Jpn. J. Infect. Dis., 66, 299-305, 2013

Original Article

Monitoring Genetic Diversity of Influenza A(H1N1)pdm09 Virus Circulating during the Post-Pandemic Period in Turkey

Dilek Guldemir[1], Atila T. Kalaycioglu[1], A. Basak Altas[1], Gulay Korukluoglu[1], and Riza Durmaz[1,2]*

[1]*Department of Microbiology Reference Laboratories, Public Health Agency of Turkey, Ankara; and*
[2]*Department of Clinical Microbiology, Faculty of Medicine, Kirikkale University, Kirikkale, Turkey*

(Received November 7, 2012. Accepted April 22, 2013)

SUMMARY: The aimes of the present study were to monitor genetic alterations in the hemagglutin (HA) gene and oseltamivir resistance-related alterations in the neuraminidase (NA) gene of influenza A(H1N1)pdm09 viral isolates detected during the post-pandemic period in Turkey. A total of 2601 clinical specimens obtained from suspected cases of influenza A(H1N1)pdm09 viral infections were analyzed by real-time reverse transcription polymerase chain reaction. Viral RNA was detected in 233 (9%) clinical specimens. Sequence analysis of the HA gene in 16 random isolates showed >98.7% homology among each other and with the A/California/07/2009 vaccine strain. These 16 isolates had common (75%–100%) amino acid substitiutions at positions P83S, D97N, S203T, R205K, I216V, V249L, I321V, and E374K in the HA gene. In addition, two additional rare mutations were also observed at positions S162N (addition of a glycosylation site, 6.25%) and A186T (receptor binding region, 6.25%). On the basis of amino acid substitutions in the HA1 domain, majority of the Turkish isolates were classified in the genetic group v and others in the genetic groups ii, iii, and vi. In the present study, we observed an increase in the variety and ratio of mutations detected in the HA1 and HA2 domains of the HA gene; however, these alterations have not yet resulted in vaccine escape mutants in Turkey. In addition, analysis of the NA regions of the isolates revealed that oseltamivir resistance was not an issue in Turkey.

INTRODUCTION

The influenza A(H1N1)pdm09 virus first emerged in North America in mid-February 2009 and spread to most other regions of the world, which compelled the World Health Organization (WHO) to declare an emergent pandemic (1). Subsequently the virus became a serious public health problem worldwide, but its effect decreased in mid-2010. Thereafter, the WHO announced that the H1N1 flu virus moved beyond the post-pandemic period. However, localized outbreaks of various magnitudes are likely to continue (2). Globally, the levels and patterns of H1N1 transmission now differ significantly from those observed during the pandemic period, and off-season outbreaks are no longer being reported in either the Northern or Southern Hemispheres. Influenza outbreaks, including those primarily caused by the H1N1 virus, have shown similar intensities to seasonal epidemics (3).

The WHO recommended that health authorities should monitor respiratory disease activity and circulation of the influenza A(H1N1)pdm09 virus during the post-pandemic period to assess important genetic, antigenic, and functional changes, such as antiviral drug sensitivity (4). In Turkey, pandemic influenza A(H1N1) viral infections were reported to the Ministry of Health and clinical specimens obtained from suspected cases were sent to the Public Health Agency of Turkey (PHAT), National Influenza Center (Ankara, Turkey), where real-time reverse transcription polymerase chain reaction (RT-PCR) was used to confirm the presence of the influenza A(H1N1)pdm09 virus. All available data regarding demographic, epidemiological, and clinical parameters were sent to the reference laboratory, together with the clinical specimens. So far, there have been only two published studies reflecting the epidemiological and molecular characteristics of influenza A(H1N1)pdm virus infections in our country (5,6). Therefore, in the present study, we aimed to monitor mutations in the receptor binding region of the hemagglutinin (HA) gene, and investigate oseltamivir resistance-related mutations in the neuraminidase (NA) gene in 16 influenza A(H1N1)pdm09 viral isolates circulating in Turkey during the 2010–2011 influenza season.

MATERIALS AND METHODS

Samples and laboratory diagnosis: A total of 2601 nasal or nasopharyngeal specimens obtained from suspected cases of influenza viral infections were analyzed from the 40th week of 2010 to the end of the 14th week of 2011. Samples were transported in viral transport medium (Virocult; Medical Wire & Equipment Co., Ltd., Wiltshire, UK) to the National Influenza Reference Laboratory of PHAT. RNA extraction and

*Corresponding author: Mailing address: Molecular Microbiology Research and Application Laboratory, Microbiology Reference Laboratories Department, Public Health Agency of Turkey, Adnan Saygun Cad. No: 55, Sihhiye, 06100, Ankara, Turkey. Tel: +90-312-565-5541, Fax: +90-312-565-5749, E-mail: rizadurmaz@ymail.com

real-time RT-PCR amplification of the influenza A(H1N1)pdm09 viral RNA were performed as described previously (6).

