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(54) Title: MARKERS OF FEBRILE SEIZURES AND TEMPORAL LOBE EPILEPSY

(57) Abstract: Described is the use of the Srp9 gene and/or Srp14 gene and/or SRP9 polypeptide and/or SRP14 polypeptide as a diagnostic marker, in particular for febrile seizures, complex febrile seizures, for complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or for temporal lobe epilepsy. Additionally, the genes and/or polypeptides may be used for identifying subjects at risk of developing febrile seizures. The invention further provides for kits for identifying subjects at risk of developing febrile seizures and methods of preventing and/or treating febrile seizures, as well as compounds suitable for these purposes.



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Title: Markers of febrile seizures and temporal lobe epilepsy

Field of the invention

The present invention is in the field of medicine, more particular in the field of neurosciences. It relates to early diagnostic markers for febrile seizures and diseases and conditions associated herewith such as complex epilepsy syndromes such as Dravet's Syndrome, epilepsies with mental retardation, and temporal lobe epilepsy, and methods of diagnosis using such markers. It also relates to a method for preventing and/or treating such febrile seizures and diseases and conditions associated therewith using gene modulation therapy, such as RNA interference. Finally, the invention provides for kits suitable for identifying a subject at risk of developing febrile seizures and diseases and conditions associated therewith.

Background

A febrile seizure (FS), also known as a fever fit or febrile convulsion, is a convulsion associated with a significant rise in body temperature. According to the ILEA it is defined as a seizure occurring in childhood after 6 months of age, associated with a febrile illness not caused by an infection of the central nervous system, without previous neonatal seizures or a previous unprovoked seizure, and not meeting criteria for other acute symptomatic seizures (1993). They most commonly occur in children between the ages of 6 months and 5 years.

There are two types of febrile seizures: simple febrile seizures (also referred to as "febrile seizure") and complex febrile seizures. A simple FS lasts less than 15 minutes, does not recur in 24 hours, and involves the entire body (classically a generalized tonic-clonic seizure). On the other hand, complex or prolonged FS last more than 15 minutes, are often recurrent, and increase the risk of developing temporal lobe epilepsy (TLE) in later life. Thirty to 50% of patients with TLE have a history of prolonged FS during childhood (French et al. 1993 Annals of Neurology 34, 774-780). Complex FS maybe part of more complex epilepsy syndromes, such as for instance Generalised Epilepsy with FS (GEFS+).

Genetic factors are known to play an important role in determining FS susceptibility. About 25% of children experiencing FS have a positive family history and concordance for FS in monozygotic twins is about 40% compared to 10% for dizygous twins. Several mutations have been identified in families with complex epilepsy syndromes with FS as a

central feature, such as GEFS+, but the genetic factors involved in sporadic FS remain elusive.

To study the genetics of FS a rat model for hyperthermia induced seizures (Chen et al. 1999. Nat. Med. Vol. 5:888-894) was adapted to mice (Van Gassen et al. 2008. Genes Brain and Behav. Vol. 7:578-586) enabling large scale phenotypic screening of inbred strains for FS susceptibility. In this model, the rise in core-body temperature of mouse induces tonic-clonic seizures, the onset of which coincides with spike-wave discharges in the hippocampus. Recently, this mouse model was used in a phenotype driven screen for FS susceptibility employing the C57BL/6J-Chr#A/NaJ chromosome substitution panel. This panel has proven to be a powerful tool in detecting quantitative trait loci (QTLs) for complex traits (Singer et al., 2004. Science 304, 445-448; Mozhui et al. 2008. PloS Genet. Vol. 4, 11; e1000260). Three chromosome substitution strains (CSS1, -10 and -13) carrying a protective QTL and three (CSS2, -6 and -X) with a susceptibility QTL for experimental FS (Hessel et al. 2009. Genes Brain and Behav. Vol. 8: 248-2550) were identified.

Complex FS occur in childhood and are considered harmful for normal brain development. Complex FS increase the risk to develop hippocampal sclerosis and TLE about tenfold. The decision to treat complex FS with anti-epileptic drug is a difficult one, because the treatment per se may affect normal brain development. Thus, an early diagnosis of children with complex FS is of paramount importance for clinical practice.

Summary of the invention

In a first aspect, the present invention provides for the use of an SRP9 protein and/or an *Srp9* nucleic acid sequence and/or an SRP14 protein and/or an *Srp14* nucleic acid sequence as a diagnostic marker. Said diagnostic marker may be for febrile seizures, complex febrile seizures, for complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or for temporal lobe epilepsy.

Also, the present invention relates to the use of an SRP9 protein and/or an *Srp9* nucleic acid sequence and/or an SRP14 protein and/or an *Srp14* nucleic acid sequence in identifying a subject at risk of developing febrile seizures (FS), a subject at risk of developing complex FS, a subject at risk of developing a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or a subject at risk of developing temporal lobe epilepsy.

The invention further pertains to a method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the steps of: a) providing a sample of a subject; b)

subject; b) determining the level of at least one biomarker selected from SRP9 polypeptide, *Srp9* mRNA, SRP14 protein and/or *Srp14* mRNA in said sample; c) comparing the level of said biomarker to a reference level; and d) determining whether the level of said biomarker is indicative of a risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. In an embodiment, said subject is an infant or child. Said sample may be selected from the group consisting of a whole blood sample, serum, and plasma. In an embodiment, an increased level of said biomarker is indicative of a risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

The present invention is further concerned with the use of a SNP in the promoter region of the *Srp9* gene, said SNP being selected from the group consisting of rs6688819 (T/C), rs12403575 (G/A), rs16845266 (G/C), rs120398148 (T/C), and rs6659660 (G/A), for determining the risk in a subject of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. Said SNP preferably is rs12403575 G/A.

