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Genetic variations of adenosine kinase as predictable biomarkers of efficacy of vagus nerve stimulation in patients with pharmacoresistant epilepsy

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Abstract

OBJECTIVE—Vagus nerve stimulation (VNS) is an alternative treatment option for individuals with refractory epilepsy, with nearly 40% of patients showing no benefit after VNS and only 6%–8% achieving seizure freedom. It is presently unclear why some patients respond to treatment and others do not. Therefore, identification of biomarkers to predict efficacy of VNS is of utmost importance. The objective of this study was to explore whether genetic variations in genes involved in adenosine kinase (ADK), ecto-5′-nucleotidase (NT5E), and adenosine A1 receptor (A1R) are linked to outcome of VNS in patients with refractory epilepsy.

METHODS—Thirty single-nucleotide polymorphisms (SNPs), including 9 in genes encoding ADK, 3 in genes encoding NT5E, and 18 in genes encoding A1R, were genotyped in 194 refractory epilepsy patients who underwent VNS. The chi-square test and binary logistic regression were used to determine associations between genetic differences and VNS efficacy.

RESULTS—A significant association between *ADK* SNPs rs11001109, rs7899674, and rs946185 and seizure reduction with VNS was found. Regardless of sex, age, seizure frequency and type,

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Author Contributions

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

antiseizure drug use, etiology, and prior surgical history, all patients (10/10 patients [100%]) with minor allele homozygosity at rs11001109 (genotype AA) or rs946185 (AA) achieved > 50% seizure reduction and 4 patients (4/10 [40%]) achieved seizure freedom. VNS therapy demonstrated higher efficacy among carriers of minor allele rs7899674 (CG + GG) (68.3% vs 48.8% for patients with major allele homozygosity).

CONCLUSIONS—Homozygous *ADK* SNPs rs11001109 (AA) and rs946185 (AA), as well as minor allele rs7899674 (CG + GG), may serve as useful biomarkers for prediction of VNS therapy outcome.

Keywords

adenosine kinase; biomarker; epilepsy; outcome; single-nucleotide polymorphism; SNP; vagus nerve simulation

Epilepsy is a chronic neurological disorder affecting over 70 million people worldwide. Approximately 36% patients with epilepsy are pharmacoresistant,[1] which means that the estimated number of patients with pharmacoresistant epilepsy worldwide is about 25 million.[2] A significant number of patients with temporal lobe epilepsy or other epilepsies with focal lesions can achieve seizure freedom with curative surgical interventions, such as resection, thermocoagulation, or disconnection, whereas the remaining patients with intractable epilepsy are poor candidates for curative surgical interventions given the presence of nonlocalized, multifocal, highly generalized, or epileptogenic zones overlapping with eloquent brain regions. Neuromodulation for epilepsy, such as with vagus nerve stimulation (VNS), has become a well-accepted palliative treatment of pharmacoresistant epilepsies not amenable to resection.

Based on the results of randomized controlled trials,[3] meta-analyses,[4] and retrospective studies,[5,6] 50%–60% of patients benefit from VNS with achievement of ≥ 50% seizure reduction and 6%–8% of patients achieve complete seizure freedom. Prediction of VNS therapy outcome is currently challenging; therefore, a better understanding of the predictors and biomarkers of success of VNS therapy is critically important.

Our previous studies found that adenosine dysfunction plays a critical role in epileptogenesis. The major adenosine-metabolizing enzyme, adenosine kinase (ADK), has been identified as a promising target for prediction and prevention of epilepsy.[7–10] The adenosine regulatory cycle consists of extracellular adenosine-generating enzymes, including 5′-nucleotidase or CD73 encoded by the *NT5E* gene. These enzymes play key roles in the breakdown of adenosine triphosphate (ATP) to adenosine and reverse ATP-generating processes, which include metabolism of adenosine through phosphorylation into adenosine monophosphate (AMP) by ADK.[11] Strict control of the adenosine regulatory cycle is important because adenosine, acting via the adenosine A1 receptor (A1R), plays a major role as an endogenous anticonvulsant and seizure terminator in the brain.[12]

Identification of biomarkers that predict efficacy of VNS for pharmacoresistant epilepsy may help to optimize candidate selection and improve outcomes. Adenosine-related single-nucleotide polymorphisms (SNPs) were previously identified as biomarkers that predict

epilepsy development after traumatic brain injury,[13–16] but associations between genetic variations in the adenosine system and VNS prognosis have not been determined. Because electrical stimulation is known to increase adenosine release,[17] we hypothesized that adenosine metabolism is critical to the outcome of VNS. Thus, genes implicated in the adenosine regulatory cycle and adenosine A1 receptor (*ADK*, *NT5E*, and *A1R*) are potential biomarkers of outcome after VNS.

