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Aetiology of acute hepatitis of unknown in children, association with AAV2 and controversies

To the Editor:

In the spring of 2022, there was an outbreak of acute severe hepatitis of unknown aetiology (ASHU) in children.[1,2] Since then, over 1,000 cases have been reported in 35 countries, resulting in 22 deaths (https://www.who.int/). Despite ongoing research, the cause of ASHU remains uncertain.

Recently, three independent groups from the US and UK have provided evidence linking the infection of adeno-associated virus 2 (AAV2), a virus that typically requires a helper virus to replicate in the liver without causing hepatitis, to ASHU.[3–5] Servellita *et al.* found AAV2 infection in 13 of the 14 human adenovirus (HAdV)-positive cases and also identified co-infection with other viruses such as Epstein-Barr virus and/or human herpesvirus 6, which may act as helper viruses.[3] The severity of the disease appears to be related to co-infections of AAV2 and its helper viruses.[3] This is similar to the fulminant liver failure caused by hepatitis D virus infection superimposed on chronic hepatitis B virus infection. However, the study did not test HAdV-negative ASHU cases. Morfopoulou *et al.* analyzed both HAdV-positive and HAdV-negative cases and detected a high rate of AAV2 positivity (27/28) in ASHU cases.[4] The study hypothesized that high levels of AAV2 replication mediated by helper viruses triggered immune-mediated liver injury.[4] Interestingly, the co-infection of AAV2 and HAdV in six immunocompromised children with acute hepatitis due to other causes, and four children with normal immune systems who had fever, did not lead to ASHU in the control groups, indicating that co-infection alone is not sufficient to cause ASHU.[4] Genetic susceptibility analysis revealed a high rate of the HLA class II DRB1*04:01 allele positivity (12/13) in these ASHU cases, which is consistent with another study conducted in the UK.[5] Ho *et al.* found an association between AAV2 infection and the host HLA class II DRB1*04:01 allele, both of which may have contributed to the development of the acute pediatric hepatitis.[5] Furthermore, a surveillance study of wastewater in Ireland found a high correlation between HAdV-F41 and AAV2 circulation in the community and ASHU cases, providing additional indirect evidence. These studies highlight that ASHU is the result of multiple essential factors including AAV2, helper viruses, and genetic predisposition.

One question that arises is why similar cases were not discovered and reported in the past, given that these factors must have co-existed in the population's history? Considering that the outbreak of acute hepatitis occurred during the COVID-19 pandemic, the pandemic-induced lockdown measures may have contributed to a decrease in social interactions among children, thereby limiting their exposure to common pathogens and reducing the collective immunity against said pathogens, and ultimately leading to the emergence of a vulnerable population susceptible to a previously harmless virus.[1,6] Also, enhanced infectious disease surveillance systems in various countries during the pandemic may have contributed to the timely discovery of the disease.[7]

However, controversies remain. Firstly, the key publications mentioned above (references 3-5) have limitations. For instance, they all recruited a small sample size of patients, which affects their persuasiveness; these retrospective case-control studies have the advantage of elucidating relationships between various variables and outcomes, and put forward the hypothesis accordingly, while they cannot determine causation. So, it is hard to determine the aetiology of the ASHU based on these observational studies. Secondly, over 1,000 cases were reported in 35 countries, and they may not share the same mechanisms. For instance, people of different races may have various genetic predispositions. In that sense, it is necessary to investigate cases outside the US and UK, such as in Japan in Asia and Mexico in South America, where a number of ASHU cases have also been reported. Thirdly, AAV is a defective virus that requires a helper virus for an active, self-limiting infection around the world (seropositive rates of 30%-80%) and has been used as a viral vector for delivering genes to people in gene therapy due to being avirulent and non-pathogenic.[8,9] The hypothesis of AAV causing hepatitis will compel individuals to reassess its safety, especially considering that AAV2 has also been reported to be associated with oncogenic insertional mutagenesis in human hepatocellular carcinoma by inducing overexpression of cancer driver genes.[9,10]

Nevertheless, based on the existing evidence, we speculate that the acute hepatitis in children is the result of a combination of multiple factors, including intensive containment measures during a pandemic resulting in vulnerable immune systems, co-infections of AAV2 and other helper viruses, and susceptible gene alleles. Further mechanistic studies (*e.g.*, cell experiments and animal models) are urgently required to confirm that AAV2 co-infection with certain viruses under a certain immune background can cause hepatocyte damage or liver injury, which would be a step closer to confirming the hypothesis proposed.

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Author names in bold designate shared co-first authorship.

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