Virulent PB1-F2 residues: effects on fitness of H1N1 influenza A virus in mice and changes during evolution of human influenza A viruses

Running title: influenza A PB1-F2 virulence

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Human seasonal H1N1 isolates

Isolation year	Presence of virulent residues at positions								No. of isolates	No. of isolates per year				
	62	66	68	69	70	75	79	82		≥62 amino acids			≤61 amino acid	Total[β]
										with virulent residues[α]	with virulent residue S66	without any virulent residue		
2011-17									0	0	0	0	11,676	11,676
2010										0	0	0	5	5
2009										0	0	0	310	310
2008					V				1	3	0	0	192	195
	L							L	1					
	L		I				R	L	1					
2007					V				5	10	0	0	377	387
	L					R			1					
	L							L	4					
2006										0	0	0	88	88
2005	L							L	1	1	0	0	26	27
2004										0	0	0	10	10
2003										0	0	0	51	51
2002										0	0	0	12	12
2001										0	0	0	125	125
2000										0	0	0	79	79
1999										0	0	0	13	13
1998	L							L	1	1	0	0	4	5
1997	L		I	L	V			L	1	1	0	0	6	7
1996										0	0	0	34	34
1995										0	0	0	34	34
1994										No isolate				
1993										0	0	0	1	1
1992										0	0	0	1	1

1991									0	0	0	2	2
1990									No isolate				
1989									0	0	0	2	2
1988	L				R			1	1	0	0	3	4
1987									0	0	0	5	5
1986	L				R	R	L	1	1	0	0	9	10
1985-84									No isolate				
1983									0	0	0	50	50
1982									0	0	0	4	4
1981									0	0	0	4	4
1980	L				R			3	3	0	0	3	6
1979	L				R			3	4	0	0	0	4
	L				R		L	1					
1978	L				R			2	2	0	0	13	15
1977									0	0	0	3	3
1976-58									No isolate				
1957									0	0	0	1	1
1956									0	0	0	3	3
1955									No isolate				
1954									0	0	0	3	3
1953									0	0	0	2	2
1952									No isolate				
1951									0	0	0	6	6
1950									0	0	0	2	2
1949	L	I	L	V	R		L	1	1	0	0	1	2
1948	L	I	L	V	R	R	L	2	2	0	0	1	3
1947	L	I	L	V	R		L	1	1	0	0	0	1
1946	L	I	L	V	R		L	1	2	0	0	0	2
	L	I	L	V	R	R	L	1					
1945	L		L	V		R	L	1	1	0	0	0	1
1944									No isolate				
	L	I	L	V	R	R	L	2					

1943	L			L				L	1	3	0	0	0	3
1942	L		I	L	V	R	R	L	1	1	0	0	0	1
1941										No isolate				
1940	L		I	L	V	R		L	1	1	0	0	0	1
1939-37										No isolate				
1936	L		I	L	V	R	R	L	1	1	0	0	0	1
1935	L		I	L	V	R	R	L	4	5	0	0	0	5
			I	L	V	R	R	L	1					
1934	L		I	L	V	R	R	L	1	1	0	0	0	1
1933			I	L	V	R	R	L	3	3	0	0	0	3
pdm1918	L	S			V	R	R	L	1	1	1	0	0	1
Total									**50**	**50**	**1**	**0**	**13,191**	**13,211**

Human H1N1 isolates of swine origin

Isolation year	**Presence of virulent residues at positions**								**No. of isolates**	**No. of isolates per year**				
	62	**66**	**68**	**69**	**70**	**75**	**79**	**82**		**≥62 amino acids**		**≤61 amino acid**	**Total[β]**	
										with virulent residues[α]	**with virulent residue S66**	**without any virulent residue**		
2016	L		I						1[a]	2	0	0	0	2
	L		I					L	1[a]					
2015	L								1[b]	3	0	0	0	3
	L		I			R			1[b]					
	L								1[b]					
2014										0	0	0	1[b]	1
2013										No isolate				
2012	L							L	1[b]	1	0	0	0	1
2011	L							L	1[b]	1	0	0	2[a]	3

2010										0	0	0	1[a]	1
2009	L							L	3[c]	6	0	0	0	6
	L							L	2[b]					
	L		I				R	L	1[a]					
2008	L							L	1[b]	1	0	0	0	1
2007-06	No isolate													
2005	L							L	1[b]	1	0	0	0	1
Total									**15**	**15**	**0**	**0**	**4**	**19**

