# Variation in Active Site Amino Residues of H1N1 Swine Flu Neuraminidase

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**Abstract.** In this paper, we report the variations of amino acid residues between H5N1 and H1N1 swine flu neuraminidase sequences at protein level. Random search in NCBI Flu database resulted in Canadian viral gene and analysis using blast technique revealed sites that are variant among sequences for which 3-dimensional structures were known. PDB summary database and multiple alignments were employed for validation of the results. Based on the mutations observed within active site region, homology derived model was constructed using swiss-pdb viewer. The residue variation observed was with respect to Tyr347 in H5N1 versus Asn344 in H1N1 neuraminidase sequence, which resulted in geometrical modification of ligand binding domain.

## 1 Introduction

Swine influenza was first proposed to be a disease related to human influenza during the 1918 flu pandemic. The H1N1 form of swine flu is one of the descendants of the strain that caused the 1918 flu pandemic [Jeffery K. Taubenberger, David M. Morens. 1918 Influenza: The mother of all pandemics. Rev Biomed 2006; 17:69-79]. The human influenza a virus continues to thrive among populations and continues to be a major cause of morbidity and mortality [Frost WH. Statistics of influenza morbidity. Public Health Rep. 1920; 35:584–97]. The virus showed various mutations [Glaser L, Stevens J, Zamarin D, Wilson IA, Garcia-Sastre A, Tumpey TM, et al. A single amino acid substitution in the 1918 influenza virus hemagglutinin changes the receptor binding specificity. J Virol. 2005; 79:11533–6] since it first originated thereby making the existing vaccines ineffective on a regular basis [Elodie Ghedin, Naomi A. Sengamalay, Martin Shumway et. al. Large-scale sequencing of human influenza reveals the dynamic nature of viral genome evolution. Nature 2005; 437, 1162-1166].

Influenza, commonly referred to as the flu, is an infectious disease caused by RNA viruses of the family Orthomyxviridae (the influenza viruses), that affects birds and mammals. The most common symptoms of the disease are chills, fever, sore throat, muscle pains, severe headache, coughing, weakness and general discomfort. Typically, influenza is transmitted through the air by coughs or sneezes, creating aerosols containing the virus. Influenza can also be transmitted by bird droppings, saliva, nasal secretions, faeces and blood. An avian strain named H5N1 raised the concern of a

Aswatha Kumar M. et al. (Eds.): Proceedings of ICAdC, AISC 174, pp. 575–583.
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new influenza pandemic, after it emerged in Asia in the 1990s. In April 2009 a novel flu strain evolved that combined genes from human, pig, and bird flu, referred as 'swine flu' [Yasushi Itoh, Kyoko Shinya, Maki Kiso et. al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature 2009; 460, 1021-1025].

## 2 Materials and Methods

The viral gene sequences were accessed and extracted from NCBI (National Centre for Biotechnology Information) Flu database [www.ncbi.nlm.nih.gov]. From the H1N1 sequences deposited in NCBI, the Canadian origin neuraminidase gene (Figure 1) was selected randomly to perform sequence comparisons.

The fasta format of the sequence selected for analysis is given below.

>gi|255734960|gb|ACU31180.1| neuraminidase [Influenza A virus (A/Canada-NS/RV1554/2009(H1N1))]

MNPNQKIITIGSVCMTIGMANLILQIGNIISIWISHSIQLGNQNQIETCNQSVIT
YENNTWVNQTYVNISNTNFAAGQSVVSVKLAGNSSLCPVSGWAIYSKDNSV
RIGSKGDVFVIREPFISCSPLECRTFFLTQGALLNDKHSNGTIKDRSPYRTLMSC
PIGEVPSPYNSRFESVAWSASACHDGINWLTIGISGPDNGAVAVLKYNGIITDT
IKSWRNNILRTQESECACVNGSCFTVMTDGPSNGQASYKIFRIEKGKIVKSVE
MNAPNYHYEECSCYPDSSEITCVCRDNWHGSNRPWVSFNQNLEYQIGYICSG
IFGDNPRPNDKTGSCGPVSSNGANGVKGFSFKYGNGVWIGRTKSISSRNGFE
MIWDPNGWTGTDNNFSIKQDIVGINEWSGYSGSFVQHPELTGLDCIRPCFWV
ELIRGRPKENTIWTSGSSISFCGVNSDTVGWSWPDGAELPFTIDK

