

# H5N1 Genetic Changes Inventory: A Tool for Influenza Surveillance and Preparedness

## Background

Since highly pathogenic avian influenza (HPAI) H5N1 viruses were first identified in humans in 1997, they have evolved rapidly and have formed multiple distinct genetic clades. During this time HPAI viruses have caused millions of deaths among birds and over six hundred deaths in humans.

Laboratory studies have implicated convergent evolution of certain molecular determinants for pathogenicity and host specificity for influenza viruses in nature; however, our understanding of the precise molecular determinants required for transmissibility and severe disease in mammals remains incomplete. It is believed that there are likely to be multiple evolutionary pathways that would increase mammalian transmissibility of avian influenza viruses, including HPAI H5N1 viruses.

In light of the expanding host range, geographic spread, and the effect of co-circulation of heterogeneous viruses on interspecies transmission of H5N1 viruses, it is critical that enhanced approaches to influenza surveillance are maintained. Early detection of the natural emergence of genetic changes that may enhance adaptation of HPAI H5N1 viruses for humans and/or other mammals or may impair antiviral interventions is an essential component of this effort. The WHO Collaborating Center for Influenza Reference and Research at the Centers for Disease Control and Prevention in Atlanta, with input from many influenza subject matter experts from around the world, has developed an inventory of amino acid mutations in H5N1 viruses that is intended to be used by the influenza surveillance and research community as a tool to inform the influenza knowledge base for surveillance and public health preparedness.

## H5N1 Inventory

The H5N1 Inventory supports a molecular-based approach for surveillance and should be used to identify genetic mutations that determine viral phenotypic characteristics of importance. The H5N1 Inventory is an inclusive compilation of amino acid changes and/or motifs identified within each viral protein that affect one or more biological properties, provided in broad categories. References to manuscripts in the relevant published literature describing these relationships are provided; citations are representative of the published literature and are not intended to be all inclusive. This H5N1 inventory will be updated periodically by the WHO CC for Influenza at the CDC in collaboration with international partners and will be available on line with a date stamp indicating when the list was updated.

## How to Use the H5N1 Inventory

The H5N1 Inventory should be used by those conducting surveillance in humans and animals as well as research on influenza H5N1 viruses as a reference for the identification of specific mutations in naturally occurring H5N1 viruses that may signal adaptation to mammalian species or alter susceptibility to existing antivirals. Surveillance and research teams that identify viruses with mutations of concern should alert a relevant WHO Collaborating Center or an H5N1 reference laboratory and submit the virus for further characterization and for risk assessment. Contact information for these laboratories can be found on the World Health Organization (WHO) website at [http://www.who.int/influenza/gisrs\\_laboratory/en/](http://www.who.int/influenza/gisrs_laboratory/en/). Please ensure that your national veterinary services are informed when influenza viruses are detected in animals. All H5N1 avian influenza viruses in poultry and all HPAI viruses in birds are notifiable to OIE; please consult [http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre\\_1.10.4.htm](http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.10.4.htm) for further information about the definition of notifiable avian influenza.

## How the H5N1 Inventory Was Created

The H5N1 Inventory is based on published and publically available information identified in two ways: i) a review of the literature by influenza subject matter experts and ii) a bioinformatics text mining process using key words to search the existing PubMed H5N1 literature to identify relevant published materials. The following criteria were used for inclusion in the H5N1 Inventory: i) studies in mammalian cell culture, human specimens or tissues; studies in laboratory animals (mice, ferrets, guinea pigs); ii) mutations or motifs that were introduced into HPAI H5N1 viruses by reverse genetics or arose through repeated passage in the above mammalian systems or were identified through genetic sequence comparison of wild type viruses with different phenotypes; iii) studies on the H5 hemagglutinin (HA) which introduced mutations into recombinant proteins, recombinant viruses or pseudo viruses bearing an H5 HA; iv) mutations that affect the following properties: receptor binding (increased binding to  $\alpha$ 2,6 sialic acid as measured by *in vitro* assays); altered binding profiles or tropism for human airway tissues; replication efficiency (plaque morphology, cell culture or tissue viral titers, temperature sensitivity); virulence and transmissibility in animal models; altered polymerase complex activity in *in vitro* assays; altered host response including cell signaling pathways that may alter host antiviral response; altered susceptibility of N1 subtype viruses (H5N1, former seasonal H1N1 and H1N1pdm) to licensed neuraminidase (NA) and M2 inhibitors in functional assays detected in field isolates or clinical case reports; and animals or cultured cells treated with drugs. H5N1 virus (A/Vietnam/1203/2004) amino acid numbering for the mature proteins has been used throughout.