Human seasonal H1N2 isolates

Isolation year	Presence of virulent residues at positions								No. of isolates	No. of isolates per year				
	62	66	68	69	70	75	79	82		≥62 amino acids			≤61 amino acid	Total[β]
										with virulent residues[α]	with virulent residue S66	without any virulent residue		
2009					V				1	1	0	0	0	1
2003									0	0	0	23	0	23
2002									0	0	0	6	0	6
Total									**1**	**1**	**0**	**29**	**0**	**30**

Human H1N2 isolates of swine origin

Isolation year	Presence of virulent residues at positions								No. of isolates	No. of isolates per year				
	62	66	68	69	70	75	79	82		≥62 amino acids			≤61 amino acid	Total[β]
										with virulent residues[α]	with virulent residue S66	without any virulent residue		
2017	L							L	1	1	0		1	2

2016	L							L	2	2	0	0	2	4
2015									0	0	0	0	1	1
2014-13	No isolate													
2012	L		I				R		1	1	0	0	0	1
2011	L							L	1	1	0	0	0	1
2010-08	No isolate													
2007	L							L	1	1	0	0	0	1
Total									**6**	**6**	**0**	**0**	**4**	**10**

1
2

3 **Human seasonal H2N2 isolates**

Isolation year	**Presence of virulent residues at positions**								**No. of isolates**	**No. of isolates per year**				
	62	**66**	**68**	**69**	**70**	**75**	**79**	**82**		**≥62 amino acids**			**≤61 amino acid**	**Total[β]**
										with virulent residues[α]	**with virulent residue S66**	**without any virulent residue**		
1968	L					R	R	L	7	7	0	0	0	7
1967	L					R	R	L	12	15	4	0	0	15
	L	S				R	R	L	4					
	L					R	R		1					
	L					R		L	1					
1966	L					R	R	L	4	4	0	0	0	4
1965	L					R	R	L	5	5	0	0	0	5
1964	L					R	R	L	3	4	0	0	1	5
	L					R	R		1					
1963	L					R	R	L	6	6	0	0	0	6
1962	L					R	R	L	2	2	0	0	0	2
1961	L					R	R	L	2	2	0	0	0	2
1960	L					R	R	L	3	3	0	0	0	3

1959	L					R	R	L	4	4	0	0	0	4
1958	L					R	R	L	8	8	0	0	0	8
pdm1957	L					R	R	L	16	17	0	0	0	17
	L					R		L	1					
Total									**77**	**77**	**4**	**0**	**1**	**78**

Human seasonal H3N2 isolates

Isolation year	Presence of virulent residues at positions								No. of isolates	No. of isolates per year				
	62	66	68	69	70	75	79	82		≥62 amino acids			≤61 amino acid	Total[β]
										with virulent residues[α]	with virulent residue S66	without any virulent residue		
2017					V				2018	2474	1	83	43	2600
					V	R			435					
			I		V				7					
						R			5					
			I		V	R			3					
					V			L	3					
			I		V		R	L	1					
					V	R		L	1					
		S			V				1					
2016					V				2225	2527	0	33	38	2598
					V	R			287					
			I		V				4					
			I		V	R			4					
					V			L	3					
	L				V				2					
				L	V				1					

	L			V		R	1				
				V			2213				
				V	R		37				
	L			V			6				
2015			I	V			5	2266	0	4	88	2358
				V		L	4				
			I	V	R		1				
				V			1006				
				V		L	14				
				V	R		9				
	L			V			4				
2014		S		V			4	1039	4	4	77	1120
			I	V			1				
	L						1				
				V			670				
		S					2				
				V		R	2				
2013		S		V			1	676	3	1	44	723
			I	V			1				
				V	R		1				
	L			V			1				
				V			501				
				V		R	1				
2012			I	V			1	504	0	0	152	656
				V	R		1				
				V			195				
2011	L						1	197	0	8	211	416
	L			V			1				
				V			229				
2010		S					1	229	1	3	195	421
				V			380				
				V	R		3				