The present invention is also directed to a method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the step of: a) determining in a sample of a subject a SNP in the promoter region of the *Srp9* gene, wherein the SNP is selected from rs12403575 G/A, rs6688819 T/C, rs16845266 G/C, rs120398148 T/C, and rs6659660 G/A, and preferably is rs12403575 G/A. Said SNP preferably is rs12403575 G/A. In an embodiment, the A allele of rs12403575 G/A is associated with an increased risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. In an embodiment, the genotype of said subject for rs12403575 is determined, and an AA genotype is associated with an increased risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. Said method may further comprise the step of providing a sample of a subject. Said sample may comprise or may be derived from blood, an amniotic fluid, cerebrospinal fluid, tissue from skin, muscle, buccal or conjunctival mucosa, placenta, gastrointestinal tract or chorionic villi.

The invention also concerns a method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy,

said method comprising the steps of: a) determining in a sample of a subject the sequence of the *Srp9* promoter sequence, wherein the presence of one or more de novo mutations is indicative of a risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

In a further aspect, the present invention relates to a kit comprising a probe or primer that distinguishes an allele of a SNP in a *Srp9* gene in a sample from a subject, wherein the allele is selected from the A or G allele in the SNP rs12403575. Said kit may be for determining an increased risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy in a subject. The probe or primer may be detectably labelled.

In another aspect, the invention provides a method for screening chemical compounds or compositions interfering with the function of the SRP complex, said method comprising the step of identifying chemical compounds or compositions that suppress expression of SRP9 and/or SRP14 polypeptide, that interact with SRP9 and/or SRP14 polypeptide and thereby prevent formation of the SRP9/SRP14 complex, and/or that interact with the SRP9/SRP14 complex and thereby prevent formation of the SRP complex.

The invention also relates to a compound capable of selectively binding to SRP9 and/or SRP14 polypeptide, preferably an antibody capable of selectively binding to SRP9 and/or SRP14 polypeptide, for use as a medicament, such as for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

In yet another aspect, the present invention is concerned with the use of therapeutic gene modulation for suppressing the level of SRP9 protein and/or SRP14 protein in a mammal, such as for preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

The invention also pertains to a *Srp9* and/or *Srp14* nucleic acid modulator selected from the group consisting of antisense DNA, siRNA, miRNA, shRNA, ribozyme specifically binding to and cleaving *Srp9* and/or *Srp14* mRNA and zinc finger nuclease specifically targeting a nucleic acid sequence in the *Srp9* and/or *Srp14* gene resulting in suppression of *Srp9* and/or *Srp14* expression for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

Further, the invention is directed to a recombinant nucleic acid construct comprising at least one transcriptional unit comprising: - a promoter; - a nucleic acid sequence encoding a gene suppressor selected from: - an antisense DNA sequence complementary to the sense strand of the *Srp9* and/or *Srp14* gene; - an interfering RNA molecule which is
 5 complementary to *Srp9* mRNA and/or *Srp14* mRNA; - a nucleic acid sequence encoding a zinc finger nuclease specifically targeting a nucleic acid sequence in the *Srp9* gene and/or *Srp14* gene; - a nucleic acid sequence encoding a ribozyme specifically binding to and cleaving *Srp9* and/or *Srp14* mRNA; and - a terminator, for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such
 10 as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

The invention further relates to a vector comprising a recombinant nucleic acid construct of the invention for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, which vector may comprise
 15 more than one transcriptional unit, as well as a cell comprising a recombinant nucleic acid construct or a vector of the invention for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

The invention further includes a method for suppressing the level of SRP9 protein and/or SRP14 protein in a cell, said method comprising the step of including an *Srp9* and/or
 20 *Srp14* nucleic acid modulator of the invention or recombinant nucleic acid construct of the invention in said cell.

Finally, the present invention provides for a kit comprising means for detecting the level of *Srp9* mRNA and/or SRP9 protein and/or *Srp14* mRNA and/or SRP14 protein in a
 25 sample derived from a subject. Said kit may be for use in identifying a subject at risk of developing febrile seizures (FS), a subject at risk of developing complex FS, a subject at risk of developing a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or a subject at risk of developing temporal lobe epilepsy. The kit may comprise a primer pair allowing selective amplification of *Srp9* and/or
 30 *Srp14* mRNA, or an antibody that specifically binds to SRP9 protein and/or SRP14 protein. Said sample derived from a subject may be a blood sample or a sample derived from blood.

Figures and Drawings

Figure 1 shows FS QTL mapping on chromosome 1. A) The FSL (s) of CSS1 (n=9; 915.3±37.5 s) is significantly longer than of C57BL/6J (n=9; 631.9±26.9 s) mice (FStrain
 35 (1,14)=11.355, $P<0.001$) showing that CSS1 is less susceptible to FS. No sex differences

were found in either strain ($F_{gender}(1,14)=0.736$, $P=0.406$, interaction effect, interaction $(1,14)=0.104$, $P=0.752$). Strains did not differ in core body-temperature increase during hyperthermia (time $F(1,14)=1478.191$, $P=0.0001$; time $\times$ strain $F(1,14)=0.715$, $P=0.511$). Data are expressed as means $\pm$ SEM. *significantly different $P<0.05$. B) Frequency histogram of FS latencies of CSS1-F2 progeny ($n=129$) shows that, as expected, the majority of these genetically unique CSS1-F2 mice have a longer FSL than C57BL/6J (black bar is C57BL/6J average). C) QTL1 (rs31997386, LOD=7.8) located at 183,756,349-187,980,365 bp (1-LOD support interval) accounts for 23.9% of the phenotypic variance. No sex or (grand) parent of origin differences in FSL were observed for QTL mapping.

Figure 2 shows brain *Srp9* mRNA expression in CSS1 is lower than in C57BL/6J. A) In situ hybridization of hippocampal gene expression (nCi/g) of candidate genes (Table 1 below) in QTL1 in CSS1 ($n=6$) and C57BL/6J mice ($n=6$). Data are expressed as means $\pm$ SEM * $P<0.006$ was considered as significant after multiple testing correction. B) Quantification shows a significant ($t_{10}=5.090$, $P=0.001$) decrease in *Srp9* expression in CSS1 (78.7 ± 7.0 nCi/g; right) compared to C57BL/6J (122.3 ± 4.5 nCi/g; left).

