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Oryzias latipes (JAPANESE MEDAKA) AS GENETIC MODEL TO STUDY CAUSATIVE GENES OF EPILEPSY DISEASE: AN IN-SILICO APPROACH

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ABSTRACT

Epilepsy is a chronic neurological disorder that has affected around 50-70 million people worldwide. Various animal models have previously been used in epilepsy research. To expand the knowledge of the disease, a new animal model is suggested to be explored considering the genetic and phenotypic heterogeneity that contributes to the complexity of the disease. This study was undertaken to analyze 14 causative genes of epilepsy disease in Japanese medaka (*Oryzias latipes*), humans, and the established model of this disease which is zebrafish (*Danio rerio*) by assessing the variation in the genes by using MEGA X and predicting the functional motif and secondary structure of the proteins by using PROSITE and GORIV respectively. Results from the variation analysis showed the lowest percentage of conserved genes in Japanese medaka was 60%.50% of the genes of Japanese medaka were found to be more conserved than zebrafish in comparison to a human. The functional motifs present in all genes in Japanese medaka showed the same motifs present in humans. All the secondary structures of Japanese medaka genes were predicted to contain the alpha helix, extended strand, and random coil. In conclusion, it can be inferred that Japanese medaka could be a reliable animal model for epilepsy disease.

Key words: Animal model, epilepsy, epilepsy genes, Japanese medaka, zebrafish

INTRODUCTION

Epilepsy is a chronic central nervous system (neurological) disorder that causes abnormal brain activity, recurrent seizures, and odd behaviors, and sometimes the patients will lose consciousness during the period. This disease is diagnosed after a person experiences repeated seizures and it can happen to everyone of different ages, races, and social classes. Epilepsy has affected around 50-70 million people worldwide (World Health Organization, 2019; Cho *et al*., 2020) and the cause of the disease remains unclear for about 50% of cases globally (World Health Organization, 2019). Epilepsy is frequently linked to genetic defects that affect brain function, structural abnormalities caused by head injury or brain development changes that occur since birth, and metabolic disorders (Wang *et al*., 2017). There are currently three main epilepsy treatments available which are antiepileptic drugs (AEDs) (French & Staley, 2012), such as carbamazepine, resective or palliative surgery (West *et al*., 2019), which is the removal of a small portion of the brain where the seizure originates and 'disconnecting' areas of the brains where the seizures begin or spread, and neurostimulation (Kwon *et al*., 2018). According to the United Kingdom National Health Service, AEDs are not able to cure the disease but only can stop seizures from happening. Meanwhile, the promising outcome of surgery requires further research (West *et al*., 2019) and neurostimulation rarely results in seizure-free status. Based on the latest findings in epilepsy research, there is no cure yet for this disease. Therefore, existing treatments available up to date are only able to manage the disease.

According to the International League Against Epilepsy (ILAE), the majority of epilepsy genes discovered exhibit phenotypic heterogeneity, and the majority of syndromes exhibit genetic heterogeneity, and this has been aided by the research in epileptic encephalopathies, which has made remarkable findings in gene discovery and demonstrates genetic heterogeneity (McTague *et al*., 2016; Scheffer *et al*., 2017; Ellis *et al*., 2020). Genetic heterogeneity implies that variation in multiple genes can result in the same phenotype. Another occurrence is phenotypic heterogeneity, which implies that a single gene may give rise to various phenotypes, such as epilepsy types and severity levels (McTague *et al*., 2016; Scheffer *et al*., 2017). The complexity of the disease has

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also been attributed to the usage of non-mammalian models in previous research as clinical trials cannot be conducted in humans due to ethical reasons. There are currently six non-mammalian models that have been used for epilepsy research which include roundworm (Locke *et al*., 2006), leech (Kristan *et al*., 2005), planaria, tadpole (Hewapathirane *et al*., 2008), fruit fly (Fogle *et al*., 2019) and zebrafish (Decui *et al*., 2020). Among all models, zebrafish has been widely utilized in epilepsy research due to the complex anatomy and behaviors that zebrafish possess including 70% similarity of its genome compared to humans (Johan Arief *et al*., 2018). Animal research has been developed over the years and the usage of the animal in disease studies varies (Tanner, 2018).

In epilepsy research, the animal model has been used for various purposes such as understanding underlying substrates and mechanisms that control normal or abnormal behavior such as behavioral analysis and brain activity recordings as well as preclinical development of novel AEDs (van der Staay *et al*., 2009; Johan Arief *et al*., 2018). Among all the animal models used, none of them can fully represent the disease and in fact, there is no single animal model that could mimic a full version of this disease as supported by Grone and Baraban (2015) due to the range of conditions present in epilepsy. According to previous studies, fruit flies, flatworm, roundworm, and zebrafish were used to test AEDs, and some were used to test proconvulsant such as flatworm, tadpole, and zebrafish. In addition, zebrafish and fruit flies had been used for transgenic and mutant studies as well (Cunliffe *et al*., 2015; Grone & Baraban, 2015; Johan Arief *et al*., 2018).

