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New variants in the *CACNA1H* gene identified in childhood absence epilepsy

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Abstract

Childhood absence epilepsy (CAE) is a common form of idiopathic generalized epilepsy with polygenic inheritance. In our previous studies, relatively high frequent variants in the T-type calcium channel gene, CACNA1H, were identified in the Chinese Han population, most of which are located in exons 6–12. The goal of this study was to identify additional variants in this region of the CACNA1H gene. To this end, exons 6–12 were sequenced in 100 newly recruited CAE trios and 191 normal controls. Thirty-nine variants were identified in CAE trios or controls, 14 of which were found only in CAE patients, including two nonsynonymous variants that were newly found. Thirteen of the 39 variants were found in both CAE patients and controls, 11 were found only in parents of CAE trios, and one was found only in controls. Twenty-eight of these variants had not been previously reported. Both permutation test and transmission/disequilibrium test (TDT) indicated that a SNP-52037C > T in intron11 was significant in association with CAE. In conclusion, these data further support the hypothesis that CACNA1H is an important susceptibility gene for CAE in the Chinese Han population.

Keywords: Childhood absence epilepsy; Variation; Single nucleotide polymorphism; Chinese; CACNA1H

Childhood absence epilepsy (CAE) is a common form of idiopathic epilepsy accounting for approximately 5–15% of all childhood epilepsies [1]. Disease onset is between the ages of 3 and 12, and affected children experience multiple daily absence seizures. On electroencephalogram, the seizures have a characteristic generalized bilateral synchronous 3 Hz spike and a slow wave discharge. Although the molecular basis of CAE, which is characterized by complex inheritance, is not yet understood, preliminary genetic evidence implicates four genes encoding neuronal calcium channel subunits as possible molecular markers for CAE in human [2,6,10,16]. Previous studies based on 118 CAE trios from the Chinese Han population identified 12 missense mutations unique to CAE-positive individuals in the T-type calcium channel gene *CACNA1H*. Ten of these mutations (83.3%) were located in exons 6–12 of *CACNA1H* [2]. These data, although based on a small sample size, suggested that *CACNA1H* may be a susceptibility gene for CAE in the Chinese Han population. In this study, exons 6–12 of *CACNA1H* were sequenced in 100 newly-recruited CAE trios. The results presented here provide further support for a statistically significant association between *CACNA1H* and CAE in the Chinese Han population.

This study included 100 Chinese Han CAE trios, recruited consecutively from our pediatric neurology clinics, and two children's hospitals in Beijing city. Thirty-nine of the patients were male and 61 were female. Cases diagnosed as CAE must fulfill the following criteria [3]: (1) onset of absence seizures at 3–12 years of age, (2) absence as the initial seizure type, (3) very frequent absence seizures occurring multiple times per day, (4) EEG patterns during absence seizures associated with

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bilateral, symmetric, and synchronous discharge of regular 3-Hz spike-and-wave complexes with normal background, (5) normal neurological exam, (6) if performed, normal neuroradiological examinations (CT or MRI). Informed consent was obtained from parents of all patients. Fifteen CAE cases had a history of febrile seizures, 10 cases developed generalized tonic clonic seizure (GTCS), five cases had a family history of febrile seizures, and three cases had a family history of epilepsy. This study utilized 191 unrelated gender-matched normal controls, which were selected from our previous research [2]. None of the controls had a family or personal history of febrile seizures and epilepsy.

Genomic DNA was extracted from peripheral blood leukocytes using the method of Miller [13]. Genomic segments of exons 6–12 and their 5′- and 3′-flanking regions of *CACNA1H* were amplified by PCR in 100 CAE trios and controls using the same sequencing primers as before [2]. PCR amplification conditions were as follows: denaturation for 15 min at 95 °C, 14 touch-down cycles of 94 °C, 62 °C and 72 °C for 30, 60 and 90 s, respectively, with the annealing temperature descending 0.5 °C for per cycle, followed by 25 cycles of 94 °C, 56 °C and 72 °C for 30, 40 and 60 s, respectively, finishing with a single step at 72 °C for 10 min. Sequence variants were determined by direct sequencing of purified PCR products using an autosequencer (ABI PRISM 3730XL DNA Analyzer, Applied Biosystems, AB, USA). Sequence analyses were carried out using phredPhrap/Consed software and by visually examining printed chromatograms to detect sequence changes [14]. All nonsynonymous/synonymous variants were verified at least twice by PCR and by sequencing with forward and reverse primers.

