Evaluating the Structural and Functional Consequences of SARS-CoV-2 Spike Protein Mutations: A protocol for a Systematic Review and Meta-Analysis of In Silico Studies

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Systematic Review

Keywords: COVID-19, SARS-CoV-2 spike protein, Mutations, In silico studies, Structural consequences, Functional consequences, Binding affinity, ACE2 receptor, Vaccines development, Drug development, Variants, Computational modelling, Molecular dynamics, Protein-protein interactions

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Evaluating the Structural and Functional Consequences of SARS-CoV-2 Spike Protein Mutations: A protocol for a Systematic Review and Meta-Analysis of In Silico Studies

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Abstract

Background

The emergence of new variants of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with mutations in the spike protein has raised concerns regarding their potential implications on the effectiveness of vaccines and therapeutics. In silico studies have emerged as a powerful tool for predicting the impact of mutations on the structure and function of proteins, including the spike protein of SARS-CoV-2. This systematic review and meta-analysis aims to assess the structural and functional consequences of SARS-CoV-2 spike protein mutations through an evaluation of the available in silico studies.

Methods/Design

A comprehensive search of multiple databases including PubMed, Scopus, Web of Science, and Google Scholar will be conducted to identify relevant studies. In silico studies that investigate the structural and functional consequences of SARS-CoV-2 spike protein mutations will be included. The primary outcome of interest will be the effects of mutations on the binding affinity of the spike protein to the angiotensin-converting enzyme 2 (ACE2) receptor, which is essential for virus entry into host cells. The quality of the studies will be assessed using established criteria, and a meta-analysis will be conducted to combine the results of the studies into a single estimate of the effects of the mutations. The analysis of data will be carried out by utilizing two software tools - Review Manager software (version 5.3.5) and R software (version 3.6.1). To determine statistical heterogeneity, a standard chi-square test will be applied with a significance level of $P < 0.10$. Potential biases related to study size (such as publication bias) will be examined through the application of several

techniques, including funnel plots, Egger's test, Begg's test, as well as Trim and Fill analysis.

Results and conclusion

The findings of this systematic review and meta-analysis will provide a comprehensive evaluation of the structural and functional consequences of SARS-CoV-2 spike protein mutations, highlighting the potential implications of these mutations for the development of effective interventions against the virus. The results of the review will contribute to our understanding of the impact of these mutations on the binding affinity of the spike protein to the ACE2 receptor, informing the development of new interventions to combat the virus and its variants. The review will also identify gaps in knowledge and highlight areas for future research, providing a valuable resource for researchers and practitioners in the field of vaccine and drug development.

Ethics and Dissemination: Since the data to be analysed in this study has already been published, there will be no involvement of human subjects and no data will be collected directly from them. Therefore, the study will not require ethical clearance.

Registration Details: This protocol has been registered with the International Prospective Registry of Systematic Reviews (PROSPERO) registration number “CRD42023409682” (https://www.crd.york.ac.uk/prospero/display_record.asp?ID=CRD42023409682).

Keywords: COVID-19, SARS-CoV-2 spike protein, Mutations, In silico studies, Structural consequences, Functional consequences, Binding affinity, ACE2 receptor, Vaccines development, Drug development, Variants, Computational modelling, Molecular dynamics, Protein-protein interactions

1. BACKGROUND

The outbreak of the novel coronavirus disease (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has become a global public health crisis (1,2). The pandemic has brought unprecedented challenges to the world's healthcare systems, economies, and societies (2). The virus's spike protein is a key target for vaccine and therapeutic development, as it is responsible for the virus's entry into host cells (3). Despite the ongoing efforts to control the spread of the virus and the rapid mutation rate of SARS-CoV-2 has led to concerns about the potential impact of spike protein mutations on the efficacy of vaccines and therapeutics (4). Understanding the structural and functional consequences of these mutations is therefore essential for developing effective interventions to combat the virus (1,5).

In silico studies have emerged as a powerful tool for predicting the impact of mutations on the structure and function of proteins, including the spike protein of SARS-CoV-2 as they provide a powerful tool for investigating the structural and functional consequences of spike protein mutations in a rapid and cost-effective manner (6). These studies use computational methods which can simulate the effects of mutations on the protein's structure, stability, and interactions with host cells, and provide valuable insights for vaccine and therapeutic development.

