Published in final edited form as:

Mamm Genome. 2009 June ; 20(6): 359–366. doi:10.1007/s00335-009-9193-6.

Fine Mapping of an Epilepsy Modifier Gene on Mouse Chromosome 19

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Abstract

Mutations in voltage-gated sodium channels are associated with several types of human epilepsy. Variable expressivity and penetrance are common features of inherited epilepsy caused by sodium channel mutations, suggesting that genetic modifiers may influence clinical severity. The mouse model *Scn2a*^{Q54} has an epilepsy phenotype due to a mutation in *Scn2a* that results in elevated persistent sodium current. Phenotype severity in *Scn2a*^{Q54} mice is dependent on the genetic background. Congenic C57BL/6J.Q54 mice have delayed onset and low seizure frequency compared to (C57BL/6J×SJL/J)F1.Q54 mice. Previously we identified two modifier loci that influence the *Scn2a*^{Q54} epilepsy phenotype designated *Moe1* (Modifier of Epilepsy 1) on Chromosome 11 and *Moe2* on Chromosome 19. We have constructed interval specific congenic strains to further refine the position of *Moe2* on Chromosome 19 to a 5 Mb region. Sequencing and expression analysis of genes in the critical interval suggested two potential modifier candidates: 1) voltage-gated potassium channel subunit subfamily V, member 2 (*Kcnn2*); and 2) SWI/SNF related matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2 (*Smarca2*). Based on its biological role in regulating membrane excitability and the association between ion channel variants and seizures, *Kcnn2* is a strong functional candidate for *Moe2*. Modifier genes affecting the epilepsy phenotype of *Scn2a*^{Q54} mice may contribute to variable expressivity and penetrance in human epilepsy patients with sodium channel mutations.

Keywords

seizures; sodium channel; potassium channel; QTL

INTRODUCTION

Mutations in voltage-gated sodium channels are responsible for several types of human epilepsy. Mutations in sodium channels *SCN1A* and *SCN1B* were first identified in Generalized Epilepsy with Febrile Seizures Plus (GEFS⁺), a benign, familial syndrome with autosomal dominant inheritance (Escayg et al, 2000; Wallace et al, 1998). *SCN1A* mutations were subsequently identified in patients with Severe Myoclonic Epilepsy of Infancy (SMEI), a sporadic, infant-onset syndrome with progressive worsening of seizures accompanied by psychomotor and mental regression (Meisler and Kearney, 2005). Over 400 mutations of *SCN1A* have been reported in patients with epilepsy. A small number of mutations have been identified in *SCN2A* and *SCN3A*. Mutations in *SCN2A* were identified in one GEFS⁺ patient and in nine patients with Benign Familial Neonatal Convulsions (BFNC) and a mutation in

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SCN3A was reported in cryptogenic pediatric partial epilepsy (Catterall et al, 2008; Holland et al, 2008). A common feature of inherited epilepsy is variable expressivity and penetrance among family members carrying the same primary mutation, suggesting that genetic modifiers may influence the clinical severity.

The transgenic mouse model *Scn2a*^{Q54} has an epilepsy phenotype due to a mutation in *Scn2a* (Kearney et al, 2001). The mutation results in persistent sodium current when measured in *Xenopus* oocytes and hippocampal neurons from *Scn2a*^{Q54} mice (Kearney et al, 2001). The level of persistent current in hippocampal neurons is comparable to that produced by mutations in *SCN1A* from GEFS+ families (Lossin et al, 2002). *Scn2a*^{Q54} mice display progressive epilepsy beginning with short duration focal motor seizures. They also exhibit hippocampal pathology that resembles human mesial temporal lobe epilepsy (Kearney et al, 2001). Temporal lobe epilepsy is observed within GEFS+ spectrum (Abou-Khalil et al, 2001; Colosimo et al, 2007).