Methods

Study Design and Patients

Our study was approved by the Institutional Review Board of the Sanbo Brain Hospital, Capital Medical University. All patients in this study provided written informed consent. For the children included in this study, we obtained written informed consent from the next of kin, caretakers, or guardians on their behalf. SNPs associated with *ADK*, *A1R* and *NT5E* were genotyped. After comprehensive presurgical evaluation, including determination of epileptic history and surgical history and evaluation of data obtained with long-term video electroencephalography, MRI, positron emission tomography, and magnetoencephalography, all patients were diagnosed with pharmacoresistant epilepsy and considered poor candidates for resection, as determined by weekly multidisciplinary team discussions at the Comprehensive Epilepsy Center, Sanbo Brain Hospital, Capital Medical University. An ad hoc task force of the International League Against Epilepsy defined drug resistance as “failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom.”[18] All 194 patients received follow-up for at least 1 year after VNS.

Demographic, Clinical, and Epileptic Characteristics

The demographic characteristics of all 194 patients were extracted from electronic medical records at Sanbo Brain Hospital, including sex, age, seizure type, seizure frequency, antiseizure drug (ASD) use, etiology (determined according to medical history and neuroimaging results), and surgical history. Efficacy of VNS was determined with questionnaires when patients were readmitted for stimulation parameter adjustment, or efficacy was evaluated with remote follow-up via telephone, WeChat, or other online approaches. The strategy for adjustment of stimulation parameters was based on available guidelines.[19]

DNA Extraction

DNA was extracted from whole blood samples that were collected in tubes containing ethylenediaminetetraacetic acid. The samples were divided into serum and blood cells by centrifugation, and then stored at −80° before DNA extraction using the Qiagen QIAamp DNA Blood Mini Kit.

SNP Selection and Genotyping

To explore the relationship between adenosine-related SNPs and outcomes of VNS, 30 SNPs—including 9 for *ADK* (rs10824094, rs10824218, rs11000980, rs11001109,

rs11001111, rs1908335, rs4746209, rs946185, and rs7899674), 18 for *A1R* (rs10920573, rs17511192, rs200239809, rs200281933, rs200325525, rs200703683, rs200961926, rs2228079, rs3766553, rs556203229, rs564412568, rs61731145, rs6701725, rs903361, rs146573702, rs201445170, rs539390179, and rs562367584), and 3 for *NT5E* (rs4431401, rs9444348, and rs9450282)—were genotyped in all patients included in this study. Adenosine-related SNPs were previously identified as biomarkers that predict epilepsy development after traumatic brain injury. We selected SNPs according to the findings of previous studies and methods.[16] SNPs were analyzed with various packages of the Sequenom Mass-ARRAY platform ("Genetics," "Genotype," and "LD heatmap"), R version 3.6.3 (The R Foundation) was used to examine linkage disequilibrium (LD) of the 194 patients, and HapMap database build 36 was used to determine the degree of LD among the 30 SNPs included in our study. In addition, all SNP locations were determined according to Genome Reference Consortium Human Build 38 (https://www.ncbi.nlm.nih.gov/assembly/GCF_000001405.26/).

Statistical Analysis

SPSS version 21 (IBM Corp.) and R version 3.6.3 (The R Foundation) were used for statistical analysis. The chi-square test and binary logistic regression were used to verify the relationships among patient characteristics, SNPs, and outcomes of VNS. Continuity correction and the Fisher's exact test. were used when appropriate. Patients were divided into two groups on the basis of VNS outcomes: those with seizure frequency reduced by less than half, and those with seizure frequency reduced by more than half. The latter group was considered to have received effective treatment. The relationships between population characteristics and outcomes of VNS were tested with the chi-square test.

There are no accredited assumptions about the genetic models for the target SNPs. Therefore, we primarily examined allele, autosomal dominant, and recessive models for all SNPs in our patients, and we used the chi-square test to determine whether any genetic model was associated with outcome of VNS. Because ecto-5′-nucleotidase (NT5E) is known to form homodimers, we contrasted the efficacy of VNS between patients with heterozygous *NT5E* SNPs and those with homozygous *NT5E* SNPs, in addition to evaluations of the allele, autosomal dominant, and recessive models.

In addition, binary logistic regression was used to examine the significance of associations between SNPs and outcome of VNS, and p values and odds ratios (ORs) (95% CIs) were calculated. Furthermore, all models were adjusted for sex, age, seizure type, seizure frequency, ASD use, etiology, and prior surgical history (Table S1). For all SNPs, the most significant genetic model was exhibited.