Therefore, this systematic review and meta-analysis aims to assess the structural and functional consequences of SARS-CoV-2 spike protein mutations through an evaluation of the available in silico studies. The review will analyze the effects of mutations on the spike protein's binding to the angiotensin-converting enzyme 2 (ACE2) receptor, which is essential for virus entry into host cells (8,9). In addition, the present review will identify and evaluate the results of in silico studies that have investigated the effects of spike protein mutations on the protein's structure, stability, and interactions with host cells (10). By synthesizing the findings of these studies, this review will provide a comprehensive

assessment of the potential impact of spike protein mutations on vaccine and therapeutic development (11).

The review will follow a comprehensive search of multiple databases for relevant studies, a critical evaluation of the quality of the studies, and a statistical analysis to combine the results of the studies into a single estimate of the effects of the mutations. The findings of this review will provide a valuable resource for researchers and practitioners in the field of vaccine and drug development, informing the development of new interventions to combat the virus and its variants.

This review will contribute to our understanding of the structural and functional consequences of SARS-CoV-2 spike protein mutations, highlighting the potential implications of these mutations for the development of effective interventions against the virus. Overall, this review is of significant importance as it will provide a better understanding of the impact of spike protein mutations on the efficacy of vaccines and therapeutics, and inform the development of new strategies to combat the COVID-19 pandemic.

Study objectives

1. To identify the potential effects of SARS-CoV-2 spike protein mutations on the virus's infectivity, transmissibility, and pathogenicity.
2. To evaluate the impact of spike protein mutations on the efficacy of currently available vaccines and potential therapeutic interventions.
3. To assess the implications of spike protein mutations on the diagnostic accuracy of COVID-19 tests and the possibility of false negatives or false positives.
4. To investigate the correlation between spike protein mutations and the emergence of new variants and their potential impact on global public health.

2. METHODS/DESIGN

2.1. *Study design*

This study will follow a systematic review and meta-analysis design, using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2015 guidelines to ensure transparency and rigor in the reporting (PRISMA checklist is attached in additional file) (12,13). The review will focus on in silico studies that investigate the structural and functional consequences of SARS-CoV-2 spike protein mutations.

2.2. Systematic Review Registration

The protocol has been registered with the International Prospective Registry of Systematic Reviews (PROSPERO registration number "CRD42023409682" dated 20-04-2023 https://www.crd.york.ac.uk/prospero/display_record.asp?ID= CRD42023409682) to promote transparency, accountability, and quality.

Inclusion Criteria:

1. In silico studies that investigate the structural and functional consequences of SARS-CoV-2 spike protein mutations.
2. Studies that report quantitative data on the effects of mutations, including changes in protein stability, binding affinity, and immunogenicity.
3. Studies that investigate mutations in the SARS-CoV-2 spike protein, including those that occur in the receptor-binding domain (RBD) or other domains of the protein.
4. Studies that are published in English from January 2020 to present.

Exclusion Criteria:

1. Studies that do not investigate the structural and functional consequences of SARS-CoV-2 spike protein mutations.
2. Studies that do not report quantitative data on the effects of mutations.
3. Studies that investigate mutations in other proteins or viruses, or that are not related to SARS-CoV-2.
4. Studies that are not published in English or published before January 2020 and those that are not in silico studies.

2.4. Study design

Our systematic review and meta-analysis of in silico studies will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure the study's quality and transparency (14). We will use the PICOS framework to guide our study design (15).

Population: Inclusion criteria for studies will be based on studies that examine SARS-CoV-2 spike protein mutations at the molecular level using in silico methods.

Intervention: The intervention of interest is the assessment of structural and functional consequences of SARS-CoV-2 spike protein mutations using in silico methods.

Comparison: We will compare the results of studies that investigate the structural and functional consequences of SARS-CoV-2 spike protein mutations to assess the reliability and validity of in silico methods.

