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Determination of inter- and intra-subtype/species variations in polymerase acidic protein from influenza A virus using amino-acid pair predictability

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ABSTRACT

The polymerase acidic protein is an important family of proteins from influenza A virus, which is classified as many different subtypes or species. Thus, an important question is if these classifications are numerically distinguishable with respect to the polymerase acidic protein. The amino-acid pair predictability was used to transfer 2432 polymerase acidic proteins into 2432 scalar data. The one-way ANOVA found these polymerase acidic proteins distinguishable in terms of subtypes and species. However, the large residuals in ANOVA suggested a possible large intra-subtype/species variation. Therefore, the inter- and intra-subtype/species variations were studied using the model II ANOVA. The results showed that the intra-subtype/species variations accounted most of variation, which was 100% in total for both inter- and intra- subtype/species variations. Our analysis threw lights on the issue of how to determine a wide variety of patterns of antigenic variation across space and time, and within and between subtypes as well as hosts.

Keywords: Amino-Acid Pair; Influenza A Virus; Inter- and Intra-; Model II ANOVA; Polymerase Acidic Protein; Species; Subtype; Variation

1. INTRODUCTION

The unpredictable mutations in proteins from influenza A virus threaten the humans with possible pandemics or epidemics, therefore it is considered important to accurately, precisely and reliably predict the mutations. In this way, the new vaccines, which would be more effective against the influenza A virus, could be manufactured [1,2,3,4,5,6].

Currently, the manufactured vaccines are designed to target the influenza A virus according to their subtypes, for example, the focus in recent year would be the H5N1 subtype of influenza A virus [7,8,9,10,11,12,13,14], and anti-flu drugs are designed to target neuraminidases and M2 protein [15,16]. It would be understandable that proteins should be different from one subtype to another. Otherwise, there would be no classification of subtype. Moreover, the proteins under the same subtype should be different one another, otherwise a single subtype would contain only a single protein. The same holds for proteins classified according to species, where the sample was obtained.

Here, an important question is if these classifications are numerically distinguishable, say, if a protein is different from species to species and from subtype to subtype in number. This is the base for prediction of mutation using mathematical modeling.

However this work has yet to be done, because the difference between proteins is different in terms of letters, which represent the amino acids in proteins. It is difficult to use any statistical method to determine these differences cross a protein family.

For this aim, it needs to transfer a protein into a datum that should be different from protein to protein. Then it would be possible to conduct an ANOVA statistics to answer the question above.

Actually there are quite a few methods, which can transfer a protein sequence into a series of numerical codes or numerical sequence for predicting its various attributes (see, e.g., [17,18,19,20,21,22,23,24,25]).

Since 1999, we have developed three approaches to transfer each amino acid in a protein as well as a whole protein (for reviews, see [26,27,28]) into either a single datum or numerical sequence, which resulted in many studies on proteins.

Afterward, another question would be the inter- and intra-subtype/species variations. This issue is important because the vaccines and anti-flu drugs manufactured based on subtype would be more efficient and effective if the difference within subtype/species would be smaller

than that between subtypes/species.

Influenza viruses replicate and transcribe their segmented negative-sense single-stranded RNA genome in the nucleus of the infected host cell. All RNA synthesizing activities associated with influenza virus are performed by the virally encoded RNA-dependent RNA polymerase that consists of three subunits, polymerase acidic protein (PA), polymerase basic proteins 1 and 2 [29]. The PA subunit is involved for the conversion of RNA polymerase from transcriptase to replicase [30] and contains the endonuclease active site. A recent study strongly implicates the viral RNA polymerase complex as a major determinant of the pathogenicity of the 1918 pandemic virus [31].

Many studies have indicated that sequence-based prediction approaches, such as protein subcellular location prediction [32,33,34], protein quaternary attribute prediction [25,35], identification of membrane proteins and their types [36,37], identification of enzymes and their functional classes [38], identification of GPCR and their types [24,39,40], identification of proteases and their types [41,42], protein cleavage site prediction [43,44,45], signal peptide prediction [46,47], and protein 3D structure prediction based on sequence alignment [48], can timely provide very useful information and insights for both basic research and drug design.

The present study was attempted to use the model II ANOVA to investigate the inter- and intra- subtype/species variations in polymerase acidic protein from influenza A virus in hope to shed lights on helping find effective drugs against influenza A virus.

2. MATERIALS AND METHODS

2.1. Data

A total of 5165 full-length PA sequences of influenza A virus sampled from 1918 to 2008 was obtained from the influenza virus resources [49]. After excluded identical sequences, 2432 PA proteins were used in this study.

