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Evaluation of phenotypic markers in full genome sequences of avian influenza isolates from California☆

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ABSTRACT

We evaluated phenotypic markers in full-genome sequences of avian influenza isolates to identify avian strains with increased potential for transmission and pathogenicity in mammals. Of 149 markers examined, 67 were positive in the consensus sequences from 206 avian isolates. Analysis of deep sequencing data in a subset of 24 isolates revealed that 344 subpopulations occurred at marker positions. Markers in subpopulations were significantly more likely to be negative (258/344) than positive (86/344), but nearly all of the marker-positive subpopulations (78/86) were associated with marker-negative consensus sequences. Our analysis revealed significant variation in important markers among avian isolates, and showed that consensus sequences do not fully convey an isolate's potential for increased transmissibility and pathogenicity in mammals.

1. Introduction

The recent emergence of highly pathogenic avian influenza (HPAI) H5N1 and pandemic H1N1 (H1N1pdm) graphically shows that genotypic and phenotypic changes in animal influenza viruses will continue to produce new human pathogens [1,2]. If we are to recognize and prepare for new emerging viruses, we must identify genotypic and phenotypic markers that indicate which animal viruses are most transmissible and pathogenic to humans. This is a formidable challenge given the tremendous genetic diversity of viruses circulating among birds and mammals in nature [3,4], and since laboratory phenotyping for even a single amino acid change can involve the use of wild-type and reverse genetic variants [5–7], culture in multiple cell lines [8], and experimental infections in rodents [8–10], guinea pigs [11,12], and ferrets [10,13,14].

Enhanced surveillance [15], coupled with advances in sequencing and bioinformatics [16], gives us the ability to develop hypotheses about which strains and subtypes pose the greatest risk and deserve further scrutiny as potential human pathogens. An important step in this process is to determine whether or not phenotypic markers deduced from sequence data are useful for assessing risk [11,17–19]. Phenotypic markers are amino acid substitutions that are suggested to alter pathogenicity and host tropism of influenza viruses to increase their likelihood to be transmitted from an avian to a mammalian host and cause disease.

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Table 1
Phenotypic markers ($n = 149$) reported from influenza A viruses that were analyzed in this study of 206 full genome sequences. The H5 numbering system was used to identify nucleotide positions in our analysis. Markers were identified by review of existing literature. Asterisks refer to positive markers in the consensus sequence (*) or subpopulation (**).

Protein	AA substitution (H5 numbering)	Phenotype	Identified in consensus (*) or subpop (**)	References
PB2	M28I	Increased polymerase activity, Increased virulence in mammals and birds	**	[37,38]
	A44S	Human host marker		[18]
	T63I (with PB1 M677T)	Pathogenic in mice	*	[39]
	M64T/I	Human host marker	*, **	[18]
	L89V	Enhanced polymerase activity, Increased virulence in mice	*	[40]
	A199S	Human host marker	**	[18,38,41]
	D256G	Enhanced polymerase activity, Mammalian host marker	**	[42]
	T271A	Human host marker (host-specific polymerase activity)	*, **	[18,38,43]
	A274T	Increased polymerase activity, Increased virulence in mammals and birds		[37]
	G309D	Enhanced polymerase activity, Increased virulence in mice	*, **	[40]
	R318K	Increased virulence in mammals	*, **	[5,44]
	T339K	Enhanced polymerase activity, Increased virulence in mice	*	[40]
	R/Q355K	Increased virulence in mammals	*, **	[5,9,45]
	Q368R	Increased polymerase activity, Increased virulence in mammals	*, **	[10,46]
	E391Q	Increased polymerase activity, Increased virulence in mammals	**	[10,46]
	H447Q	Increased polymerase activity, Increased virulence in mammals	*, **	[10,46]
	L475M	Human host marker (host-specific polymerase activity)	*	[18,41,43]
	R477G	Enhanced polymerase activity, Increased virulence in mice	*	[40]
	I495V	Enhanced polymerase activity, Increased virulence in mice	*	[40]
	K526R	Increased polymerase activity, Increased virulence in mammals and birds (host-specific polymerase activity)		[37,43]
	I553V	Increased polymerase activity, Increased virulence in mammals and birds	*	[37]
	D567N	Human host marker	*	[18,41]
	A588I	Human host marker (host-specific polymerase activity)		[18,38,43]
	GQ590/591SR	Increased polymerase activity, Human host adaptation	*	[47]
	Q591K	Increased virulence in mammals		[8]
	L607V	Increased polymerase activity, Increased virulence in mammals and birds	**	[37]
	E/D627K/N	Human host marker, Enhanced polymerase activity, Increased virulence in mammals	**	[5,8,9,12,13,18,41,42,48–72]
	A661T	Enhanced Transmission	*, **	[38]
	V667I	Enhanced Transmission	*	http://www.fludb.org
	A/S674T	Human host marker		[18,38]
	A676T	Enhanced polymerase active, Increased virulence in mice	*, **	[40]
	D701N	Increased polymerase activity, Increased virulence in mammals, Mammalian host marker		[8,11,12,43,47,58,71,73–77]
	K702R	Human host marker		[18,38,41]
	S714R	Increased polymerase activity, Increased virulence in mammals, Mammalian host marker	**	[74,77]
PB1	A3V	Increased polymerase activity, Increased virulence in mammals	*	[10,46]
	L13P	Increased polymerase activity, Increased virulence in mammals, Mammalian host marker	*	[74,77]
	M51T	Increased virulence in ducks		[78]
	A56V	Increased virulence in ducks		[78]
	G87E	Increased virulence in ducks		[78]
	H99Y	Airborne transmissible in mammals		[13]
	R207K	Increased polymerase activity in mammalian cells	*, **	[79]
	M/V317I	Increased virulence in mammals	*	[9,44,45,80]
	K328N	Increased polymerase activity, Increased virulence in mammals	*	[10,46]

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