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Genetic Analysis of Influenza A (H3N2) Viruses from Index Patients and Family Contacts

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Background/Aims: Influenza hemagglutinin (HA) is a highly evolving surface glycoprotein responsible for viral attachment and entry. Mutational HA drift among circulating human viruses during the 2007/08 season prompted a change in HA proteins used in the current 2008/09 vaccine. Analysis of influenza genes from index patients and family contacts were performed to: 1) evaluate genetic homology to vaccine strains, and 2) determine the extent of genetic mutation in confirmed family transmissions. **Methods:** Genetic analysis of the influenza HA1 hemagglutinin and matrix genes (MA) were performed on viruses obtained from seven families, including an index patient and at least one influenza-positive household family contact. **Results:** All index and transmission strains were genetically more similar to the 2008/09 vaccine Strain, A/Brisbane/10/2007 compared to previous H3N2 vaccine strains. HA analysis of viral strains revealed 100% protein homology from index patient to family contact in four of seven families. Of the three families with HA1 sequence variation, two exhibited one amino acid change, and one family revealed 5 amino acid changes compared to the index strain. The MA and M2 ectodomain were highly conserved among family transmissions, and all strains contained the mutation conferring resistance to adamantane drugs. **Conclusions**: Genetic analysis reveals that all family strains from this cohort are similar to the current A/Brisbane/10/2007 vaccine strain compared to last season's A/Wisconsin/67/2005 strain. HA changes were observed between families and within family transmissions. The results of this study suggest that the HA protein is highly evolving and can drift within a single human transmission.

Results. Genetic analysis of the influenza HA1 hemagglutinin and matrix genes were performed on viruses obtained from seven families. The analysis includes seven confirmed pediatric (index) patients and at least one influenza-positive household family (transmission) contact. Gene comparisons of viral strains were analyzed for mutation (genetic drift) among and within families comprising this cohort, and also compared to current and older vaccine reference strains. All index and transmission strains were

genetically more similar to the 2008/09 vaccine Strain A/Brisbane/10/2007 compared to last season's A/Wisconsin and older H3N2 vaccine strains, indicating better genetic coverage to the A/Brisbane vaccine strain (Figure 1).