2.2. Transferring Symbolized PA Proteins into Scalar Data

Among these methods developed by us, the amino-acid pair predictability is the simplest, which was thus used in this study. According to the permutation, the adjacent amino-acid pairs in a protein can be classified as predictable and unpredictable, which provided a measure to distinguish protein one another and was used in many our previous studies (for example 2008, [28,50,51,52,53,54]).

For example, there was an avian influenza virus (strain A/quail/Hong Kong/1721-20/99(H6N1)) and its PA was composed of 716 amino acids (accession number CAC84865). The first and second amino acids could be counted as an amino-acid pair, the second and third as another amino-acid pairs, the third and fourth, until the 715th and 716th, thus there were 715 amino-acid pairs. Then, how many amino-acid pairs can be explained by the permutation or random mechanism in this PA? This can be determined using the percentage of predictable and unpredictable amino-acid pairs.

There were 37 aspartic acids "D" and 76 glutamic acids "E" in CAC84865 PA. If the permutation could explain the appearance of amino-acid pair DE, it could appear four times in this PA (37/716×76/715×715=3.927). Actually there were 4 DEs in this PA. Thus, the appearance of DE could be explained by permutation or predicted by random mechanism. By clear contrast, there were 50 isoleucines "I" in the PA. If the permutation could explain the appearance of IE, it could appear five times (50/716×76/715×715=5.307). However, it appeared 12 times in realty, which could not be explained by permutation or randomly unpredictable. In this way, all amino-acid pairs in this PA could be classified as predictable and unpredictable. For this particular PA, its predictable and unpredictable portions were 25.45% and 74.55%.

Taking another PA (accession number CAC84866) as example, this PA had only one amino acid different from CAC84865 PA at position 437. However, its predictable and unpredictable portions were 25.59% and 74.41%. Thus, the amino-acid pair predictability distinguished the difference between different PA proteins as a very sensitive measure.

2.3. Difference among Subtypes/Species

Influenza A viruses are classified by the serological subtypes of the primary viral surface proteins. Currently, there are 16 haemagglutinin subtypes from H1 to H16 and 9 neuraminidase subtypes from N1 to N9 [55]. Also, influenza A viruses can be classified according to their host.

After computation of 2432 PA proteins, the predictable portions of PA proteins were grouped according to their classifications of subtypes and species.

As there were more than two subtypes and two species, and the number of PA proteins was highly different from subtype to subtype, and from species to species, the one-way ANOVA followed by the Holm-Sidak's comparison test was used to compare the difference among and between subtypes/species using the SigmaStat software [56]. $P < 0.05$ was considered statistically significant.

2.4. Inter- and Intra-Subtype Variation

The single classification model II ANOVA with unequal sample sizes [57] was used to determine the inter- and intra-subtype/species variations.

3. RESULTS AND DISCUSSION

The one-way ANOVA showed that there were statistically significant difference among HA subtypes (**Figure 1**), NA subtypes (**Figure 2**) and species (**Figure 3**). Even the statistical difference was found between subtypes and between species. The detailed results were listed in Supplementary results.

