[Frontiers In Bioscience, Scholar, 10, 229-237, January 1, 2018]

A protocol for investigating the association of vaccination and anti-NMDA receptor encephalitis

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TABLE OF CONTENTS

1. ABSTRACT

Anti-N-methyl-D-aspartate (Anti-NMDA) receptor encephalitis is an acute autoimmune neurological disorder that can be triggered by virus, H1N1/tetanus/diphtheria/pertussis and polio vaccines or by presence of a tumor. The association between anti-NMDA receptor encephalitis and Japanese encephalitis vaccination was examined and a general protocol of phylogenetic method which details the steps and code and an example of its utility is provided. The approach used here is potentially useful for analyzing the relationship between vaccines and diseases.

2. INTRODUCTION

Anti-N-methyl-D-aspartate (Anti-NMDA) receptor encephalitis is an acute autoimmune disorder prevalent more in females than in males and which is caused by antibodies produced by the immune system binding N-methyl-D-aspartate (NMDA) receptors (Figure 1). This disorder presents a multi-stage illness progressing from initial psychiatric symptoms to memory disturbances, seizures, dyskinesia and catatonia (1, 2). The treatments involve first-line immunotherapies with steroids, intravenous immunoglobulin (IVIG) or plasmapheresis (or plasma exchange), and second-line immunotherapy with drugs such as rituximab or cyclophosphamide (2, 3).

A proportion of patients with anti-NMDA receptor encephalitis were reported having tumors, especially of ovarian origin (4). Most patients with tumors have substantial improvement after tumor resection. As a result, tumors may be one of the triggers of this disease. Several cases have been reported to be related to the herpes simplex virus (5, 6) or vaccination. A few anti-NMDA receptor encephalitis cases related to the vaccination have been reported (7, 8). However, for patients without detectable tumors, or history of vaccination or infection, the triggering mechanism is not known.

A Japanese encephalitis (JE) vaccination-related case of a 2-year-old girl was also reported (9). This patient fully recovered one and half year after onset of the disease. Although there was no direct evidence, based on microRNA (miRNA) phylogenetic tree analysis, an association was proposed between anti-NMDA receptor encephalitis and vaccination. miRNAs are small approximately 22 nucleotide RNA molecules that can upregulate or downregulate their target gene expression (10-12). The expression of miRNAs changes in these patients and for this reason they are used as potential prognostic factors, as biomarkers of diseases and in response to treatment (13-15). Microarray data analysis is a useful method to find miRNA biomarkers for diseases (10, 16-18). In addition to using microarray expression to find miRNA biomarkers, the phylogenetic structure of miRNAs can be used to increase the accuracy of miRNA biomarker prediction (19). The phylogenetic tree based on nucleotide substitution models is a widely-used tool to classify nucleotide s equences (20-23). As a result, the previous studies reveal that the phylogenetic analysis of miRNAs can provide helpful information for disease investigation.