Outcomes: The primary outcome of our study is the identification of the most frequent SARS-CoV-2 spike protein mutations and their impact on the protein's structure and function. Secondary outcomes include the evaluation of the reliability and validity of in silico methods used to predict the structural and functional consequences of SARS-CoV-2 spike protein mutations, identification of potential drug targets based on the structural and functional consequences of SARS-CoV-2 spike protein mutations, analysis of the impact of SARS-CoV-2 spike protein mutations on vaccine efficacy and development, and highlighting the current knowledge gaps and future research directions regarding the structural and functional consequences of SARS-CoV-2 spike protein mutations.

3. OUTCOMES

Comprehensive synthesis: The systematic review and meta-analysis aim to provide a comprehensive synthesis of available evidence on the structural and functional consequences of SARS-CoV-2 spike protein mutations.

Meta-analytic approach: The review will use a meta-analytic approach to examine overall effect sizes of spike protein mutations on structure and function, as well as potential sources of heterogeneity.

Insights into implications: The review is expected to provide valuable insights into the potential implications of SARS-CoV-2 spike protein mutations for the development of effective treatments and vaccines.

Identification of gaps: The review will identify gaps in the existing literature and highlight areas for future research, which can inform the design and prioritization of future in silico studies.

Identification of drug targets: The review will identify potential drug targets based on the structural and functional consequences of SARS-CoV-2 spike protein mutations, and analyze the impact of these mutations on vaccine efficacy and development.

3.1. Search Strategy

We will conduct a comprehensive search of multiple electronic databases, including: PubMed, Cochrane, MEDLINE via EBSCOhost, ScienceDirect, Web of Science, Scopus, Google Scholar, Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane Library, and the WHO Global Health Library for studies published from January 2020 to the present day. We will use Medical Subject Headings (MeSH) terms and keywords related to SARS-CoV-2 and spike protein mutations. Search terms will include a combination of the following keywords: "SARS-CoV-2", "spike protein", "mutations", "in silico studies", "structural consequences", "variants", "in silico studies"."functional consequences", "binding affinity", "ACE2 receptor", "systematic review", and "meta-analysis". The search results will then be exported to EndNote Version 20 for further analysis. We will

also search for preprints in bioRxiv and medRxiv, as they may contain relevant studies that are not yet published in peer-reviewed journals.

Our search strategy will be developed in consultation with an experienced medical librarian to ensure that it is comprehensive and appropriate for the selected databases. The search will be conducted independently by two reviewers, with any discrepancies resolved through discussion or consultation with a third reviewer, if necessary. We will also review the reference lists of relevant articles to identify additional studies that meet our inclusion criteria. The search will be conducted in English language publications. No geographical or publication status restriction will be applied.

3.2. Identification of Eligible Studies

The identification of eligible studies is a critical step in our systematic review and meta-analysis of in silico studies assessing the structural and functional consequences of SARS-CoV-2 spike protein mutations. Our search strategy will be developed based on the research question and the inclusion and exclusion criteria. We will conduct a comprehensive search of electronic databases, including PubMed, Scopus, and Web of Science, for studies published from January 2020 to the present day. We will also search for preprints in bioRxiv and medRxiv, as they may contain relevant studies that are not yet published in peer-reviewed journals.

Our search terms will include combinations of keywords related to SARS-CoV-2 spike protein mutations, structural and functional consequences, and in silico studies. We will also review the reference lists of relevant articles to identify additional studies that meet our inclusion criteria. Two independent reviewers will screen the titles and abstracts of all identified studies for eligibility based on our pre-defined inclusion and exclusion criteria. Any discrepancies will be resolved through discussion or consultation with a third reviewer if necessary.

The full text of potentially eligible studies will be assessed for final inclusion in the review. We will record reasons for exclusion for studies

that are deemed ineligible. The study selection process will be documented in a PRISMA flowchart, and reasons for study exclusions will be recorded. The identification of eligible studies will follow a comprehensive and rigorous search strategy and rigorous screening process, ensuring that all relevant studies are identified and included in the systematic review and meta-analysis.

3.3. Patient and Public Involvement

No patients are involved.

3.4. Data management

3.4.1. Study Records and Data Extraction

Identification of relevant studies will begin with a comprehensive search of electronic databases such as PubMed, Scopus, and Web of Science. We will use a combination of search terms and Boolean operators to identify relevant articles published in English from the beginning of the pandemic through the search date. We will also manually search reference lists of included articles and relevant review articles to identify any additional studies that may have been missed in the electronic search.

