JOURNAL OF VIROLOGY, Apr. 2008, p. 3809–3810 Vol. 82, No. 7
0022-538X/08/$08.00+0 doi:10.1128/JVI.02457-07

# Letter to the Editor

## Human Adenovirus Type 52: a Type 41 in Disguise?

Recently, Jones et al. described a new human adenovirus (HAdV) (3) and claimed it to be a "new type" and even a "new species": "human adenovirus 52 or HAdV-52 of species G." We dispute both claims.

By virus neutralization (VN), they showed it to be different from its phylogenetically closest relative, simian adenovirus 1. Surprisingly, they failed to provide additional VN data corroborating their claim of a novel serotype. Consequently, the new adenovirus may later, on the basis of VN data, prove to be a known serotype, e.g., HAdV-40 or HAdV-41.

In their attempts to show that the virus was sufficiently distinct from HAdV-40 and HAdV-41 to be considered a new type, they embarked on phylogenetic analyses. These viruses were accepted as separate serotypes by the ICTV (1) on the basis of differences in their DNAs and their antigenic distances as measured by VN (1, 2), complying with the definition of serotypes (or "types") as follows.

Adenovirus serotypes are differentiated on the basis of neutralization assays. A serotype is defined as one which either exhibits no cross-reaction with others, or shows a homologous/heterologous titer ratio greater than 16 (in both directions). For homologous/heterologous titer ratios of 8 or 16, a serotype assignment is made if either the viral haemagglutinins are unrelated (as shown by lack of cross-reaction in haemagglutination-inhibition tests), or if substantial biophysical, biochemical or phylogenetic differences exist (1).

Jones et al. also claim that the new adenovirus belongs to a novel species, named "G." They argue that the phylogenetic distance between "HAdV-52" and HAdV-40 and HAdV-41 is "at least as great as those differentiating the other HAdV species." This species designation would also be unprecedented. The phylogenetic distance between HAdV-40 and HAdV-41 is also at least as great as those differentiating other HAdV species, and also these two adenoviruses are not considered different species (1).

In summary, according to current taxonomical criteria and the data presented, the newly identified adenovirus should not be considered a novel serotype, let alone a novel species.

### REFERENCES

1. **Benkö, M., B. Harrach, G. W. Both, W. C. Russell, B. M. Adair, É. Ádam, J. C. de Jong, M. Hess, M. Johnson, A. Kajon, A. H. Kidd, H. D. Lehmkuhl, Q.-G. Li, V. Mautner, P. Pring-Akerblom, and G. Wadell.** 2005. Family *Adenoviridae*, p. 213–228. *In* C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (ed.), Virus taxonomy. Eighth report of the International Committee on the Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA.
2. **de Jong, J. C., R. Wigand, A. H. Kidd, G. Wadell, J. G. Kapsenberg, C. J. Muzerie, A. G. Wermenbol, and R. G. Firtzlaff.** 1983. Candidate adenoviruses 40 and 41: fastidious adenoviruses from human infant stool. J. Med. Virol. **11:**215–231.
3. **Jones, M. S., II, B. Harrach, R. D. Ganac, M. M. A. Gozum, W. P. dela Cruz, B. Riedel, C. Pan, E. L. Delwart, and D. P. Schnurr.** 2007. New adenovirus species found in a patient presenting with gastroenteritis. J. Virol. **81:**5978–5984.

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### Authors' Reply

Thank you very much for your letter, which gives us the opportunity to discuss publicly one of the hottest problems of present-day adenovirus taxonomy, namely, the difficulty of transitioning from serology to molecular/sequence data in the classification of viruses.

Historically, the virus neutralization test (VN) has been the gold standard method for typing new adenoviruses. This method required successful isolation and propagation of the virus, as well as the availability of a full panel of hyperimmune sera against every known (approved) serotype. It is obviously difficult to provide a reliable prototype strain and serum collection to every laboratory not only because of the high number (>100) of existing adenovirus types but also because of the recent restrictions concerning the intercontinental transport of microbiological samples. In the case of a candidate novel human adenovirus (HAdV) type, for example, this method would require more than 100 VN tests. Sequence analysis is a quicker and less cumbersome alternative for adenovirus typing. Negotiations regarding the target genome region for PCR and sequencing methods that replace serology are in progress. An excellent example for how phylogenetic analysis can replace serology by comparing sequences responsible for neutralization and hemagglutination determinants was recently published in the *Journal of Virology* (5). DNA sequencing and phylogenetic calculations have opened a relatively easy way to acquire quantitative data on the relatedness of two or more viruses, even if only a partial genome sequence is determined.