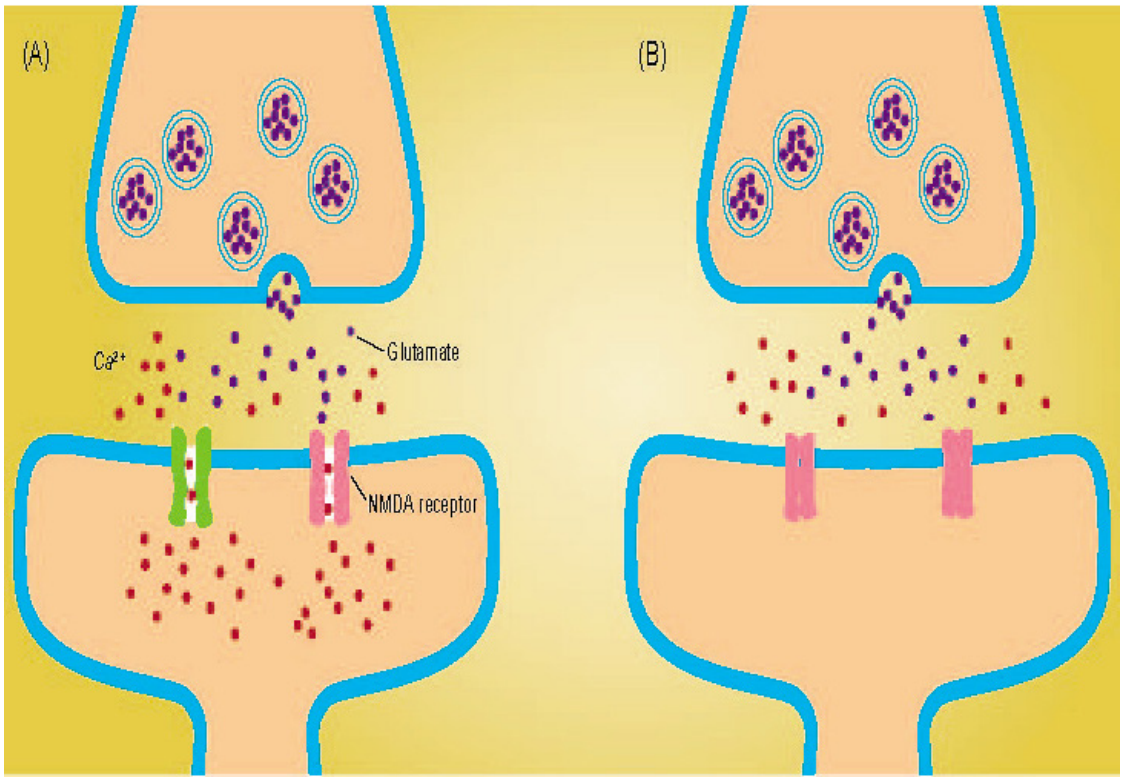

Figure 1. (A) NMDA receptors are under normal condition (B) NMDA receptors are blocked in anti-NMDA receptor encephalitis patients

In this article, a general protocol based on a previous report to explore the relationship between vaccines and this disorder is provided (9). This approach is potential to be a useful tool for analyzing the causal relationship between vaccines and other diseases.

3. RESULTS AND DISCUSSION

This study was approved by the Research Ethics Committee for Human Subject at National Chiao Tung University, Taiwan.

A general protocol to investigate the association between anti-NMDA receptor encephalitis and vaccination is provided as follows.

3.1. Protocol

3.1.1. miRNA Biomarkers

(i) The miRNAs related to the anti-NMDA receptor encephalitis were obtained from literature or database. Let Group I denote the set that consists of these miRNAs related to the anti-NMDA receptor encephalitis.

(ii) The miRNAs related to the vaccine viruses or bacteria were obtained from literature or database. Let Group II denote the set that consists of these miRNAs related to the vaccine viruses or bacteria.

3.1.2. Nucloeotide sequences of miRNAs

(i) The miRBase (24) (http://www.mirbase.org/) is a database of published miRNA sequences and annotation.

(ii) The nucloeotide sequences of miRNAs from miRBase in Group I and those in Group II, were identified. There are usually three types of sequence: stem-loop sequence, mature -5p sequence and mature -3p sequence. There is no special recommendation of the sequence type, but the stem-loop sequence including mature -5p sequence and mature -3p sequence may provide more information than the other two types of sequence.

3.1.3. Phylogenetic trees

(i) There are many types of phylogenetic trees (22, 23). We can select several methods to plot phylogenetic trees, and then combine these results to reach a conclusion.

(ii) Although several software such as Matlab, MEGA , *etc* can be used to plot phylogenetic trees (25), we used the Matlab code as an example to plot trees

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>> clear
delete('m*')
fastawrite('m323','mir-323','UUGGUACUUGGAGAGAGGUGGUCCGUGGCGCGUUCGCUUUAUUUAUGGCGCACAUUACACGGUCGACCUCUUUGCAGUAUCUAAUC');
R323=fastaread('m323');

fastawrite('m491','mir-491','UUGACUUAGCUGGGUAGUGGGGAACCCUUCCAUGAGGAGUAGAACACUCCUUAUGCAAGAUUCCCUUCUACCUGGCUGGGUUGG');
R491=fastaread('m491');

fastawrite('mlet7c','let-7c','GCAUCCGGGUUGAGGUAGUAGGUUGUAUGGUUUAGAGUUACACCCUGGGAGUUAACUGUACAACCUUCUAGCUUUCCUUGGAGC');
Rlet7c=fastaread('mlet7c');