Clinical severity of the *Scn2a*^{Q54} phenotype is influenced by genetic background. *Scn2a*^{Q54} mice congenic on the C57BL/6J background (B6.Q54) exhibit a low occurrence of spontaneous seizures with delayed age of onset, and increased survival compared with (C57BL/6J × SJL/J)F1.Q54 mice. This suggests that strain SJL/J contributes dominant alleles that modify the phenotype. We previously performed a N2 backcross to strain C57BL/6J and mapped two loci that influence the *Scn2a*^{Q54} epilepsy phenotype, *Moe1* (Modifier of Epilepsy) on Chromosome 11 and *Moe2* on Chromosome 19 (Bergren et al, 2005).

In the present study we carried out fine mapping of the *Moe2* locus on Chromosome 19, narrowing its position to 5 Mb using interval specific congenic strains. Within this interval, we performed sequence and expression analysis of brain-expressed genes, identifying two candidate genes with non-synonymous coding sequence variations between C57BL/6J and SJL/J.

MATERIALS AND METHODS

Mice

Scn2a^{Q54} transgenic mice (TgN54Mm) were generated by microinjection of (C57BL/6J × SJL/J)F2 oocytes as described (Kearney et al, 2001). The congenic line C57BL/6J.Q54 (abbreviated B6.Q54) was established as described (Bergren et al, 2005) and is maintained by continued backcrossing of hemizygous transgenic males to C57BL/6J females. All studies were approved by the Institutional Animal Care and Use Committees in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Interval-Specific Congenic Strains

Previously we showed that a modifier locus affecting epilepsy severity was captured within the SJL introgressed interval of Chromosome 19 in doubleridge (dblr) mice (Bergren et al, 2005). The doubleridge line was used to generate interval specific congenic strains (ISC) for high resolution mapping. B6.dblr mice were backcrossed to C57BL/6J and offspring were monitored for recombinations within the SJL-derived interval. Once established, ISCs were maintained by backcrossing to B6 and genotyping the Chr 19 interval.

Genotyping

DNA was prepared from tail biopsies by phenol:chloroform extraction and ethanol precipitation. *Scn2a*^{Q54} and dblr mice were genotyped as previously described (Bergren et al, 2005). Microsatellite genotyping was performed by analysis of PCR products on 7% non-denaturing acrylamide gels or an ABI 3730 Automated Sequencer (University of Michigan

DNA Sequencing Core, Robert Lyons, Director). SNP genotyping was performed by RFLP analysis, conformation-sensitive gel electrophoresis (CSGE) (Plummer et al, 1998) or sequencing.

Phenotyping

Mice were tail biopsied on postnatal day 14 and genotyped. Non-transgenic mice were discarded. We utilized the same phenotyping paradigm that was used for low resolution mapping of the *Moe2* locus (Bergren et al, 2005). Briefly, *Scn2a*^{Q54} transgenic mice were observed for visible seizures during 30-min observation periods at 3 and 6 weeks of age. Mice were transferred to a clean observation cage (7 3/4 in. W × 12 in. D × 6 1/2 in. H) just before the observation session. Observations were performed by a blinded observer between 1:00 and 4:00pm. Assessment of visible partial motor seizures was based on prior extensive video-EEG monitoring that demonstrated coincident stereotyped behavioral and EEG abnormalities (Kearney et al, 2001). On both the B6 congenic and (B6×SJL)F1 backgrounds, *Scn2a*^{Q54} mice exhibit partial motor seizures with behavioral arrest, tonic deviation of the head and forelimb clonus. When crossed with Chromosome 19 interval specific congenics, mice exhibiting seizures had an average seizure frequency of 4 seizures per 30 min regardless of their genotype in the *Moe2* region. Animals exhibiting one or more seizures during the observations were classified as having seizures. The proportion of mice with a seizure frequency of >1 per 30 minutes by 6 weeks of age was compared between genotypes using Fisher's Exact Test ($n \geq 14$ per group).