Nucleotide sequencing: Thirty-one isolates randomly selected among laboratory-confirmed cases were propagated in Madin-Darby canine kidney (MDCK) cells, of which 16 isolates were succesfully sequenced and resulted in sufficient sequence data.

Complete HA and partial NA gene sequences and primers were deduced on the basis of a publication from the WHO Collaborating Centre for influenza (http:// www.who.int / csr / resources / publications / swineflu/ GenomePrimers_20090512.pdf). Briefly, viral RNA was amplified with specific primers using the in-house one-step RT-PCR system (Invitrogen, Carlsbad, Calif., USA). After purification of the amplicon using the Agencourt AMPure XP PCR purification system (Beckman Coulter, Inc., Beverly, Mass., USA), sequence reaction was performed using the Dye Terminator Cycle Sequencing Quick Start Kit (Beckman Coulter, Inc.) as described previously (6). Sequence results were analyzed using the BLAST algorithm available from the National Center for Biotechnology Information GenBank.

Statistical analysis: Homology between the sequence results obtained in this study, those reported during the pandemic period in Turkey (6), and the A/California/ 07/2009 strain, were compared using the MEGA software program (version 4.1 beta; Alignment Explorer) (7).

Phylogenetic analysis: Amino acid sequences from the HA1 region of the influenza A(H1N1)pdm09 isolates were aligned using Clustal W (8) and BioEdit version 7.0.0 DNA analytical software (9) with recommended representative sequences (10). Phylogenetic analyses were conducted using the neighbor-joining method (1000 bootstraps) included with the MEGA software package.

RESULTS

A total of 2601 specimens were obtained and analyzed from suspected cases of influenza viral infections from the 40th week of 2010 to the end of the 14th week of 2011 by the PHAT, National Influenza Center. Of these specimens, 1045 yielded positive PCR results for any respiratory virus, of which 404 (38.7%) were influenza A-positive. Of the influenza A-positive specimens, 233 (22.3%) were identified as influenza A(H1N1)pdm09, and 171 (16.4%) as seasonal influenza A(H3N2). When analyzing the overall occurrence rate, a total of 233 laboratory-confirmed influenza A(H1N1)pdm viral infections (8.96%) were among the 2601 clinical specimens collected during the post-pandemic period.

A total of 16 isolates among the positive cases from different parts of Turkey collected during the winter of 2011, were randomly selected and subjected to sequence analyses. None of these pandemic viral isolates were collected from deceased patients. Demographic characteristics of the patients are shown in Table 1. BLAST analysis of the sequencing results of these 16 strains indicated that all isolates were influenza A(H1N1)pdm09. MEGA analysis showed that the sequence of the HA genes of the 16 influenza A(H1N1)pdm09 viruses and the A/California/07/2009 (H1N1) vaccine strain yielded a very high homology rate (98.71%–99.12%; Table 1).

Amino acid substitutions coded by various HA gene sequences are shown in Table 2. The isolates had virtually similar (75%–100%) amino acid substitutions at positions P83S, D97N, S203T, R205K, I216V, V249L, I321V, and E374K. Three mutations (P83S, S203T, and I321V) were identified in isolates collected from both pandemic and post-pandemic seasons. Five mutations in the HA coding sequence (D97N, R205K, I216V, V249L, and E374K) were found predominately in samples collected during the post-pandemic season. According to a technical document published by the Community Net-

Table 1. Data of the 16 influenza A(H1N1)pdm09 viruses analyzed in the current study