seqs = [R323, R491, Rlet7c];
distances = seqpdist(seqs,'method','jukes-cantor','Alphabet', 'NT');
%distances = seqpdist(seqs,'method','alignment-score','Alphabet', 'NT');
%distances = seqpdist(seqs,'method','p-distance','Alphabet', 'NT');
phylotree = seqlinkage(distances,'single',seqs);
%phylotree = seqlinkage(distances,'average',seqs);
%phylotree = seqlinkage(distances,'median',seqs);
%xlabel('Evolutionary distance')
view(phylotree)

fx >>
```

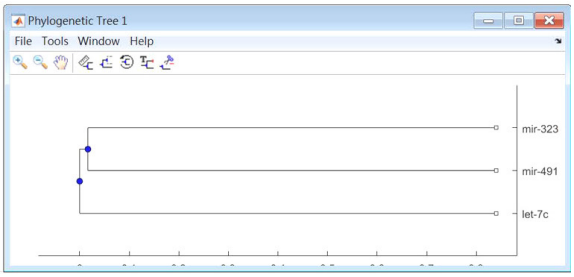

Figure 2. Matlab code to plot trees for stem-loop sequences of 3 miRNAs.

Table 1. miRNAs related to vaccination or encephalitis

Encephalitis or Vaccine	mircoRNA
Anti-NMDA receptor encephalitis	let-7a, let-7b, let-7d, and let-7f
H1N1	miR-323, miR-491, miR-654, miR-10a, let-7c, let-7f, miR-31, miR-29a, miR-148a, miR-146a
Pertussis	miR-202, miR-342, miR-206, miR-487b, miR-576
Poliomyelitis	miR-555
Herpes simplex virus	miR-145, miR-101
Japanese encephalitis virus	miR-19b-3p, miR-33a-5p, miR-155, miR-29b, miR-146a

for stem-loop sequences of 3 miRNAs. A short version code is provided in Figure 2. In this code, we need to select a distance method to calculate the pairwise distances between two sequences and to select a linkage method to build a tree. The distance method can select *p-distance* method*, jukes-cantor* method*, alignment-score* method