Genetic analysis of post-pandemic influenza A(H1N1)pdm09 haemagglutinin virus variants that caused mild, severe and fatal infections in N. Greece

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Introduction

- Genetic analysis of post-pandemic influenza A(H1N1)pdm09 strains isolated in northern Greece
- Molecular analysis of HA1 sequences to identify variations and any possible relation to severity of illness.
- Comparison with data obtained during the pandemic period.

Materials and methods

- 1870 pharyngeal swabs - RNA extraction (Qiagen viral RNA mini kit)
- Real time RT-PCR for detection of A(H1N1)pdm09 influenza (CDC protocol) - HA1 gene of 102 strains sequenced
- Phylogenetic analysis: MEGA 5.1, ClustalW, GISAID.

Results

•The D222G variation was observed only in fatal cases (6). None of the examined strains had the Q293H variation.

•N-linked glycosylation sites: two 2010-11 strains had thesubstitution N287S, but this did not change the neutral and polar properties of the side chain. Interestingly, the same sites was only altered in two 2009-10 circulating strains.

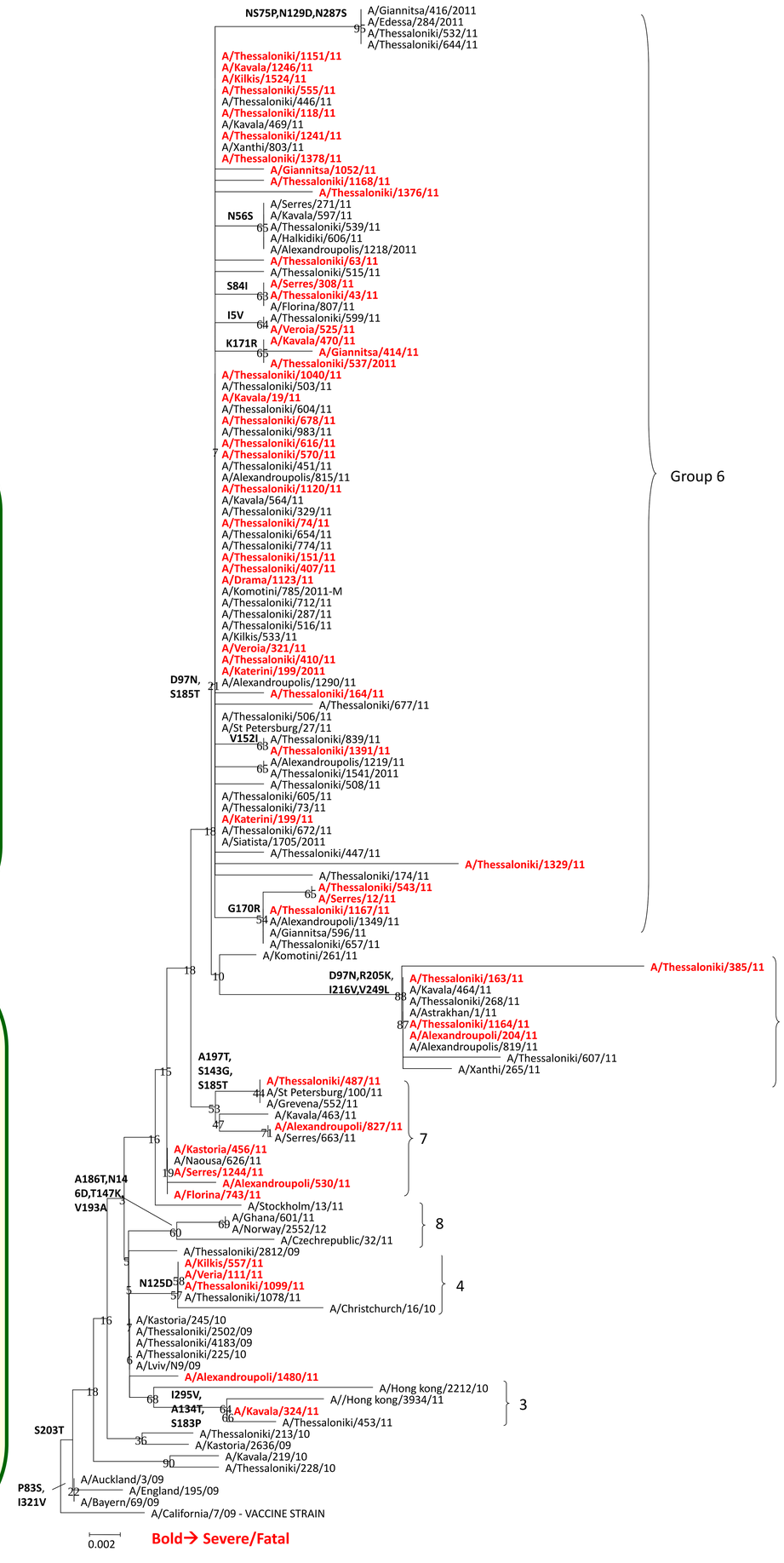
•Most viruses clustered with group 6 viruses, whereas a number of viruses clustered with groups 3,4,5 and 7. None clustered with groups 1,2 or 8.

•Genetic analysis showed that the HA amino acid sequences were largely similar to the vaccine strain, with sequence similarity of 96-99%.

•Certain signature amino acid changes, such as P83S, D97N, S185T, S203T and I321V, persisted from the pandemic period, indicating that they offer some selective advantages to the virus.

•Interestingly, some persistent variations were observed at amino acid residues in the vicinity of antigenic or receptor binding sites, such as I116V, S143G, V152I, K171R, S185T, S203T, D223R.

Influenza Season	2010-2011
Total examined	1870
Total positive samples	848(45.3%)
Positive influenza A(H1N1)	821 (96.8%)
Positive influenza A(H3N2)	13 (1.53%)
Positive influenza B	14 (1.65%)
Fatal cases	49



Conclusions

- High risk groups were considered to be pregnant women and obese individuals, whereas the elderly were much less affected than expected, probably due to the extensive vaccination of this age group and to the increased level of pre-existing antibodies that were cross-reactive with the pandemic influenza virus. However, the median age of severe and fatal cases was higher, 44 and 54 years during the post-pandemic period, compared to 47 years during the pandemic.
- Overall the 2010-2011 circulating strains had a close match with the vaccine strain A/California/7/2009, with nucleotide similarity between 96-99%, whereas in 2009-10 between 98-99.7%.
- There is an obvious trend of the new virus strains to accumulate amino acid changes on their antigenic sites and form new phylogenetic clades. The coexistence of different clusters indicated co-circulation of lineages in northern Greece.
- Epidemiological analysis revealed that the post-pandemic period 2010-11 was a severe influenza season with increased morbidity and mortality rates, even though exact rates cannot be determined because of the increased number of severe cases that are tested at the NIC.
- Molecular analysis revealed a number of variations at the HA1 sequences of northern Greek circulating strains.
- Constant epidemiological and molecular surveillance is important to monitor the efficacy of the vaccine and assess the severity of each influenza season.