Table 2. Primer pairs and probes used in this study.

Application	Host	Target	Orientation	Sequence (5′ to 3′)
TaqMan	Homo sapiens	18S	Sense	GTAACCCGTTGAACCCCATT
			Anti-sense	CCATCCAATCGGTAGTAGCG
			Probe	GTGCGTTGATTAAGTCCCTGCCCTTTGTA
	Homo sapiens	IRF7	Sense	CAGAGTCTTCTTCCAAGAGCTGG
			Anti-sense	TGCGTGCCCTCTAGGTGC
			Probe	CCAGGGTTCCAGCTTCACCAGGACC
	Homo sapiens	IRF3	Sense	GCTCGTGATGGTCAAGGTTGTG
			Anti-sense	GAGTGGGTGGCTGTTGGAAATG
			Probe	CCACAGTATTCTCCAGGGAGGAGGCACC
	Canis lupus familiaris	18S	Sense	GCCCGAAGCGTTTACTTTGAAA
			Anti-sense	ATGGCCTCAGTTCCGAAAACC
			Probe	CGAGCCGCCTGGATACCGCAGC
		IRF7	Sense	TCAGCACGTTCTTCCGAGAGAT
			Anti-sense	TAGATGGTGTAGTATGGGGAGCC
			Probe	CCGGCGAGCCCGGAACTCGG
		IRF3	Sense	ACCACGCTACACCCTCTGG
			Anti-sense	CCCTCTAACCGTGCCATTTCC
			Probe	CGTGGGAACAACCTTGAGCATCACCAGC
	Influenza A virus (PR8)	M	Sense	TGCACTTTGACATTGTGGATTCTTG
			Anti-sense	CCCTCATAGACTTTGGCACTCC
			Probe	GCCGTAGAAGGCCCTCCTTTCAGTCCG
	Influenza A virus (NR1)	M	Sense	CACCTGATATTGTGGATTACTGATCG
			Anti-sense	CACTCTGCTGTTCCTGTTGATATTC
			Probe	CCTCATGGACTCAGGCACTCCTTCCG
	Homo sapiens	IFNA	Sense	GGTCACGCTTTCATGAATTCTGT
			Anti-sense	GTGTAAAGGTGCACATGACGTTA
			Probe	TCACCCCTGCTATAACTATGACCATGCTGA
	Homo sapiens	IFNB	Sense	GCTACAACTTGCTTGGATTCCTAC
			Anti-sense	TCCTGTCCTTGAGGCAGTATTC
			Probe	AGCCTCCCATTCAATTGCCACAGGAGC
	Canis lupus familiaris	IFNA	Sense	GCTCTTGTGACCACTACACCA
			Anti-sense	AAGACCTTCTGGGTCATCACG
			Probe	CGCCTCCTGGAGCCGCTGGC
	Canis lupus familiaris	IFNB	Sense	GGATGGAATGAGACCACTGTCG
			Anti-sense	ACGTCCTCCAGGATTATCTCCA
			Probe	GTTCCTTCTGCCAGTGGAGCTTCACAA
PCR	Canis lupus familiaris	IRF7	Sense	ATGGAGCCATACCAGCCACG
			Anti-sense	GGCTCTACCTCCATGAGGAAGT
SYBR	Canis lupus familiaris	IRF7	Sense	TCAGCACGTTCTTCCGAGAGAT
			Anti-sense	TAGATGGTGTAGTATGGGGAGCC
	Canis lupus familiaris	IRF3	Sense	CAGTACCTCGGATACCCAGGAAG
			Anti-sense	GAATGGGGTCAAGACCATGTCAC
	Canis lupus familiaris	MyD88	Sense	GCTTGATGATCCCTCAGGGCAAA
			Anti-sense	CGCTGGGGCAGTAGCAGAT
	Canis lupus familiaris	DDX58	Sense	CAGAATGATCCAAACCAGAGGCA
			Anti-sense	GTTAGAAGGAAGCACTTGCTACC

doi:10.1371/journal.pone.0059892.t002

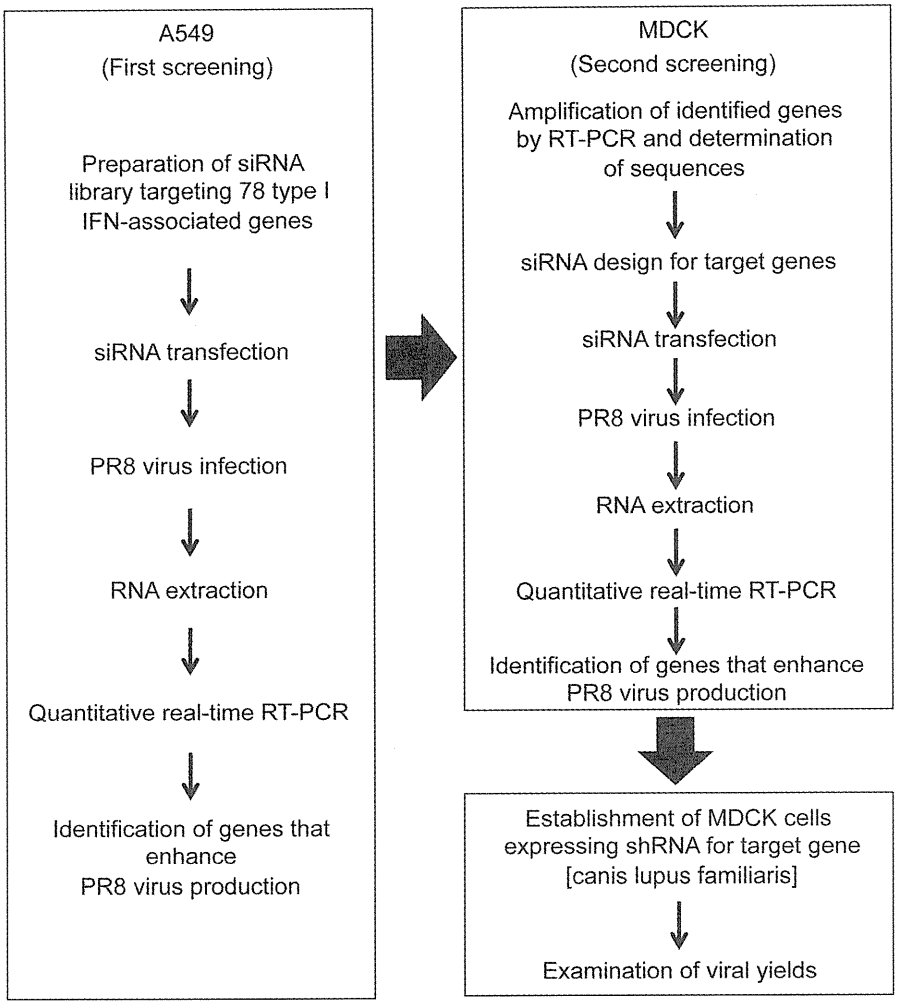

Figure 1. Overview of the study design for the establishment of modified MDCK cells.
doi:10.1371/journal.pone.0059892.g001

RIG-I/IPS-1 signaling pathway enhance the production of PR8 virus in A549 cells when knocked down by siRNA. Regarding the molecules upstream of IRF7, knockdown of DDX58 (also known as RIG-I) or MyD88 did not affect the viral production (Figs. 2A and S1). It is interesting to note that knockdown of IRF3 did not affect the production of PR8 virus. However, knockdown of NOD2, MAVS (also known as IPS-1, VISA or Cardif) or IRF7 significantly enhanced the production of PR8 virus (Figs. 2A and S1). Of these highly potential candidates, IRF7 was the most promising molecule because knockdown of IRF7 showed the greatest enhancement of PR8 virus production in A549 cells (data not shown). Thus, we examined whether the same thing holds true for MDCK cells.

Knockdown of IRF7 Enhances the Production of PR8 Virus in MDCK Cells

To identify a specific gene(s) in canine MDCK cells, we performed second screening of a set of 9 genes involved in RIG-I/IPS-1 signaling pathway for enhancement of virus production in MDCK cells by siRNA-mediated knockdown. Since the sequences for IFN-related genes of canine MDCK cells are not available, we first performed RT-PCR of mRNA for target genes using specific primers designed based on GenBank or Ensemble sequence database. Then, we determined the entire or partial coding sequences of each gene and designed siRNAs for each target gene in MDCK cells. A number of genes showed some sequence variations from the reference sequence (data not shown). Regarding IRF7, canine IRF7 (Transcript ID: ENSCAFT00000010569 in Ensembl data base) was identified as the most similar gene to human IRF7 by BLAST. The RT-PCR product of IRF7 gene in MDCK cells showed a single band with the amplicon size (855 bp) expected from the database of canis lupus familiaris genome and had almost identical nucleotide sequences to those included in the database. However, the sequence of the part encoding N-terminal portion of IRF7 with length of about 70 nucleotides was difficult to determine. Based on the obtained sequences, we designed siRNAs targeting three different sites at nucleotide 454, 449 and 380 in the coding region of IRF7.

