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Phylogenetic Analysis of H1N1 Swine Flu Virus Isolated In India

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Abstract

The H1N1 subtype of Influenza A virus is the causative agent of swine flu. The 2009 outbreak caused by subtype H1N1 in humans is due to transfer of Swine Influenza Virus from pig to human. Thus to analyze the origin of this novel virus we compared the 42 nucleocapsid sequences of H1N1 viruses of different origins. Phylogenetic analysis of these sequences was carried out along with bootstrap analysis of 100 replicates. The phylogenetic tree constructed revealed that Indian H1N1 strain showed the highest homology with Iowa H1N1 strain and also with Wisconsin H1N1 strain. Further the H1N1 strains analyzed using NP sequences of different Indian origins showed highly close sequence similarity. Hence, in future this study will be helpful for knowing the taxonomy and evolution of influenza viruses.

Keywords: H1N1; Homology; Phylogenetic analysis; Evolution

Introduction

Swine influenza is an acute respiratory disease caused by influenza A virus within the Orthomyxoviridae family. It is named so because some of the genes (NA) normally occurring in North American swine were also reported in the patients. The primary clinical manifestations of viral infection are fever and acute respiratory distress. Influenza A viruses infects many animal species including birds, seals, whales, humans, horses and swine[1-3]. Influenza A virus strains are assigned an H number and an N number base on which forms of these two proteins the strain contains. There are 16 H and 9 N subtypes known in birds, but only H 1, 2 and 3, and N 1 and 2 are commonly found in humans. Influenza A (H1N1) virus is a subtype of influenza virus A and the most common cause of influenza (flu) in humans. Due to the viral genome being fragmented there is a possibility of reassortment between different strains of the same subtypes. The genome of Influenza A (H1N1) virus consists of negative sense single stranded segmented RNA which encodes 11 structural proteins (HA, NA, PB1, PB2, PA, M1, M2, NP, NS1 and NS2, NEP).In this study the focus is mainly on Nucleocapsid protein. Nucleocapsid protein (NP) is encoded by RNA segment 5. It is transported into the infected cell nucleus, where it binds to and encapsidates viral RNA. In addition to its structural role, NP is believed to play a role in the switching of viral RNA polymerase activity from mRNA synthesis to cRNA and vRNA synthesis. NP is abundantly synthesized in infected cells and is the second most abundant protein in the influenza virus virion [4-5]. It is phosphorylated; the pattern of phosphorylation is host cell dependent and may be related to viral host range restriction. NP is also a major target of the host cytotoxic T-cell immune response. Phylogenetic analysis is the technique to determine the evolutionary relationships between organisms. The results of an analysis can be drawn in a hierarchical diagram called a cladogram or phylogram (phylogenetic tree). The branches in a tree are based on the hypothesized evolutionary relationships (phylogeny) between organisms. Each member in a branch, also known as a monophyletic group, is assumed to be descended from a common ancestor. Originally, phylogenetic trees were created using morphology, but now, determining evolutionary relationships includes matching patterns in nucleic acid and protein sequences. To reveal differential evolutionary trends of A (H1N1) NP, the phylogenetic analysis of various NP sequences of Influenza A (H1N1) virus was carried out.

Materials and Methodology

The 42 protein sequences of nucleocapsid of H1N1 Influenza A Virus were retrieved from the biological database- National Centre for Biotechnology Information (NCBI) cited at http://www.ncbi.nlm.nih.gov and the UniProt KB Database in ExPASy Proteomics Server available at http://www.uniprot.org/. Multiple sequence alignments of the given NP sequences were performed by using the Clustal W Program with default parameters in MEGA 4.0.2 version. Phylogenetic tree was built by Maximum Parsimony method in MEGA 4.0.2 version. The NP tree was rooted by an unrelated H3N2 duck influenza virus, A/duck/Korea/S72/2007. Phylogenies were determined by Bootstrap Analysis of 100 replicates in MEGA 4.0.2 version.

Result and Discussion

"Mixing Vessels" that is pigs play an important role in interspecies transmission of influenza viruses. The new H1N1 strain which appeared in 2009 outbreak with high pathogenecity to human was originated as a result of reassortment. Thus to trace out the origin of this novel human H1N1 virus, we performed the phylogenetic analysis of nucleocapsid protein. (Figure 1).