Protein	Amino acid position/motif <sup>a</sup>	Phenotypic consequences <sup>b</sup>	H5N1 virus tested <sup>c</sup>	References <sup>d</sup>	PMID <sup>e</sup>	
PB2	Ile63Thr	Decreased pathogenicity in mice	A/Vietnam/1194/2004	Li et al., 2011	21367983	
	Asp256Gly	Enhanced polymerase activity, mammalian host adaptation	A/chicken/Yamaguchi/7/2004	Manzoor et al., 2009	19052090	
	Gln591Lys	Enhanced replication efficiency and increased virulence in mice	A/chicken/Indonesia/UT3091/2005, A/Indonesia/UT3006/2005	Yamada et al., 2010	20700447	
	Lys627Glu	Reduced polymerase activity		Webster, personal communication		
	Glu627Lys		Increased replication efficiency in cell culture and enhanced virulence in mice	A/Vietnam/1203/2004, A/Vietnam/1204/2004	Hatta et al., 2007	17922570
			Increased virulence in mice	A/chicken/Vietnam/NCVD5/2003	Kim et al., 2010	20016035
			Increased virulence in mice	A/Hong Kong/483/97, A/Hong Kong/486/97	Chen et al., 2007	17521765
			Enhanced polymerase activity and mammalian host adaptation	A/chicken/Yamaguchi/7/2004	Manzoor et al., 2009	19052090
			Increased virulence in mice	A/Hong Kong/483/97, A/Hong Kong/486/97	Hatta et al., 2001; Shinya et al., 2004	11546875; 15016548
			Increased virulence in mice	A/Hong Kong/483/97, A/Hong Kong/486/97	Fornek et al., 2009	19692471
			Mammalian host adaptation	A/Vietnam/1203/2004, A/Vietnam/1204/2004, A/Vietnam/3030/04	Le et al., 2009	19264775
			Mammalian host adaptation, increased virulence in mice	A/chicken/Yamaguchi/7/2004	Mase et al., 2006	17098982
			Enhanced polymerase activity	A/Vietnam/1203/2004	Bortz et al., 2011	21846828
			Mammalian host adaptation	A/swan/Germany/R65/2006	Bogs et al., 2011	21849466
		H5 virus transmissible among ferrets <sup>f</sup>	A/Indonesia/05/2005	Herfst et al., 2012	22723413	
	Asp701Asn		Enhanced replication efficiency and transmission in guinea pigs	A/duck/Guangxi/22/2001, A/duck/Guangxi/35/2001	Gao et al., 2009	20041223
			Mammalian host adaptation	A/Vietnam/1203/2004, A/Vietnam/1204/2004, A/Vietnam/3030/04	Le et al., 2009	19264775
			Enhanced replication efficiency; increased virulence and transmission in guinea pigs	A/Vietnam/1203/2004	Steel et al., 2009	19119420
			Mammalian host adaptation, increased virulence in mice	A/duck/Guangxi/22/2001, A/duck/Guangxi/352/2001	Li et al., 2005	16140781
	Met28Ile, Ala274Thr, Lys526Arg, Ile553Val, Leu607Val <sup>f</sup>	Decreased polymerase activity	A/duck/Fujian/01/2002, A/duck/Guangxi/53/2002	Leung et al., 2010	20211480	
	Leu89Val, Gly309Asp, Thr339Lys, Arg477Gly, Ile495Val, Lys627Glu, Ala676Thr <sup>f</sup>	Enhanced polymerase activity and increased virulence in mice	A/wild duck/Hunan/021/2005	Li et al., 2009	19393699	
Arg368Gln, Gln391Glu, Gln447His, Lys627Glu <sup>f</sup>	Reduction in replication efficiency in cell culture and virulence in mice and ferrets	A/Vietnam/1203/2004, A/chicken/Vietnam/C58/2004	Salomon et al., 2006	16533883		