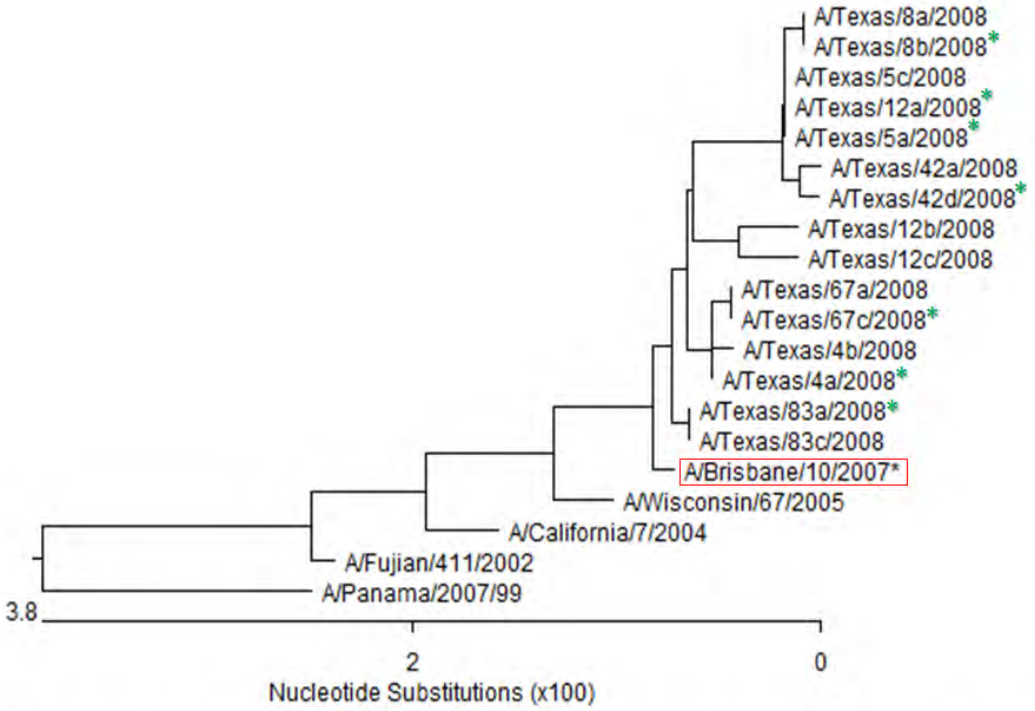

Figure 1. Phylogenetic analysis of the influenza (HA1) hemagglutinin from seven families with a pediatric index and family (household) transmissions. Amino acid changes were observed in 3 of 7 family transmissions. All family strains were genetically more similar to A/Brisbane/10/2007 (H3N2), selected as the 2008/09 vaccine component , compared to older vaccine strains since 2000. Red Box denotes 2008/09 vaccine strain; Green asterisks (*) denote pediatric index cases.

Genetic comparison of viral HA1 strains within families, i.e., from pediatric index patients to household family members, revealed 100% protein homology from index patient to family contact within four of the seven families analyzed. Of the three families exhibiting HA1 sequence variation, two families (42 and 4), exhibited one amino acid change (Table 1), and one family (12) exhibited 5 amino acid changes in comparison to the index strain (Table 1).

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The Pediatric index strain A/Texas/12a/2007 shares 99.1% and 98.6% nucleotide hemagglutinin homology to (family transmission) strains A/Texas/12b/2007 and A/Texas/12c/2007, respectively. Translation of the respective HA1 revealed five amino acid differences compared to the 12a index strain; of these five, two (S157L and N173E) were observed within both transmissions (Table 1).

The amino acid change at hemagglutinin positions 173 is located within known antibody binding site D, and is distinct from residues in the A/Brisbane H3 vaccine strain. The Substitution mutation at position 157 observed between the 12a index and 12b and 12c transmissions is located within antibody binding site B.

Table 1. Influenza A (H3N2) transmissions with at least one amino acid substitution within a pediatric index patient and family contact.

	HA1 Hemagglutinin Position						
Family transmissions	**3**	**83**	**123**	**157**	**173**	**208**	**300**
A/Texas/12a/2008*	F	N	E	S	N	R	I
A/Texas/12b/2008	F	N	E	L	E	R	V
A/Texas/12c/2008	L	K	E	L	E	R	I
A/Texas/42d/2008*	F	N	E	S	N	R	I
A/Texas/42a/2008	F	N	G	S	N	R	I
A/Texas/4a/2008*	L	K	E	L	Q	R	I
A/Texas/4b/2008	L	K	E	L	Q	G	I
A/Brisbane/10/2007	**L**	**K**	**E**	**L**	**K**	**R**	**I**

* Virus isolated from pediatric index patient.

The portions of gene segment 7 were sequenced, including the complete 252 amino acid M1 gene sequence and the alternatively spliced M2 minor surface ectodomain (M2e) to determine if any mutations were present among transmission cases in this highly conserved influenza gene. Sequences comprising the M1 was conserved across all family strains sequenced with the exception of a two mutations: 1) N85Y present in the A/Texas/5c/2008 (family contact), and 2) R174K present in A/Texas/67a/2008 (Family contact) and A/Texas/4a/2008 (index) strains. All family index and transmission strains contained an asparagine (N) at position 31 in the M2 protein indicating resistance to the adamantine class of drugs.

Influenza A segment 7 is also transpliced into the M2 minor proton pump surface protein, a portion of which consists of the 23 amino acid M2e. The M2e of all family transmission strains were conserved and consisted of **SLLTEVETPIRNEWGCRCNDSSD** amino acid protein sequence.

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Overarching Conclusions

- The influenza virus analysis reveals that all transmission strains from this cohort are a better genetic match to the current 2008/09 A/Brisbane/10/2007 vaccine strain compared to last season's A/Wisconsin/67/2005 and older vaccine strains (Figure 1).

- HA1 hemagglutinin mutations were observed within index strains compared to transmission strains from contacts in 3 of 7 families analyzed. This was particularly noted within Family 12 (a-c), where 5 mutations were observed in family transmissions compared to the pediatric index case (Table 1).

- Mutations transmission strains located at positions 157 and 173 are located within antibody binding sites B and D, respectively.

- With the exception of 2 mutations noted in two transmission strains, the M1 gene remained conserved within families. All index and family contact strains were adamantane resistant. The M2e minor surface antigen showed 100% homology across all transmission strains.

- The results of this work suggest that the HA1 hemagglutinin protein is a highly evolving viral protein that can mutate even among passage within a single human host.