Protein families and domains were analyzed using ScanProsite (http://www.expasy.org/). Sequence alignments of the human *CACNA1H* gene product, newly identified nonsynonymous protein variants and protein homologues from other species were performed using ClustalW (Clustalw, http://www.ebi.ac.uk/clustalw/). Computer-based predictive models the impact of *CACNA1H* variants were generated using online resources. These resources were used to analyze protein secondary structure (Nnpredict, http://www.cmpharm.ucsf.edu/), transcription factor binding sites (NIH, http://thr.cit.nih.gov/molbio/), potential splicing sites (SPL, http://softberry.com/berry/), charge status (SAPS, http://www.ch.embnet.org/) and potential phosphorylation sites (NetPhos, http://www.cbs.dtu.dk/).

Hardy–Weinberg equilibrium was assessed by Fisher's exact test using the program HWE in R (http://www.r-project.org) [8]. A Chi-square test for statistical significance was carried out using the SPSS12.0 software package. A permutation test was performed to test the genotype distribution difference of *CACNA1H* variants between cases and controls in R. TDT test was performed to analyze the association between the variants presenting different distribution between cases and controls and CAE. TDT test takes the form of the X^2 statistic: $X^2 = (b - c)^2/(b + c)$. b indicates the number of heterozygous parents who transmitted an allele. c indicates the number of heterozygous parents who did not transmit the allele [7]. Statistical significance was established at the $P < 0.05$ level, alternatively, was set to be $P < 0.05$/time of multiple tests level by Bonferroni correction when multiple statistical tests were performed.

1. Fourteen variants in the sequenced region of *CACNA1H* were observed in DNA samples from 100 CAE cases but not from 191 normal controls, 12 of which were not previously reported. The variants P314S, P492S, N345N, L602L and S619S were in the coding region of *CACNA1H* (Table 1, Fig. 1). The frequencies of these five variants were low and each of them was observed only once except for N345N, which had been reported in one CAE patient in previous study [2].
2. Eleven variants were observed only in parents of CAE trios but not in CAE patients and 191 normal controls, all of them were not previously reported, including four nonsynonymous variants, five synonymous variants and two intronic variants (Table 2). The frequencies of these variants were low and each of them was observed only once.
3. One variant 47454A > C in intron7 were observed only in normal controls, but not in CAE trios.
4. Thirteen variants were observed in both CAE cases and normal controls. P277S, M313V and S677L were not previously reported. The minor allele frequencies of 10 SNPs (M313V; rs8044363; rs8043905; rs9922076; rs2407083; rs9934839; P640L; rs3751664; rs2745150; rs11646893) were all above 6%. These 10 variants were in accordance with Hardy–Weinberg equilibrium. Thus, they were utilized as genetic markers to conduct case-control analysis. A SNP-52037C > T in intron11 was found in 17 CAE patients. However, 52037C > T present neither in the 191 controls nor the previous 118 CAE patients detected by PCR and sequencing protocol using the same primers [2]. Permutation test (100000 times) indicated that the genotype of 52037C > T between 100 cases and 191 controls was significant different ($P < 0.0001$). 52037C > T was also significant in TDT in CAE trios (Table 1).
5. Protein sequence analysis indicated that the nonsynonymous P492S variant affected a relatively well-conserved amino acid in the $Ca_v3.2$ calcium channel protein. In contrast, the P314S variant affected a poorly conserved residue. Bioinformatics analysis was used to predict the functional implications of the variants occurred only in CAE patients (Table 3).