The discovery of various types of epilepsy in each patient shows a wide phenotypic spectrum of epilepsy genes. Due to its variability of affected genes and phenotype in different patients, Japanese medaka (*Oryzias latipes*) is therefore chosen to broaden the spectrum of models available for analysis of human epilepsy genes. Japanese medaka is a small freshwater fish native to East Asia and it has been used in experiments since the early 20th century (Matsumoto *et al*., 2009). Other than that, wild medaka populations have been also preserved as bio-resources in some universities and research institutes since 1985 with funding from the Japanese government (Katsumura *et al*., 2019). In this study, Japanese medaka was selected as a well-characterized species and has been recognized as a potential animal model in research due to its similar features and advantages to zebrafish for example short generation time, complete genome analysis, and well-established transgenic technology (Naruse *et al*., 2016).

Thus, this present study aimed to determine whether Japanese medaka could be a preliminary model for epilepsy research by investigating the presence of human epilepsy genes in this species and analyzing the variation of the epilepsy genes between humans, medaka, and zebrafish by comparing the percentage of conserved sites and variable sites. This study was done to expand the knowledge on the functional similarity of the gene by predicting the protein motifs and the secondary structures of Japanese medaka. The exploration of a new animal model to use for further research is crucial in understanding epilepsy disease and the identification of the epilepsy genes in the model can provide significant input in determining Japanese medaka (*Oryzias latipes*) as a preliminary model for human epilepsy gene research in the future.

MATERIALS AND METHODS

Retrieval of epilepsy gene sequences in human, Japanese medaka, and zebrafish

Nucleotide sequences and amino acids of 14 epilepsy genes in humans (*Homo sapiens*), Japanese medaka (*Oryzias latipes*), and zebrafish (*Danio rerio*) were retrieved from National Centre for Biotechnology Information (NCBI) and a list of all 14 genes that were chosen with their full name can be referred in Table 1.

Variation analysis of epilepsy genes in human, Japanese medaka, and zebrafish

Variation analysis was conducted to compare the percentage of conserved sites (C) and variable sites (V) of epilepsy genes in humans, Japanese medaka, and zebrafish by using Sequence Data Explorer in MEGA X (Kumar *et al*., 2008). These statistical attributes from the analysis were used to conduct the comparison between human – Japanese medaka and human–zebrafish to observe the percentage difference between Japanese medaka and zebrafish on the conserved sites and variable sites.

Prediction of protein motifs and protein secondary structures of the epilepsy genes

Protein motifs of all the 14 epilepsy genes in humans, Japanese medaka, and zebrafish were predicted using PROSITE software by the Swiss Institute of Bioinformatics (de Castro *et al*., 2006; Ikpeme *et al*., 2016) and the protein structures were then predicted by using GOR secondary structure prediction method version IV by PRABI Lyon Gerland *(*Garnier *et al*., 1996; Ikpeme *et al*., 2016).

RESULTS AND DISCUSSION

Variation analysis of epilepsy genes in human, Japanese medaka, and zebrafish

In this study, variation analysis was conducted on 14 epilepsy genes in Japanese medaka and zebrafish in which conserved sites (C), variable sites (V), and percentage of conservation of the nucleotide sequences were generated by conducting

Table 1. List of all 14 genes studied and their full name

Gene symbol	Gene full name
SCN1A	sodium voltage-gated channel alpha subunit 1
KCNJ10	potassium inwardly-rectifying channel subfamily J member 10
KCNQ3	potassium voltage-gated channel subfamily Q member 3
STX1B	syntaxin 1B
STXBP1	syntaxin binding protein 1
CACNA1A	calcium voltage-gated channel subunit alpha1 A
CHD2	chromodomain helicase DNA binding protein 2
LGI1	leucine-rich glioma inactivated 1
GABRA1	gamma-aminobutyric acid type A receptor subunit alpha1
DEPDC5	DEP domain containing 5, GATOR1 subcomplex subunit
UBE3A	ubiquitin protein ligase E3A
ALDH7A1	aldehyde dehydrogenase 7 family member A1
PLPBP	pyridoxal phosphate binding protein
TSC2	TSC complex subunit 2

pairwise comparison using Sequence Data Explorer in MEGA X. Conserved sequences refer to similar or identical sequences found in nucleic acids such as DNA or RNA, or either protein sequences across species or within different molecules produced by the same organism. Hence, variation analysis gives crucial information on the similarities and differences between Japanese medaka-human and zebrafish-human. According to Figure 1, the percentage of conservation of all 14 genes in Japanese medaka was over 60% when compared with human sequences, and 50% out of 14 Japanese medaka epilepsy genes were found to be more conserved compared to zebrafish.