Results

Study Population

We included 194 patients with pharmacoresistant epilepsy to analyze the associations between adenosine cycle–related SNPs and efficacy of VNS. Figure 1 illustrates our overall experimental workflow. The mean ± SD (range) age at VNS implantation was 16.38 ±

10.44 (1.6–55.2) years, 64.9% of the entire study cohort were children, and 65.5% were male patients. In terms of seizure type, 19.6% had focal seizure, 29.9% had focal secondary generalized seizure, and 50.5% had generalized seizure. Consistent with the findings of previous studies,[20,21] patients with focal seizure had the best therapeutic outcomes, and 63.2% of these patients achieved more than 50% seizure reduction. The majority of the 194 included patients (37.1%) had serious epilepsy with > 30 seizures per month, whereas 26.8% of patients had 5–30 seizures per month and 23.7% had 1–5 seizures per month. Given the presence and complexity of pharmacoresistant epilepsy, especially for pediatric patients, 45.9% of patients had an unknown etiology; encephalitis was the second most common etiology (15.5%). Prior surgical history was not an exclusion criterion, and 15.5% of patients had prior surgical history. These population characteristics were not associated with outcome of VNS (Table 1).

Evaluation of SNPs and VNS Prognosis

Detailed information about the target SNPs for all 194 patients, including allele, gene, chromosome position, genotype distribution, and minor allele frequency, is listed in Table 2. The linkage map for loci rs11001109 and rs946185 in the *ADK* gene was assembled using Haploview. As shown in Fig. 2A, the D value between rs11001109 and rs946185 was 0.98. LD analysis of SNPs within the *A1R* and *NT5E* genes are shown in Fig. 2B and Fig. 2C, respectively. SNPs of *A1R*, *NT5E*, and *ADK* were tagged to evaluate the associations of these genes with outcome after VNS according to the chi-square test and binary logistic regression.

Regarding the *ADK* gene, all patients homozygous for the minor alleles at rs11001109 (genotype AA) ($p = 0.010$, chi-square test) and rs946185 (AA) ($p = 0.010$) responded to VNS, and there were significant differences in the ORs of the autosomal dominant model of rs7899674 after adjustment for sex, age, seizure type, seizure frequency, ASD use, etiology, and prior surgical history (OR 2.252, $p = 0.016$). The efficacy of VNS for carriers with minor allele rs7899674 (CG + GG) was 68.3%, which was greater than the efficacy of 48.8% for patients with major allele homozygosity (CC) (Table 3). These results showed that patients with pharmacoresistant epilepsy and genotype AA (rs11001109 and rs946185) and genotype CG or GG (rs7899674) had better VNS response than patients with other genotypes.

Furthermore, the allele models of patients with SNPs rs11001109 (A), rs946185 (A), and rs7899674 (G) showed significant differences in prognosis of VNS. The ORs and p values of these three sites were 1.693 and $p = 0.027$ (rs11001109), 1.602 and $p = 0.047$ (rs946185), and 2.006 and $p = 0.019$ (rs7899674). These values indicate that carriers of allele A (rs11001109 and rs946185 vs carriers of allele G) and carriers of allele G (rs7899674 vs carriers of allele C) may have higher response rates to VNS (Table 4); these results were consistent with the results of the genetic models listed above. In addition, patients who were minor allele carriers of rs4746209 (GT + TT) demonstrated marginally better response to VNS than those with major allele homozygosity (GG) ($p = 0.049$), but this finding was not significant according to the allele model.

Homozygous *ADK* SNPs rs11001109 and rs946185 as Predictive Biomarkers of Efficacy of VNS Therapy

As mentioned before, patients homozygous for *ADK* variants rs11001109 and rs946185 showed a 100% response rate to VNS; this finding qualifies these 2 SNPs as predictive biomarkers of VNS outcome. In addition, minor allele carriers of rs7899674 had better prognosis for VNS. The rate of rs11001109 homozygosity was 5.15% (10/194 patients) and the rate of rs946185 homozygosity was 5.21% (10/192), whereas the rate of rs7899674 homozygosity was 32.81% (63/192) among minor allele carriers (Table 3). The sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) for both rs11001109 and rs946185 were 9.3%, 100%, 100%, and 46.7%, respectively. The sensitivity, specificity, PPV, and NPV for rs7899674 were 40.6%, 76.7%, 68.3%, and 51.2%. In addition, the seizure freedom rates among patients with rs11001109 homozygosity and rs946185 homozygosity were 40%, or approximately 5 times higher than the average seizure freedom rate after VNS therapy.[4]

Discussion

In this study, we investigated SNPs of the *ADK*, *A1R*, and *NT5E* genes to explore the relationships between genetic variations within essential components of the adenosine regulatory cycle and therapeutic outcome after implantation of VNS devices in 194 patients with refractory epilepsy. Importantly, homozygous *ADK* SNPs rs11001109 (AA) and rs946185 (AA), as well as minor allele rs7899674 (CG + GG), were significantly associated with therapeutic efficacy of VNS. To our knowledge, this is the first report on the identification of predictive genetic biomarkers for efficacy of VNS therapy in patients with epilepsy.