ClustalW program [www.ebi.ac.uk/clustalw] was utilized to perform multiple sequence alignments. The template 3D structures were downloaded from Protein Data Bank [www.recsb.org/pdb]. PDB summary database [www.ebi.ac.uk/pdbsum] was employed to study active site residue region. Viral neuraminidase structure was built using Swiss-PdbViewer. Initially the sequence (H1N1 neuraminidase) to be modelled is loaded from Swiss model menu and then the option move raw sequence into the structure followed by move structure into raw sequence is performed. Then the reference sequence (3CL2) is loaded from open pdb file option of the file menu and performed iterative magic fit of the fit menu by which the target sequence and the template structure fits into each other.

GenBank: GQ465699.1

**Influenza A virus (A/Canada-NS/RV1554/2009(H1N1)) segment 6 neuraminidase (NA) gene, complete cds**

Comment Features Sequence

```
LOCUS       GQ465699              1422 bp    cRNA    linear   VRL 11-AUG-2009
DEFINITION  Influenza A virus (A/Canada-NS/RV1554/2009(H1N1)) segment 6
            neuraminidase (NA) gene, complete cds.
ACCESSION   GQ465699
VERSION     GQ465699.1  GI:255734959
DBLINK      Project:37813
KEYWORDS    .
SOURCE      Influenza A virus (A/Canada-NS/RV1554/2009(H1N1))
  ORGANISM  Influenza A virus (A/Canada-NS/RV1554/2009(H1N1))
            Viruses; ssRNA negative-strand viruses; Orthomyxoviridae;
            Influenzavirus A.
REFERENCE   1  (bases 1 to 1422)
  AUTHORS   Bastien,N., Graham,M., Tyler,S., Van Domselaar,G., Drebot,M.,
            Plummer,F., Aranda,C.A., Zavala,E.P., Eshaghi,A., Gubbay,J.,
            Guyard,C., Guthrie,J., Duncan,C., Elgngihy,N., Tijet,N.,
            Farrell,D., Drews,S.J., Hatchette,T., Davidson,R., Sarwal,S.,
