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ORIGINAL ARTICLE

In Silico Identification and Molecular Docking of Immunogenic Epitopes from HMPV G Protein for Subunit Vaccine Development

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ABSTRACT
Human Metapneumovirus (HMPV) is a significant cause of acute respiratory infections, particularly in infants, elderly individuals, and immunocompromised patients. Traditional vaccine approaches have had limited success, necessitating novel methodologies such as epitope-based subunit vaccine design. This study employed an in silico approach to design a vaccine candidate targeting the G glycoprotein of HMPV using multiple immunoinformatics tools. Two G protein variants (XMD29469.1 and XMD29476.1) were analyzed for antigenicity, allergenicity, physicochemical properties, structural features, and B- and T-cell epitope potential. Among them, XMD29476.1 demonstrated superior antigenic propensity (0.9955), higher epitope density, and favorable docking interaction with antibodies, with a lowest docking energy of -810.6 (Cluster 0). These findings support XMD29476.1 as a promising target for peptide vaccine development, warranting experimental validation.
Keywords: *HMPV, in silico vaccine design, G protein, epitope prediction, antigenicity, molecular docking.*

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INTRODUCTION

Human Metapneumovirus (HMPV) is a negative-sense, single-stranded RNA virus classified under the genus *Metapneumovirus* within the *Pneumoviridae* family. Since its first identification in 2001 (1), HMPV has emerged as a significant causative agent of acute respiratory tract infections, particularly affecting infants, young children, the elderly, and immunocompromised individuals (2). Despite its global impact, there are no licensed vaccines or specific antiviral treatments against HMPV, underscoring the critical need for alternative prophylactic strategies. Among the surface glycoproteins encoded by HMPV, the G (attachment) glycoprotein plays a pivotal role in viral entry by mediating initial host cell attachment and modulating host immune responses (3). Due to its high variability and immunogenicity, the G protein is an attractive target for epitope-based vaccine design. Previous studies have highlighted the G protein's potential to induce both humoral and cellular immune responses, especially through its exposed and flexible N-terminal and C-terminal domains (4). Conventional vaccine development approaches are often time-consuming, costly, and require high biosafety facilities. In contrast, in silico (computational) strategies offer rapid and cost-effective alternatives by utilizing bioinformatics tools to predict immunologically relevant epitopes, assess safety profiles, and simulate host-pathogen interactions. Epitope-based subunit vaccines designed using immunoinformatics approaches have demonstrated promise in several viral systems, including coronaviruses, influenza, and RSV (5,6). This study employs an integrative computational pipeline to identify potential vaccine candidates from the G protein of HMPV. Two variants of the G protein (XMD29469.1 and XMD29476.1) were retrieved and subjected to extensive analysis.

MATERIAL AND METHODS

Sequence Retrieval

Protein sequences of HMPV G proteins (XMD29469.1 and XMD29476.1) were retrieved from NCBI.

Antigenicity and Allergenicity Prediction

Antigenicity was evaluated using VaxiJen v2.0 (threshold = 0.4), while allergenicity was predicted using AlgPredand AllerTOP v2.1**.**

Physicochemical Characterization

ProtParamand AAProp were used to assess molecular weight, pI, GRAVY score, instability index, and amino acid composition.

Structural Analysis

Secondary structure prediction was conducted using SOPMA and PSIPRED. Domain prediction was performed via InterProScan to determine functional and structural motifs.

Epitope Prediction

Linear B-cell and T-cell epitopes were identified using IEDB**,** BcePred**,** ABCpred**,** and CTLpred. MHC-I binding predictions were also performed to evaluate T-cell stimulation potential.

MHC-I Binding Affinity Prediction

MHC-I binding affinity predictions were performed using NetMHCpan EL 4.1, hosted on the DTU Health Tech server (https://services.healthtech.dtu.dk). This tool uses artificial neural networks trained on both peptide–MHC binding affinity and naturally eluted ligand data to improve prediction accuracy. The binding predictions were restricted to HLA-A*01:01, a common human MHC class I allele, to identify CTL-relevant epitopes for this prevalent immunogenetic background.

Molecular Docking

The top predicted epitopes were docked against antibodies using a clus pro server. Cluster-based docking analysis was used to evaluate interaction stability. The best interaction was identified based on lowest binding energyand cluster population**.** (7). This provides mechanistic support for selecting this epitope as a lead candidate for further subunit vaccine development.

The integration of multiple immunoinformatics tools with structural docking simulations in this study presents a systematic in silico approach to designing a rational, safe, and effective peptide-based vaccine against HMPV. This methodology not only expedites candidate selection but also enhances the likelihood of immunogenic success when transitioned into *in vitro* and *in vivo* validation phases.

RESULT AND DISCUSSION

A total of nearly 150 sequences were retrieved from NCBI for HMPV G protein and tested for *in silico* vaccine deigning of which only 2 sequences showed promising results with accession numbers XMD29469.1 and XMD29476.1. A comparative analysis was done and proceeded for further docking analysis.