Strain name	Gender/ age	Sampling date	Localization	Outcome	GenBank accession no.	Available sequence length (base pair)	Homology with A/California/ 07/2009 (%)[1)]	Mutation at partial NA gene
A/Ankara/01/2010	M/19	30.12.2010	Ankara	Recovered	JN613301	1778	98.82	No
A/Ankara/02/2010	M/19	29.12.2010	Ankara	Recovered	JN613302	1760	99.06	No
A/Ankara/03/2010	M/20	30.12.2010	Ankara	Recovered	KC311433	1769	98.94	No
A/Ankara/04/2010	M/20	30.12.2010	Ankara	Recovered	KC311434	1777	98.88	No
A/Cankiri/01/2011	F/27	19.01.2011	Cankırı	Recovered	KC311435	1778	98.71	No
A/Ankara/02/2011	M/41	20.01.2011	Ankara	Recovered	KC311436	1782	98.94	No
A/Tokat/03/2011	M/26	21.01.2011	Tokat	Recovered	KC311437	1781	99.00	No
A/Ankara/04/2011	F/37	10.01.2011	Ankara	Recovered	KC311438	1766	99.06	No
A/Tekirdag/05/2011	M/36	03.02.2011	Tekirdag	Recovered	KC311439	1782	99.06	No
A/Samsun/06/2011	M/49	11.02.2011	Samsun	Recovered	KC311440	1732	98.94	No
A/Kastamonu/07/2011	F/54	15.02.2011	Kastamonu	Recovered	KC311441	1777	99.12	No
A/Trabzon/08/2011	F/11	06.01.2011	Trabzon	Recovered	KC311422	1766	98.94	No
A/Ankara/09/2011	F/53	28.01.2011	Adana	Recovered	KC311443	1766	99.06	No
A/Konya/10/2011	F/22	03.02.2011	Konya	Recovered	KC311444	1775	99.12	No
A/Antalya/11/2011	M/31	10.02.2011	Antalya	Recovered	KC311445	1781	98.94	No
A/Izmir/12/2011	F/1	15.02.2011	Izmir	Recovered	KC311446	1781	98.88	No

[1)]: The homology was calculated using available nucleotide sequence length of hemagglutinin gene and the corresponding region of vaccine strain A/California/07/2009.
NA, neuraminidase.

Table 2. Comparison of the amino acid alteration in HA1 and HA2 domains of the HA gene between the pandemic and post-pandemic isolates

Amino acid alterations	Influenza A (H1N1)pdm season in 2009 (6)	Influenza A (H1N1)pdm season in 2010–2011	6 main genetic groups[1)]
I5R	No	(1/16, 6.25%)	
N31D	No	(1/16, 6.25%)	Genetic group vi
D35G	(1/29, 3.45%)	No	
P83S	**(29/29, 100%)**	**(16/16, 100%)**	
D97N	(3/29, 10.34%)	**(14/16, 87.5%)**	Genetic group ii, v
Y98Stop	(1/29, 3.45%)	No	
L105W	No	(1/16, 6.25%)	
F117V	(2/29, 6.9%)	No	
K119Q	(1/29, 3.45%)	No	
S143G	No	(1/16, 6.25%)	Genetic group iii
S162N	No	(1/16, 6.25%)	Genetic group vi (addition of a glycosylation site)
S185T	No	(3/16, 18.75%)	Genetic group ii, iii
A186T	No	(1/16, 6.25%)	Genetic group vi (receptor binding region)
A197T	No	(1/16, 6.25%)	Genetic group iii
S203T	**(29/29, 100%)**	**(16/16, 100%)**	
R205K	No	**(12/16, 75%)**	Genetic group v
I216V	No	**(12/16, 75%)**	Genetic group v
N228D	(2/29, 6.9%)	(1/16, 6.25%)	
D222E/N	(9/25, 37.5%)	No	
V234L	No	(1/16, 6.25%)	
V249L	(1/29, 3.45%)	**(12/16, 75%)**	Genetic group v
V272I	No	(1/16, 6.25%)	
T277A	No	(1/16, 6.25%)	
K283N	(1/29, 3.45%)	No	
I321V	**(29/29, 100%)**	**(16/16, 100%)**	
E374K	No	**(15/16, 93.75%)**	
K443E	No	(1/16, 6.25%)	
S451N	No	(3/16, 18.75%)	
V527I	No	(3/16, 18.75%)	
Common	**3**	**8**	
Non-common	9	15	

Common mutantions and their frequency are indicated as bold.
1): Influenza A(H1N1)pdm09 strain has divided into 6 main genetic groups, according to Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe; May–June 2011 technical document.

work of Reference Laboratories (CNRL) for Human Influenza in Europe, the WHO reference laboratory in London classified the A/H1N1 (pdm) viral strains into 6 main genetic groups (10). The amino acid substitutions were compared to the data in the CNRL document, which showed that the majority of the Turkish isolates (12/16; 75%) had common alterations (D97N, R205K, I216V, and V249L) and were classified in genetic group v, wheres two isolates were classified as genetic group ii, one isolate as genetic group iii, and one isolate as genetic group vi. The same classification pattern was also obtained by phylogenetic analysis (Table 2, Fig. 1). There were no amino acid substitutions associated with genetic groups i and iv. Some isolates identified in the present study also had low amino acid substitution rates (6.3%–18.8%) at positions I5R, L105W, N228D, V234L, V272I, T277A, K443E, S451N, and V527I, and a considerably high amino acid substitution rate (93.8%) at position E374K (Fig. 2, Table 2).