Recent genetic studies implicated four genes encoding subunits of voltage-dependent calcium channels in CAE. Briefly, the genetic evidence is summarized as follows: (1) *CACNG3*, encoding high-voltage-activated (HVA) calcium channel γ3 subunit, was positively linked with CAE in a European population [16], (2) A missense mutation (C104F) in *CACNB4*, encoding calcium-channel beta4-subunit, was identified in a family with idiopathic absence epilepsy, but not in 255 unaffected controls or normal family members [6], (3) A heterozygous point mutation, C5733T, in the Q-type voltage-gated calcium channel α1 subunit gene *CACNA1A* was found in an 11-year-old boy of European descent with a complex phenotype including absence epilepsy and the mutation was linked to a biochemical defect in channel function [10]. Because the findings for these three

Table 1
CACNA1H variants found only in CAE patients, only in controls or in both CAE patients and controls[a]

Location	Nucleotide substitution	Amino acid substitution	refSNP ID in NCBI	Structural location	Genotype/Allele	Cases ($n=100$)	Control ($n=191$)	P-value	Parents ($n=200$)			
									Distribution	Trans	Un-trans	P-value
	45537G > A				GG/GA/AA	99/1/0			199/1/0			
Intron6	45650C > T				CC/CT/TT	98/2/0			198/2/0			
	46925T > C				TT/TC/CC	99/1/0			186/14/0			
	47041C > T	P277S		IS5-IP-loop	CC/CT/TT	99/1/0	189/2/0	NO	198/2/0			
	47149A > G	M313V		IS5-IP-loop	AA/AG/GG	84/14/2	172/17/2	0.379	172/28/0			
					A/G	182/18	361/21	0.118	372/28	18	10	NO
Exon7	47152C > T	P314S		IS5-IP-loop	CC/CT/TT	99/1/0			199/1/0			
	47247C > T	N345N		IS5-IP-loop	CC/CT/TT	99/1/0			199/1/0			
	47319T > C	I369I	rs8044363	IP-loop	TT/TC/CC	74/23/3	164/25/2	0.032	154/45/1			
					T/C	171/29	353/29	0.013	353/47	29	18	NO
	47381G > A				GG/GA/AA	99/1/0			199/1/0			
Intron7	47401C > G				CC/CG/GG	100/0/0	187/4/0	NO	198/2/0			
	47454A > C				AA/AC/CC	100/0/0	179/12/0	NO	200/0/0			
	48322G > A		rs8043905		GG/GA/AA	76/19/5	172/17/2	0.003	157/32/11			
Intron8					G/A	171/29	361/21	<0.0001*	346/54	29	25	0.586
	48563C > T	S451S	rs9922076	linker I-II	CC/CT/TT	83/14/3	172/17/2	0.176	171/27/2			
					C/T	180/20	361/21	0.059	369/31	20	11	NO
	48684C > T	P492S		linker I-II	CC/CT/TT	99/1/0			199/1/0			
	48878C > T	P556P	rs2407083	linker I-II	CC/CT/TT	82/16/2	172/17/2	0.142	171/28/1			
					C/T	180/20	361/21	0.059	370/30	20	10	NO
	49016C > G	L602L		linker I-II	CC/CG/GG	99/1/0			199/1/0			
Exon9	49019A > G	R603R	rs9934839	linker I-II	AA/AG/GG	70/26/4	172/17/2	<0.0001*	152/43/5			
					A/G	166/34	361/21	<0.0001*	347/53	34	19	0.039
	49067A > G	S619S		linker I-II	AA/AG/GG	99/1/0			199/1/0			
	49129C > T	P640L		linker I-II	CC/CT/TT	5/38/57	6/41/144	0.004	9/59/132			
					C/T	48/152	53/329	0.003	77/323	152	171	NO
	49201T > C	V664A	rs4984636	linker I-II	TT/TC/CC	98/2/0	190/1/0	NO	198/2/0			
Intron9	49271G > T				GG/GT/TT	99/1/0			199/1/0			
	50797C > T	S677L		linker I-II	CC/CT/TT	99/1/0	173/18/0	0.002	196/4/0			
Exon10	51129C > T	R788C	rs3751664	linker I-II	CC/CT/TT	78/22/0	161/30/0	0.189	162/38/0			
					C/T	178/22	352/30	0.222	362/38	22	16	NO
Intron10	51268C > T				CC/CT/TT	98/2/0			198/2/0			
	52037C > T		rs2745150		CC/CT/TT	83/15/2		<0.0001*	177/23/0			
					C/T	181/19		<0.0001*	377/23	19	4	0.00176#
Intron11	52845C > T		rs11646893		CC/CT/TT	84/15/1	177/12/2	0.061	177/22/1			
					C/T	183/17	366/16	0.038	376/24	17	7	NO
	53059A > G				AA/AG/GG	99/1/0			198/2/0			
Intron12	53061C > G				CC/CG/GG	99/1/0			199/1/0			