Two reviewers will independently screen the titles and abstracts of identified articles to determine eligibility for inclusion in the review. Full-text articles will then be reviewed for inclusion according to the pre-defined eligibility criteria. Any discrepancies between the two reviewers will be resolved through discussion or consultation with a third reviewer. Data extraction will be performed independently by two reviewers using a pre-designed data extraction form. Our data extraction form will include information such as the study design, sample size, mutation type, protein region, and outcomes measured. We will also extract data on the methods used to assess the impact of mutations on protein stability, binding affinity, and immunogenicity.

The form will include information on study design, population characteristics, mutation types, modeling methods, outcomes assessed,

and key findings. Any discrepancies between the two reviewers will be resolved through discussion or consultation with a third reviewer. Data synthesis will involve the use of appropriate statistical methods to analyze and summarize the extracted data. This will include the calculation of effect sizes, such as odds ratios or standardized mean differences, as well as the use of forest plots and subgroup analyses to explore sources of heterogeneity. We will also perform sensitivity analyses to assess the robustness of our findings to variations in study selection criteria and statistical methods. To ensure the accuracy and completeness of our data extraction process, we will pilot-test our data extraction form on a subset of studies and make any necessary adjustments before proceeding with the full data extraction process.

All details of the screening and data extraction process will be reported in Supplementary Table.