All designed siRNAs were introduced into MDCK cells separately and the efficiency of virus production was monitored (Fig. 3A). As in A549 cells, the siRNA which enhanced virus production more than 2-fold compared with control was regarded positive (shown as a gray box in Fig. 3A). Inhibition of IRF7, MAVS, and TRAF5 by siRNA-mediated knockdown reproduc-

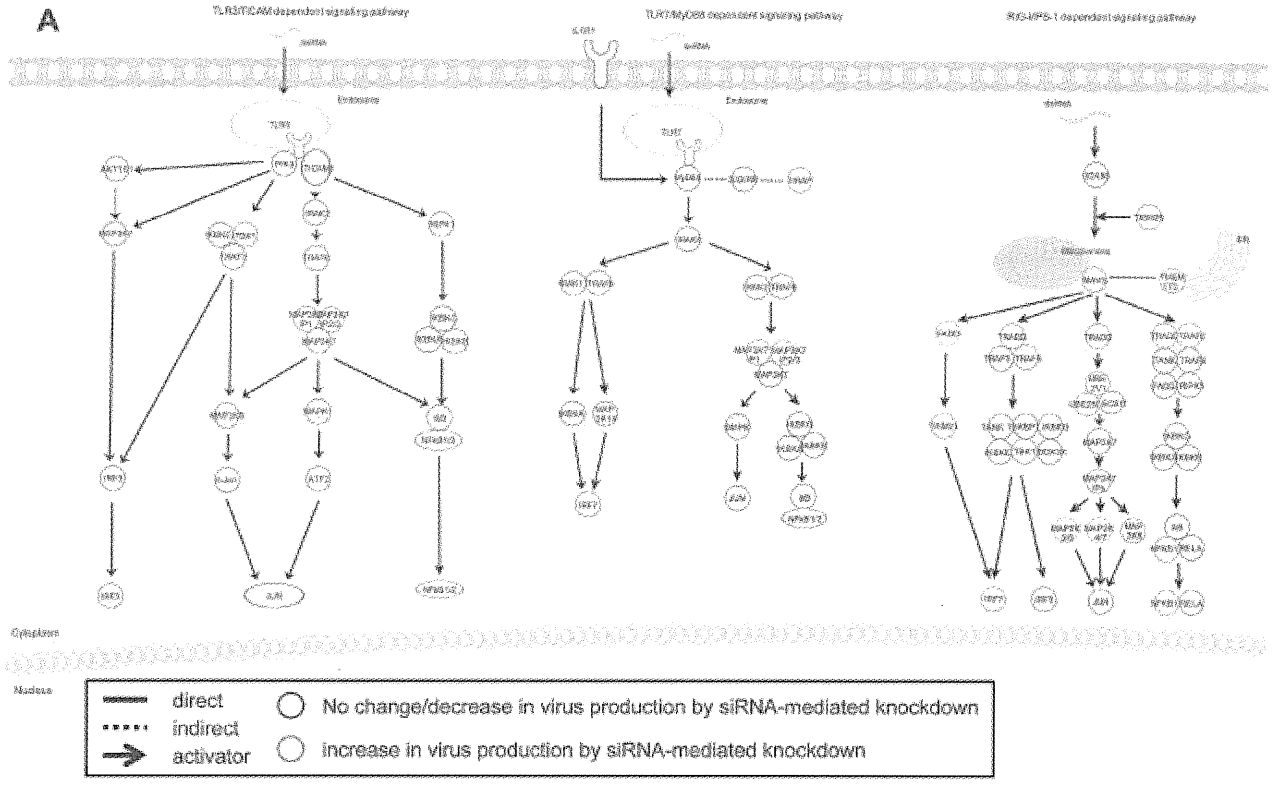

Figure 2. Mapping of the hit genes in the main pathways regarding type I IFN. (A) Ingenuity Pathway Analysis (IPA) was employed to map hit genes that when knocked down by siRNA affected viral production in A549 cells. The core networks show representative molecules related to three main type I IFN signaling pathways. Knockdown of target gene with siRNA in PR8-infected cells increased (red circles), decreased or did not change (black circles) PR8 virus production compared with control siRNA. Each assay was performed three times in independent experiments. (B) The Venn diagram indicates the number of genes overlapping and unique in TLR3/TICAM, TLR7/MyD88 and RIG-I/IPS-1 pathway. The 23 genes were selected from the first screening and most target genes were grouped in RIG-I/IPS-1 pathway.
doi:10.1371/journal.pone.0059892.g002

ibly increased virus production. Particularly, IRF7 showed the highest reproducibility amongst the three candidate genes. Therefore, we focused on the IRF7 for further validation. As shown in Fig. 3B, introduction of siRNA for IRF7 reduced the endogenous expression of this gene (90–70% reduction), and the viral titer showed about 4-fold increase in the culture supernatant of cells transfected with specific siRNAs targeting IRF7 (Fig. 3C). As in A549 cells, we also examined whether knockdown of IRF3, MyD88 or DDX58 affects the efficiency of the viral production in MDCK cells for comparison with IRF7. Knockdown of IRF3, MyD88 and DDX58 by siRNAs was confirmed (Fig. 3B), but did not affect the viral production in MDCK cells (Fig. 3C). In summary, these results indicate that IRF7 inhibits the multiplication of PR8 virus and knockdown of this gene enhances virus production in MDCK cells.

MDCK Cells Stably Expressing shRNA for IRF7 Show Enhancement of Influenza Virus Production

To examine whether stable knockdown of IRF7 enhances the production of influenza A virus in MDCK cells, we established

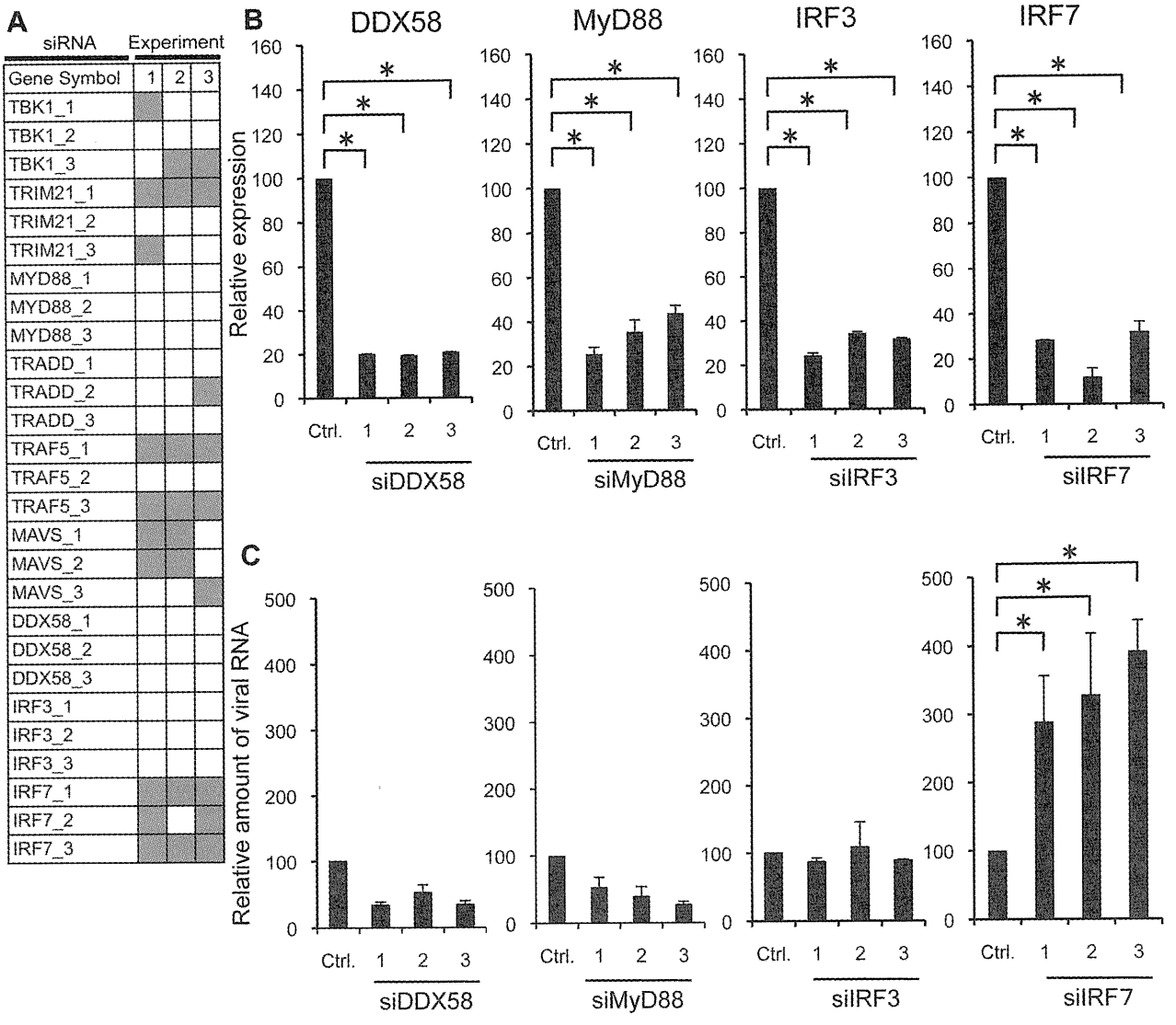

Figure 3. Knockdown of IRF7 enhances PR8 virus production in MDCK cells. (A) MDCK cells were transfected with siRNAs targeting the indicated genes. At 48 h post-transfection, the cells were infected with PR8 virus at a MOI of 0.01. After 24 h of incubation, viral RNA in culture supernatant was measured by quantitative real-time RT-PCR. The gray boxes in the panel mean that siRNA which targets indicated gene showed more than 2-fold enhancement of the virus production compared with negative control siRNA. (B) Total RNA from cells was extracted to monitor knockdown efficiency of target gene in the cells. The endogenous expression of target genes was measured by quantitative real-time RT-PCR with SYBR green. (C) MDCK cells were transfected with three siRNAs targeting genes or negative control siRNA. At 48 h post-transfection, the cells were infected with PR8 virus at a MOI of 0.01. After 24 h of incubation, the RNA from culture supernatant was isolated to examine the amounts of a specific viral RNA. The data are representative results of three independent experiments. Asterisks indicate statistically significant differences compared with the control (*$P<0.05$).

doi:10.1371/journal.pone.0059892.g003

MDCK-based cells, which were stably expressing short hairpin RNA (shRNA) for IRF7 by lentiviral transduction. To know the knockdown efficiency, endogenous mRNA of IRF7 was examined by quantitative real-time RT-PCR. The MDCK cells transduced with shIRF7 vector (MDCK-shIRF7) showed an 80% reduction in the transcription of IRF7 compared with the cells transduced with shControl vector (MDCK-shCtrl) (Fig. 4A). The MDCK cells expressing shRNA for either IRF7 or control have the same morphological and physiological characteristics as parental MDCK cells. The cell viability of the parental and the transduced MDCK cells expressing each shRNA showed at least 90% throughout the culture period. To determine the viral growth curve in transduced MDCK cells and parental MDCK cells, the cells were infected with PR8 virus at a MOI of 0.0003 for 1 h. After washing extensively, the cells were incubated for 72 h and the viral RNA in the culture supernatant was measured by real-time RT-PCR. MDCK-shIRF7 cells showed about 3-fold increase in titer of PR8 virus compared with MDCK-shCtrl cells (Fig. 4B).

