LETTER TO THE EDITOR

Diverse human rhinoviruses A and C from children with respiratory infections in Kuala Lumpur, Malaysia

Dear Editor,

We read with interest the recent paper by Xiang and colleagues describing the molecular epidemiology of human rhinovirus in China. Human rhinoviruses (HRVs) were first discovered more than 50 years ago. HRVs are a common cause of upper respiratory tract infections, but are also associated with asthma exacerbations and more severe respiratory infections. HRVs can be divided into three groups, A, B and C, with the latter, HRV-C, only reported in 2007. There are more than 100 types of HRV-A and B, while discovery of new HRV-C still continues. The high genetic and antigenic heterogeneity amongst the HRV results in lack of protective immunity and repeated infections. With the wider availability of molecular diagnostic methods, there is great interest in determining the global range of HRV variants, for subsequent development of antivirals and vaccines. As data from tropical Asian countries is relatively lacking, we report here the clinical manifestations of HRV infection and the molecular typing of HRV detected at the University Malaya Medical Centre, a major 900-bed referral and teaching hospital in Kuala Lumpur, Malaysia.

As part of ongoing studies on respiratory virus infections in children seen at our hospital, nasopharyngeal aspirates or throat swabs collected from children with respiratory signs and symptoms between June 2007 and May 2011 were tested for HRV. Over the four years, 1096 samples were tested and 87 (7.9%) were positive for HRV. Viral RNA was extracted from 30 randomly selected specimens using QIAamp viral RNA kit (Qiagen, Germany) and stored at −80°C. RNA was first transcribed to cDNA using SuperScript III (Invitrogen, USA). PCR was performed using previously published VP4/VP2 primers. The same primers were used for sequencing on an ABI 3730XL Genetic Analyzer (Applied Biosystems, USA). The partial VP4/VP2 sequences have been shown to correlate with serotype classification. Sequences of 400 nucleotides obtained were aligned with all reference HRV genomes available in GenBank, using Clustal X version 2.12. Phylogenetic trees were constructed using the neighbour-joining method with 1000 bootstrap replicates and depicted with MEGA version 5. This study was approved by the hospital’s Medical Ethics Committee (no. 788.3).

Based on the sequences of partial VP4/VP2 obtained from 30 children, 40% (n = 12) were HRV-A and 60% (n = 18) were HRV-C, and no HRV-B was identified. These sequences have been deposited into GenBank (accession numbers JQ356547–JQ356576). The absence of HRV-B is consistent with the findings of others where HRV-B are often the least commonly detected species (0–11%). However, it could also be due to the small number of HRV sequenced in the present study. In most other studies, however, the predominant species reported were HRV-A, followed by HRV-C.

Among the HRV positive patients, 60% (n = 18/30) were males and the median age was 1.1 years (range 2 months–10 years). Medical records were available for 23 of the patients. Ten (43.5%) had pre-existing medical conditions, including 6 with asthma. The commonest (82.6%) presentation was wheezing and this was as previously described, either as an exacerbation of asthma or bronchiolitis. Other frequently reported symptoms were coryza (69.6%), fever (43.5%), and diarrhoea or vomiting (34.8%). One patient died during admission, but this was most likely due to underlying leukaemia. Excluding two nosocomial cases, the average duration of admission was 3.5 days.

Pairwise sequence alignment against the reference HRV-A and HRV-B types in the VP4/VP2 region suggests 75–88% and 75–89% sequence similarities amongst all the isolates, respectively. The nucleotide sequence divergence of the 12 HRV-A from this study ranged from 4 to 11%, and belonged to 11 different subtypes, including HRV-49 (n = 2), HRV-2, HRV-10, HRV-12, HRV-15, HRV-21, HRV-46, HRV-55, HRV-56 and HRV-60 (Fig. 1). One isolate had 90% nucleotide similarity to both HRV-29 and HRV-44, and requires further confirmation using VP1 or 5′ untranslated region sequences. Comparison with 20 previously published HRV-A sequences from Malaysia from 2009 showed little overlap, except for clusters of 5 and 2 strains of HRV-12 and HRV-56, respectively.

Currently, the molecular classification of HRV-C is still not agreed upon. Using the proposed classification with a cut-off of >10% nucleotide divergence in partial
VP4/VP2 sequences, we classified the 18 HRV-C into 15 different subtypes (Fig. 2), with nucleotide divergence ranging from 2 to 10%. The HRV-C types identified were HRV-C10 (n = 2), HRV-C22 (n = 2), HRV-C23 (n = 2), HRV-C32 (n = 2), HRV-C2, HRV-C6, HRV-C12, HRV-C14, HRV-C17, HRV-C26, HRV-C37, HRV-C40 and HRV-C42, and the provisionally-assigned types, HRV-C_pat15 and HRV-C_pat16. Comparison with 14 available HRV-C from Malaysia detected in 2009 also showed the presence of HRV-C10, HRV-C17, HRV-C26 and HRV-C_pat16.

Although the number of HRV sequences analyzed in our centre was small, we found a wide variation by year in HRV-A and HRV-C types, even over a short period of several years. The comparison with other published Malaysian sequences also suggests extensive HRV diversity within Malaysia, as in other countries. The heterogeneity of HRVs causing respiratory infections among Malaysian patients, with no predominant subtypes, suggests that sustained community transmission is driven by many different subtypes, rather than a small number of outbreak strains. More detailed studies of the incidence...
and epidemiology of HRV and other emerging respiratory viruses in Malaysia are necessary, and are ongoing at our centre.

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**References**


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