-1 inhibitors and to gain insight into potential underlying biological differences. Furthermore, researchers have increasingly turned to bioinformatics and machine learning (ML) techniques to discover more precise biomarkers by analyzing large-scale genomic and molecular data. Among ML techniques, deep neural networks (DNNs) are particularly well suited for these tasks due to their ability to process and analyze vast, high-dimensional datasets. The use of ML in this research is

indispensable for tackling the complexity of RNA-seq data and addressing the limitations of traditional analytical methods. Traditional statistical methods, such as ANOVA and *t* tests, rely on assumptions such as a normal distribution of the data, which is generally violated in gene expression data. Furthermore, as sample sizes and feature dimensions expand, these approaches also face computational constraints. In contrast, deep learning (DL) methods are particularly well suited to capturing the complex patterns present in genomic data [18]. Such models enable the identification of high-impact biomarkers, uncover nonlinear relationships in gene expression, and generate robust predictions for patient responses to PD-1 immunotherapy.

Several DL approaches have previously been proposed to predict immunotherapy outcomes, including survival-focused models such as DeepSurv and attention-based architectures designed to capture complex transcriptomic interactions [19-23]. These models demonstrate the growing interest in applying advanced DL to immunogenomics. We build upon this foundation by integrating interpretability into our approach. Furthermore, other existing approaches typically rely heavily on imaging-based methods, which can suffer from scanner or protocol heterogeneity and spurious correlation, among others. This study highlights the potential of ML techniques, particularly DNNs, in advancing precision medicine for patients with NSCLC undergoing PD-1 immunotherapy. We applied permutation importance in conjunction with DeepImmunoGene, which identified 98 important genes from a large RNA-seq dataset of 19,911 genes in the Gene Expression Omnibus (GEO) Repository [24]. We trained the DeepImmunoGene model on these genes, which outperformed linear models, achieving an accuracy of 87% and an area under the receiver operating characteristic curve (AUC) of 95%. This model identified a set of 36 upregulated genes in patients with NSCLC who are responders, which may serve as potential biomarkers for predicting responses to PD-1 immunotherapy for this group. Additionally, it identified another set of 62 upregulated genes in patients with NSCLC who are nonresponders, which could act as potential biomarkers for developing ICI therapy for this subgroup. These findings not only offer a foundation for improving patient stratification but also provide insights for tailoring therapeutic strategies. Despite significant advancements in treatment over the past decade, including the development of immunotherapy as a promising strategy for NSCLC, the prognosis for many patients remains poor [25,26]. Although ICIs targeting PD-1 and PD-L1 have shown potential as immunotherapy for patients with NSCLC, only a small fraction of patients respond to PD-1 inhibitors [24].

This underscores the need for more reliable biomarkers to accurately identify patients who will benefit from PD-1 inhibitors. The core work tries to answer 2 research questions (RQs) as follows:

- RQ1: How do ML models perform in predicting patient response to PD-1 immunotherapy based on differentially expressed genes (DEGs)?
- RQ2: What are the key biomarkers identified through feature selection and DL that predict patient response to

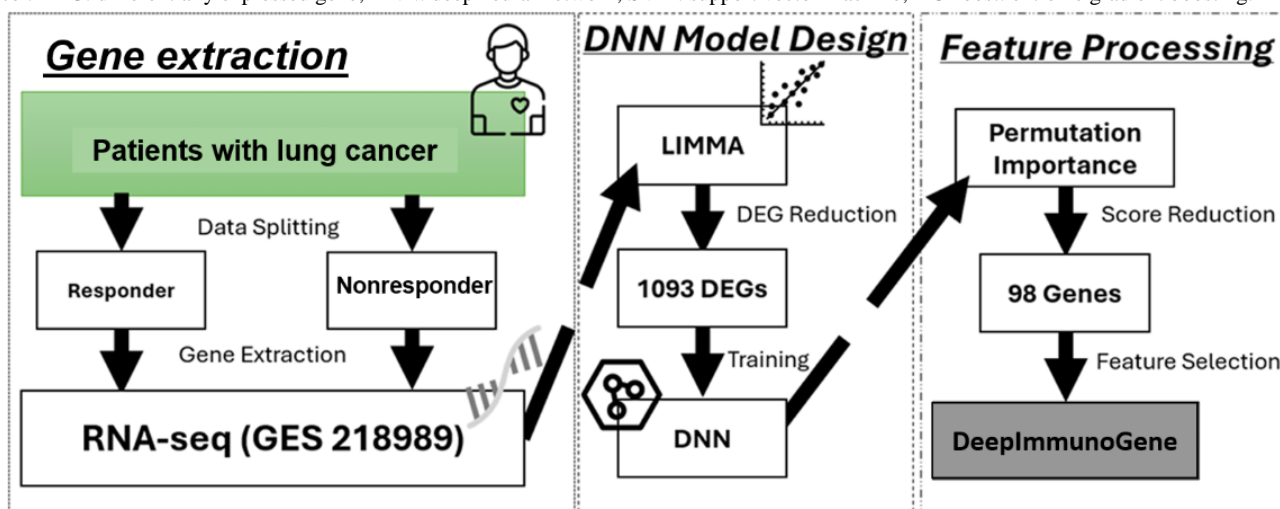
PD-1 immunotherapy, and how do they contribute to model performance?

Methods

Overview

The study was carried out according to the workflow presented in Figure 1. This workflow delineates the steps, beginning with

Figure 1. Workflow for identifying biomarkers and predicting programmed cell death receptor-1 immunotherapy response in non-small cell lung cancer. DEG: differentially expressed gene; DNN: deep neural network; SVM: support vector machine; XGBoost: extreme gradient boosting.



Data Acquisition and Preprocessing

We used one RNA-Seq dataset (GSE218989) from the GEO public database GEO Repository [24]. This dataset included gene expression data for 19,911 genes across 355 patients with lung cancer who were treated with either PD-1 or PD-L1 inhibitors. It consisted of 187 nonresponders and 168 responders. Responsiveness was determined by Kang et al [24] using Response Evaluation Criteria in Solid Tumors (RECIST; version 1.1) [28]. Progression-free survival [29] was measured from the start of PD-1/PD-L1 inhibitor therapy to either documented disease progression or death from any cause. Overall survival was measured from the start of PD-1/PD-L1 inhibitor therapy to death from any cause [24]. A responder is therefore classified as a patient who showed improvement under the RECIST criteria or, in other words, a patient who experienced improvements after the PD-1 immunotherapy was administered. At the same time, a nonresponder is a patient who did not meet the criteria showcased by a worsening or stable disease.

The raw gene expression count data were already normalized in the transcripts per million (TPM) value for the 19,911 protein-coding genes. We first identified the DEGs between the responders and nonresponders using the LIMMA package [30] in R (version 4.4.1; Bioconductor, USA). LIMMA was used to create a linear function to model the entire dataset and to develop correlations with response status as the main variable in the design matrix. Empirical Bayes moderation was performed to model and stabilize the gene-wise variances using a prior marginal distribution of the data [30]. Genes with a LIMMA-calculated P value less than .05 were considered significantly differentially expressed and were selected for all subsequent analyses and modeling. For model training and

the identification of significant DEGs from RNA-seq data [27] using the LIMMA package and culminating in the application of the DeepImmunoGene framework to identify and validate key genes associated with the response to PD-1 immunotherapy in patients with NSCLC.

testing, the data were further processed by performing a \log_2 (TPM+1) transformation on each gene expression value to stabilize the variance in gene expression.

