

Observed association between the HA1 mutation D222G in the 2009 pandemic influenza A(H1N1) virus and severe clinical outcome, Norway 2009-2010

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Infection with the recently emerged pandemic influenza A(H1N1) virus causes mild disease in the vast majority of cases, but sporadically also very severe disease. A specific mutation in the viral haemagglutinin (D222G) was found with considerable frequency in fatal and severe cases in Norway, but was virtually absent among clinically mild cases. This difference was statistically significant and our data are consistent with a possible causal relationship between this mutation and the clinical outcome.

The 2009 influenza A(H1N1) pandemic has been characterised by mild and self-limiting disease in the overwhelming majority of cases. However, severe and fatal cases, many of them with primary viral pneumonia, have been occurring in age groups where such clinical outcomes are very rarely seen in seasonal influenza [1,2]. It is important to better understand what viral and host-related factors determine this dichotomy.

Genetic characterisation of clinical specimens

As part of the intensified surveillance carried out during the current influenza pandemic, the national reference laboratory for human influenza at the Norwegian Institute of Public Health collected a large number of respiratory specimens from verified and possible cases of pandemic influenza. In the present study we analysed 61 respiratory specimens from severe and fatal cases that occurred between July and December 2009, as well as from 205 cases with mild clinical outcomes collected between May 2009 and January 2010. Genetic characterisation was performed using conventional sequencing, or with a pyrosequencing assay subsequently developed to detect the particular mutations described below and which facilitated investigation of a large number of specimens.

Here we report the occurrence of an amino acid substitution, aspartic acid to glycine in position 222 (D222G) in the HA1 subunit of the viral haemagglutinin, in clinical specimens from 11 out of 61 cases analysed in Norway with severe outcome. Such mutants were not observed

in any of 205 mild cases investigated (Table), thus the frequency of this mutation was significantly higher in severe (including fatal) cases ($p < 0.001$, Fisher's exact test, two-sided) than in mild cases. D222G mutants were detected throughout the sampling period, from the first recorded severe cases in July until early December. The frequency of another substitution in the same position, D222E, did not differ significantly between mild and severe cases ($p = 0.772$). Yet another substitution, D222N, was observed in a very few cases ($n = 4$), and at a higher rate than expected among severe cases (three of four cases, $p = 0.039$). The wild type 222D was, not surprisingly, significantly less frequent in severe than in mild cases ($p < 0.001$).

In several of the patients where D222G mutant viruses were found, they coexisted with wildtype 222D viruses. Further analysis of this phenomenon is ongoing.

The cases infected with the D222G-mutated virus were not epidemiologically related to each other, and the mutated viruses do not cluster together in phylogenetic analysis (data not shown).

Validity and limitations of the analysis

Cases with severe clinical outcomes were much more likely to be included in our study for several reasons: they are more likely to seek healthcare, they are more likely to be prioritised for virological testing, and their specimens are more likely to be forwarded to the national reference laboratory where they have a higher chance of being selected for detailed analysis than viruses from mild cases. Because of this, we chose to record the frequency of a given genotype in each severity group and compare it with the corresponding frequency in other severity groups. This approach is not expected to have a selection bias.

Cases were classified as mild, severe non-fatal and fatal based on the patient information that was available to us. Some seemingly mild cases may later have exacerbated to severe outcomes without our knowledge, or the presented patient information may have

been incomplete, but we think these cases must be few. On the other hand, all severe and fatal cases were confirmed as non-mild. Thus, the fact remains that only cases confirmed as severe outcomes exhibited the D222G mutation in our investigation.

The sampling period for the cases analysed spans from the initial detections of the pandemic H1N1 virus in early May 2009 until early January 2010. The first severe and fatal cases occurred in July. By the end of December, the epidemic in Norway had largely passed, and a large proportion of cases in our data set is from the peak period in October and November. At all times an effort was made to include a reasonable number of non-severe cases in our analyses, and such cases were well represented throughout the pandemic. The fractions of severe/fatal cases among all analysed cases during the two-month periods July/August (n=21), September/October (n=84), and November/December (n=149), were within the range of 23% to 26%. Severe outcomes were not recorded among the few cases in May and June (n=11) and in January (n=1). We thus do not see a trend over time in the composition of severe versus mild cases in our dataset that could lead to an artificial difference in the frequency of the D222G substitution. Furthermore, the D222G substitution was represented also among the earliest fatal and severe cases in July and August.

