Analysis of *Mycoplasma pneumoniae* p1 genes across variants.

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Background

- *Mycoplasma pneumoniae* is a leading cause of community aquired pneumonia in children over five years old [(1)].
- *M.pneumoniae* is noted to have a highly conserved genome and a small genomic size (~800kbp)[(2)].
- The p1 gene encodes p1 adhesin protein, whose primary role is in adhesion to host cell surfaces[(3)].
- Variable regions in the p1 gene have been noted and characterised into variant subgroups.
- Variation between p1 types is attributed to homologous recombination events of repetitive elements across the genome[(4)].

To date, 13 distinct variants have been characterised. Previous studies have shown phenotypic differences between p1 type 1 and type 2 variants[(5,6)], and surveillance efforts have shown dominant variants appear in 10 year cycles[(7,8)]. Genetic analysis and analysis of protein structure between all variants including subtypes however is minimal.

Key Aims of Study

1. Assess and further understand recorded *M.pneumoniae* variants using phylogenetic trees and 3D protein modelling.
2. Determine sites of variation that may confer physiologically relevant differences between variants.

Methodology

Deposited p1 gene sequences searched for on PubMed and taken from NCBI GenBank. Sequences were aligned and translated. Phylogenetic trees were generated with Geneious (2024.0.5).

3D structures and similarity reports were generated using SWISS-MODEL.
3D structure variation analysis was completed using PyMol open source software (ver. 2.6.2). Using the type 1 sequence as the reference, all variations in amino acid sequences were highlighted for each variant.

Results

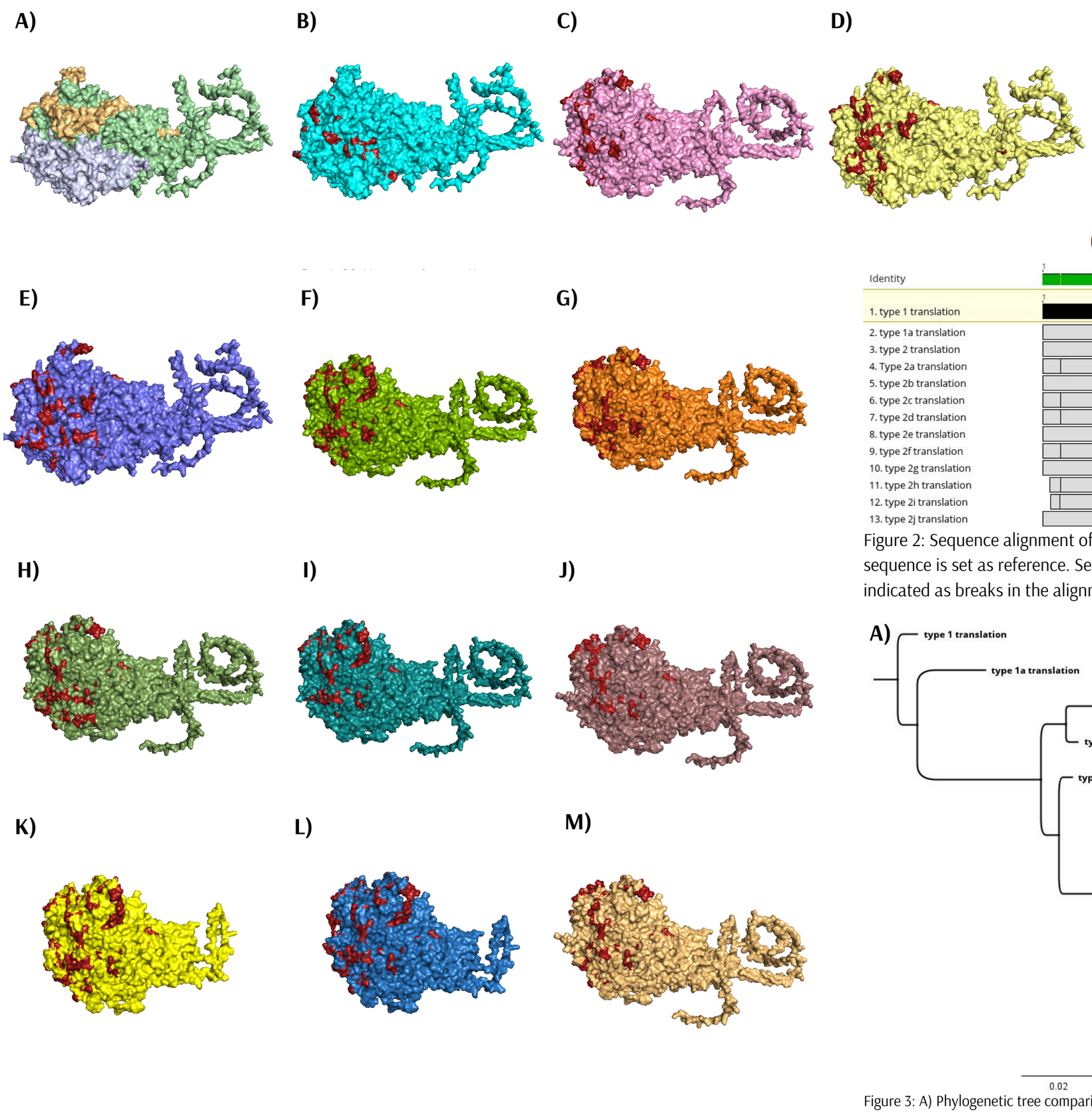


Figure 1 (left): 3D p1 protein structures of all 13 p1 variants generated with SWISS-MODEL. Variation to the P1 type 1 reference sequence highlighted in red. **A)** Type 1. RepMp4 region highlighted in orange, RepMp2/3 region highlighted in purple, non-repetitive elements highlighted in green **B)** Type 1a **C)** Type 2 **D)** Type 2a **E)** Type 2b **F)** Type 2c **G)** Type 2d **H)** Type 2e **I)** Type 2f **J)** Type 2g **K)** Type 2h **L)** Type 2i **M)** Type 2j

