TITLE:

Pipeline to explore information on genome editing using large language models and genome editing meta-database

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ABSTRACT:

Genome editing (GE) is widely recognized as an effective and valuable technology in life sciences research. However, certain genes are difficult to edit depending on some factors such as the type of species, sequences, and GE tools. Therefore, confirming the presence or absence of GE practices in previous publications is crucial for the effectively design and establishment of research using GE. Although the Genome Editing Meta-database (GEM: https://bonohu.hiroshima-u.ac.jp/gem/) aims to provide as comprehensive GE information as possible, it does not indicate how each registered gene is involved in GE. In this study, we developed a systematic method for extracting essential GE information using large language models from the information based on GEM and GE-related articles. This approach allows for a systematic and efficient investigation of GE information that cannot be achieved using the current GEM alone. In addition, by converting the extracted GE information into metrics, we propose a potential application of this method to prioritize genes for future research. The extracted GE information and novel GE-related scores are expected to facilitate the efficient selection of target genes for GE and support the design of research using GE.

Database Tool URLs:

https://github.com/szktkyk/extract_geinfo,

https://github.com/szktkyk/visualize_geinfo

INTRODUCTION:

Genome editing (GE) enables the induction of specific modifications at targeted locations in the genome[1], promoting the advancements in genomic science. The development of Zinc-finger nuclease[2], followed by numerous GE technologies including transcription activator-like effector nuclease (TALEN)[3], CRISPR-Cas9[4], base editor[5], and prime editor[6], has allowed for the accurate and efficient functional analysis of specific regions in genomes. GE is also applied for the transient modification of gene expression using technologies such as CRISPR activation and CRISPR inhibition[7]. Additionally, the high-throughput GE method, CRISPR screen[8], has advanced the comprehensive investigations of

genes related to specific traits using a forward genetics approach. As increase in the number of publications in which researchers use GE in their experiments[9,10] indicates the impact of GE in life science research and how well GE technologies are accessible for application in research. Efficient and appropriate use of GE has the potential to accelerate life science research.

However, it has been reported that GE does not always work effectively at any genomics location due to various factors such as chromatin accessibility[11,12], DNA repair mechanisms depending on the species and cell-types[13,14,15], and target sequences[11,16,17]. These challenges stem from the mechanisms of how GEs work[16], including the requirement for GE tools to bind to the target sequence, reliance on endogenous genome repair mechanisms to achieve the desired editing outcome, and potential toxicity of the GE tools themselves. For example, TALEN has been reported to be more effective than CRISPR-Cas9 in heterochromatin regions owing to the target specificity of TALE protein's character. In another example, a previous study revealed that 41% of GEs with CRISPR-Cas9 did not show cleavage of the targeted genome locus when they attempted to induce GE in vitro for 218 times for 153 distinct genomic loci across 18 chromosomes in the mouse genome[11]. As implied by previous studies, selecting appropriate GE tools and protocols for specific target regions is crucial to avoid repeated failures in GE experiments, especially when targeting difficult genes. In this context, access to comprehensive prior information regarding GE experiments conducted on specific genes and species serves as a valuable reference.

Several public databases provide GE-related information, including the Human Genome Editing Registry, Plant Genome Editing Database[18], and European Sustainable Agriculture Through Genome Editing (eu-sage: https://www.eu-sage.eu/genome-search). The genome editing meta-database (GEM) is one such database, characterized by a large number of GE metadata entries from an integration of various public databases, and its focus on PubMed literature-based data collection[10]. The GEM systematically compiles and publishes metadata related to GE studies, drawing from PubMed, PubMed Central, NCBI gene, PubTator[19], MeSH, NCBI taxonomy, and EXTRACT2.0[20,21], integrating these public databases and services to provide as comprehensive data as possible. The GEM allows users to search for 46,039 GE-related articles as of September 18, 2024, linked with associated GE tools, genes, species, cell types, mutation types, diseases, BioProject IDs, and Gene Expression Omnibus (GEO) IDs. As each article may be linked to a few genes, the current GEM as of September 18, 2024, contained 92,182 entries in total, with the data visualized according to GE tools, species categories, and years. Such a comprehensive database is useful for exploring various research scenarios, including identifying genes that are accessible or efficiently targeted by GE, genes that remain understudied in the GE research field (i.e., genes that have not been studied or paid attention to), and GE cases involving orthologs of the gene of interest. However, the GEM is currently in the

prototype stage, and several issues limit its use for these purposes. One issue is that all collected genes linked to GE articles in GEM are displayed without indicating their specific role because of the data collection methodology used for GEM. As a result, it is unclear from the current GEM whether a gene was the target of GE, if it was a gene that reported as altered expression due to GE of other genes, or if the entry was a result of a data collection error.

