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[1] Tong Zhou, Eun A. Ko, Wanjun Gu, Inja Lim, Hyoweon Bang, and Jae-Hong Ko. Non-silent story on synonymous sites in voltage-gated ion channel genes. *PLOS ONE*, 7(10):1–8, 10 2012.

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Non-Silent Story on Synonymous Sites in Voltage-Gated Ion Channel Genes

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Abstract

Synonymous mutations are usually referred to as "silent", but increasing evidence shows that they are not neutral in a wide range of organisms. We looked into the relationship between synonymous codon usage bias and residue importance of voltage-gated ion channel proteins in mice, rats, and humans. We tested whether translationally optimal codons are associated with transmembrane or channel-forming regions, i.e., the sites that are particularly likely to be involved in the closing and opening of an ion channel. Our hypothesis is that translationally optimal codons are preferred at the sites within transmembrane domains or channel-forming regions in voltage-gated ion channel genes to avoid mistranslation-induced protein misfolding or loss-of-function. Using the Mantel-Haenszel procedure, which applies to categorical data, we found that translationally optimal codons are more likely to be used at transmembrane residues and the residues involved in channel-forming. We also found that the conservation level at synonymous sites in the transmembrane region is significantly higher than that in the non-transmembrane region. This study provides evidence that synonymous sites in voltage-gated ion channel genes are not neutral. Silent mutations at channel-related sites may lead to dysfunction of the ion channel.

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Introduction

Ion channels are membrane protein complexes that help establish and control the voltage gradient across biological membranes by allowing the flow of ions down their electrochemical gradient. Ion channels play vital roles in diverse cellular processes such as cardiac, skeletal, and smooth muscle contraction, epithelial transport of nutrients and ions, T-cell activation and pancreatic beta-cell insulin release, hormonal secretion, and osmotic regulation of blood pressure [1,2,3,4,5]. Ion channel dysfunction can have profound physiological effects [6]. Therefore, ion channels are frequently considered as drug targets [7,8,9].

The ion channel conformational change between the closed and open states is called gating. Ion channels can be classified by gating, such as the chemical or physical modulator that controls their opening or closing activity. Voltage-gated ion channels open or close depending on the voltage gradient across the plasma membrane. It has been found that the amino acid sequences involved in pore-forming are highly conserved in voltage-gated ion channel proteins [10,11,12]. Even a single-site mutation in these regions may lead to a change in channel conductance, voltage dependence, or activity level [6], which suggests that the non-synonymous sites in transmembrane domains of voltage-gated ion channel genes are under stronger purifying selection than the sites in other regions in the same genes [13]. However, the effect of synonymous mutations in voltage-gated ion channel genes is still unknown.

Synonymous mutations (so-called silent mutations) are the change of one base for another in an exon of a gene, but the coded amino acid is not changed. When a synonymous or silent mutation occurs, the change is often assumed to be neutral, meaning that it does not affect the fitness of the individual carrying the new gene to survive and reproduce. However, increasing evidence shows that synonymous mutations are not neutral in a wide range of organisms. For example, selection on synonymous sites has been linked to transcription, splicing, DNA secondary structure, messenger RNA secondary structure and stability, and protein expression [14,15,16,17,18,19,20,21,22,23]. More importantly, selection on synonymous sites for translation with high fidelity has been observed in bacteria, plants, yeast, flies, worms, and even mammals [24,25,26,27].

Translation is an error-prone process [28]. Translation errors occur at frequencies of several misincorporations *per* 10,000 codons translated; precise error rates vary over nearly an order of magnitude among codons [29]. At this error rate, 15% of average-length protein molecules will contain at least one misincorporated amino acid [28]. According to the mistranslation-induced-protein-misfolding hypothesis, selection should prefer high-fidelity codons (optimal codons) at sites at which translation errors are structurally

disruptive and lead to protein misfolding, aggregation or dysfunction [30]. For example, the usage of optimal codons was found to be increased in putative zinc-finger and homeodomain regions of transcription factors [24]. Also, optimal codons were reported to be more likely to encode residues in the core of proteins to minimize the misfolding of mistranslated proteins [26].

Here, we investigate whether synonymous codon usage is linked to key residues in voltage-gated ion channel proteins. Specifically, we test whether translationally optimal codons are associated with transmembrane segments or channel-forming regions, i.e., sites that are particularly likely to be involved in the closing and opening of ion channels. Our hypothesis is that translationally optimal codons are preferred at sites within the transmembrane domains or channel-forming regions in voltage-gated ion channel genes. We consider three mammalian organisms: human, rat, and mouse. Using the Mantel-Haenszel procedure, which applies to categorical data, we find that translationally optimal codons are more likely to be used at transmembrane residues and the residues involved in channel-forming. We also find that the conservation level at synonymous sites in transmembrane regions is significantly higher than that in non-transmembrane regions.