Candidate Gene Sequencing

Positional candidate genes were identified from the publicly available mouse genome sequence (www.ensembl.org) and filtered by tissue expression. Genes were amplified from B6 and SJL by RT-PCR of brain RNA or by PCR of exon sequence from genomic DNA. PCR products were gel-purified and sequenced on an ABI 3730 Automated Sequencer in The University of Michigan DNA Sequencing Core Facility (Robert Lyons, Director) or The Vanderbilt University DNA Sequencing Facility (Alfred L. George, Jr., Director). Sequences were compared using Sequencher software (GeneCodes, Ann Arbor, MI).

Real-Time RT-PCR

Whole brain RNA was isolated from 6 week old male C57BL/6J and SJL/J mice ($n=5$ per strain) using the RNeasy Kit with on-column DNaseI digestion according to the manufacturer's instructions (Qiagen). Following total RNA isolation a second DNase treatment was performed with Turbo DNase according to the manufacturer's instructions (Ambion). First strand cDNA was synthesized from 1–2 micrograms of template RNA using oligo(dT)₂₀ primers with the Superscript III first strand synthesis system (Invitrogen). Real-time PCR was performed in triplicate with QuantiTect primer assays and QuantiTect SYBR green pcr mix (Qiagen) on an ABI 7900HT system. All reported assays exhibited a single peak in dissociation analysis and no detectable signal in no-RT controls. Relative gene expression was analyzed using the $2^{-\Delta\Delta C_T}$ method with TATA binding protein (TBP) as a reference gene (Livak and Schmittgen, 2001). Statistical comparison between groups was assessed by the Pair Wise Fixed Reallocation Randomisation Test (Pfaffl et al, 2002).

RESULTS

Interval Specific Congenics

The B6.dblr mouse strain carries an SJL/J-derived Chromosome 19 segment that spans the *Moe2* 1-LOD support interval from D19Mit86 to D19Mit19 (B6-SJL-D19Mit86-D19Mit19). Previously we showed that B6.Q54 mice carrying the SJL-derived dblr interval exhibited a

higher occurrence of seizures by 6 weeks of age than B6.Q54 mice (Bergren et al, 2005). In order to generate ISCs for fine mapping of the *Moe2* locus, we backcrossed the B6.dblr strain to B6 and screened for recombinants. We generated two ISCs that divided the *Moe2* locus into proximal and distal segments: B6-SJL.D19Mit86-dblr and B6.dblr-D19Mit19 (Figure 1a).

Phenotyping

Hemizygous B6.Q54 mice were crossed with heterozygotes from the ISCs to generate offspring with and without the SJL-derived interval. Mice were genotyped for *Scn2a*^{Q54} and microsatellite markers spanning the Chr 19 interval. All *Scn2a*^{Q54} transgene-positive offspring underwent 30 minute observations for spontaneous seizures at 3 and 6 weeks of age. An increased proportion of B6.Q54 mice carrying the SJL-derived Chr 19 interval from D19Mit86-D19Mit19 or D19Mit86-dblr exhibited spontaneous seizures at a frequency of >1 per 30 minutes by 6 weeks of age compared with B6.Q54 littermate controls (Figure 1b). B6.Q54 mice carrying the distal SJL-derived Chromosome 19 interval from Dblr-D19Mit19 were not different from B6.Q54 littermate controls (Figure 1b). These results localize *Moe2* to a 5 Mb interval of Chromosome 19 between D19Mit40 and the dblr mutation 21.8 kb upstream of *Dkk1* (Figure 1) (MacDonald et al, 2004).

Candidate Gene Identification

We identified 42 positional candidate genes in this *Moe2* region between D19Mit40 and the dblr mutation (21.8 kb upstream of *Dkk1* (MacDonald et al, 2004)) from the publicly available mouse genome sequence (www.ensembl.org). Candidate genes were prioritized based on evidence of brain expression by identification of brain-derived cDNAs or ESTs, or positive in situ hybridization signal in the Allen Brain Atlas (<http://mouse.brain-map.org>). Within the *Moe2* critical interval we identified 24 genes for analysis, 22 with evidence of brain expression and 2 predicted genes with no expression information available (Table 1). We previously sequenced 2 of these candidate genes following low resolution mapping (Bergren et al, 2005).