In this study, we identified one of the metadata issues in the GEM and proposed a novel pipeline to improve metadata by addressing this issue. First, we analyzed a subset of the GEM dataset to clarify this issue. By analyzing 266 annotation pairs of gene ID and PubMed ID as a case study, we identified four different types of genes in the GEM. This makes it difficult for users to search for specific GE-related genes. To address this issue, in which the current GEM does not specify the role of each gene in GE, we developed a method that utilizes large language models (LLMs). LLMs, such as OpenAI's ChatGPT and Meta's Llama, have been reported to exhibit strong capacities for a wide range of complex tasks beyond text generation[22], including the extraction of metadata from research literature[23]. Therefore, we selected LLMs to address the challenges posed in this study. The pipeline developed in this study (Figure 1) allows users to access genes targeted by specific species and GE tools more accurately than the current GEM dataset. Additionally, it enables users to roughly estimate the number of cases "a gene was targeted by GE (GE_target_gene)" and the number of articles "a gene reported as altered expression due to GE of other genes" (GE_deg)". This study proposes a novel methodology to explore GE information through a systematic and comprehensive PubMed literature-based analysis facilitated by a combination of GEM data and LLM.

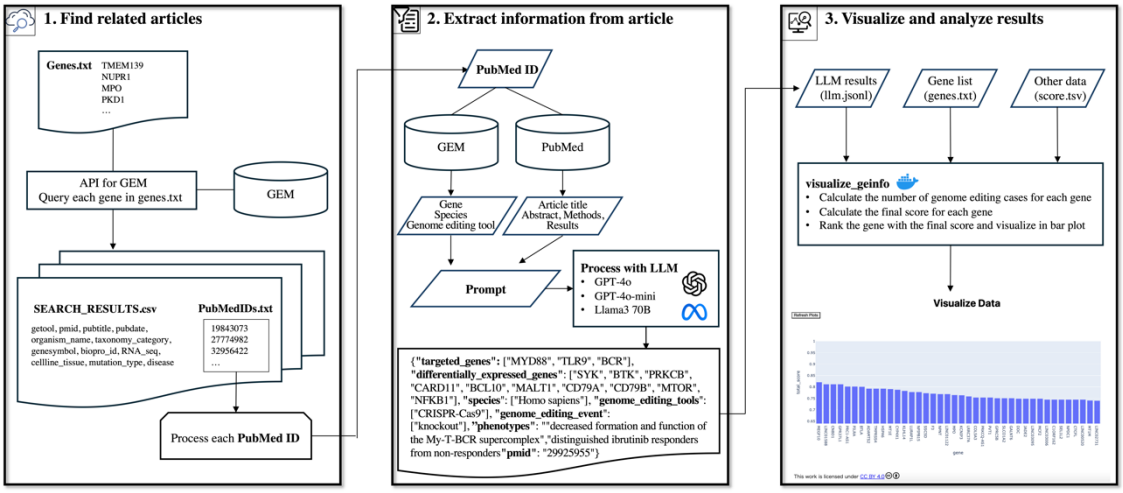

Figure 1. Overview of the pipeline for exploring information on genome editing using the genome editing meta-database and a large language model. The pipeline comprised three major steps: 1. Find related articles, 2. Extract information from article, 3. Visualize and analyze results.

MATERIALS AND METHODS

Overview of the pipeline

The pipeline developed in this study systematically collects novel metadata related to GE by utilizing the GEM and literature as reference materials processed through LLM. Figure 1 shows an overview of the entire pipeline. In Step 1, "Find related articles" selects relevant GE articles for subsequent analysis based on a group of gene IDs or symbols of interest. In Step 2, "Extract information from article", applies LLM-based processing to the selected articles to extract GE-related information. In Step 3, "Visualize and analyze results," visualizes and analyzes the extracted information (Figure 2). A detailed explanation of each step is provided in the subsequent sections.

Step 1: Find related articles

We conducted a case study focusing on the GEM entries related to the 168 genes (See Table S1 in the Supplementary File). These 168 genes were identified in a previous meta-analysis conducted by our group as being associated with oxidative stress in Parkinson's disease[24]. Each GEM entry comprises a GE-related article linked to specific genes and other associated metadata. To retrieve GEM entries for the 168 genes, we used the "gem_api" (https://github.com/szktkyk/gem_api) to query the GEM dataset. A text file containing the NCBI Gene IDs or gene symbols, separated by line breaks, was prepared, and the script "01_search_gem.py" from the "extract_geinfo" repository (https://github.com/szktkyk/extract_geinfo) was executed to retrieve relevant GEM entries. Out of the 168 genes, 146 had an associated NCBI Gene ID, and 158 had a gene symbol as a result of using NCBI command-line tools (https://www.ncbi.nlm.nih.gov/datasets/docs/v2/download-and-install/) (See Table S1 in the Supplementary File). Therefore, the number of genes was reduced when the GEM dataset was queried.

In the first attempt, conducted in May 2024, queries were made against the GEM data file 20240507_ge_metadata.csv (https://doi.org/10.6084/m9.figshare.27245733.v1). In the second attempt, conducted in August 2024, queries were directed against the updated GEM data file, 20240710_ge_metadata_all.csv (https://doi.org/10.6084/m9.figshare.27245742.v1). Despite using different GEM data file for first and second attempts, it was confirmed that, there were no differences in the GEM entries retrieved from either dataset for the 168 genes in this case study.

