广西科学院学报
Journal of Guangxi Academy of Sciences

2010,26(1):45~51
Vol. 26,No. 1 February 2010

Statistical Evidence for Cross-Species Infection and Cross-Subtype Mutation in Matrix Protein 2 Family of Influenza A Virus*

甲型流感病毒基质蛋白2家族的跨种属感染和跨亚型变异的统计学证据

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Abstract: The species and subtype differences were analyzed in the matrix 2 (M2) proteins of influenza A viruses from a statistical viewpoint. First, the amino-acid pair predictability was used to convert 1129 M2 proteins into 1129 scalar data; Second, the model Ⅰ ANOVA was used to analyze these data in terms of species and subtype in order to find if they are distinguishable; Third, the model Ⅱ ANOVA was used to determine the inter- and intra-species/subtype variations to further trace the reason for cross-species infection and cross-subtype mutation. The results provide three pieces of statistical evidence to demonstrate why the cross-species infection and cross-subtype mutation are possible because the barriers between species and between subtypes are not strong enough, which leads a mutation to easily jump to another species or subtype.

Key words: amino-acid pair, influenza A virus, matrix protein 2, species, subtype, variation, ANOVA

摘要:从统计学视角分析甲型流感病毒基质蛋白2(M2)的种属差异和亚型差异。首先,用氨基酸对的可预测性将1129个M2蛋白质转换成1129标量数据,然后用Ⅰ型ANOVA分析这些数据,寻找是否存在种属和亚型的区别,最后用Ⅱ型ANOVA确定组间和组内的差异,进一步追踪跨种属感染和跨亚型突变的原因。得到3条统计学证据,说明甲型流感病毒可能发生跨种属感染和跨亚型突变,其原因是由于各种属和亚型之间的屏障不够强壮,导致突变易于跨越到其它种属或亚型。

关键词:氨基酸对　甲型流感病毒　基质蛋白2　种属　亚型　变异　方差分析

中图法分类号:R373.1^{+}3　　**文献标识码**:A　　**文章编号**:1002-7378(2010)01-0045-07

收稿日期:2009-11-01

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* This study was partly supported by National Science and Technology Platform Construction (2008FU115XB), Guangxi Science Foundation (0907016 and 0991080) and Guangxi Academy of Sciences (09YJ17SW07).

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The unpredictable mutations of influenza A viruses threaten the humans with possible flu pandemics or epidemics, therefore the accurate, precise and reliable prediction of mutations becomes more and more important, by which we can manufacture new vaccines more effective against the influenza A virus[1~6].

The design of vaccines is generally based on the virus subtype, for example, the focus in recent year was directed to the H5N1 subtype of influenza A virus[7,8]. Understandably, the proteins of influenza A viruses are different from subtype to subtype, otherwise there would be no classification of subtypes. Moreover, the proteins of influenza A viruses under the same subtype are different one another, otherwise a single subtype would contain only a single protein. The same consideration should be held for the proteins classified according to species, where the sample was obtained. These are so called a wide variety of patterns of antigenic variation across space and time, and within and between subtypes as well as hosts[9].

Here, an important question raised is if these classifications are numerically distinguishable, say, if a protein is different from species to species or from subtype to subtype in number. If distinguishable, it would mean that the barrier between species and between subtypes is strong enough to prevent cross-species infection and cross-subtype mutation; if indistinguishable, we would deduce an opposite consequence, which could explain why the H1N1 swine flu pandemic currently privileges although our focuses were misplaced on the highly pathogenic H5N1 avian viruses[6~8].

Statistically, it is not difficult to determine if proteins are distinguishable in terms of species and subtype because ANOVA can do the job. However, this job is not easy because ANOVA deals with only numbers but proteins are sequenced in terms of letters, which represent amino acids. Therefore, it is necessary to convert a protein as a number in order to conduct statistical analysis. In this study, we use the amino-acid pair predictability to convert a protein into a single number because we have developed three computational mutation approaches, which can convert a protein into a scalar datum or a numeric sequence, and then we can use them to study various issues[10~14].