ML Models

Overview

The application of ML is vital in this research due to the complexity, scale, and dimensionality of RNA-seq data, as well as the intricate, nonlinear biological mechanisms underlying immunotherapy response in patients with NSCLC [31]. Traditional statistical methods struggle with high-dimensional datasets, such as the 19,911-gene RNA-seq data used here, often succumbing to the “curse of dimensionality” and failing to capture subtle gene interactions. ML models such as support vector machines (SVMs) [32], extreme gradient boosting (XGBoost) [33], and DNN [34] overcome these challenges by effectively handling high-dimensional inputs, modeling complex nonlinear relationships, and identifying important gene features through built-in feature selection techniques. This enables the discovery of meaningful gene patterns that differentiate responders from nonresponders while enhancing predictive power and model generalizability.

Moreover, ML methods excel in managing noise and variability inherent in biological data, offering robust performance through techniques such as regularization and early stopping [35,36]. Their scalability and automation allow for efficient analysis of massive RNA-seq datasets, ensuring accuracy and rapid processing, essential for clinical translation. By integrating advanced techniques for hyperparameter tuning, ML provides a unified, systematic workflow that optimizes predictive performance [37]. These capabilities facilitate the identification

of potential predictive biomarkers from gene expression data, which may serve as a foundation for future precision medicine efforts aimed at tailoring immunotherapy strategies in patients with NSCLC. This study used several ML models, including SVM, XGBoost, and DNN [11]. Their predictive performance was evaluated to identify the model that worked best. We built the SVM model using the Python package Scikit-learn (sklearn); for XGBoost, we used the XGBoost Python package [38]; and for the DNN, we used the Keras and TensorFlow Python packages [11]. The details about each ML approach are further described below.

Table . Summary of model architectures' hyperparameter settings.

Model	Key hyperparameters tuned	Final settings	Optimization approach
SVM ^a	C, kernel, gamma	C=0.1, kernel=linear, gamma=0.1	GridSearchCV (5-fold CV ^b)
XGBoost ^c	n_estimators, max_depth, learning_rate, sampling	n_estimators=300, max_depth=100, learning_rate=0.1, sampling=uniform	GridSearchCV (5-fold CV)
DNN ^d	batch_size, epochs, initializer, optimizer, activation, dropout, layers, nodes	Input=256; hidden layers=[128, 100, 100]; activation=ELU ^e ; optimizer=Adam; dropout=0; epochs=100; batch size=100	Multistage GridSearchCV

^aSVM: support vector machine.

^bCV: cross-validation.

^cXGBoost: extreme gradient boosting.

^dDNN: deep neural network.

^eELU: exponential linear unit.

XGBoost

XGBoost is an ensemble learning algorithm that builds gradient-boosted decision trees one by one and passes the residuals of the previous tree to train the following model. It uses the second partial derivative of the loss function and adds an L1 and L2 regularization term to reduce overfitting [41]. Similar to SVM, we optimized the hyperparameters using GridSearchCV to evaluate a combination of parameters. The hyperparameter settings are given in Table 1.

Deep Neural Network

DNN is a nonlinear model that combines neurons that simulate the human brain to make predictions [41,42]. It consists of 3 layers: the input layer, hidden layers, and output layer, which are linked by weights to allow the model to understand complex patterns in the data. We used a DNN because they have been previously applied for genomic-based predictions for diseases [43]. Similar to the previous 2 models, we started with hyperparameter optimization using GridSearchCV. As the DNN has more parameters to tune, we split the Grid Search into 3 stages: (1) batch size and epoch; (2) weight initializer, optimizer, and activation function; and (3) hidden layers, nodes per hidden layer, and dropout optimization. The resulting network consisted of an input layer with 256 nodes, 3 hidden layers with 128 nodes, 100 nodes, and 100 nodes, respectively, an exponential linear unit activation function, Adam optimizer, zero dropout, and normal initializer. The details are summarized in Table 1. We applied the binary cross-entropy loss function as shown in Equation 1 so that the model minimizes to learn the optimal

Support Vector Machine

SVM is a kernel-based binary classifier that separates key data features linearly into 2 groups in a high-dimensional space called the feature space [38,39]. It searches for the optimal decision boundary (hyperplane) to separate the features by maximizing the margin between the hyperplane and the nearest training data. SVM effectively extracts key but subtle patterns in a complex dataset, allowing for low-error, high-precision sample classification [40]. The model architecture's hyperparameter settings are given in Table 1.

weights for each gene to classify responder and nonresponder patients.

$$(1) LBCE = -1/N \sum_{i=1}^N [y_i \times \log(p(y_i)) + (1-y_i) \times \log(1-p(y_i))]$$

The model was trained for 100 epochs with a batch size of 100 based on the GridSearchCV results. After identifying these optimal hyperparameters for the DNN, we used it to construct the architecture for the DeepImmunoGene network.

Permutation Importance

To develop the DeepImmunoGene framework, we used the permutation importance method from scikit-learn to identify the subset of genes that most significantly contributed to the DNN's prediction of patient outcomes to PD-1 immunotherapy [11]. Basically, this technique improves model accuracy by removing the "noisy" genes. First, we used the original DNN trained on the 1093 gene expression data to establish a baseline performance using the accuracy score. Then, we randomly shuffled each gene's expression values across the 71 testing patients one at a time to disrupt any existing association between that gene and the response classification. After shuffling a gene, the DNN was run again to recalculate the accuracy. If the accuracy decreased after shuffling, that gene was important for predicting the response. Conversely, if the accuracy increased or did not change after shuffling, that gene showed little to no correlation with response prediction. Given the nonlinearity of PD-1 immunotherapy genetics, a standard linear model, such as least absolute shrinkage and selection operator or stepwise regression, is unable to capture the noise in the genes. Feature permutation ignores this weakness by using a direct DNN

architecture to quantify the decrease in performance due to a change in the feature. By exploring the performance of the model directly, we remove the uncertainty of a linear model and guarantee the importance of the features in the deployed solution. To evaluate the stability of the features identified, we ran the analysis 3 additional times, each with 50 iterations. We then compared the resulting gene sets to quantify their overlap. We also trained and evaluated the model using each gene set to determine the superior cohort for all subsequent analyses. Equation 2 was used to calculate the importance score assigned to each gene.

$$(2) \text{Importance score} = \text{accuracy}_{\text{baseline}} - \text{accuracy}_{\text{permutation}}$$

Training and Testing

We executed our code for the ML models in Google Colab notebooks [44] using an NVIDIA T4 GPU [45] operating with 15 GB of RAM. For all models, 284 patients were used for training, and 71 patients were used for testing. This provided an 80/20 percentage split of the data. For the DNN, an additional validation split of 10% was applied to the training data to monitor model performance during training. This validation set was extracted from the training data, leaving the test set of 71 patients unchanged. During the training of the DNN, an early stopping method was used to monitor the validation loss after each epoch to stop training if the model's performance diminished. The state of the model was saved after each epoch so that it could revert to the optimal state for testing. This was done to mitigate any overfitting that might occur during training. All ML models were executed 15 times.

Evaluation Metrics

To evaluate the models' performance, we used accuracy, AUC score, recall, specificity, precision, and F_1 -scores [46], which are standard metrics used to assess classification performance. These metrics can be found using the confusion matrix, a 2×2 matrix with the number of true positives, true negatives, false positives, and false negatives that the model predicts, with the equations listed below to calculate each metric.

$$(3) \text{Accuracy} = \frac{TP + TN}{TP + TN + FP + FN} \in [0, 1]$$

$$(4) \text{Recall} = \frac{TP}{TP + FN} \in [0, 1]$$

$$(5) \text{Specificity} = \frac{TN}{TN + FP} \in [0, 1]$$

$$(6) \text{Precision} = \frac{TP}{TP + FP} \in [0, 1]$$

$$(7) F_1 = 2 \times \frac{\text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \in [0, 1]$$

Accuracy (Equation 3) measures the overall correct predictions out of all predictions made. Recall evaluates the model's ability to correctly identify PD-1 responders as positive out of all PD-1 responders, as shown in Equation 4. Specificity (Equation 5) is the opposite; it measures the model's ability to correctly identify PD-1 nonresponders out of all nonresponders. Precision (Equation 6) is the ratio of all correctly identified positive PD-1 respondents to all the patients the model assigns as positive, and the F_1 -score (Equation 7) is a harmonic mean of precision and recall that penalizes extreme values [47]. AUC measures the trade-off between specificity and recall [38,48].