Total 42 nucleocapsid sequences of H1N1 were used to construct the phylogenetic tree. The multiple sequence alignment revealed that all these sequences were genetically very close, so Maximum Parsimony method was selected for the phylogenetic tree construction [6-8]. Phylogenies were determined using bootstrap analysis of 100 replicates in MEGA 4.0.2 version. The tree was rooted by an unrelated H3N2 Influenza virus A/duck/korea/S72/2007. The results of the tree constructed suggested that Indian H1N1 strain showed the highest homology (%identity) of 98.19% with Iowa H1N1 strain (1988) (Accession no. P68042) and the same % identity with Wisconsin H1N1 strain (1988) (Accession no. P68043) (as shown in Table 1) [9]. Further the H1N1 strains analyzed using NP sequences of different Indian origins showed very close sequence similarity. Finally it concludes that this study will be helpful for knowing the taxonomy and evolution of newer influenza viruses[10].

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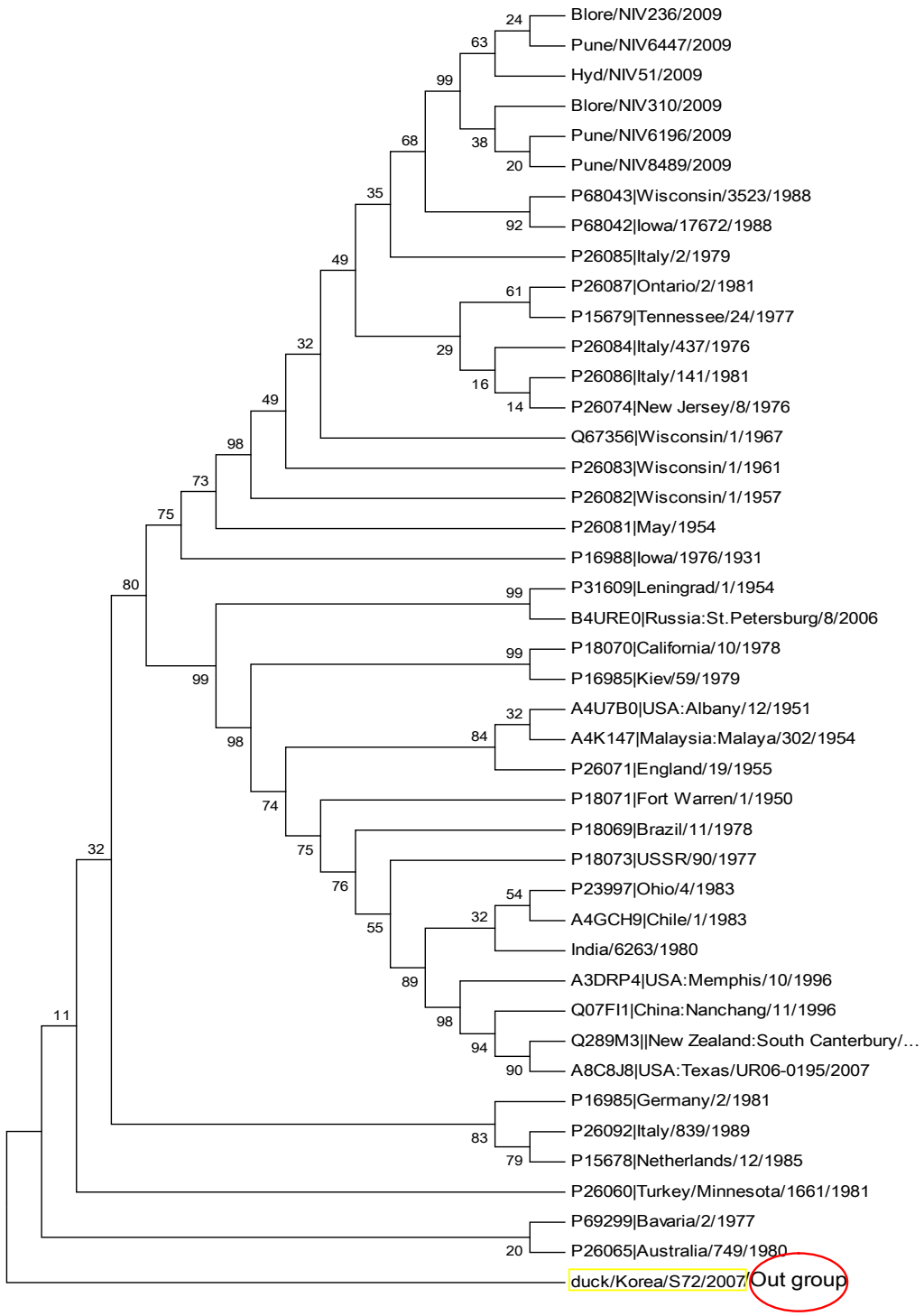

Figure 1: A Maximum Parsimony Phylogenetic tree of NP protein sequences of H1N1 Influenza A virus of various origins.