We also examined the efficiency of propagation of A/Narita/1/2009 (NR1; A(H1N1)pdm09) virus in the modified MDCK cells by real-time RT-PCR. The amount of viral RNA from the culture supernatant of MDCK-shIRF7 cells was 5-fold greater than that from MDCK-shCtrl cells at 3 days post-infection (Fig. 4C).

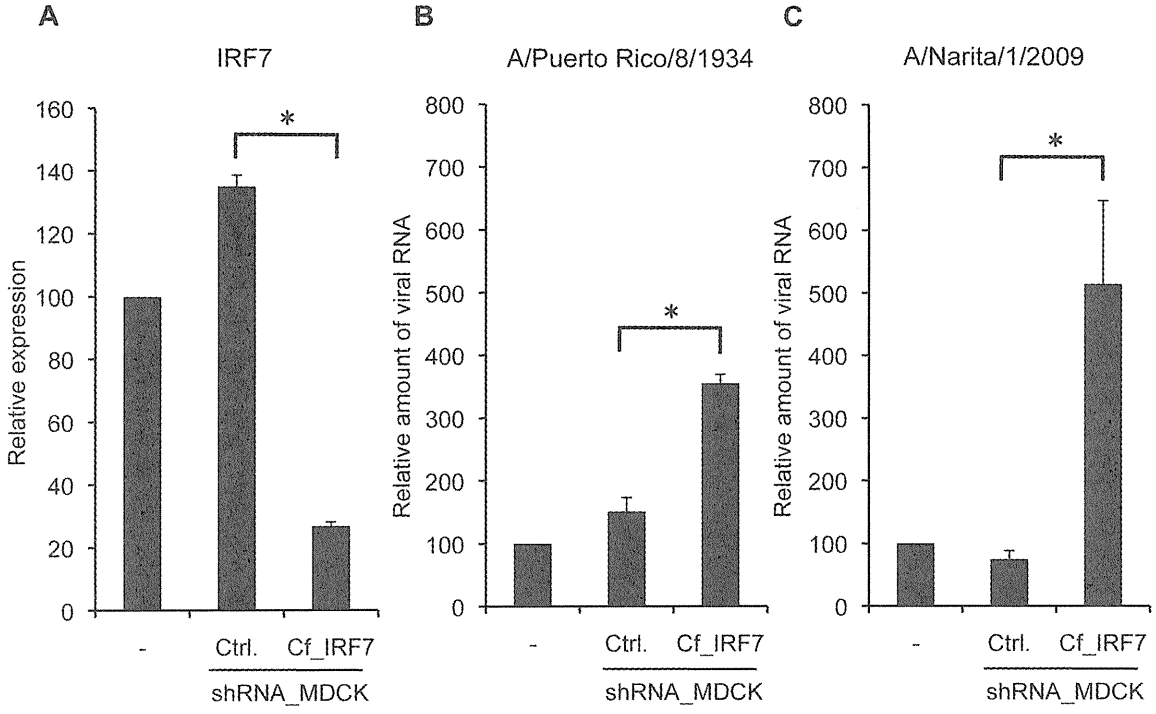

Figure 4. MDCK cells stably expressing shRNA for IRF7 enhances influenza A virus production. (A) The total RNA from parental or transduced MDCK cells was isolated and subjected to quantitative real-time RT-PCR. The knockdown efficiency of IRF7 mRNA expression level by transduced shRNA showed an 80% reduction. (B). The MDCK cells were infected with PR8 virus at a MOI of 0.0003. On day 3 after PR8 infection, the amount of viral RNA in culture supernatant was determined by quantitative real-time RT-PCR. (C) The MDCK cells were infected with A/Narita/1/2009 (A (H1N1) pdm09) virus at a MOI of 0.0003. On day 3 after virus infection, the amount of viral RNA in culture supernatant was determined by quantitative real-time RT-PCR. The relative viral RNA levels were normalized to values for 18S rRNA which was included in carrier RNA, and expressed as the relative (n-fold) value to the level of RNA from control. The data are representative results of three independent experiments. Asterisks indicate statistically significant differences compared with the control (*$P<0.05$).
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To confirm that more viral antigen is actually produced from MDCK cells expressing shRNA for IRF7, we performed hemagglutination (HA) assays for quantification of H1N1 viruses. The HA titers of PR8 and NR1 produced from MDCK-shIRF7 cells was twice to 8 times higher than those from MDCK-shCtrl cells (Table 3). To verify that production of hemagglutinin of A(H3N2) virus and B virus is enhanced in MDCK-shIRF7 cells, we performed HA assays for quantification of A/Victoria/361/2011 (VC361; H3N2) and B/Florida/4/2006 (FL4; type B, Yamagata lineage) produced from modified MDCK cells. The HA titers of VC361 and FL4 from MDCK-shIRF7 cells were 4 to 8 times higher than those from MDCK-shCtrl cells (Table 3). These results indicate that the production of influenza viruses is significantly enhanced in the MDCK cells with lower expression level of IRF7.

Table 3. HA titers of influenza viruses produced from modified MDCK cells.

Experiment No.	Influenza virus	Subtype/Lineage	sh-Control	sh-IRF7	Input virus titer/well	Time after infection
#1	A/Puerto Rico/8/1934	H1N1	8	16	100 pfu	48 h
	A/Narita/1/2009	H1N1pdm09	4	16	1000 $TCID_{50}$	96 h
	A/Victoria/361/2011	H3N2	4	16	100 $TCID_{50}$	72 h
	B/Florida/4/2006	Yamagata	4	16	100 pfu	72 h
#2	A/Puerto Rico/8/1934	H1N1	8	16	100 pfu	48 h
	A/Narita/1/2009	H1N1pdm09	8	32	1000 $TCID_{50}$	96 h
	A/Victoria/361/2011	H3N2	4	32	100 $TCID_{50}$	72 h
	B/Florida/4/2006	Yamagata	8	32	100 pfu	72 h

doi:10.1371/journal.pone.0059892.t003

Enhancement of Viral Propagation by siRNA for IRF7 is not Associated with Inhibition of Type I IFN Induction

Since knockdown of IRF7 enhanced PR8 virus production in infected MDCK cells, we next examined whether this enhanced viral production by siRNA for IRF7 might be due to the reduced expression of IFN-α/β. To investigate the effects of knockdown of IRF7 on expression levels of IFN-α/β in virus-infected MDCK cells, siRNA for IRF3 or IRF7 was transfected into MDCK cells and the cells were infected with PR8 virus. The efficient knockdown of IRF3 or IRF7 mRNA (70% reduction) was confirmed by quantitative real-time RT-PCR at 48 h post-transfection (Fig. 5A). The viral RNA from culture supernatant and total RNA from cell lysate were extracted at 24 h post-infection (hpi). The relative expression level of endogenous IFN-α/β was quantified by real-time RT-PCR. Notably, while knockdown of IRF7 significantly increased PR8 virus production by about 9-fold compared with the control at 24 hpi (Fig. 5B), the level of IFN-α/β induced in the infected MDCK cells with siIRF7 was not significantly impaired compared with those in MDCK cells with control siRNA (Fig. 5C). On the other hand, knockdown of IRF3 did not affect the virus production (Fig. 5B) and the expression levels of IFN-α/β remained unchanged after infection of siIRF3-transfected MDCK cells (Fig. 5C). IFN-α/β proteins in the supernatants, which were quantified by ELISA (PBL Biomedical Laboratories, Piscataway, NJ), were below the detectable level (<12.5 pg/ml) at 24 hpi in infected-A549 cells (data not shown). Collectively, these results suggest that knockdown of IRF7 enhances PR8 virus production by an unknown mechanism including pathways independent of IFN-α/β.

Discussion

Influenza virus is currently being produced in embryonated hen's eggs, but a well-defined cell line is considered to have merit for production of influenza virus to address concerns about the limitations of egg-based influenza vaccines. Potential advantages of cell-culture technology over conventional egg-based technology are as follows: elimination of the long lead time required for egg-based production systems, ease of supply of a substrate that is not susceptible to virulent virus strains, a more controlled production process involving closed-system bioreactors with reduced risk of microbial contamination, and the isolation and replication of influenza viruses without significant egg passage-dependent antigenic changes [4].

The aim of this study is to establish engineered MDCK cells that support efficient influenza virus propagation for better vaccine production. Because inhibition of IFN signaling seems to be a simple and efficient way to increase viral yield [16–18], we first screened, to identify target genes for such inhibition, a series of siRNAs for 78 human type I IFN-associated cellular genes (Fig. S1). We used A549 human lung adenocarcinoma cell line for the first screening and MDCK cells for the second screening because human genome database has much more information than canine genome database and pre-designed siRNA libraries are available for human genes but not for canine genes. By siRNA-mediated knockdown, a number of genes that are involved in the RIG-I/IPS-1 signaling pathway enhanced the production of PR8 virus in A549 and MDCK cells (Figs. 2 and 3). From the screening experiments, we identified the human IRF7 gene in A549 cells and a canine IRF7 in MDCK cells as a target gene for the knockdown-induced enhancement of influenza A virus propagation (Figs. 2, 3, 4 and S1). IRF7 seems to be reasonable as a gene to enhance virus propagation when knocked down by siRNA because it is located at the most downstream of the pathways for transcriptional activation of type I IFN and it is the common gene in three major signaling pathways.