The findings revealed that seven genes in Japanese medaka specifically *KCNJ10*, *KCNQ3*, *STX1B*, *STXBP1*, *LGI1*, *GABRA1*, and *ALDH7A1* were discovered to be more conserved than those in zebrafish. Besides, all genes also had less than 33% of the variable percentage for both species. Subsequently, ubiquitin protein ligase E3A (*UBE3A*) in zebrafish showed a higher percentage of conservation than Japanese medaka compared to other genes while *UBE3A* and *CACNA1A* in Japanese medaka have more than 5% of the variable percentage than zebrafish. Although the variable percentage of *UBE3A* and *CACNA1A* in Japanese medaka was more than 5%, the genes can still be considered in the study.

The genes in Japanese medaka that were found to be more conserved than those in zebrafish play an important role in this study because conserved sequences play critical biological roles in cellular processes such as basic cellular stability, functional conservation, and evolutionary relationships between sequences. Aside from that, protein-coding sequence conservation is useful for sustaining proteins or domain structure and function. All genes that are conserved by more than 60% demonstrated a high level of nucleotide sequence conservation as reported by Isenbarger *et al*. (Isenbarger *et al*., 2008), and the genes are suggested to have their functions similar to humans. Thus, the results from this variation analysis hold an important value for Japanese medaka to be useful in epilepsy research because the genes in Japanese medaka are highly conserved as zebrafish which has been a remarkable animal model in epilepsy research.

Comparison of protein motifs of the epilepsy genes

Protein motifs are short conserved sequences that are involved with the function of the protein and they could be an indicator of any specific function of the gene (Ikpeme *et al*., 2016). The prediction of protein motif was performed to observe the functionality of the protein of the same gene in target species and humans as a reference to check structural sites that are required to perform a particular function. Few motifs were repeatedly found in most of the genes as it is said to ensure the system is functioning correctly (Ikpeme *et al*., 2016). A total of 39 motifs were discovered, and a list of all motifs can be referred to in Table 2. Figure 2 presents the summary of motifs percentage that were predicted in all genes among Japanese medaka, zebrafish, and humans. The figure depicts the motifs that were found to be repeatedly present in all genes. Among 14 genes that were observed in the presence of the motifs in Japanese medaka with humans as the reference, there were two genes in Japanese medaka that have all the motifs predicted in humans which were syntaxin 1B (*STX1B*) and leucine-rich glioma inactivated 1 (*LGI1*). As shown in Table 3 and Table 4, zebrafish's STX1B and LGI1 genes also have all the motifs predicted in humans, as well as zebrafish's UBE3A and ALDH7A1 as shown in Table 5 and Table 6. Among all the motifs predicted in these four genes, few significant motifs have been linked to and are important in epilepsy disease.

The t-SNARE coiled-coil homology domain profile is one of the predicted motifs in *STX1B*, which plays a role in encoding syntaxin-1B, a