Figure 3 shows that down-regulation of *Srp9* mRNA in C57BL/6J *in vivo* reduces FS susceptibility. A) ICV injection of *Srp9* antisense oligonucleotide (AS *Srp9*) probe in C57BL/6J pups 20 hours prior to hyperthermia significantly ($t_{10}=-3.411$, $P=0.007$) prolonged FSL (s) in C57BL/6J ($n=7$; 899.0 ± 77.7 s) mice compared to scrambled (Scr) probe injected littermate controls ($n=5$; 574.0 ± 23.5 s). This *in vivo* treatment resulted in down-regulation of *Srp9* transcript levels (-16.7%; $t_{10}=2.533$, $P=0.032$) in the cortex compared to controls. B) IP injection of cycloheximide (CHX, $n=9$; 769.1 ± 41.1 s) 20 min prior to hyperthermia delayed FSL ($t_{16}=-2.141$, $P=0.048$) compared to vehicle controls ($n=9$; 662.6 ± 28.0 s). IP injection of rapamycin (RAP, $n=9$; 708.1 ± 35.9 s) 30 min prior to hyperthermia delayed FSL ($t_{16}=-2.700$, $P=0.018$) compared to controls ($n=9$; 591.9 ± 11.4 s). Data are expressed as means $\pm$ SEM; *significantly different $P<0.05$. ICV and IP injections did not affect temperature curves during hyperthermia induction.

Figure 4 demonstrates *SRP9* expression and sequencing in mTLE patients with and without FS. A) *SRP9* mRNA levels (normalized qPCR data: Rn) in hippocampal homogenates of autopsy control patients ($n=5$; $Rn=40.4\pm6.1$), mTLE patients without hippocampal sclerosis (non-HS; $n=16$; $Rn=100.0\pm14.9$) and mTLE patients with hippocampal sclerosis (HS; $n=20$; $Rn=169.3\pm17.0$) ($F_{2,38}=10.175$, $P=0.0001$; non-HS vs HS: $P=0.008$; autopsy vs HS: $P=0.001$; Autopsy vs non-HS: $P=0.240$). *SRP68* mRNA levels did not differ between non-HS and HS groups (autopsy $Rn=38.7\pm7.8$; non-HS $Rn=100.0\pm5.4$; HS $Rn=113.7\pm11.8$; $F_{2,13}=18.601$, $P=0.0001$; non-HS vs HS: $P=0.898$). B) *SRP9* mRNA levels

in hippocampal homogenates of HS patients without ($n=13$; $Rn=144.7\pm19.2$) and with ($n=7$; $Rn=215.0\pm26.8$) FS ($t_{18}=2.150$, $P=0.045$). Data are expressed as means $\pm$ SEM. $*P<0.025$ was considered as significant after multiple testing correction. C) Sequencing of *SRP9* exons, intron/exon boundaries and promoter region (5 kb) in healthy controls ($n=169$), mTLE patients ($n=368$) and mTLE patients with FS ($n=91$) revealed a significant ($P<0.01$ bold) difference in allelic frequencies of one common promoter SNP (rs12403575 G/A) in mTLE patients. D) Further single SNP analysis using a larger control cohort ($n=730$) identified a significant association between rs12403575 and mTLE ($P=0.01$, ODDS-ratio (OR)=1.238) and mTLE+FS ($P=0.045$, ODDS-ratio=1.321) E) Patients with AA genotype had a significantly ($F(2,33)=4.096$, $P=0.026$) higher hippocampal *SRP9* expression ($Rn=180.4\pm23.8$) than patients with AG genotype ($Rn=105.9\pm16.7$, $P=0.022$). Data are expressed as means $\pm$ SEM. *significantly different $P<0.05$.

Figure 5 demonstrates that CSS2 mice are more susceptible to FS than C57BL/6J mice. A) febrile seizure latency (FSL) of C57BL/6J and CSS2 mice. B) C57BL/6J and CSS2 mice do not differ in the time course of temperature increase. Data are expressed as means $\pm$ SEM ($n=9$). * significantly different $p<0.05$.

Figure 6 represents FS QTL mapping on chromosome 2. QTL analysis was performed on $n=144$ CSS2-F2 mice. A) CSS2-F2 phenotypic histogram showing a right shift toward shorter FSL (black bar indicates C57BL/6J average). B) Identification of 2 FS QTLs on mouse chromosome 2. QTL2a (D2Mit156; LOD=3.6; 1-LOD support interval 52,581,428–66,278,629 bp), which is homologous to the human *FEB3* locus, was responsible for 13.5% of the F2 phenotypic variance and the effect was recessive with respect to the A/J grandparents allele. QTL2b (D2Mit277; LOD=6.2; 1-LOD support interval 114,875,854–133,817,501 bp) was responsible for 19.8% of the F2 phenotypic variance and the effect was also recessive for the A/J grandparents allele. 1-LOD support intervals are indicated by the dotted lines. C) Standard genetic map of murine chromosome 2 showing positions of markers (Cox et al., Genetics 182, 1335-1344, 2008).

Figure 7 shows *Srp14* mRNA expression levels in mouse and human hippocampus as determined by quantitative PCR. A) Hippocampal *Srp14* expression is higher in CSS2 than in C57BL/6J mice. B) Hippocampal *Srp14* expression is higher in TLE patients with hippocampal sclerosis (HS; $n=6$) than in patients without hippocampal sclerosis (non-HS; $n=6$) and autopsy controls ($n=7$). Data are expressed as means $\pm$ SEM. * significantly different $p<0.02$. *Srp68* is another protein subunit of the SRP complex.