Bioinformatics and Statistical Analysis

All computations and analyses in this study were performed in Google Colab notebooks using Python (version 3.10) and R (version 4.4.1). Differentially expressed genes were analyzed with LIMMA in R [30]. Upregulated genes were classified for responders and nonresponders by calculating log fold changes (LogFC). Accuracy, AUC, recall, specificity, precision, F_1 -score, true positives, true negatives, false positives, and false negatives were calculated using sklearn Metrics. Statistical analyses were conducted using GraphPad Prism (version 5.01; GraphPad Software). The Kruskal-Wallis nonparametric test, followed by the Dunn post hoc multiple comparison test, was used to compare predictive performance between the models. A P value less than .05 was considered statistically significant.

The next section delves into the detailed analysis of the genes identified through the DeepImmunoGene framework and their relevance in predicting immunotherapy response. It outlines how the permutation importance method was used to isolate key genes associated with positive or negative treatment outcomes and discusses the biological significance of these genes in the context of immune response modulation in NSCLC. Additionally, the section provides an in-depth comparison of the ML models' performance, highlighting the strengths and limitations of each approach, and evaluates their potential applications in clinical settings for improving patient stratification and personalized treatment strategies. By integrating these findings, the study aims to contribute to our understanding of molecular biomarkers that may inform future efforts to optimize the use of PD-1 inhibitors in cancer therapy.

External Validation

To externally validate the biomarkers identified by DeepImmunoGene, we obtained a bulk RNA-seq dataset (GSE207422) from the GEO public database. This dataset included gene expression data for 58,387 genes across 24 patients with NSCLC who were treated with PD-1 inhibitors combined with chemotherapy [49]. Patient responsiveness was determined using RECIST, where complete response and partial response were considered responders, whereas stable disease was considered a nonresponder. The cohort comprised 17 responders and 7 nonresponders. This external dataset was processed using the aforementioned workflow applied to the training dataset. The Mann-Whitney U test was used to determine whether the difference in gene expression between responders and nonresponders was statistically significant. We generated violin plots of the top-ranked responder and nonresponder biomarkers identified by DeepImmunoGene to assess whether their expression patterns in the test set were consistent with the model's predictions using the ggplot2 package [50].

Ethical Considerations

This study used only publicly available or fully deidentified secondary data; therefore, institutional review board approval and informed consent were not required. No personal identifiers were accessed, and privacy and confidentiality were strictly maintained.

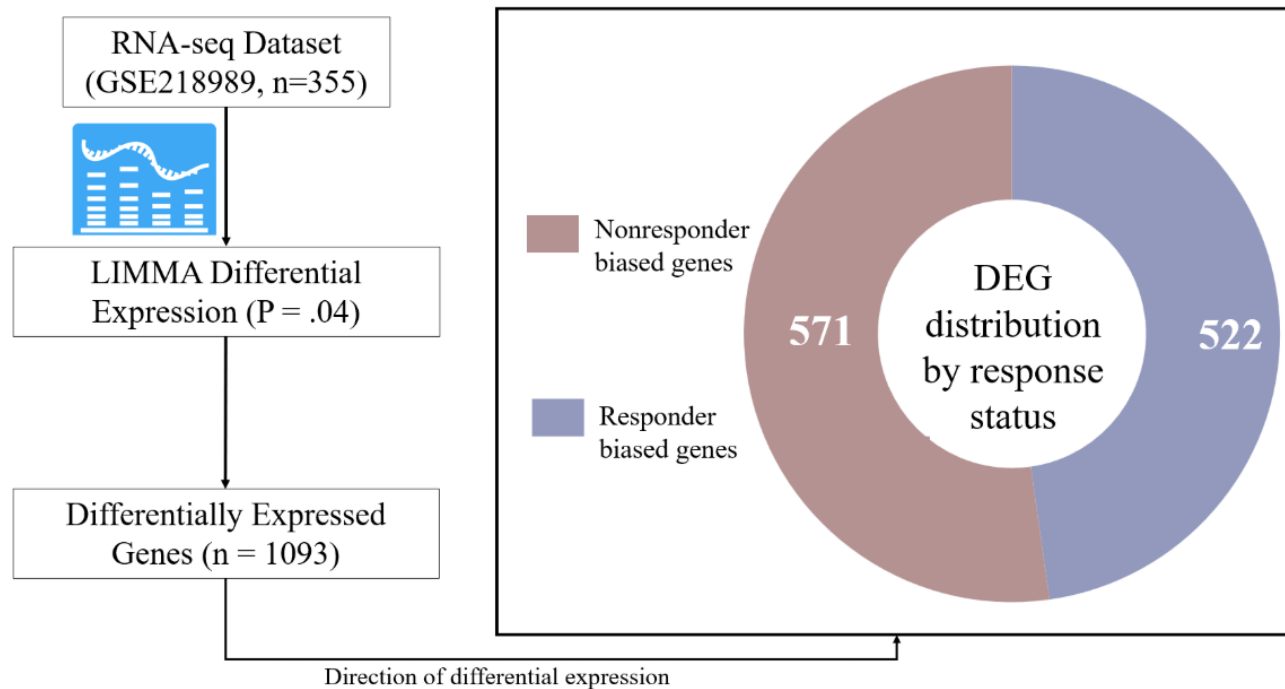
Results

ML Predicts Response to PD-1 Immunotherapy (RQ1)

DEGs were identified using LIMMA power analysis of bulk

RNA-seq data (GSE218989) from the GEO public database GEO Repository. LIMMA identified 1093 important DEGs from a total of 19,911 genes in patients with lung cancer, where 522 genes were upregulated in responders, and 571 genes were upregulated in nonresponders ($P=.04$), as shown in Figure 2.

Figure 2. Identification and stratification of differentially expressed genes associated with programmed cell death receptor-1 immunotherapy response in non-small cell lung cancer. Bulk RNA-seq data from 355 patients (GSE218989) were analyzed using LIMMA differential expression analysis ($P=.04$), identifying 1093 differentially expressed genes. These genes were stratified by direction of differential expression into responder-upregulated ($n=522$) and nonresponder-upregulated ($n=571$) gene sets, forming the initial feature space for downstream machine learning analyses. DEG: differentially expressed gene.



Here, we trained SVM and XGBoost models using the 1093 identified DEGs to predict patient response to PD-1 immunotherapy. The performance of the models was evaluated using several metrics, including accuracy, AUC, recall, specificity, precision, and F_1 -score [46]. First, we applied SVM, and our data showed that it achieved an accuracy of 68% and an AUC score of 76% with recall, specificity, precision, and F_1 -score values of 0.70, 0.65, 0.77, and 0.71, respectively (Figure 3A, 3B and Table 1). Next, we used XGBoost to see if its ensemble learning method could yield higher accuracy and AUC scores. Our data showed that XGBoost performed slightly

better than SVM, with an accuracy of 72%, an AUC score of 77%, a recall of 0.73, a specificity of 0.71, a precision of 0.76, and an F_1 -score of 0.74 (Figure 3A, 3B and Table 2). The suboptimal performance of these 2 models may be due to the large dataset, suggesting that a more complex and nonlinear approach, such as a DNN, is necessary for accurately predicting patient responses. We used SVM and XGBoost as baseline classifiers commonly applied in gene expression studies to provide context for the performance of our DNN. While these models differ in complexity from DNNs, the comparison helps demonstrate the added value of capturing nonlinear interactions in gene expression data.