Step 2: Extract information from article

Using the custom-developed scripts "extract_geinfo" (https://github.com/szktkyk/extract_geinfo), we performed information extraction using LLMs. A total of 259 or 742 GE-related articles retrieved in Step 1 (259 for querying with 146 NCBI Gene IDs and 742 for querying with 158 Gene Symbols)

were used as target articles for LLM-based information extraction in Step 2. For each of the 259 or 742 articles, a specific prompt was created incorporating reference information, including metadata from the GEM entries and textual data from the article. This process was repeated 259 or 742 times, corresponding to the number of articles, to enable comprehensive information extraction using all GE-related articles associated with all the queried genes archived in the GEM.

Specifically, we prepared a text file containing PubMed Central IDs (PMCIDs) separated by line breaks was prepared. By running the “02_get_pubdetails.py” script from the extract_geinfo repository, we collected detailed information such as the textual content of each article. Subsequently, executing “03_run_gpt.py” facilitated information extraction from the textual data of PubMed Central articles by referencing the metadata from GEM entries.

Two different prompts were tested for Step 2. In the first attempt, the following prompt was used to extract eight types of metadata: “targeted genes of GE,” “genes reported as altered expression due to GE of other target genes,” “Species studied using GE,” “GE tools used,” “GE events induced,” “Study context,” “Key findings,” and “Implications suggested.”

PROMPT1 (Table 1)

TABLE 1: the prompt used for the first attempt of genome editing information extraction

※ The sections marked with {} indicate that different text is inserted for each LLM processing with an article.

Extract key genome editing related data from the provided ABSTRACT and TITLE, referencing the predicted genes and species ({GENES from GEM} by {SPECIES from GEM}) involved in this research. Analyze the ABSTRACT to:

1. categorize the genes into "targeted gene of genome editing" or "differentially expressed gene by genome editing".
2. confirm the species or organisms studied.
3. describe the genome editing event (e.g., knockout, knockin, knockdown, frameshift, SNP, expression modulation etc) and specify the genome editing tools used (e.g., CRISPR-Cas9, TALEN, Prime editor, etc).

If information on any of the above points is not provided in the text, state "Not mentioned".

Summarize findings in this JSON format:

{{

"targeted_genes": [], // List of targeted genes of genome editing

"differentially_expressed_genes": [], // List of genes altered expression by genome editing

"species": [], // List of species or organisms studied with genome editing

"genome_editing_tools": [], // List of genome editing tools used

"genome_editing_event": [], // List of editing events described

"study_context": "", // Study context in short one sentence

"key_findings": "", // Key findings in short one sentence

"implications": "" // Implications in short one sentence

}}

Please fill out the JSON structure based on the information from the research ABSTRACT and TITLE provided below:

Abstract:

{ABSTRACT from PubMed Central}

Title:

{TITLE from PubMed Central}

In the second attempt, we refined the prompt as follows and extracted six types of metadata: “targeted genes of GE,” “genes reported as altered expression due to GE of other target genes,” “Species studied using GE,” “GE tools used,” “GE events induced” and “Phenotypes resulting from GE.”

PROMPT2 (Table 2)

TABLE2: the prompt used for the second attempt of genome editing information extraction

※ The sections marked with {} indicate that different text is inserted for each LLM processing with an article.

Extract key genome editing related information from the provided texts (ABSTRACT, TITLE, METHODS, and RESULTS of the research article), referencing the predicted genes, species, and genome editing tools (GENES: {GENES from GEM} by {SPECIES from GEM}, genome editing tools:{genome editing tools from GEM}) involved in this research.

Analyze the provided texts to:

1. identify the targeted genes of genome editing
2. identify the differentially expressed genes by genome editing
3. confirm the species or organisms studied using genome editing
4. identify the genome editing events (e.g., knockout, knockin, knockdown, frameshift, SNP, expression modulation etc)
5. specify the genome editing tools used (e.g., CRISPR-Cas9, TALEN, Prime editor, etc).
6. identify phenotypes observed using genome editing in the study

If information on any of the above points is not provided in the text, state "Not mentioned".

If genome editing is not mentioned in the text, state "Not mentioned".