Figure 8 shows sequencing of the human *SRP9* gene in TLE patients, FS patients and healthy controls. A) Known transcripts of the *Srp9* gene. B) On top the known variants (available on the web). At the bottom the newly discovered variants in the human *SRP9* gene

gene detected in TLE patients (n>300), FS patients (n>300) and healthy controls (n>400). The amplicons represent the sequenced domains (black and gray bars).

Definitions

5 As used herein, the term "SRP9" refers to a SRP9 polypeptide, whereas "*Srp9*" refers to a *Srp9* nucleic acid such as the *Srp9* gene or *Srp9* RNA, such as *Srp9* mRNA. Similarly, the term "SRP14" refers to a SRP9 polypeptide, whereas "*Srp14*" refers to a *Srp14* nucleic acid such as the *Srp14* gene or *Srp14* RNA, such as *Srp14* mRNA. When one refers to the human *Srp9* gene, reference may be made to "*hSrp9*", whereas the mouse *Srp9* gene may
10 be referred to as "*mSrp9*".

The term "complex epilepsy syndromes" as used in the context of the present invention refers to epilepsy syndromes with a variety of epileptic phenotypes, one of which is febrile seizures, preferably complex febrile seizures. The term includes, without limitation, Dravet's Syndrome, genetic epilepsy with febrile seizures plus (GEFS+), and epilepsies with
15 mental retardation. The skilled person is well aware which diseases and conditions are encompassed by the term "complex epilepsy syndromes".

A "nucleic acid", "nucleic acid sequence", or "nucleotide sequence" according to the present invention may include any polymer or oligomer of pyrimidine and purine bases, preferably cytosine, thymine, and uracil, and adenine and guanine, respectively (See Albert
20 L. Lehninger, *Principles of Biochemistry*, at 793-800 (Worth Pub. 1982) which is herein incorporated by reference in its entirety for all purposes). The present invention contemplates any deoxyribonucleotide, ribonucleotide or peptide nucleic acid component, and any chemical variants thereof, such as methylated, hydroxymethylated or glycosylated forms of these bases, and the like. The polymers or oligomers may be heterogenous or homogenous
25 in composition, and may be isolated from naturally occurring sources or may be artificially or synthetically produced. In addition, the nucleic acids may be DNA or RNA, or a mixture thereof, and may exist permanently or transitionally in single-stranded or double-stranded form, including homoduplex, heteroduplex, and hybrid states.

The term "gene" means a DNA sequence comprising a region (transcribed region),
30 which is transcribed into an RNA molecule (e.g. an mRNA) in a cell, operably linked to suitable regulatory regions (e.g. a promoter). A gene may thus comprise several untranslated operably linked sequences, such as a promoter, a 5' leader sequence comprising e.g. sequences involved in transcription initiation and regulation, a (protein) coding region (cDNA or genomic DNA) and a 3' non-translated sequence (3'UTR) comprising e.g. transcription
35 termination and regulatory sites.

As used herein, the term "promoter" refers to a nucleic acid sequence that functions to control the transcription of one or more genes, located upstream with respect to the direction of transcription of the transcription initiation site of the gene, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art to act directly or indirectly to regulate the amount of transcription from the promoter. Optionally the term "promoter" includes herein also the 5'UTR region (e.g. the promoter may herein include one or more parts upstream (5') of the translation initiation codon of a gene, as this region may have a role in regulating transcription and/or translation. A "constitutive" promoter is a promoter that is active in most tissues under most physiological and developmental conditions. An "inducible" promoter is a promoter that is physiologically (e.g. by external application of certain compounds) or developmentally regulated. A "tissue specific" promoter is only active in specific types of tissues or cells. A "promoter active in plants or plant cells" refers to the general capability of the promoter to drive transcription within a plant or plant cell. It does not make any implications about the spatiotemporal activity of the promoter.

A "3' UTR" or "3' non-translated sequence" (also often referred to as 3' untranslated region, or 3'end) refers to the nucleic acid sequence found downstream of the coding sequence of a gene, which comprises for example a transcription termination site and (in most, but not all eukaryotic mRNAs) a polyadenylation signal (such as e.g. AAUAAA or variants thereof). After termination of transcription, the mRNA transcript may be cleaved downstream of the polyadenylation signal and a poly(A) tail may be added, which is involved in the transport of the mRNA to the cytoplasm (where translation takes place).

A "5' UTR" or "leader sequence" or "5' untranslated region" is a region of the mRNA transcript, and the corresponding DNA, between the +1 position where mRNA transcription begins and the translation start codon of the coding region (usually AUG on the mRNA or ATG on the DNA). The 5'UTR usually contains sites important for translation, mRNA stability and/or turnover, and other regulatory elements.

"Expression of a gene" refers to the process wherein a DNA region, which is operably linked to appropriate regulatory regions, particularly a promoter, is transcribed into an RNA, which is biologically active, i.e. which is capable of being translated into a biologically active protein or peptide (or active peptide fragment) or which is active itself (e.g. in posttranscriptional gene silencing or RNAi). An active protein in certain embodiments refers to a protein being constitutively active. The coding sequence is preferably in sense-orientation and encodes a desired, biologically active protein or peptide, or an active peptide fragment. In gene silencing approaches, the DNA sequence is preferably present in the form

of an antisense DNA or an inverted repeat DNA, comprising a short sequence of the target gene in antisense or in sense and antisense orientation.

When reference is made to the “expression level” of a gene, the expression level is to be understood as the amount of RNA transcript that is transcribed by a gene and/or the amount of protein that may be translated from an RNA transcript, e.g. mRNA. Furthermore, in case the 3'UTR sequence is from a particular gene, the amount of Luciferase expression also correlates with the expression level of the particular gene. The amount of protein that may be translated from an RNA transcript may also be measured to determine the expression level of a gene. Methods are known in the art, such as ELISA and Western blot. Alternatively, the expression level of a protein may also be indirectly measured. For example, in case the expression level of an RNA transcription factor needs to be determined, a reporter gene construct may be used which comprises the RNA transcription factor binding site. The expression level may then be subsequently determined by measuring the reporter gene construct expression, e.g. in case GFP is used, green fluorescence intensity may be determined as a measure of the expression level of the RNA transcription factor.

