Molecular epidemiology of rhinovirus among hospitalised patients, Singapore

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Summary

Human rhinovirus (HRV) is the most prevalent respiratory etiological agent in the world. Over 100 genotypes have been characterised using molecular genotyping techniques. Here, we characterised the molecular epidemiology of the circulating rhinoviruses among hospitalised patients in Singapore by sequencing 134 rhinovirus-positive respiratory specimens that were collected in the period between 2013 and 2015. Each sequence was assigned a genogroup and a genotype using the Enterovirus Genotyping Tool Version 0.1 and phylogenetic reconstruction, respectively. In this study, HRV-A (n=88) and HRV-C (n=38) were identified as the dominant genogroups in Singapore. HRV-A28 (n=7) was the dominant genotype in HRV-A while both HRV-C2 (n=8) and HRV-C11 (n=8) were the dominant genotypes in HRV-C. HRV-B was observed to have the lowest number of positive detections in our study population (n=8). The result is interesting as another group had previously found HRV-B to be the second most common genogroup in Singapore after HRV-A.

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Introduction

The human rhinoviruses (HRVs, family *Picornaviridae*, genus *Enterovirus*) are positive-sense, single-stranded-RNA viruses and are responsible for more than 50% of all common cold incidences (2). HRV exhibits high genetic diversity and over 100 genotypes have been categorised. Previously, HRVs were serotyped using serotype-specific antisera to differentiate between HRV-A and HRV-B. Recently, a hitherto unreported genogroup, HRV-C, has been identified using molecular genotyping techniques. The traditional serotyping method is unable to detect this genogroup as it cannot be cultured (1).

To the best of our knowledge, there has only been one Singapore-based study that examined the molecular epidemiology of HRVs (6). The study was conducted in the period between 2005 and 2007 and the study population involved hospitalised children only. In the present study, we aimed to characterise the more recent molecular epidemiology of the circulating HRVs among hospitalised patients in Singapore. One-hundred and thirty-four HRV-positive specimens, collected in the period between 2013 and 2015, were included in the study. The specimens were genotyped using a previously described method (3).

Materials and Methods

This study received local institutional ethics approval (from the National Healthcare Group Domain-Specific Review Board A, reference number: 2016/00044). The study was carried out on 134 clinical respiratory specimens that were submitted to the Molecular Diagnosis Centre, Singapore National University Hospital, for respiratory virus detection in the period between January 2013 and June 2015. These clinical specimens were archived specimens that were previously identified as HRV positive by a laboratory-developed multiplex RT-PCR respiratory viral panel. In the current study, a set of primers was used to amplify a 390-bp region within the 5'UTR as previously described (3). The RT-PCR products were then purified with the QIAGEN QIAquick gel extraction kit (Qiagen, Hilden, Germany) and sequenced bi-directionally using the Applied Biosystems 3130xl genetic analyzer (Thermo Fisher Scientific, Wohlen, Switzerland). Assembly of the sequences was performed using the Assign™ ATF software (Conexio Genomics, Fremantle, Australia). A web-based typing website, Enterovirus Genotyping Tool Version 0.1 was used for genogroup assignment (4). Genotype assignment was conducted based on phylogenetic inference using minimum-evolution subtree-pruning-regrafting and maximum-likelihood nearest-neighbour

interchanges with 1000 bootstrapped replicates. The evolutionary distances were computed based on the general time reversible nucleotide substitution model with FastTree 2 (5). One hundred and fifty-six HRV complete genomes were obtained from GenBank to serve as reference genotypes in the phylogenetic analysis.

Results

All of the 134 specimens were successfully sequenced. The phylogenetic reconstruction was able to correctly classify the 156 GenBank sequences according to their respective genotypes. The 134 clinically isolated HRV specimens were also fully assigned a genotype based on the phylogenetic analysis. A total of 60 unique genotypes were identified. Of these unique genotypes, 66.7% (40/60) belonged to HRV-A, 11.7% (7/60) belonged to HRV-B, and 21.7% (13/60) belonged to HRV-C. For genogroups, 65.7% (88/134) were HRV-A, 6.0% (8/134) were HRV-B, and 28.3% (38/134) were HRV-C.

Discussions and Conclusions

In summary, we described the use of a partial 5UTR region of the HRV genome for accurate typing of 134 rhinovirus-positive specimens that were collected from hospitalised patients over a period of three years. In this study, HRV-A and HRV-C were identified as the dominant genogroups in Singapore. HRV-B has the lowest number of positive detections in our study population. This is interesting as another group had previously found HRV-B to be the second most common genogroup in Singapore (6). This discrepancy may be attributed to the different recruitment criteria of the study population (hospitalised children *vs.* hospitalised patients of all age groups) and sampling periods (2005-2007 *vs*. 2013-2015). HRV-A28 (n=7) was the dominant genotype in HRV-A while both HRV-C2 (n=8) and HRV-C11 (n=8) were the dominant genotypes in HRV-C. None of the HRV-B genotypes appears to be dominant due to the low prevalence of HRV-B.

There have been several reports on the association of HRV-C infection with asthma attacks, wheezing occurrence, and severe pneumonia (6-8). Therefore, the ability to provide genotype information for the HRV strain that is infecting a patient may present an advantage for the prevention and control of HRV-associated acute respiratory diseases.

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