ADK is a ribokinase that metabolizes adenosine by phosphorylation to AMP. The *ADK* gene in humans is located on chromosome 10q11.q24 and is 546 kb long. ADK is mainly expressed in astrocytes of the brain to drive the metabolic clearance of adenosine.[22,23] Overexpression of ADK is a pathological hallmark of temporal lobe epilepsy and results from astrocyte activation, as demonstrated in both animal models and human brain tissue obtained from patients with temporal lobe epilepsy.[24–26] Indeed, pathological overexpression of ADK plays a key role in epileptogenesis,[10,27] and SNPs in the *ADK* gene have been identified as predictive biomarkers for posttraumatic epilepsy development.[16] During epileptogenesis, an epilepsy-triggering brain injury causes transient downregulation of ADK, with an accompanying increase in adenosine as an acute protective response mechanism of the brain.[28] However, inflammatory processes involved in the epileptogenic cascade drive microglia and astrocyte activation,[29] which results in chronic overexpression of ADK.[10,27,30] Overexpression of ADK was shown to drive epileptogenesis through an epigenetic mechanism.[27] It is now well established that ADK is a target for the prediction and prevention of epilepsy.[10,27,31]

ADK SNP rs11001109 is located within intron 10, whereas rs946185 and rs7899674 are located within intron 9 of the *ADK* gene. Importantly, homozygous rs11001109 was previously associated with increased risk of posttraumatic epilepsy and time to first seizure after traumatic brain injury.[16] In our present study, patients homozygous for SNP

rs11001109 (AA) responded to VNS therapy (100% response rate, as defined by > 50% seizure reduction), with 40% achieving complete seizure freedom. However, it is currently unknown how variations within intronic sequences of the *ADK* gene may affect gene function, mRNA stability, or genetic splicing. Further investigation is required to determine the underlying mechanism of how variations of SNPs rs11001109, rs946185, and rs7899674 affect response to VNS therapy in epilepsy patients.

In addition, A1R and NT5E are key components in the adenosine regulatory system. NT5E is an adenosine-producing nucleotidase that hydrolyzes extracellular AMP to adenosine.[11] A1R is one of four G protein–coupled adenosine receptors, and as an endogenous anticonvulsant and seizure terminator in the brain, it is the main receptor that mediates the antiseizure activity of adenosine.[32] In line with this physiological role of A1R, mice with genetic deletion of this receptor have spontaneous electrographic seizures.[7] To study the potential roles of SNPs in *NT5E* and *A1R* as predictors of responsiveness to VNS therapy, we selected 3 loci in *NT5E* and 18 loci in *A1R* for SNP analysis. Heterozygous rs9444348 within *NT5E* was related to increased incidence of epilepsy after traumatic brain injury.[16] However, none of these SNPs were associated with outcome after VNS therapy in patients of pharmacoresistant epilepsy.

VNS has been widely used in clinical practice as a means for safe and effective neuromodulation in patients with pharmacoresistant epilepsy; however, the underlying therapeutic mechanism is not clearly understood, and no reliable predictors of clinical response to VNS have been determined. A retrospective single-center study of 58 children concluded that therapeutic efficacy was better in patients with focal epileptiform discharges.[33] However, a prospective single-center analysis of 85 patients proposed that type of seizure, frequency of seizures, and previous surgery did not affect outcome of VNS therapy.[34] Therefore, there is an important clinical need to identify a biomarker capable of predicting therapeutic response to VNS therapy. In our study, we identified homozygous *ADK* SNPs rs11001109 (AA) and rs946185 (AA), as well as minor allele rs7899674 (CG + GG), as predictive biomarkers of favorable outcome of VNS therapy. Although a small minority of the overall population was homozygous for SNPs rs11001109 (AA) and rs946185 (AA), the response rate of these patients was 100%. This finding is significant for clinical applications.

We also need to point out several limitations of our study. First, our single-center design limited the number of included patients. The proportions of patients homozygous for minor alleles rs11001109 and rs946185 (AA) were 5.15% (10/194 patients) and 5.21% (10/192), respectively, whereas the proportions of the Chinese Han population homozygous for these alleles are both 8.7% (https://www.ensembl.org/). As the next step, we hope to perform multicenter cooperative research in order to expand the sample size. Second, future studies need to address how variations of intronic SNPs affect the underlying mechanism of therapeutic VNS within the context of the adenosine regulatory system.

Conclusions

Our study of 194 patients with pharmacoresistant epilepsy who underwent VNS therapy found that 10 patients with minor allele homozygosity at rs11001109 (AA) or rs946185 (AA) achieved > 50% reduction of seizures, and 4 of these patients achieved seizure freedom. In addition, minor allele carriers of rs7899674 (CG + GG) showed a higher rate of VNS efficiency than patients with major allele homozygosity. In this study, we provided evidence that homozygous *ADK* SNPs rs11001109 (AA) and rs946185 (AA), as well as minor allele rs7899674 (CG + GG), may serve as useful biomarkers for outcome prediction in patients undergoing VNS therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

