A community cluster of influenza A(H1N1)pdm09 virus exhibiting cross-resistance to oseltamivir and peramivir in Japan, November to December 2013

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Six influenza A(H1N1)pdm09 viruses were detected in Sapporo, Japan, between November and December 2013. All six viruses possessed an H275Y substitution in the neuraminidase protein, which confers cross-resistance to oseltamivir and peramivir. No epidemiological link among the six cases could be identified; none of them had received neuraminidase inhibitors before specimen collection. The haemagglutinin and neuraminidase genes of the six viruses were closely related to one another, suggesting clonal spread of a single resistant virus.

Detection of mutant H275Y influenza A(H1N1)pdm09 viruses

Between September and December 2013, 76 influenza A(H1N1)pdm09 viruses were detected in 20 local public health institutes in Japan and then screened by allelic discrimination [1] and/or neuraminidase (NA) gene sequencing to detect an H275Y substitution, which confers resistance to oseltamivir and peramivir [2] (Figure 1). This is part of our nationwide monitoring for antiviral-resistant influenza viruses in cooperation with 74 local public health institutes [2]: such surveillance is important for public health planning and clinical recommendations for antiviral use. We found that seven of the 76 influenza A(H1N1)pdm09 viruses possessed the H275Y substitution. Six of the seven H275Y mutant viruses were detected in Sapporo, the capital city of Hokkaido, the second-largest island in Japan. The seventh case was detected from another part of the country. In Sapporo, six influenza A(H1N1)pdm09 viruses were detected during weeks 46–50, and all six viruses possessed the H275Y substitution. Elsewhere in Hokkaido, nine influenza viruses were detected: all were influenza A(H3N2) viruses. In this article, we focus on the analysis of the six H275Y mutant viruses detected in Sapporo.

Isolate details from the six cases in Sapporo are shown in Table 1. Clinical specimens of the patients

were collected in three paediatric clinics (serving outpatients only) and two general hospitals (serving outpatients and inpatients). Five of the six patients were male and four were children (aged up to 10 years). Five male patients showed mild symptoms and received only outpatient care, but a woman in her late 30s without underlying disease was hospitalised for severe pneumonia. She was admitted to an intensive-care unit because of acute respiratory distress syndrome and is currently in critical condition. All six cases occurred sporadically and no epidemiological link among them could be identified. None of them had received NA inhibitors before specimen collection. The nucleotide sequences of the haemagglutinin (HA) and NA genes of the six viruses were closely related to one another (Figure 2). These results suggest the clonal spread of a single H275Y mutant virus in Sapporo.

Antiviral susceptibility of H275Y mutant viruses

We analysed the susceptibility of five of the six H275Y mutant viruses detected in Sapporo to four NA inhibitors approved in Japan: oseltamivir, peramivir, zanamivir and laninamivir (Table 2); the sixth virus could not be cultured. A/Perth/261/2009 and A/Perth/265/2009 [3] were used as reference H275Y mutant and 275H wild-type A(H1N1)pdm09 viruses, respectively. Oseltamivir carboxylate, peramivir and zanamivir were purchased from Sequoia Research Products (Pangbourne, United Kingdom) and laninamivir was kindly provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). The susceptibility of these viruses to NA inhibitors was determined by fluorescent enzyme inhibition assay with the NA-Fluor Influenza Neuraminidase Assay Kit (Applied Biosystems, California, United States). Results were expressed as the drug concentrations required to inhibit NA activity by 50% (IC₅₀). The IC₅₀ values were calculated using MikroWin 2000 software (Mikrotek Laborsysteme GmbH, Overath, Germany). To interpret the NA inhibitor susceptibility, the World Health

FIGURE 1

Detection of influenza A(H1N1)pdm09 viruses with H275Y substitution, September–December (weeks 36–51)^a, Japan (n=76)

Weekly reports of influenza A(H1N1)pdm09 virus isolation/detection by local public health institutes under the National Epidemiological Surveillance of Infectious Diseases.

^a Week 36 started on 2 September 2013.

Organization criteria based on the fold change of IC_{50} values compared with reference IC_{50} values were applied [4]. For influenza A viruses, normal (<10-fold increase), reduced (10–100-fold increase) or highly reduced (>100-fold increase) inhibition were defined. All five H275Y mutant viruses showed more than 600- and 170-fold increased IC_{50} values to oseltamivir and peramivir, respectively, compared with the 275H reference virus. However, the IC_{50} values of the H275Y mutants to zanamivir and laninamivir were comparable to those of the 275H reference virus. These results indicate that the five H275Y mutant viruses tested exhibit highly reduced inhibition by oseltamivir and peramivir, but remain fully susceptible to zanamivir and laninamivir.