Summarize findings in this JSON format:

```
{{
"targeted_genes": [], // List of targeted genes of genome editing
"differentially_expressed_genes": [], // List of differentially expressed genes by genome editing
"species": [], // List of species or organisms studied using genome editing
"genome_editing_tools": [], // List of genome editing tools used
"genome_editing_event": [], // List of editing events identified
"phenotypes": [] // List of phenotypes observed using genome editing
}}
```

Please fill out the JSON structure based on the information from the texts (ABSTRACT, TITLE, METHODS, and RESULTS of the research article) provided below:

TITLE:

{TITLE from PubMed Central}

ABSTRACT:

{ABSTRACT from PubMed Central}

METHODS:

{METHODS from PubMed Central}
RESULTS:
{RESULTS from PubMed Central}

In the first attempt, we utilized the following information: the genes and species linked to the GEM entries as well as the titles and abstracts of the articles. In the second attempt, we incorporated additional information, including the GE tools registered in the GEM entries and the textual content from the methods and results sections of the article.
The outputs from the LLM were standardized into a unified JSON format using the “output_parser” function from the LangChain library. Three LLMs were tested: GPT-4, GPT-4o, GPT-4o-mini, and Llama3-70b. Based on its accuracy, GPT-4o was selected for further use. The primary libraries and tools used to query the LLM API were LangChain (https://www.langchain.com/) and Groq (https://pypi.org/project/groq/).

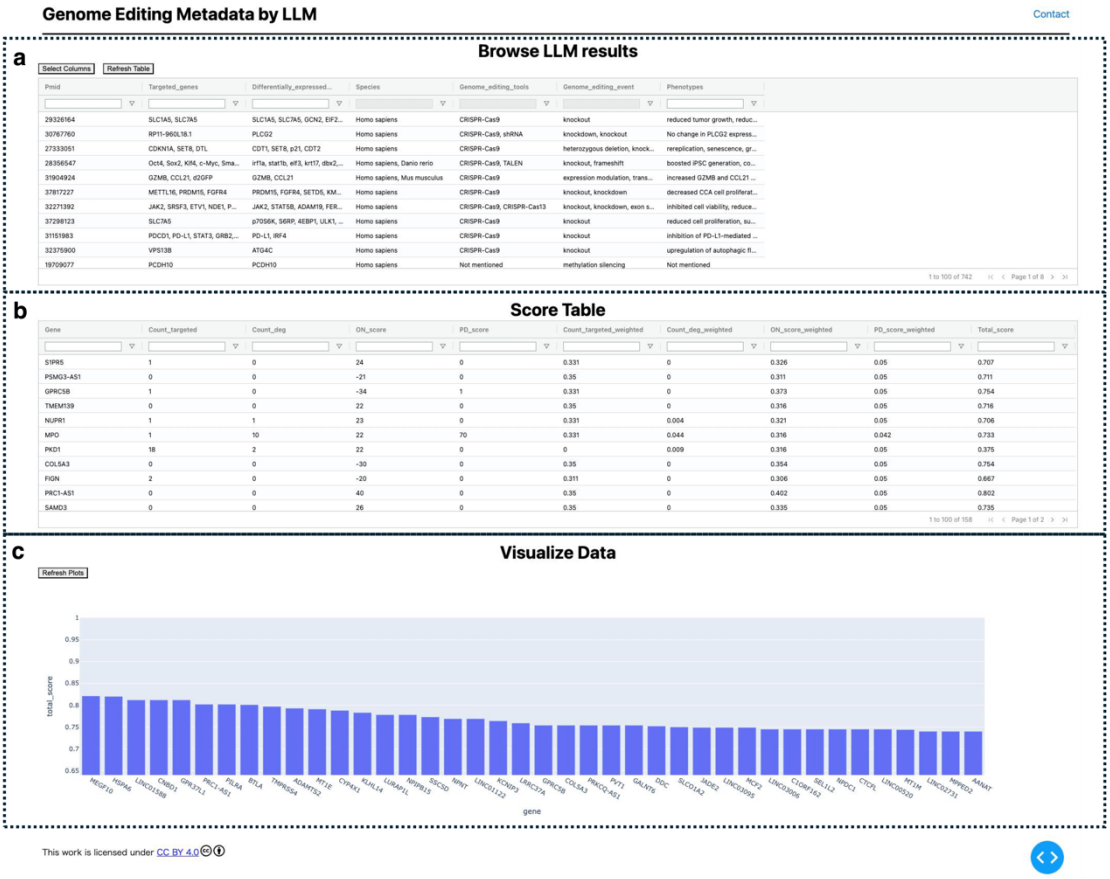

Figure 2. Visualization of extracted genome editing information. (a) Extracted information provided in a searchable table. (b) genome editing related metrics, and additional custom scores are provided as a table. In addition, the normalized scores were calculated and are listed in table. (c) The top 40 ranked genes are visualized using a bar plot with their scores.