Haplotype Analysis

It has been suggested that strain modifiers are more likely to reside in regions where the strains show divergent haplotypes (Grupe et al, 2001; Park et al, 2003; Pletcher et al, 2004). We performed *in silico* haplotype analysis of the *Moe2* region on mouse Chromosome 19 using SNP genotypes available in the Mouse Phenome Database (<http://aretha.jax.org/pub-cgi/phenome/mpdcgi?rtn=snp/door>). Analysis of SNP alleles at ≤0.2 Mb intervals indicates that B6 and SJL share a common haplotype block that extends from 27.7 – 32.1 Mb. This shared haplotype block includes the distal 2.9 Mb of the *Moe2* critical interval which is the most gene dense portion. Of the 24 candidate genes, only 4 reside in the divergent haplotype block (25.4–27.6 Mb). These genes were assigned highest priority for analysis. Although the results of this analysis assisted us in prioritizing candidate genes for analysis, it is possible that a modifier may result from a more recent mutation in an inbred strain (Buchner et al, 2003). Genes within the shared haplotype block were given lower priority but were not eliminated as candidates based on this criterion.

Candidate Gene Sequencing

Of the 24 candidate genes, we identified 3 genes with coding sequence differences between B6 and SJL (Table 1). Many of the polymorphisms identified had been reported in SNP databases but had not previously been genotyped in SJL. Within the shared haplotype block we identified a single synonymous coding polymorphism in *Gldc*, encoding glycine decarboxylase (rs33888238). This synonymous SNP is not predicted to affect an exonic splicing enhancer and is most likely a neutral polymorphism (Cartegni et al, 2003).

All other coding polymorphisms were identified in the divergent haplotype block in the proximal portion of the *Moe2* interval (25.4–27.6 Mb) (Table 1). In SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2 (*Smarca2*) there were 2 synonymous coding variants (rs31012540; novel Chr 19–26,846,048 bp) and a 5 amino acid expansion of an imperfect polyglutamine repeat in strain SJL/J (Q₂₄PQ₆PQ₂PQ₃AQAQPQ₉) relative to B6 (Q₂₆PQ₂PQ₃AQAQPQ₉). In *Kcnv2*, encoding the voltage gated potassium channel subunit Kv8.2, there were 2 non-synonymous and 3 synonymous coding variants (Table 1). The non-synonymous variants both affect amino acids in the N-terminal cytoplasmic region of the channel (Figure 2a). Arginine 208 shows strong evolutionary conservation (Figure 2b) and substitution of a histidine at this position is predicted to be probably damaging by PolyPhen analysis, while substitution of glutamine for arginine at position 252 is predicted to be benign (Ramensky et al, 2002).

Altered ion channel function has been shown to be associated with epilepsy, making *Kcnv2* a strong functional candidate. Therefore, we examined the strain distribution of the *Kcnv2* variants using SNP data from the publicly available Mouse Phenome Database (<http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rt=snps/door>) and imputed SNP data from the Center for Genome Dynamics at the Jackson Laboratory (<http://cgd.jax.org/imputedSNPData/v1.1>). We identified 3 major haplotypes in the *Kcnv2* locus that correlate with the ancestral history of the mouse strains (Table 2) (Beck et al, 2000). Strains sharing the SJL haplotype have lower thresholds to induced seizures relative to B6 mice (Frankel et al, 2001; Kitami et al, 2004).