A: alanine; C: cysteine; I: isoleucine; L: leucine; M: methionine; N: asparagines; P: proline; R: arginine; S: serine; V: valine; The allele distribution of the variants with very low frequency in cases is not listed here. NO indicates nonsense to perform the statistical test. Significant value for comparison of cases and controls: *$P < 0.05/20 = 0.0025$; for comparison of cases and parents by TDT: #$P < 0.05/3 = 0.01667$.

[a] Protein families and domains were analyzed using ScanProsite (http://www.expasy.org/), the numbering of the nucleotide acids, amino acids is according to the system used in NC_000016 and NP_001005407, respectively. Twenty-eight variants were identified, 14 of which were found only in CAE patients, 13 variants were found in both CAE patients and controls, and one was found only in controls.

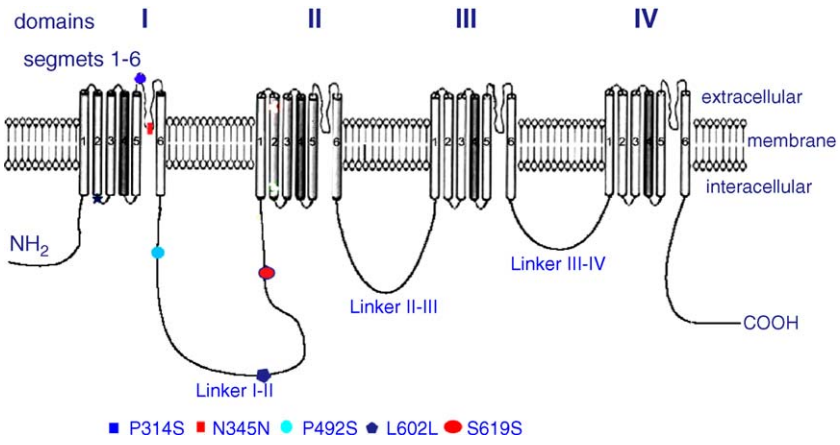

Fig. 1.

Table 2
CACNA1H variants found only in parents of CAE trios, but not in CAE patients or normal controls[a]

Location	Nucleotide substitution	Amino acid substitution	Structural location	Genotype	Distribution in parents
Exon9	48581C > T	Y457Y	linker I-II	CC/CT/TT	199/1/0
	48590G > T	L460L	linker I-II	GG/GT/TT	199/1/0
	48591C > T	L461L	linker I-II	CC/CT/TT	199/1/0
	48748C > T	S513L	linker I-II	CC/CT/TT	199/1/0
	48804A > G	S532G	linker I-II	AA/AG/GG	199/1/0
	49059C > T	L617L	linker I-II	CC/CT/TT	199/1/0
Exon10	51049G > A	R761D	linker I-II	GG/GA/AA	199/1/0
Intron10	51223G > A			GG/GA/AA	199/1/0
	51834C > T			CC/CT/TT	199/1/0
Exon11	52006G > A	G862S	II-S3	GG/GA/AA	199/1/0
Exon12	52940C > T	L894L	II-S4	CC/CT/TT	199/1/0