Step 3: Visualize and analyze results

The information extraction results using LLM and GEM were visualized locally as a table_vis1 (Figure 2a) using the custom-developed tool "visualize_geinfo" (https://github.com/szktkyk/visualize_geinfo), which allows for automated calculation of GE related metrics and facilitates easy searching of the results. During visualization, the number of GE targeted cases (GE_target_count) and the number of articles reporting gene expression changes due to GE of other genes (GE_degs_count) for each of the queried genes (158 genes in this case) were automatically calculated and displayed as table_vis2 (Figure 2b). Additional custom-related scores and metrics can be incorporated into table_vis2 (Figure 2b). In this study, we added two metrics: scores from a transcriptomics meta-analysis (meta-analysis score), and the number of articles reporting an association with Parkinson's disease for each gene (PD_count) (Figure 2b). We also

applied max-min normalization to scale each value between 0 and 1 (equation1), and calculated a cumulative score, which is presented in table_vis2 (Figure 2b). In total, 158 genes were ranked in descending order based on cumulative scores. For metrics in which lower values indicated stronger target candidates (i.e., GE_target_count and PD_count), the scoring system adjusted the ranking by subtracting each value from the overall maximum (equation2). The score is automatically calculated through executing the "weight_score.py" in visualize_geinfo repository. Two important settings - addition of custom-related scores to the table_vis2 and indication of specific metrics where lower values indicating stronger target candidates, can be configured by editing the "config.py" file in the repository. The top 40 ranked genes were visualized using a bar plot (Figure 2c). The visualize_geinfo tool can be executed in a containerized environment by loading a Docker file from the repository, ensuring that it can run on any computer platform.

$$eauation\ 1: X' = \frac{X - (\min the\ column)}{(\max the\ column) - (\min the\ column)}$$

$$eauation\ 2:\ X' = \frac{((\max the\ column) - X) - (\min the\ column)}{(\max the\ column) - (\min the\ column)}$$

Preparation of evaluation data

After querying 146 NCBI Gene IDs against the GEM dataset, 266 annotation pairs between the NCBI Gene ID and PubMed ID were retrieved from 259 articles. We manually curated the role of each gene from 266 annotation pairs by reviewing the contents of the annotated articles.

The curation results are summarized in the Table S2 of the Supplementary File. If the gene was identified as a target of GE in the article, "1" was entered in the "curation_gene" column of the curation file. If the gene was not a target, "0" was recorded. For genes targeted in non-human species, "2" was entered, with the correct species was noted in the memo column.

If the gene was reported in the article to have altered expression due to GE of other genes, "1" was entered in the "deg" column of the curation file, and "0" if not. If a gene was both a GE target and mentioned altered expression, "2" was entered in the deg column, allowing for its exclusion in the subsequent process of performance evaluation of LLM information extraction.

Performance evaluation of information extraction by large language model

The evaluation of LLM-based information extraction was conducted for two types of extracted metadata: genes targeted by GE (GE_target) and genes reported as having altered expression due to the GE of other genes (GE_deg).

For GE_target, the evaluation followed these criteria: if the evaluation data (curated csv file) was labeled "1" and the LLM output included the gene, it was classified as a true positive (TP); if the gene

was missing from the LLM output, it was a false negative (FN). If the evaluation data was labeled "0" and the LLM output did not include the gene, it was considered a false positive (FP), while inclusion of the gene was treated as a true negative (TN). For cases labeled "2," it was considered a TP if the gene was present in the LLM output and the species mentioned in the LLM results matched the curated species in memo column.

For GE_deg, 163 annotation pairs were evaluated, excluding the 103 annotation pairs curated as GE_target. The same criteria used for GE_target applied: if the evaluation data was labeled "1" and the gene was included in the LLM output, it was classified a TP, while omission of the gene was an FN. For cases labeled "0," if the gene was absent from the LLM output, it was considered a TN; otherwise, it was classified as an FP.

The accuracy, precision, recall, and F1 scores were calculated using the following formulas:

Accuracy = (TP+TN)/(TP+TN+FP+FN)

Precision = TP/(TP+FP)

Recall = TP/(TP+FN)

F1 score = 2*(Precision*recall)/(Precision+Recall)

The calculation was executed by running "evaluate_targetedgenes.py" and "evaluate_deg.py" from extract_geinfo repository (https://github.com/szktkyk/extract_geinfo).

RESULTS

Proportion of genome editing targeted genes in Genome Editing Meta-database

As mentioned in the introduction, the Genome Editing Meta-database (GEM) dataset includes various types of genes linked to GE related literature owing to the nature of its data collection system. To investigate the types of genes linked to GE-related article, we manually curated a subset of the GEM dataset. Following Step 1 of the pipeline (Figure 1, method step1), we queried 168 genes against the GEM dataset and retrieved the GE-related articles associated with these 168 genes. The 168 genes used in this case study were identified through data-driven analyses in a previous study and have been reported to respond to oxidative stress in Parkinson's disease. Consequently, 266 gene ID (NCBI gene ID) with article ID (PubMed ID) annotation pairs (referred to as 266_GE_annotation_pairs) were retrieved. As some articles were linked to multiple genes, the total number of GE-related articles retrieved was 259 (referred to as 259_GE_articles).

Manual curation of the 266_GE_annotation pairs using the method outlined in preparation of evaluation data, revealed four major categories of genes: genes targeted by GE (38.72%, 103 of 266_GE_annotation_pairs), genes reported as altered expression due to GE of other genes (24.44%, 65 of 266_GE_annotation_pairs), genes studied in the article but not related to GE (10.53%, 28 of 266_GE_annotation_pairs), and genes collected due to extraction errors (8.27%, 22 of

266_GE_annotation_pairs).