To confirm that stable knockdown of IRF7 in MDCK cells enhances production of influenza viruses, we established MDCK cells expressing shRNA for IRF7. By quantitative RT-PCR, MDCK-shIRF7 cells were shown to produce more PR8 viruses than MDCK-shCtrl cells (Fig. 4). HA assays for PR8 and other viruses including H3N2 and B viruses demonstrated that more viral antigens were produced from MDCK-shIRF7 cells than from MDCK-shCtrl cells (Table 3). These results suggest that MDCK-shIRF7 cells can propagate a variety of influenza viruses more efficiently than MDCK-shCtrl cells.

In addition to IRF7, knockdown of MAVS, but not MyD88 or DDX58, enhanced the production of PR8 virus from the siRNA-transfected cells (Figs. 3A and S1). Knockdown of NOD2 also exhibited enhancement of PR8 virus production (data not shown). Sabbah et. al. demonstrated that NOD-like receptors such as NOD2 could act as a PRR for RSV and influenza A virus [19]. Therefore, we speculate that there may be a reciprocal cross talk between NOD2 and MAVS signaling pathways.

In contrast to IRF7, knockdown of IRF3 did not affect the viral production (Figs. 3C and 5B) even though IRF3 and IRF7 are considered to play essential roles in innate immune responses to virus infections. Of the IRF family, IRF7 plays an important role in anti-viral responses and can be activated in a similar manner to IRF3 during viral infection [20,21]. IRF7 is largely responsible for IFN production in response to viral infection, as evidenced by the abrogation of IFN production in *Irf7* −/− mice, but not in *Irf3* −/− mice [22]. Importantly, although the virus production was significantly enhanced in siIRF7 cells compared with siCtrl cells and siIRF3 cells at 24 hpi (Fig. 5B), there was no statistically significant difference ($P>0.05$) in the expression levels of IFN-α/β mRNA among cells transfected with siRNA for Ctrl, IRF3 and IRF7 under the conditions described (Fig. 5C). These results suggest that RNAi-mediated knockdown of IRF7 in MDCK cells enhances virus propagation through an unknown process that includes mechanisms other than inhibition of IFN-α/β induction.

Many viruses have evolved mechanisms to escape the IFN system, an antiviral defense of host cells [23,24]. Influenza A viruses have at least 3 viral proteins which counteract the IFN signaling: NS1, PB1-F2 and PB2. NS1 binds directly to Cleavage and Polyadenylation Specificity Factor 30 (CPSF30) and inhibits maturation of IFN, and other cellular mRNAs in the nucleus [25]. NS1 can associate with RIG-I, as well as TRIM25, a ubiquitin ligase required for RIG-I activation, to prevent its downstream activation of the IFN-β promoter [9,26–28]. Both IRF3 translocation and NFκB activation are impaired in the presence of NS1, which blocks the induction of pro-inflammatory cytokines and IFNs [29,30]. PB2, a subunit of the influenza virus RNA polymerase, interacts with MAVS and inhibits IFN-β expression [31]. The viral PB1-F2 inhibits RIG-I mediated induction of IFN by suppressing MAVS function [32]. Induction of IFN-α/β was not observed in MDCK cells by 24 h after infection with PR8 virus (Fig. 5C). This may be due to functions of viral proteins which block production of IFN-β. In our screening of siRNA library for human and canine genes, knockdown of RIG-I showed no significant increase in propagation of influenza virus (Figs. 3A and S1. RIG-I is shown as DDX-58 in these figures.). It is possible to consider that in both siCtrl-transfected cells and siRIG-I-transfected cells, the function of RIG-I was strongly blocked by viral proteins. It seems reasonable to assume that when the function of some cellular gene product is sufficiently inhibited by virus-encoded protein(s) unrelated to siRNA, additional inhibition of its function by siRNA may not show a significantly different

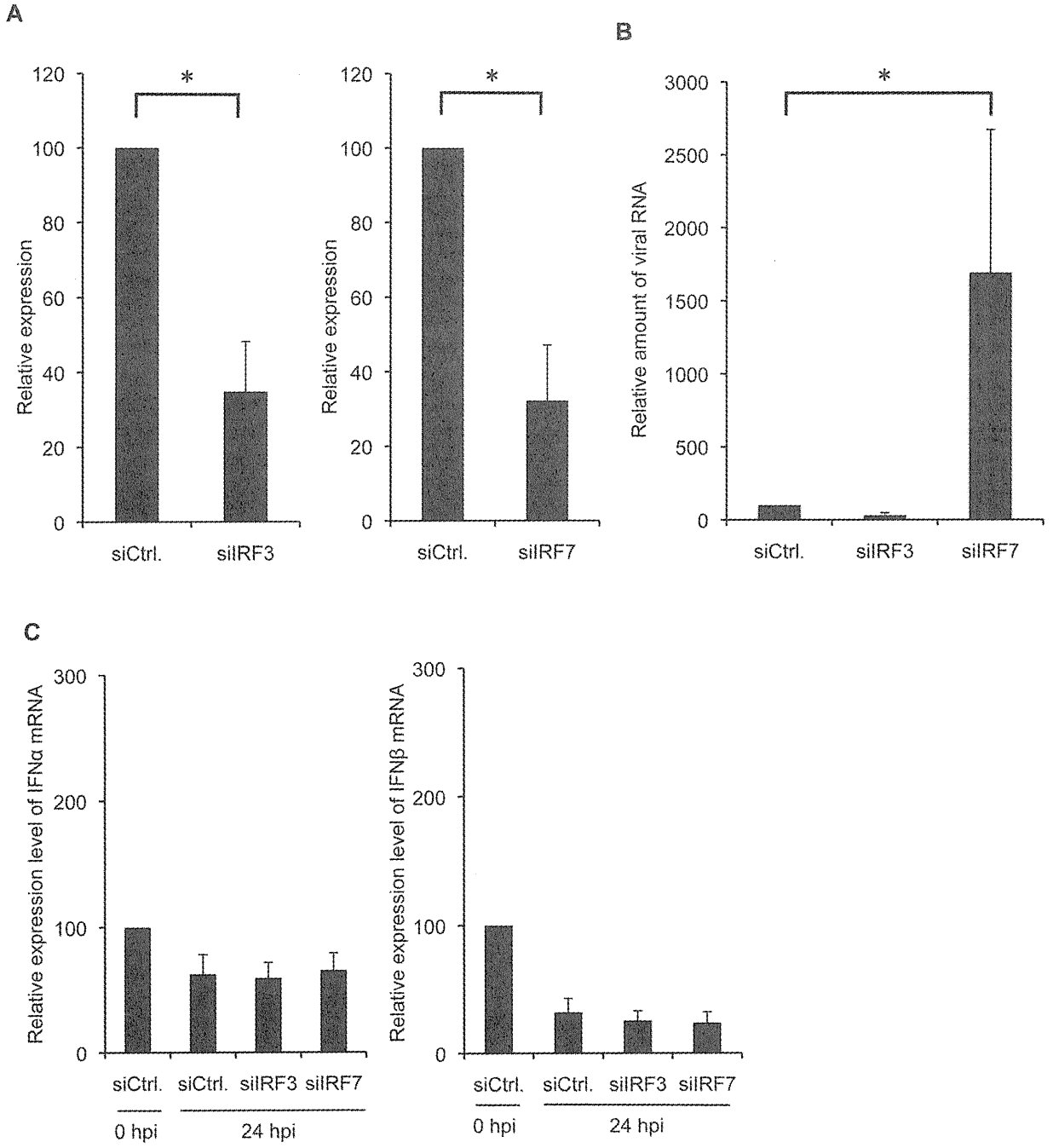

Figure 5. Enhancement of viral propagation with siIRF7 is not mediated by inhibition of type I IFN induction. (A) The total RNA from siRNA-transfected MDCK cells was isolated and subjected to quantitative real-time RT-PCR. (B) The siRNA transfected MDCK cells were infected with PR8 virus at a MOI of 0.03. The amount of viral RNA in culture supernatant was determined by quantitative real-time RT-PCR. Data are shown as fold change in amounts of viral RNA from siRNA-transfected cells compared with that from control cells. (C) The expression of mRNA for each IFN-α/β was measured at indicated time points after infection by quantitative real-time RT-PCR. Data are shown as fold change in expression of mRNA for each IFN-α/β compared with that of control siRNA-transfected cells and normalized to the values for 18S rRNA. The data are representative results of three independent experiments. Asterisks indicate statistically significant differences compared with the control (*$P<0.05$).
doi:10.1371/journal.pone.0059892.g005

phenotype. Inhibition of IFN signaling by viral proteins would explain, at least in part, the reason why knockdown of some IFN-related genes did not show enhancement of virus replication. Furthermore, it could also be considered that the IFN-related genes, that show enhancement of virus propagation triggered by siRNA-mediated knockdown, may have another function different from and independent of the IFN signaling so far described. Considering the observation in the present paper, this could be consistent with the idea that the enhancement of influenza virus propagation by siRNA for IRF7 is caused by an unknown cellular

process that includes mechanisms other than inhibition of IFN-α/β induction.

Cell-based manufacturing has many potential merits over egg-based manufacturing, but the major drawback of cell-based technique at present may be cost. The cost of cell-based vaccine would be high due to the scale required to generate sufficient products. If yield of viral antigen from cell substrate is improved, it will contribute to reduction of the culture scale and the cost for vaccine production. We consider that our modified MDCK cells could be one of the potential remedies for the shortcoming of cell-based manufacturing. Increase in virus yield by manipulation of cell substrates would turn mammalian cell manufacturing into a more viable approach.

Here, our results demonstrate that the novel MDCK cells expressing shRNA for IRF7 can produce twice to 8 times more influenza viruses than control cells and they will be useful for larger and more rapid production of influenza vaccines. Furthermore, these cells will have an advantage for isolating influenza viruses in a small amount in clinical specimens and thus preparing seed viruses for vaccine production. The novel MDCK cells could contribute to expanding availability of influenza vaccine to a large number of people worldwide.

