

## Identification of a Point Mutation Causing Splitting of Antigenic Domain in M1 Protein of H5N1 Strain from 2006 Outbreak in India

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### Abstract

Influenza virus shows great variation in virulence. Numerous influenza virulence studies have sought to define the roles of each viral gene in disease production. To understand M1 protein it is necessary to study the functional properties of its distinct domains. Until now, in silico studies on M1 protein of Influenza A H5N1 virus are limited. The purpose of this study was to analyze the antigenic domains of the M1 protein of H5N1 strains found epidemic in Navapur (ABG88883) and Jalgaon, India (ABJ96491) during 2006 outbreak, and to identify antigenic differences between M1 proteins of other strains from different origin. It was noticed that the splitting of antigenic domain within position 48 to 69 of M1 was due to point mutation at position 59 from Isoleucine to Methionine. The antigenic regions identified here might be important for the development of diagnostic test for Influenza A H5N1 infection showing discrimination between different antigenic types of M1 protein of Influenza A H5N1.

**Keywords:** M1 protein; H5N1; Antigenic domain

### Introduction

Influenza virus is a typical enveloped (Schulze, 1973 and Laver, 1973) negative-strand RNA virus which is composed of eight single-stranded genomic segments coding for 10 or more polypeptides (Anwar et al., 2006; Lamb, 1983; Lamb and Choppin, 1983). The M1 matrix protein of influenza virus is a multifunctional factor involved in several steps of the life cycle of the virus (Helenius, 1992; Martin and Helenius, 1991). It is the most abundant protein found within the virion and has been shown to play a central role in virus assembly (Allen et al., 1980; Lamb, 1983; Lamb and Choppin, 1983; Lamb et al., 1981; Lamb and Zebedee, 1985). This protein is located at the inner surface of the lipid bilayer of the virion envelope in close proximity to the ribonucleocapsid protein (RNP) complex (Apostolov and Flewett, 1969; Compans and Choppin, 1975; Compans et al., 1979; Schulze, 1972). As with all RNA viruses, the influenza virus lacks a proofreader for replication, allowing the virus to mutate quickly. The host immune system selects for mutants by making antibodies to the original strain of virus. This leads to antigenic drift, whereby the virus gradually changes its types or sub-type. Antigenic variation in influenza comes in a multitude of forms, enabling it to effectively evade the immune system. In hu-

mans, changes in certain genes can lead to increasing virulence. Interestingly, antigenic drift in avian influenza is at a standstill; mutant viruses contain only silent changes in amino acid sequences [[http://www.brown.edu/Courses/Bio\\_160/Projects1999/av/](http://www.brown.edu/Courses/Bio_160/Projects1999/av/)]. The most important aspect in the production of antibodies or drug is the design of the peptide-antigen. The peptide-antigen is a small segment (15-18 amino acids) of the protein sequence of interest. These peptide-antigens can be used for immunization in order to produce antibodies against the protein for which the peptide is formed or the peptides can be used as a basis for small-molecule/drug targeting. There is a renewed interest to understand the antigenic diversity of influenza virus because of recent outbreaks of influenza epidemics (CDC, 2006). The knowledge thus gained will play a decisive role in influenza vaccine development (Amexis et al., 2001). We have undertaken this study to compare the antigenic properties of the Indian isolates of Influenza H5N1 with the homologous region of different subtypes of the Avian flu virus. The predicted antigenic sites of the matrix protein (M1) of H5N1, found in India, have been compared with the antigenic sites of the homologous domains in other subtypes, H5N1.

Materials and Methods

Antigenic site analysis

The sequences of Matrix protein (M1) of Influenza A Virus, spread in India during 2005-06 were obtained, which includes sub-types A/chicken/Navapur/Maharashtra/India/7972/2006 (H5N1) [GenBank: ABG88883] and A/chicken/Jalgaon/India/9386/2006 (H5N1) [GenBank: ABJ96491] isolated from chicken were analysed in the present work. Sequences similar to M1 of A/chicken/Navapur/Maharashtra/India/7972/2006 (H5N1) were extracted from NCBI FTP server ftp://ftp.ncbi.nih.gov/genomes/INFLUENZA/ from the file influenza.faa. Protein sequences of the sub-type H5N1 were sorted out for analysis. The sequences selected for the study are given in table 1. Antigenic sites were predicted using the tool Antigenic at Emboss available at <http://bio.dfci.harvard.edu/Tools/EMBOSS/>.