Figure 3. Predictive performance comparison of support vector machine (SVM), extreme gradient boosting (XGBoost), and deep neural network (DNN) models. (A) Accuracy scores and (B) receiver operating characteristic (ROC) curve analysis demonstrate that the DNN model outperformed both SVM and XGBoost. The DNN achieved an accuracy of 82% and an area under the curve (AUC) of 90%, compared to 68% and 76% for SVM and 72% and 77% for XGBoost. These results highlight the advantage of deep learning for modeling complex, high-dimensional gene expression data.

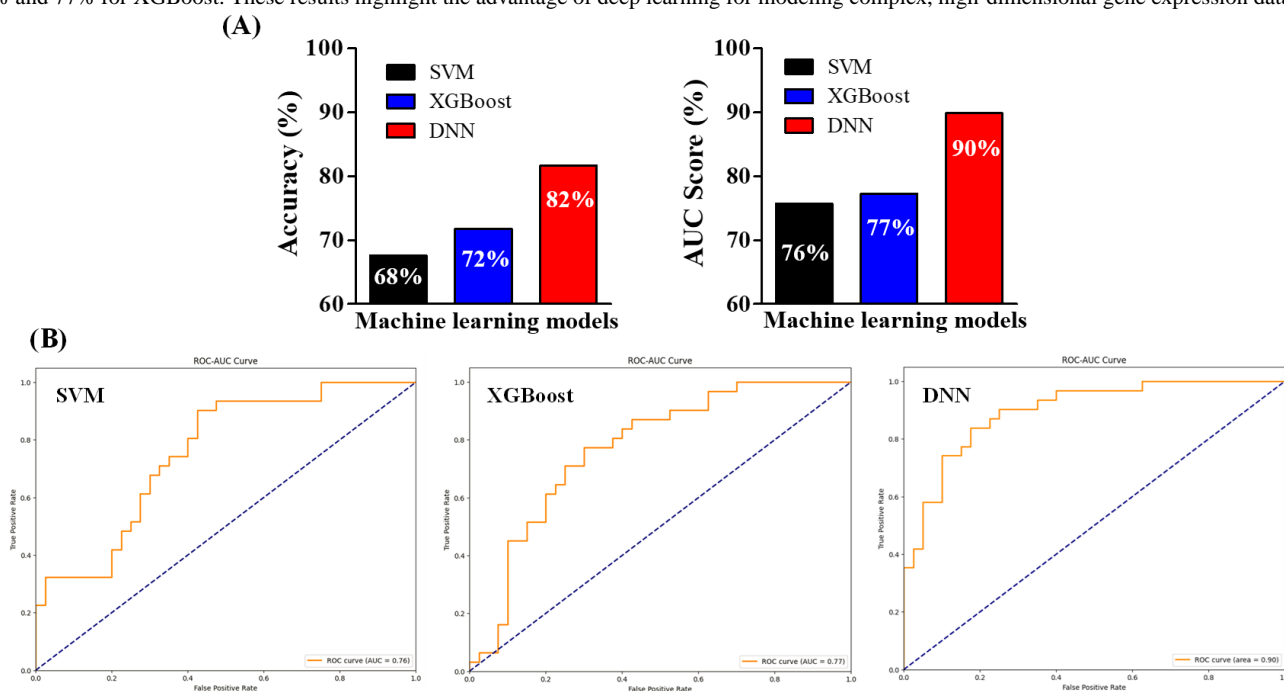


Table . Performance comparison of machine learning models for predicting response to programmed cell death receptor-1 immunotherapy.

Models	Accuracy	AUC ^a	Recall	Specificity	Precision	F_1 -score
SVM ^b (1093 genes)	0.68	0.76	0.70	0.65	0.77	0.71
XGBoost ^c (1093 genes)	0.72	0.77	0.73	0.71	0.76	0.74
DNN ^d (1093 genes)	0.82 ^e	0.90 ^e	0.85 ^e	0.78 ^e	0.81	0.84 ^e
SVM (98 genes)	0.65	0.75	0.65	0.65	0.70	0.68
XGBoost (98 genes)	0.77	0.81	0.80	0.74	0.80	0.80
DeepImmunoGene (98 genes)	0.87 ^e	0.95 ^e	0.87 ^e	0.89 ^e	0.93 ^e	0.89 ^e

^aAUC: area under the receiver operating characteristic curve.

^bSVM: support vector machine.

^cXGBoost: extreme gradient boosting.

^dDNN: deep neural network.

^eA statistically significant difference from DeepImmunoGene when compared to SVM or XGBoost.

DNN Predicts Response to PD-1 Immunotherapy With Higher Accuracy

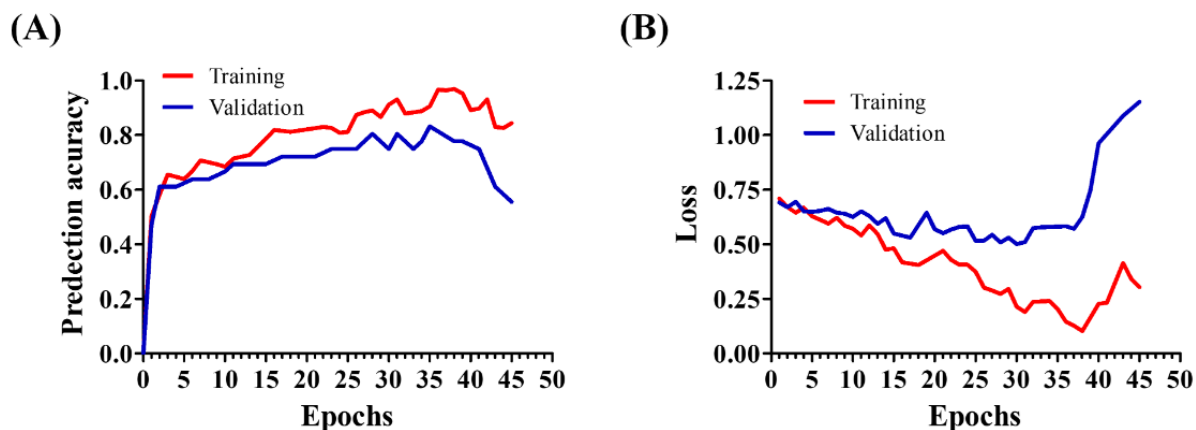
Given that the RNA-seq data includes the expression of more than 1000 genes, we implemented a DNN to enhance predictive accuracy. First, we set the DNN training for 100 epochs, but it stopped at 45 epochs due to early stopping, and the model was then reverted to the optimal state reached at 35 epochs (Figure 4). During the training process, both training and validation accuracy and loss were monitored. We found that the accuracy increased until it exhibited an asymptotic behavior (Figure 4A).

Conversely, the training loss decreased steadily, while the validation loss showed some fluctuations (Figure 4B). These findings suggest that training the model for additional epochs would not further improve its performance. Next, we tested the predictive performance. Our data revealed that the DNN achieved excellent predictive performance compared to both SVM and XGBoost, achieving an accuracy of 82%, an AUC score of 90%, a recall of 0.85, a specificity of 0.78, a precision of 0.81, and an F_1 -score of 0.84 (Figure 3A, 3B and Table 2). Given the nature of the data, DNN can analyze multidimensional genetic information more accurately than existing linear models.

This is showcased with a 21% accuracy improvement over more linear models, such as SVM, and a 14% improvement over XGBoost in our experiments. As a result, we can showcase that

to capture the intricacies of the data, it is important to use a model capable of supporting complex multidimensional relationships such as a DNN architecture.

Figure 4. Deep neural network training and validation performance. (A) Training and validation accuracy over epochs shows a steady increase until convergence, with early stopping triggered at epoch 45 and the model reverting to optimal weights from epoch 35. (B) Training loss decreased continuously, whereas validation loss fluctuated slightly before stabilizing, indicating that further training would not significantly improve model performance.