A1R	adenosine A1 receptor
ADK	adenosine kinase
AMP	adenosine monophosphate
ASD	antiseizure drug
ATP	adenosine triphosphate
LD	linkage disequilibrium
NPV	negative predictive value
NT5E	ecto-5′-nucleotidase
PPV	positive predictive value
SNP	single-nucleotide polymorphism
VNS	vagus nerve stimulation

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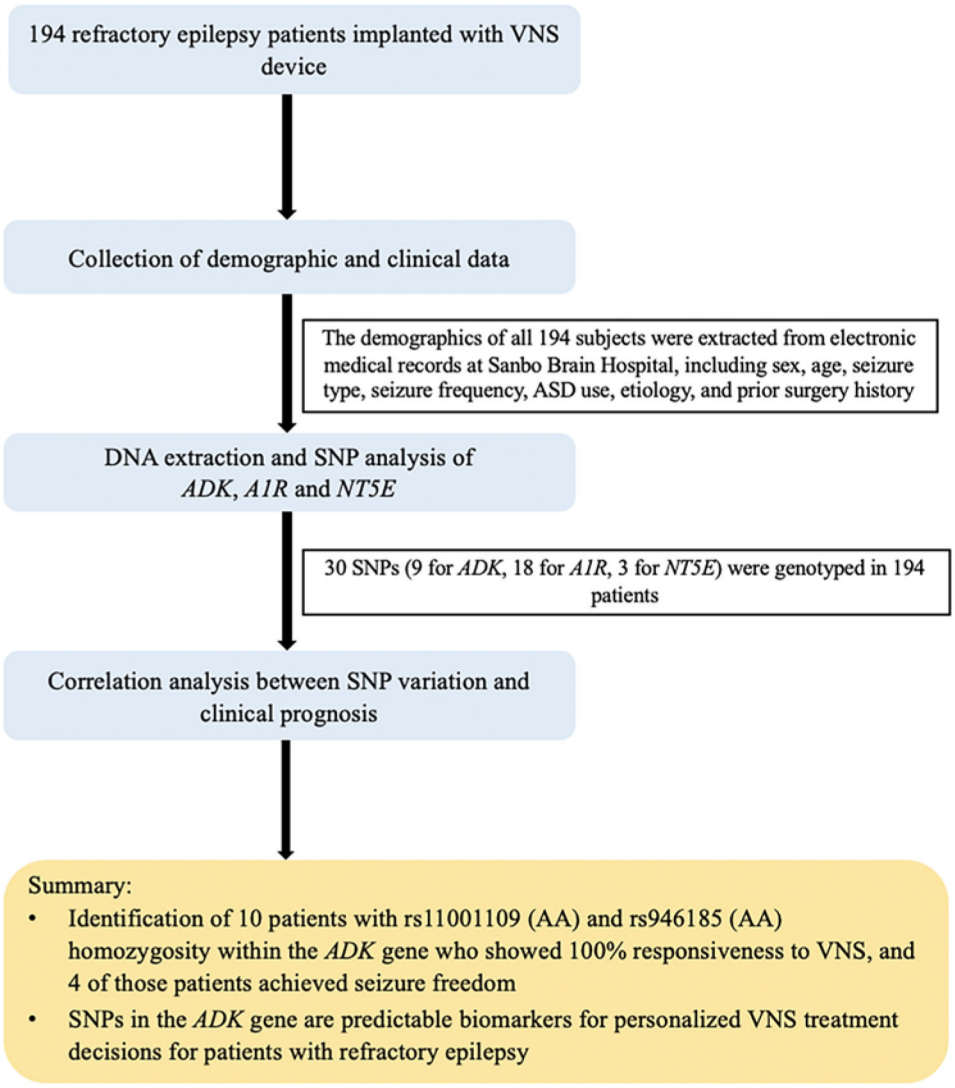

FIG. 1.

Experimental workflow of this study. In total, 194 patients were included and genetic analysis was performed as shown. Figure is available in color online only.