Supporting Information

Figure S1 Screening of siRNA library for human typeI interferon-related genes. A549 cells were transfected with siRNAs targeting 78 different genes at a final concentration of 10 nM. At 48 h post-transfection, A549 cells were infected with PR8 virus at a MOI of 0.01. At 24 hpi, the viral RNA from culture supernatant was extracted for quantitative real-time RT-PCR. The relative amount of viral RNA was normalized to the values for 18S rRNA which was included in carrier RNA. The gray box means that siRNA targeting the indicated gene increases the amount of viral RNA more than 2-fold compared with control siRNA in A549 cells. Each assay was performed three times in independent experiments.
(TIF)

Acknowledgments

The authors thank Dr. H. Miyoshi (RIKEN Tsukuba Institute, Tsukuba, Japan) for pCS-CDF-EG-PRE, pCAG-HIVgp, and pRSV-Rev plasmids.

Author Contributions

Conceived and designed the experiments: NY MT. Performed the experiments: IH HT NY. Analyzed the data: IH NY. Contributed reagents/materials/analysis tools: HT NY. Wrote the paper: IH HT MT NY.

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Scandinavian Journal of Infectious Diseases, 2013; Early Online: 1–9

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ORIGINAL ARTICLE

Safety evaluation of laninamivir octanoate hydrate through analysis of adverse events reported during early post-marketing phase vigilance

TAKASHI NAKANO[1], AKIHISA OKUMURA[2], TAKUYA TANABE[3], SHIMPEI NIWA[4], MASATO FUKUSHIMA[4], RIE YONEMOCHI[4], HISANO EDA[4] & HIROYUKI TSUTSUMI[5]

From the [1]Department of Pediatrics, Kawasaki Medical School, Okayama, [2]Department of Pediatrics, Juntendo University, Tokyo, [3]Tanabe-Kadobayashi Children's Clinic, Osaka, [4]Pharmacovigilance Department, Daiichi Sankyo Co., Ltd, Tokyo, and [5]Department of Pediatrics, Sapporo Medical University School of Medicine, Hokkaido, Japan

Abstract

Background: Abnormal behavior and delirium are common in children with influenza. While abnormal behavior and delirium are considered to be associated with influenza encephalopathy, an increased risk of such neuropsychiatric symptoms in patients receiving neuraminidase inhibitor treatment is suspected. Laninamivir octanoate hydrate, recently approved in Japan, is a long-acting neuraminidase inhibitor. It is important to establish a safety profile for laninamivir early, based on post-marketing experiences. *Methods:* Spontaneous safety reports collected in the early post-marketing phase vigilance were analyzed. Adverse events of interest such as abnormal behavior/delirium, dizziness/vertigo, respiratory disorders, shock/syncope, and any other serious events were intensively reviewed by the Safety Evaluation Committee. *Results:* Abnormal behavior/delirium was a frequently reported event. Almost all the reported cases were considered to be due to influenza and not laninamivir. There were 32 cases of abnormal behavior/delirium that could lead to dangerous accidents, and these were observed more frequently in males and teenagers. Syncope probably related to the act of inhalation per se of laninamivir was reported during this survey. *Conclusions:* This safety review revealed that the safety profile of laninamivir for abnormal behavior/delirium and syncope was similar to that of other neuraminidase inhibitors. As stated in the labeling, teenage patients inhaling laninamivir should remain under constant parental supervision for at least 2 days and should be closely monitored for behavioral changes to prevent serious accidents associated with abnormal behavior/delirium. Furthermore, to avoid syncope because of inhalation, patients should be instructed to inhale in a relaxed sitting position.

Keywords: *Laninamivir, neuraminidase inhibitor, abnormal behaviors, syncope, influenza*

Introduction

Influenza is one of the most common infections and also a major cause of hospitalization of children during epidemics. Based on experience with the novel H1N1 influenza 2009 [1] in Japan during the 2009–2010 epidemic, it is believed that implementation of early treatment with neuraminidase inhibitors may lower the mortality rate of pediatric patients with influenza [2].

Neuropsychiatric events such as abnormal behavior and delirium are well known events in influenza patients and have been reported mostly in Japan [3]. There has been much discussion about a possible causal relationship between these events and the use of neuraminidase inhibitors [4–6]; however, the relationship remains unclear since these events are accompanied by complications of influenza such as encephalopathy and febrile delirium [7,8]. To date there has been no study conducted to clarify the causal association between them.

Laninamivir octanoate hydrate (hereinafter 'laninamivir') was first approved in Japan for the treatment of influenza in September 2010. It is a long-acting neuraminidase inhibitor that can treat

Correspondence: S. Niwa, Pharmacovigilance Department, Daiichi Sankyo Co., Ltd, 3-5-1, Nihonbashi Honcho, Chuo-ku, Tokyo 103-8426, Japan. Tel: +81 3 6225 1036. Fax: +81 3 6225 1919. E-mail: niwa.shimpei.f2@daiichisankyo.co.jp

(Received 8 July 2012; accepted 27 December 2012)

ISSN 0036-5548 print/ISSN 1651-1980 online
DOI: 10.3109/00365548.2012.763104

influenza with a single inhalation [9,10]. The accumulation of information on its efficacy and safety has become indispensable, since this enables physicians to select the appropriate treatment for patients. Although the safety of laninamivir has been demonstrated in clinical trials [11,12], it is difficult to identify very rare adverse events (AEs) in clinical trials because of the limited number of patients and the limited target population. Therefore, analysis of safety information in the early post-marketing period is considered to be essential to identify early signs of any AEs linked to the drug [13,14].

Daiichi Sankyo Co., Ltd (Tokyo, Japan), which manufactures and sells laninamivir in Japan, collected information on all the AEs that occurred during the early post-marketing phase vigilance (EPPV); this information was then used to analyze and evaluate the safety of laninamivir. Furthermore, the Safety Evaluation Committee (SEC), consisting of medical experts, was established to conduct an intensive review of AEs of interest. This is the first report of the safety evaluation of laninamivir during the early post-marketing period and is aimed at providing safety information to facilitate the proper use of laninamivir in clinical practice.

Methods

A process chart depicting the collection of data on AEs, selection, individual case review, AE categorization, and the cumulative review is shown in Figure 1.

AEs collected during the EPPV

Daiichi Sankyo collected individual AE cases in the use of laninamivir in Japan intensively between October 2010 and April 2011, in compliance with the EPPV guidelines [15]. All the cases reported by healthcare professionals (HCPs) during the EPPV were included in the data collection. Daiichi Sankyo collected precise and detailed medical information on each case from HCPs whenever possible. In all the collected AE cases, the diagnosis of influenza was confirmed by a HCP based on the results of a rapid diagnostic kit and/or influenza-like symptoms during the clinical course. Laninamivir was prescribed to patients who had the ability to inhale the drug. Collected AEs were coded according to the System Organ Class and Preferred Terms of the Medical Dictionary for Regulatory Activities (MedDRA/J version 14.0).

Estimated patient exposure during the EPPV

The precise number of patient exposures to laninamivir in the EPPV was unknown. Therefore, the number of patients was estimated using information from a medical claims database maintained by the Japan Medical Data Center Co., Ltd (Tokyo, Japan). The database is comprised of medical claims data covering 600,000 patients from multiple company-provided health insurance programs [16]. The estimated patient exposure to laninamivir during the EPPV was about 1.8 million. The estimated exposure to laninamivir in patients aged ≤ 9, 10–19, and ≥ 20 y was 14.4% (0.26 million), 33.3% (0.60 million), and 52.2% (0.94 million), respectively.

Selection of AEs for evaluation

All serious AEs and the events described below were selected by the SEC as AEs for evaluation: (1) abnormal behavior/delirium, (2) dizziness/vertigo, (3) respiratory disorders, and (4) shock/syncope (Figure 1).

Safety Evaluation Committee

The SEC was established to conduct intensive reviews of individual cases and to perform a cumulative review of AEs. The committee comprised 3 reviewers: 1 pediatric infectious disease expert (T. Nakano) and 2 pediatric neuropsychiatry experts (A. Okumura and T. Tanabe). SEC members evaluated all the available information on the selected AEs that had been collected by Daiichi Sankyo.

For the individual case reviews, an evaluation sheet for each AE was prepared in addition to instructions for evaluating the AE. All the reviewers validated the reported term and selected contributing factors and the most plausible contributing factor based on the instructions and the evaluation sheet when evaluating the AEs (Figure 2).

For the cumulative review, the AEs were categorized into the following 5 groups by the SEC: (1) abnormal behavior/delirium, (2) dizziness/vertigo, (3) respiratory disorders, (4) shock, and (5) syncope. AEs categorized into the abnormal behavior/delirium group were further divided into 2 subgroups. The 2 subgroups were category A and other than category A, i.e., categories B, C, D, and E (Figure 2). Category A was defined in accordance with the criteria used in an epidemiological study conducted in Japan to encompass the following events: sudden running, dangerous acts, violent acts, movements in a vertical direction or to an unexpected location, and other dangerous acts [17]. The events that were assessed as category A by at least 1 reviewer in the individual case evaluation were reassessed by all members to determine the validity of the classification of an event into category A.