Key Biomarker Identification (RQ2)

We applied DeepImmunoGene with scikit-learn permutation importance to a set of 1093 genes. To mitigate variability in feature importance estimates and to ensure the identification of robust features, this procedure was repeated 3 additional times with 50 iterations each. We then compared the gene sets identified across all 4 total runs and observed a high degree of overlap, with an average of 85.5% consistency among them. The resulting analysis (Figure 5) identified a final set of 98 genes with nonzero importance scores and ranked them according to their level of importance (Figure 6). Although individual gene importance scores below 0.0025 may appear low, the combined contribution of these genes accounts for approximately 18% of the total model importance, indicating they meaningfully improve the model's predictive performance. These 98 genes were subsequently used to train DeepImmunoGene. Testing this model revealed an accuracy of 0.87 and an AUC of 0.95, a recall of 0.87, a specificity of 0.89, a precision of 0.93, and an F_1 -score of 0.89, demonstrating superior performance across all metrics. To validate the necessity of a DL approach for our feature selection and to better contextualize the significant performance improvement of DeepImmunoGene, we conducted a comparative analysis with the traditional ML models. We trained and tested both SVM and XGBoost on the same 98 genes identified via permutation importance. The 98-gene SVM model attained an accuracy of

65%, an AUC of 75%, a recall and specificity of 0.65, a precision of 0.70, and an F_1 -score of 0.68. The 98-gene XGBoost model achieved an accuracy of 77%, an AUC of 81%, a recall of 0.80, a specificity of 0.74, a precision of 0.80, and an F_1 -score of 0.80 (Table 2). This indicates that DeepImmunoGene outperformed all other models in every metric (Table 2). Genes with a LogFC greater than 0 were considered upregulated in responders, whereas genes with a LogFC less than 0 were considered upregulated in nonresponders. We discovered that 36 genes were upregulated in patients with NSCLC who responded to PD-1 immunotherapy, with the top 10 most significant being GSTT2B, HMGA2, AC135050.2, ANKRD33B, MMP13, PLA2G2D, RASGEF1A, BIRC7, DCAF4L2, and CHMP7 (Figure 7). These genes may serve as potential biomarkers for predicting response to PD-1 immunotherapy. Additionally, we identified 62 upregulated genes in nonresponder patients with NSCLC, with the top 10 most important being SPINK1, FEZF1, THBS4, BEST3, TESC, C6orf226, TSSK2, SFRP2, C1GALT1C1L, and RARRES1 (Figure 7).

The top 10 most significant upregulated genes were identified for both responder and nonresponder patients with NSCLC based on the DeepImmunoGene model. In responders, genes such as GSTT2B, HMGA2, and MMP13 were prominent, whereas SPINK1, FEZF1, and THBS4 were among the top in nonresponders. These genes may serve as potential predictive biomarkers for PD-1 treatment outcomes.

Figure 5. Workflow for identifying predictive biomarkers using DeepImmunoGene. Schematic of the DeepImmunoGene model pipeline. The 1093 differentially expressed genes were subjected to permutation importance analysis to extract the 98 most informative features, which were then used to train the final model. This approach enabled identification of key genes associated with programmed cell death receptor-1 (PD-1) immunotherapy response.

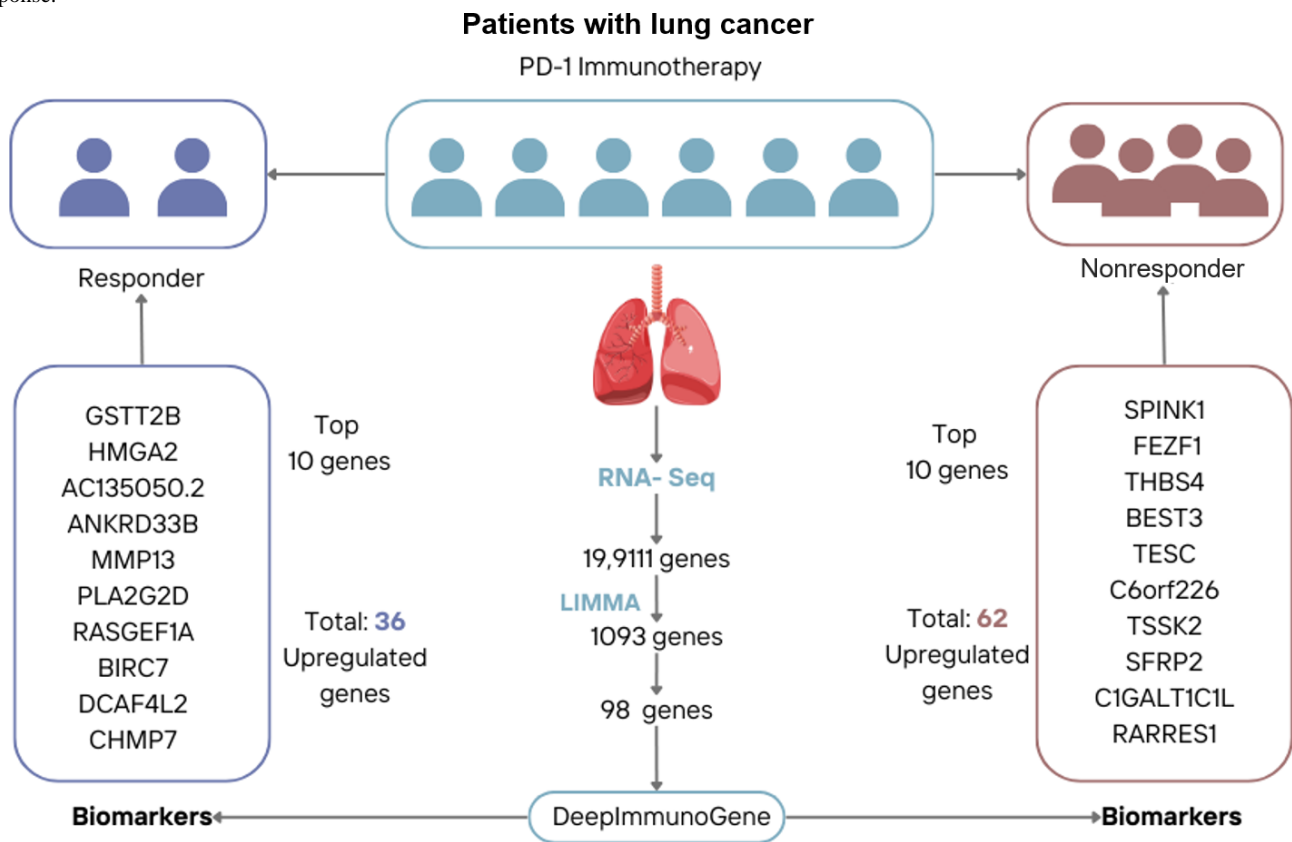


Figure 6. Gene importance ranking using permutation analysis. Permutation importance applied to the 1093 differentially expressed genes using the DeepImmunoGene model identified 98 genes with nonzero importance scores. These genes were ranked based on their contribution to model prediction performance, highlighting their potential as key features for programmed cell death receptor-1 response classification in patients with non-small cell lung cancer.