D: aspartic acid; G: glycine; L: leucine; R: arginine; S: serine; Y: tyrosine.
[a] Protein families and domains were analyzed using ScanProsite (http://www.expasy.org/), the numbering of the nucleotide acids, amino acids is according to the system used in NC_000016 and NP_001005407, respectively.

genes did not involve patients in typical CAE families, the major phenotypes involved were episodic or progressive ataxia. This is consistent with the hypothesis that defects in these three calcium channel genes are more likely to cause ataxia than absence epilepsy. The last of the four calcium channel genes implicated in CAE is (4) *CACNA1H*, encoding the T-type Ca^{2+} channel $Ca_v3.2$ (α1H). This protein channel localizes to the neuronal membrane, is activated by low-voltage, and consists only of a functional α1 subunit [18]. Evidence from animal models and pharmacological studies strongly suggest that T-type Ca^{2+}

Table 3
Bioinformatics prediction of *CACNA1H* variants found only in CAE patients[a]

Location	Nucleotide substitution	refSNP ID in NCBI	Amino acid substitution	Comments/Prediction
Intron6	45537G > A			Transcription factor binding site
	45650C > T			Alter potential splicing site
	46925T > C			Transcription factor binding site
Exon7	47152C > T		P314S	Alter secondary structure, phosphorylation site
	47247C > T		N345N	Alter potential splicing sites
Intron7	47381G > A			Transcription factor binding site, alter potential splicing sites
Exon9	48684C > T		P492S	Change charge status, phosphorylation site
	49016C > G		L602L	Transcription factor binding site
	49067A > G		S619S	Transcription factor binding site, alter potential splicing sites
Intron9	49271G > T			Alter potential splicing sites
Intron10	51268C > T			Alter potential splicing sites
Intron11	52037C > T	rs2745150		Alter potential splicing sites,
Intron12	53059A > G			Transcription factor binding site
	53061C > G			Alter potential splicing sites

L: leucine; N: asparagines; P: proline; S: serine.
[a] The numbering of the nucleotide acids, amino acids is according to the system used in NC_000016 and NP_001005407, respectively.

channel genes are the most important candidate genes for CAE [11,17].

Our previous study identified 68 variants in exons 3–35 and the exon-intron boundaries of *CACNA1H* in 118 sporadic Han Chinese patients with CAE. Twenty-nine of these variants were found in CAE patients but not in 230 unrelated controls. These included 12 missense mutations in highly conserved residues in 14 patients [2]. In vitro studies demonstrated that 11 of the 12 missense $Ca_v3.2$ variants caused a functional defect in channel gating which was detectable by whole-cell patch-clamp studies [19]. The present study identifies two novel nonsynonymous variants in *CACNA1H*, P492S and P314S, in two CAE patients without GTCS, febrile seizure history or a family history of epilepsy. P492S affects a conserved amino acid in the linker I-II region and may alter charge status of the channel, while P314S is predicted to change the secondary structure of the $Ca_v3.2$ channel. Both P314 and P492 are potential phosphorylation sites for kinases such as brain Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Thus, these variants may cause changes in calcium channel phosphorylation status, which could potentially increase excitability in neurons in epilepsy patients, In addition, the activity of T-type calcium $Ca_v3.2$ channel can be stimulated by CaMK II [5,11,20]. Therefore, we speculate that P314S and P492S may lead to an imbalance in neuronal Ca^{2+}/calmodulin-dependent phosphorylation/dephosphorylation systems during calcium channel activity with pathological consequences. Future studies are planned to test this idea.

In general, our previous findings of multiple rare *CACNA1H* missense variants associated with CAE fit well to the multiple rare-variants/common-disease hypothesis, if these missense variants do not reflect chance findings of rare population variants. In this study, however, we also found that some rare variants only occurred in parents of CAE trios or in normal controls. Since the frequencies of these variants were very low and there were no nonsynonymous variants only occurred in normal controls, it was still ambiguous and should be further investigated whether those variants only found in CAE patients were chance findings. To determine whether the *CACNA1H* gene is significantly associated with CAE, we analyzed those common variants observed in both CAE patients and controls. By Bonferroni correction, both permutation test and TDT test showed that a SNP-52037C > T in intron11 was close related to CAE, indicating that the *CACNA1H* gene might be associated with CAE.