A “nucleic acid construct” or “vector” is herein understood to mean a man-made nucleic acid molecule resulting from the use of recombinant DNA technology and which is used to deliver exogenous DNA into a host cell. Vectors usually comprise further genetic elements to facilitate their use in molecular cloning, such as e.g. selectable markers, multiple cloning sites and the like.

The term “polymorphism” refers to the presence of two or more variants of a nucleotide sequence in a population. A polymorphism may comprise one or more base changes, an insertion, a repeat, or a deletion. A polymorphism includes e.g. a simple sequence repeat (SSR) and a single nucleotide polymorphism (herein referred to as “SNP”), which is a variation, occurring when a single nucleotide: adenine (A), thymine (T), cytosine (C) or guanine (G) - is altered. A variation must generally occur in at least 1% of the population to be considered a SNP. SNPs make up e.g. 90% of all human genetic variations, and occur every 100 to 300 bases along the human genome. Two of every three SNPs substitute Cytosine (C) with Thymine (T). Variations in the DNA sequences of e.g. humans or plants can affect how they handle diseases, bacteria, viruses, chemicals, drugs, etc.

As used herein, the term “allele(s)” means any of one or more alternative forms of a gene at a particular locus. In a diploid cell of an organism, alleles of a given gene are located at a specific location, or locus (loci plural) on a chromosome. One allele is present on each chromosome of the pair of homologous chromosomes.

The term “de novo mutations” refers to mutations that occur spontaneously in a nucleotide sequence within the gene, which has not previously been reported.

“rs12403575” represents an internationally known coding system to identify each SNP in the dbSNP of the NCBI’s Entrez system. The original database with additional
5 information of the SNP rs number is available at the website of <http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp>. The rs number can also be queried in the Entrez database such as pubmed (<http://www.ncbi.nlm.nih.gov/pubmed/>) and GenBank (<http://www.ncbi.nlm.nih.gov/>). The SNP rs12403575 is located in the promoter of the *Srp9* gene. The nucleotide at its position can be either adenine (A) or guanine (G). If adenine is
10 present in SNP rs12403575, it is called the A allele when it is present in this polymorphism. If guanine is present in SNP rs12403575, it is called the G allele when it is present in this polymorphism. Since each individual has one pair of the same chromosome, each individual has the SNP rs12403575 genotype either AA, AG, or GG.

The term “primer” refers to an oligonucleotide complementary to a nucleic acid strand
15 for initiating the nucleic acid synthesis in the presence of four nucleoside triphosphates, a polymerase and buffer in a hybridization condition. The term “probe” refers to an oligonucleotide that selectively hybridizes to a target nucleic acid under a suitable condition.

The terms “protein” or “polypeptide” are used interchangeably and refer to molecules consisting of a chain of amino acids, without reference to a specific mode of action, size, 3
20 dimensional structure or origin. A “fragment” or “portion” of a protein may thus still be referred to as a “protein”. An “isolated protein” is used to refer to a protein which is no longer in its natural environment, for example *in vitro* or in a recombinant bacterial or plant host cell.

The term “antibody” used herein refers to any immunoglobulin or fragment thereof,
25 and encompasses any polypeptide comprising an antigen-binding site with at least one complementarity determining region (CDR). The term includes, but is not limited to, polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, chimeric, human, single-chain, synthetic, recombinant, hybrid, mutated, grafted and *in vitro* generated antibodies. The term “antibody” also includes antibody fragments such Fab, F(ab')₂, Fv, scFv,
30 Fd, dAb, and other antibody fragments or other constructs comprising CDRs that retain antigen-binding function. Typically, such fragments would comprise an antigen-binding domain. The details of the preparation of such antibodies are well known to those skilled in the art. The antibodies may be neutralizing antibodies. The term “neutralizing” refers to the ability of an antibody to inhibit (i.e., eliminate or reduce) at least one activity of another
35 compound or molecule. The antibody or fragment thereof may be any of the known antibody isotypes and their conformations, for example, IgA, such as IgA1 or IgA2, IgD, IgE, IgG, such as IgG1, IgG2a, IgG2b, IgG3, IgG4, or IgM class, or may constitute mixtures thereof in any

thereof in any combination, such as a mixture of antibodies from the IgG1 and IgG2a class.

The antibody may display specific binding to SRP9 and/or SRP14. "Specific binding" of the antibody to SRP9 and/or SRP14 means that the protein and the antibody form a complex that is relatively stable under physiological conditions. Specific binding is characterized by a high affinity and a low to moderate capacity as distinguished from non-specific binding which usually has a low affinity with a moderate to high capacity. Typically, binding is considered specific when the affinity constant K_a is higher than 10^6 M^{-1} . If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions in an *in vitro* test. In the present invention an antibody that specifically binds to SRP9 and/or SRP14 is an antibody in which SRP9 and/or SRP14 is the only target that is bound with high affinity, i.e. other polypeptides are not bound by said specific antibody or bound only with low affinity (non-specific binding).

As used herein, the term "effective amount" refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, e.g., an amount which results in the treatment and/or prevention of, febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. In the context of therapeutic or prophylactic applications, the amount of antibody administered to the subject will depend on the type and severity of the disease or condition and on the characteristics of the subject, such as general health, age, sex, body weight and tolerance to drugs. It will also depend on the degree, severity and type of disease or condition. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The antibody can also be administered in combination with one or more additional therapeutic compounds. For example, a "therapeutically effective amount" of the binding member is meant levels in which the physiological effects of a disease or condition associated with febrile seizures are, at a minimum, ameliorated. The skilled person will be capable of determining when such disease or condition has been treated and/or prevented.