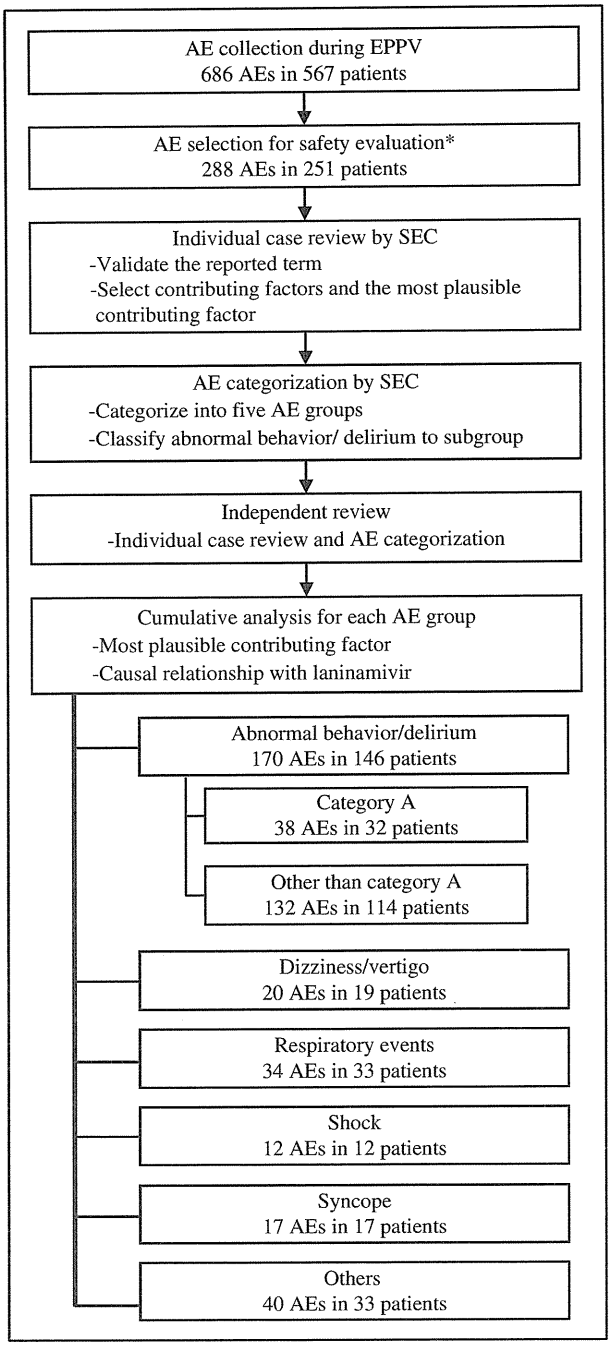

Figure 1. Process chart for the handling and evaluation of adverse events (AEs). *All serious AEs and the AEs described below were selected as AEs for evaluation: (1) Abnormal behavior/delirium: numerous case reports have described abnormal behavior/delirium in patients receiving neuraminidase inhibitors in the same class as laninamivir, which have led to serious accidents, such as jumping or falls. (2) Dizziness/vertigo: this AE had a high incidence in clinical trials of laninamivir in adult patients. (3) Respiratory disorders: as laninamivir is administered by dry-powder inhalation, the respiratory organs are directly exposed to the drug, with the possibility of the development of events related to the respiratory system. (4) Shock/syncope: shock/syncope has been reported in patients after the inhalation of zanamivir, which is in the same class as laninamivir.

As an independent review, individual case reviews and AE categorization were also performed by another pediatric infectious disease expert (H. Tsutsumi) in accordance with the protocol used by the SEC.

The dataset for the cumulative review was merged with the results of both the SEC and the independent review to analyze all the available data. A cumulative review of abnormal behavior/delirium was conducted by comparing the gender, age, and time of onset of events between category A and other than category A subjects. Plausible contributing factors and the causal relationship with laninamivir according to the AE groups were further analyzed. The causality was divided into 3 categories: 'probable', 'possible', and 'unrelated', as follows: (1) probable: evaluated as the most plausible contributing factor by at least 1 reviewer; (2) possible: not evaluated as the most plausible contributing factor by any of the reviewers, but evaluated as a contributing factor by at least 1 reviewer; (3) unrelated: not evaluated as a contributing factor by any of the reviewers.

Results

Overview of AEs collected during the EPPV

A total of 686 AEs in 567 patients were reported during the EPPV. The most frequently reported AEs were gastrointestinal disorders (27.0%), psychiatric disorders (26.8%), and skin disorders (18.7%). When comparing by age, the most frequently reported AEs in patients aged <20 y were psychiatric disorders (40.3%), and for those aged ≥ 20 y were gastrointestinal disorders (29.2%) (Table I).

There were 44 serious cases that included 6 cases of abnormal behavior, 4 cases of loss of consciousness, and 4 fatal cases. All fatal cases were assessed to be unrelated to laninamivir by all reviewers based on the clinical course. Most of the remaining serious cases recovered from their serious AEs.

Cumulative review

Two hundred and eighty-eight AEs in 251 patients were selected for the individual case evaluation. The AEs were classified into 5 groups for the cumulative review, and most events were classified into the group of abnormal behavior/delirium (Figure 1 and Supplementary Material Table I to be found online at http://informahealthcare.com/doi/abs/10.3109/00365548.2012.763104).

Of the 170 AEs in the abnormal behavior/delirium group seen in 146 patients, 38 events involving 32 patients were classified as category A and the remaining 132 events involving 114 patients were

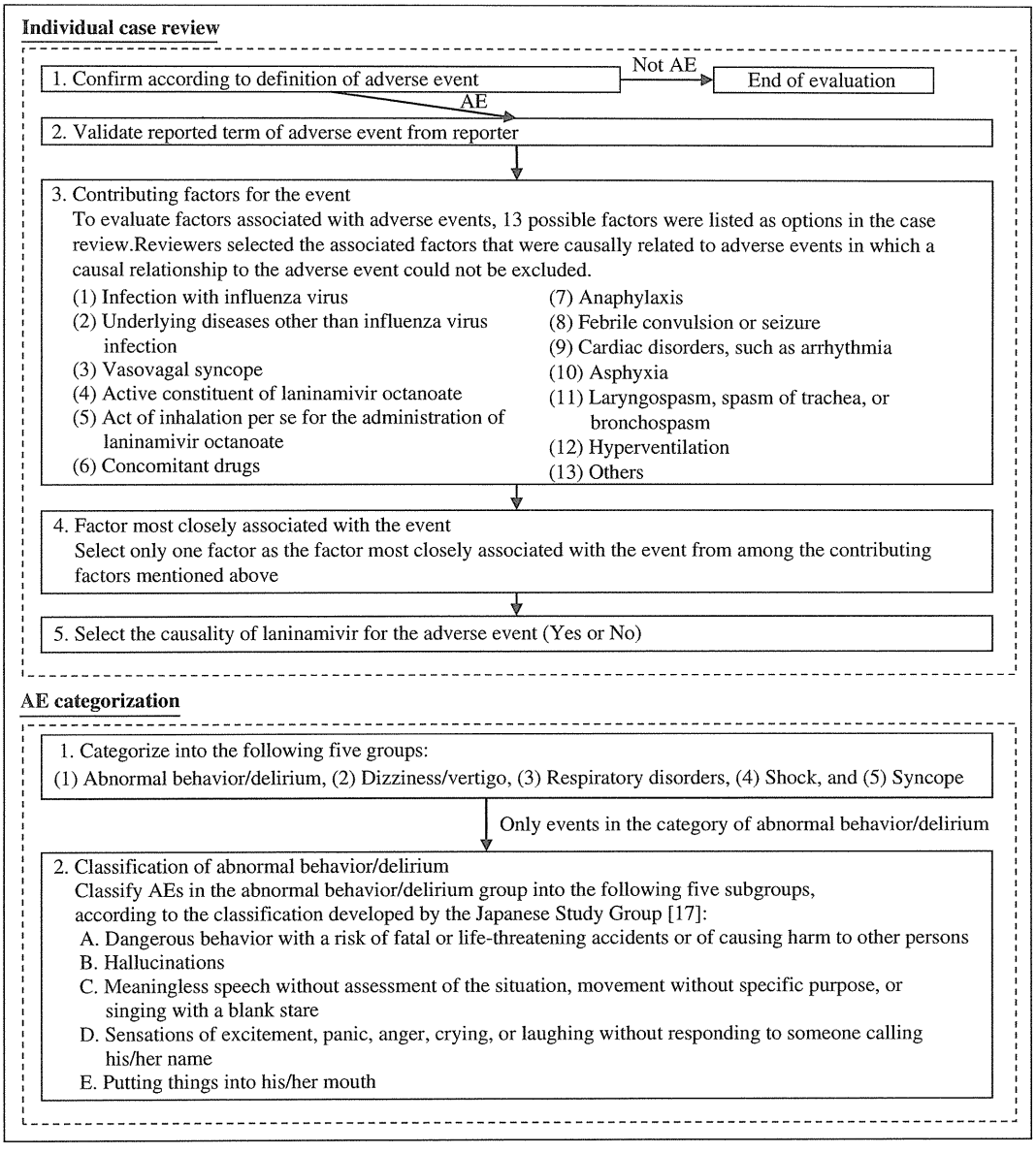

Figure 2. Scheme for individual case review and adverse events (AE) categorization.

classified as other than category A. The symptoms in category A included sudden running, vertical movements including running down the stairs and falling down stairs, violent acts including beating family members, and dangerous acts including vehicular accidents and banging one's head against a pole (Table II). Analysis according to gender and age revealed that the events in category A were more frequently seen in males (71.9%) and teenagers (62.5%) than events in other than category A. The events in both subgroups developed within 1 or 2 days of drug inhalation (category A events: 89.5%, other than category A events: 86.4%), as shown in Table II.

A total of 680 evaluations in the abnormal behavior/delirium group were available for the analysis of the most plausible contributing factor for the event, based on an intensive review. Influenza per se was considered to be the factor most closely associated with the development of abnormal behavior/delirium (92.8%). Because this analysis was based on spontaneously reported cases, 4.6% of the total evaluation data was assessed as cases with insufficient data to provide an assessment of contributing factors. Influenza per se was assessed as the most contributing factor in the dizziness/vertigo (83.8%) and respiratory disorders (47.1%) groups. Vasovagal syncope was assessed as the most contributing factor in the syncope group (35.3%). In the shock group, both influenza per se (22.9%) and vasovagal syncope (22.9%) were assessed as the most plausible contributing factors (Table III).

In the analysis of the causal relationship with the act of inhalation, 16% of all events for intensive review were assessed as 'probable' or 'possible'

Table I. Distribution of adverse events (AEs) by age group.