Results

Antigenic site analysis

Distinct antigenic domains identified in H5N1 strain of Indian origin (ABG88883 & ABJ96491), are reported in table 1. On comparing the antigenic domains in Indian strain, ABG88883 with the antigenic domains of Turkey, CAJ01905, it was noticed that in Turkey, CAJ01905 there was a single domain at position 48 to 69 while in our India strain, ABG88883, the antigenic domain at this position was splitted into two domains within the same position viz., 48->55 and 60->69. We, then compared Indian strain, ABG88883 with another strain of Indian origin i.e., strains from Jalgaon (ABJ96491). It was found that Jalgaon strain also showed two antigenic domains within the same position, 48->69. To find out the reason for this discrimination of being splitting into two different antigenic domains in some of the strains, 68 different H5N1 strains were analysed for the antigenic domain of M1 protein at 48 -> 69 (Table. 2). Through in silico analysis of this antigenic domain revealed that the strains that were having mutation at position 59 (M59I) have two antigenic domains (48->55 and 60->69), within the same region, while strains with single domain (48 -> 69) found to have Isoleucine at position 59. Moreover, it was also no-

Position	Antigenic Domain	No. of Residues	Score
139-154	TTEVAFGLVCATCEQI	16	1.208
4->32	LTEVETYVLSIIPSGPLKAEIAQKLEDVF	29	1.168
60->69	LGFVFTLTVP	10	1.145
96->102	AVKLYKK	7	1.119
112->130	AKEVALSYSTGALASCMGL	19	1.113
48->55	TRPILSPL	8	1.1
178->184	RMVLAST	7	1.084
234->240	LENLQAY	7	1.065
169->174	TNPLIR	6	1.03
A/Hatay/2004			
48->69	TRPILSPLTKGILGFVFTLTVP	22	1.145

**Table 1:** Predicted antigenic domains in Influenza A virus of Indian origin during the outbreak of 2006 in Navapur and Jalgaon ( ABG88883 and ABJ96491)

ticed that the strains from Germany, Sudan, Russia and Nigeria found to have two antigenic domains within position 48 to 69.

Discussion

To understand the function of M1 protein it is necessary to study the functional properties of its distinct domains (such

as antigenic domains). Antigenic sites represent potential candidates for peptide vaccine against the virus; this type of study can help in better understanding of Influenza A H5N1 isolates spread in India. In this work, we identified distinct antigenic regions in the M1 protein of Influenza A H5N1 virus and compared these domains with other H5N1 strains for M1 protein. Sequence based analyses of M1 pro-

Strain	Location	Acc. No.	Base at position 59	Antigenic Site
A/chicken/Navapur/Maharashtra/India/7972/2006	India	ABG88883	M	48->55, 60->69
A/chicken/Jalgaon/India/9386/2006	India	ABJ96491	M	48->55, 60->69
A/cat/Germany/606/2006	Germany	ABF61764	M	48->55, 60->69
A/grebe/Tyva/Tyv06-1/2006	Russia, Asia	ABI34120	M	48->55, 60->69
A/chicken/Dovolnoe/03/2005	Russia	ABG20477	M	48->55, 60->69
A/chicken/Sudan/1784-10/2006	Sudan	ABI95350	M	48->55, 60->69
A/chicken/Nigeria/957-20/2006	Nigeria	ABI95328	M	48->55, 60->69
A/Cygnus olor/Astrakhan/Ast05-2-2/2005	Russia	ABC94731	M	48->55, 60->69
A/chicken/Kurgan/3/2005	Russia	ABC48793	M	48->55, 60->69
A/Hatay/2004/	Turkey, Asia	CAJ01905	I	48->69
A/Goose/Guangdong/1/96	China	AAD51928	I	48->69
A/chicken/Hubei/wn/2003	China, Asia	ABI96761	I	48->69
A/chicken/Vietnam/132/2004	Vietnam	ABF01904	I	48->55, 60->69
A/Pheasant/Hong Kong/FY155/01	Hong Kong	AAO52887	I	48->69
A/Duck/Hong Kong/573.4/01	Hong Kong	AAO52907	I	48->69
A/Goose/Hong Kong/ww100/01	Hong Kong	AAO52906	I	48->69
A/Goose/Hong Kong/76.1/01	Hong Kong	AAO52905	I	48->69
A/Chicken/Hong Kong/893.2/01	Hong Kong	AAO52904	I	48->69
A/chicken/Hubei/327/2004	China	AAT90836	I	48->69
A/Hong Kong/485/97	Hong Kong	AAF74336	I	48->69
A/Vietnam/CL119/2005	Vietnam	ABF01924	I	48->69
A/Indonesia/CDC326/2006	Indonesia	ABI36062	I	48->69
A/chicken/Henan/01/2004	China	AAX53511	I	48->69
A/swan/Guangxi/307/2004	China	AAX53523	I	48->69
A/wild duck/Guangdong/314/2004	China	AAX53521	I	48->69
A/chicken/Henan/16/2004	China	AAX53519	I	48->69
A/crow/Osaka/102/2004	Japan	BAD89349	I	48->69
A/crow/Kyoto/53/2004	Japan	BAD89339	I	48->69
A/chicken/Oita/8/2004	Japan	BAD89319	I	48->69
A/chicken/Yamaguchi/7/2004	Japan	BAD89309	I	48->69
A/chicken/Jiangsu/cz1/2002	China	ABI96764	I	48->69
A/chicken/Kulon Progo/BBVet-XII-2/2004	Indonesia	ABF01806	I	48->69
A/chicken/Tarutung/BPPVI/2005	Indonesia	ABF01804	I	48->69
A/chicken/Deli Serdang/BPPVI/2005	Indonesia	ABF01802	I	48->69
A/chicken/Dairi/BPPVI/2005	Indonesia	ABF01800	I	48->69
A/chicken/Tebing Tinggi/BPPVI/2005	Indonesia	ABF01798	I	48->69
A/chicken/Simalanggang/BPPVI/2005	Indonesia	ABF01796	I	48->69
A/turkey/Kedaton/BPPV3/2004	Indonesia	ABF01794	I	48->69

