Molecular and phylogenetic analyses of influenza B viruses isolated from pediatric inpatients in South Korea during the 2011–2012 winter season

Jeong-Hyun Nam,† Eun-Jung Song,† Daesub Song,† Erica España, Sang-Mu Shim, Seo-Hee Jeong, Robert G. Webster, Woo-Joo Kim,* and Jeong-Ki Kim*

Abstract

Influenza B virus remains a major cause of respiratory diseases worldwide. Because of limited epidemiological and genetic data, the local and global transmission patterns of influenza B virus are not fully understood. Here we report the molecular and phylogenetic characterization of 163 influenza B virus isolates from pediatric inpatients with influenza-like illness in the winter of 2011–2012 in South Korea. Analysis of haemagglutinin and neuraminidase genes of the influenza B isolates revealed that both B/Victoria (62 %) and B/Yamagata lineages (38 %) co-circulated during that influenza season, and a considerable number of the isolates carried several amino acid substitutions in the four major antigenic epitopes of their haemagglutinin protein.

Influenza viruses belong to the family Orthomyxoviridae, which consists of segmented, single-stranded negative sense RNA viruses. They can be mainly classified into three types: influenza A, B and C. Among these, type A and B viruses are considered major causes of respiratory infections by influenza virus, contributing to morbidity and mortality in humans globally [1–5]. Generally, children are more susceptible to influenza infection owing to their naïve immunological state [6, 7]; however, community-based surveillance programmes have shown that influenza A H3N2 subtype virus is more common among adults, whereas influenza A H1N1 subtype and influenza B viruses are detected more frequently in children [8].

Influenza A virus infects a wider range of species, such as humans, swine, avian and equine, than influenza B virus, which primarily infects humans [9]. In contrast to influenza A virus, which has different subtypes that cause human infection (e.g. H1, H2 and H3), influenza B virus has no known subtypes [10]. Influenza virus evolves via two mechanisms: antigenic drift and genetic shift [9]. Antigenic drift is brought about by amino acid substitutions within the antibody-binding sites in both haemagglutinin (HA) and neuraminidase (NA) [11], and genetic shift is caused by gene reassortment among viruses within a given subtype or between different subtypes [12]. Although influenza A virus can undergo antigenic shift due to genetic reassortment between different subtypes or strains, influenza B virus variants are generated through antigenic drift commonly by insertion, deletion, and substitution [11, 13]. This antigenic drift of influenza B virus enables the virus to evade host immunity and facilitates evolution without antigenic shift [13].

Currently, influenza B virus can be subdivided into two major lineages, namely, Victoria and Yamagata, which have evolved distinct genetic and antigenic characteristics [14, 15]. These two lineages, B/Victoria/2/87-like and B/Yamagata/16/88-like, have been co-circulating in many regions of the world since 1983 [14, 15]. Studies have shown that vaccination against one lineage results in little or no cross-protection from the other [16, 17]. Therefore, various studies suggest that the use of quadrivalent vaccines that include both influenza B lineages can be more effective than the traditional trivalent seasonal influenza vaccine, which contains only one influenza B lineage [18, 19].

Clinical surveillance was conducted here using the clinical influenza-like illness (ILI) criteria. A total of 1662
nasopharyngeal swab specimens were collected from children hospitalized with an ILI in Korea University Guro Hospital, South Korea, during the 2011–2012 winter season. Among those, a total of 167 specimens were influenza B-positive according to a rapid diagnostic test (SD Bioline Influenza Antigen Test, Standard Diagnostic, South Korea). Virus isolation was performed in Madin–Darby canine kidney (MDCK) cells, and 163 influenza B virus isolates (97.6% of the influenza B-positive specimens) were successfully recovered. Viral RNA was extracted by means of the RNeasy Mini kit (Qiagen, Valencia, CA). RT-PCR was carried out to amplify the viral gene segments using the One-Step RT-PCR kit (Qiagen, Valencia, CA) with primers described previously [20–23]. The amplified gene segments were commercially sequenced at Cosmogenetech (Seoul, South Korea).