Expression Analysis

We performed real-time RT-PCR on whole brain RNA to compare relative expression of *Moe2* candidate genes between B6 and SJL. Within the *Moe2* interval we determined relative expression levels of 19 genes which had brain expression at 6 weeks of age. Of those 19 genes, none had a statistically significant difference in relative expression between the 2 strains (Table 1). Several genes exhibited a trend toward differential expression, with average fold differences of less than 2. However, it is difficult to achieve statistical significance with fold differences of <2 due to biological and technical variation inherent in qRT-PCR assays.

DISCUSSION

In the present study we constructed a set of ISCs to fine map the *Moe2* locus on mouse Chromosome 19. Our results localize the *Moe2* critical region to a 5 Mb interval flanked by D19Mit40 and the *dbl*r mutation, 21.8 kb upstream of *Dkk1*. Within the *Moe2* critical interval, we identified 24 candidate genes with evidence of brain expression. Sequencing of those genes revealed non-synonymous coding sequence polymorphisms between B6 and SJL in *Smarca2*, and *Kcnv2*.

Smarca2, also known as Brahma (Brm), encodes the SWI/SNF related matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 2. Brm is a member of the SWI/SNF multi-protein chromatin-remodeling complex that is involved in regulation of transcription, oncogenesis and cell cycle control. Homozygous *Smarca2*^{-/-} null mice are viable, exhibit partial infertility and abnormal cellular proliferation (Reyes et al, 1998). A hotspot mutation in *Smarca2* was recently associated with melanoma in human patients (Moloney et al, 2008). The two synonymous SNPs that we identified in *Smarca2* are not predicted to affect exonic splicing enhancers and are most likely neutral polymorphisms (Cartegni et al, 2003). Strain SJL/J harbors a 5 amino acid expansion of an imperfect polyglutamine repeat in *Smarca2* compared to B6. The significance of this expansion is unknown. However, the polyglutamine repeat length is polymorphic in mouse strains (data not shown) as well as humans (Pandey et al, 2004).

Kcnv2 encodes the potassium channel subunit Kv8.2 which forms heterotetramers with Kv2 family members and alters their activity (Czirjak et al, 2007; Ottschytsch et al, 2002). Kv2.1 channels are important regulators of neuronal excitability (Misonou et al, 2005). We previously demonstrated a modifier effect of another voltage-gated potassium channel subunit, *Kcnq2* on *Scn2a*^{Q54}-associated epilepsy. The mouse mutant allele *Kcnq2*^{V182M} has a reduced threshold to electroconvulsive seizures but no spontaneous seizures. Combining the *Kcnq2*^{V182M} allele with *Scn2a*^{Q54} in double mutant mice resulted in a severe epilepsy syndrome with early onset and lethality by 3 weeks of age (Kearney et al, 2006). Within the *Kcnv2* locus, we identified three major haplotypes correlated with the ancestral history of the mouse strains (Beck et al, 2000). Strains that share the SJL ancestral haplotype have reduced thresholds to induced seizures compared to B6 (Frankel et al, 2001). This supports *Kcnv2* as a candidate modifier, although it does not rule out other genes in linkage disequilibrium with *Kcnv2*. Taken together, these observations suggest that the voltage-gated potassium channel *Kcnv2* is a strong candidate for *Moe2*.

Within the *Kcnv2* coding sequence, we identified 3 synonymous and 2 non-synonymous variants between B6 and SJL. The 3 synonymous variants are not predicted to affect exonic splicing enhancers and are likely to be neutral polymorphisms (Cartegni et al, 2003). One of the non-synonymous variants results in substitution of histidine for arginine in strain SJL at position 208. This residue exhibits a high degree of evolutionary conservation and substitution with histidine is predicted to be “probably damaging” by PolyPhen analysis (<http://genetics.bwh.harvard.edu/pph/>). Arginine 208 is located within the T1 tetramerization domain, a functionally important domain of the channel that is involved in co-assembly of subunits to form a functional heteromeric channel. Future analysis of the coding variants will be required to determine their effect on protein function.