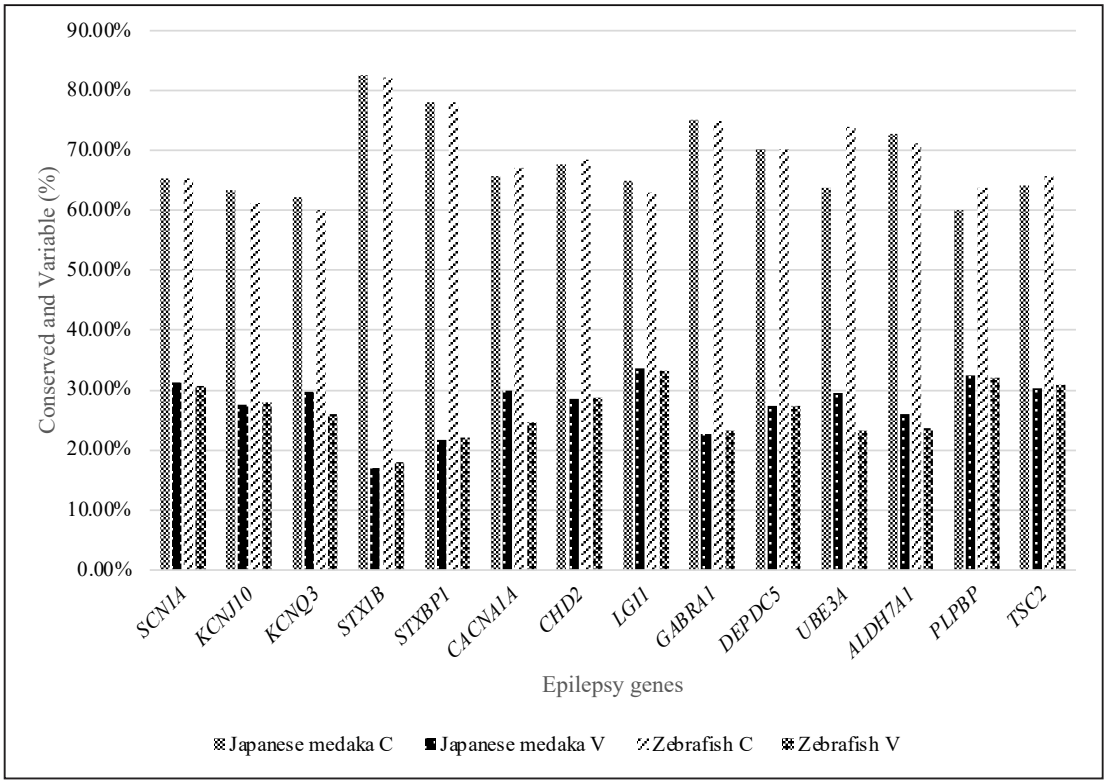

Fig. 1. Variation analysis of the nucleic acid sequence of Japanese medaka and zebrafish which showed the conserved (C) and variable (V) percentage of both species in comparison to humans.

presynaptic protein involved in the SNARE complex. It contributes to the exocytosis of calcium-dependent synaptic vesicles (Wolking *et al*., 2019), which explains the presence of the t-SNARE coiled-coil homology domain profile in the predicted motif of humans. This crucial motif is present in both Japanese medaka and zebrafish as it will also assist in the membrane fusion events (Weimbs *et al*., 1997). The EAR repeat profile (epilepsy-associated repeat) is the next significant motif in the LGI1 protein. The EAR repeat is a conserved repeated region encoded by LGI1, an ion channel whose mutation is linked to the majority of hereditary idiopathic epilepsies (Mulley *et al*., 2003). Another notable finding in this section was that, although Japanese medaka does not have all motifs predicted in UBE3A and ALDH7A1 as zebrafish, functional motifs related to the neurological disorders, such as HECT domain profile and aldehyde dehydrogenases glutamic acid active site in UBE3A and ALDH7A1, were detected. The HECT (Homologous to the E6-AP Carboxyl Terminus) domain profile is a member of the E3 ubiquitin-protein ligase family, which is responsible for transferring ubiquitin to a lysine residue on the substrate (George *et al*., 2018). HECT encoded by UBE3A is imprinted in neurons of the central nervous system and mutation studies have been conducted about Angelman syndrome, as E3 ligase mutations cause over 90% of the cases reported (George *et al*., 2018).

Aside from the HECT domain profile, aldehyde dehydrogenases glutamic acid active site was also found in the ALDH7A1 protein in the three species previously mentioned. Past research on ALDH7A1 mutations has found that this motif was frequently associated with pyridoxine-dependent epilepsy (PDE), a common epileptic encephalopathy that can be treated with pyridoxine supplementation (Coughlin *et al*., 2019). Aldehyde dehydrogenases (ALDH) are dependent enzymes encoded by the *ALDH7A1* gene, and the conserved residues found in ALDH are critical in positional requirements for the enzyme's comprehensive catalysis. Furthermore, ALDH catalysis activity is associated with conserved residues such as lysine and glutamic acid (Shortall *et al*., 2021), which explains the presence of aldehyde dehydrogenase glutamic acid active side motif.

Based on the results obtained from the prediction of protein motifs on the genes in the target species, protein function was able to be identified by using this method (de Castro *et al*., 2006; Ikpeme *et al*., 2016). Each PROSITE profile found is highly related to the UniProtKB/SwissProt database which has been remarked to provide up to 190 million protein sequences (UniProt, 2021). Hence, the approach taken was able to identify functional motifs related to the protein function and the presence of all the motifs discussed gives an insight that mutation studies could