A phylogenetic analysis was performed on their HA gene segments to identify and characterize the genetic lineages of the isolates. The nucleotide sequences of their HA gene segments were edited in the Lasergene sequence analysis software, package version 5.0 (DNASTAR, Madison, WI), and aligned in CLUSTAL V [24]. Rooted phylograms were produced by the neighbour-joining (NJ) method and then plotted in the NJ Plot software [25]. The branch lengths were proportional to the sequence divergence on the scale of 0.01 nucleotide changes per site. As shown in Fig. 1, 101 (62 %) isolates belong to the influenza B/Victoria lineage, and 62 (38 %) isolates belong to the B/Yamagata lineage, meaning

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**Fig. 1.** Phylogenetic relations of the HA gene of our influenza B isolates and vaccine strains. The phylogenetic tree was constructed by means of the nucleotide sequences of the HA gene from our isolates and from the vaccine strains that were available in GenBank. The phylograms were generated by the neighbour-joining (NJ) method with 1000 bootstrapped replicates; the NJ percentage bootstrap value (> 600) for each node is shown in the tree. Branch lengths were proportional to the sequence divergence on the scale of 0.01 nucleotide changes per site. It includes the vaccine strains that have been recommended from 2006 to 2015: B/Malaysia/2506/2004 (MYS/2506), B/Florida/4/2006 (FL/4), B/Brisbane/60/2008 (Bris/60), B/Wisconsin/01/2010 (WI/01) and B/Massachusetts/02/2012 (MA/02).
that the two major lineages co-circulated in the 2011–2012 winter season in South Korea with comparable incidence rates. In the analysis of the HA gene, the isolates of the Victoria lineage were clustered into one mono-phylogenic clade and showed a close relationship with the B/Brisbane/60/2008 vaccine strain (Victoria lineage) used from 2009 to 2012 (Fig. 1). In the Yamagata lineage, on the other hand, the isolates were clustered into two genetic clades. One clade with only six isolates revealed high sequence homology with the B/Wisconsin/01/2010 vaccine strain (Yamagata lineage) used in the winter of 2012–2013 (Fig. 1). This clade shared the amino acid substitutions K63R, A123P, N131K, S165I, N181Y, A197T, N218S and G245D in the HA gene that are not found in the other Yamagata-lineage isolates. Thus, according to our phylogenetic characterization of the influenza B virus isolates from the 2011–2012 winter season in South Korea, 101 out of 163 isolates (62%) were highly similar to the influenza B vaccine strain (B/Brisbane/60/2008) of the corresponding year, while the rest of the isolates were quite different from the vaccine strain.

Amino acid sequence variations in the HA surface glycoprotein affect both antigenic and receptor-binding epitopes, and a previous study reported that four major antigenic epitopes play critical roles in the epidemic potential of influenza B virus: the 120-loop (positions 116–137), the 150-loop (141–150), the 160-loop (162–167) and the 190-helix (194–202) [26, 27]. Among these, the 120-loop epitope was found to be the most frequently mutated region [28]. The 150-loop epitope is specific to the Yamagata lineage, whereas the 160-loop epitope is specific to the Victoria lineage [26]. Furthermore, the 160-loop epitope is known to be the only region in the HA of influenza B virus with insertions, deletions and single amino acid substitutions in field isolates [13, 26, 29]. The 190-helix, which is a part of the receptor-binding site (RBS) of the HA of the influenza B virus, is considered to be one of the most important epitopes on the protein [26]. In our molecular characteristic study, a comparison of the amino acid sequences in major antigenic epitopes of the HA1 region with the representative influenza B virus vaccine strains in use from 2006 to 2013 uncovered several amino acid substitutions (Table 1). We confirmed some amino acid mutations in these significant regions. In detail, in the Victoria lineage, we found T136A and H137Y in the 120-loop region in one isolate each out of 101 isolates, and A142T in the 150-loop region in one isolate. In the Yamagata lineage, we detected P123A and N131K in the 120-loop region in 56 and 6 out of 62 isolates, respectively. We also found T197A in 56 isolates and E198G in one isolate within the 190-helix loop region (Table 1). All isolates of the Yamagata lineage had a deletion at amino acid position 178 of HA1 instead of Asparagine shown in the Victoria lineage isolates (data not shown). This deletion of the amino acid residue 178 was known as a determinant of the antigenic difference between the two major lineages (Victoria and Yamagata) [30]. We observed that most of the amino acid substitutions were located in the 120-loop and 190-helix, and Yamagata isolates seemed to be more diverse than the Victoria isolates (Table 1).

