Journal of Clinical Virology 107 (2018) 11–18

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

History of matrix genes mutations within PCR target regions among circulating influenza H3N2 clades over ten-plus-years

Kathleen A. Stellrecht

Department of Pathology and Laboratory Medicine, Albany Medical Center Hospital and Albany Medical College, MC-22 43 New Scotland Ave., Albany, NY 12208, United States

ARTICLE INFO

Keywords:
Influenza
Genetic drift
Clades
Matrix gene
Diagnostic
Testing
PCR

ABSTRACT

Background: Emerging influenza A/H3N2 clades have been associated with M1 gene mutations which affect the performance of commercial PCR assays.
Objectives and study design: The evolution and prevalence of problematic M1 mutations, and their associated viral clades, were investigated. All European and USA isolates from the GISAID database with both HA and M1 sequences available, collected during the respiratory seasons from the Fall of 2007 through January of 2018, were analyzed.
Results: Five M1 target region patterns, designated A–E, were observed in more than 10% of the isolates during a season, with patterns that appeared sequentially, each having one additional mutation. The C153T mutation was universal. Pattern A, which only had the single mutation, predominated between 2007/08 and 2009/10. Dual- and triple-mutation patterns (B and C) emerged in 2010/11 and 2011/12 respectively, and pattern C predominated for one season (2012/13). In 2012/13, the problematic quadruple-mutation containing C163T first appeared in 3C.2 viruses. Seasons 2013/14 and 14/15 were associated with significant viral diversity with five clades and four M1 patterns co-circulating, with different rates in Europe and the USA. Since 2014, clade 3C.2a with M1 pattern D has emerged as the predominant type. During 2016/17 season, a new quintuplet mutation pattern (E) emerged in cluster 3C.2a1 isolates.
Conclusions: M1 target region mutations have been prevalent for more than ten years, with the number of mutations continually increasing. Often population inferences of M1 mutations can be made based on viral clade. However, gene segment reassortment can affect predictive abilities.

1. Background

The World Health Organization (WHO) Global Influenza Surveillance Network has defined seven genetic groups for influenza A(H3N2) viruses based on haemagglutinin (HA) gene sequences since 2009 [1]. Before then, the A/Perth/16/2009 and A/Victoria/208/2009 genetic clades predominated. These clades were redefined and subdivided into clades 1 and 2 and clades 3–7, respectively [2]. More recently, clade 3 viruses have predominated and have formed subgroups. Group 3C had three subdivisions, 3C.1, 3C.2 and 3C.3, which were antigenically similar [3,4]. In 2014, three new genetic subgroups with unique HA mutations emerged: 3C.2a, 3C.3a and 3C.3b. Antigenic drift was demonstrated in subgroups 3C.2a and 3C.3a. Indeed, the 2014/15 influenza season in the United States of America (USA) was characterized by widespread circulation of multiple clades of H3N2 viruses, with the majority being antigenically different from the H3N2 vaccine component (clade 3C.1A/Texas/50/2012), leading to reduced vaccine effectiveness [5,6]. Again in 2017, clusters of further diversity have been observed among the 3C.2a subclade [7], with a proposed 3C.2a2 cluster being linked to vaccine failure in some countries [8].

Besides reduced vaccine efficacy, these emerging H3N2 clades were associated with mutations in the matrix protein (M1) gene affecting the performance of commercial nucleic acid amplification tests [9–12]. Although exact primer and probe sequences for most commercial assays are proprietary, it is presumed they follow WHO recommendations (M1 target region). During the 2014/15 season, co-circulation of 4 patterns of mutations were observed within this region. These patterns included the single C153T mutation, which has become universal in A(H3N2) strains since 2005 [9]; a dual-mutation of C153T and G183A; a triple-mutation of C153T, G180A and G189T; and the highly problematic quadruple-mutation containing C163T, in addition to the C153T, G180A and G189T mutations [13,14]. The C163T mutation resulted in a mismatch with the probe from one commercial assay [10] and the quadruple-mutation pattern was associated with false negative or weak

E-mail addresses: stellrk@mail.amc.edu, @KathyStellrk.

https://doi.org/10.1016/j.jcv.2018.08.002
Received 11 March 2018; Received in revised form 25 June 2018; Accepted 5 August 2018

positive results by many commercial kits [10,12]. In addition, assays with lower sensitivities during the 2014/15 season also demonstrated much higher lower-limits of detection with viruses harboring the C163T mutation in the M1 gene [12].