Table 2. List of motifs found in all 14 genes

No.	Motif names
1.	Bacterial ribonuclease P protein component signature
2.	Casein kinase II phosphorylation site
3.	N-myristoylation site
4.	N-glycosylation site
5.	Protein kinase C phosphorylation site
6.	cAMP- and cGMP-dependent protein kinase phosphorylation site
7.	Tyrosine kinase phosphorylation site 1
8.	Tyrosine kinase phosphorylation site 2
9.	ATP/GTP-binding site motif A (P-loop)
10.	IQ motif profile
11.	Amidation site
12.	Glycine-rich region profile
13.	Leucine zipper pattern
14.	Cell attachment sequence
15.	t-SNARE coiled-coil homology domain profile
16.	Bipartite nuclear localization signal profile
17.	Syntaxin/epimorphin family signature
18.	Arginine-rich region profile
19.	Histidine-rich region profile
20.	Glutamine-rich region profile
21.	Proline-rich region profile
22.	Chromo and chromo shadow domain profile
23.	Superfamilies 1 and 2 helicase ATP-binding type-1 domain profile
24.	Superfamilies 1 and 2 helicase C-terminal domain profile
25.	Serine-rich region profile
26.	Glutamic acid-rich region profile
27.	Lysine-rich region profile
28.	Chromo domain signature
29.	Aspartic acid-rich region profile
30.	EAR repeat profile
31.	Leucine-rich repeat profile
32.	Neurotransmitter-gated ion-channels signature
33.	DEP domain profile
34.	ATP synthase alpha and beta subunits signature
35.	HECT domain profile
36.	Legume lectins beta-chain signature
37.	Uncharacterized protein family UPF0001 signature
38.	Rap GTPase activating proteins domain profile
39.	HEAT repeat profile

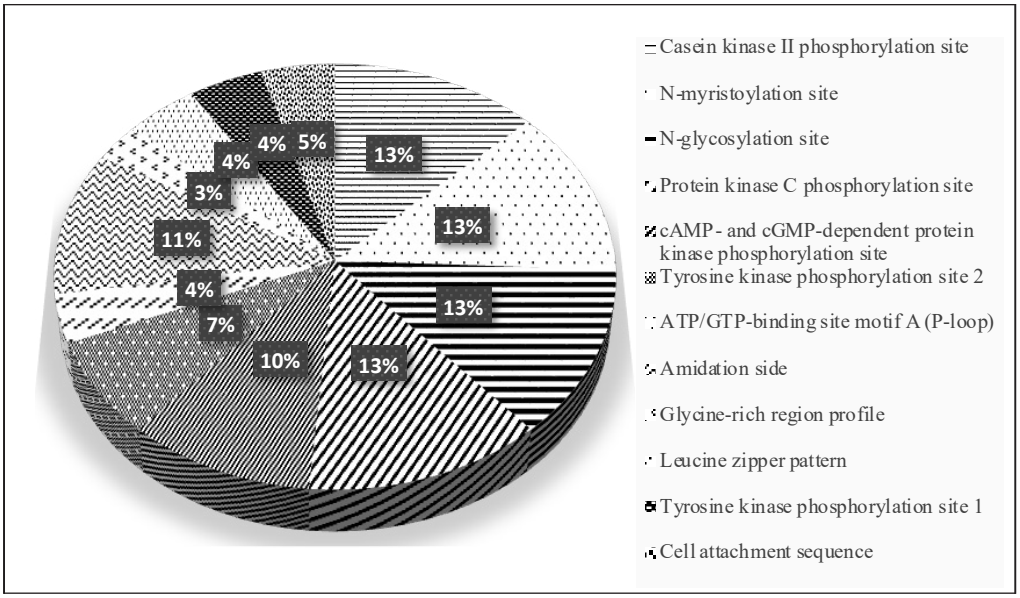

Fig. 2. The summary of the motif percentage of mostly present motifs in all 14 epilepsy genes in humans, Japanese medaka, and zebrafish based on PROSITE, Swiss Institute of Bioinformatics (SIB) database.

Table 3. Motifs present in STX1B protein in human, Japanese medaka and zebrafish

Motifs in STX1B protein	Presence of the motifs		
	Japanese Medaka	Zebrafish	Human
t-SNARE coiled-coil homology domain profile	/	/	/
Bipartite nuclear localization signal profile	/	/	/
Syntaxin / epimorphin family signature	/	/	/
Protein kinase C phosphorylation site	/	/	/
Casein kinase II phosphorylation site	/	/	/
N-myristoylation site	/	/	/
N-glycosylation site	/	/	/

(/) indicates the presence of motif, (-) indicates the absence of motif while (//) indicates the motif only present in Japanese medaka or zebrafish.

Table 4. Motifs present in LGI1 proteins in humans, Japanese medaka, and zebrafish

Motifs in LGI1 protein	Presence of the motifs		
	Japanese Medaka	Zebrafish	Human
EAR repeat profile	/	/	/
Protein kinase C phosphorylation site	/	/	/
Amidation site	/	/	/
Casein kinase II phosphorylation site	/	/	/
N-myristoylation site	/	/	/
N-glycosylation site	/	/	/
Leucine-rich repeat profile	//	//	-
Leucine zipper pattern	-	//	-
ATP/GTP-binding site motif A (P-loop)	//	-	-

(/) indicates the presence of motif, (-) indicates the absence of motif while (//) indicates the motif only present in Japanese medaka or zebrafish.