If these results reflect a similar trend across the entire GEM dataset, which contains 92,182 entries as of September 18, 2024, this suggests that only approximately 39% of the search results represent the intended entries if a user searches for GEM to investigate whether a gene has been targeted by GE. Additionally, approximately 35% of the search hits would consist of unrelated studies or extraction errors. This indicates that the GEM dataset, in its current form, may be an unreliable dataset for such purposes.

Proportion of genome editing targeted genes after information extraction by large language model

To address the challenge of identifying the role of each gene in a GE study, we explored the application of large language models (LLMs). In Step 2 of the pipeline, we utilized information on genes, species, GE tools from the GEM, along with textual data from the articles, combined with custom prompts, as input to the LLMs to systematically extract GE metadata.

In May 2024, as part of the first attempt, we used genes and species data from the GEM, and the titles and abstracts of relevant articles. Several LLMs were tested to extract "targeted genes by GE" and their extraction performance, cost, and processing time were compared. As outlined in the 'Materials and Methods: Step 2: Extract information from the article', 259_GE_articles were processed using LLMs, and the extracted information was compared with manually curated evaluation data (See Table S2 in the Supplementary File). The results show that GPT-4 achieved 84.96% accuracy, a cost of $9.9, and a processing time of 3000 seconds; GPT-4o achieved 90.23% accuracy, a cost of $1.4, and a processing time of 960 seconds; and Llama3 70B achieved 83.83% accuracy, a cost of $1.5 (the cost of using GPT-4 with LangChain's output_parser library), and a processing time of 4,440 seconds (Table 3). Llama3-70B was tested using the Groq's API Demo version. Based on these results, GPT-4o, which had the highest accuracy and a relatively low cost, was selected.

In August 2024, during the second attempt, the prompt was refined to improve accuracy, and with the release of GPT-4o-mini, we conducted a comparison between GPT-4o and GPT-4o-mini. In the second attempt, our custom prompts for LLMs included information about genes, species, and GE tools from the GEM, and textual data from the title, abstract, methods, and results sections of the article. Metadata extraction was performed using GPT-4o and GPT-4o-mini to extract six types of metadata: targeted_genes (genes targeted by GE, referred to GE_target), differentially_expressed_genes (genes reported as altered expression due to GE of other genes, referred to GE_deg), species (organisms studied using GE), genome_editing_tools (tools used for GE in the study), genome_editing_event (events induced by GE), and phenotypes (phenotypes observed as a result of GE). As listed in Table 4, GPT-4o outperformed GPT-4o-mini and the models from the first attempt in terms of extraction accuracy. Therefore, the results of GPT-4o on the second attempt were selected to use for further

analysis.

For GE_target, the results from the second attempt demonstrated that the LLM was able to interpret the context of GE-related genes described in the article, with an F1 score of 0.9384. For GE_deg, the F1 score was 0.831.

In the task of extracting GE_target from the article and GEM data using LLM, precision had the lowest value among the measured metrics. Of the 266_GE_annotation_pairs, 13 genes were incorrectly extracted, including 9 false positives (FP) and 4 false negatives (FN) (Table 5). 6 of the 9 FP cases resulted from the LLM incorrectly annotating GE_deg as GE_target. Similarly, precision was the lowest metric in the GE_deg extraction task with 19 FP and 6 FN cases (Table 6). In the evaluation of the two types of GE-related metadata in this study, a trend was observed in which precision was lower compared to accuracy and recall (Tables 5 and 6).

The output files include the results of the GPT-4o and GPT-4o-mini for the second attempt (See Tables S3 and S4 in the Supplementary File). The evaluation results for GE_target and GE_deg for the second attempt using GPT-4o are available in the Tables S5 and S6 Supplementary File.

	gpt-4	gpt-4o	llama3-70b
number of articles processed	259	259	259
accuracy for 259 articles	0.8496	0.9023	0.8383
precision for 259 articles	0.7890	0.9326	0.7679
recall for 259 articles	0.8349	0.8058	0.8350
f1 for 259 articles	0.8113	0.8646	0.8000
price	$9.90	$1.40	$1.50
time	3000 (s)	960 (s)	4440 (s)

TABLE3: Evaluation for extraction of GE_target (genes targeted by GE) using large language models for first attempt (20240510-16)

	gpt-4o-mini (GE_targeted)	gpt-4o (GE_targeted)	gpt-4o (GE_deg)
number of articles processed	742	742	742
accuracy for 259 articles	0.8797	0.9511	0.8528
precision for 259 articles	0.7840	0.9167	0.7662
recall for 259 articles	0.9615	0.9612	0.9077
f1 for 259 articles	0.8596	0.9384	0.8310
price	$0.76	$26.55	$26.55
time	2050 (s)	4339 (s)	4339 (s)