2009	L		V			2	388	2	1	12	401
		S	V			2					
			I V			1					
2008			V			240	254	13	4	3	264
		S	V			10					
			V		L	3					
		S				3					
	L		V			1					
2007			V			291	293	0	1	4	298
			V	R		1					
			V		L	1					
2006			V			110	112	0	0	0	112
	L		V	R		2					
2005			V			298	306	1	2	2	310
			V		L	3					
		I	V			2					
			V	R		1					
	L		V			1					
		S	V			1					
2004			V			278	281	1	48	1	330
			V		L	1					
				R		1					
		S	V			1					
2003			V			69	73	11	276	1	361
		S				11					
					L	2					
			V		L	1					
				R		1					
2002					L	85	89	1	105	1	196
			V			4					
		S				1					
			V			3					

			I					1					
2001							L	1	5	0	47	0	52
	L							89					
2000				V				4	93	1	70	1	165
		S						1					
						R		6					
		S				R		3					
1999				V				1	12	3	96	1	109
			I					1					
	L							1					
						R		2					
1998							L	1	3	0	59	1	63
1997	L							11	11	0	48	4	63
	L							17					
1996	L						L	16	35	1	47	0	82
		S				R		1					
				V				1					
	L							33					
1995	L		I				L	23	75	0	0	1	76
	L						L	19					
	L							120					
1994-92	L						L	73	193	0	0	4	197
1991-90	L							46	46	0	1	2	49
	L							16					
1989	L				R			2	19	1	0	1	20
	L	S						1					
	L				R			12					
1988	L							1	13	0	0	0	13
1987	L							5	5	0	0	0	5
1986	L				R			7	7	0	0	1	8
	L				R			10					
					R			4					

1985	L				R	R		2	16	0	0	0	16
1984					R			3	4	0	0	0	4
	L				R			1					
1983	L				R			6	7	1	0	0	7
	L	S			R			1					
1982	L				R			7	8	0	0	0	8
	L				R	R		1					
1981-78	L				R			26	26	0	0	0	26
1977	L				R			6	7	0	0	1	8
	L				R	R	L	1					
1976	L				R	R		13	15	0	0	0	15
	L				R			1					
	L		I		R	R		1					
1975	L				R	R		6	9	0	0	0	9
	L				R			3					
1974	L				R	R		5	10	0	0	0	10
	L				R			4					
					R	R		1					
1973	L				R	R		5	7	0	0	0	7
	L				R	R	L	2					
1972	L				R	R		6	15	0	0	0	15
	L				R	R	L	5					
					R	R		2					
	L	I			R	R		2					
1971	L				R	R	L	9	11	0	0	0	11
	L				R		L	2					
1970-69	L				R	R	L	19	19	0	0	0	19
pdm1968	L				R	R	L	17	15	0	0	0	15
Total								**12,415**	**12,396**	**45**	**941**	**889**	**14,245**

Human H3N2 isolates of swine origin

Isolation year	Presence of virulent residues at positions								No. of isolates	No. of isolates per year				
	62	66	68	69	70	75	79	82		≥62 amino acids			≤61 amino acid	Total[β]
										with virulent residues[α]	with virulent residue S66	without any virulent residue		
2017	L							L	10[d]	10	0	0	0	10
2016	L						R		12[d]	17	0	0	0	17
	L							L	5[d]					
2015	L							L	2[d]	2	0	0	0	2
2014	L					R	R		1[d]	4	0	0	0	4
	L		I				R	L	1[e]					
	L					R		L	1[d]					
	L								1[d]					
2013	L							L	6[d]	8	0	0	0	8
	L		I				R		1[d]					
	L		I					L	1[d]					
2012	L							L	88[d]	89	0	0	0	89
	L						R	L	1[d]					
2011	L							L	6[d]	7	0	0	0	7
	L								1[d]					
2010-09	L							L	6[d]	6	0	0	1	7
2008-06	No isolate													
2005	L							L	1[d]	1	0	0	0	1
2004-00	No isolate													
1999	L					R	R	L	1[e]	1	0	0	0	1
Total									**145**	**145**	**0**	**0**	**1**	**146**