Recent studies suggested that intronic or synonymous exonic variants could lead to catastrophic splicing abnormalities, including exon skipping or activation of cryptic splice sites. These variants alter the ratio of alternatively spliced mRNA isoforms, and have the potential to cause pathological changes and ensuing disease [15]. Structural changes in proteins caused by missense mutations may also differentially affect the activity of alternative gene products. A very recent study showed that *CACNA1H* has 12-14 alternative splice sites, and alternative splicing pathways produce both functional and non-functional $Ca_v3.2$ isoforms, which are likely to affect membrane firing [21]. These effects were comparable to or larger than effects reported for previously studied mutations, suggesting that the changes in *CACNA1H* alternative splicing might be an important pathway underlying CAE. In this work, Bioinformatics predicted that 52037C > T might alter potential splicing sites of *CACNA1H* gene. Future studies are needed to determine the impact of 52037C > T on alternative splicing of *CACNA1H* mRNA.

However, other two relative studies failed to find any of the variants reported by us in CAE patients in Australian population or European population, respectively, as well as to find any evidences supporting that the *CACNA1H* gene was associated with CAE [2,4,9]. The discrepancy between their results and the present study could involve several factors. First, the common-disease/common-variant (CDCV) hypothesis for CAE is supported by a meta-analysis of 25 association analyses reported in 301 papers [12], which suggests that high frequency SNPs in *CACNA1H* should be taken into account. Second, small sample size in their or our studies could have interfered with accurate prediction of association between *CACNA1H* and CAE. Lastly, the differences in the genetic background of the study population may partly explain conflicting results in different studies on the association between *CACNA1H* and CAE [4,9].

In conclusion, Regardless whether or not those rare variants in the *CACNA1H* gene identified only in CAE patients are chance findings, a common polymorphism 52037C > T being statistically significant associated with CAE further support our hypothesis that *CACNA1H* is an important susceptibility gene for CAE in the Chinese Han population. However, it is likely that other as yet unknown factors also play direct and/or indirect roles in the pathology of CAE.

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References

[1] S.F. Berkovic, R.A. Howell, D.A. Hay, J.L. Hopper, Epilepsies in twins: genetics of the major epilepsy syndromes, Ann. Neurol. 43 (1998) 435–445.

[2] Y. Chen, J. Lu, H. Pan, Y. Zhang, H. Wu, K. Xu, X. Liu, Y. Jiang, X. Bao, Z. Yao, K. Ding, W.H. Lo, B. Qiang, P. Chan, Y. Shen, X. Wu, Association between genetic variation of *CACNA1H* and childhood absence epilepsy, Ann. Neurol. 54 (2003) 239–243.

[3] Commission on classification and terminology of the International League against Epilepsy, Proposal for revised classification of epilepsies and epileptic syndromes, Epilepsia 30 (1989) 389–399.

[4] B. Chioza, K. Everett, H. Aschauer, O. Brouwer, P. Callenbach, A. Covanis, O. Dulac, M. Durner, O. Eeg-Olofsson, M. Feucht, M. Friis, A. Heils, M. Kjeldsen, K. Larsson, A.E. Lehesjoki, R. Nabbout, I. Olsson, A. Sander, R. Siren, M. Robinson, M. Rees, R.M. Gardiner, Evaluation of CACNA1H in European patients with childhood absence epilepsy, Epilepsy Res. 25 (2006) [Epub ahead of print].