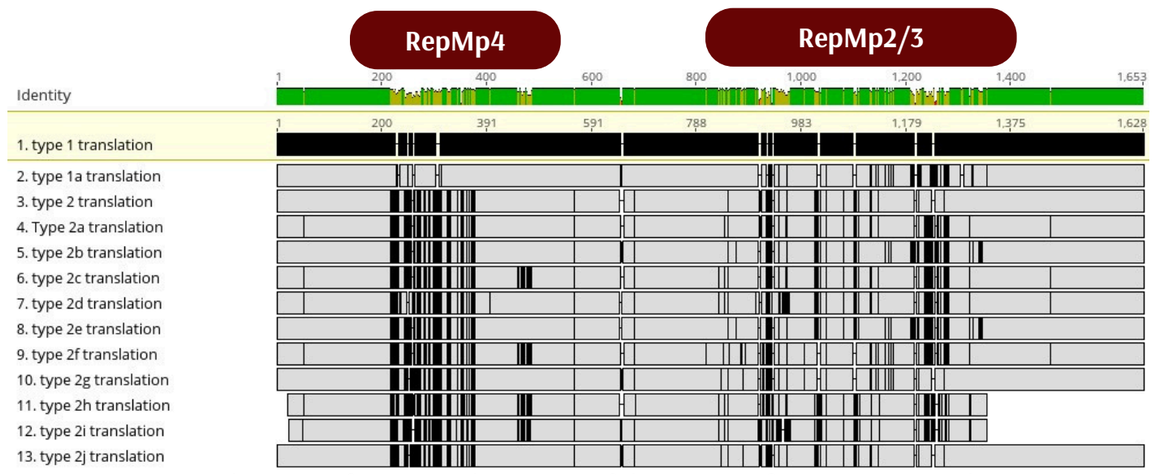
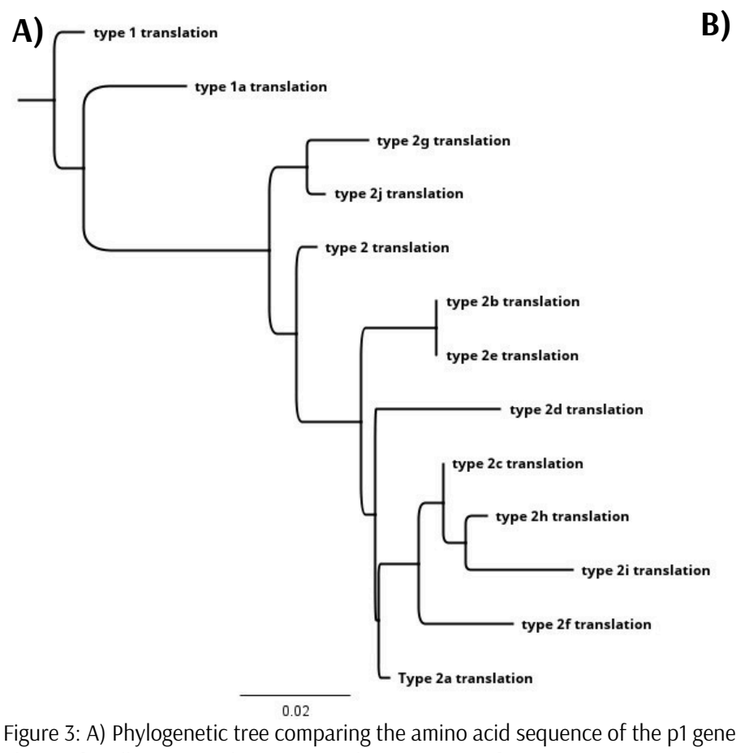


Figure 2: Sequence alignment of amino acid sequence of the p1 gene of 13 p1 variants with p1 Type 1 sequence is set as reference. Sequence mismatches are indicated in black, with deletions/insertions indicated as breaks in the alignment bars. Positions of both repetitive elements are indicated in red.



B)

Variant	P1 % sequence identity to T1
T1	100
T1a	97.11
T2	95.19
T2a	93.51
T2b	92.72
T2c	92.58
T2d	93.07
T2e	92.82
T2f	92.46
T2g	94.83
T2h*	90.60
T2i*	88.80
T2j	94.77

Figure 3: A) Phylogenetic tree comparing the amino acid sequence of the p1 gene for all 13 p1 variants. B) % sequence identity for all variants when compared to p1 type 1 reference sequence generated during 3D structure analysis. Sequences marked with * contained both repetitive elements but were not full p1 sequences.

Conclusions

- Distinct variation exists between all types, with many variants sharing combinations of similar variable regions which causes clusters in the phylogenetic trees.
- Variable regions are mostly expressed on the surface of the p1 gene in the N-terminal domain, likely indicating that the variation plays a direct role in binding to human alveolar membranes.
- Further study of the surface variation, specifically variant antigenic regions may aid in vaccine development.
- For most p1 types, few reference p1 sequences and whole genomes have been deposited. More work should be done in the future to sequence all variants whole genomes for further comparison of variation at the nucleotide level.
- Additionally, phenotypic testing on all variants should be carried out to determine how genetic variation translates in vivo.

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