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PB1	Lys207Arg	Decreased polymerase activity in mammalian cells	A/Vietnam/1203/2004	Hulse-Post et al., 2007	17553873
	Tyr436His	Decreased polymerase activity and virulence in mallards, ferrets and mice	A/Vietnam/1203/2004	Hulse-Post et al., 2007	17553873
	Thr677Met	Decreased virulence in mice	A/Vietnam/1194/2004	Li et al., 2011	21367983
	Val3Ala, Asn328Lys, Asn375Ser <sup>f</sup>	Decreased replication efficiency and virulence in ferrets	A/Vietnam/1203/2004, A/chicken/Vietnam/C58/2004	Salomon et al., 2006	16533883
	His99Tyr, Ile368Val <sup>f</sup>	H5 virus transmissible among ferrets	A/Indonesia/05/2005	Herfst et al., 2012	22723413
	Val473Leu, Pro598Leu <sup>f</sup>	Decreased polymerase activity and replication efficiency	A/Cambodia/P0322095/2005	Xu et al., 2011	22090209
PB1-F2	Asn66Ser	Increased virulence, replication efficiency and antiviral response in mice	A/Vietnam/1203/2004	Schmolke et al., 2011	21852950
		Increased virulence and antiviral response in mice	A/Hong Kong/156/1997	Conenello et al., 2007	17922571
		Increased virulence and antiviral response in mice	A/Hong Kong/156/1997	Conenello et al., 2011	21084483
PA	Thr515Ala	Decreased polymerase activity in mammalian cells	A/Vietnam/1203/2004	Hulse-Post et al., 2007	17553873
	Pro149Ser, Arg266His, Lys357Ile, Thr515Ser <sup>f</sup>	Decreased polymerase activity in mammalian cells	A/duck/Fujian/01/2002, A/duck/Guangxi/53/2002	Leung et al., 2010	20211480

Protein	Amino acid position/motif <sup>a</sup>	Phenotypic consequences <sup>b</sup>	H5N1 virus tested <sup>c</sup>	References <sup>d</sup>	PMID <sup>e</sup>
HA	Asp94Asn	Increased virus binding to $\alpha$ 2-6; enhanced virus fusion	A/chicken/Fujian/1042/05	Su et al., 2008	19020946
	Ser121Asn	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Wang et al., 2010	20427525
	Ser133Ala	Increased psuedovirus binding to $\alpha$ 2-6	A/Thailand/KAN-1/2004	Yang et al., 2007	17690300
	Ala134Val	Increased infectivity in SIAT Cells ; emerged in the course of virus replication in a patient (fatal case)	A/Cambodia/408008/2005 & A/Thailand/KAN-1/2004	Naughtin et al., 2011; Kongchanagul et al., 2008	21343450; 18632950
	Gly139Arg	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Ser155Asn	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Wang et al., 2010	20427525
	Thr156Ala	Increased virus binding to $\alpha$ 2-6 and increased transmission in guinea pigs	A/Vietnam/1203/2004 & A/duck/Guangxi/35/2001 & A/bar-headed goose/Qinghai/3/2005	Wang et al., 2010; Gao et al., 2009	20427525
	Asn182Lys/Asn182Asp	Increased virus binding to $\alpha$ 2-6 ; emerged in the course of virus replication in a patient (fatal case)	A/Vietnam/1194/2004 & A/Indonesia/05/2005 & A/Thailand/KAN-1/2004	Yamada et al., 2006 ; Chutinimitkul et al., 2010 ; Kongchanagul et al., 2008	17108965; 20392847
	Asp183Gly	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Chen et al., 2012	22056389
	Glu186Gly	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Chen et al., 2012	22056389
	Thr188Ile	Increased psuedovirus binding to $\alpha$ 2-6	A/Thailand/KAN-1/2004	Yang et al.2007	17690300
	Lys189Arg	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Wang et al., 2010	20427525
	Gln192Arg	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004 & A/chicken/Indonesia/N1/2005 & A/Vietnam/1203/2004	Yamada et al., 2006 ; Chen et al., 2012	17108965; 22056389
	Gln192His	Increased virus binding to $\alpha$ 2-6	A/duck/Egypt/D1Br12/2007	Watanabe et al., 2011	21637809
	Asn193Lys	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004 & A/chicken/Indonesia/N1/2005	Yamada et al., 2006	17108965
	Val210Ile	Increased virus binding to $\alpha$ 2-6	A/duck/Egypt/D1Br12/2007	Watanabe et al., 2011	21637809
	Lys218Glu	Altered pathogenicity and tissue tropism in mice, emerged in the course of virus replication in a patient (fatal case)	A/Thailand/KAN-1/2004	Manz et al., 2010 ; Kongchanagul et al., 2008	20519408; 18632950
	Gln222Leu	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004 & A/Hong Kong/156/1997 & A/Indonesia/05/2005	Chutinimitkul et al., 2010	20392847
	Ser223Asn	Increased virus binding to $\alpha$ 2-6, emerged in the course of virus replication in a patient (fatal case)	A/Hong Kong/212/2003 & A/Hong Kong/213/2003 & A/Vietnam/1203/2004 & A/Indonesia/05/2005	Gambaryan et al., 2006; Shinya et al., 2010; Chutinimitkul et al., 2010; Chen et al., 2012; Kongchanagul et al., 2008	16226289; 20130132; 20392847; 22056389; 18632950
	Gly224Ser	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004 & A/Hong Kong/156/1997 & A/Indonesia/05/2005	Stevens et al., 2006 ; Chutinimitkul et al., 2010; Wang et al., 2010	16543414; 20392847; 20427525