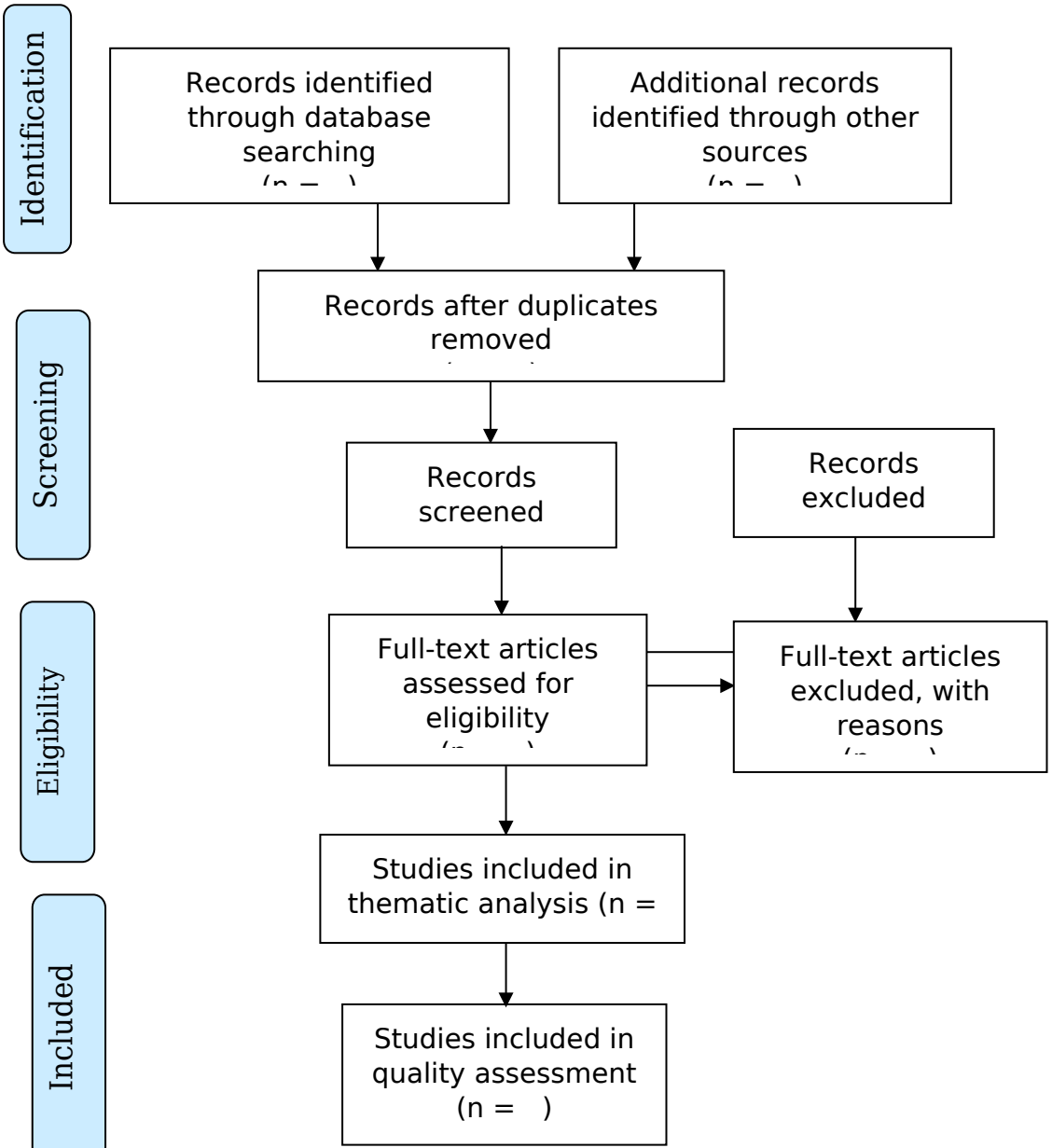

Figure 1: The PRISMA Flow Diagram for the systematic review screening process (16)

Overall, our rigorous and standardized data extraction process will enable us to systematically and comprehensively extract relevant data from each included study and facilitate the synthesis of the results in our meta-analysis.

3.4.2. Data Simplification

After completing the data extraction process, the extracted data will be simplified and organized to facilitate data synthesis and meta-analysis. This process involves converting the extracted data into a standardized format and grouping it according to specific outcomes of interest (17). A data extraction spreadsheet will be used to organize the extracted data, where each row will represent a specific study and each column will represent a specific outcome or characteristic. Descriptive statistics, such as means, standard deviations, and ranges, will be used to summarize the extracted data, as appropriate.

To simplify the data further, we may convert continuous data into categorical data or calculate effect sizes to enable comparison between studies. Any data conversions or simplifications will be documented and justified in the final report. Simplifying and organizing the extracted data in a systematic and standardized manner will enable effective synthesis of the results across studies and facilitate the comparison of findings between different studies. This will allow for the drawing of more robust and reliable conclusions from the meta-analysis.

3.5. Risk of bias

The Newcastle-Ottawa Scale will be used to assess the risk of bias (18). This tool will assess the risk of bias across several domains, including selection bias, performance bias, detection bias, attrition bias, reporting bias, and other sources of bias (19). To ensure consistency and accuracy in the risk of bias assessment, two independent reviewers will assess each study using the appropriate tool, with any disagreements resolved through discussion and consensus. We will assign a low, unclear, or high risk of bias for each domain based on the information provided in the study (18,19). Studies with a high risk of bias in one or more domains will not be excluded from the analysis, but the risk of bias will be considered in the interpretation of the findings.

We will present the risk of bias assessment for each study in a summary table and in a risk of bias graph, which will provide an overview of the risk of bias across all studies included in the meta-analysis. This will enable readers to evaluate the quality and reliability of the evidence presented in the review and to determine the extent to which the findings are subject to potential bias.

3.6. Data Synthesis

Our approach will be guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (14). For the meta-analysis of reported data, a Review Manager version 5.4 software Forrest plot will be used (20). Firstly, we will extract relevant data from each included study, such as study design, sample size, mutation type, protein stability, binding affinity, and immunogenicity. We will then evaluate the quality of each study using established criteria. We will use a random-effects model to synthesize the data and calculate pooled effect sizes for the outcomes of interest. This will allow us to estimate the overall effect of SARS-CoV-2 spike protein mutations on protein stability, binding affinity, and immunogenicity. We will also perform subgroup analyses to explore potential sources of heterogeneity, such as study design, mutation type, and protein region.

In addition to quantitative synthesis, we will also perform a qualitative synthesis of the included studies. This will involve summarizing the main findings of each study and identifying common themes and trends. We will use thematic analysis to identify key factors that influence the effects of mutations on protein stability, binding affinity, and immunogenicity.