The matrix protein 2 (M2) of influenza A virus forms a proton channel in the virion and is essential for infection[15,16]. The blockers for M2 ion channel have been used to treat influenza virus infections[17~20], however, the usage of M2 inhibitors is impaired by high frequencies of their resistance among currently circulating strains[21~23]. In addition, the M2 protein of influenza A virus serves as a prototype for designing vaccine based on the conserved ectodomain in M2 protein[24], thus it is very practical to analyze if M2 proteins are distinguishable from both vaccine-design and pandemic-analysis viewpoints, which is the aim of this study.

1 Materials and methods

1.1 Data

6017 full-length M2 proteins of influenza A viruses sampled from 1918 to 2008 were obtained from the influenza virus resources[25]. After excluded identical sequences, 1129 M2 proteins are actually used in this study.

1.2 Conversion of lettered M2 proteins into scalar data

We use the amino-acid pair predictability to convert a protein into a single number. According to the permutation, the adjacent amino-acid pairs in a protein can be classified as predictable and unpredictable, which provides a measure to distinguish protein one another, and we have used it in many our previous studies[26-31].

For example, ABB86897 M2 protein from a swine influenza virus, strain A/swine/Ontario/55383/04(H1N2), has 97 amino acids. The first and second amino acids can be counted as an amino-acid pair, the second and third as another amino-acid pair, the third and fourth, until the 96th and 97th, thus there are totally 96 amino-acid pairs. There are 10 leucines "L" in ABB86897 M2 protein. If the permutation can predict the appearance of amino-acid pair LL in this protein: it must appear once ($10/97 \times 9/96 \times 96 = 0.93$); actually there is only one LL in it, so the appearance of LL is predictable. By clear contrast, there are 9 isoleucines "I" in this protein. If the permutation can predict the appearance of amino-acid pair IL in this M2 protein: it must appear once ($9/97 \times 10/96 \times 96 = 0.93$); but it appears 3

times in realty, so the appearance of IL appearance is unpredictable. In this way, all amino-acid pairs in an M2 protein can be classified as predictable and unpredictable. For this particular M2 protein, its predictable and unpredictable portions are 18.18% and 81.82%.

Taking another M2 protein (accession number ABB86927) as the second example, it has only one amino acid different from ABB86897 M2 protein at position 95. However, its predictable and unpredictable portions are 20.46% and 79.54%. Thus, the amino-acid pair predictability distinguishes the difference between different M2 proteins in numbers rather than in letters, which represent amino acids in proteins.

1.3 Statistics

After computed 1129 M2 proteins, the predictable portions of M2 proteins were grouped according to their classifications of subtypes and species. The data were presented as mean±SD. The model I ANOVA followed by the Holm-Sidak's comparison test was used to compare the difference among and between subtypes/species using the SigmaStat software[32]. $P < 0.05$ is considered statistically significant. The single classification model II ANOVA with unequal sample sizes[33] was used to determine the inter- and intra-subtype/species variations.

2 Results and discussion

Figure 1 shows the statistically significant difference in M2 proteins among HA subtypes, which simply means that there are barriers among HA subtypes. This figure can be read as follows, for example, the first bar represents the mean±SD of predictable portion of amino-acid pairs from 237 M2 proteins that are classified as H1 subtype isolated from all the species, and the similar reading can be applied to other bars. From this figure, we can see that the use of predictable portion helps to conduct statistical analysis.

However, the Holm-Sidak comparison test indicates the statistical difference in only 21 pairs of subtypes (legend to Fig. 1), thus there is no statistical difference in the rest of pairs of subtypes such as H1 versus H2, which means that there are no barriers between many pairs of subtypes, and consequently a cross HA subtype mutation would easily occur.