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HA	Pro235Ser	Increase in SA $\alpha$ 2,6Gal binding	A/duck/Egypt/D1Br12/2007	Watanabe, 2011	21637809
	Glu251Lys	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Chen et al., 2012	22056389
	323 to 330 (R-X-R/K-R)	Polybasic cleavage motif sequence required for high pathogenicity of H5N1 avian influenza viruses	All H5N1 viruses <sup>e</sup>	Bosch et al., 1981 ; Perdue et al., 1997 ; Webster & Rott 1987 ; Subbarao et al., 1998 ; Horimoto & Kawaoka 1994 ; Schrauwen et al., 2012 ; Sugitan et al., 2012 ; Zhang et al., 2012	7023022; 9213392; 3304656; 9430591; 8151777; 22278228; 22205751; 22496231
	Lys388Ile (Lys58Ile in HA2)	Decreased pH of fusion, increased HA stability, increased replication efficiency in mice	A/Vietnam/1203/2004	Reed et al., 2009 ; Krenn et al., 2011	19193808; 21490925
	Glu435Lys	Decreased pH of fusion	A/chicken/Vietnam/C58/2004	Reed, 2009	19193808
	Asn444Lys	Increased pH of fusion	A/chicken/Vietnam/C58/2004	Reed, 2009	19193808
	Glu75Lys/Ser123Pro <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Glu75Lys/Ser123Pro/Arg497Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Glu75Lys/Ser123Pro/Asn193Lys/Arg497Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Glu75Lys/Asn193Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Glu75Lys/Asn193Lys/Arg497Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Glu75Lys/Arg497Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	His103Tyr, Thr156Ala, Gln222Leu, Gly224Ser <sup>f</sup>	H5 virus transmissible among ferrets	A/Indonesia/05/2005	Herfst et al., 2012	22723413
	Ser123Pro/Asn193Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Ser123Pro/Asn193Lys/Arg497Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Ser123Pro/Arg497Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
	Leu129Val, Ala134Val <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Thailand/676/2005	Auewarakul et al., 2007	17626098
	Leu129del, Ile151Thr <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/duck/Egypt/D1Br12/2007	Watanabe et al., 2011 ; Auewarakul et al., 2007	20427525; 17626098
	Ser133Ala/Thr188Ile <sup>f</sup>	Increased pseudovirus binding to $\alpha$ 2-6	A/Thailand/KAN-1/2004	Yang et al.2007	17690300
	Gly139Arg, Asn182Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004 & A/Indonesia/05/2005	Yamada et al., 2006; Chutinimitkul et al., 2010	17108965; 20392847
Asn154Asp, Asn220Lys, Gln222Leu, Thr315Ile <sup>f</sup>	H5 HA virus transmissible among ferrets	A/Vietnam/1203/2004 HA in H1N1p background (A/California/04/09)	Imai et al., 2012	22722205	
Asn154Ser, Gln222Leu <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Ilyushina et al., 2008	18404209	
Asn154Ser, Gln222Leu, Asn244Asp <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Ilyushina et al., 2008	18404209	

