Human Rhinovirus Species and Season of Infection Determine Illness Severity

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Supplementary Method and Data

456 words

HRV molecular typing. HRV samples were analyzed with molecular typing assay performed as described (1) or with a new simplified method that used a modified PCR protocol to generate a longer PCR fragment for direct sequencing. Briefly, the new PCR protocol replaces the P2-1, P2-2 and P2-3 primers used in the second PCR of the original protocol (1) with primer P3-1 (1). This new protocol produces a longer 390 nt (instead of the original 300 nt) PCR fragment that can be sequenced directly with primer P3-1 to generate the P1-P2 sequences for typing. An isolate is assigned as a classical serotype (prefixed with R) or a new type (W) by phylogenetic tree analysis as described (1). Briefly, if the new sequence clusters with the sequence of one of the 101 classsical serotypes, it is assigned as that serotype, and if the new sequence has 9% pairwise nucleotide divergence from the nearest serotype, it was designated as a new type. The species assignment of each new type was confirmed by sequencing representative samples at the 420-nt VP4-VP2 coding region as described.(2)

Notably, the molecular typing assay was even more sensitive for HRV detection than the respiratory multicode assay (RMA). It detected HRV in 79 of the 820 samples that were tested negative for HRV by RMA.

In this study, we identified a total of 97 types: 47 classical A serotypes, 10 classical B serotypes, 3 new A types and 37 C types. Two new A types and 34 HRV-C types have been reported previously. (1, 3-7) Only HRV-A-W22 and HRV-C-W5, -W34 and -W40 were new detections.

Although some other investigators are promoting the use of VP4-2 sequence for HRV typing (8), we chose to use 5' NCR sequence for several reasons. First, typing with 5' NCR sequence is much more sensitive than with VP4-2 sequence. In this study, our typing method was successful in 98.7% of HRV samples. This provided us an unbiased data set required for testing the relationships between species, type, and illness outcome. In the early stage of our study, we

tested VP4-2 typing method (2) and found its sensitivity was only about 50% of the 5’ NCR method for our clinical samples. Second, it has been shown that the 5' NCR sequence is as accurate as the VP1 (1) and VP4-2 (2) sequences for identifying individual HRV types. Reference 14 tested 79 samples, all 5'NCR typing results were in agreement with the VP1 typing results (Table 1 of reference 14)(1). In reference 23, only 1 of the 71 5'NCR typing results disagreed with the VP4-2 typing results (Table 2 of reference 23)(2).

We acknowledge that the 5' NCR and VP4-2 sequences produce differences in determining the phylogenetic grouping of HRV(1, 2, 8). Since 2005, we have used the 5' NCR sequence to type more than 1500 HRV samples and identified a total of 46 HRV-C types. According to the 5’NCR sequences, 39 HRV-Cs clearly segregate from HRV-A types and 7 HRV-Cs (W2, W7, W15, W24, W27, W29 and W36) cluster with 3 HRV-A serotypes (R12, R45 and R78) (data not shown). The biological reason for this difference is not understood.

Relationship between our new types and designations of Simmonds et al. Simmonds et al (9) have proposed a nomenclature for HRV-C that is based on nucleotide sequence similarity in segments of coding region of capsid proteins (VP4-VP2 and VP1). To relate our W types to Simmonds’ assignments, the nucleotide sequences of VP4-VP2 coding regions were compared. Table 1 shows the conversion between our W types and Simmonds’ types.

Table E1. Conversion of designations of new HRV type*

W type	Simmonds type	W type	Simmonds type
W01	HRV-C36	W27	HRV-C39
W02	HRV-C3	W28	HRV-A**
W03	HRV-C14	W29	HRV-C21
W04	HRV-C44	W30	HRV-C31
W05	HRV-C25	W31	HRV-C9
W06	HRV-A**	W32	HRV-C37
W07	HRV-C13	W33	HRV-C49
W08	HRV-C18	W34	HRV-C24
W09	HRV-C12	W35	HRV-C_pat17
W10	HRV-C15	W36	HRV-C43
W11	HRV-C40	W37	HRV-C26
W12	HRV-C2	W38	HRV-C16
W13	HRV-C45	W39	HRV-C47
W14	HRV-A**	W40	HRV-C35
W15	HRV-C22	W41	HRV-C19
W16	HRV-C42	W42	HRV-C30
W17	HRV-C23	W43	HRV-C50
W18	HRV-C_pat19	W44	HRV-C27
W19	HRV-C46	W45	HRV-C29
W20	HRV-C28	W46	HRV-C5
W21	HRV-C_pat22	W47	HRV-C_pat28
W22	HRV-A**	W48	HRV-A**
W23	HRV-C11	W49	HRV-A**
W24	HRV-C6	W50	HRV-C34
W25	HRV-C_pat20	W51	HRV-C_pat22
W26	HRV-C_pat10	W52	HRV-C_pat21

* The table lists conversion of our designations to those of Simmonds et al (9) based on 5'UTR and VP4-VP2 sequences.
** Indicates HRV-A types that have not previously been described.

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