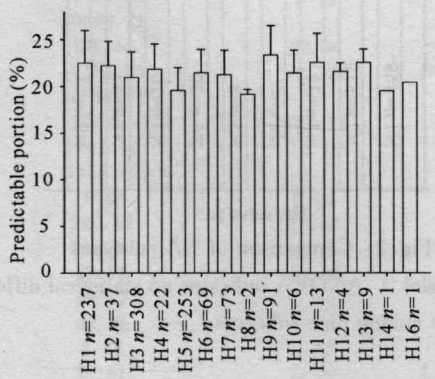

Fig. 1 Comparison of HA subtypes

The model I ANOVA indicates a statistically significant difference ($P < 0.001$) among fifteen subtypes, and the Holm-Sidak comparison test indicates the significantly statistical difference between any two subtypes as follows ($P < 0.05$): H1 versus H3, H1 versus H5, H1 versus H6, H1 versus H7, H2 versus H3, H2 versus H5, H3 versus H5, H4 versus H5, H6 versus H5, H7 versus H5, H9 versus H1, H9 versus H2, H9 versus H3, H9 versus H4, H9 versus H5, H9 versus H6, H9 versus H7, H9 versus H8, H11 versus H3, H11 versus H5, and H13 versus H5.

Figure 2 can be read in the same way as done in Figure 1. This figure indicates no statistical difference in M2 proteins among NA subtypes, suggesting that there are no barriers between any pair of NA subtypes at all so that a cross NA subtype mutation would totally easily occur. As the neuraminidase is a target for anti-flu drugs, the direct implication is that the drug designed to target M2 is better according to NA subtype because there is no statistical difference cross NA subtypes.

Figure 3 demonstrates the statistically significant difference in M2 proteins among species. This figure can be read in a similar way as done in Figures 1 and 2, but each bar includes various subtypes that were sampled in the same species. Actually, the difference between species can only be found between avian and human using the Holm-Sidak's comparison test, which implies that the cross-species inflection between avian and human is not easy to occur as we previously thought. On the other hand, the cross-species inflection related to any other pairs of species

is not difficult to occur as we previously thought. This conclusion can be supported by the study done by other research group[34].

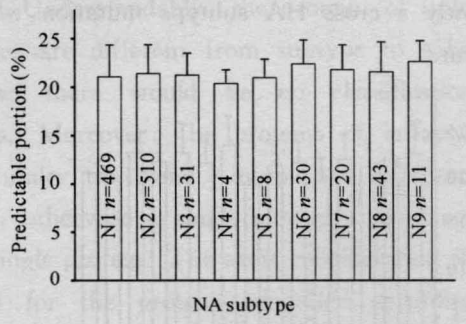

Fig. 2 Comparison of NA subtypes

The model I ANOVA indicates no statistical difference ($P = 0.231$) among nine subtypes.

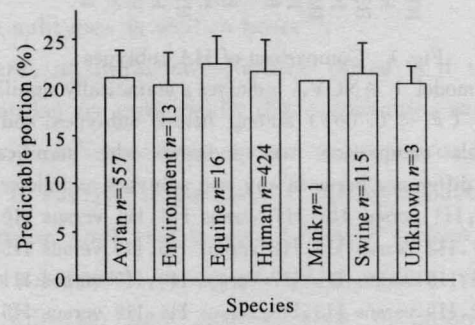

Fig. 3 Comparison of species

The model I ANOVA indicates a statistically significant difference ($P = 0.012$) among seven species, and the Holm-Sidak's comparison test indicates a statistically significant difference between avian and human ($P = 0.001$).

Figure 4 shows the comparison in terms of three major species, which are the focus of current pandemic. From this figure, we can see how easy for the cross-species infection to occur because the statistical difference is found only in few cases either using ANOVA or using the Holm-Sidak's comparison test following ANOVA.

Thus, Figures 1 to 4 provide the first piece of statistical evidence for cross-species infection and cross-subtype mutation.

The fact that no barriers exist between subtypes and species in many cases requires us to have a close look at the standard ANOVA table. This table can be read as follows: the first column indicates the objective studied by ANOVA; the second column divides the variation as inter-subtype/species and intra-subtype/specie in terms of model Ⅱ ANOVA (or between group and residual in terms of model I ANOVA); the third column shows the degree of freedom, for example, ANOVA studied fifteen HA subtypes whose degree of freedom of inter-subtype is 14, and the degree of freedom of intra-subtype is the difference between 1126 M2 proteins and 15 HA subtypes; the fourth column displays the variations in terms of the sum of squares; the fifth column is mean squares obtained by dividing the sum of squares by the degree of freedom; and the sixth column is F value obtained by dividing mean squares of inter-subtype/species by mean squares of intra-subtype/species, by which and the degree of freedom we can judge if the comparison is statistically significant.