As used in the context of the invention, the term "RNA interference" refers to a process of sequence-specific, post-transcriptional gene silencing (PTGS). RNA interference (RNAi) is a system within living cells that takes part in controlling which genes are active and how active they are. RNAs are the direct products of genes, and these small RNAs can bind to other specific RNAs (mRNA) and decrease their activity, for example by preventing a messenger RNA from producing a protein. RNA molecules capable of RNA interference include, without limitation, siRNA, shRNA, and miRNA.

In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, the verb "to consist" may be replaced by

"to consist essentially of" meaning that a composition of the invention may comprise additional component(s) than the ones specifically identified, said additional component(s) not altering the unique characteristics of the invention.

In addition, reference to an element by the indefinite article "a" or "an" does not
5 exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

The terms "increased level" and "decreased level" as used throughout this document refers to a significantly increased level or significantly decreased level. Generally, a level in a
10 test sample is increased or decreased when it is at least 5%, such as 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% higher or lower, respectively, than the corresponding level in a control sample or reference sample.

15 Detailed description of the invention

The present inventors mapped a FS susceptibility QTL on chromosome 1 by QTL analysis and showed differential expression of *Srp9*, a gene within this protective QTL which is part of the ubiquitous signal recognition particle (SRP) which is involved in regulating ER-dependent protein synthesis (secreted and membrane proteins). Partial knock-down of *Srp9*
20 expression in C57BL/6J mice *in vivo* significantly reduced FS susceptibility, thus identifying *Srp9* as a new FS susceptibility gene in mouse. The *Srp9* gene in the C57BL/6J and chromosome substitution strain 1 (CSS1) was sequenced. The sequence of the *Srp9* gene in the C57BL/6J strain is set forth in SEQ ID NO:1, whereas the *Srp9* gene of the CSS1 strain is set forth in SEQ ID NO:2. The differences in sequences are depicted in Table 6 below. A
25 deletion of approximately 200 bp in the promoter region was identified which most likely is responsible for the differential expression in the two mice strains.

In TLE patients with FS the inventors found increased hippocampal *Srp9* mRNA levels compared to TLE patients without FS. These findings provide new insight into the genetics of FS and open new roads to the early diagnosis and treatment of complex FS and
30 associated temporal lobe epilepsy.

In a cohort of 368 Dutch mTLE patients (compared to 730 healthy controls) an association was found of a single *Srp9* promoter SNP (rs12403575 G/A) with FS and mTLE. Patients with AA genotype had a significantly higher hippocampal *Srp9* expression than those with AG genotype, indicating that this SNP contributes to regulation of *Srp9*
35 expression. The human *Srp9* gene sequence is set forth in SEQ ID NO:3.

It was also found that in TLE patients the frequency of de novo mutations in the *Srp9* promoter region was significantly higher than in the control population. It could be concluded that the presence of one or more de novo mutations in the *Srp9* promoter indicated a propensity to develop FS in a subject.

5 The inventors further mapped 2 FS susceptibility QTLs to mouse chromosome 2 one of which (QTL2b) contains the *Srp14* gene. SRP14 is the binding partner of SRP9 in the SRP complex and thus also involved in regulation of ER-dependent protein synthesis. Hippocampal *Srp14* mRNA expression was found to be up-regulated in CSS2 mice compared to C57BL/6J and in TLE patients with hippocampal sclerosis (which have a high
10 incidence of antecedent FS) compared to non-sclerotic TLE patients and autopsy controls. The human *Srp14* gene sequence is set forth in SEQ ID NO:4.

In an aspect, the present invention relates to the use of an SRP9 protein and/or an *Srp9* nucleic acid sequence and/or an SRP14 protein and/or an *Srp14* nucleic acid sequence as a diagnostic marker. It was found that both the SRP9 polypeptide and *Srp9* mRNA and/or
15 both the SRP14 polypeptide and *Srp14* mRNA could be used as diagnostic markers, particularly for diagnosing and/or identifying an increased risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's Syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy (TLE; herein also referred to as "mesial temporal lobe epilepsy; "mTLE").

20 The nucleotide sequence of the human *Srp9* gene sequence is set forth in SEQ ID NO:3, whereas the amino acid sequence of human SRP9 polypeptide is set forth in SEQ ID NO:5. The nucleotide sequence of the human *Srp14* gene sequence is set forth in SEQ ID NO:4. The amino acid sequence of human SRP14 polypeptide is set forth in SEQ ID NO:6. The nucleotide sequences of the human *Srp9* and *Srp14* genes and the amino acid
25 sequences of the human SRP9 and SRP14 polypeptides can also be found in GenBank.

In another aspect, the present invention relates to use of an SRP9 protein and/or an *Srp9* nucleic acid sequence and/or an SRP14 protein and/or an *Srp14* nucleic acid sequence in identifying a subject at risk of developing febrile seizures (FS), a subject at risk of developing complex FS, a subject at risk of developing a complex epilepsy syndrome such
30 as Dravet's Syndrome and epilepsies with mental retardation, and/or a subject at risk of developing temporal lobe epilepsy. In one embodiment of the invention, the subject is at increased risk of developing FS, complex FS, a complex epilepsy syndrome including FS, and/or TLE, preferably when compared to the risk of a healthy subject of developing these conditions and/or diseases.

35 In a further aspect, the present invention pertains to a method for identifying identifying a subject at risk of developing febrile seizures, complex febrile seizures, a

complex epilepsy syndrome including FS, such as Dravet's Syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the steps of:

- a) providing a sample derived from a subject;
- b) determining the level of at least one biomarker selected from SRP9 polypeptide,

5 *Srp9* mRNA, SRP14 polypeptide and/or *Srp14* mRNA in said sample;

- c) comparing the level of said biomarker to a reference level; and

d) determining whether the level of said biomarker is indicative of a risk of developing febrile seizure, complex febrile seizure, a complex epilepsy syndrome including FS, such as Dravet's Syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

10 In an embodiment, an increased level of said biomarker is indicative of a risk, preferably an increased risk, of developing febrile seizures, complex febrile seizures, and/or temporal lobe epilepsy. This method of the invention is preferably performed postnatally, such as in a child or infant. After establishing a risk of developing the conditions or diseases referred to herein, said subject may optionally be started on therapy capable of preventing
15 febrile seizures, such as complex febrile seizures, and associated temporal lobe epilepsy, complex epilepsy syndrome including FS, such as Dravet's Syndrome and epilepsies with mental retardation.