A comparison of the mutations between the 2009 pandemic season and 2010–2011 post-pandemic season indicated some remarkable variations (Table 2). While the D97N substitution rate in genetic groups ii and v was 10.34% in isolates collected during the 2009 influenza A H1N1 season, it was 87.5% in those during the 2010–2011 season. Although the S185T substitution in genetic groups ii and iii was not detected in samples collected during the 2009 season, it occurred in 18.75% of the isolates collected durig the 2010–2011 season. The S143G and A197T substitutions in genetic group iii were not detected in samples from 2009, but their rate elevated to 6.25% in samples collected during the 2010–2011 season. While R205K and I216V substitutions in genetic group v were not detected and the V249L substitution in this genetic group was detected in 3.45% of the samples collected in 2009 season, the rate of all three mutations was 75% in samples collected during the 2010–2011 season. The N31D, S162N (addition of a glycosylation site), A186T (receptor binding region) substitutions in genetic group vi were not detected in samples collected during the 2009 season, however, they occurred in 6.25% of the samples collected during the 2010–2011 season.

H275Y and N295S mutations responsible for resistance to NA inhibitors (38,39) were not detected in any of the 16 isolates analyzed in this study (Table 1).

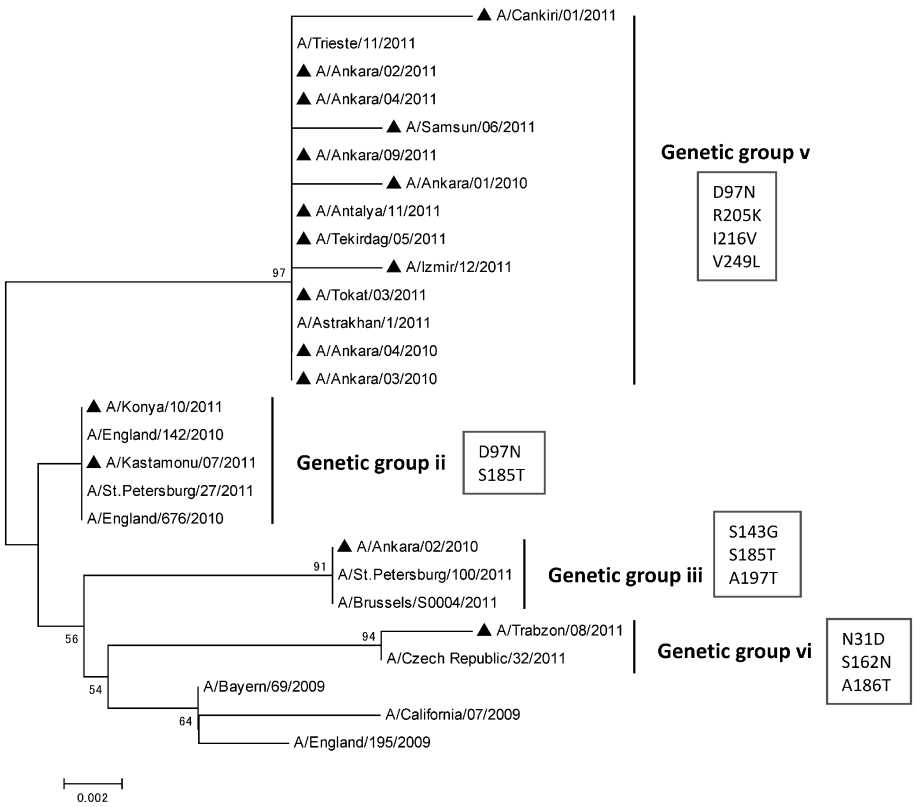

Fig. 1. Phylogenetic relationship of HA1 domain of the HA genes of influenza A(H1N1)pdm09 viruses circulating in Turkey during 2010–2011 season. The dendrogram was constructed using the neighbor-joining method with Kimura two-parameter distances using MEGA 4 software. Bootstrap confidence limits were based on 1000 replicates. Numbers at the nodes indicate bootstrapping values and bars represent amino acid substitutions (H1 numbering) per position. Isolates from Turkey subjected for the present study are indicated by a triangle. Representative strains for genetic groups ii, iii, v, and vi were obtained from the WHO Collaborating Centre at London, CNRL, May–June 2011 technical document.

DISCUSSION

The purpose of the present study was to analyze the characteristics of influenza A(H1N1)pdm09 viral infections in Turkey between December 29, 2010 and February 15, 2011, and to identify mutations in the HA gene and resistance to oseltamivir among the 16 randomly selected isolates. Our results determined that only 9% of the clinical specimens collected during the post-pandemic period carried influenza A(H1N1)pdm09 viral RNA. However, our previous study regarding the 2009–2010 pandemic influenza season, an analysis of 19973 clinical specimens obtained from suspected cases of pandemic influenza A(H1N1) viral infections, revealed that 47.3% were positive for influenza A(H1N1) viral RNA (6). As in Turkey, the pandemic influenza A virus positivity rate rapidly increased worldwide during the pandemic (11,12). However, in contrast to the pattern observed during the pandemic, the virus now co-circulates with other influenza viruses and is no longer the predominant influenza A virus in many countries (13).