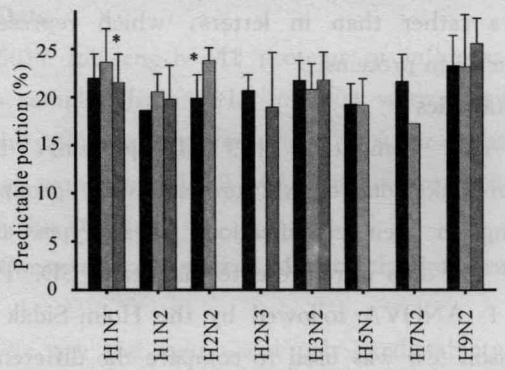

Fig. 4 Comparison of difference among three species under different subtypes.

The model I ANOVA indicates a statistically significant difference ($P < 0.001$) among species in H1N1 and H2N2 subtypes. * indicates the statistical difference compared with human at $P < 0.001$ level (the Holm-Sidak's comparison test).

■ :Avian; ■ :Human; ■ :Swine

Except for the species in H2N2 subtype, we can see the intra-subtype/species variation is larger than inter-subtype/species variation in Table 1, which makes a mutation easily jump from one species to another species, and from one subtype to another subtype.

So, Table 1 provides the second piece of statistical evidence for cross-species infection and cross-subtype mutation.

This again suggests that we need to have a good concept on the intra- and inter-subtype/species variations in terms of percentage for better comparison, whose computation should be conducted using model Ⅱ ANOVA[35, 36]. The model Ⅱ ANOVA defines the total variation as 100%, which

Table 1 Standard ANOVA table regarding subtype and species

Subtype/Species	Source of Variation	Degree of Freedom	Sum of Squares	Mean Squares	F value
HA subtypes	Inter-subtype	14	1601.03	114.36	14.14
	Intra-subtype	1111	8987.18	8.09	
	Total	1125	10588.21		
NA subtypes	Inter-subtype	8	98.96	12.37	1.32
	Intra-subtype	1117	10489.25	9.39	
	Total	1125	10588.21		
Species	Inter-species	6	137.68	22.94	2.46
	Intra-species	1122	10461.75	9.32	
	Total	1128	10599.41		
Species in H1N1	Inter-species	2	207.42	103.71	9.62
	Intra-species	206	2221.46	10.78	
	Total	208	2428.89		
Species in H1N2	Inter-species	2	4.49	2.24	0.21
	Intra-species	23	240.47	10.46	
	Total	25	244.96		
Species in H2N2	Inter-species	1	49.72	49.72	20.43
	Intra-species	20	48.67	2.43	
	Total	21	98.39		
Species in H2N3	Inter-species	1	2.97	2.97	0.17
	Intra-species	2	35.01	17.51	
	Total	3	37.98		
Species in H3N2	Inter-species	2	25.29	12.64	1.74
	Intra-species	270	1960.60	7.26	
	Total	272	1985.88		
Species in H5N1	Inter-species	1	0.16	0.16	0.03
	Intra-species	203	1019.13	5.02	
	Total	204	1019.29		
Species in H7N2	Inter-species	1	18.65	18.65	2.39
	Intra-species	23	179.59	7.81	
	Total	24	198.23		
Species in H9N2	Inter-species	2	24.74	12.37	1.24
	Intra-species	85	850.05	10.00	
	Total	87	874.79		

is further divided into inter- and intra-subtype/species variations with respect to this study. Table 2 lists the inter- and intra-subtype/species variations. As seen in Table 2, the intra-subtype/species variation is far much larger than the inter-subtype/species variation, except for the species in H2N2 subtype. For example, the NA intra-subtype variation accounted for 99.64% whereas NA inter-subtype variation was only 0.36%.