Materials and Methods

Genomic Data

The definition of an ion channel gene for human, rat, and mouse was obtained from IUPHAR-DB [31]. We collected the coding sequence for each orthologous ion channel gene from the Reference Sequence (RefSeq) database [32]. In total, 141 orthologous genes from 9 categories of voltage-gated ion channel were involved in this study (Table 1 and Table S1). We built multiple alignments of orthologous sequences based on the peptide sequences with MUSCLE [33]. For each ion channel protein, we considered the residues as transmembrane if they were within the segments annotated as "transmembrane-region" by RefSeq. We also assigned the residues as channel-forming if they were within the region annotated by RefSeq as "BK_channel_a", "Ion_trans_2", "Ion_trans", "KCNQ_channel", "Kv2channel", "PKD_channel", "PLN03192", "pore-forming domain", "Potassium_chann", "Shal-type", "SK_channel", "TRP_2", or "Selectivity filter".

Identifying Optimal Codons

To identify which codons are translationally optimal in each species, we calculated the codon use frequency for each codon for all of the annotated coding sequences in each genome. The effective number of codons (*ENC*) of each gene was also calculated, which measured the overall codon bias of that gene [34]. A lower *ENC* value indicates stronger overall codon bias. We assumed that genes with stronger codon bias were more likely to use optimal codons. We then calculated the Spearman's rank correlation between the frequency of each codon within each gene and *ENC* of that gene. We defined codons as "optimal" if they showed a statistically significant increase in frequency in the genes with stronger codon bias, which was identified by a significant negative correlation ($P<0.05$ after Benjamini-Hochberg adjustment) between codon frequency and *ENC*. We defined codon optimality as the multiplication product of -1 and the correlation coefficient between codon frequency and *ENC*, calculated separately for each codon.

Mantel-Haenszel Procedure

For pairs of discrete variables (e.g., optimal vs. non-optimal codons and transmembrane vs. non-transmembrane sites), we stratified the data by gene and synonymous codon family within each gene, and constructed a separate 2×2 contingency table for each stratum. We then combined either the tables for all genes and a given codon family or the tables for all genes and all codon families into an overall analysis, using the Mantel-Haenszel procedure [35]. The null hypothesis in this analysis assumes that the status of the site (e.g., transmembrane or non-transmembrane sites) is independent of the codon type in any given stratum. Because the Mantel-Haenszel procedure yields undefined results on contingency tables whose sum of all four entries is less than 2 (i.e., 0 or 1), we excluded all such tables from the analyses.

Table 1. Voltage-gated ion channel genes involved in this study.

Channel type	Gene[a]
Calcium-activated potassium channel	*KCNMA1, KCNN1, KCNN2, KCNN3, KCNN4, KCNT1, KCNT2, KCNU1*
CatSper and two-pore channel	*CATSPER1, CATSPER2, CATSPER3, CATSPER4, TPCN1, TPCN2*
Cyclic nucleotide-regulated channel	*CNGA1, CNGA2, CNGA3, CNGA4, CNGB1, CNGB3, HCN1, HCN2, HCN3, HCN4*
Inwardly rectifying potassium channel	*KCNJ1, KCNJ2, KCNJ3, KCNJ4, KCNJ5, KCNJ6, KCNJ8, KCNJ9, KCNJ10, KCNJ11, KCNJ12, KCNJ13, KCNJ14, KCNJ15, KCNJ16*
Transient Receptor Potential channel	*TRPA1, TRPC1, TRPC2, TRPC3, TRPC4, TRPC5, TRPC6, TRPC7, TRPM1, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, TRPM8, MCOLN1, MCOLN2, MCOLN3, PKD2, PKD2L1, PKD2L2, TRPV1, TRPV2, TRPV3, TRPV4, TRPV5, TRPV6*
Two-P potassium channel	*KCNK1, KCNK2, KCNK3, KCNK4, KCNK5, KCNK6, KCNK7, KCNK9, KCNK10, KCNK12, KCNK13, KCNK15, KCNK16, KCNK17, KCNK18*
Voltage-gated calcium channel	*CACNA1S, CACNA1C, CACNA1D, CACNA1F, CACNA1A, CACNA1B, CACNA1E, CACNA1G, CACNA1H, CACNA1I*
Voltage-gated potassium channel	*KCNA1, KCNA2, KCNA3, KCNA4, KCNA5, KCNA6, KCNA7, KCNA10, KCNB1, KCNB2, KCNC1, KCNC2, KCNC3, KCNC4, KCND1, KCND2, KCND3, KCNF1, KCNG1, KCNG2, KCNG3, KCNG4, KCNQ1, KCNQ2, KCNQ3, KCNQ4, KCNQ5, KCNV1, KCNV2, KCNS1, KCNS2, KCNS3, KCNH1, KCNH2, KCNH3, KCNH4, KCNH5, KCNH6, KCNH7, KCNH8*
Voltage-gated sodium channel	*SCN1A, SCN2A, SCN3A, SCN4A, SCN5A, SCN8A, SCN9A, SCN10A, SCN11A*

[a]Only human gene symbols are listed.

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