Protein	Amino acid position/motif <sup>a</sup>	Phenotypic consequences <sup>b</sup>	H5N1 virus tested <sup>c</sup>	References <sup>d</sup>	PMID <sup>e</sup>
HA	Ser155Asn, Thr156Ala <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Wang et al., 2010	20427525
	Ser155Asn, Thr156Ala, Ser223Asn <sup>f</sup>	Increased virus binding to $\alpha$ 2-6, reduced lethality and systemic spread in mice	A/Vietnam/1203/2004	Yen et al., 2009	19116267
	Thr156Ala, Gln222Leu <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Wang et al., 2010	20427525
	Thr156Ala, Gln222Leu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004 & A/Indonesia/05/2005	Stevens et al., 2008 ; Wang et al., 2010	18672252; 20427525
	Thr156Ala, Ser223Asn <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Wang et al., 2010	20427525
	Asn182Lys, Gln192Arg, Gln222Leu, Ser223Asn, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Indonesia/05/2005	Chutinimitkul et al., 2010	20392847
	Asn182Lys, Gln222Leu, Ser223Asn, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Indonesia/05/2005	Chutinimitkul et al., 2010	20392847
	Asn182Lys, Gln222Leu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Indonesia/05/2005	Chutinimitkul et al., 2010	20392847
	Glu183Gly, Glu186Asp, Lys189Ser, Gln222Leu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Hong Kong/486/1997	Maines et al., 2011	21397290
	Glu183Gly, Gln222Leu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Egret/Egypt/1162/NAMRU-3/2006	Chen et al., 2012	22056389
	Asp183Gly, Ser223Asn <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Chen et al., 2012	22056389
	Glu186Gly, Gln222Glu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Egret/Egypt/1162/NAMRU-3/2006	Chen et al., 2012	22056389
	Lys189Arg, Gln222Leu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004	Stevens et al., 2008 ; Maines et al., 2011	18672252; 21397290
	Gln192Arg, Gln222Leu, Ser223Asn, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Indonesia/05/2005	Chutinimitkul et al., 2010	20392847
	Gln192Arg, Gln222Leu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Egret/Egypt/1162/NAMRU-3/2006 & A/Indonesia/05/2005	Chutinimitkul et al., 2010; Chen et al., 2012	20392847; 22056389
	Gln192Arg, Ser223Asn <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1203/2004 & A/Vietnam/1194/2004 & A/Indonesia/05/2005	Chutinimitkul et al., 2010; Chen et al., 2012	20392847; 22056389
	Asn193Lys/Arg497Lys <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Vietnam/1194/2004	Yamada et al., 2006	17108965
Gln222Leu, Ser223Asn, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6	A/Indonesia/05/2005	Chutinimitkul et al., 2010	20392847	
Gln222Leu, Gly224Ser <sup>f</sup>	Increased virus binding to $\alpha$ 2-6; decreased antiviral response in host; reduced tissue tropism in guinea pigs	A/Vietnam/1203/2004 & A/Indonesia/05/2005 & A/Egret/Egypt/1162/NAMRU-3/2006 & A/Hong Kong/156/1997 & A/Hong Kong/486/1997 & A/duck/Guangxi/35/2001 & A/Vietnam/1194/2004	Harvey et al., 2004; Stevens et al., 2006; Stevens et al., 2008; Ilyushina et al., 2008; Maines et al., 2011; Wang et al., 2010; Chutinimitkul et al., 2010; Ramos et al., 2011; Chen et al., 2012; Gao et al., 2009; Ayora-Talavera et al., 2009	14671130; 16543414; 18672252; 18404209; 21397290; 20427525; 20392847; 21345953; 22056389; 20041223; 19924306	