**Table 2:** Comparison of antigenic domains at position 48 to 69 within 68 different strains of Influenza virus AH5N1 for M1 protein from different geographic regions.

Continued

A/chicken/Pangkalpinang/BPPV3/2004	Indonesia	ABF01792		48->69
A/chicken/Kupang-1-NTT/BPPV6/2004	Indonesia	ABF01790		48->69
A/duck/Parepare/BBVM/2005	Indonesia	ABF01784		48->69
A/chicken/Mangarai-NTT/BPPV6/2004	Indonesia	ABF01782		48->69
A/chicken/Jembrana/BPPV6/2004	Indonesia	ABF01780		48->69
A/chicken/Bangli Bali/BBPV6-1/2004	Indonesia	ABF01778		48->69
A/quail/Tasikmalaya/BPPV4/2004	Indonesia	ABF01774		48->69
A/chicken/Purwakarta/BBVet-IV/2004	Indonesia	ABF01772		48->69
A/chicken/Kulon Progo/BBVW/2005	Indonesia	ABF01770		48->69
A/quail/Yogyakarta/BBVet-IX/2004	Indonesia	ABF01766		48->69
A/chicken/Purworejo/BBVW/2005	Indonesia	ABF01764		48->69
A/chicken/Gunung Kidul/BBVW/2005	Indonesia	ABF01768		48->69
A/quail/Boyolali/BPPV4/2004	Indonesia	ABF01762		48->69
A/chicken/Sragen/BPPV4/2003	Indonesia	ABF01760		48->69
A/chicken/Pekalongan/BPPV4/2003	Indonesia	ABF01758		48->69
A/chicken/Ngawi/BPPV4/2004	Indonesia	ABF01756		48->69
A/chicken/Magetan/BBVW/2005	Indonesia	ABF01754		48->69
A/chicken/Malang/BBVet-IV/2004	Indonesia	ABF01752		48->69
A/R(duck/Mongolia/54/01- duck/Mongolia/47/01	Mongolia, Asia	BAE96571		48->69
A/crested eagle/Belgium/01/2004	Belgium	ABB54696		48->69
A/duck/Yokohama/aaq10/2003	China	BAE07159		48->69
A/turkey/England/50-92/1991	UK: England	ABI85163		48->69
A/chicken/Scotland/1959	UK:Scotland	ABI85107		48->69
A/duck/Minnesota/1525/1981	USA: Minnesota	ABI84609		48->69
A/duck/Hokkaido/Vac-1/04	Japan	BAE94703		48->69
A/chicken/Yunnan/K001/2004	China	AAV53536		48->69
A/chicken/Jilin/9/2004	China	AAT76159		48->69
A/human/Zhejiang/16/2006	China: Zhejiang Province	ABG23663		48->69
A/duck/Korea/ESD1/03	Korea	AAV97612		48->69
A/duck/China/E319-2/03	China	AAR99627		48->69
A/Ck/Indonesia/5/2004	Indonesia	AAT70511		48->69

**Table 2:** Comparison of antigenic domains at position 48 to 69 within 68 different strains of Influenza virus A H5N1 for M1 protein from different geographic regions.

tein revealed that although there exists very high sequence similarity at protein level, various strains have acquired certain mutations which confer strain-specific properties. It is known that a phenotypic property such as antigenicity is the result of 'spatio-temporal' hierarchical processes. Exact mapping of the same at molecular level is difficult due to the fact that there exists complexity in terms of intermolecular interactions which may be discrete or continuous (Flower et al., 2003). Thus, a bioinformatics approach was utilized for this analysis. It was found out that the reason for splitting of the antigenic domain is the point mutation at position 59 (M59I). Our data shows that the strains which have Isoleucine at position 59 show single antigenic domain while the strains with methionine at this position are having two antigenic domains within the region 48 to 69 (Table 2). It was also noticed that the two strains isolated from Indian subcontinent are showing similarity for the presence of antigenic domain (48 -> 69).

## Conclusion

Antigenic sites represent potential candidates for the peptide vaccine against H5N1. In silico approach has the potential to become an important tool to understand disease pathogenesis and designing antigen-specific therapies. The peptides, derived from antigenic sites may serve as ideal antigens to develop site-specific immunoassays for serological diagnosis.

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