AEs[a]	Number of cases[b] ($n = 567$)	Age group, y: <20 ($n = 345$)	≥20 ($n = 209$)	Unknown[c] ($n = 13$)
Gastrointestinal disorders	153 (27.0%)	90 (26.1%)	61 (29.2%)	2 (15.4%)
Psychiatric disorders	152 (26.8%)	139 (40.3%)	12 (5.7%)	1 (7.7%)
Skin disorders	106 (18.7%)	45 (13%)	57 (27.3%)	4 (30.8%)
Nervous system disorders	78 (13.8%)	31 (9%)	45 (21.5%)	2 (15.4%)
General disorders	38 (6.7%)	15 (4.3%)	19 (9.1%)	4 (30.8%)
Others	102 (18.0%)	52 (15.1%)	46 (22%)	4 (30.8%)

n, number of cases.
[a]AEs were classified in accordance with the Medical Dictionary for Regulatory Activities (MedDRA version 14.0) System Organ Class. 'Skin disorders' indicates skin and subcutaneous tissue disorders; 'General disorders' indicates general disorders and administration site conditions.
[b]Patients who developed more than 1 AE were included, therefore the sum of all the rows does not equal 567.
[c]Ten patients whose exact age was unknown but who were known to be over 10 y old were included.

(probable: 8%, possible: 8%, unrelated: 84%). With respect to the abnormal behavior/delirium group, all events were assessed as unrelated to the act of inhalation (100%). As for the other groups, both the respiratory disorders group and the shock group had a higher rate of AEs that were assessed as 'probable' or 'possible', yielding rates of 73.5% and 75.0%, respectively (Table IV).

In the analysis of the causal relationship with the active ingredient of laninamivir, 12.5% of the 288 events were assessed as 'probable' or 'possible' (probable: 2.8%, possible: 9.7%, unrelated: 87.5%). Only 1 event of the abnormal behavior/delirium group was assessed as possible, and all other events were assessed as unrelated (99.4%). As for the other groups, both the dizziness/vertigo and shock groups had a higher rate of AEs assessed as 'probable' or 'possible', yielding rates of 75.0% and 50.0%, respectively (Table IV).

Table II. Summary of abnormal behavior/delirium according to subgroups.

Gender	Category A[a] ($n = 32$)	Other than category A ($n = 114$)
Male	23 (71.9%)	61 (53.5%)
Female	9 (28.1%)	48 (42.1%)
Unknown	0 (0.0%)	5 (4.4%)

Age (y)	Category A[a] ($n = 32$)	Other than category A ($n = 114$)
≤9	12 (37.5%)	62 (54.4%)
10–19	20 (62.5%)	42 (36.8%)
20–64	0 (0.0%)	8 (7.0%)
≥65	0 (0.0%)	1 (0.9%)
Unknown	0 (0.0%)	1 (0.9%)

Time to onset (days)	Category A[a] ($n_e = 38$)	Other than category A ($n_e = 132$)
1–2	34 (89.5%)	114 (86.4%)
3–4	2 (5.3%)	7 (5.3%)
5–7	0 (0.0%)	2 (1.5%)
≥8	1 (2.6%)	0 (0.0%)
Unknown	1 (2.6%)	9 (6.8%)

n, number of cases; n_e, number of adverse events.
[a]Definition of category A was dangerous behavior with a risk of fatal or life-threatening accidents, or harm to other persons. The symptoms in category A included sudden running, vertical movements including running down the stairs and falling down stairs, violent acts including beating family members, and dangerous acts including vehicular accidents and banging one's head against a pole.

Discussion

The major AEs reported during the EPPV were abnormal behavior, delirium, nausea/vomiting, diarrhea, urticaria, and rash. None of them were newly identified events that had not been observed in the laninamivir clinical trials conducted during the pre-approval phase [11,12]. The overall review of serious AEs revealed that abnormal behavior and loss of consciousness were more frequently reported than other AEs. The analysis from various aspects regarding the remaining serious AEs identified no specific safety profile to be noted. None of 4 fatal cases was assessed as related to the therapy with laninamivir by both reviewers in the individual case evaluation and the reporter of the case.

Abnormal behavior/delirium was the most frequently reported AE during the EPPV. In Japan, the incidence of abnormal behavior/delirium in influenza patients aged less than 18 y, regardless of the use of neuraminidase inhibitors, has been shown to be approximately 12% [17]. Most of the safety data

Table III. Most plausible contributing factors according to adverse event (AE) group.

Contributing factor	Abnormal behavior/delirium (n_d = 680[b])	Dizziness/vertigo (n_d = 80)	Respiratory events (n_d = 136)	Shock (n_d = 48)	Syncope (n_d = 68)	AEs for evaluation[c] (n_d = 1152)
Influenza per se	631 (92.8%)	67 (83.8%)	64 (47.1%)	11 (22.9%)	17 (25.0%)	876 (76.0%)
Laninamivir	0 (0.0%)	5 (6.3%)	32 (23.5%)	4 (8.3%)	7 (10.3%)	48 (4.2%)
Vasovagal syncope	0 (0.0%)	1 (1.3%)	0 (0.0%)	11 (22.9%)	24 (35.3%)	38 (3.3%)
Others	18 (2.6%)	5 (6.3%)	32 (23.5%)	18 (37.5%)	18 (26.5%)	135 (11.7%)
Not assessable[a]	31 (4.6%)	2 (2.5%)	8 (5.9%)	4 (8.3%)	2 (2.9%)	55 (4.8%)

n_d, number of evaluation data for the primary contributing factor.
[a]There was insufficient information about this event for assessment of the most closely associated factor.
[b]Evaluation by 4 experts for each of the 170 events in the abnormal behavior/delirium group.
[c]AEs for evaluation: AEs that were not classified into one of the 5 groups for the cumulative review were included.

used for our intensive review were from spontaneous reports. In general, the number of spontaneous reports is stimulated by awareness of an event in association with the use of a product [18,19]. One of the reasons for the high number of reports of abnormal behavior/delirium during the EPPV for laninamivir could, therefore, be strong public awareness of its occurrence in pediatric patients with influenza among the Japanese.

The result from our cumulative review of AEs in the abnormal behavior/delirium group showed that influenza per se could be the most plausible contributing factor toward the events. Almost all cases with these events were assessed as unrelated to the active ingredient of laninamivir. There have been several reports of abnormal behavior/delirium observed in patients with influenza who have not taken any medication such as neuraminidase inhibitors for the treatment of influenza, and therefore, these events were probably attributable to influenza per se [20–22]. Abnormal behavior/delirium also sometimes develops as a manifestation of influenza encephalopathy [7]. In our review, some events in the abnormal behavior/delirium group were also judged to be probably related to influenza manifestations associated with the central nervous system.

In the cumulative review of the subgroup of abnormal behavior/delirium, category A was reported at a high frequency among males and teenagers. In an observational study it was reported that abnormal behavior/delirium potentially leading to serious accidents was observed more frequently in teenage males [17]. Our findings in the intensive review for abnormal behavior/delirium of category A were similar to those published in previous reports.

Table IV. Causal relationship with laninamivir according to the adverse event (AE) group.

	Abnormal Behavior/ delirium	Dizziness/vertigo	Respiratory events	Shock	Syncope	All AEs of interest[a]
	(n_e = 170)	(n_e = 20)	(n_e = 34)	(n_e = 12)	(n_e = 17)	(n_e = 288)
Causality of the act of inhalation						
Probable[b]	0 (0.0%)	1 (5%)	16 (47.1%)	1 (8.3%)	5 (29.4%)	23 (8%)
Possible[c]	0 (0.0%)	7 (35%)	9 (26.5%)	8 (66.7%)	0 (0.0%)	23 (8%)
Unrelated[d]	170 (100%)	12 (60%)	9 (26.5%)	3 (25.0%)	12 (70.6%)	242 (84%)
Causality of the active ingredient, laninamivir						
Probable[b]	0 (0.0%)	4 (20%)	1 (2.9%)	1 (8.3%)	0 (0.0%)	8 (2.8%)
Possible[c]	1 (0.6%)	11 (55%)	3 (8.8%)	5 (41.7%)	1 (5.9%)	28 (9.7%)
Unrelated[d]	169 (99.4%)	5 (25%)	30 (88.2%)	6 (50%)	16 (94.1%)	252 (87.5%)

n_e, number of adverse events.
[a]All AEs of interest: AEs that were not classified into the 5 groups for cumulative review were included.
[b]Probable: the relevant contributing factor (i.e., the act of inhalation per se of laninamivir or of the active drug ingredient) was evaluated as the most contributing factor by at least 1 reviewer.
[c]Possible: the relevant contributing factor was evaluated as the most contributing factor by none of the reviewers, but assessed as one of the contributing factors by at least 1 reviewer.
[d]Unrelated: the relevant contributing factor was evaluated as a contributing factor by none of the reviewers.

No literature for a comparison of the incidence of the events among neuraminidase inhibitors was found by our intensive literature search. The only information we obtained in the search was the number of cases with serious abnormal behavior reported to the Japanese regulatory authorities during the 2010–2011 season for each neuraminidase inhibitor [23]. According to the information disclosed by the Ministry of Health, Labor and Welfare [23], there were 5 reported cases for laninamivir, 16 cases for oseltamivir, 8 cases for zanamivir, and 1 case for peramivir. Based on the published prescription data estimates for neuraminidase inhibitors, the most commonly prescribed was oseltamivir, and other patients were prescribed laninamivir or zanamivir in approximately the same percentage [20,24]. Since only limited data are available and also the reporting rate is not comparative data, it is difficult to directly compare the incidence rates of abnormal behavior. Considering these data, however, it may suggest that the reporting rate of serious abnormal behavior in the use of laninamivir is included in the range of the reporting ratio of the other neuraminidase inhibitors such as oseltamivir and zanamivir.

It has been reported that dangerous abnormal behavior/delirium, such as jumping from heights, most often develops within 2 days of the onset of influenza [17]. The finding in our analysis of the time of onset of abnormal behavior/delirium is consistent with the observation in the epidemiology study on influenza-associated symptoms. In addition, case reports of dangerous abnormal behavior/delirium have been reported even in patients not receiving neuraminidase inhibitor treatment [22]. Although the possible effects of neuraminidase inhibitor treatment on the increase in the risk of abnormal behavior/delirium have not yet been clarified, HCPs should be cautioned about this possibility in the use of neuraminidase inhibitors, including laninamivir [25–27]. In Japan, the labeling of neuraminidase inhibitors states that pediatric or teenage influenza patients should be closely monitored and not left alone for at least 2 days after the start of treatment to prevent serious accidents due to abnormal behavior [6]. There were some cases of potentially dangerous abnormal behavior/delirium that developed after laninamivir treatment, and also some cases suggesting that a serious outcome could be successfully avoided if there was close monitoring of the patient's behavior by family members. From the viewpoint of preventing serious accidents, it would seem desirable to continue implementing the safety measures currently adopted in Japan.