Table 5. Motifs present in UBE3A protein in humans, Japanese medaka, and zebrafish

Motifs in UBE3A protein	Presence of the motifs		
	Japanese Medaka	Zebrafish	Human
HECT domain profile	/	/	/
Tyrosine kinase phosphorylation site 2	-	/	/
N-myristoylation site	/	/	/
Protein kinase C phosphorylation site	/	/	/
N-glycosylation site	/	/	/
Casein kinase II phosphorylation site	/	/	/
cAMP- and cGMP-dependent protein kinase phosphorylation site	/	/	/
Amidation site	/	/	/
Legume lectins beta-chain signature	//	-	-

(/) indicates the presence of motif, (-) indicates the absence of motif while (//) indicates the motif only present in Japanese medaka or zebrafish.

Table 6. Motifs present in ALDH7A1 protein in humans, Japanese medaka, and zebrafish

Motifs in ALDH7A1 protein	Presence of the motifs		
	Japanese Medaka	Zebrafish	Human
Aldehyde dehydrogenases glutamic acid active site	/	/	/
Protein kinase C phosphorylation site	/	/	/
Tyrosine kinase phosphorylation site 2	-	/	/
N-myristoylation site	/	/	/
N-glycosylation site	/	/	/
Casein kinase II phosphorylation site	/	/	/
cAMP- and cGMP-dependent protein kinase phosphorylation site	/	/	/
Amidation site	//	-	-

(/) indicates the presence of motif, (-) indicates the absence of motif while (//) indicates the motif only present in Japanese medaka or zebrafish.

be done in Japanese medaka in further research.

Comparison of protein secondary structures of the epilepsy genes

Previously, the functional motifs of the proteins were predicted to determine the protein function of the proteins encoded by the epilepsy genes. Protein secondary structure prediction was performed in this section of the study as it can provide information about protein activity, relationships, and functions, and it can also serve as the first step toward tertiary structure prediction (Ma *et al*., 2018). The GOR method version IV was used to predict the secondary structures of the proteins in Japanese medaka, zebrafish, and humans. There are three secondary structures present in the proteins which include alpha helix, extended strand, and random coil. In general, there are only four zebrafish genes and two Japanese medaka genes that have a higher percentage of the alpha helix in their structures, as shown in Figure 3. For zebrafish, these genes were *STX1B*, *STXBP1*, *LGI1*, and *DEPDC5*, while for Japanese medaka, they were *SCN1A* and *PLPBP*. The presence of an alpha helix is significant because it indicates the role of the protein in mediating protein-protein interactions and determining the overall structure and function of the protein (Haimov & Srebnik, 2016). The comparison of alpha helix percentage between only Japanese medaka and zebrafish showed that 50% of Japanese medaka genes were higher than zebrafish which involved *SCN1A, KCNJ10*, *KCNQ3*, *CHD2*, *ALDH7A1*, *PLPBP,* and *TSC2*.

The second element observed was the extended strand. The extended strand is important because it is said to aid in the stabilization of both the original secondary structure and the resulting tertiary structure (Degreve *et al*., 2014). Figure 4 illustrates that the five human and Japanese medaka genes have the highest percentage of extended strands among the 14 genes studied. In zebrafish, only four genes out of 14 have

the highest percentage of extended strands compared to human and Japanese medaka, and that includes *KCNQ3*, *CHD2*, *PLPBP,* and *TSC2*. Apart from that, six genes in Japanese medaka have a higher percentage of extended strand than zebrafish which includes, *STXBP1*, *CACNA1A*, *LGI1*, *GABRA1*, *UBE3A*, and *ALDH7A1*. Next, a random coil was discovered in the secondary structure of the proteins, in addition to the alpha helix and extended strand. The random coil was said to contain critical information for understanding the structural properties of polypeptide chains, as well as protein folding and molecular design (Smith *et al*., 1996). As can be seen in Figure 5, it is apparent that five genes of Japanese medaka and zebrafish had a high percentage of the random coil out of the 14 genes compared to humans. *KCNJ10*, *KCNQ3*, *STX1B*, *STXBP1*, and *UBE3A* were Japanese medaka genes with a high percentage of the random coil, whereas