In yet another aspect, the present invention is concerned with a method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy
20 syndrome including FS, such as Dravet's Syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the step of:

- a) determining in a sample of a subject a SNP in the promoter region of the *Srp9* gene, wherein the SNP is selected from rs12403575 G/A, said SNP being selected from the group consisting of rs6688819 (T/C), rs12403575 (G/A), rs16845266 (G/C), rs120398148
25 (T/C), and rs6659660 (G/A).

There are many methods for determining polymorphisms in the *Srp9* gene. One of ordinary skill in the art will select the appropriate method and protocol to use. These and many other methods will be readily apparent to those of ordinary skill in the art.

In an embodiment, the A allele of rs12403575 G/A, is associated with an increased
30 risk of developing febrile seizure, complex febrile seizure and/or temporal lobe epilepsy, whereas the G allele of rs12403575, is not. Thus the A allele of rs12403575 G/A may be considered the risk allele. The method may comprise a further step of providing a sample of a subject. The latter method is considered particularly suitable for prenatal screening purposes. Once the genotype of the allele has been established, the baby may be further
35 screened after birth for SRP9/*Srp9*, e.g., in accordance with the methods taught herein, and may optionally be started on therapy capable of preventing febrile seizures, such as complex febrile seizures, and associated complex epilepsy syndromes, temporal lobe epilepsy and

epilepsy and hippocampal sclerosis.

The method may also comprise the step of determined the genotype of said subject for SNP rs12403575, wherein an AA genotype is associated with an increased risk of developing FS, complex FS, complex epilepsy syndromes and/or TLE.

5 Other SNPs in the *Srp9* gene as depicted in fig. 4C, which provides sequence data identifying 5 promotor SNPs in the human *Srp9* gene, may also be useful for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's Syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. Moreover, detection of de novo mutations in the *Srp9* gene
10 sequence, not found in the control population, may also be useful for identifying persons at risk for the above diseases and conditions. Thus, the present invention also provides for a method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's Syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising
15 the step of: a) determining in a sample of a subject a SNP in the *Srp9* gene, wherein the SNP is selected from among those depicted in fig. 4C.

The method may further comprise a step of providing a sample derived from a subject. Preferably, the sample comprises or is derived from blood, amniotic fluid, cerebrospinal fluid, tissue from skin, muscle, buccal or conjunctival mucosa, placenta,
20 gastrointestinal tract, or chorionic villi.

The risk of developing the above-referenced conditions may be increased for such subject, in particular compared to one or more healthy subjects. Healthy subjects as referred to herein do not suffer neurological disorders, particularly not from febrile seizures, and preferably have no positive family history of febrile seizures, complex febrile seizures and/or
25 temporal lobe epilepsy. Preferably, a healthy subject does not suffer from any conditions or diseases.

The uses and methods of the invention encompass *ex vivo* (or *in vitro*) analyzing a sample derived from a subject for the level of one, two, three, or four of the biomarkers of the invention, SRP9 protein, *Srp9* nucleic acid sequence, SRP14 protein and/or *Srp14* nucleic
30 acid sequence. The level of such biomarker or biomarkers may then be compared to a reference level. A reference level may be a level of said biomarkers or biomarkers in a healthy subject or may be a default reference level determined by taking the average level of said biomarker(s) of a plurality of healthy subjects. Preferably, the reference level is age-matched to the subject. It is not necessary to determine the level of said biomarker(s) each
35 time a sample is measured; once the reference level of said biomarker(s) is reliably determined, the reference level may be stored, e.g., in a computer, and used for the

comparative purposes herein set forth. Alternatively, the reference level may be known to the skilled physician and compared mentally. It is to be noted that the level of each biomarker is measured independently and compared to the reference level of the same biomarker in order to establish whether the level in said subject is increased, similar, or decreased.

5 Moreover, SRP9 protein and/or *Srp9* nucleic acid sequence may be used as a biomarker in the application of the present invention, SRP14 protein and/or *Srp14* nucleic acid sequence may be used as a biomarker in the applications of the present invention, or one of SRP9 protein and *Srp9* nucleic acid sequence and one of SRP14 protein and *Srp14* nucleic acid sequence, or three or all of SRP9 protein and/or *Srp9* nucleic acid sequence and/or SRP14
10 protein and/or *Srp14* nucleic acid sequence may be used as biomarkers in the applications and uses of the present invention.

When SRP9 protein and/or *Srp9* nucleic acid sequence is employed as a biomarker in the present invention, either levels of SRP9 polypeptide and/or levels of *Srp9* mRNA may be determined. Measurement of the levels of either SRP9 polypeptide or *Srp9* mRNA would
15 suffice for the present invention. However, both of the levels of either SRP9 polypeptide and *Srp9* mRNA could be measured for the purposes of the present invention. Similarly, when SRP14/*Srp14* is employed as a biomarker in the present invention, either levels of SRP14 polypeptide and/or levels of *Srp14* mRNA may be determined. Measurement of the levels of either SRP14 polypeptide or *Srp14* mRNA would suffice for the present invention. However,
20 both of the levels of either SRP14 polypeptide and *Srp14* mRNA could be measured for the purposes of the present invention.

In the context of the invention, a subject may be an animal or a human being. In principle, any subject could be diagnosed using any of the methods or uses of the invention. The methods and uses of the invention may be applied as often as necessary in a subject.
25 Preferably, the subject is a human being. In a suitable embodiment, the subject is an infant, such as in the ages of between about 1 day and about 8 years, for example about 2 months and about 7 years, or about 6 months and about 6 years. The subject may have a positive family history of febrile seizures, complex febrile seizures and/or temporal lobe epilepsy. Alternatively, the invention may be used for prenatal screening for a subject at risk of
30 developing FS, complex FS, a complex epilepsy syndrome including FS, such as Dravet's Syndrome and epilepsies with mental retardation and/or TLE. Thus, the subject may also be a foetus.