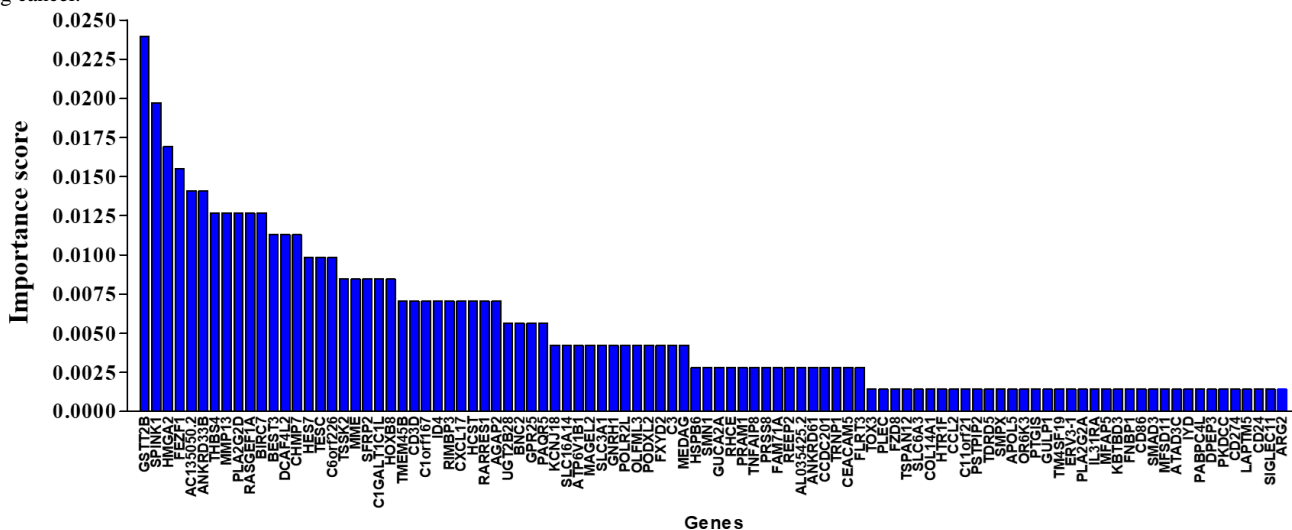
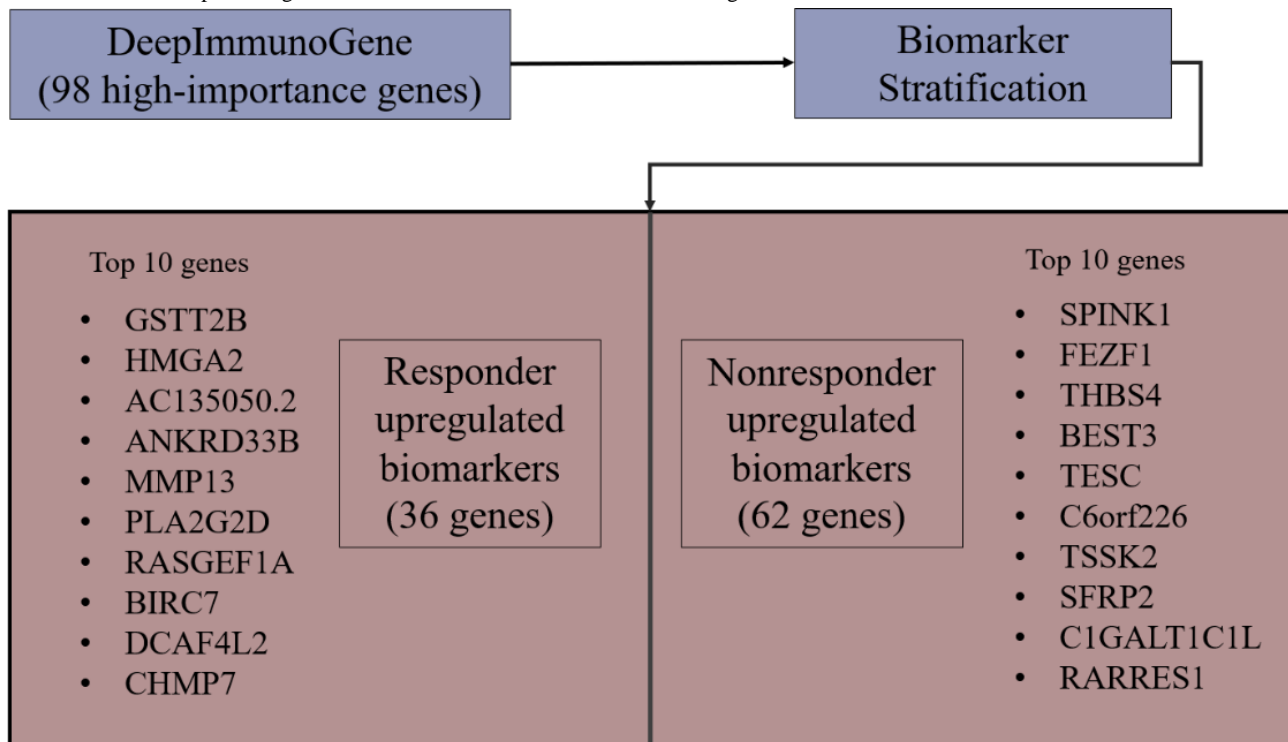


Figure 7. DeepImmunoGene-based stratification of predictive biomarkers associated with programmed cell death receptor-1 (PD-1) immunotherapy response. Using permutation importance and deep neural network modeling, 98 high-importance genes were identified and stratified based on direction of differential expression. Thirty-six genes were upregulated in responders and 62 in nonresponders. The top 10 genes in each group are shown as candidate biomarkers for predicting PD-1 treatment outcomes in non-small cell lung cancer.



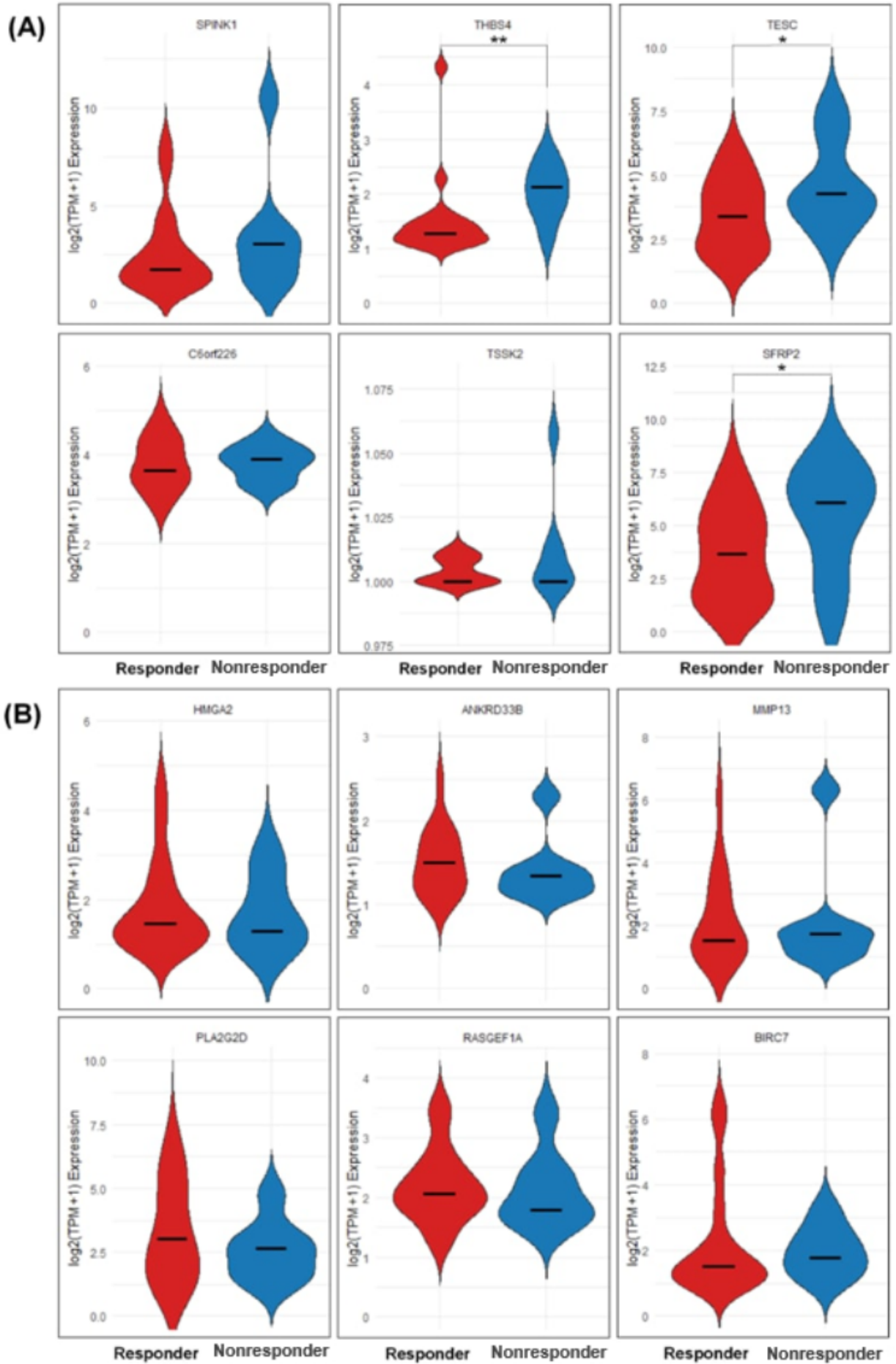
External Validation of Biomarkers Identified by DeepImmunoGene