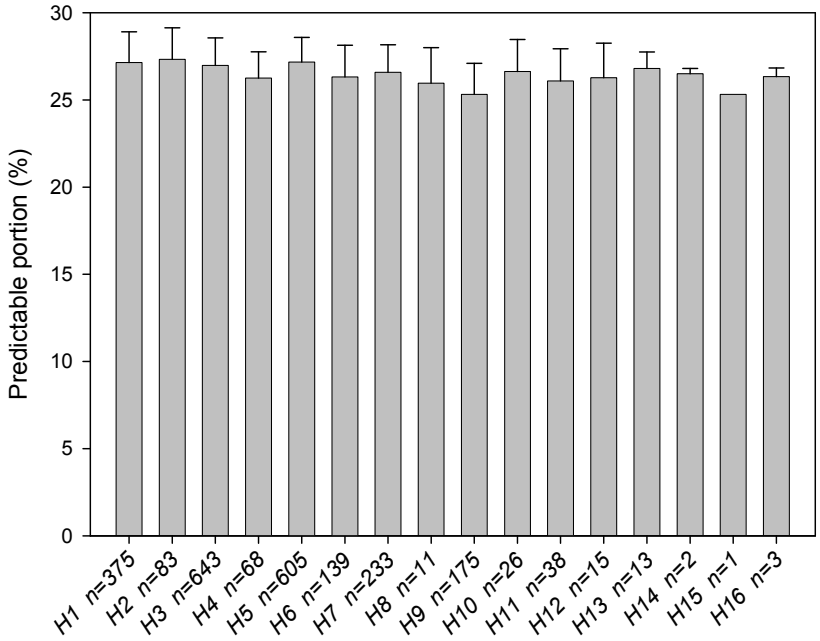

Figure 1. HA subtype comparison of PA proteins from influenza A viruses. The one-way ANOVA indicated a statistically significant difference ($P < 0.001$) among sixteen subtypes, and the Holm-Sidak's comparison test indicated the statistical difference between two subtypes as follows: H5 versus H9, H1 versus H9, H3 versus H9, H2 versus H9, H7 versus H9, H5 versus H6, H6 versus H9, H1 versus H6, H5 versus H7, H2 versus H6, H5 versus H4, H3 versus H6, H1 versus H4, H1 versus H7, H2 versus H4, H4 versus H9, H5 versus H11, H2 versus H11, H10 versus H9, H1 versus H11, H2 versus H7, H3 versus H4, H3 versus H11, H13 versus H9, H3 versus H7, H11 versus H9, H2 versus H8, H5 versus H8, H1 versus H8, H2 versus H12, H12 versus H9, H5 versus H12, H5 versus H3, H3 versus H8, and H1 versus H12.

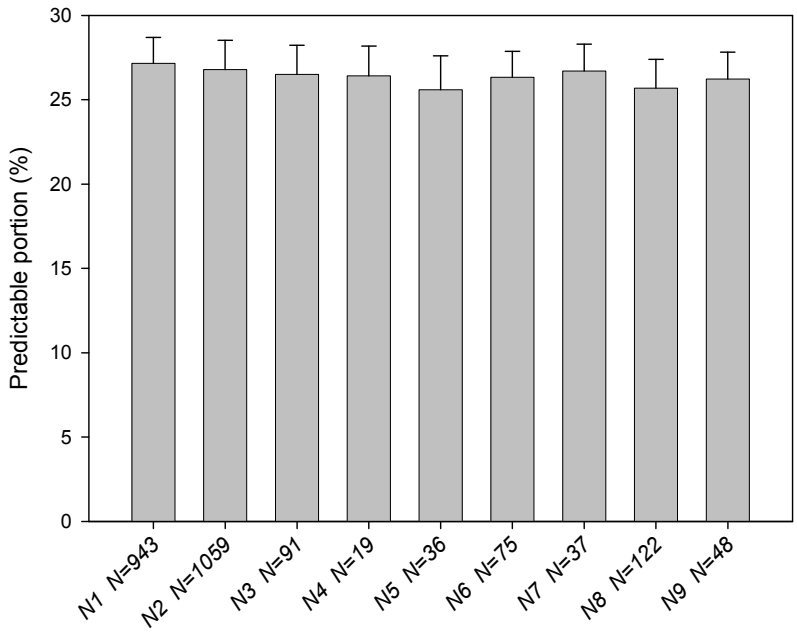

Figure 2. NA subtype comparison of PA proteins from influenza A viruses. The one-way ANOVA indicated a statistically significant difference ($P < 0.001$) among nine subtypes, and the Holm-Sidak's comparison test indicated the statistical difference between two subtypes as follows: N1 versus N8, N2 versus N8, N1 versus N5, N1 versus N2, N2 versus N5, N1 versus N6, N1 versus N9, N1 versus N3, N3 versus N8, N7 versus N8, N7 versus N5, N3 versus N5, N6 versus N8, N2 versus N6, N2 versus N9 and N6 versus N5.