Table 1. Comparison of amino acid sequences of the HA1 regions (four major antigenic epitopes)

<table>
<thead>
<tr>
<th>Residues at site</th>
<th>Vaccine strains</th>
<th>2011–2012 isolates$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B/Malaysia/2506/04$^{a}$</td>
<td>B/Brisbane/60/08$^{b}$</td>
</tr>
<tr>
<td>120-loop (residues 116–137)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>123</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>131</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>136</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>137</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>150-loop (residues 141–150)</td>
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</tr>
<tr>
<td>141</td>
<td>N</td>
<td>N</td>
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<tr>
<td>142</td>
<td>A</td>
<td>A</td>
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<tr>
<td>144</td>
<td>N</td>
<td>N</td>
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<tr>
<td>160-loop (residues 162–167)</td>
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<tr>
<td>163</td>
<td>N</td>
<td>N</td>
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<tr>
<td>164</td>
<td>G</td>
<td>G</td>
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<tr>
<td>165</td>
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<td>N</td>
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<tr>
<td>190-helix (residues 194–202)</td>
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<tr>
<td>197</td>
<td>T</td>
<td>T</td>
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<tr>
<td>198</td>
<td>E</td>
<td>E</td>
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</tbody>
</table>

$a$, Victoria vaccine strain used from 2006 to 2008.
$b$, Victoria vaccine strain used from 2009 to 2012.
$c$, Yamagata vaccine strain used from 2008 to 2009.
$d$, Yamagata vaccine strain used from 2012 to 2013.
$e$, The number in parenthesis is the number of isolates.
uncovered amino acid substitutions in the 120-loop regions of both influenza B virus lineages, suggesting that this site is the most frequently mutated HA region, in agreement with findings in other studies [28, 31]. The PI23A, N131K, T136A, H137Y and A142T detected in this study suggest potential differences in antigenicity between the samples and the vaccine strains. Isolates with point mutations at the 190-helix (T197A and E198G) obtained here might also have alterations in the receptor-binding proteins compared to the vaccine strains.

Among the eight influenza B virus genomic segments, the NA performs an important function in virus replication by releasing viral progenies [32]. The NA activity inhibitors, such as oseltamivir and zanamivir, play key roles in the control of influenza B virus infection during outbreaks [33]. It has been reported that E117V/A, D197N/E/Y, I221T, H273Y, R292K and R374K mutations of the influenza B virus NA gene could reduce viral susceptibility to oseltamivir and zanamivir [34–36]. However, the NA protein sequences that we analysed in all of the influenza B isolates did not bear these mutations. Therefore, all these isolates might be sensitive to NA inhibitors.

As shown by our results, influenza B viruses of the Victoria and Yamagata lineages co-circulated in South Korea in the winter of 2011–2012 with similar prevalence. Notably, however, only the B/Victoria/87 lineage (B/Brisbane/60/2008) was recommended by the WHO as the influenza vaccine for that season. Such cases, where a vaccine targets only one of two circulating influenza B virus lineages or where the vaccine is a complete mismatch to the predominant lineage, have caused a significant public health burden due to influenza B virus infection [19, 37]. This situation highlights the need to use quadrivalent vaccines for improved vaccine-mediated protection as recommended by more in-depth studies [18].

In summary, we described the phylogenetic and molecular characteristics of the influenza B viruses isolated from pediatric inpatients in Seoul, South Korea during the 2011–2012 winter season. In particular, we focussed on the HA gene and protein to identify differences between the isolates and vaccine strains, including the vaccine strain of the corresponding year. Data on the influenza B clinical isolates in this study suggested the co-circulation of the Victoria and Yamagata lineage strains and showed several amino acid substitutions in the four major antigenic epitopes of the HA. Moreover, no amino acid substitutions were detected at the NAI sites in all the isolates. This study might provide significant information on the incidences of influenza B virus infection especially in South Korea and contribute to the decision-making regarding the prophylaxis and/or treatment of influenza B virus infections, especially regarding the ideal choice for the design of the seasonal influenza vaccine.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

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