Protein	Amino acid position/motif <sup>a</sup>	Phenotypic consequences <sup>b</sup>	H5N1 virus tested <sup>c</sup>	References <sup>d</sup>	PMID <sup>e</sup>
NA	49-68 deletion <sup>h</sup>	Enhanced virulence in mice	Conserved among contemporary H5N1 viruses <sup>g</sup>	Matsuoka et al., 2009; Zhou et al., 2009	19225004; 19609439
	Val96Ala (116 in N2)	Reduced susceptibility to zanamivir and oseltamivir		Hurt et al., 2007; Boltz et al., 2010; Ilyushina et al., 2010	17112602; 20016036; 20523902
	Ile97Val (117 in N2)	Reduced susceptibility to oseltamivir		Hurt et al., 2007; Ilyushina et al., 2010; Le et al., 2008	17112602; 20523902; 18836532
	Glu99Ala/Gly/Val (119 in N2)	Reduced susceptibility to zanamivir, oseltamivir and/or peramivir		Ilyushina et al., 2010; 2012; Hurt et al., 2009; Pizzorno et al., 2011; Abed et al., 2006	20523902; 22379077; 19651908; 21148493; 17302366
	Gln116Leu/Lys/Arg (136 in N2)	Reduced susceptibility to zanamivir and oseltamivir		Hurt et al., 2010; Okomo-Adhiambo et al., 2009; Hurt et al., 2009	20603155; 19917319; 19641000
	Val129Ala (149 in N2)	Reduced susceptibility to zanamivir		Naughtin et al., 2011	21343450
	Arg136Lys (156 in N2)	Reduced susceptibility to oseltamivir, zanamivir, and peramivir		Ilyushina, 2012	22379077
	Asp179Gly (198 in N2)	Reduced susceptibility to zanamivir and oseltamivir		Hurt et al., 2009; Pizzorno et al., 2011	19651908; 21148493
	Ile203Met/Val/Leu/Lys/Arg (222 in N2)	Reduced susceptibility to oseltamivir		Hurt et al., 2009; Pizzorno et al., 2011; Wibawa et al., 2011; Boltz et al., 2010; Nguyen et al., 2010	19651908; 21148493; 17302366; 20016036; 20858074
	Ser227Asn (246 in N2)	Reduced susceptibility to oseltamivir		Boltz et al., 2010; Hurt et al., 2011	20016036; 21679678
	His254Tyr/Arg (274 in N2)	Reduced susceptibility to oseltamivir and peramivir		Gubareva et al., 2001; Le et al., 2005; de Jong et al., 2005; Ilyushina et al., 2008; Hill et al., 2009; Hurt et al., 2009; Govorkova et al., 2007	1170976; 16228009; 16371632; 17296744; 18368779; 19022400; 19651908
	Glu258Gln (277 in N2)	Reduced susceptibility to oseltamivir		Govorkova et al., 2007	17296744
	Asn275Ser (295 in N2)	Reduced susceptibility to oseltamivir		Le et al., 2005; Yen et al., 2007; Earhart et al., 2009; Kiso et al., 2011; Hill et al., 2009; Pizzorno et al., 2011	16228009; 17855542; 20701864; 21367898; 19022400; 21148493



