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Incidence of matrix genes mutations affecting PCR tests among influenza H3N2 clades circulating during the 2014/15 season

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ABSTRACT

Influenza circulating during the 2014/15 season was associated with significant drift and the emergence of new H3N2 subgroups. It was also known that mutations were accumulating in the WHO-recommended amplification region of the M1 gene, affecting the performance of many commercial polymerase chain reaction assays. However, the prevalence of M1 target mutations was unknown. To investigate this, isolates from the GISAID database from Australia (n=100), Europe (n=473), and the USA (n=1175) were analyzed. The predominate clade was 3C.2a (75%), with a higher representation among USA isolates (81%). The M1 target demonstrated four primary sequence patterns, designated A–D. Pattern D was most often observed (84%). Some clades correlated with M1 target patterns, as seen between subclades 3C.2a and 3C.3a and patterns D and C, respectively. 3C.3 isolates were more diverse, with three patterns represented. Among the 3C.3b isolates, pattern B predominated in non-USA viruses, while pattern D predominated among USA isolates.

1. Introduction

The World Health Organization (WHO) Global Influenza Surveillance Network has defined seven genetic groups for influenza A (H3N2) viruses based on HA gene sequences, with more recent isolates being from the genetic group 3C. This group has three subdivisions: 3C.1, 3C.2, and 3C.3, which are antigenically similar (Broberg et al., 2015). 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 (European Centre for Disease Prevention and Control, 2015). 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.1 A/Texas/50/2012), leading to reduced vaccine effectiveness (Appiah et al., 2015; Flannery et al., 2015).

Besides affecting vaccine efficacy, influenza evolution has been associated with changes in diagnostic test sensitivity, particularly with rapid immunoassays (RIDT) (Busson et al., 2014; Centers for Disease Control and Prevention, 2009; Drexler et al., 2009; Ginocchio et al., 2009). Cell culture reliability has also been influenced at times, with changes in cell-line permissiveness (Frank et al., 1979; Memoli et al., 2009). Nucleic acid amplification tests for influenza A are designed to detect highly conserved genomic targets, generally in the matrix protein 1 (M1) gene (Ward et al., 2004; World Health Organization (WHO), 2009, 2011). However, mutations affecting the performance of commercial assays have long been observed with individual A(H1N1)pdm09 isolates (Binnicker et al., 2013; Dhiman et al., 2010; Zheng et al., 2010).

More recently, M1 mutations affecting the performance of polymerase chain reaction (PCR) tests with populations of H3N2 viruses have been reported in Taiwan, Belgium, Germany, and the USA (Huzly et al., 2016; Overmeire et al., 2016; Stellrecht et al., 2017; Yang et al., 2014), with a C163T mutation being the most problematic (Overmeire et al., 2016; Stellrecht et al., 2017). Although exact primer and probe sequences for most commercial assays are proprietary, it is presumed that they follow WHO recommendations. It was reported that C163T mutations in 3C.2a isolates from Belgium resulted in a mismatch with the probe from one commercial assay and subsequent false-negative test results (Overmeire et al., 2016). Previously, my laboratory compared the limit of detection of five commercial assays among an assortment of viral isolates from different clades and demonstrated that assays having the lowest clinical sensitivity with specimens from the 2014/15 season also had the lowest analytical sensitivity with isolates harboring the C163T point mutation (Stellrecht et al., 2017).

The manufacturer of one commercial kit modified their package insert in 2015 to indicate assay limitations with A/New York/1/2015 (H3N2), but they reported that this issue was restricted to viruses circulating in a discrete region of New York State. Indeed, it was unknown how common the M1 C163T mutation was among influenza isolates or even how conserved the M1 target region was among circulating viral clades during the 2014/15 season. It was also unknown if particular

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M1 mutations were associated with the various co-circulating subclades. Hence, H3N2 isolates from three distinct locations across the world were investigated to assess the incidence of mutations within the M1 target region during the 2014/15 respiratory virus season. From these data, mutation patterns in the target region were determined, and attempts were made to associate these patterns with H3N2 clades.

2. Materials and methods

2.1. HA and M1 sequences

Sequence data were obtained from the Global Initiative on Sharing Avian Influenza Database (GISAID) EpiFlu. This dataset is comprised of influenza sequences uniquely submitted from contributors such as the Office International des Epizooties; National Reference Laboratories; and all the WHO Collaborating Centers for Surveillance, Epidemiology, and Control of Influenza for the semiannual vaccine strain selection (Shu and McCauley, 2017). Included were all unique human H3N2 isolates for which both HA and M1 sequences were available and collected from Australia in 2014 (n=100) and from Europe and USA during the 2014/15 respiratory virus season (collection date: September 1 through April 30, n=473 and 1175, respectively; accession numbers shown in Supplemental Table 1). Three other well-characterized isolates, A/Texas/50/2012, A/Switzerland/9715293/2013, and A/Hong Kong/5738/2014, were included in the phylogenetic tree (see below) for benchmarking purposes. Likewise, four European clade 3C.2 isolates from the spring of 2014 were included in the phylogenetic tree to improve clustering of the clade (Broberg et al., 2015).