Specimens from both the lower and upper respiratory tract were analysed. Lower respiratory tract specimens were available from severe/fatal cases only, and in some cases they were the only materials available. However, in all cases where we had paired upper and lower airway specimens (five cases with 222D and four cases with 222G), the wildtype-versus-D222G pattern was matching between the locations. We have therefore no reason to believe that this difference in proportion of lower airway specimens distorted the analysis.

Discussion

Amino acid position 222 resides in the receptor binding site of the HA protein and may possibly influence the binding specificity and thus the cellular tropism of the virus. The corresponding difference between

two viruses from the 1918 Spanish influenza pandemic correlates to a shift in receptor preference [3], which conceivably could make the virus prone to infect a wider range of cells in the lower respiratory tract [4,5]. However, the effect of a mutation depends on the molecular context and it is unclear whether the binding properties are affected likewise in the present pandemic virus as they were in the 1918 influenza virus.

Our observations are consistent with an epidemiological pattern where the D222G substitution is absent or infrequent in circulating viruses, with the mutation arising sporadically in single cases where it may have contributed to severity of infection. This may aid in filling some knowledge gaps identified in a recent preliminary review of this and other mutations in the pandemic virus [6]. The correlation between presence of the D222G substitution and a severe clinical outcome may reflect an increase in pathogenicity caused by the mutation, possibly related to 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. These two possibilities are not mutually exclusive. A large proportion of the fatal and severe cases had underlying risk conditions. However, some of the D222G cases manifested themselves as a rapid unexpected deterioration after a period of mild symptoms in previously healthy subjects, and we consider it likely that there is a causal relationship between the occurrence of the D222G mutation in this virus and severe disease.

It should be borne in mind, however, that the majority of severe and fatal cases investigated did not carry the D222G substitution and, clearly, this mutation is not required for a severe outcome.

Conclusions

To our knowledge, this is the first identification of a change in the pandemic virus that correlates with a severe clinical outcome. However, whereas our data lend statistically significant support to an association between the D222G mutation and severity, the number

TABLE

Pandemic influenza A(H1N1) viruses characterised for amino acid position 222 of the haemagglutinin HA1 domain, by clinical outcome, Norway, May 2009–January 2010 (n=266)

HA1 position 222 genotype ^b	Clinical outcome ^a				
	Mild (n=205)	Severe (n=34)	Fatal (n=27)	Severe plus fatal (n=61)	All cases (n=266)
222D (wt)	92% (189)	82% (28)	59% (16)	72% (44)	88% (233)
222G	0% (0)	8.8% (3)	30% (8)	18% (11)	4.1% (11)
222E	7.3% (15)	2.9% (1)	7.4% (2)	4.9% (3)	6.8% (18)
222N	0.5% (1)	5.9% (2)	3.7% (1)	4.9% (3)	1.5% (4)
Total	100%	100%	100%	100%	100%

^a Clinical outcome based on patient information, assigned into categories by a medical specialist according to WHO guidance criteria [1].

^b Percentage of genotype within each clinical category is given, with number of cases per category in parentheses.

of mild cases would need to be larger to determine whether mutant viruses are indeed circulating at a very low frequency also in non-severe cases. Provided that D222G mutant viruses are not circulating, i.e. that they are less transmissible, the immediate public health impact of this finding is limited. However, it may have implications for the management of severe cases where the virus, if transmitted through massive exposure, may be more virulent than the commonly circulating variant. Furthermore, it may serve as a reminder that the generally very low virulence of the current pandemic virus is not a fixed characteristic, and that there is no reason for complacency in carrying out measures that limit infection with this virus at individual and population level.

Further 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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