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M1	Asn30Asp	Increased virulence in mice	A/duck/Fujian/01/2002	Fan et al., 2009	19117585
	Thr139Ala	Increased virulence in mice	Conserved among contemporary H5N1 viruses <sup>g</sup>	Smeenk, 1996; Brown, 1999	8879138; 10426210
	Thr215Ala	Increased virulence in mice	A/duck/Fujian/01/2002	Fan et al., 2009	19117585
M2	Leu26Phe	Reduced susceptibility to amantadine and rimantadine		Lan et al., 2010; Abed et al., 2005	20834097; 15673732
	Val27Ala	Reduced susceptibility to amantadine and rimantadine		Lan et al., 2010; Abed et al., 2005; Cheung et al., 2006; Ilyushina et al., 2005	20834097; 15673732; 16703504; 16081121
	Ala30Val/Thr/Ser	Reduced susceptibility to amantadine and rimantadine		Bean et al., 1989; Cheung et al., 2006	2723453; 16703504
	Ser31Asn/Gly	Reduced susceptibility to amantadine and rimantadine		Lan et al., 2010; Cheung et al., 2005; Bean et al 1989; He et al., 2008; Cinatl et al., 2007; Ilyushina et al., 2005; Puthavathana et al 2005; Buranathai et al., 2007	20834097; 16703504; 2723453; 17897729; 17431677; 16081121; 15659762; 17494553
	Gly34Glu	Reduced susceptibility to amantadine and rimantadine		Abed et al., 2005	15673732
NP	Asn319Lys	Enhanced replication efficiency	Conserved among contemporary H5N1 viruses <sup>g</sup>	Gabriel et al., 2008	18248089
	Gln357Lys (with PB2 627K) <sup>f</sup>	Enhanced virulence in mice	A/chicken/Vietnam/NCVD5/2003	Kim et al., 2010	20016035
	Arg99Lys, Ser345Asn <sup>f</sup>	H5 virus transmissible among ferrets	A/Indonesia/05/2005	Herfst et al., 2012	22723413

Protein	Amino acid position/motif <sup>a</sup>	Phenotypic consequences <sup>b</sup>	H5N1 virus tested <sup>c</sup>	References <sup>d</sup>	PMID <sup>e</sup>
NS1	Pro42Ser	Increased virulence in mice	A/Duck/Guangxi/12/03 ; A/Duck/Guangxi/27/03; conserved among contemporary H5N1 viruses <sup>g</sup>	Jiao et al., 2008	18032512
	80-84 deletion <sup>i</sup>	Increased virulence in mice	Conserved among contemporary H5N1 viruses <sup>g</sup>	Long et al., 2008 ; Seo et al., 2002	18317917; 12195436
	Asp87Glu	Increased virulence in mice	E conserved among contemporary H5N1 viruses <sup>g</sup>	Long et al., 2008 ; Seo et al., 2002	18317917; 12195436
	Leu98Phe	Increased virulence in mice	A/Hong Kong/483/1997; conserved among contemporary H5N1 viruses <sup>g</sup>	Kuo and Krug 2009 ; Spesock et al., 2011	19052083; 21593152
	Ile101Met	Increased virulence in mice	A/Hong Kong/483/1997; conserved among contemporary H5N1 viruses <sup>g</sup>	Kuo and Krug 2009 ; Spesock et al., 2011	19052083; 21593152
	Asn200Ser (when coupled with NS2 Thr47Ala)	Decreased antiviral response in host	A/Vietnam/UT3062/04; conserved among contemporary H5N1 viruses <sup>g</sup>	Imai et al., 2010	20862325
	Gly205Arg (when coupled with NS2 Met51Ile)	Decreased antiviral response in host	A/Vietnam/UT3062/04	Imai et al., 2010	20862325
	222-225 (presence of PDZ ligand domain)	Increased virulence in mice	ESEV is consensus among contemporary H5N1 viruses <sup>g</sup>	Jackson et al., 2008	18334632
NS2	Thr47Ala (when coupled with NS1 Asn200Ser)	Decreased antiviral response in host	A/Vietnam/UT3062/04	Imai et al., 2010	20862325
	Met51Ile (when coupled with NS1 Gly205Arg)	Decreased antiviral response in host	A/Vietnam/UT3062/04	Imai et al., 2010	20862325

- a** The numbering of each amino acid/motif is relative to A/Vietnam/1203/2004 except where noted.
- b** The mutation to the right of the amino acid position confers the phenotype describe below.
- c** The specific virus or virus backbone below was tested experimentally to measure the phenotype described.
- d** References include representative papers describing experiments which tested the mutation/motif of interest.
- e** PMID: Pubmed identifier of references cited.
- f** Experiments where a combination of mutations was associated with the phenotype described and the role of individual mutations were not identified.
- g** The mutation or motif described is conserved in contemporary highly pathogenic H5N1 viruses.
- h** The numbering of the NA deletion is relative to A/goose/Guangdong/1/1996.
- i** The numbering of the NS1 deletion is relative to A/goose/Guangdong/1/1996.