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LETTER TO THE EDITOR

Oseltamivir Resistance Depends on the Amino Acid at the 273rd Position of Neuraminidase of Influenza A Virus (H1N1) Circulating in the Human Population

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Abstract—The structure of neuraminidase of influenza A virus (H1N1) spreading in the human population was analyzed. The results indicate a significant correlation between the virus sensitivity to oseltamivir and the nature of amino acids at positions 274 and 273 of neuraminidase. Phenylalanine at position 273 indicates a higher propensity of the influenza virus to mutation H274Y, which leads to resistant strains. It is suggested that the mutation at position 273 may be one of the characteristics that allow the type-A influenza virus to be ascribed to a pandemic or a seasonal type.

Keywords: influenza A virus, neuraminidase, diagnostics, antiviral drugs, oseltamivir, resistance

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Two types of antiviral drugs are currently used: blockers of M2 protein and inhibitors of the influenza-A virus. The influenza virus circulating in the human population is highly resistant to antiviral drugs of the first group. Therefore, special attention is paid to the preparations of the second group, one representative of which is oseltamivir [1]. However, strains of the influenza-A virus resistant to oseltamivir has begun to appear in recent years. Studies have shown that resistance is caused by the amino acid substitution H274Y in neuraminidase (according to N2 nomenclature) [2].

Strains resistant to oseltamivir belong mainly to seasonal influenza. At the same time, the pandemic version of H1N1 influenza spread around the world in 2009 was generally sensitive to oseltamivir, but resistant isolates among them also contained the H274Y mutation [3]. According to [4], D79G, S247G, and S247N mutations in neuraminidase are also typical for oseltamivir-resistant strains of the influenza virus. For seasonal flu, these variations are insignificant. Neuraminidase of seasonal flu contained mainly 79D and 247S mutations typical for wild strains (only two isolates had 247N mutation), while 79S and 247N mutations were characteristic for the pandemic flu [4]. These relationships in the neuraminidase structure were the basis for the creation of the diagnostic systems [5–8]. Therefore, the search for relevant mutations in the influenza-A virus genome is of undoubted interest.

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In this paper, we present data on the neuraminidase mutation at position 273, which is adjacent to the amino acid residue that determines the virus sensitivity to oseltamivir.

Changes in the primary structure of neuraminidase in close proximity to the binding site with oseltamivir should obviously have some impact on the degree of their interaction. In this work, we analyzed influenza A virus H1N1 spreading in the human population. Protein and nucleotide sequences were taken from the NCBI database for the influenza virus (http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html). We selected 7317 isolates of the influenza-A virus for the period from 1918 to 2010 (table). It should be noted that we used the entire data set without excluding isolates identical in the neuraminidase structure. For example, all data for the A/Puerto Rico/8/1934 isolate sequenced by various groups that have different access numbers are included in the review, so this isolate is presented in the table several times.

In general, amino acids were shown to change at positions 273 and 274 of neuroaminidase in the region of the binding site with oseltamivir. Adjacent positions are highly conservative for most isolates: the 275Y, 276E, 277E motif is presented in 7239 isolates. The remaining 78 isolates either almost entirely represent incomplete sequences or the nature of one of the amino acids is not uniquely determined. Only five isolates have a mutation in one of the positions. Similarly, motif 270A, 271P, 272N is typical for 7208 isolates, and the remaining 109 isolates are, in most cases,

Representation of isolates of influenza A virus of H1N1 subtype circulating in the human population