Table 2 Inter- and intra-subtype/species variations

Classification	Intra-subtype/species variation(%)	Inter-subtype/species variation(%)
HA subtype	83.29	16.71
NA subtype	99.64	0.36
Species	98.73	1.27
Species in H1N1	86.48	13.52
Species in H1N2	100.00	0.00
Species in H2N2	25.20	74.80
Species in H2N3	100.00	0.00
Species in H3N2	97.28	2.72
Species in H5N1	100.00	0.00
Species in H7N2	58.04	41.96
Species in H9N2	96.51	3.49

Hence, Table 2 provides the third piece of statistical evidence for cross-species infection and cross-subtype mutation.

The results in this study are consistent with our previous studies on the influenza A virus hemagglutinns[37], M1 proteins[38] and polymerase acidic proteins[39]. All the statistical evidence supports the idea to develop a vaccine that generates effective heterosubtypic immunity based on immune recognition of influenza A virus antigens conserved across all viral strains[40, 41].

In conclusion, this study provides three pieces of statistical evidence for cross-species infection and cross-subtype mutation of M2 proteins from influenza A viruses because the barriers between species and between subtypes are not strong enough to confine the infection within a single species as well as the mutation within a single subtype.

References:

[1] Capuaa I, Alexander D J. Avian influenza vaccines and vaccination in birds[J]. Vaccine, 2008, 26 Suppl 4: D70-

D73.

[2] Hehme N,Colegate T,Palache B,et al. Influenza vaccine supply: building long-term sustainability [J]. Vaccine,2008,26 Suppl 4:D23-D26.

[3] Hoelscher M,Gangappa S,Zhong W,et al. Vaccines against epidemic and pandemic influenza [J]. Expert Opin Drug Deliv,2008,5:1139-1157.

[4] Palache B. New vaccine approaches for seasonal and pandemic influenza[J]. Vaccine,2008,26:6232-6236.

[5] Tilburt J C,Mueller P S,Ottenberg A L,et al. Facing the challenges of influenza in healthcare settings: the ethical rationale for mandatory seasonal influenza vaccination and its implications for future pandemics [J]. Vaccine,2008,26 Suppl 4:D27-D30.

[6] Peyre M,Fusheng G,Desvaux S,et al. Avian influenza vaccines:a practical review in relation to their application in the field with a focus on the Asian experience[J]. Epidemiol Infect,2009,137:1-21.

[7] Hampson A W. Vaccines for pandemic influenza. The history of our current vaccines,their limitations and the requirements to deal with a pandemic threat[J]. Ann Acad Med Singapore,2008,37:510-517.

[8] Skeik N,Jabr F I. Influenza viruses and the evolution of avian influenza virus H5N1[J]. Int J Infect Dis,2008,12:233-238.

[9] Lipsitch M,O'Hagan J J. Patterns of antigenic diversity and the mechanisms that maintain them[J]. J R Soc Interface,2007,4:787-802.

[10] Wu G,Yan S. Randomness in the primary structure of protein:methods and implications[J]. Mol Biol Today,2002,3:55-69.

[11] Wu G,Yan S. Mutation trend of hemagglutinin of influenza A virus: a review from computational mutation viewpoint[J]. Acta Pharmacol Sin,2006,27:513-526.

[12] Wu G,Yan S. Fate of influenza A virus proteins[J]. Protein Pept Lett,2006,13:377-384.

[13] Wu G,Yan S. Lecture notes on computational mutation[M]. New York: Nova Science Publishers,2008.

[14] Yan S M,Wu G. Introduction of computational mutation[J]. J Guangxi Acad Sci(in press).

[15] Betakova T. M2 protein-a proton channel of influenza A virus[J]. Current Pharm Des,2007,13:3231-3235.

[16] Pinto L H,Lamb R A Controlling influenza virus replication by inhibiting its proton channel[J]. Mol Biosyst,2007,3:18-23.

[17] Kelly M L,Cook J A,Brown-Augsburger P,et al. Demonstrating the intrinsic ion channel activity of virally encoded proteins[J]. FEBS Lett,2003,552:61-67.

[18] Hayden F G. Antivirals for influenza:historical perspectives and lessons learned[J]. Antiviral Res,2006,71:372-378.