When full-genome sequences are available, this allows for complete species analysis as opposed to species determination based on one of three genes expressed on the outer coat of the virus. Species determination based on the whole genome is a superior method because it includes the genome organization, especially in the least conserved E3 and E4 regions. It is a horrible mistake to suppose that HAdV-52 may actually be identical with HAdV-41 (as stated in the title of the letter by de Jong and Osterhaus). Moreover, de Jong and Osterhaus were incorrect to use "species G" instead of "HAdV-G species," as animal adenovirus species are also lettered. Furthermore, a simple glance at the difference in the genetic organizations (4) as well as the lack of amino acid similarity that exists between HAdV-52 and HAdV-41 demonstrates that there is a chasm that divides these two viruses. The phylogenetic and genomic distances point to the need to place these viruses into different species.

In modern taxonomy, it is more appropriate to use the term "type" instead of "serotype" when referring to an adenovirus (e.g., human adenovirus type 2 in the current ICTV report coauthored by Jan de Jong). This change was necessary to

reflect the genetic reality, as serology is no longer the decisive criterion in classification. Taxonomists widely accept that the main criterion is genetic distance, which can be best measured by phylogenetic analysis. These new techniques have not failed because they are based on whole-genome comparisons. In contrast, when VN tests are performed, the only information that is acquired suggests the relatedness of a few important antigens (the hexon and fiber-related epitopes for adenoviruses). It is well known that the two most common and serologically distinct HAdV types, namely, 2 and 5, share 98% identity on the nucleotide sequence level. This justifies the classification of these viruses into the same species, *Human adenovirus C*, and underlines the relevance of species as opposed to serotypes (1).

Moreover, there are a number of examples for recombination between hexon genes that result in contradictory serological findings; a good example is HAdV-16 (2). Therefore, it is possible that a virus showing serological reactions characteristic of a certain serotype will prove to belong to a very different type based on the rest of its genome. Unfortunately, the hexon of HAdV-41 also seems to be the result of a recombination event (3). Although an annotated full genome for HAdV-41 is not available, its complete nucleotide sequence can now be found in GenBank. If one compares other genes, HAdV-40 and -41 are much closer to each other than has been shown on hexon-based trees. By the way, shortly after their discovery, HAdV-40 and -41 were even considered candidates for two separate species (called "groups" at that time), F and G, but finally (and wisely) have been placed into one common species.

As is obvious from our paper, the complete genomic sequence of HAdV-41 was also included in our analyses. The phylogenetic distance of HAdV-52 from HAdV-F species (HAdV-40 and -41) was calculated based on many genes, and these findings were shown in Fig. 4 for three of them (pTP, pIIIa, penton base), whereas a hexon-based tree was omitted because of the above-mentioned problem. On the trees in Fig. 4, HAdV-52 is clearly separated from HAdV-40 and -41. Three significant characteristics of the HAdV-52 genome include the presence of an integrin-binding site (RGD motif) on the penton base and the presence of genes for the 12.5-kilodalton E3 protein and the dUTPase, all of which are absent in HAdV-40 and -41 (4, 6).

Although the attention of our prestigious colleagues is flattering, we believe that after careful analysis of our paper, one can easily realize that the unique characteristics of HAdV-52 are unmistakable; they have also recently been confirmed by Madisch et al. (6).

## REFERENCES


1. **Benkö, M., B. Harrach, G. W. Both, W. C. Russell, B. M. Adair, É. Ádam, J. C. de Jong, M. Hess, M. Johnson, A. Kajon, A. H. Kidd, H. D. Lehmkuhl, Q.-G. Li, V. Mautner, P. Pring-Akerblom, and G. Wadell.** 2005. Family *Adenoviridae*, p. 213–228. *In* C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger, and L. A. Ball (ed.), Virus taxonomy. Eighth report of the International Committee on the Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA.
2. **Ebner, K., W. Pinsker, and T. Lion.** 2005. Comparative sequence analysis of the hexon gene in the entire spectrum of human adenovirus serotypes: phylogenetic, taxonomic, and clinical implications. J. Virol. **79:**12635–12642.
3. **Harrach, B.** Adenoviruses. *In* B. W. J. Mahy and M. H. W. van Regenmortel (ed.), Encyclopedia of virology, 3rd ed. Academic Press, New York, NY, in press.
4. **Jones, M. S., II, B. Harrach, R. D. Ganac, M. M. A. Gozum, W. P. dela Cruz, B. Riedel, C. Pan, E. L. Delwart, and D. P. Schnurr.** 2007. New adenovirus species found in a patient presenting with gastroenteritis. J. Virol. **81:**5978–5984.
5. **Madisch, I., G. Harste, H. Pommer, and A. Heim.** 2005. Phylogenetic analysis of the main neutralization and hemagglutination determinants of all human adenovirus prototypes as a basis for molecular classification and taxonomy. J. Virol. **79:**15265–15276.
6. **Madisch, I., S. Hofmayer, C. Moritz, A. Grintzalis, J. Hainmueller, P. Pring-Akerblom, and A. Heim.** 2007. Phylogenetic analysis and structural predictions of human adenovirus penton proteins as a basis for tissue-specific adenovirus vector design. J. Virol. **81:**8270–8281.



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