The WHO reported that the influenza A(H1N1)pdm09 viruses circulated in the Northern Hemisphere during the 2010–2011 influenza season were antigenically and genetically similar to the A/California/07/2009 vaccine strain (14). In the present study, our genetic analysis of the influenza A(H1N1)pdm09 isolates revealed that there was >98.7% nucleotide sequences homology with the vaccine strain. Notably, a similar range of homology (>98.9%) was observed in our previous study conducted during the pandemic influenza season (6).

Although influenza A(H1N1)pdm09 virus is no longer considered as a pandemic threat, it continues to circulate worldwide; therefore further studies are warranted to monitor mutations in H1N1 strains, which have the potential to result in further virulence and resistance (15–17). Paticularly, it is vital to monitor mutations in the HA1 domain of the HA gene, essential for viral attachment and immunization (15,16). Phylogenetic analysis defined 6 different genetic groups (genetic group i–vi) according to specific mutations in the HA gene sequence that have been co-circulating since the beginning of the 2010–2011 flu season (18). Although the genetic groups showed specific changes in different antigenic epitopes of the HA gene, these genetic substitutions did not result in antigenic differences in the A/California/07/2009 vaccine strain (10,19–21). Phylogenetic analysis of the most common amino acid substitutions observed in the HA1 domain showed that majority of the Turkish isolates were classified in genetic group v. The remaining four isolates were classified in genetic groups ii, iii, and vi. Some Turkish isolates, such as the strains A/Cankiri/01/2011 and A/Samsun/06/2011, were classified in genetic group v, characterized by additional rare mutations (I5R, L105W, N228D, V234L, and T277A). However, there is currently no report regarding the significance of these alterations.