Other than abnormal behavior/delirium, the safety assessment revealed that dizziness/vertigo and shock were major events with a probable causal relationship with the active ingredient of laninamivir. Of these events, serious anaphylactic shock was reported in 1 case. The possibility of the onset of anaphylactic shock cannot be excluded for any drug. Although the incidence of anaphylactic shock associated with the use of neuraminidase inhibitors has not been reported until now, the incidence has been estimated to be 0.7–10% with penicillins and 0.22–1% with contrast medium [28]. Although the incidence of events associated with the use of laninamivir cannot be calculated, the reporting frequency during the period covered by this EPPV was estimated as 1 per 1.8 million laninamivir treatments. This finding suggests that the incidence of anaphylactic shock associated with the use of laninamivir may not be as high as the incidence reported for other drugs.

Because laninamivir is a dry powder for inhalation, we evaluated the influence of the inhalation. Twenty-three events were assessed as having a 'probable' relationship with the act of inhalation, and these events developed immediately after inhalation. The most frequent of these events were cough and loss of consciousness. Regarding loss of consciousness, it was suggested that in patients with influenza, who are often in a poor general condition, inhalation causes elevation of the intrathoracic pressure, potentially resulting in the onset of vasovagal syncope.

For the use of laninamivir, multiple inhalation sessions are required due to the design of the container [27]. Although the incidence of loss of consciousness in relation to the number of laninamivir inhalations is unknown, we cannot exclude the possibility that the need for multiple inhalations from a single container may be a contributing factor for loss of consciousness after inhalation. When zanamivir is used, 2 blisters need to be inhaled simultaneously, thus necessitating multiple inhalations. Reports on syncope after the inhalation of zanamivir have also been published, and it has been advised that the drug be inhaled under relaxed conditions [25]. For laninamivir, inhalation under relaxed conditions also seems to be desirable.

As treatment with laninamivir is completed in a single dose, the drug may be administered effectively at a medical facility under the guidance of a HCP. Therefore, compliance with the dosing instructions is expected to be better for this drug than for drugs that require daily dosing.

Based on the information collected during the EPPV, the labeling of laninamivir has been revised to warn HCPs of the possible onset of syncope and shock immediately after inhalation and to urge them to exercise caution. Furthermore, the labeling has been revised to add a caution for anaphylactic symptoms based on the reported case with anaphylactic shock [27].

In conclusion, the safety evaluation of laninamivir during the early post-marketing period revealed that the available information on abnormal behavior/delirium and loss of consciousness was similar to previously published reports for the other neuraminidase inhibitors. While using laninamivir, the following measures are recommended: (1) children or teenagers should be closely monitored and not left alone for at least 2 days after the start of treatment to prevent serious accidents following the development of abnormal behavior; (2) physicians and other HCPs should guide patients to inhale this drug under relaxed conditions to prevent the onset of vasovagal syncope, which has been reported in some patients, immediately after the inhalation of this drug.

Acknowledgements

The authors thank all healthcare professionals for their case report contributions.

Declaration of interest: Dr Takashi Nakano has received consulting fees and payment for lectures from Daiichi Sankyo, Glaxo Smith Kline, Pfizer, Takeda Pharmaceutical, MSD, Drs Akihisa Okumura, Takuya Tanabe, and Hiroyuki Tsutsumi have received fees for reviewing data from Daiichi Sankyo; Dr Hiroyuki Tsutsumi has received consulting fees from Glaxo Smith Kline, Shimpei Niwa, Masato Fukushima, Rie Yonemochi, and Hisano Eda are employees of Daiichi Sankyo Co. Ltd, which manufactures and sells laninamivir octanoate hydrate in Japan.

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Supplementary material available online

Supplementary Table I showing the number of events in each AE group.

Supplementary material for Nakano T, et al. Safety evaluation of laninamivir octanoate hydrate through analysis of adverse events reported during early post-marketing phase vigilance, Scandinavian Journal of Infectious Diseases, 2013; doi: 10.3109/00365548.2012.763104.

Supplementary Table I. Number of events in each AE group.

AE group[a] (Total N_e = 288)	N_e	AE group[a] (Total N_e = 288)	N_e
Abnormal behavior/delirium	170	Respiratory disorder	34
Abnormal behavior	90	Cough	9
Delirium	24	Dyspnoea	5
Hallucination	17	Asthma	4
Inappropriate affect	5	Epistaxis	4
Restlessness	5	Dysphonia	2
Agitation	3	Choking sensation	1
Fear	3	Hyperventilation	1
Hallucination, visual	3	Nasal dryness	1
Depressed level of consciousness	2	Obstructive airways disorder	1
Altered state of consciousness	2	Oropharyngeal pain	1
Confusional state	2	Pneumonitis, chemical	1
Crying	2	Respiratory failure	1
Hallucination, auditory	2	Rhinitis, allergic	1
Illusion	2	Rhinorrhoea	1
Feeling abnormal	1	Sputum retention	1
Delusion	1	Shock	12
Fall	1	Dyspnoea	4
Phobia	1	Anaphylactic shock	2
Sleep disorder	1	Shock	2
Speech disorder	1	Respiratory failure	1
Syncope	1	Anaphylactoid reaction	1
Unresponsive to stimuli	1	Cold sweat	1
Dizziness/vertigo	20	Peripheral coldness	1
Dizziness	14	Syncope	17
Vertigo	4	Loss of consciousness	9
Dizziness postural	2	Syncope	3
		Depressed level of consciousness	2
		Altered state of consciousness	2
		Petit mal epilepsy	1

Ne, number of adverse events.

[a]AEs were coded according to the Medical Dictionary for Regulatory Activities (MedDRA, version 14.0) Preferred Terms. Forty AEs that were not categorized into any of the five groups were not included in this table. Five AEs were included in both the Respiratory disorder group and the Shock group.

原　著

小児におけるインフルエンザHAワクチン接種量変更による効果と安全性の検討

[1]三重県保健環境研究所，[2]独立行政法人国立病院機構三重病院，[3]まつだ小児科クリニック，[4]アクエア・メディカル・ステーション，[5]白子クリニック，[6]すずかこどもクリニック，[7]落合小児科医院，[8]さかとく小児科，[9]かとう小児科，[10]一般財団法人阪大微生物病研究会

高橋　裕明[1]　矢野　拓弥[1]　福田　美和[1]　山内　昭則[1]
大熊　和行[1]　庵原　俊昭[2]　中野　貴司[2]　松田　正[3]
鳥越　貞義[4]　二井　立恵[5]　伊佐地真知子[5]　渡辺　正博[6]
落合　仁[7]　酒徳　浩之[8]　加藤　孝[9]　前田　一洋[10]
奥野　良信[10]　神谷　齊[2]

（平成24年3月8日受付）
（平成25年1月9日受理）

Key words: influenza, vaccination, efficacy, safety

要　旨

小児におけるインフルエンザHAワクチンの年齢別1回接種量の規定を見直す基礎資料を得る目的で，WHO推奨用量と同一の年齢別接種量に増量した場合の有効性，安全性を検討した．国内で承認されているワクチンとして阪大微生物病研究会製，対照群としてサノフィパスツール社製を用い，6カ月～13歳未満児を対象に0.25mL接種群と0.5mL接種群を設定し，罹患状況，副反応発現状況等を調査し，接種前，2回接種後のHI抗体価を測定した．また，HI抗体価の結果を補完するため中和抗体価を測定した．その結果，A/H1N1型のHI抗体価は両接種群でビケン製，サノフィ製ともに良好な上昇傾向を示し，中和抗体価も同傾向を示した．A/H3N2型のHI抗体価はビケン製では全体に低値であったが，中和抗体価はビケン製，サノフィ製とも良好な上昇傾向を示した．B型のHI抗体価はビケン製，サノフィ製とも顕著な上昇傾向を示さなかったが，中和抗体価はともに良好な上昇傾向を示した．当該シーズンのインフルエンザ流行がA/H1N1型のほぼ単独流行であったことからA/H1N1型について発症に関する要因解析を行ったところ，発症リスクを下げる要因として接種後HI抗体価が40倍以上に上昇していることが有意となった．また，接種後HI抗体価40倍以上上昇群に対する同20倍以下群の発熱に関する相対危険が有意に高く，抗体価の高い群で発熱の程度が抑制される傾向がみられたことから，本研究の接種量で用いた両ワクチンとも発症予防効果を有すると認められ，さらに接種時に重篤な副反応の発現を認めず，同等の安全性を有すると考えられた．以上のことから，小児に対してインフルエンザHAワクチンをWHO推奨用量で接種した際の有効性，安全性を確認することができた．

〔感染症誌　87：195～206，2013〕

序　文

わが国におけるインフルエンザHAワクチンの小児における接種量は，2011/12シーズンからWHO推奨用量（6カ月～3歳未満：0.25mL，3歳以上：0.5mL）[1]に増量されたところであるが，それ以前は科学的根拠も不明確なまま，全粒子ワクチンの接種規定（6カ月～1歳未満：0.1mL，1～6歳未満：0.2mL，6～13歳未満：0.3mL，13歳以上：0.5mL）が適用されていた．わが国のワクチンは小児に対して効果が低いとされていたが，欧米のワクチンは小児に対しても効果があるとされ，ワクチンの質および接種量の違いが原因と考えられていた[2]．加えて，細分化された接種規定では新型インフルエンザのパンデミック発生時に混乱の元となることも危惧されていた．そこで，WHO推

別刷請求先：（〒512-1211）三重県四日市市桜町3684―11
三重県保健環境研究所　高橋　裕明

平成25年3月20日