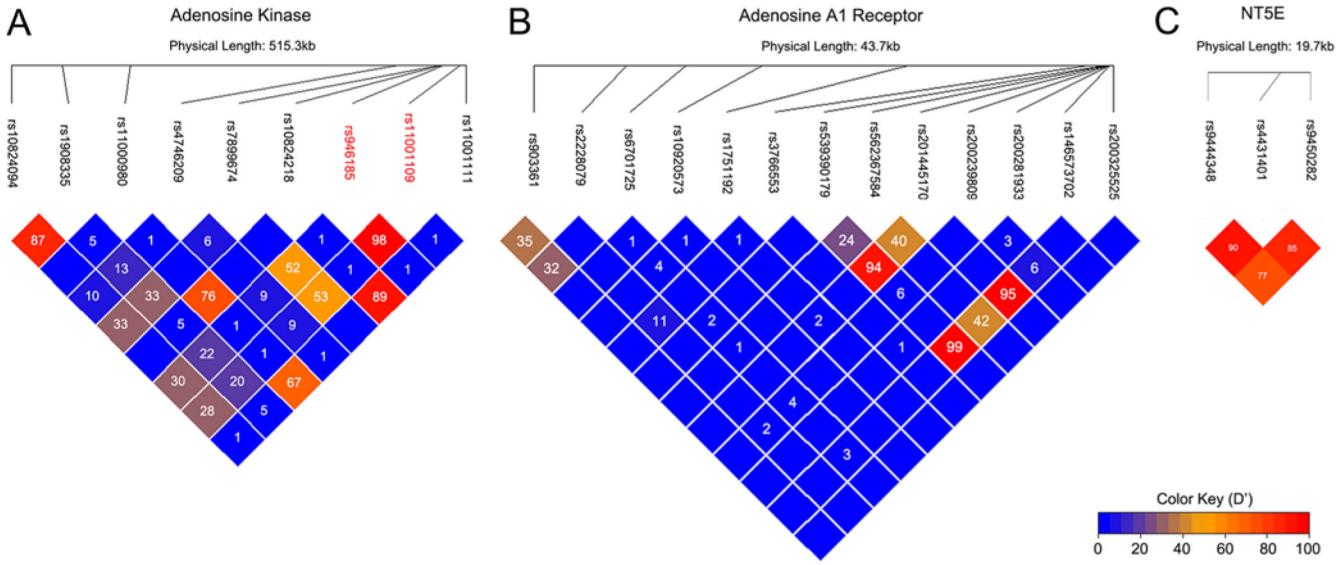

FIG. 2.

LD maps of D values for *ADK*, *A1R*, and *NT5E*. LD maps (created with R software) of D values (within *diamonds*) are shown as a logarithm of the odds (LOD) heatmap. LOD scores (from 0 [*blue*] to 100 [*red*]) are shown for *ADK* (**A**), *A1R* (**B**), and *NT5E* (**C**). Figure is available in color online only.

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TABLE 1.

Comparison of demographic and presurgical characteristics between patients who did respond to VNS and those who did not

Characteristic	Adenosine Genetics Population (n = 194)		
	≥50% Reduction	<50% Reduction	p Value
Sex			0.831
Male	70 (55.1)	57 (44.9)	
Female	38 (56.7)	29 (43.3)	
Age			0.387
Child	73 (57.9)	53 (42.1)	
Adult	35 (51.5)	33 (48.5)	
Seizure type			0.583
Focal	24 (63.2)	14 (36.8)	
Generalized	53 (54.1)	45 (45.9)	
Focal & secondary generalized	31 (53.4)	27 (46.6)	
Seizure frequency, no./mo			0.985
<1	13 (54.2)	11 (45.8)	
1–5	26 (56.5)	20 (43.5)	
5–30	28 (53.8)	24 (46.2)	
>30	41 (56.9)	31 (43.1)	
No. of antiepileptic drugs			0.469
1	26 (61.9)	16 (38.1)	
2–3	58 (55.8)	46 (44.2)	
>3	18 (46.2)	21 (53.8)	
None	6 (66.7)	3 (33.3)	
Etiology			0.950 *
Encephalitis	17 (56.7)	13 (43.3)	
Trauma	10 (55.6)	8 (44.4)	
Hemorrhage	4 (57.1)	3 (42.9)	
Hypoxic ischemic encephalopathy	15 (62.5)	9 (37.5)	

Characteristic	Adenosine Genetics Population (n = 194)		
	≥50% Reduction	<50% Reduction	p Value
Febrile convulsion	6 (50.0)	6 (50.0)	
Malformation of cortical dysplasia	1 (25.0)	3 (75.0)	
Other	5 (50.0)	5 (50.0)	
Unknown	50 (56.2)	39 (43.8)	
Surgical history			0.604
Yes	18 (60.0)	12 (40.0)	
No	90 (54.9)	74 (45.1)	

Values are shown as number (percent) unless indicated otherwise.

* Determined using the Fisher's exact test.

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TABLE 2.