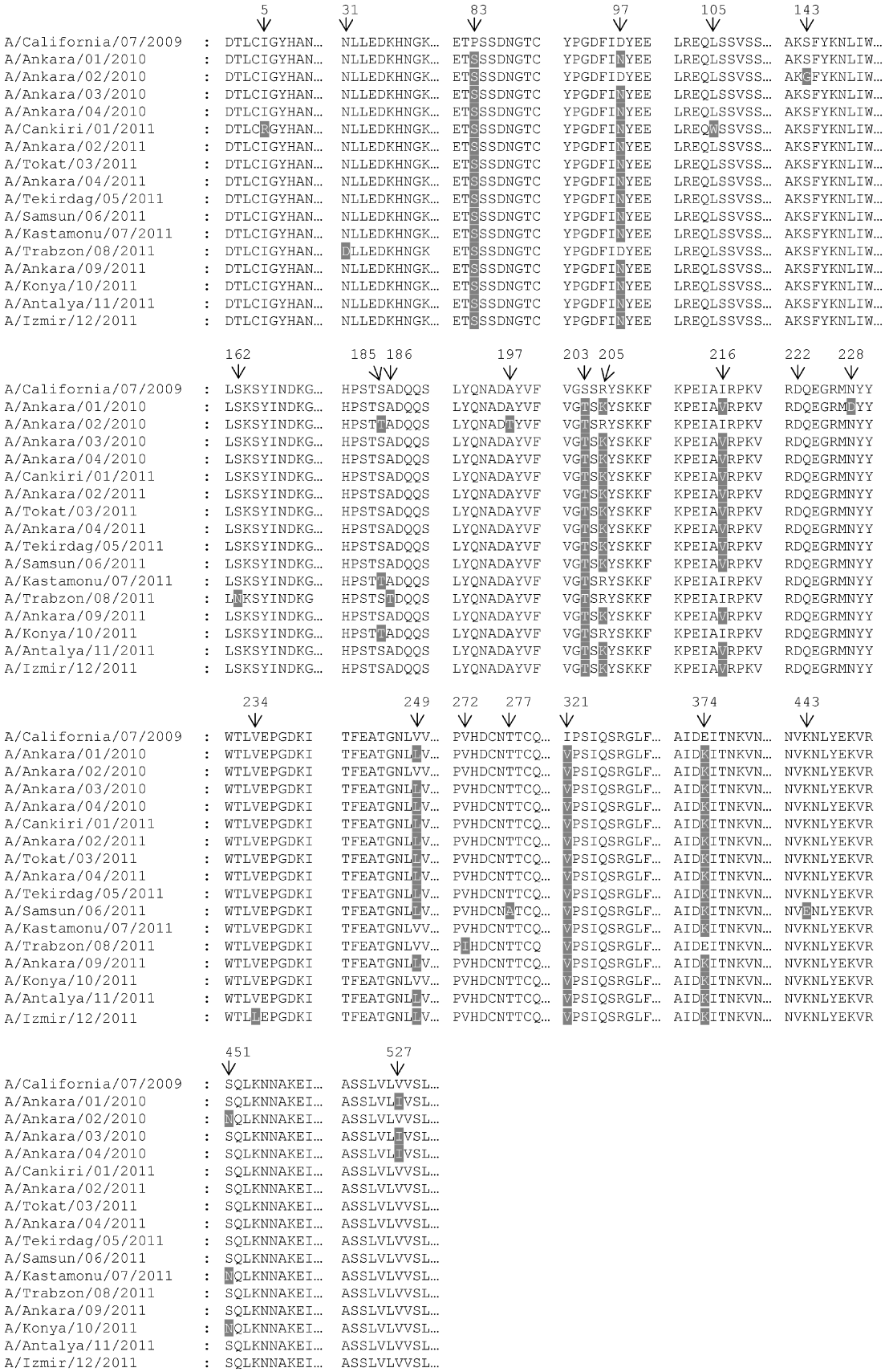

Fig. 2. Amino acid sequences of the HA1 domain and HA2 domain of the A/California/07/2009 (H1N1) strain and 16 clinical isolates. Only mutation carrying segments are given and the alterations are indicated as gray background.

The genetic group i, characterized by the N125D substitution located in the E antigenic region (22,23), was identified during the winter of 2010 in the Southern Hemisphere (24) and between March and December 2010 in Hong Kong (25) and the United Kingdom (UK) (26). However, this mutation was not detected in the viral isolates collected in Turkey during this study period. The genetic group ii, characterized by the D97N and S185T substitutions in the B antigenic site (22,23) and represented by the strains A/England/142/2010 (27), A/England/676/2010; or A/St. Petersburg/27/2011 (19,28), was previously identified in the Northern Hemisphere (25,26). The genetic group iii, characterized by the S143G, S185T, and A197T alterations, was represented by the strains A/Baden–Wurtemburg/14/2010 and A/Brussels/S0004/2011. In the Turkish viral isolates, specific mutations of genetic groups ii and iii were absent or occurred at a very low rate (10.3%) during the pandemic season, however, the mutation rates ranged from 6.3% to 87.5% during the post-pandemic period. This distribution pattern was similar to that reported in UK (26). The genetic group iv was characterized in the UK by the A134T and S183P substitutions (26). None of the Turkish isolates tested in this study included the mutations found in this genetic group. The genetic group v, represented by the strains A/Trieste/11/2011 and A/Astrakhan/1/2011, was characterized during the pandemic season (19,20), and it exclusively carried the D97N, R205K, I216V, and V249L substitutions, which were observed in 75%–87.5% of isolates collected in Turkey during the 2010–2011 season. The genetic group vi, represented by the strain A/Czech Republic/32/2011, has been recently defined on the basis of the presence of a combination of N31D, S162N, and A186T substitutions (10,19). While these substitutions were not detected during the 2009 season, they were observed in 6.25% of the isolates collected in Turkey during the 2010–2011 season.

The D222G/N/E mutations in the HA gene were reported among various pandemic influenza A(H1N1) viral strains during the 2009 pandemic season in several countris (6,17,26,29). It is postulated that the D222G alteration in the HA1 domain occurred more frequently in viruses isolated from patients suffering with severe diseases (30,31). In the present study, we investigated these mutations in 16 isolates from recovered patients and none had a substitution at position 222.

Recently, sequence analysis of the pandemic H1N1 viruses isolated in Hong Kong, Singapore, New Zealand and the UK revealed the emergence of the E374K substitution, which is located at the stalk region of HA2 and is an important site for membrane fusion (32–34). A report from Malaysia indicated that the E374K substitution was associated with a severe disease state (31). However, there was no significant association reported between the E374K substitution and the severity of clinical outcome in Taiwan (34). The E374K substitution was observed at a very high rate (93.8%) during the post-pandemic season in Turkey, but its association with disease status remains unclear.

Influenza A(H1N1)pdm09 strains are mainly susceptible to NA inhibitors, but resistant to adamantine (35). Infections with oseltamivir-resistant strains have been reported in both sporadic cases and a limited number of clusters during the post-pandemic period in several studies (36,37). Oseltamivir resistance-related mutations, such as NA-H275Y and NA-N295S, have been reported elswhere (38,39). To date, reports have indicated that only a limited number of influenza A(H1N1)pdm09 strains (<1%) showed resistance to neuraminidase inhibitors (40). Oseltamivir resistance-related mutations reported at positions H275Y and N295S, were not detected in influenza A(H1N1)pdm09 strains tested in the present study or in our previos study (6); therefore, it can be assumed that oseltamivir resistance was not an issue in Turkey during these periods.