In the United States, the Centers for Disease Control and Prevention reported that 10 (1.3%) of 768 influenza A(H1N1)pdm09 viruses were resistant to oseltamivir in the 2013/14 season, as of week 51 2013 [5]. Five of the 10 resistant viruses were detected in Louisiana and Mississippi (Table 3), suggesting a cluster of resistant viruses. The largest cluster of influenza A(H1N1)pdm09 viruses with the H275Y substitution occurred in Newcastle, Australia, in 2011: 29 (15%) of 191 influenza A(H1N1)pdm09 viruses possessed the H275Y substitution [6].

For comparison with the six H275Y mutant viruses detected in Sapporo, HA and NA gene sequences of the H275Y mutant viruses isolated in the United States and Australia were downloaded from the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID) (Table 3). The HA and NA genes of the

TABLE 1

Influenza A(H1N1)pdm09 viruses with H275Y substitution detected in Sapporo, Japan, November–December 2013 (n=6)

GISAID isolate ID	Isolate name	Collection date
EPI_ISL_152910	A/Sapporo/107/2013	2013-11-15
EPI_ISL_152927	A/Sapporo/114/2013	2013-11-24
EPI_ISL_152931	A/Sapporo/TH1/2013	2013-11-25
EPI_ISL_152928	A/Sapporo/116/2013	2013-11-26
EPI_ISL_152929	A/Sapporo/119/2013	2013-12-07
EPI_ISL_152930	A/Sapporo/120/2013	2013-12-09

GISAID: Global Initiative on Sharing All Influenza Data.

FIGURE 2

Phylogenetic analysis of the neuraminidase gene of the six H275Y mutant influenza A(H1N1)pdm09 viruses isolated in Sapporo, Japan, and the United States in 2013 and in Australia in 2011

Multiple alignment was constructed using the CLUSTAL W algorithm. The tree was constructed using the neighbor-joining method with bootstrap analyses of 1,000 replicates in CLUSTAL W. The H275Y mutant viruses are shown in red. Amino acid substitutions relative to the A/California/07/2009 virus are shown on the left of the nodes. The gene sequences of the H275Y mutant viruses isolated in the United States and Australia were downloaded from the EpiFlu database of the Global Initiative on Sharing All Influenza Data (GISAID).

TABLE 2

Susceptibility of five influenza A(H1N1)pdm09 viruses with H275Y substitution to neuraminidase inhibitors, detected in Sapporo, Japan, November–December 2013

Isolate name	NA substitution	IC ₅₀ (nM)			
		Oseltamivir	Peramivir	Zanamivir	Laninamivir
A/SAPPORO/107/2013	H275Y	240.60	35.28	0.50	0.81
A/SAPPORO/114/2013	H275Y	193.05	22.86	0.50	0.63
A/SAPPORO/116/2013	H275Y	257.10	23.97	0.43	0.53
A/SAPPORO/119/2013	H275Y	189.25	23.19	0.43	0.58
A/SAPPORO/120/2013	H275Y	192.44	22.35	0.45	0.54
Reference isolates ^a					
A/PERTH/261/2009	H275Y	257.88	34.30	0.30	0.35
A/PERTH/265/2009	275H	0.31	0.13	0.30	0.29

IC₅₀: drug concentrations required to inhibit NA activity by 50%; NA: neuraminidase.

^a A/PERTH/261/2009 is the H275Y reference virus. A/PERTH/265/2009 is the 275H wild-type influenza A(H1N1)pdm09 reference virus.

TABLE 3

Accession numbers of the H275Y mutant influenza A(H1N1)pdm09 viruses detected in the United States in 2013 and in Australia in 2011

GISAID Isolate ID	Isolate name	Collection date	Originating laboratory	Submitting laboratory
EPI_ISL_150042	A/Louisiana/07/2013	2013-10-07	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_150043	A/Louisiana/08/2013	2013-10-09	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_150298	A/Louisiana/10/2013	2013-10-21	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_151839	A/Mississippi/11/2013	2013-11-04	Mississippi Public Health Laboratory	Centers for Disease Control and Prevention
EPI_ISL_151838	A/Louisiana/13/2013	2013-11-11	Louisiana Department of Health and Hospitals	Centers for Disease Control and Prevention
EPI_ISL_95596	A/Newcastle/2/2011	2011-05-31	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95595	A/Newcastle/17/2011	2011-06-20	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95597	A/Newcastle/37/2011	2011-06-23	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101526	A/Newcastle/125/2011	2011-06-30	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95598	A/Newcastle/53/2011	2011-06-30	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95600	A/Newcastle/82/2011	2011-07-01	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95599	A/Newcastle/62/2011	2011-07-01	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101511	A/Newcastle/86/2011	2011-07-02	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101527	A/Newcastle/129/2011	2011-07-03	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95602	A/Newcastle/89/2011	2011-07-04	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95601	A/Newcastle/85/2011	2011-07-04	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101525	A/Newcastle/102/2011	2011-07-05	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95593	A/Newcastle/132/2011	2011-07-10	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_95594	A/Newcastle/151/2011	2011-07-11	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101540	A/Newcastle/168/2011	2011-07-12	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101564	A/Newcastle/179/2011	2011-07-15	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza
EPI_ISL_101565	A/Newcastle/212/2011	2011-08-02	John Hunter Hospital, Virology Unit, Clinical Microbiology	WHO Collaborating Centre for Reference and Research on Influenza

GISAID: Global Initiative on Sharing All Influenza Data.