Beyond coding sequence differences, modifier effects could be the result of differences in expression levels and/or copy number variations (CNVs) between the strain backgrounds. We did not observe any significant difference in relative expression of the 19 genes interrogated at the whole brain level at 6 weeks of age. However, differences in regional or developmental expression have not been ruled out. It has been suggested that strain differences in regional gene expression in the brain may underlie phenotypic QTLs (Letwin et al, 2006). Cutler et al (2007) examined genome-wide CNVs in 42 inbred mouse strains. Based on their report, there is no evidence of CNV in the *Moe2* region of Chromosome 19 (Cutler et al, 2007).

In addition to identifying candidate modifier genes, our current study eliminates the *Dblr* mutation as a candidate. The *Dblr* mutation results in decreased expression of the Wnt signaling inhibitor *Dkk1* to ~20% of wildtype levels in heterozygotes (Adamska et al, 2003; MacDonald et al, 2004). Previously, we indirectly demonstrated that the *Dkk1*^{*Dblr*} mutation was not likely to underlie the modifier effect, based on the observation that 50% reduction of *Dkk1* did not influence the epilepsy phenotype (Bergren et al, 2005). Our current results eliminate the possibility that the *Dblr* mutation is responsible for the modifier effect since the ISC strain B6.dblr-D19Mit19 carries the *Dblr* mutation but did not influence seizure occurrence.

Over 400 mutations in voltage-gated sodium channels have been identified in patients with epilepsy. Variable expressivity among family members is a common feature of inherited epilepsy due to sodium channel mutations, suggesting that modifier genes may influence the clinical manifestation of human epilepsy. Identification of epilepsy modifier genes that influence susceptibility and disease progression will provide insight into the molecular events of epileptogenesis, and may identify novel therapeutic targets for the treatment of human patients.

Acknowledgments

We thank Rebecca Somershoe for technical assistance and Dr. Miriam Meisler for providing the Dbhr mice. SKB present address: Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY

GRANTS. This work was supported by National Institutes of Neurological Disorders and Stroke Grant NS053792 (JK) and The Partnership for Pediatric Epilepsy Research (JK).

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Figure 1.

Fine mapping of *Moe2* on Chromosome 19 with interval specific congenic strains. **A.** ISC lines with varying SJL-derived Chr 19 segments. The SJL-derived interval is shown in black, and grey denotes the interval containing the recombination. **B.** Percentage of animals with seizures in Q54 mice carrying the SJL-derived Chr 19 segments compared to B6.Q54 (* $p < 0.05$, Fishers Exact Test).

Figure 2. Candidate gene analysis. **A.** Non-synonymous SNPs in the voltage-gated potassium channel gene *Kcnv2* alter 2 amino acids in the cytoplasmic N-terminus within or near the T1 tetramerization domain. **B.** Evolutionary conservation of Arginine 208 and Glutamine 252.

Table 1

Sequencing and Expression Analysis of Brain-expressed Candidate genes

Gene	Position (Mb) [†]	Coding Polymorphisms	SNP Accession Numbers	B6 allele	SJL allele	Relative Expression SJL v B6 (SE)
<i>Smarca2</i>	26.679650– 26.852811	Trinucleotide repeat polymorphism; 2 synonymous SNPs	novel rs31012540	C A	T G	1.43 (0.99–2.05)
* <i>Vldlr</i>	27.292074– 27.324109	None				1.39 (0.89–2.18)
<i>Kcmv2</i>	27.397078– 27.411669	2 non-synonymous SNPs 3 synonymous SNPs	rs31282999 rs36587909 rs37032500 rs31139921 rs31156241	A G A G C	G A G A T	1.02 (0.70–1.49)
<i>D19Bwg1357e</i>	27.463192– 27.504310	None				1.27 (0.95–1.68)
<i>Rfx3</i>	27.842635– 28.085630	None				-
<i>Glis3</i>	28.336938– 28.754567	None				0.79 (0.49–1.27)
XP_897581.1	28.837349– 28.838039	None				-
* <i>Slc1a1</i>	28.979940– 28.986161	None				1.27 (1.03–1.57)
<i>Ppapdc2</i>	29.038410– 29.041284	None				1.03 (0.73–1.45)