TABLE4: Evaluation for extraction of GE_target and GE_deg (genes reported as altered expression due to GE of other genes) using GPT-4o for second attempt (20240802)

		LLM result		
		positive	negative	total
evaluation data	positive	99	4	103
	negative	9	154	163
	total	108	158	266

TABLE5: Confusion matrix for the GE_target of second attempt with GPT-4o (20240802)

		LLM result		
		positive	negative	total
evaluation data	positive	59	6	65
	negative	18	80	98
	total	77	86	163

TABLE6: Confusion matrix for the GE_deg results of second attempt with GPT-4o (20240802)

Calculation of genome editing-related metrics for genes

By utilizing an LLM-based information extraction process, we were systematically able to obtain novel GE metadata that the current GEM cannot achieve. Although the evaluation was conducted on only 259_GE_articles, we expanded the dataset to include 742 articles retrieved by querying the GEM with 158 gene symbols rather than NCBI Gene IDs. The searching for gene symbols in the GEM enables the inclusion of articles linked to orthologous genes in non-human species, resulting in an increased number of hits. We processed these 742 articles following Step 2 of the pipeline to collect GE metadata. From the LLM results for these 742 articles, two GE-related metrics were calculated for each gene: (1) the number of cases in which the gene was targeted by the GE tool (GE_target_count), and (2) the number of articles reporting altered gene expression due to GE of other genes (GE_deg_count). These metrics were automatically calculated by running the custom-developed visualize_geinfo tool (https://github.com/szktkyk/visualize_geinfo), which calls search_gem_llm.py to compute how frequently each gene was identified as "GE_target_count" or "GE_deg_count" based on the LLM output.

The top 10 genes with the highest GE_target_count values among the 158 genes were: *PKD1, FOS, SLC7A5, TXNIP, STAT6, SPP1, SLC3A2, TUBB3, SLC26A4,* and *CARD11*. These genes are considered to have a higher likelihood of being well-studied for effective GE within the 158 gene set. In contract, 103 of the 158 genes had a GE_target_count of zero according to the GEM and LLM, suggesting that they have not yet been extensively studied using GE or have limited examples. These are challenging candidate genes, potentially leading to novel discoveries through GE experiments.

The top 10 genes with the highest GE_deg_count values among the 158 genes were: *FOS, SPP1, TXNIP, MPO, CDKN1C, CRYAB, CD74, STAT6, CPT1A,* and *PKD1*. These genes have been suggested

to have potential responsiveness or exhibit certain phenotypes in response to GE.

Utilization of genome editing-related metrics

As an experimental approach for utilizing the two GE related metrics, we scored and ranked the 158 genes. In ranking the 158 genes, we incorporated two custom metrics: a meta-analysis score derived from gene expression data (meta-analysis_score) and the number of studies reporting an association with Parkinson's disease (PD_score), along with two GE-related metrics: GE_target_count and GE_deg_count. The custom-developed visualize_geinfo tool allows the addition of custom scores to the default metrics (GE_target_count and GE_deg_count) in the table. All four metrics were normalized to a 0-1 scale using max-min normalization, with a configuration file specifying how to handle the metrics, where lower values indicate higher priority. Weights were assigned to each metric based on their importance: "GE_target_count:0.35," "GE_deg_count:0.15," "meta-analysis_score:0.45," and "PD_score:0.05," ensuring that the total sum of weights equals 1.

After calculating the total score for each gene, the top 40 genes were ranked and visualized in a bar plot (Figure 2c). The table shown in Figure 2b displays all 158 genes sorted by score. The top 10 ranked genes were *MEGF10, HSPA6, LINC01588, CNBD1, GPR37L1, PRC1-AS1, PILRA, BTLA, TMPRSS4,* and *ADAMTS2*. The scores for the 158 genes are listed in the Table S7 of the Supplementary File.

These genes can be hypothesized as potential targets for future research, as they scored highly in the meta-analysis_score based on gene expression meta-analysis and the GE_deg_count based on literature interpretation by the LLM. In addition, they have low GE_target_count and PD_score values, indicating that they are understudied in the GE and PD research fields. This suggests that they may lead to new discoveries on in-depth investigations. However, these top-ranked genes have not yet been experimentally validated.

DISCUSSIONS

This study identified a key issue with the existing genome editing meta-database (GEM), in which genes linked to GE-related literature in the GEM can be classified into four major categories; however, the current GEM does not allow for determining which category each gene belongs to. To address this limitation, we employed large language models (LLMs). In a case study involving 259_GE_articles associated with the test 168 genes (including 146 NCBI Gene IDs and 158 Gene Symbols), we extracted information on GE contexts, including targeted genes of GE (GE_target) and genes reported as having altered expression due to GE of other genes (GE_deg). The results showed that GE_target was identified with an F1 score of 0.9384, and GE_deg with an F1 score of 0.8310. This demonstrates that combining LLMs with existing GEM can provide novel GE metadata that could not be systematically collected previously. By leveraging this enhanced GE information, we calculated two

new metrics for each gene: "number of GE targeted cases (GE_target_count)" and "number of articles reporting differential expression due to GE of other genes (GE_deg_count)." These metrics were then used in an experimental approach to score and prioritize the 158 genes.