            Watson-Creed,G., Preiksaitis,J., Pabbaraju,K., Wong,S. and Li,Y.
  CONSRTM   Unknown Pathogen Investigation Collaborative Team (UPICT) and
            Instituto de Diangnostico y Referencia Epidemiologicos (inDRE)
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**Fig. 1.** H1N1 Neuraminidase gene selected for analysis

## 3 Results and Discussion

Initially BLAST analysis was employed to evaluate the percent identities, similarities and number of gaps. Apart from this, based on PAM and BLOSUM matrices, considering Score and E-value, alignments are chosen for structure predictions.

The neuraminidase belongs to sialidase superfamily and the data from NCBI suggests that Sialidases or neuraminidases function to bind and hydrolyze terminal sialic acid residues from various glycoconjugates as well as playing roles in pathogenesis, bacterial nutrition and cellular interactions. They have a six-bladed, beta-propeller fold with the non-viral sialidases containing 2-5 Asp-box motifs (most commonly

Ser/Thr-X-Asp-[X]-Gly-X-Thr- Trp/Phe). This Conserved Domain also includes eubacterial, eukaryotic, and viral sialidases.

BLAST analysis was carried out with default parameters and the scores, top alignments are given in Figures 2 and 3.

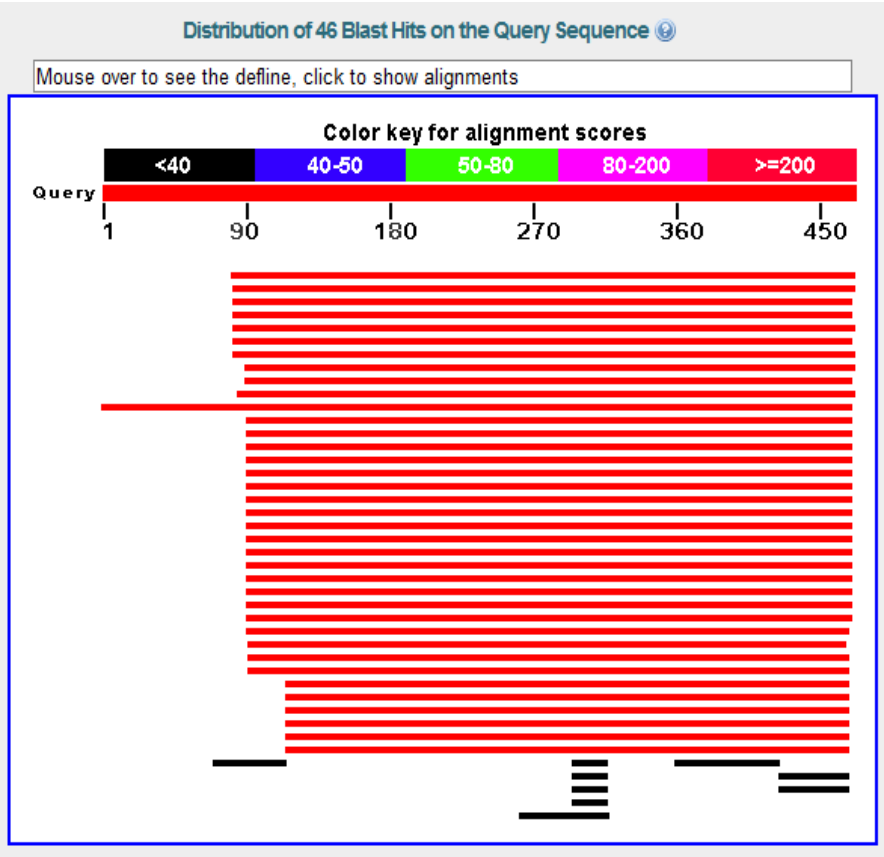


**Fig. 2.** BLAST analysis graphical representation

From the above top two and below alignments, although 3CL2, a H5N1 neuraminidase was considered for further work because 3CL2 was bound with oseltamivir. Therefore, structural and sequential differences between 3CL2 and H1N1 sequences were performed (Figure 4).

Sequences producing significant alignments:

| Accession | Description | Max score | Total score | Query coverage | E value | Links |
|---|---|---|---|---|---|---|
| 3NSS_A | Chain A, The 2009 Pandemic H1n1 Neuraminidase N1 Lacks The 150- | 797 | 797 | 82% | 0.0 | S |
| 2HTY_A | Chain A, N1 Neuraminidase >pdb\|2HTY\|B Chain B, N1 Neuraminidase | 750 | 750 | 82% | 0.0 | S |
| 3CL2_A | Chain A, N1 Neuraminidase N294s + Oseltamivir >pdb\|3CL2\|B Chain E | 744 | 744 | 82% | 0.0 | S |
| 3CKZ_A | Chain A, N1 Neuraminidase H274y + Zanamivir >pdb\|3CL0\|A Chain A, | 743 | 743 | 82% | 0.0 | S |
| 3CYE_A | Chain A, Cyrstal Structure Of The Native 1918 H1n1 Neuraminidase F | 737 | 737 | 82% | 0.0 | S |
| 3BEQ_A | Chain A, Neuraminidase Of ABREVIG MISSION11918 H1N1 STRAIN >p | 733 | 733 | 82% | 0.0 | S |
| 2HTV_A | Chain A, N4 Neuraminidase >pdb\|2HTV\|B Chain B, N4 Neuraminidase | 558 | 558 | 82% | 0.0 | S |
| 2HT5_A | Chain A, N8 Neuraminidase >pdb\|2HT7\|A Chain A, N8 Neuraminidase | 468 | 468 | 81% | 6e-164 | S |
| 3O9J_A | Chain A, Influenza Na In Complex With Compound 5 >pdb\|3O9K\|A Ch | 466 | 466 | 80% | 4e-163 | S |
| 3SAL_A | Chain A, Crystal Structure Of Influenza A Virus Neuraminidase N5 >p | 453 | 453 | 82% | 4e-158 | S |
| 1NMB_N | Chain N, The Structure Of A Complex Between The Nc10 Antibody A | 382 | 382 | 99% | 3e-129 | S |
| 1NNA_A | Chain A, Three-Dimensional Structure Of Influenza A N9 Neuraminida | 363 | 363 | 80% | 7e-123 | S |
| 1XOE_A | Chain A, N9 Tern Influenza Neuraminidase Complexed With (2r,4r,5r)- | 363 | 363 | 80% | 8e-123 | S |
| 1NCA_N | Chain N, Refined Crystal Structure Of The Influenza Virus N9 Neuram | 363 | 363 | 80% | 9e-123 | S |
| 1A14_N | Chain N, Complex Between Nc10 Anti-Influenza Virus Neuraminidase | 363 | 363 | 80% | 1e-122 | S |