Human H5N1 and H5N6 isolates of avian origin

Isolation year	Presence of virulent residues at positions								No. of isolates	No. of isolates per year			
	62	66	68	69	70	75	79	82		≥62 amino acids		≤61 amino acid	Total[β]
										with virulent residues[α]	with virulent residue S66	without any virulent residue	
2017									No isolate				
2016	L					R	R	L	4	5	0	0	1	6
						R			1				
2015	L					R	R	L	9	9	0	0	0	9
2014	L					R	R	L	3	3	0	2	7	12
2013	L					R	R	L	9	15	0	0	0	15
	L						R	L	5					
	L					R	R		1					
2012	L	S					R	L	3	3	3	0	2	5
2011	L						R	L	17	23	3	0	4	27
	L	S					R	L	3					
	L						R	L	3					
2010	L					R	R	L	16	17	1	0	1	18
	L	S					R	L	1					
2009	L					R	R	L	22	26	0	0	2	28
	L		I			R	R	L	3					
	L					R	R		1					
2008	L					R	R	L	15	16	0	0	0	16
	L						R	L	1					
2007	L					R	R	L	28	30	0	0	1	31
	L					R	R		2					
	L					R	R	L	59					

2006	L					R	L	5	67	0	0	0	67
	L				R		L	2					
	L				R	R		1					
2005	L				R	R	L	25	29	0	0	0	29
	L				R		L	2					
	L		I		R	R	L	2					
2004	L				R	R	L	19	29	0	0	0	29
	L				R		L	10					
2003	L				R	R	L	5	5	0	0	0	5
2002-00	No isolate												
1999	L					R	L	1	1	0	0	0	1
1998	L				R	R	L	1	1	0	0	0	1
1997	L					R	L	3	6	2	0	1	7
	L	S				R	L	2					
	L				R	R	L	1					
Total								**285**	**285**	**9**	**2**	**19**	**306**

Human H7N2, H7N3, H7N7, and H7N9 isolates of avian origin

Isolation year	Presence of virulent residues at positions								No. of isolates	No. of isolates per year			
	62	66	68	69	70	75	79	82		≥62 amino acids		≤61 amino acid	Total[β]
										with virulent residues[α]	with virulent residue S66	without any virulent residue	
2017	L					R	R	L	45	93	2	0	19	112
						R			19					
	L					R		L	12					
	L		I			R	R	L	11					
						R	R	L	4					
	L	S				R	R	L	2					

2016	L				R	R	L	22	51	1	0	9	60
	L		I		R	R	L	15					
					R	R	L	10					
	L					R	L	2					
	L	S		L			L	1					
					R			1					
2015	L				R	R	L	92	101	0	0	17	118
	L				R		L	3					
					R			3					
	L					R	L	2					
	L		I		R		L	1					
2014	L				R	R	L	121	205	3	0	25	230
	L		I		R	R	L	77					
	L				R	R		2					
	L	S			R	R	L	3					
	L				R		L	1					
	L					R	L	1					
2013	L				R	R	L	84	101	0	0	13	115
	L		I		R	R	L	12					
	L				R			3					
	L					R	L	2					
	L					R		1					
2012	L	S			R	R	L	1	1	1	0	0	1
2011-05	No isolate												
2004	L	S			R	R	L	1	1	1	0	0	1
2003						R		3	6	1	0	0	6
	L					R		2					
	L	S			R	R	L	1					
Total								**560**	**560**	**9**	**0**	**83**	**643**

SUPPLEMENTARY TABLE 1 Frequency of Virulent PB1-F2 Residues in Human Influenza A Virus Isolates. Highlighted in: yellow – the residues enhancing the cytotoxicity of A/Puerto Rico/8/1934(H1N1) PB1-F2,[31] turquoise – the residues enhancing inflammatory properties of pandemic H3N2 A/Hong Kong/1/1968(H3N2) PB1-F2,[16] and pink – the reside linked to the higher virulence of pandemic 1918(H1N1) and HPAIV(H5N1).[12,13] The annotation of swine origin viruses in H3N2 dataset is based on the data from the Centers for Disease Control and Prevention (Atlanta, GA).

[α]Cytotoxic (I68, L69, and V70)[31] and inflammatory (L62, R75, R79, and L82)[16] residues (according to PB1-F2 amino acid numbering).

[β]The S66 was not included in the total count when combined with other virulent residues.

[a]Eurasian avian-like lineage

[b]North American triple reassortant lineage

[c]Reassortant strains with human seasonal HA and NA

[d]European lineage

[e]North American lineage

Note, the number of human cases infected by IAVs is higher than counts included in our analyses because of the unavailability of complete PB1-F2 ORF sequences in public databases. Reassortment in swine viruses is very common and complex than human strains and out of the scope of this study.