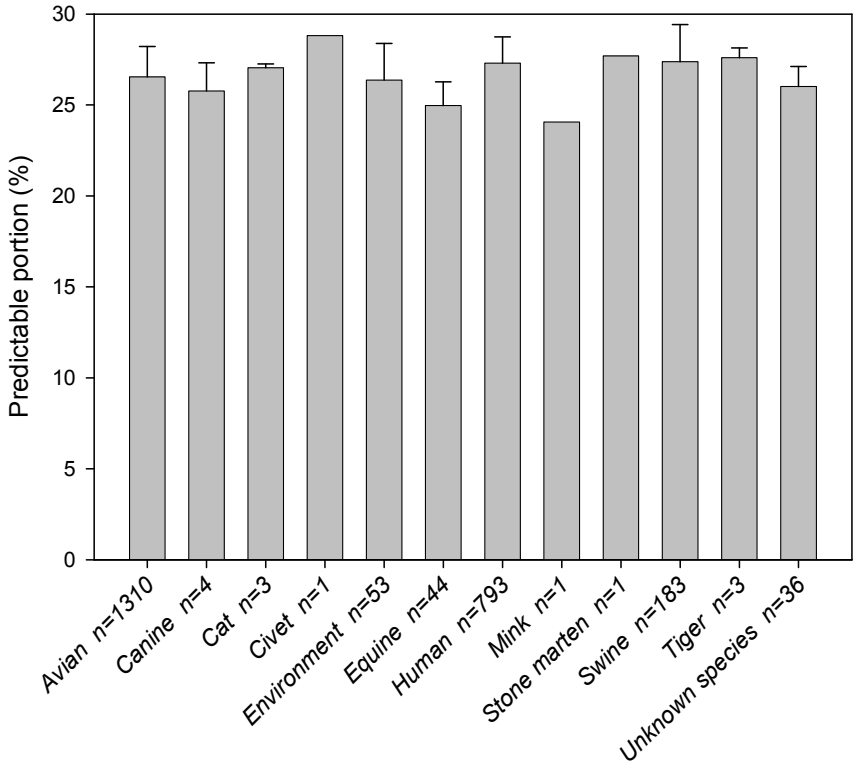

Figure 3. Species comparison of PA proteins from influenza A viruses. The one-way ANOVA indicated a statistically significant difference ($P < 0.001$) among ten species, and the Holm-Sidak's comparison test indicated the statistical difference between two species as follows: human versus avian, human versus equine, swine versus equine, swine versus avian, avian versus equine, human versus unknown, swine versus unknown, environment versus equine, human versus environment, swine versus environment, unknown versus equine, tiger versus equine, civet versus equine, cat versus equine, civet versus mink, swine versus mink, human versus mink, and swine versus canine.

During the one-way ANOVA test, a particular phenomenon got our attention, i.e. the residual was very large in standard ANOVA table. For example, the sum of squares (SS) was 668.97 and 6293.13 for between groups and residual under HA subtype (Table 1).

Table 1 suggested that there were very large variations in PA proteins within each subtype or species, which further suggested that the model II ANOVA was in need to determine the inter- and intra-subtype/species variations.

Table 2 listed the inter- and intra-subtype/species variations. The model II ANOVA defined the total variation as 100%, which was further divided into 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. For example, the inter-subtype HA variation was 10.71% while the intra-subtype HA variation was 89.28%.

Table 1. Standard ANOVA table regarding HA subtype, NA subtype and species of PA proteins from influenza A viruses.

	Source of variation	Degree of freedom	Sum of Squares	Mean Square	F
HA subtype	Between groups	16	668.97	41.81	16.05
	Residual	2415	6293.13	2.61	
	Total	2431	6962.10		
NA subtype	Between groups	9	377.13	41.90	15.41
	Residual	2422	6584.97	2.72	
	Total	2431	6962.10		
Species	Between groups	11	547.64	49.79	18.78
	Residual	2420	6414.46	2.65	
	Total	2431	6962.10		

Table 2. Inter- and intra-subtype/species variations of PA proteins from influenza A viruses.

Classification	Inter-subtype/ species variation	Intra-subtype/ species variation
HA subtype	10.71%	89.28%
NA subtype	7.54%	92.46%
Species	11.88%	88.12%

At this point, one might wonder why the statistical difference was found among subtypes and species while there were so large variations within subtype and species. These results in fact were very reasonable. In plain words, there would be statistical difference between males and females in performing a sport, for example, however the difference between male sportsman and ordinary male would be also huge, thus it could be possible this variation would be larger than that between males and females.

In fact, the single classification model II ANOVA has many important applications although this method is less familiar with most researchers [58,59]. For example, it is better to know the inter- and intra-patient variations before planning clinical experiments, say, if an experimental design should include two parallel groups (inter- patients) or a two-part crossover design (intra-patients).

In the context of this study, generally, a small intra-subtype/species variation suggested a cost-effective way in collecting of samples, it said, not many samples for a particular subtype/species were in need, by clear contrast, many samples were in need regarding a particular subtype/species if there was a large intra- subtype/species variation.

Actually, the inter- and intra-subtype/species variations were the mutations occurred in the same subtype or species, and occurred cross subtypes or species. There was a wide variety of patterns of antigenic variation across space and time, and within and between subtypes as well as hosts and we did not yet understand the determinants of these different patterns [60]. Our analysis could shed lights on this issue.

The far much large intra-subtype/species variation found in this study suggested: 1) much more PA proteins belonging to the same subtype/species were needed in order to better understand the mutation pattern in the same subtype/species, and 2) the current classification was based on the surface proteins from influenza A virus, while under the same subtype, the PA mutations were quite large.

On the other hand, the statistically significant difference between subtypes suggested the current classification valid even for the PA, which was an internal protein. The most important requirement for producing vaccines against viruses displaying antigenic diversity is a method of measuring antigenic distances between strains and developing an understanding of how these distances relate to cross-protection [61]. The current results supported the idea to develop vaccines and anti-flu drugs that generate effective heterosubtypic immunity based on immune recognition of influenza A virus antigens conserved across all viral strains [62,63].

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