| 5NN9_A | Chain A, Refined Atomic Structures Of N9 Subtype Influenza Virus Ne | 362 | 362 | 80% | 2e-122 | S |
| 1NCB_N | Chain N, Crystal Structures Of Two Mutant Neuraminidase-Antibody | 362 | 362 | 80% | 2e-122 | S |
| 1L7H_A | Chain A, Crystal Structure Of R292k Mutant Influenza Virus Neuramir | 361 | 361 | 80% | 3e-122 | S |
| 3NN9_A | Chain A, Refined Atomic Structures Of N9 Subtype Influenza Virus Ne | 361 | 361 | 80% | 4e-122 | S |
| 1INY_A | Chain A, A Sialic Acid Derived Phosphonate Analog Inhibits Different | 361 | 361 | 80% | 5e-122 | S |
| 6NN9_A | Chain A, Refined Atomic Structures Of N9 Subtype Influenza Virus Ne | 361 | 361 | 80% | 5e-122 | S |
| 1NCC_N | Chain N, Crystal Structures Of Two Mutant Neuraminidase-Antibody | 360 | 360 | 80% | 7e-122 | S |
| 4NN9_A | Chain A, Refined Atomic Structures Of N9 Subtype Influenza Virus Ne | 360 | 360 | 80% | 8e-122 | S |
| 1NMA_N | Chain N, N9 Neuraminidase Complexes With Antibodies Nc41 And Nc1 | 360 | 360 | 80% | 9e-122 | S |
| 1NCD_N | Chain N, Refined Crystal Structure Of The Influenza Virus N9 Neuram | 360 | 360 | 80% | 1e-121 | S |
| 1L7G_A | Chain A, Crystal Structure Of E119g Mutant Influenza Virus Neuramir | 360 | 360 | 80% | 2e-121 | S |
| 2B8H_A | Chain A, ANWSWHALEMAINE184 (H1N9) REASSORTANT INFLUENZA V | 360 | 360 | 80% | 2e-121 | S |
| 1V0Z_A | Chain A, Structure Of Neuraminidase From English Duck Subtype N6 | 355 | 355 | 79% | 1e-119 | S |

**Fig. 3.** BLAST analysis result

However, a careful observation of active site lining residues resulted in residue mutation in human H1N1 sequence. In other words, the residue variation was observed with respect to Tyr347 in H5N1 versus Asn344 in H1N1 neuraminidase sequence. Owing to the active site residue mutation, the protein model was built using SPDBV software.