etc. The linkage method can select *median* method*, single* method*,* and *average* method *etc* (22, 23). In Figure 2, 3 sequences were chosen as an example, and we adopted *Juke–Cantor* distance as the distance method and *single* method as the linkage function.

3.2. Phylogenetic tree analysis

From the obtained phylogenetic trees, to investigate whether a specific vaccine might induce anti-NMDA receptor encephalitis, we can examine whether the miRNAs related to this vaccine are closer to the miRNAs related to anti-NMDA receptor encephalitis than the miRNAs related other vaccines. If most of the miRNAs related to this vaccine are close to the miRNAs related to anti-NMDA receptor encephalitis compared with the miRNAs related other vaccines, we may conclude that this vaccine is associated with anti-NMDA receptor encephalitis. Otherwise, there is no significant evidence to conclude this result.

By applying this method, we present two examples. One is to show a case that the corresponding vaccine may be relevant to anti-NMDA receptor encephalitis, and the other one shows a case that the corresponding vaccine is not relevant to this disorder. The miRNAs corresponding to anti-NMDA receptor encephalitis or vaccines in the reported cases (9) are presented in Table 1. Here, a tool was used to show whether vaccine A and vaccine B, can induce anti-NMDA receptor encephalitis.

We make the assumption that miRNA A1 and miRNA A2 correspond to vaccine A and miRNA B1 and miRNA B2 correspond to vaccine B, respectively (Table 2). We can apply the method to plot the phylogenetic trees of all the miRNAs in Table 1 or corresponding to vaccine A (or vaccine B) based on different distance methods and linkage functions. Figure 3 shows the

Table 2. miRNAs related to vaccine A or vaccine B

Vaccine	mircoRNA
A	miRNA A1, miRNA A2
B	miRNA B1, miRNA B2

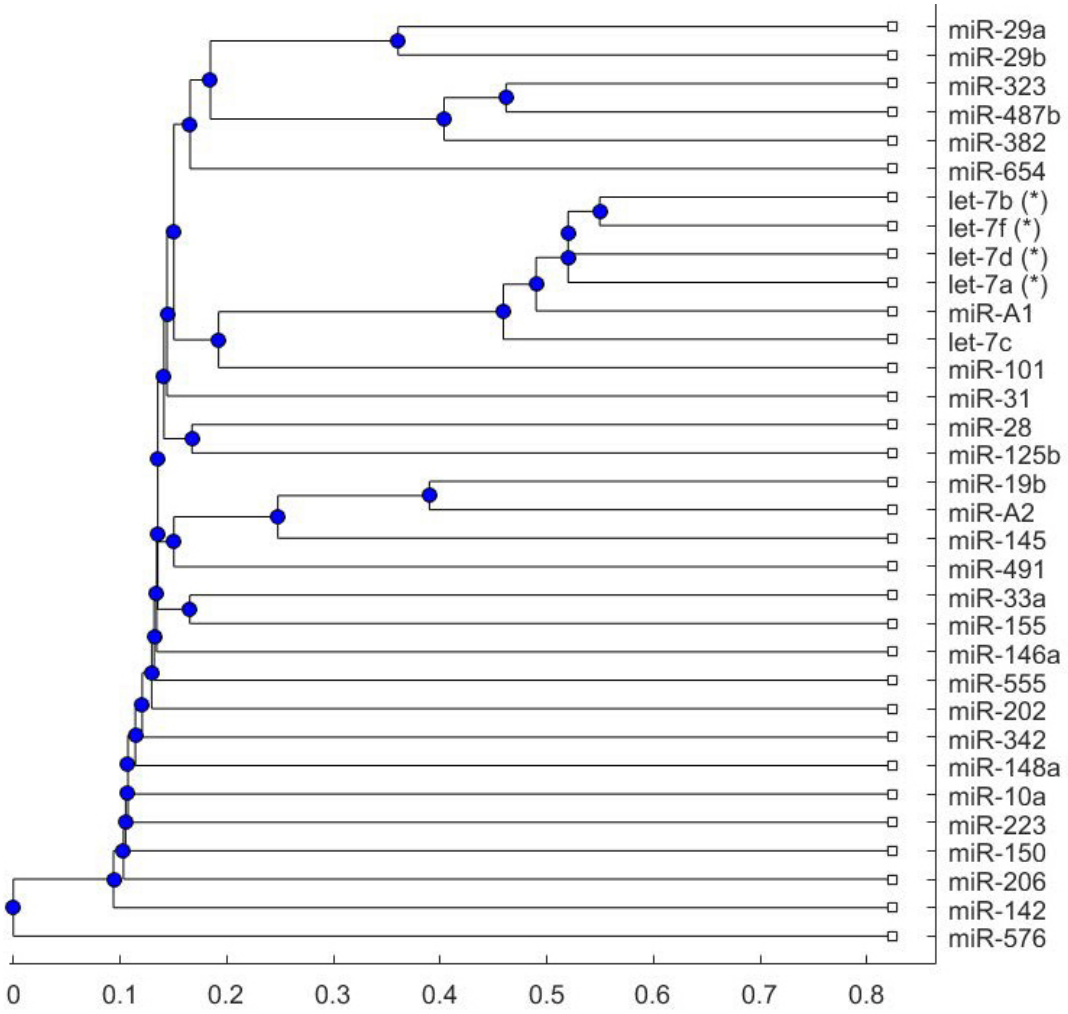

Figure 3. The phylogenetic tree of miRNA A1 and miRNA A2 and miRNAs in table 1 based on the *Juke–Cantor* distance and the *single* linkage function.

phylogenetic trees for investigating the association between vaccine A and this disorder based on the *Juke–Cantor* distance and the *single* linkage function; Figure 4 shows the phylogenetic trees based on the *alignment score* distance and the *average* linkage function; Figure 5 shows the phylogenetic trees based on the *p-distance* and the *median* linkage function. The results for vaccine B are presented in Figures 6-8. The miRNAs, let-7 family, corresponding to anti-NMDA receptor encephalitis are marked by the notation "*" in Figures 3-8.