After normalizing the scores, we ranked the 158 genes and examined the characteristics of some of the top-ranked genes. For example, *LINC01588*, ranked third, had a score of zero for GE_target_count, GE_deg_count, and PD_score. Its high ranking was driven by its relatively strong oxidative stress (OS) meta-analysis_score. A PubMed search for "LINC01588" returned five articles, one of which reported that knocking down *LINC01588* enhances oxidative stress through its interaction with HNRNPL[25]. *LINC01588* encodes for a non-coding RNA, which may explain the limited research on this gene. The second-ranked gene, *HSPA6*, encodes the molecular chaperone heat shock 70kDa Protein 6, which responds to stress. It had three cases of GE, and one study linking to PD. With the value of 2 for GE_deg_count and a meta-analysis_score of 50, *HSPA6* ranked in the second position. As one of the GE articles also explained *HSPA6* as an upregulated gene in PD[26], *HSPA6* is an already established target gene for PD and can be considered a positive control for this ranking. *CNBD1*, ranked fourth, was downregulated in OS conditions. The protein encoded by cyclic nucleotide-binding domain-containing 1 remains relatively understudied, with limited available information. Research on *CNBD1* in PD or OS is sparse, no GE cases were found using our method, and no PubMed hits querying with "*CNBD1*" were identified, making *CNBD1* as one of the potential new research target gene for the future PD or OS study.

A high score on these metrics does not necessarily imply that the genes are functionally linked to OS or PD. However, by incorporating GE information, this method can be seen as a way to prioritize candidate genes for further experimental functional analysis, particularly focusing on genes with limited prior research, but notable gene expression profiles. There is a growing concern regarding research bias toward well-studied human genes[27,28]. In response, approaches aimed at promoting the investigation of understudied genes and fostering new discoveries, such as omics-based methods as "Unknomics", have gained attention[29–31]. Unknomics seeks to advance research on novel genes. This scoring method, which integrates GE_target_count and PD_score in this study, can be viewed as a form of unknomics.

In the end, we outline the three major limitations of the pipeline developed in this study. First, the GEM dataset was collected based only on the literature from PubMed. As of September 18, 2024, the GEM contained 46,039 GE-related articles retrieved from PubMed using a custom search query (https://github.com/szktkyk/gem/blob/main/config.py). However, there is a possibility that irrelevant literature unrelated to GE was included, and relevant literatures may have been missed. Articles that

were not indexed in PubMed were excluded from the GEM dataset. Second, the number of articles processed using LLMs was limited in this study. As a feasibility study, only 742 of the 46,039 articles in the GEM dataset were processed using the pipeline. The evaluation data were limited to 259 of the 46,039 articles, indicating that the performance of the LLM was assessed only on a subset of the data registered in the GEM. The LLM performance on a large scale remains untested. Finally, there is always a risk of error in the LLM output. Information extraction for GE_target and GE_deg using LLM achieved accuracies of 95.11 and 85.28%, respectively, indicating error rates of 4.89 and 14.72% for GE_target and GE_deg. Thus, potential errors in the LLM output cannot be entirely ruled out. Although this pipeline shows the potential for systematically collecting a large amount of GE metadata for future research, the results generated by the LLM should not be regarded as definitive.

CONCLUSION

In this study, we explored a method to address the metadata issues in GEM using LLMs. Through this approach, we were able to systematically collect metadata that could not be obtained through the conventional GEM. Furthermore, we developed a method to rank genes based on newly collected data by leveraging the concept of unknomics. The findings of this study are expected to contribute to the efficient design of research using GE. However, because the information extracted by the LLM and the gene rankings may contain errors, it is essential for users to manually verify the data as a final step.

SUPPLEMENTARY DATA

- SUPPL1_168genes.xlsx
- SUPPL2_evaluation_data.xls
- SUPPL3_gpt4o_llm_results_240802.jsonl
- SUPPL4_gpt4o_mini_llm_results_240802.jsonl
- SUPPL5_eval_results_targetedgenes_gpt4o.xls
- SUPPL6_eval_results_deg_gpt4o.xls
- SUPPL7_scores.xls

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DATA AVAILABILITY

The Supplementary File and the source codes for this research are available at https://github.com/szktkyk/extract_geinfo, https://github.com/szktkyk/visualize_geinfo, and figshare repository (https://doi.org/10.6084/m9.figshare.c.7497327.v1).

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Contributions

T.S. was responsible for the data curation, software development, pipeline analysis, draft of the original manuscript. T.S and H. B were responsible for the study design, conceptualization, methodology manuscript review and editing. H.B. was responsible for the project administration, funding acquisition. All the authors have read and approved the final version of the manuscript.

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ETHICS DECLARATIONS

Competing interests

The authors declare no competing interests.

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