In conclusion, although we observed an increase in the variety and frequency of mutations detected in the HA1 domain, these genetic drifts only resulted in a limited substitution rate (1.3%) within the A/California/07/2009 vaccine strain. Monitoring these mutations is likely important to predict future pandemics. On the basis of amino acid substitutions observed in the HA1 domain, the majority of Turkish isolates were classified as genetic group v; however, oseltamivir resistance-related mutations were not detected during the post-pandemic period in Turkey.

Conflict of interest None to declare.

REFERENCES

1. Childs, R.A., Palma, A.S., Wharton, S., et al. (2003): Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray. Nat. Biotechnol., 27, 797–799.
2. World Health Organization (2010): H1N1 now in the post-pandemic period. Online at <http://www.who.int/csr/disease/swineflu/en/>.
3. World Health Organization (2010): H1N1 in post-pandemic period. Director-General's opening statement at virtual press conference-10 August 2010. Online at <http://www.who.int/mediacentre/news/statements/2010/h1n1 _ vpc _ 20100810/en/index.html>.
4. World Health Organization (2010): WHO recommendations for the post-pandemic period. Online at <http://www.who.int/csr/disease/swineflu/notes/briefing_20100810/en/index.html>.
5. Ciblak, M.A., Albayrak, N., Odabas, Y., et al. (2009): Cases of influenza A(H1N1)v reported in Turkey, May–July 2009. Euro Surveill., 14(33):pii = 19313.
6. Ertek, M., Durmaz, R., Guldemir, D., et al. (2010): Epidemiological, demographic, and molecular characteristics of laboratory-confirmed pandemic influenza A (H1N1) virus infection in Turkey, May 15–November 30, 2009. Jpn. J. Infect. Dis., 63, 239–245.
7. Tamura, K., Dudley, J., Nei, M., et al. (2007): MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol., 24, 1596–1599.
8. Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucl. Acids Res., 22, 4673–4680.
9. Hall, T.A. (1999): BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser., 41, 95–98.
10. European Centre for Disease Prevention and Control (2011): Influenza virus characterization surveillance report. Summary for Europe. Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe; May–June 2011. Online at <http://www.ecdc.europa.eu/en/publications/Publications/1106_Influenza_virus_characterisation.pdf>.
11. World Health Organization (2009): Pandemic (H1N1) 2009-update 77. Online at <www.who.int/csr/don/2009_12_04/en/index.html>.