In Figures 3-5, miRNA A1 and miRNA A2 are close to let-7 family. As a result, we might conclude that there is a chance that vaccine A may induce the anti-NMDA receptor encephalitis. Figures 6-8 show that miRNA B1 or miRNA B2 is in an outer branch away from the let-7 family. Thus, we might conclude that the chance of vaccine B inducing the anti-NMDA receptor encephalitis is not high.

Anti-NMDA receptor encephalitis is an acute autoimmune disease. Most patients with anti-

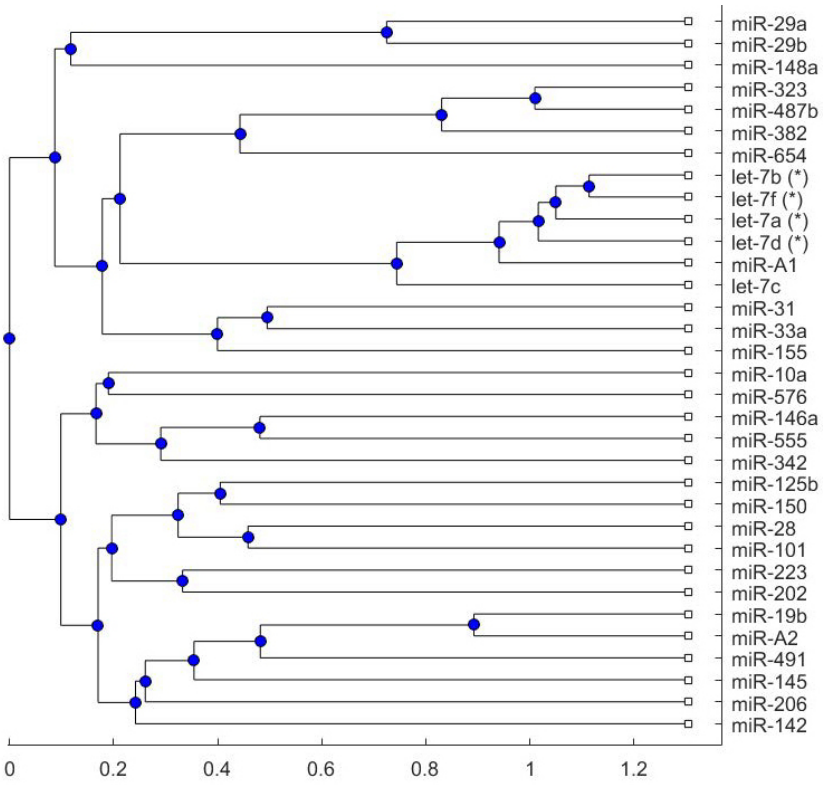

Figure 4. The phylogenetic tree of miRNA A1 and miRNA A2 and miRNAs in table 1 based on the *alignment score* distance and the *average* linkage function.

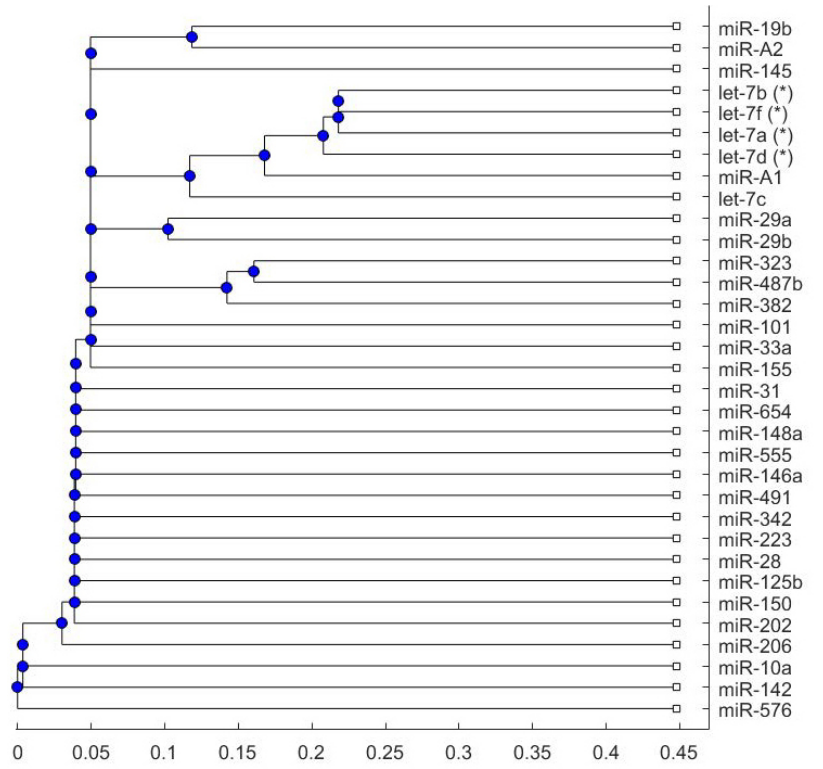

Figure 5. The phylogenetic tree of miRNA A1 and miRNA A2 and miRNAs in table 1 based on the *p-distance* and the *median* linkage function.

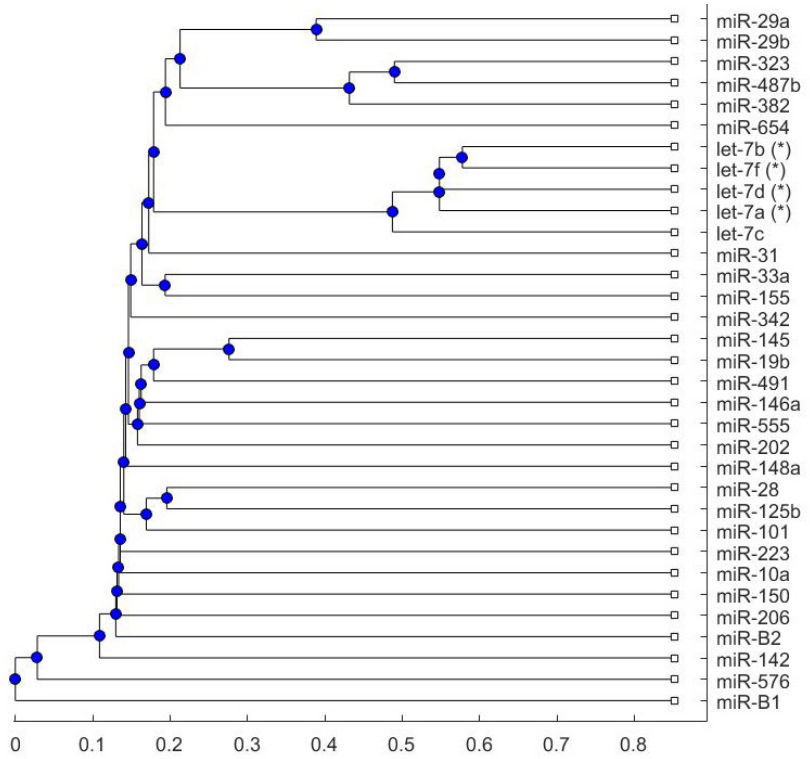

Figure 6. The phylogenetic tree of miRNA B1 and miRNA B2 and miRNAs in table 1 based on the *Juke–Cantor* distance and the *single* linkage function.

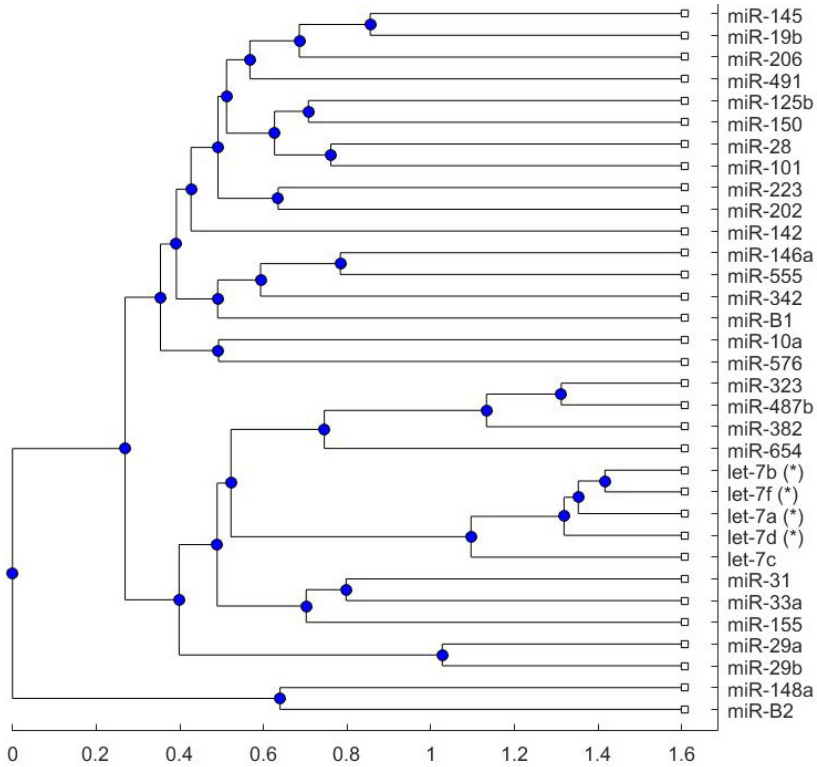

Figure 7. The phylogenetic tree of miRNA B1 and miRNA B2 and miRNAs in table 1 based on the *alignment score* distance and the *average* linkage function.

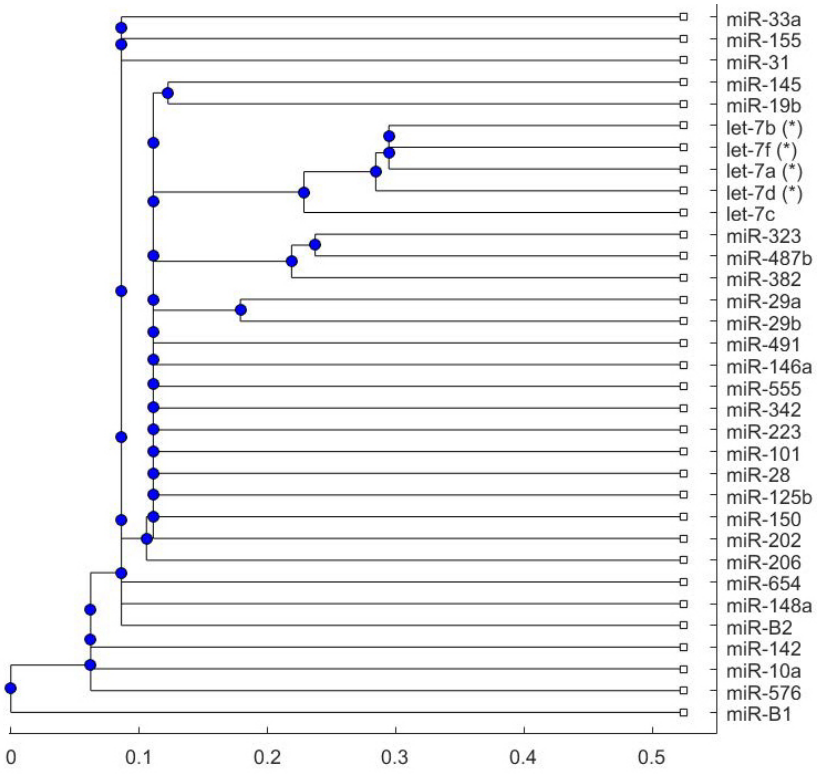

Figure 8. The phylogenetic tree of miRNA B1 and miRNA B2 and miRNAs in table 1 based on the *p-distance* and the *median* linkage function.

NMDA receptor encephalitis need critical care, and the recovery takes several months to serval years. To prevent the occurrence or relapse of this disease, investigating the cause of this disease is very important.

Except for cases that a tumor may be a main factor to cause this autoimmune disorder, the significance of other factors associated with this condition is not clear. Although there are several cases that have been reported to be related with vaccination, it has been hard to provide direct evidence for such an association. The approach provided here provides the opportunity to study the relationship between the anti-NMDA receptor encephalitis and vaccination. By applying this method, many types of phylogenetic trees can be adopted that can demonstrate the relationship between vaccines and diseases.

4. ACKNOWLEDGMENTS

This work has been supported by the Ministry of Science and Technology 105-2118-M-009-001-MY2, Taiwan. The author declares no conflict of interest.

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Key Words: Anti-NMDA Receptor Encephalitis, Autoimmune, microRNA, Phylogenetic Tree, Vaccination, Virus

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