H275Y mutant viruses in Sapporo, the United States and Australia were distinct from one another (Table 4).

Discussion

During the 2007/08 influenza season, an oseltamivir-resistant former seasonal influenza A(H1N1) virus emerged in Europe and became the majority of A(H1N1) viruses within a year [7]. This oseltamivir-resistant

A(H1N1) virus possessed the H275Y substitution; some additional amino acid substitutions were also reported for the virus that could make the mutant virus biologically stable [8,9]. Since the H275Y substitution would destabilise the mutant virus, the oseltamivir-resistant A(H1N1) virus probably acquired the capacity for efficient human-to-human transmission through these stabilising substitutions.

TABLE 4

Characteristic amino acids of H275Y mutant influenza A(H1N1)pdm09 viruses isolated in Sapporo, Japan, in 2013 (n=6) and the United States in 2013 and in Australia in 2011

HA gene of isolated viruses	Amino acid position										
	15	97	112	143	163	197	222	223	256	283	499
Reference virus A/PERTH/265/2009	T	D	E	S	K	A	D	Q	A	K	E
Sapporo, Japan, 2013	S	N	E	S	Q	A	D	Q	T	D	K
United States, 2013	T	N	E	S	Q	A	D	Q	T	E	K
Australia, 2011	T	D	K	G	K	T	D	Q	A	K	E

NA gene of isolated viruses	Amino acid position											
	34	62	82	106	200	241	275	321	369	386	397	432
Reference virus A/PERTH/265/2009	I	V	S	I	N	V	H	I	N	N	N	K
Sapporo, Japan, 2013	V	V	S	V	S	I	Y	V	K	K	N	E
United States, 2013	V	V	P	V	S	I	Y	V	K	N	K	E
Australia, 2011	I	I	S	I	N	I	Y	I	K	S	N	K

HA: haemagglutinin; NA: neuraminidase.

In the case of H275Y mutants of influenza A(H1N1)pdm09 viruses, three substitutions (V241I, N369K and N386S) in the NA protein may offset the destabilising effect of the H275Y mutation [6]. The influenza A(H1N1)pdm09 virus that appeared in 2009 as a pandemic virus had none of these stabilising substitutions, whereas the A(H1N1)pdm09 viruses that have been circulating since 2011 to date have acquired two of the three substitutions. The H275Y mutant viruses detected in a community cluster in 2011 in Newcastle, Australia, contained these three substitutions [6]. Furthermore, the same substitutions were detected in H275Y mutant viruses isolated from Dutch travellers returning from Spain in 2012 [10]. In our study, all H275Y mutant viruses detected in Sapporo possessed V241I, N369K and N386K substitutions (Table 4); H275Y mutant viruses found in the United States possessed only V241I and N369K substitutions (Table 4). Before the 2013/14 influenza season, we had not detected any H275Y mutant viruses with V241I, N369K and N386K substitutions in Japan. The effect of the N386K substitution – at the same position but with an amino acid residue that differs from N386S previously reported for H275Y mutant viruses – remains to be clarified.

D222G and Q223R substitutions in the HA protein of influenza A(H1N1)pdm09 viruses are known to cause a switch in receptor-binding preference from human-type α -2,6 to avian-type α -2,3 sialic acid [11–13]. All H275Y mutant viruses detected in Sapporo and the United States in the 2013/14 season did not contain these substitutions that would be associated with increased pathogenicity (Table 4). The reason why the patient in

her late 30s in Sapporo developed severe pneumonia has yet to be studied.

It has been shown that oseltamivir-resistant influenza A(H1N1) virus infection reduced the effectiveness of oseltamivir and this tendency was more apparent in children 0 to 6 years old [14–16]. Among patients from whom oseltamivir- and peramivir-resistant A(H1N1)pdm09 viruses have been detected in Japan, the percentage with no known exposure to NA inhibitors has increased significantly, from 16% during the pandemic period to 44% during the post-pandemic period [2]. These observations may suggest that human-to-human transmission with H275Y mutant viruses has increased gradually in the post-pandemic period. Consequently, surveillance of antiviral-resistant influenza viruses should be continued and strengthened, particularly for the choice of antiviral drugs.

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Conflict of interest

None declared.

Authors' contributions

Designed the analyses: ET, AO, HN, TO, MT. Analysed and interpreted data: ET, ME, RI, MM, AO, HN, TO, MT. Drafted the article: ET. Revised the article: TO, MT.

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