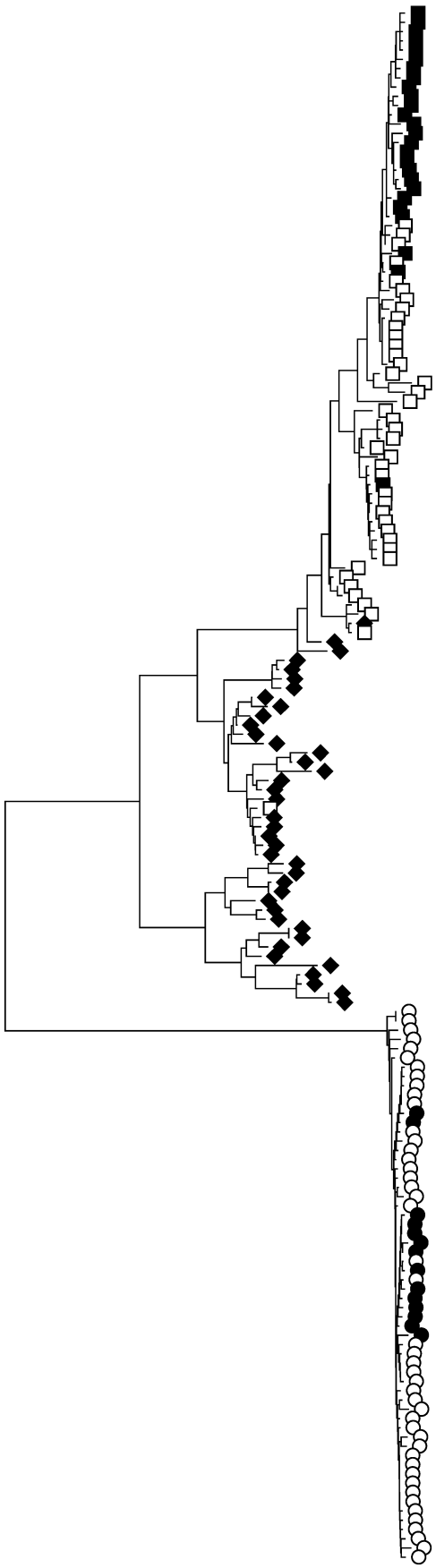
Year	273/274					
	FH	FY	SH	YH	YY	Total
1915–1990	1	0	131	7	–	139
1991	–	–	–	3	–	3
1992	–	–	–	1	–	1
1993	–	–	–	–	–	–
1994	–	–	–	1	–	1
1995	1	–	1	22	–	24
1996	–	–	13	15	–	28
1997	–	–	4	3	–	7
1998	–	–	2	–	–	2
1999	8	–	–	–	–	8
2000	69	–	–	13	–	82
2001	121	–	1	6	–	128
2002	42	–	1	–	–	43
2003	26	–	–	–	–	26
2004	16	–	–	–	–	16
2005	75	–	–	2	–	77
2006	213	7	–	–	–	220
2007	510	58	2	–	–	570
2008	455	318	3	1	–	777
2009	16	509	–	4288	65	4878
2010	–	–	–	136	8	144
Total	1553	892	158	4497	73	7173

* Isolates bearing 273S274Y mutations were not found in NCBI database.

incomplete amino acid sequences, where amino acids at positions 273 and 274 are not determined. Only a small number of isolates have a different structure of tripeptide 270–272. Tripeptides APS, ASN, and SPN are presented 9, 3, and 2 times, respectively; the remaining tripeptides VPN, APD, and API are presented once each.

Positions 273 and 274 are more variable. Position 273 in isolates is presented by three amino acids: serine, phenylalanine, and tyrosine. There are also four isolates containing histidine in this position (273H). Position 274 for all isolates is presented by two variants, H and Y, which determine the sensitivity to oseltamivir.

It is noteworthy that according to data reported before 1991, serine was almost exclusively present at position 273 of neuraminidase (273S). The literature describes only one isolate containing phenylalanine at this position (273F) and seven isolates containing tyrosine (273Y) including the isolate of the pandemic A/Brevig Mission/1/1918 flu. Significant numbers of isolates containing phenylalanine or tyrosine at position 273 have been revealed since 1995. Until 2009, the 273F variant was mostly determined. Only in 2009,



Phylogenetic tree for nucleotide sequences of the neuraminidase gene of the influenza-A virus (H1N1) circulating in the human population and bearing mutations at positions 273 and 274 of neuraminidase: 273F274H (white squares), 273F274Y (black squares), 273S274H (black diamonds), 273Y274H (white circles), 273Y274Y (black circles). Analysis was carried out using the Mega version 4.0 program of Neighbor-Joining cluster method.