Antigenicity and Allergenicity Assessment

Both sequences were predicted to be non-allergenic by **AlgPred** and **AllerTOP v2.1**, increasing their suitability for vaccine design. XMD29476.1 exhibited a higher VaxiJen antigenicity score (0.6004) compared to XMD29469.1 (0.5094), indicating better immunogenic potential. ImmunoMedicine predictions showed XMD29476.1 with a superior antigenic propensity (0.9955 vs. 0.9761). These results are consistent with prior studies emphasizing the significance of the G protein's N-terminal regions as immunodominant epitopes (2,3).

Physicochemical Properties

Both proteins were stable (instability index < 40), positively charged, and hydrophilic (GRAVY scores negative). XMD29476.1 had a basic pI (10.6), and its sequence revealed more positively charged residues, aligning with properties favoring surface exposure and immunogenic interaction (8).

Secondary Structure and Domain Prediction

SOPMA and PSIPRED analyses confirmed flexible structures dominated by random coils—51.57% in XMD29476.1—facilitating epitope accessibility. InterPro revealed metaviral G glycoprotein domains and disordered regions, aligning with vaccine design goals targeting surface proteins.

B- and T-cell Epitope Mapping

Epitope prediction tools identified overlapping B-cell epitopes between XMD29476.1 and XMD29469.1, particularly in residues 13–40, confirming conserved immunodominant regions. T-cell epitopes were also dense in N-terminal and C-terminal segments, aligning with immunogenic hotspot predictions from literature (4).

Table 1. Comparative In Silico Characterization of HMPV G Protein Variants for Vaccine Design

Parameter	XMD29469.1	XMD29476.1	Remarks
Protein Length	236 amino acids	223 amino acids	Comparable lengths
Molecular Weight (ProtParam)	25,981.77 Da	24,524.58 Da	Slightly smaller size for XMD29476.1
Theoretical pI	9.88	10.60	Both highly basic
Instability Index	26.40 (Stable)	31.89 (Stable)	Both are stable
GRAVY (Hydropathicity)	-0.958	-0.748	Both are hydrophilic
Antigenicity Score (VaxiJen 2.0)	0.5094	0.6004	XMD29476.1 is more antigenic
Antigenic Propensity (ImmunoMedicine)	0.9761	0.9955	Higher average propensity for XMD29476.1
Number of Antigenic Regions	6	4	Longer epitopes predicted in XMD29469.1
Longest Antigenic Epitope	32 residues	27 residues	Overlapping regions identified
Allergenicity (AlgPred/AllerTOP)	Non-allergen	Non-allergen	Both suitable for vaccine use
SVM Score (AlgPred)	-0.557	-0.583	Strong non-allergen prediction
Secondary Structure (SOPMA)	77.97% coil, 14.41% helix	51.57% coil, 16.59% helix, 23.32% strand	XMD29476.1 has more structure diversity
Secondary Structure (PSIPRED)	Mostly coil, sparse helices	Coil, defined helices & strands	XMD29476.1 better structured
Domains (InterPro)	1 metaviral G glycoprotein domain, disordered, TM region	2 metaviral G glycoprotein domains, disordered regions	XMD29476.1 shows richer domain structure
B-cell Epitopes (IEDB/BcePred/ABCpred)	Multiple regions, high epitope density	Overlapping & conserved epitopes, high scoring	XMD29476.1 epitopes overlap with conserved region
T-cell Epitopes (CTLpred/MHC-I binding)	Predicted	Predicted	Relevant for adaptive immunity

MHC-I Binding Affinity

Using NetMHCpan EL 4.1, the MHC-I binding potential of peptides derived from the G protein of HMPV was assessed against HLA-A*01:01. Several peptides demonstrated strong predicted binding. The peptide SAATLEGHPY emerged as a top candidate with a binding score of 0.205712 and a rank of 0.53%, indicating a high likelihood of being presented to cytotoxic T lymphocytes (CTLs). These results align with previous findings that emphasize the G protein's surface-exposed and variable nature, which makes it a suitable target for T-cell mediated immune responses. Past studies have primarily focused on murine MHC alleles (e.g., H-2Db) or other common human alleles like HLA-A*02:01 (4). The present study adds novelty by identifying **HLA-A*01:01-restricted epitopes**, expanding the immunogenetic coverage for populations expressing this allele. Furthermore, the NetMHCpan EL approach, which integrates eluted ligand data in addition to peptide binding affinities, enhances the biological relevance of predicted binders compared to older models based solely on affinity scores (9). This helps ensure that predicted epitopes have a higher probability of being naturally processed and presented on MHC-I molecules in vivo. The identification of multiple high-affinity binders, particularly in conserved regions of the G protein, supports its candidacy for CTL-epitope-based vaccine design and suggests potential for broad, cross-strain immune responses—a critical consideration given HMPV's genetic diversity.