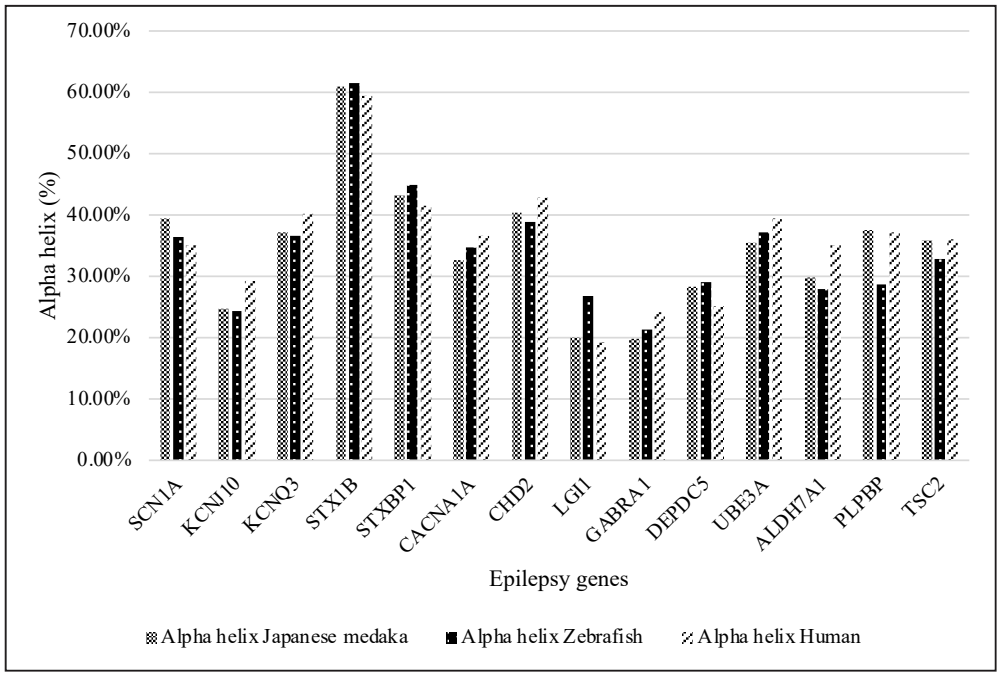

Fig. 3. Comparison of alpha helix percentage among Japanese medaka, zebrafish, and humans in 14 different epilepsy genes.

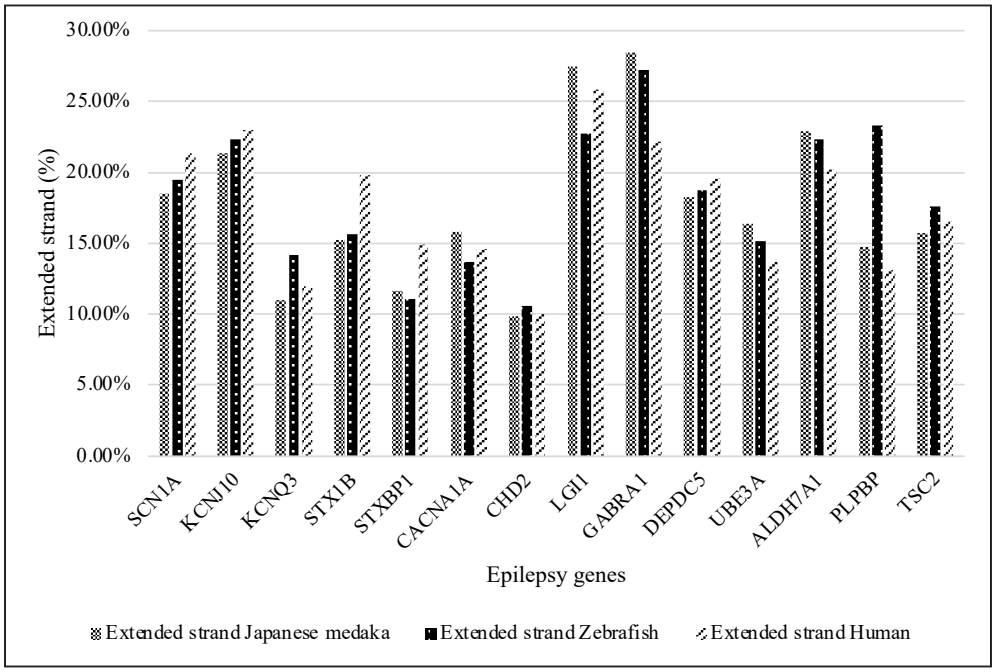

Fig. 4. Comparison of extended strand percentage among Japanese medaka, zebrafish, and humans in 14 different epilepsy genes.