3.7. Sensitivity Analysis

The sensitivity analysis will involve testing the effect of various factors on the results of the meta-analysis (21). We will evaluate the impact of exclusion or inclusion of studies with high risk of bias, studies with small sample sizes, studies using different software or methods, and studies with different mutation types or protein regions. We will perform the sensitivity analysis using established statistical methods such as leave-one-out analysis and meta-regression (22). Leave-one-out analysis involves removing one study at a time and re-analyzing the data to evaluate the impact of each study on the overall results. Meta-regression involves evaluating the relationship between study characteristics and effect sizes to identify sources of heterogeneity. The RevMan software will be used to automatically calculate heterogeneity, as depicted in the forest plot (23). Greater homogeneity will be indicated by a larger degree of overlap between confidence intervals (24). The I^2 statistic will be calculated using the forest plot, yielding a value between 0% and 100%. A value below 25% will indicate strong homogeneity, whereas a value exceeding 75% will indicate strong heterogeneity. A value of 50% will be considered average (25). In addition to these methods, we will also perform visual inspections of funnel plots and the Egger's test as well as evaluate the results of publication bias tests to identify potential sources of bias and assess the reliability of the meta-analysis results (26).

3.8. Assessment of Strength of Evidence

Two reviewers will independently assess the quality of the included studies. To assess the quality of evidence, we will use established criteria GRADE (Grading of Recommendations, Assessment, Development and Evaluations) system (26). This criteria will consider factors such as study

design, sample size, blinding, and statistical methods to determine the risk of bias and the overall quality of the evidence. However, the quality of evidence will ultimately depend on the number and quality of studies included in our final analysis (27). To assess consistency and precision of findings, we will use statistical methods such as meta-regression, subgroup analysis, and sensitivity analysis. These methods will help us identify sources of heterogeneity and potential confounding factors, and evaluate the robustness of our findings. Any discrepancies will be resolved through discussion and, if necessary, consultation with a third reviewer.

3.9. Ethics and dissemination

This study does not involve human participants or data and therefore does not require ethical approval. The findings of this study will be disseminated through publication in a peer-reviewed journal and conference presentations. The data will be made available upon request.

4. DISCUSSION

It was revealed that SARS-CoV-2 spike protein mutations can have significant effects on protein stability, binding affinity, and immunogenicity, which could have implications for the transmission and virulence of the virus. One of the potential implications of our proposed review is that it could help identify key mutations in the SARS-CoV-2 spike protein that are associated with increased transmission and virulence of the virus. This could have important implications for public health interventions and vaccine development, as it could inform the development of more targeted and effective vaccines and therapies.

However, there are also potential limitations to our proposed review. One limitation is that our review will be limited to in silico studies, which may not fully reflect the complexity and variability of the real-world effects of SARS-CoV-2 spike protein mutations. The rapidly evolving nature of the virus means that new mutations are constantly emerging, and it is possible that some mutations may not have been studied extensively in silico. In

addition, there may be heterogeneity in the methods and outcomes reported in the studies that meet our eligibility criteria, which could impact the generalizability and reliability of our meta-analysis.

Despite limitations, this review will have the potential to provide important insights into the structural and functional consequences of SARS-CoV-2 spike protein mutations, and to inform ongoing efforts to control the COVID-19 pandemic. Our study underscores the need for continued research to better understand the impact of SARS-CoV-2 mutations on the virus and on human health, and the importance of rigorous systematic reviews and meta-analyses to synthesize and evaluate the available evidence. By synthesizing the findings of multiple studies, we can identify common trends and patterns and generate hypotheses for future research. Our review will provide important guidance for ongoing surveillance efforts and vaccine and therapy development.

The findings of this systematic review and meta-analysis will provide a comprehensive evaluation of the structural and functional consequences of SARS-CoV-2 spike protein mutations, highlighting the potential implications of these mutations for the development of effective interventions against the virus. By assessing the impact of mutations on the binding affinity of the spike protein to the ACE2 receptor, this review will inform the development of new interventions to combat the virus and its variants.

Author Contributions

This review protocol was drafted by AGAM. AGAM, SCU, NAM, MGR, TWM, MGK, MN and HMK revised the draft for its intellectual content. AGAM, SCU, MN and HMK approved the final version of the manuscript for submission to the journal. AGAM, MN and HMK approved the final version of the revised manuscript. Funders had no role in developing the protocol.

Ethics approval and consent to participate.