2.2. Phylogenic analysis and clade determinations

Sequence alignments, motif searches, and phylogenetic trees were performed with MEGA version 7.0 (Biodesign Institute, Tempe, AZ). A phylogenetic tree of the HA genes was constructed using the maximum-likelihood method with a Hasegawa–Kishino–Yano (HKY) + gamma nucleotide substitution model and 2000 bootstrap replications. The ability to perform clade designations based on signature amino acids as compared to A/Texas/50/2012-like A/H3N2-like clade 3C.1 viruses (European Centre for Disease Prevention and Control, 2015) was confirmed with the 64 isolates depicted in the final tree, and extended to the other 1684 isolates. Specifically, the amino acid changes associated with antigenic drift in clade 3C.2 (A/Hong Kong/146/2013-like) were N145S and V186G; L3I, N144S, N145S, F159Y, K160T, V186G, N225D, and Q311H in subgroup 3C.2a (A/Hong Kong/5738/2014-like); T128A, R142G, N145S, and V186G in clade 3C.3 (A/Samara/73/2013-like); T128A, A138S, R142G, N145S, F159S, V186G, and N225D in subgroup 3C.3a (A/Switzerland/9715293/2013-like); and E62K, K83R, N122D, T128A, A138S, R142G, N145S, L157S, V186G, and R261Q in subgroup 3C.3b (A/Newcastle/22/2014-like). NCBI BLAST was used to determine if A/Wisconsin/24/2014 was a variant H3N2 (vH3) isolate with swine origin.

2.3. M1 pattern determinations

Nucleotides 144–251 from the WHO-recommended amplification target region in the M1 gene were aligned, and unique sequences were counted. Previously published patterns and those representing more than 1% of the population were considered for investigation.

2.4. Statistical analysis

Cross-tabulation with column proportions tests and Bonferroni-adjusted P values was performed using SPSS Statistics version 21 (IBM Corp., Armonk, NY). First, Mahalanobis' distance was used to determine and remove multivariate outliers, which were the vH3 isolate and the M1 pattern A isolate (A/New York/07/2015).

3. Results

3.1. Influenza H3N2 clade determination

In an analysis of the HA genes, all viruses except the vH3 (not shown) clustered into the 3C subgroups (Fig. 1, Table 1). The predominant clade in this sample set was 3C.2a at 75%. 3C.3b was the second most prevalent clade at 16%. However, the clade distribution was skewed by overrepresentation of the population with USA data. Each location demonstrated different clade rates. Isolates from Australia were equally distributed over four clades, with the rates for 3C.2a, 3C.3, and 3C.3b ranging from 26% to 28% and the rate for 3C.3a being 16%. Clades 3C.3 and 3C.3a represent a small percentage of the European and USA populations. The USA data also further skewed the overall total data with a disproportionally higher number of 3C.2a isolates and lower number of 3C.3b isolates.

3.2. M1 gene target region patterns

Nucleotides 144–251 from the M1 gene target region from all 1748 influenza isolates were evaluated. Thirty-seven unique patterns were observed; however, only four patterns, designated A–D, were observed in more than 1% of the population or were of historical significance (pattern A). All 34 other unique patterns contained 1 of the 4 designated patterns and were classified accordingly. All patterns have a C153T substitution as compared to the WHO-recommended forward primer sequence (Table 2). Pattern A has no additional substitutions. Pattern B has a G183A substitution, whereas patterns C and D have both G180A and G189T substitutions. Pattern D also has a fourth C163T mutation which was associated with reduced sensitivity for many commercial PCR tests (Overmeire et al., 2016; Stellrecht et al., 2017).

The quadruple-mutation pattern D predominated (84%, Table 1), but again, this rate was skewed by the USA data where pattern D was observed in 94% of the isolates. Each location demonstrated different pattern rates. Pattern D also predominated in Europe, but there was an equal distribution of patterns B–D in Australia. Only one isolate had pattern A, a pattern which historically was very common prior to 2014 (data not shown).

3.3. Correlation between viral clades and M1 target region patterns

Correlations between M1 target region patterns and influenza clades were variable (Table 3). For example, most 3C.2a isolates demonstrated pattern D, and all 3C.3a isolates demonstrated the triple-mutation pattern C. However, M1 target region patterns in 3C.3 isolates were more diverse, with patterns B, C, and D being observed. Interestingly, clade 3C.3b was associated with pattern B in non-USA isolates and with pattern D in USA isolates.

3.4. Monthly trends in clade and M1 target region pattern rates

Analysis of the monthly rates for both viral clade and M1 target region patterns provided an interesting perspective. In Australia, co-circulation of the clades 3C.2a, 3C.3, and 3C.3b continued throughout their respiratory virus season (March–October), with no clear trend emerging except that 3C.3a viruses were more prevalent earlier in the season while the opposite was true for clade 3C.3b viruses. In Europe, the respiratory virus season began with a strong predominance of clade 3C.2a viruses, but as the season went on, the rates of 3C.3b increased (Fig. 2B). In the USA, clade 3C.2a predominated all season, but initially, a high rate of clade 3C.3 was observed in September (Fig. 2C). Clade 3C.3b appeared between November and February.

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