Gene	Position (Mb) [†]	Coding Polymorphisms	SNP Accession Numbers	B6 allele	SJL allele	Relative Expression SJL v B6 (SE)
<i>Cdc37ll</i>	29.064984– 29.092259	None				1.08 (0.85–1.36)
<i>Ak3</i>	29.095323– 29.122451	None				1.94 (1.31–2.97)
<i>Rcll</i>	29.175912– 29.218301	None				1.32 (0.96–1.83)
<i>Jak2</i>	29.326318– 29.387570	None				1.22 (0.83–1.81)
<i>Pdcdllg2</i>	29.485409– 29.545647	None				1.3 (1.17–1.45)
<i>C030046E11Rik</i>	29.596772– 29.680411	None				0.95 (0.68–1.34)
<i>Ermp1</i>	29.684373– 29.722910	None				1.91 (1.24–3.06)
<i>9930021J03Rik</i>	29.790498– 29.792027	None				1.48 (1.08–2.03)
<i>XP_619580.1</i>	29.799481– 29.801250	None				-
<i>Ranbp6</i>	29.884123– 29.887440	None				1.68 (1.12–2.53)
<i>Il33</i>	29.999604– 30.035194	None				0.92 (0.59–1.45)
<i>Trpd52l3</i>	30.078280– 30.080508	None				-

Gene	Position (Mb) [†]	Coding Polymorphisms	SNP Accession Numbers	B6 allele	SJL allele	Relative Expression SJL v B6 (SE)
<i>Uhrf2</i>	30.105003– 30.168212	None				1.89 (1.14–3.15)
<i>Gluc</i>	30.172939– 30.249908	1 synonymous SNP	rs33888238	T	C	1.30 (1.01–1.68)
<i>Dkk1</i>	30.620366– 30.624009	None				

* Sequencing for these genes was previously reported (Bergren et al, 2005)

[†] Positions from NCBI m37 mouse assembly. Shaded region indicates the shared haplotype block between B6 and SJL.

Table 2

Strain survey of *Kcnv2* variants observed in SJL/J.

Strain	rs31282999	rs36587909 R208H	rs37032500 Q252R	rs31139921	rs31156241
C57BL/6J	A/A	G/G	A/A	G/G	C/C
129S1/SvImJ	A/A	G/G	A/A	G/G	C/C
BALB/cByJ	A/A	G/G	A/A	G/G	C/C
BTBR T + tf/J	A/A	G/G	A/A	G/G	C/C
C3H/HeJ	A/A	G/G	A/A	G/G	C/C
DBA/2J	A/A	G/G	A/A	G/G	C/C
KK/HIJ	A/A	G/G	A/A	G/G	C/C
NOD/LtJ	A/A	G/G	A/A	G/G	C/C
NZW/LacJ	A/A	G/G	A/A	G/G	C/C
WSB/EiJ	A/A	G/G	A/A	G/G	C/C
MOLF/EiJ	G/G	G/G	G/G	G/G	C/C
PWD/PhJ	g/g*	g/g*	G/G	G/G	C/C
CAST/EiJ	G/G	G/G	G/G	G/G	C/C
A/J	G/G	G/G	G/G	A/A	T/T
AKR/J	g/g*	A/A	G/G	A/A	T/T
FVB/NJ	G/G	A/A	G/G	A/A	T/T
PL/J	G/G	A/A	g/g*	a/a*	t/t*
SJL/J	G/G	A/A	G/G	A/A	T/T

* lower case letters represent imputed genotypes with a confidence level ≥ 0.9 from the Center for Genome Dynamics at the Jackson Laboratory (<http://cgd.jax.org/ImputedSNPData/v1.1/>) (Szatkiewicz et al, 2008)