12. Cao, B., Li, Z.-W., Mao, Y., et al. (2009): Clinical features of the initial cases of 2009 pandemic influenza A (H1N1) virus infection in China. N. Engl. J. Med., 361, 2507–2517.
13. World Health Organization (2011): Summary review of the 2010–2011 northern hemisphere winter influenza season. Online at <http://www.who.int/influenza/surveillance_monitoring/2010_2011_GIP_surveillance_seasonal_review/en/>.
14. World Health Organization (2010): WHO–recommended viruses for influenza vaccines for use in the 2010–2011 Northern Hemisphere influenza season. 19 Feb 2010. Online at <http://ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f-77d4-4ad8-b6d6-bf0f23083f30&ID=732&MasterPage=1>.
15. Wiley, D.C. and Skehel, J.J. (1987): The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Ann. Rev. Biochem., 56, 365–394.
16. Matrosovich, M., Tuzikov, A., Bovin, N., et al. (2000): Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. J. Virol., 74, 8502–8512.
17. World Health Organization (2009): Pandemic (H1N1) 2009–update 78. Online at <http://www.who.int/csr/disease/swineflu/laboratory11_12_2009/en/index.html>.
18. Ledesma, J., Pozo, F., Reina, G., et al. (2012): Spanish Influenza Surveillance System (SISS). Genetic diversity of influenza A(H1N1)2009 virus circulating during the season 2010–2011 in Spain. J. Clin. Virol., 53, 16–21.
19. European Centre for Disease Prevention and Control (2011): Influenza virus characterization surveillance report. Summary for Europe. Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe; July 2011. Online at <http://www.ecdc.europa.eu/en/publications/Publications/1108_SUR_Influenza_virus_characterisation_July.pdf>.
20. European Centre for Disease Prevention and Control (2011): Influenza virus characterization surveillance report. Summary for Europe. Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe; March 2011. Online at <http://www.ecdc.europa.eu/en/publications/Publications/1304_Influenza_virus_characterisation_2011_April.pdf>.
21. European Centre for Disease Prevention and Control (2010): Influenza virus characterization surveillance report. Summary for Europe. Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe; December 2010. Online at <http://www.ecdc.europa.eu/en/publications/Publications/1012_Influenza_virus_characterisation_2010_December.pdf>.
22. Caton, A.-J., Brownlee, G.-G., Yewdell, J.-W., et al. (1982): The Antigenic structure of influenza virus A/PR/8/34 hemagglutinin (H1 subtype). Cell, 31, 417–427.
23. Deem, M.-W. and Pan, K. (2009): The epitope regions of H1-subtype influenza A, with application to vaccine efficacy. Protein Eng. Des. Sel., 22, 543–546.
24. Barr, I.-G., Cui, L., Komadina, N., et al. (2010): A new pandemic influenza A(H1N1) genetic variant predominated in the winter 2010 influenza season in Australia, New Zealand and Singapore. Euro Surveill., 15(42): pii=19692.
25. Mak, G.-C., Leung, C.-K., Cheng, K.-C., et al. (2011): Evolution of haemagglutinin gene of the influenza (AH1N1)2009 virus isolated In Hong Kong, 2009–2011. Euro Surveill., 16(9): pii=19807.
26. Ellis, J., Galiano, M., Pebody, R., et al. (2011): Virological analysis of fatal influenza cases in the United Kingdom during the early wave of influenza in winter 2010/11. Euro Surveill., 16(1): pii=19760.
27. World Health Organization (2011): EuroFlu Weekly Electronic Bulletin, Week 4: 24.01.11–30.01.11, 04 February 2011. Number 387. Online at <http://www.euroflu.org/bulletins pdf/eiss 2011 4 bulletin.pdf>.
28. European Centre for Disease Prevention and Control (2012): Influenza virus characterization surveillance report. Summary for Europe. Community Network of Reference Laboratories (CNRL) for Human Influenza in Europe; November 2012. Online at <http://www.ecdc.europa.eu/en/publications/Publications/influenza-virus-characterisation-november–2012l.pdf>.
29. Kilander, A., Rykkvin, R., Dudman, S.-G., et al. (2010): Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome. Norway 2009–2010. Euro Surveill., 15(9): pii=19498.
30. World Health Organization (2010): Preliminary review of D222G amino acid substitution in the haemagglutinin of pandemic influenza A (H1N1) 2009 viruses. Wkly. Epidemiol. Rec., 85, 21–28.
31. Balraj, P., Sidek, H., Suppiah, J., et al. (2011): Molecular analysis of 2009 pandemic influenza A(H1N1) in Malaysia associated with mild and severe infections. Malays. J. Pathol., 33, 7–12.
32. de la Rosa-Zamboni, D., Vázquez-Pérez, J.A., Avila-Ríos, S., et al. (2012): Molecular Characterization of the Predominant Influenza A(H1N1)pdm09 Virus in Mexico, December 2011-February 2012. PLoS One, 7, e50116.
33. Wang, W., Suguitan, A.L., Jr., Zengel, J., et al. (2012): Generation of recombinant pandemic H1N1 influenza virus with the HA cleavable by bromelain and identification of the residues influencing HA bromelain cleavage. Vaccine, 30, 872–878.
34. Kao, C.L., Chan, T.C., Tsai, C.H., et al. (2012): Emerged HA and NA mutants of the pandemic influenza H1N1 viruses with increasing epidemiological significance in Taipei and Kaohsiung, Taiwan, 2009–10. PLoS One, 7, e31162.
35. Chen, H., Cheung, C.L., Tai, H., et al. (2009): Oseltamivir resistant influenza A pandemic (H1N1) 2009 virus, Hong Kong, China. Emerg. Infect. Dis., 15, 1970–1972.
36. World Health Organization (2009): Oseltamivir-resistant pandemic (H1N1) 2009 influenza virus, October 2009. Wkly. Epidemiol. Rec., 84, 453–458.
37. World Health Organization (2010): Update on oseltamivir-resistant pandemic A (H1N1) 2009 influenza virus, January 2010. Wkly. Epidemiol. Rec., 85, 37–39.
38. Morlighem, J.É., Aoki, S., Kishima, M., et al. (2011): Mutation analysis of 2009 pandemic influenza A(H1N1) viruses collected in Japan during the peak phase of the pandemic. PLoS One, 6, e18956.
39. Esposito, S., Molteni, C.G., Daleno, C., et al. (2010): Clinical importance and impact on the households of oseltamivir-resistant seasonal A/H1N1 influenza virus in healthy children in Italy. Virol. J., 7, 202.
40. Karthick, V., Shanthi, V., Rajasekaran, R., et al. (2013): In silico analysis of drug-resistant mutant of neuraminidase (N294S) against oseltamivir. Protoplasma, 250, 197–207.