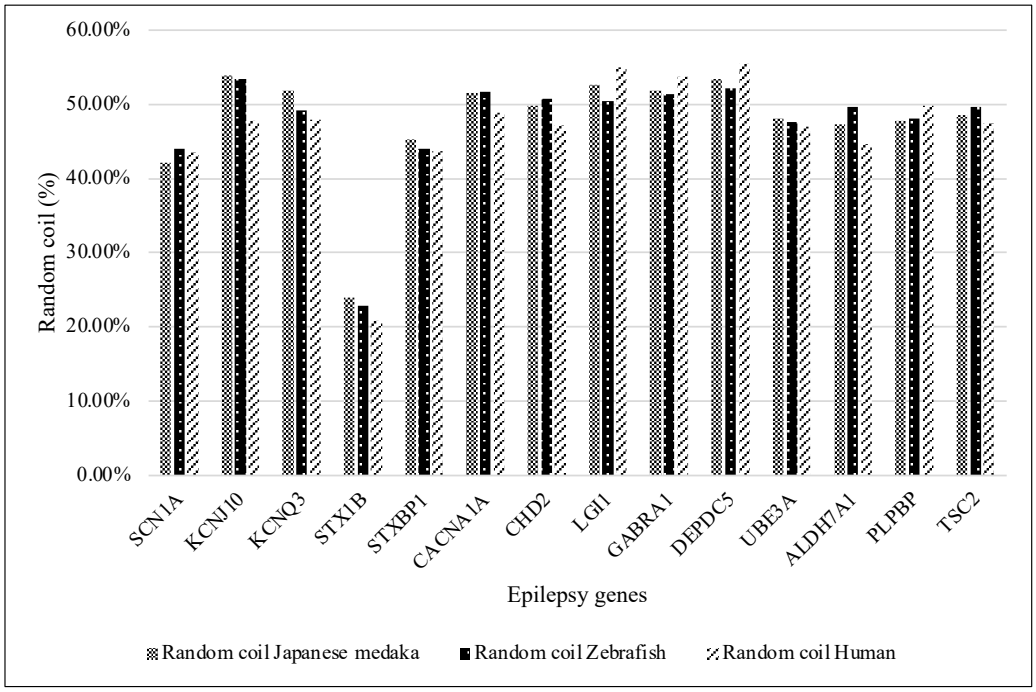

Fig. 5. Comparison of random coil percentage among Japanese medaka, zebrafish, and humans in 14 different epilepsy genes.

for zebrafish were *SCN1A*, *CACNA1A*, *CHD2*, *ALDH7A1*, and *TSC2*. Another significant analysis from the figure was the differences in random coil percentage between Japanese medaka and zebrafish, respectively. On top of the Japanese medaka genes that were listed previously, *LGI1*, *GABRA1*, and *DEPDC5* were recorded higher compared to zebrafish genes as well. Thereby, the presence of all three structures in Japanese medaka, to be a specific alpha helix, extended strand, and random coil, implies the folding stability and function of all the proteins observed in Japanese medaka (Ikpeme *et al*., 2016). Although the findings in this study suggest that Japanese medaka could be used as a model for epilepsy disease in the future, there are a few limitations to this study. First and foremost, the samples used in the study were insufficient to represent all the causative genes of epilepsy disease; thus, additional research on other causative genes is required, as many other genes have been identified to be associated with the disease. Other than that, the conservation study was done on the nucleotide sequence, so it is recommended to perform future works in 3-D structure by using software such as EXPASY as it could be used to provide extensive information in protein prediction. In addition, redundancy of the protein motifs found may result in insignificant findings in the study; hence, redundant motifs should be excluded from the method.

Despite these limitations, extensive research on the similarity and functionality of the Japanese medaka genes could be conducted to gain a better understanding of the importance of using this species as an animal model. With the current findings of this study, Japanese medaka can be used to investigate external factors that could be a possible cause of epilepsy disease or natural product effects on Japanese medaka in epilepsy disease to discover potential treatments. Aside from that, a variant study on the disease's mutations is recommended to be conducted to analyze the effect of the mutation on the species as it could contribute more knowledge on the epilepsy mechanism.

CONCLUSION

The preliminary study to expand the candidate of animal model for epilepsy disease reveals remarkable findings through the comparison study done in Japanese medaka, humans, and the established animal model for the disease, zebrafish. The percentage of conservation of all 14 genes in Japanese medaka was found to be more than 60%, with 50% of Japanese medaka epilepsy genes being more conserved than zebrafish. The prediction of motifs and secondary structure shows the functional similarity and stability of the proteins in Japanese medaka genes as compared with humans. These findings suggest that Japanese medaka could be the potential animal model for epilepsy; therefore, additional research on Japanese medaka is recommended to investigate the potential causes of epilepsy and its therapeutic strategies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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