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RAPID COMMUNICATION

Haemagglutinin D222G mutation found in a fatal case of pandemic (H1N1) flu in Tunisia

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Abstract Recently, the D222G substitution was observed in the HA of pandemic (H1N1) 2009 viruses isolated from fatal cases in several countries. We made a similar observation in one fatal case in Tunisia showing a D222G substitution in a virus isolate. The man was 47 years old and had no other subjacent pathologies or any known risk factors. He died after three days, suffering from severe respiratory symptoms of flu. The causal link of the D222G substitution in Tunisia with virulence must be verified. Further study is warranted to elucidate the intriguing relationship between the D222G substitution and severe disease. Constant molecular surveillance is important to monitor the pathogenicity of circulating strains and evaluate vaccine efficacy.

Abbreviations

HA	Haemagglutinin
D	Aspartic acid
G	Glycine

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On November 20, 2009, The Norwegian Institute of Public Health reported to the World Health Organization a possible correlation between the presence of a mutation in the haemagglutinin (HA) of pandemic (H1N1) 2009 influenza virus, consisting in a change of aspartic acid (D) to glycine (G) at position 222 (225 in H5 numbering), and severe clinical outcome. It was found with considerable frequency in fatal and severe cases in Norway [1]. The same mutation has also been detected in other European, Asian and American countries [2–4].

The haemagglutinin D222G substitution is known to cause a shift from α2,6 receptor to mixed α2,3/α2,6 receptor specificity which increases affinity for the α2,3 receptor [5–8] and contributes to the severity of disease, rendering the virus more pneumotropic.

As part of the intensified surveillance carried out in Tunisia during this influenza pandemic, respiratory specimens from three severely ill Tunisian patients with confirmed pandemic (H1N1) 2009 virus were subjected to PCR amplification and subsequent sequencing of a partial segment of the HA1 region of the haemagglutinin gene [9] in cooperation with the National Influenza Centre-Madrid (Spain). Sequences were submitted to the GenBank database with the accessions numbers HM590676 and HQ174255. The D222G substitution was found only in one respiratory specimen (1 positive/3 samples studied, 33 %) collected on January 18, 2010, from one fatal case. The man was 47 years old, living in Tunis, and had no other subjacent pathologies or any known risk factors. He died after three days, suffering from severe respiratory symptoms of flu. Autopsy revealed pulmonary oedema and a large amount of mucous secretion but no cardiac inflammation. No other respiratory or seasonal influenza viruses were found in clinical samples investigated by PCR done in our lab (xTAG® RVP FAST with Luminex® system for

other respiratory viruses and an in-house PCR method for influenza virus). These clinical and biological findings suggest that pandemic (H1N1) influenza virus was the most probable etiological agent responsible for his death, and the D222G substitution may be the main cause of the appearance of the lung complications described. In Norway, some of the D222G cases manifested themselves as a rapid, unexpected deterioration after a period of mild symptoms in previously healthy subjects [1], and we consider it likely that in the Tunisian case there is also a causal relationship between the occurrence of the D222G substitution in this virus and a change in cellular tropism, rendering the virus more pneumotropic. Conversely, it is possible that the likelihood of such mutations arising is higher in patients who fail to fight off the virus rapidly and have virus already colonising the lower respiratory tract. This is the first identification of a change in the pandemic virus in Africa that correlates with a severe clinical outcome. Although it is not easy to deduce a causal relationship between D222G and mortality in Tunisia because this observation has been made only in one case, it may aid in filling some knowledge gaps in a preliminary review of this and other mutations in the pandemic virus [10]. Also, the D222G substitution has been found in previously reported mild cases [11], so the significance of this mutation in terms of pathogenicity needs to be verified [12].

In conclusion, to confirm this hypothesis, it is necessary to do more investigation to detect the D222G substitution by sequencing the haemagglutinin genes of isolates from mild, severe non-fatal and fatal cases. The molecular characterization of the pandemic H1N1 haemagglutinin should enable the medical and public-health research community to develop improved intervention approaches to control and prevent influenza morbidity and mortality as this virus becomes endemic in human populations. Furthermore, virological, clinical and epidemiological investigations are needed to ascertain the role of this and other mutations that may alter the virulence and transmissibility of the pandemic influenza A (H1N1) virus.

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Conflict of interest None of the authors has a financial or personal conflict of interest related to this study.

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