Severe and fatal influenza A(H1N1) infections during the pandemic and post-pandemic periods in N. Greece: epidemiological and molecular analysis

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Introduction

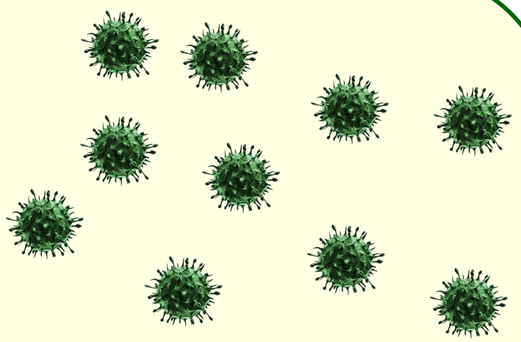
- Epidemiological and molecular analysis of post-pandemic 2010-2011 influenza A(H1N1)pdm09.
- Molecular analysis of HA1 sequences to identify variations and any possible relation to severity of illness.
- Comparison with data obtained during the pandemic period.

Materials and methods

- 1870 pharyngeal swabs
- RNA extraction (Qiagen viral RNA mini kit)
- Real time one step RT-PCR for detection of A(H1N1)pdm09 influenza (CDC protocol)
- The HA1 gene of 44 strains amplified and sequenced
- Phylogenetic analysis: MEGA 5.1, ClustalW, GISAID.

Results

Samples	Pandemic 2009-10	Post-pandemic 2010-11
Examined	4391	1870
A(H1N1)pdm09	1647 (37.5%)	823 (44%)
Severe pneumonia	115 (7%)	91 (11%)
Fatal	33 (2%)	58 (7%)



- Most common variations :
  P83S (44/44), S203T (44/44), D97N (36/44), S185T (35/44), V321I (43/44), D222G (5/44), V249L (4/44), I216V (4/44), R205K (4/44), G170R (4/44) and N56S (4/44)
- Some were observed :
  - in two of the examined strains: I5V, S75P, S84I, S143G and N287S
  - or in only one examined strain: D86G, D94N, R113M, N125D, N129D, T133S, A134T, K171R, S183P, A186S, A197T, F210S, M227I, D269D, A285S, K311N, C4R, A48T, S69L, Y78C
- The D222G variation was observed only in fatal cases. None of the examined strains had the Q293H variation.
- Regarding the N-linked glycosylation sites, two 2010-11 strains had the substitution 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.

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 96.33-98.5%, 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.
- 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 with 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.

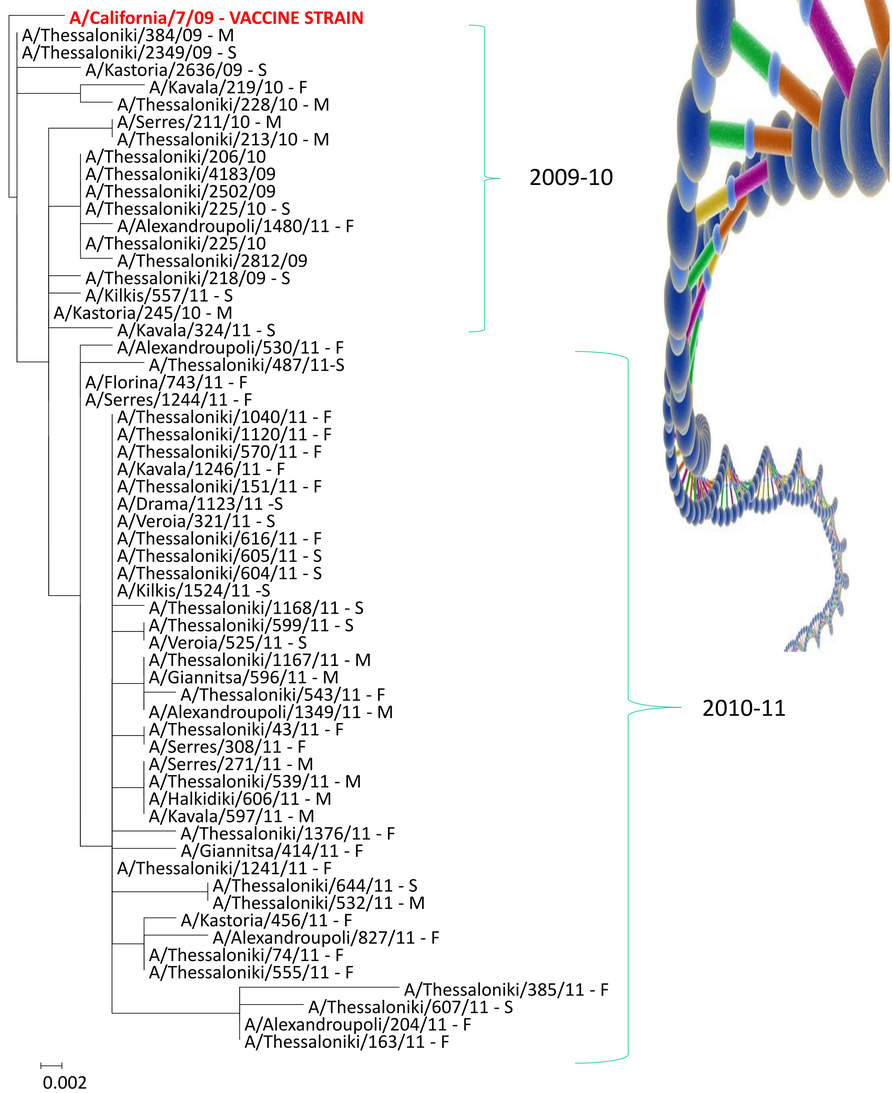


Figure. Phylogenetic relationships of the HA1 peptide of 15 representative pandemic and 44 post-pandemic influenza A(H1N1) viruses that circulated in northern Greece since 2009. [M],[S],[F] indicate mild, severe and fatal infections respectively. The tree was constructed with the Neighbor-Joining method, bootstrapped with 1000 replicates.