SNP information

SNP	Allele	Gene	Chromosome Position	No. of Pts	Genotype Frequency	Minor Allele Frequency (%)
rs10824094	C/T	*ADK*	Chr10:74174751	193	0.46/0.48/0.06	30.1
rs10824218	A/T	*ADK*	Chr10:74661458	193	0.95/0.04/0.01	2.6
rs11000980	A/G	*ADK*	Chr10:74309059	192	0.96/0.04/0.00	2.1
rs11001109	G/A	*ADK*	Chr10:74683339	194	0.49/0.46/0.05	27.8
rs11001111	G/A	*ADK*	Chr10:74690055	194	0.95/0.05/0.00	2.3
rs1908335	C/A	*ADK*	Chr10:74231883	193	0.46/0.48/0.06	30.3
rs4746209	G/T	*ADK*	Chr10:74612279	133	0.55/0.45/0.00	22.6
rs946185	G/A	*ADK*	Chr10:74663981	192	0.49/0.46/0.05	28.1
rs7899674	C/G	*ADK*	Chr10:74660091	192	0.67/0.32/0.01	16.9
rs10920573	T/C	*A1R*	Chr1:203139380	194	0.43/0.39/0.18	37.4
rs17511192	C/T	*A1R*	Chr1:203150363	163	0.96/0.03/0.01	2.1
rs200239809	G/A	*A1R*	Chr1:203165567	194	0.00/1.00/0.00	50.0
rs200281933	C/T	*A1R*	Chr1:203165615	194	0.95/0.05/0.00	2.6
rs200325525	T/C	*A1R*	Chr1:203165873	194	0.99/0.01/0.00	0.3
rs200703683	G/A/T	*A1R*	Chr1:203165750	194	1.00/0.00/0.00	0.0
rs200961926	G/A	*A1R*	Chr1:203165489	194	1.00/0.00/0.00	0.0
rs2228079	T/G	*A1R*	Chr1:203129147	194	0.69/0.28/0.03	17.0
rs3766553	A/G	*A1R*	Chr1:203163914	194	0.53/0.39/0.08	27.6
rs556203229	C/T	*A1R*	Chr1:203129051	194	1.00/0.00/0.00	0.0
rs564412568	T/C	*A1R*	Chr1:203165363	194	1.00/0.00/0.00	0.0
rs61731145	G/A/T	*A1R*	Chr1:203128847	194	1.00/0.00/0.00	0.0
rs6701725	G/A	*A1R*	Chr1:203133600	194	0.60/0.36/0.04	21.9
rs903361	A/G	*A1R*	Chr1:203122146	194	0.41/0.44/0.15	37.1
rs146573702	C/T	*A1R*	Chr1:203165633	189	0.89/0.11/0.00	5.3
rs201445170	G/A	*A1R*	Chr1:203165495	189	0.89/0.10/0.01	5.6
rs539390179	G/A	*A1R*	Chr1:203165222	190	0.89/0.11/0.00	5.3
rs562367584	G/A	*A1R*	Chr1:203165294	173	0.98/0.00/0.02	2.3

SNP	Allele	Gene	Chromosome Position	No. of Pts	Genotype Frequency	Minor Allele Frequency (%)
rs4431401	T/C	*NT5E*	Chr6:85479802	194	0.45/0.45/0.10	32.7
rs9444348	G/A	*NT5E*	Chr6:85465856	194	0.46/0.44/0.10	32.0
rs9450282	A/G	*NT5E*	Chr6:85485580	193	0.42/0.45/0.13	35.2

Pts = patients.

TABLE 3.

Comparison of SNPs associated with VNS outcomes*

				Univariate Logistic Regression		Multivariate Logistic Regression	
SNP	**No. of Pts**	**Response Rate to VNS (%)**	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value†**
ADK_rs10824094							
CC+CT	182	54.4	0.143‡	3.773 (0.793–17.948)	0.095	3.683 (0.752–18.048)	0.108
TT	11	81.8					
ADK_rs10824218							
AA	184	56.5	0.712‡	0.615 (0.160–2.366)	0.480	0.649 (0.161–2.609)	0.542
AT+TT	9	44.4					
ADK_rs11000980							
AA	184	56.5	0.486†	0.462 (0.107–1.989)	0.300	0.459 (0.102–2.066)	0.311
AG+GG	8	37.5					
ADK_rs11001109							
GG+GA	184	53.3	**0.010**‡	—	—	—	—
AA	10	100.0		—		—	
ADK_rs11001111							
GG	185	56.2	0.726*	0.623 (0.162–2.395)	0.491	0.654 (0.162–2.632)	0.550
GA+AA	9	44.4					
ADK_rs1908335							
CC+CA	181	54.1	**0.049**	4.235 (0.902–19.874)	0.067	4.464 (0.928–21.466)	0.062
AA	12	83.3					
ADK_rs4746209							
GG	73	58.9	0.074	0.534 (0.267–1.065)	0.075	0.476 (0.227–0.996)	**0.049**
GT+TT	60	43.3					
ADK_rs946185							
GG+GA	182	53.3	**0.010**‡	—	—	—	—
AA	10	100.0		—		—	