a large number of 273Y isolates appeared. An interesting fact is that the resistance to oseltamivir appears to a greater extent in 273F isolates. In 2006, the 273F274H variant exceeded the variant 273F274Y by a factor of almost 30, while, in 2009, the opposite pattern was observed; the variant 273F274Y predominated over the variant 273F274H by the same factor. At the same time, the resistant variant (273Y274Y pair) appeared only in 2009, although the 273Y274H amino acid pair has been observed in sufficient amounts since 1995. The presence of phenylalanine at position 273 probably favors the selection of isolates bearing the 274Y mutation, despite the fact that the structures of amino acids at position 273 differ insignificantly; phenylalanine differs from tyrosine only by the hydroxyl group at position 4 of the benzene ring. For all that, 273F strains correspond to seasonal H1N1 influenza, while tyrosine at position 273 is typical for isolates of 2009 pandemic influenza A. This is confirmed by data [3], which describes the increased resistance of seasonal influenza A compared with pandemic flu.

It is interesting also to note that the Brevig Mission/1/1918 strain of the influenza-A virus, which was the cause of the 1918 flu pandemic ("Spanish flu"), has a pair of amino acids, 273Y274H, that allows one to assign this strain to a pandemic-type (273Y mutation) sensitive, however, to oseltamivir (274H mutation).

The figure presents a phylogenetic tree on the basis of nucleotide sequences of isolates bearing S, F, and Y mutations at position 273. The sampling of RNA sequences was fulfilled so that all geographic variants were presented most completely. Analyzed sequences are uniquely divided into S, F, and Y Clydes. This indicates that variations of amino acid at position 273 correspond to changes in the whole structure of neuraminidase. As expected, S Clyde is the most heterogeneous; F Clyde is heterogeneous to a lesser extent and genetically more similar to S Clyde, than to Y Clyde. It should be emphasized that the subdividing into clydes is mainly determined by the nature of the amino acid at position 273, rather than at position 274. Thus, our analysis indicates a significant association between the sensitivity to oseltamivir and the nature of the amino acid not only at position 274, but also at position 273 of neuraminidase. In addition, we can assume that it is a mutation at position 273, which may be one of the characteristics for classification of the influenza A virus as the pandemic or seasonal type.

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REFERENCES

1. Weinstock, D.M., Gianna Zuccotti G, *J. Amer. Med. Assoc.*, 2009, vol. 301, pp. 1066–1069.
2. Ives, J.A., Carr, J.A., Mendel, D.B., Tai, C.Y., Lambkin, R., Kelly, L., Oxford, J.S., Hayden, F.G., and Roberts, N.A., *Antiviral Res.*, 2002, vol. 55, pp. 307–317.
3. Hurt, A.C., Holien, J.K., Parker, M.W., and Bar, I.G., *Drugs,* 2009, vol. 69, pp. 2523–2531.
4. Sheu, T., Deyde, V., Okomo-Adhiambo, M., Garten, R., Xu, X., Bright, R.A., Wallis, T.R., Cox, N., Klimov, A., and Gubareva, L.V., *Antimicrob. Agents Chemother.*, 2008, vol. 52, pp. 3284–3292.
5. Bolotin, S., Robertson, A.V., Eshaghi, A., De Lima, C., Lombos, E., Chong-King, E., Burton, L., Mazzulli, T., and Drews, S.J., *J. Virol. Methods*, 2009, vol. 158, pp. 190–194.
6. Carr, M.J., Sayre, N., Duffy, M., Connell, J., and Hall, W.W., *J. Virol. Methods,* 2008, vol. 153, pp. 257–262.
7. Hata, M., Tanaka, S., Yasui, Y., Fujiwara, N., Kobayashi, S., and Minagawa, H., *J. Clin. Microbiol.*, 2010, vol. 48, pp. 1983–1984.
8. Belyakova, N.V., Al'khovskii, S.V., Shevchenko, E.S., Prilipov, A.G., Abramov, D.D., Kolobukhina, L.V., Merkulova, L.N., Trushakova, S.V., and Burtseva, E.I., *Vopr. Virusol.*, 2010, no. 5, pp. 10–13.