An active site residue mutation was identified on comparison with H5N1 avian flu Neuraminidase enzyme. Hence, the 3D structure of H1N1 neuraminidase was built which can aid in detecting more potent binding inhibitor using computer-aided drug binding and screening studies (Figures 4-7).

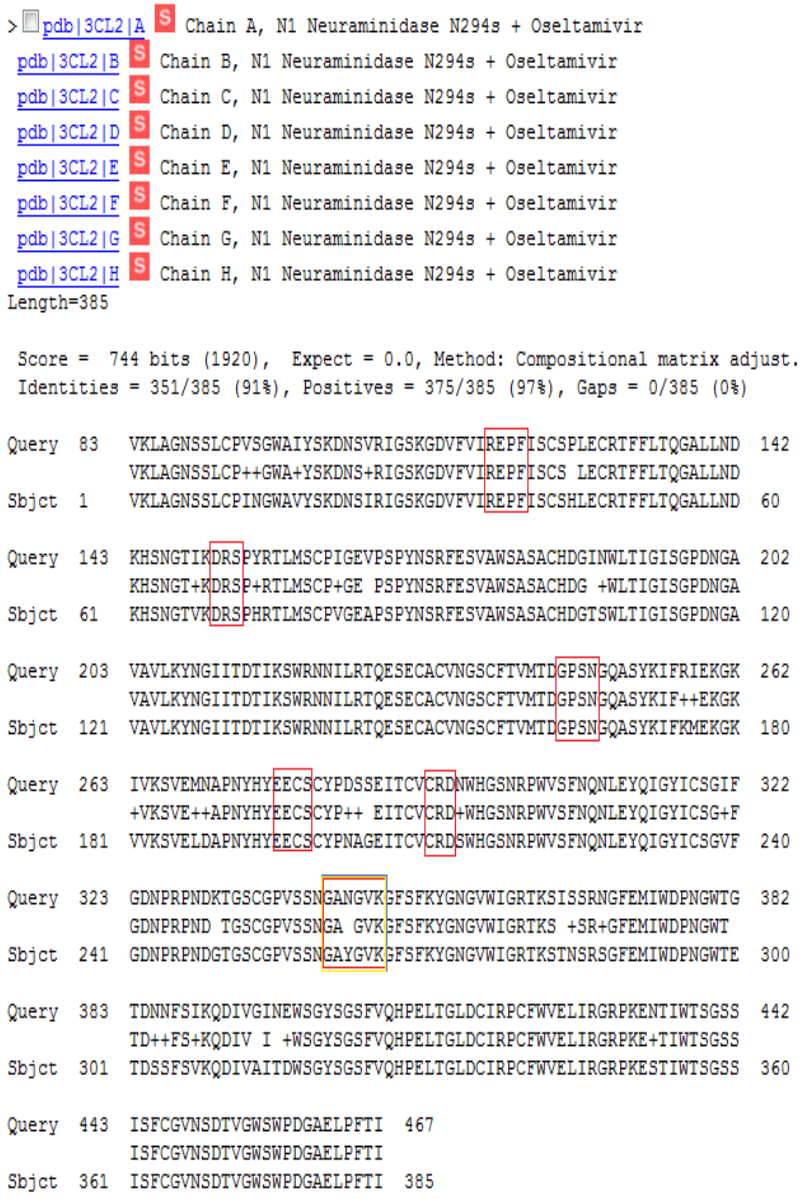


**Fig. 4.** Variations of amino acid residues between H5N1 and H1N1 neuraminidase sequences

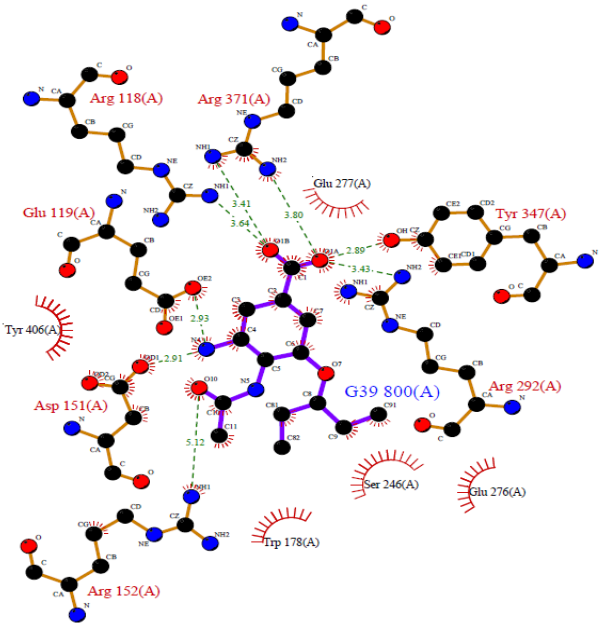


**Fig. 5.** Active site region of 3CL2 bound to oseltamivir

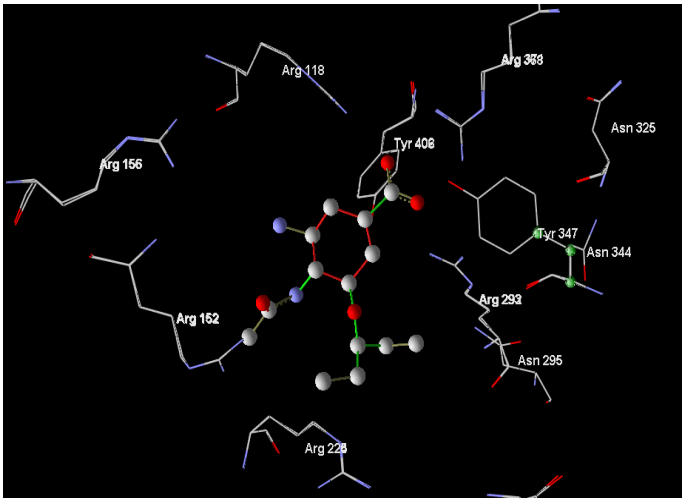


**Fig. 6.** Comparison of docked images of superimposed H5N1 and H1N1 acitve site regions showing Tyr347 of H5N1 replaced by Asn344 in H1N1

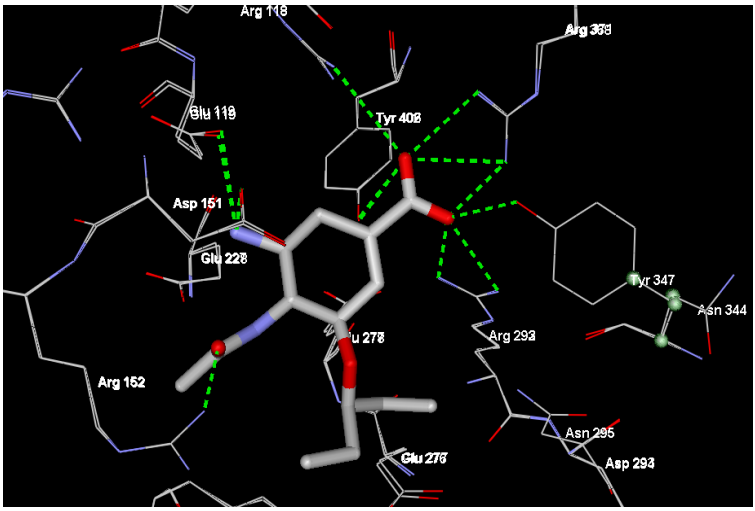


**Fig. 7.** Modelled protein with interacting H-bonds

## Conclusion

Literature reports suggest the importance of computational tools in finding few features that are relevant and important in understanding the structure and function of various mutational events in genes or proteins. One such study reported in this paper suggested the fact that with few computational efforts, variations in amino acid residue regions within the protein sequence can be known and accurate homology models can be built within short period of time. In this work, an active site residue mutation was identified in H1N1 neuraminidase upon comparison with H5N1 avian flu Neuraminidase enzyme. Hence, the 3D structure of H1N1 neuraminidase was built which can aid in detecting more potent binding inhibitor using computer-aided drug binding and screening studies.

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