Here, we sought to determine whether DeepImmunoGene's predicted biomarkers showed consistent expression patterns in an independent dataset. We generated violin plots comparing \log_2 (TPM +1) gene expression between responders and nonresponders. Of the top 10 nonresponder-upregulated biomarkers identified by DeepImmunoGene, 6 genes were present in the independent dataset and analyzed. We found that 4 of these 6 genes (SPINK1, THBS4, TESC, and SFRP2) showed a consistent trend of higher median expression in nonresponders (Figure 8A). Of these, 3 genes (THBS4, TESC,

and SFRP2) demonstrated statistically significantly higher expression ($P=.04$) in nonresponders.

Of the top 10 responder-upregulated biomarkers identified, 6 genes were present in the independent dataset and analyzed. We found that 4 of these 6 genes (HMGA2, ANKRD33B, PLA2G2D, and RASGEF1A) showed higher median expression in responders (Figure 8B). BIRC7 and MMP13 had similar median expression in both groups; however, their violin plots displayed extended upper tails, indicating that some patients exhibited markedly higher expression levels. While these patterns suggest differences in expression between responders and nonresponders, statistical significance was not reached in this analysis.

Figure 8. Validation of biomarkers identified by DeepImmunoGene. Violin plots showing differences in the expression of (A) 6 nonresponder-upregulated biomarkers and (B) 6 responder-upregulated biomarkers. *P* values determined by Mann-Whitney *U* test. **P*=.05, ***P*<.01.



Discussion

Principal Findings

We aimed to identify DEGs associated with response to PD-1 immunotherapy in patients with lung cancer using a DNN model to explore the biological mechanisms underlying immunotherapy response. Here, we developed DeepImmunoGene, a computational framework that uses an advanced neural network with an integrated approach to predict patient response to PD-1 immunotherapy with high accuracy. Our model identified 36 upregulated genes, including the top 10 (GSTT2B, HMGA2, AC135050.2, ANKRD33B, MMP13, PLA2G2D, RASGEF1A, BIRC7, DCAF4L2, and CHMP7), which were associated with positive responses to PD-1 immunotherapy in patients with NSCLC. However, apart from the 10 described, our model was able to find approximately 96 total critical genes. If we were to leverage only differential gene expression rather than DeepImmunoGene, more than 1000 genes would be present, many of which are not significant biomarkers for identifying responders. As a result, we deployed a permutation importance feature selector to identify from the potential 1000 expressive genes the ones that are critical in the identification of the patient, reducing the quantity of noisy biomarkers in the dataset. These findings suggest that these genes could serve as the candidate biomarkers for predicting patients who respond to PD-1 inhibitors. Some of these genes (HMGA2, MMP13, BIRC7, and PLA2G2D) have been reported to be overexpressed in various cancers, including lung adenocarcinoma, and are associated with tumor progression and metastasis [51-54], supporting their potential as biomarkers for PD-1 immunotherapy. We can identify these genes by ranking based on feature importance. We identify the most important genes, given the decrease in performance once permuted. The 10 most critical genes show the greatest decline in model accuracy once they are shifted. Furthermore, existing literature has shown many of these genes to be capable identifiers of immunotherapy. Genes such as HMGA2 and MMP13 are currently in the literature to identify a high likelihood of therapy success [55,56]. Our primary contribution lies not in introducing a novel DL architecture, but in developing DeepImmunoGene, a framework that complements prior frameworks, integrating interpretability and ML with the novelty to identify key genomic markers for PD-1 immunotherapy response.

In addition to their differential expression patterns, several of the top-ranked genes identified in our model have established roles in cancer-related biological processes. HMGA2 is a well-characterized architectural transcription factor associated with epithelial-mesenchymal transition and metastatic progression [57]. MMP13 contributes to extracellular matrix degradation and tumor invasion [55]. BIRC7 (also known as Livin) has been implicated in the inhibition of apoptosis and immune evasion mechanisms in solid tumors [58]. PLA2G2D is known for its involvement in inflammatory signaling and has been shown to modulate dendritic cell function and T-cell recruitment in the tumor microenvironment [59]. These functional insights, drawn from existing literature, suggest that many of the identified genes may influence immunotherapy response through diverse oncogenic and immune-related

pathways. Although a formal pathway enrichment analysis was not performed, the biological relevance of these genes supports their potential as markers of therapeutic response.

Our analysis began with the application of the LIMMA method to bulk RNA-seq data, which identified 1093 DEGs from a total of 19,911 genes in patients with lung cancer [24]. LIMMA is a widely used tool for differential gene expression analysis, facilitating the identification of genes linked to disease pathogenesis, particularly in RNA-seq and microarray data [30]. We evaluated these 1093 genes using 3 different ML models, including SVM, XGBoost, and DNN, to assess their predictive performance. The SVM showed moderate performance in classifying patient response with an accuracy of 0.68 and an AUC of 0.76, suggesting that it was unable to effectively capture the underlying correlations between gene expression and patient response. This may be due to the nonlinear nature of gene expression data, which likely hindered the SVM model's ability to generalize its predictions across patients [11,60]. While XGBoost outperformed SVM by a slight margin (0.04 for accuracy and 0.01 for AUC), there is no significant difference between these models, indicating that neither model could provide sufficiently robust predictions. These findings suggest that the high dimensionality, small sample size, and categorical imbalance of RNA-seq data pose significant challenges for traditional ML approaches [61].

To address the limitations of traditional ML models, we applied a DNN, a nonlinear model capable of capturing complex relationships within large gene expression datasets by mimicking the information-processing patterns of the human brain to generate predictions [11,40,60]. Unlike traditional models such as SVM and XGBoost, the DNN consists of multiple layers of neurons connected by weighted links, which allow the model to learn intricate patterns within the data. DNNs have shown strong performance in genomic predictions for various diseases [43]. The DNN model using the 1093 DEGs significantly outperformed both SVM and XGBoost. It exceeded SVM by 14% in both accuracy and AUC and outperformed XGBoost by 10% in accuracy and 13% in AUC. This improved performance of the DNN is attributed to its ability to capture and learn from the high-dimensional, nonlinear interactions inherent in gene expression data, which are challenging for traditional linear models to predict accurately [61]. This capability allows the DNN to generalize more effectively across diverse patient data, leading to more accurate and robust predictions than those made by more basic, linear computational models.