				Univariate Logistic Regression		Multivariate Logistic Regression	
SNP	**No. of Pts**	**Response Rate to VNS (%)**	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value[†]**
ADK_rs7899674							
CC	129	48.8	**0.011**	2.252 (1.196–4.241)	**0.012**	2.252 (1.166–4.350)	**0.016**
CG+GG	63	68.3					
A1R_rs10920573							
TT	83	50.6	0.219	1.432 (0.807–2.540)	0.220	1.490 (0.823–2.698)	0.188
TC+CC	111	59.5					
A1R_rs17511192							
CC	157	53.5	0.828[‡]	1.738 (0.309–9.766)	0.530	1.509 (0.248–9.171)	0.655
CT+TT	6	66.7					
A1R_rs200239809[§]							
GA	194	55.7	—	—	—	—	—
A1R_rs200281933							
CC	184	56.0	0.965[‡]	0.786 (0.220–2.810)	0.711	0.827 (0.226–3.021)	0.774
CT+TT	10	50.0					
A1R_rs200325525							
TT	193	56.0	0.443[¶]	—	—	—	—
TC+CC	1	0.0		—		—	
A1R_rs200703683[**]							
GG	194	55.7	—	—	—	—	—
A1R_rs200961926[**]							
GG	194	55.7	—	—	—	—	—
A1R_rs2228079							
TT	133	51.9	0.117	1.644 (0.881–3.067)	0.118	1.702 (0.895–3.236)	0.105
TG+GG	61	63.9					
A1R_rs3766553							
AA+AG	179	55.3	0.725	1.212 (0.414–3.549)	0.726	1.135 (0.372–3.464)	0.824
GG	15	60.0					

				Univariate Logistic Regression		Multivariate Logistic Regression	
SNP	**No. of Pts**	**Response Rate to VNS (%)**	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value[†]**
A1R_rs556203229[**]							
CC	194	55.7	—	—	—	—	—
A1R_rs564412568[**]							
TT	194	55.7	—	—	—	—	—
A1R_rs61731145[**]							
GG	194	55.7	—	—	—	—	—
A1R_rs6701725							
GG	117	57.3	0.581	0.850 (0.477–1.516)	0.582	0.846 (0.469–1.528)	0.580
GA+AA	77	53.2					
A1R_rs903361							
AA	80	58.8	0.469	0.808 (0.454–1.440)	0.470	0.825 (0.458–1.486)	0.522
AG+GG	114	53.5					
A1R_rs146573702							
CC	169	56.2	0.340	0.637 (0.251–1.618)	0.343	0.730 (0.278–1.922)	0.524
CT+TT	20	45.0					
A1 R_rs201445170							
GG	169	56.2	0.340	0.637 (0.251–1.618)	0.343	0.729 (0.276–1.927)	0.524
GA+AA	20	45.0					
A1R_rs539390179							
GG	170	56.5	0.329	0.631 (0.248–1.601)	0.332	0.723 (0.275–1.902)	0.511
GA+AA	20	45.0					
A1R_rs562367584							
GG	169	56.2	0.811[≠]	2.337 (0.238–22.926)	0.466	2.304 (0.223–23.845)	0.484
GA+AA	4	75.0					
NT5E_rs4431401							
TT+TC	174	54.6	0.375	1.544 (0.588–4.058)	0.378	1.598 (0.602–4.237)	0.346
CC	20	65.0					

				Univariate Logistic Regression		Multivariate Logistic Regression	
SNP	**No. of Pts**	**Response Rate to VNS (%)**	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value[†]**
NT5E_rs9444348							
GG	89	51.7	0.304	1.348 (0.763–2.382)	0.304	1.299 (0.723–2.334)	0.382
GA+AA	105	59.0					
NT5E_rs9450282							
AA+AG	168	53.6	0.176	1.842 (0.754–4.500)	0.180	1.805 (0.730–4.465)	0.201
GG	25	68.0					

— = no result.

Boldface type indicates statistical significance ($p < 0.05$).

[*] There are no accredited assumptions about the genetic models for the target SNPs; therefore, the allele, autosomal dominant, and recessive models were primarily examined (Table S1). The most significant genetic model is exhibited for all SNPs, and associations between SNPs and outcomes of VNS were determined with the chi-square test and binary logistic regression.

[†] Adjusted for sex, age, seizure type, seizure frequency, ASD use, etiology, and surgical history.

[‡] Corrected for continuity.

[§] This SNP is heterozygous.

[¶] Determined with the Fisher's exact test.

[**] This SNP is homozygous.

TABLE 4.

Allele models of rs11001109, rs946185, and rs7899674*

				Univariate Logistic Regression		Multivariate Logistic Regression	
SNP	**No. of Alleles**	**Response Rate to VNS (%)**	**p Value**	**OR (95% CI)**	**p Value**	**OR (95% CI)**	**p Value†**
ADK_rs11001109							
G	280	52.1	**0.024**	1.691 (1.068–2.676)	**0.025**	1.693 (1.062–2.699)	**0.027**
A	108	64.8					
ADK_rs946185							
G	276	52.5	**0.044**	1.598 (1.011–2.528)	**0.045**	1.602 (1.005–2.552)	**0.047**
A	108	63.9					
ADK_rs7899674							
C	319	52.4	**0.013**	2.048 (1.157–3.624)	**0.014**	2.006 (1.120–3.594)	**0.019**
G	65	69.2					

Boldface type indicates statistical significance ($p < 0.05$).

*Associations between SNPs and outcomes of VNS in the allele model were determined with the chi-square test and binary logistic regression.

†Adjusted for sex, age, seizure type, seizure frequency, ASD use, etiology, and surgical history.