[5] Y. Dong, H.C. Rosenberg, Prolonged changes in Ca^{2+}/calmodulin-dependent protein kinase II after a brief pentylenetetrazol seizure; potential role in kindling, Epilepsy Res. 58 (2004) 107–117.
[6] A. Escayg, M. De Waard, D.D. Lee, D. Bichet, P. Wolf, T. Mayer, J. Johnston, R. Baloh, T. Sander, M.H. Meisler, Coding and noncoding variation of the human calcium-channel beta4-subunit gene *CACNB4* in patients with idiopathic generalized epilepsy and episodic ataxia, Am. J. Hum. Genet. 66 (2000) 1531–1539.
[7] W.J. Ewens, R.S. Spielman, The transmission/disequilibrium test: history, subdivision, and admixture, Am. J. Hum. Genet 57 (1995) 455–464.
[8] S.W. Guo, E.A. Thompson, Performing the exact test of Hardy–Weinberg proportion for multiple alleles, Biometrics 48 (2) (1992 Jun) 361–372.
[9] S.E. Heron, H.A. Phillips, J.C. Mulley, A. Mazaeib, M.Y. Neufeld, S.F. Berkovic, I.E. Scheffer, Genetic variation of *CACNA1H* in idiopathic generalized epilepsy, Ann. Neurol. 55 (2004) 595–596.
[10] P. Imbrici, S.L. Jaffe, L.H. Eunson, N.P. Davies, C. Herd, R. Robertson, D.M. Kullmann, M.G. Hanna, Dysfunction of the brain calcium channel CaV2.1 in absence epilepsy and episodic ataxia, Brain 127 (2004) 2682–2692.
[11] L. Lacinova, Pharmacology of recombinant low-voltage activated calcium channels, Curr. Drug. Targets CNS Neurol. Disord. 3 (2004) 105–111.
[12] K.E. Lohmueller, C.L. Pearce, M. Pike, E.S. Lander, J.N. Hirschhorn, Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease, Nat. Genet. 33 (2003) 177–182.
[13] S.A. Miller, D.D. Dyhes, H.F. Poleskey, A simple saiting out procedure for extracting DNA from human nucleated cells, Nucleic. Acid. Res. 16 (1988) 1215.
[14] D.A. Nickerson, V.O. Tobe, S.L. Taylor, PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing, Nucl. Acids Res. 5 (1997) 2745–2751.
[15] F. Pagani, F.E. Baralle, Genomic variants in exons and introns: identifying the splicing spoilers, Nat. Rev. Genet. 5 (2004) 389–396.
[16] R. Robinson, N. Taske, T. Sander, A. Heils, W. Whitehouse, F. Goutieres, J. Aicardi, A.E. Lehesjoki, A. Siren, M. Laue Friis, M.J. Kjeldsen, C. Panayiotopoulos, C. Kennedy, C. Ferrie, M. Rees, R.M. Gardiner, Linkage analysis between childhood absence epilepsy and genes encoding GABAA and GABAB receptors, voltage-dependent calcium channels, and the ECA1 region on chromosome 8q, Epilepsy Res. 48 (2002) 169–179.
[17] H. Su, D. Sochivko, A. Becker, J. Chen, Y. Jiang, Y. Yaari, H. Beck, Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus, J. Neurosci. 22 (2002) 3645–3655.
[18] E.M. Talley, L.L. Cribbs, J.H. Lee, A. Daud, E. Perez-Reyes, D.A. Bayliss, Differential distribution of three members of a gene family encoding low voltage-activated (T-type) calcium channels, J. Neurosci. 19 (1999) 1895–1911.
[19] I. Vitko, Y. Chen, J.M. Arias, Y. Shen, X.R. Wu, E. Perez-Reyes, Functional characterization and neuronal modeling of the effects of childhood absence epilepsy variants of *CACNA1H*, a T-type calcium channel, J. Neurosci. 25 (2005) 4844–4855.
[20] S. Yechikhov, E. Morenkov, T. Chulanova, O. Godukhin, T. Shchipakina, Involvement of cAMP- and Ca(2+)/calmodulin-dependent neuronal protein phosphorylation in mechanisms underlying genetic predisposition to audiogenic seizures in rats, Epilepsy Res. 46 (2001) 15–25.
[21] X. Zhong, J.R. Liu, J.W. Kyle, D.A. Hanck, W.S. Agnew, A profile of alternative RNA splicing and transcript variation of *CACNA1H*, a human T-channel gene candidate for idiopathic generalized epilepsies, Hum. Mol. Genet. 15 (2006) 1497–1512.