To reduce the number of genes and enhance the reliability of our model, we performed a permutation importance analysis using the scikit-learn framework. This analysis was repeated 4 times, each with 50 iterations to ensure the identification of a robust gene set to build DeepImmunoGene on. This subsequently reduced the set of 1093 genes to 98 genes based on nonzero importance scores, which were correlated with the response to PD-1 inhibitors and ranked according to their importance [62]. The DeepImmunoGene model was then trained using this refined set of 98 genes. Compared to our previous models, DeepImmunoGene demonstrated superior performance and robustness across all metrics (Table 2), indicating that the

application of permutation importance effectively eliminated irrelevant, noisy genes, allowing the model to focus exclusively on the most relevant genes without interference during training, such as overfitting. However, we also observed that specificity was consistently slightly lower than recall across all models, indicating that the models had more difficulty discerning nonresponders. This suggests that nonresponders may not have responded to immunotherapy due to external factors, such as the tumor microenvironment, age, or gender [24]. The comparative analysis with traditional ML models using the 98-gene subset found through permutation importance validates the core framework of DeepImmunoGene. The results highlight a specific synergistic effect between our feature selection method and the DNN, which is critical for achieving superior predictive performance. Although reducing the feature set to 98 genes improved computation efficiency no less, the fact that SVM and XGBoost trained on this same reduced feature set still failed to achieve comparable performance suggests that the DNN is better suited to capture the complex, nonlinear relationships and subtle gene-gene interactions underlying the RNA-seq data. Ultimately, the strength of DeepImmunoGene lies in this integrative approach of first identifying the most influential genes for accurate prediction and then leveraging a sophisticated DL model to interpret their combined predictive signal.

Further analysis revealed that 36 genes were upregulated ($\text{LogFC} > 0$) in patients who responded to PD-1 immunotherapy, whereas 62 genes were upregulated ($\text{LogFC} < 0$) in nonresponders [63]. These results suggest that DeepImmunoGene could serve as a robust ML-based tool for predicting immunotherapy outcomes in patients with lung cancer. The identification of these genes linked to responders and nonresponders not only offers potential biomarkers for predicting immunotherapy success but also enhances our understanding of the molecular mechanisms underlying the immune response in cancer. This could help guide more personalized treatment strategies, ultimately reducing unnecessary side effects and financial burdens for patients and health care systems, as immunotherapy is currently administered without prior knowledge of its effectiveness or safety for each patient [24,26]. Recent studies showed that only approximately 25% of patients show a positive response to immunotherapy, as PD-1/PD-L1 expression is not a sufficient biomarker to select patients who are likely to benefit [25,26]. Therefore, in addition to PD-1/PD-L1 expressions, these genes could be used as clinically actionable biomarkers for predicting response to ICIs with high accuracy.

Finally, we externally validated the predictive biomarkers identified by DeepImmunoGene using an independent bulk RNA-seq dataset of patients with NSCLC treated with PD-1 inhibitors (GSE207422) [49]. Given the small size of the external validation cohort ($n=24$) and the notable class imbalance (17 responders vs 7 nonresponders), we anticipated limited statistical power to detect meaningful differences (67). Additionally, the dataset itself includes patients receiving PD-1 inhibitors in combination with chemotherapy, which introduces treatment heterogeneity that may cause much of the variations observed in the expression patterns. Despite these limitations inherent to the available data, our analysis found that 4 of 6

nonresponder-upregulated genes showed higher median expression in nonresponders, with 3 achieving statistically significant differences in the predicted direction ($P < .05$). Similarly, 4 of 6 responder-upregulated genes demonstrated higher median expression in responders, although none reached statistical significance. This partial agreement offers encouraging evidence that the model-identified biomarkers capture biologically meaningful expression trends even in an independent, clinically realistic cohort. While these results should be interpreted cautiously, given the small sample size, class imbalance, and treatment variability, they support the potential utility of these gene markers for predicting immunotherapy response. Future validation in larger, well-annotated cohorts with consistent PD-1 treatment protocols is warranted to confirm their clinical relevance, fully validate the model's predictive classification performance, and further refine the list of biomarkers.

To contextualize DeepImmunoGene among existing approaches, we compared our method to previously published biomarker studies in NSCLC using PD-1 datasets. For example, Hwang et al [64] developed immune gene signatures derived from small patient cohorts with a limited number of features, which can restrict the model's ability to generalize to diverse patient populations or capture variability in gene expression. In contrast, Ravi et al [65] applied regression-based linear models that assume compounding, independent effects of genes on treatment response, which may fail to capture complex, nonlinear gene-gene interactions. By leveraging a DNN architecture, DeepImmunoGene is designed to learn these nonlinear dependencies across large-scale gene expression data, enabling more comprehensive and potentially generalizable biomarker discovery for predicting immunotherapy response. Other approaches, such as Lee et al [66], propose an ensemble method incorporating different models for the classification from gene expression profiles and additional information. This adds informative features, which may not always be available; in contrast, DeepImmunoGene reduces the feature space of RNA sequencing, helping isolate and detect features that are more likely to carry correct information.

Conclusions

Our DeepImmunoGene predictive model identified 36 upregulated genes in patients with NSCLC who responded to PD-1 immunotherapy. Among these, the 10 most significant genes (GSTT2B, HMGA2, AC135050.2, ANKRD33B, MMP13, PLA2G2D, RASGEF1A, BIRC7, DCAF4L2, and CHMP7) may serve as potential genomic biomarkers for predicting which patients with NSCLC are most likely to respond to PD-1 immunotherapy. Our external validation on an independent cohort supported several of the model-identified biomarkers, demonstrating partial agreement with DeepImmunoGene's predicted expression patterns despite the small sample size and class imbalance. These findings offer a promising foundation for future research aiming to improve patient stratification for PD-1 immunotherapy. Further validation in larger, well-annotated datasets and biological systems is needed to confirm their correlation with PD-1 inhibitors, which could lead to the development of more personalized and effective immunotherapies for lung cancer. Although the

DeepImmunoGene model demonstrated promising predictive performance, this study has several limitations. First, the analysis was conducted on a relatively small cohort of 355 patients with lung cancer. Second, we relied on a single publicly available RNA-seq dataset, which limited our ability to perform external validation. Third, key demographic and clinical variables, such as cancer stage, NSCLC subtype, age, and sex, were not available in the dataset. These factors are known to influence both immune response and gene expression, and their absence restricts the model's robustness assessment across patient subgroups. As a result, we were unable to evaluate the potential influence of demographic biases on model predictions. Future work with more comprehensive and diverse datasets is essential to validate the model's generalizability and to assess its consistency across clinically relevant subpopulations. We plan to conduct a follow-up study using external datasets when available and collaborate with clinics to validate our findings and further refine the list of biomarkers.

We also acknowledge that more advanced DL models exist for this task. Future work will involve evaluating DeepImmunoGene against state-of-the-art architectures, incorporating multimodal data, and validating performance on larger and more diverse cohorts. In this study, while DeepImmunoGene demonstrated strong performance metrics, future research should focus on improving the model's robustness through external validation across diverse datasets, including those from different geographical regions, patient demographics, and cancer stages. This would help assess how well the model generalizes beyond the current cohort of 355 patients. Moreover, the bias-variance tradeoff is crucial in this context. Our current model, which is highly sophisticated (DNN), likely strikes a balance between bias and variance, but there may still be room for improvement. High bias could occur if the model is overly simplified, missing important patterns in the data, whereas high variance could result from overfitting the model to the training data, leading to poor performance on new, unseen data.

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Data Availability

The patient data used can be found from the Gene Expression Omnibus public database GEO Repository (accessed on August 26, 2024).

Conflicts of Interest

None declared.

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Abbreviations

AUC: area under the receiver operating characteristics curve
DEG: differentially expressed gene
DL: deep learning
DNN: deep neural network
GEO: Gene Expression Omnibus
ICI: immune checkpoint inhibitor
LogFC: log fold changes
ML: machine learning
NSCLC: non-small cell lung cancer
PD-1: programmed cell death receptor-1
PD-L1: programmed cell death-ligand 1
RECIST: Response Evaluation Criteria in Solid Tumors
RQ: research question
SCLC: small cell lung cancer
SVM: support vector machine
TPM: transcripts per million
XGBoost: extreme gradient boosting

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