2. Objectives

This manuscript details the analysis of the WHO target region in A/H3N2 isolates from Europe and the USA over a ten-plus-year period to determine if additional mutational patterns developed. It also illustrates the evolution and rates of M1 mutation patterns and how these mutations evolved with H3N2 clades.

3. Study design

3.1. HA and M1 sequences

Sequence data were obtained from the Global Initiative on Sharing Avian Influenza Database (GISAID) EpiFlu. This database is comprised of influenza sequences for the semiannual vaccine strain selection uniquely submitted from contributors such as the Office of International des Epizooties; National Reference Laboratories and all WHO Collaborating Centers for Surveillance; Epidemiology and Control of Influenza [15]. Included were all unique human H3N2 isolates from Europe and USA collected during the respiratory virus season (September 1 through April 30, except 2018) for which both HA and M1 sequences were available over a 10-plus-year period (Fall of 2007 through January of 2018 as of Feb. 5, 2018).

3.2. Phylogenic analysis and clade determinations

Sequence alignments, motif searches and phylogenetic trees were performed with MEGA version 7.0 (Biodesign Institute, Tempe, AZ). Clade, subclade and cluster designations were based on signature amino acids deviations from A/Perth/16/2009 (clade 1) as reported as viral clades have evolved (Supplemental Tables S1 and S2) [1–3,7,8]. Phylogenetic trees of HA genes, constructed using the maximum-likelihood method with a Jones-Taylor-Thornton (JTT) + gamma amino acid substitution model and 2000 bootstrap replications, were used for clade determinations on isolates with partial changes in signature amino acids and to evaluate C3.2a clusters. NCBI BLAST was used to identify swine origin H3N2 variants (vH3) isolates in the USA and A/Sydney/5/97(H3N2)-like isolates (Sydny 97) observed in Heidelberg in 2015.

3.3. M1 pattern determinations

Nucleotides 144–251 from the WHO-recommended amplification target region in the M1 gene were aligned and unique sequences were counted. M1 target region patterns representing more than 10% of the population within a season were considered for analysis. Mutation rates were calculated as the average number of M1 target region mutations observed per isolate.

4. Results

4.1. M1 target region patterns

Nucleotides 144–251 from the M1 gene target region from all influenza isolates were evaluated. Ninety-eight unique patterns were observed (Supplemental Table S1); however, only five patterns, designated A–E, were observed in more than 10% of the A(H3N2) population during any given season (Table 1). All other unique patterns contained one of the five designated patterns and were classified accordingly. All patterns had a C153T substitution as compared to the WHO-recommended forward primer sequence. Pattern A only had this single-mutation pattern. Pattern B (dual-mutation pattern) also had a G183A

Table 1
WHO and CDC M1 Gene PCR Target Region Patterns. Underlined bases represent the recommended forward and reverse primer sequences and the bold font bases represent the recommended probe sequences. Shaded nucleotides represent divergence from the reference sequence (A/Wyoming/01/2003(H3N2)).

Pattern	Sequence (144–251)
WHO	AAGACCAATCCTGTCACCTCTGACTAAGGGGATTTTGGGG**TTTGTGTTCACGCTCACCGT**GCCCAGTGAGCGAGGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCT
CDC	-GACC**R**ATCCTGTCACCTCTGAC——————————————**CGTGCCCAGTGAGCGAGGACTGCA**—TAGACG**M**TTTGTCC**AR**AATGCCCT
A	——————T———
	———
B	——————T———
	A———
C	——————T——————**A**——————————————————————————————————
	T———
D	——————T——————T——————**A**——————————————————————————
	T———
E	——————T——————T——————**A**——————T————————————————————
	A———