[19] Hsieh H P,Hsu J T. Strategies of development of antiviral agents directed against influenza virus replication[J]. Curr Pharm Des,2007,13:3531-3542.

[20] Beigel J,Bray M. Current and future antiviral therapy of severe seasonal and avian influenza[J]. Antiviral Res,2008,78:91-102.

[21] Regoes R R. Bonhoeffer S Emergence of drug-resistant influenza virus: population dynamical considerations [J]. Science,2006,312:389-391.

[22] Basler C F. Influenza viruses:basic biology and potential drug targets[J]. Infect Disord Drug Targets,2007,7:282-293.

[23] Hayden F. Developing new antiviral agents for influenza treatment:what does the future hold[J]. Clin Infect Dis,2009,48 Suppl 1:S3-S13.

[24] Schotsaert M,De Filette M,Fiers W,et al. Universal M2 ectodomain-based influenza A vaccines: preclinical and clinical developments [J]. Exp Rev Vaccines,2009,8:499-508.

[25] The National Center for Biotechnology Information. Influenza virus resources [EB/OL]. [2009-08-28] http://www. ncbi. nlm. nih. gov/genomes/FLU/Database/multiple. cgi.

[26] Yan S,Wu G. Determination of mutation pattern in human androgen receptor by means of amino-acid pair predictability [J]. Protein Pept Lett,2009,16:289-296.

[27] Yan S,Wu G. Determination of mutation patterns in human ornithine transcarbamylase precursor [J]. J Clin Monit Comput,2009,23:51-57.

[28] Yan S,Wu G. What these trends suggest[J]. Am J Appl Sci,2009,6:1116-1121.

[29] Yan S,Wu G. Prediction of mutation position,mutated amino acid and timing in hemagglutinins from North America H1 influenza A virus[J]. J Biomed Sci Eng,2009,2:117-122.

[30] Yan S,Wu G. Describing evolution of hemagglutinins from influenza A viruses using a differential equation [J]. Protein Pept Lett,2009,16:794-804.

[31] Yan S,Wu G. Trends in global warming and evolution of polymerase basic protein 2 family from influenza A virus[J]. J Biomed Sci Eng,2009,2:457-464.

[32] SPSS Inc. SigmaStat for Windows [CP]. Version 3.00,1992-2003.

[33] Sokal R R,Rohlf F J. Biometry:The principles and practices of statistics in biological research [M]. 3nd ed. New York:W H Freeman,1995:203-218.

[34] Yu H,Zhang PC,Zhou YJ,et al. Isolation and genetic characterization of avian-like H1N1 and novel ressortant H1N2 influenza viruses from pigs in China [J]. Biochem Biophys Res Commun,2009,386:278-283.

[35] Wu G,Baraldo M,Furlanut M. Inter-patient and intra-patient variations in the baseline tapping test in patients with Parkinson's disease [J]. Acta Neurol Belg,1999,99:182-184.

[36] Furlanut M,Wu G,Perucca E. Variability in the metabolism of levodopa and clinical implications[M]// Ed G M Pacifici, O Pelkonen. Interindividual Variability in Drug Metabolism in Man London and New York:Tayler & Francis,Chapter 7:181-227.

[37] Yan S,Wu G. Determination of inter- and intra-subtype variations in polymerase acidic protein from influenza A virus using amino-acid pair predictability [J]. J Biomed Sci Eng,2009,2:273-279.

[38] Yan S,Wu G. Rationale for cross-species infection and cross-subtype mutation in hemagglutinins from influenza A virus [J]. Interdisc Sci Comput Life Sci,2009(1):303-307.

[39] Yan S,Wu G. Evidence for cross-species infection and cross-subtype mutation in matrix protein 1 family from influenza a virus [J]. Viral Immunol,2010,23:105-111.

[40] Carrat F,Flahault A. Influenza vaccine:the challenge of antigenic drift[J]. Vaccine,2007,25:6852-6862.

[41] Grebe K M,Yewdell J W,Bennink J R. Heterosubtypic immunity to influenza A virus:where do we stand[J]. Microbes Infect,2008,10:1024-1029.

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