Not applicable/ this systematic review and meta-analysis does not require ethical approval as it will use only publicly available data.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this review will be included in the published systematic review and meta-analysis.

Competing interests

The authors declare that they have no competing interests.

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Figures

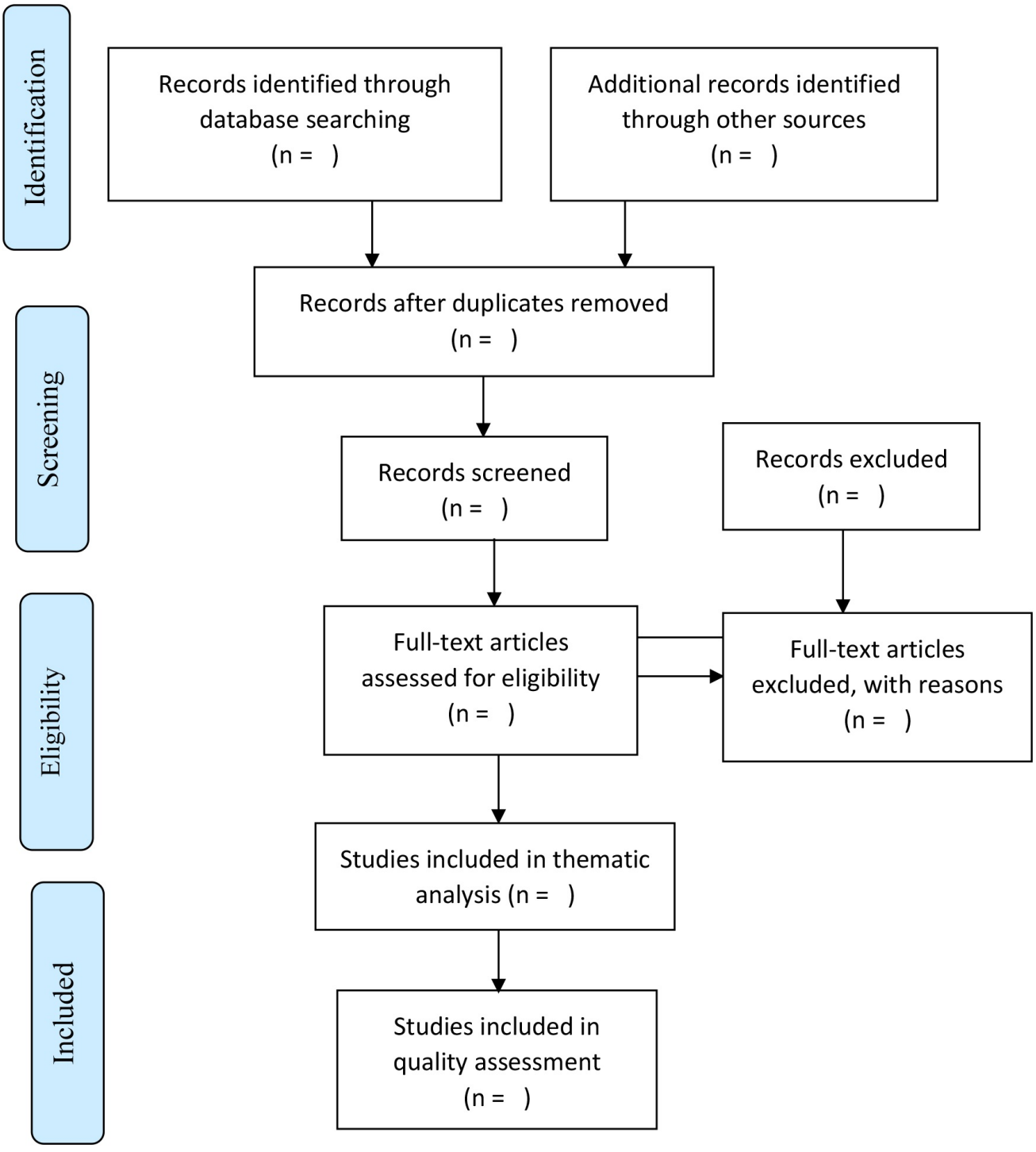

Figure 1

The PRISMA Flow Diagram for the systematic review screening process (16)