Our analysis substantiate the value of molecular screening and phylogenetic assessment for understanding the evolution of influenza viruses and, most importantly, for the early detection of emerging novel viruses that could lead to influenza pandemics. Our data also suggest that the virus will remain sensitive to the pre-existing therapeutic strategies. The conserved regions of nucleocapsid protein obtained through phylogenetic analysis play an important role in synthesis of drugs vaccination and for primer designing. From the phylogenetic tree, the branching pattern of different strains of Swine Influenza virus can be determined[11-12]. The time of origin of the species can be determined. Monitoring and characterizing influenza viruses in swine are important in preventing interspecies transmission. This effort prioritizes the use of genetic distinctness as a marker for the detection of novel viruses that could lead to influenza pandemics.

Sr No.	Strains to be Matched	Refrence Strain : (>Pune/Niv6196/2009) % Identity
1	Q67356/Wisconsin/1/1967	97.2
2	Q289M3/New Zealand: South Canterbury/35/2000	89.7
3	Q07FI/|China:Nanchang/11/1996	89.7
4	P69299/Bavaria/2/1977	94.6
5	P68043/Wisconsin/3523/1988	98.19
6	P68042/Iowa/17672/1988	98.19
7	P31609/Leningrad/1/1954	91.76
8	P26092/Italy/839/1989	93.37
9	P26087/Ontario/2/1981	97.18
10	P26086/Italy/141/1981	97.38
11	P26085/Italy/2/1979	97.79
12	P26084/Italy/437/1976	97.59
13	P26083/Wisconsin/1/1961	96.98
14	P26082/Wisconsin/1/1957	96.78
15	P2608/May/1954	94.78
16	P2607/New Jersey/8/1976	96.58
17	P26071/England/19/1955	91.36
18	P26065/Australia/749/1980	94.38
19	P26060/Turkey/Minnesota/1661/1981	93.57
20	P23997/Ohio/4/1983	90.16
21	P18073/USSR/90/1977	90.36
22	P18071/Fort Warren/1/1950	90.96
23	P18070/California/10/1978	90.96
24	P18069/Brazil/11/1978	90.36
25	P16988/Iowa/1976/1931	94.18
26	P1698/Germany/2/1981	93.77
27	P16985/Kiev/59/1979	91.16
28	P15679/Tennessee/24/1977	97.39
29	P15678/Netherlands/12/1985	93.57
30	B4URE0/Russia:St.Petersburg/8/2006	91.16
31	A8C8J8/USA:Texas/UR06-0195/2007	89.76
32	A4U7B0/USA:Albany/12/1951	91.16
33	A4K147/Malaysia:Malaya/302/1954	91.36
34	A4GCH9/Chile/1/1983	90.16
35	A3DRP4/USA:Memphis/10/1996	80.16
36	Blore/NIV236/2009	99.79
37	Blore/NIV310/2009	100
38	Hyd/NIV51/2009	99.79
39	Pune/NIV6447/2009	99.79
40	Pune/NIV8489/2009	100
41	India/6263/1980	89.95
42	duck/Korea/S72/2007	93.17

Table 1: Protein Identity of Pune/NIV6196/2009 with the strains to be matched.

Conclusion

In the present study, phylogenetic analysis of only one of the protein that is NP out of 8 proteins consisting the viral genome was done. Further how the other proteins of the virus other than NP, cluster and what is the difference obtained in the tree can be focused. Moreover, by making a comparative study of H1N1 with other subtypes of Influenza A viruses, the genetic relatedness between them can be determined which would give the strong indication of reassortment. By knowing the amino acid sequences of different proteins the conserved pattern of receptor binding site can be determined in the present H1N1. Further studies are required to determine the degree of genetic divergence, distribution and pathogenic potential of this novel subgroup.

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