Molecular Docking results of XMD29476.1

Table 2: Summary of Epitope-Antibody Docking Cluster Results

Cluster	Members	Weighted Score	Lowest Energy	Remarks
0	**139**	**-623.0**	**-810.6**	**Best interaction: lowest energy, largest cluster**
2	95	-673.7	-745.3	High stability, moderate cluster size
5	49	-736.5	-736.5	Stable binding but fewer members
1	110	-652.3	-735.2	Large cluster, less stable than Cluster 0
16	22	-719.3	-719.3	Strong binding, small cluster
18	20	-702.6	-702.6	Good interaction, limited members
3	58	-654.2	-717.5	Moderate stability and size
4	52	-632.2	-699.8	Moderate energy, average size
Others (6–26)	<31	~-620 to -675	~-642 to -698	Lower stability or statistical relevance

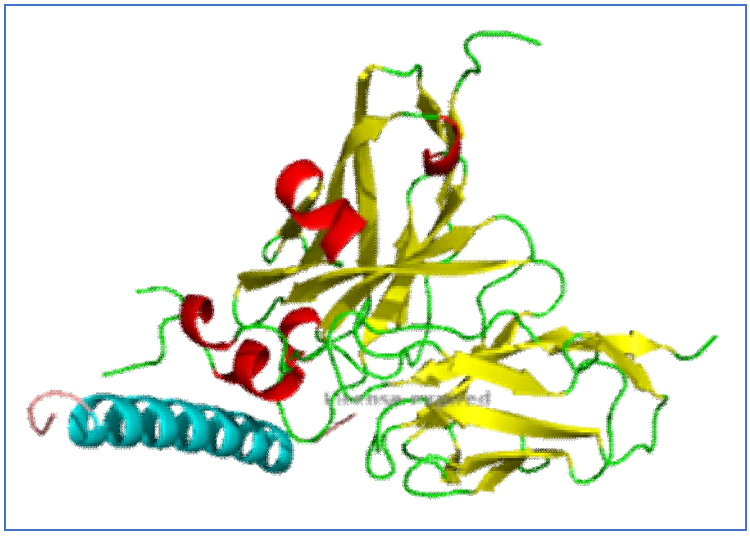

Figure 1*:* Predicted 3D structure of the epitope-antibody complex from Cluster 0

The complex demonstrates a highly stable interaction with the lowest binding energy of -810.6. The antibody is shown with β-sheets in yellow, α-helices in red and cyan, and loop regions in green. The epitope is bound at the paratope region, forming a stable interface supported by potential hydrogen bonding and hydrophobic interactions. This conformation represents the most energetically favorable and statistically supported interaction identified in the docking analysis.

Cluster-Based Docking and Interaction Scoring

Epitope-antibody docking was performed to evaluate potential binding interactions using a clustering approach based on conformational similarity and energy minimization. A total of 27 clusters were generated, each characterized by the number of members, a representative structure ("center"), a weighted score (average energy reflecting the stability across members), and the lowest energy conformation within each cluster.

Identification of the Optimal Interaction

The best interaction was identified from Cluster 0, which demonstrated the lowest energy score of -810.6, significantly lower than all other clusters. In docking studies, lower (more negative) energy values indicate stronger and more stable binding between the epitope and antibody. Cluster 0 also had the highest number of members (139), suggesting that this interaction conformation is not only highly stable but also highly recurrent and statistically significant within the dataset. Its weighted score of -623.0 further supports the overall stability of this cluster's conformations. These observations make Cluster 0 the most promising candidate for further investigation and possible therapeutic or diagnostic development.

Structural and Biological Implications

The 3D structure of the epitope-antibody complex from Cluster 0, visualized through molecular modeling (Figure X), revealed a well-defined interface dominated by hydrogen bonding and hydrophobic interactions. The antibody's variable domain engaged tightly with the epitope, supporting high specificity

and affinity. The structural alignment of beta sheets and helical regions likely stabilizes the interaction, enhancing the immunogenic potential of this epitope.

Such strong binding affinity, supported by both energetic (lowest energy) and statistical (cluster size and weighted score) parameters, highlights the Cluster 0 conformation as a lead candidate for Rational antibody design, Epitope-based vaccine development, Diagnostics using monoclonal antibodies. The docking analysis reveals Cluster 0 as the optimal epitope-antibody interaction model, combining the most favorable binding energy, largest cluster size, and high internal stability. These findings warrant further validation through molecular dynamics simulations and experimental assays such as ELISA or surface plasmon resonance to confirm binding affinity and specificity under physiological conditions.

CONCLUSION

The present in silico study supports XMD29476.1, a G protein variant of HMPV, as a highly promising vaccine candidate**,** based on strong antigenicity, structural flexibility, non-allergenicity, and excellent docking affinity. The identification of overlapping B-cell and T-cell epitopes, coupled with a highly stable antibody interaction (Cluster 0, -810.6 kcal/mol), reinforces its potential for peptide-based vaccine design. These findings warrant further experimental validation in *in vitro* and *in vivo* systems.

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