The uses and methods of the invention are carried out *ex vivo*, i.e., on an *ex vivo* sample. Said sample may be any sample derived from a subject, but is preferably a
35 biological sample. As used herein, a "biological sample" refers to a biological tissue or biological fluid from a subject. A variety of samples can be useful in practicing the invention

including, for example, blood, amniotic fluid, cerebrospinal fluid, tissue or cells from skin, muscle, buccal or conjunctival mucosa, brain, placenta, gastrointestinal tract or chorionic villi. For prenatal screening purposes (using the genotyping method of the invention), the sample is preferably selected from amniotic fluid, tissue or cells from placenta and/or tissue or cells
5 from chorionic villi. For postnatal screening purposes (using the levels of SRP polypeptide and/or *Srp* mRNA), the sample is preferably selected from a whole blood sample, plasma, and serum.

The difference in the level of said biomarkers in said sample compared to a reference level may be determined as the fold increase/decrease, or alternatively as the relative
10 increase or decrease of biomarker in the sample of said subject as compared to biomarker in a reference level.

In the context of the invention, the level of proteins or polypeptides may be determined by methods well known in the art, e.g., using an enzyme immunoassay or western blotting. A number of monoclonal or polyclonal antibodies can be generated that specifically recognize
15 SRP9 polypeptide or SRP14 polypeptide. Utilizing current antibody detection techniques that can quantitate the binding of monoclonal antibodies, one can determine the level or amount of said SRP9 or SRP14 polypeptide in a sample obtained from a subject.

The term "enzyme immunoassay" ("EIA"), also called enzyme-linked immunosorbent assay (ELISA), is a biochemical technique that is well known in the art. It is used mainly in
20 immunology to detect the presence of an antibody or an antigen in a sample. In ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal. Thus in the case of fluorescence ELISA, when light of the appropriate wavelength is
25 shone upon the sample, any antigen/antibody complexes will fluorescence so that the amount of antigen in the sample can be inferred through the magnitude of the fluorescence signal.

Performing an ELISA involves at least one antibody or other binding partners with high affinity for the biomarker with specificity for a particular antigen. The sample with an
30 unknown amount of antigen may be immobilized on a solid support (for example, a polystyrene microtiter plate) either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself
35 be detected by a secondary antibody which is linked to an enzyme through bioconjugation. Between each step the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step the plate is

is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample. The substrates may utilize chromogenic substrates, though fluorogenic substrates and chemoluminescent substrates are used more commonly as they enable higher sensitivity. For some of the biomarkers of the invention, monoclonal
5 antibodies may be commercially available from various suppliers all of which may be successfully applied, taking into account the manufacturers recommendations for use.

Alternatively, the level of polypeptide biomarker or biomarkers may be determined using any other routine techniques known to the skilled person, including, but not limited to, capillary action, precipitation, turbidimetric, diffusion, agglutination, potentiometric,
10 amperometric, piezoelectric and evanescent-wave immunosensors, or any combination of the methods recited herein.

The level of *Srp9* or *Srp14* mRNA may be determined using routine techniques, including, without limitation, hybridization, and amplification reactions such as quantitative PCR or LCR.

15 For clinical diagnostics the use of nucleic acid arrays is highly advantageous as it couples accuracy and speed to quantitative analysis. Nucleic acid arrays are ordered sequences of DNA or RNA that can be used to selectively isolate and later on quantify specific nucleic acid sequences in complex mixtures – by changing the hybridization and washing conditions the specificity of the detected nucleic acid duplexes can be modulated.

20 The oligonucleotide sequences used to detect a target sequence, whether on nucleic acid arrays or in solution, will be referred to hereinbelow as a “probe”.

Suitable hybridisation conditions (i.e. buffers used, salt strength, temperature, duration) can be selected by the skilled person, on the basis of experience or optionally after some preliminary experiments. These conditions may vary, depending on factors such the size of the
25 probes, the G+C-content of the probes and whether the probes are bound to an array as described below.

Suitable hybridisation conditions are for instance described in Sambrook et al., *Molecular Cloning: A Laboratory manual*, (1989) 2nd. Ed. Cold Spring Harbour, N.Y.; Berger and Kimmel, "Guide to Molecular Cloning Techniques", *Methods in Enzymology*", (1987), Volume 152,
30 Academic Press Inc., San Diego, CA; Young and Davis (1983) *Proc. Natl. Acad. Sci.(USA)* 80: 1194; *Laboratory Techniques in Biochemistry and Molecular Biology, Vol.24, Hybridization with Nucleic Acid Probes*, P. Thijssen, ed., Elsevier, N.Y. (1993).

The hybridisation conditions are preferably chosen such that each probe will only form a hybrid (duplex) with a target sequence with which the probe is essentially complementary, if such
35 a target sequence is present, and otherwise will not form any hybrid. The term “essentially complementary” as used herein does not mean that the complementarity of a probe to a target

target sequence should be perfect, and mismatches up to 2 nucleotides can be envisaged.

Each probe should at least in part be complementary to a specific target sequence. The probe may be any nucleic acid (i.e. DNA or RNA) but is preferably DNA. The probe will generally have a size of about 10 to 100 base pairs, preferably about 10 to 40 base pairs. The probes may
5 all be of the same size, or may be of different sizes. The probes can be obtained in any suitable manner, e.g., using an automated DNA-synthesizer or in any other manner known per se. Also, solid phase nucleic acid synthesis techniques may be used, which may result directly in an array with the desired probes. Furthermore, the probes may be obtained using techniques of genetic engineering, for instance by primer extension using the target sequence as a template, and/or by
10 using one or more restriction enzymes, optionally using amplification.

Also, the probes may comprise one or more "alternative nucleosides". Examples thereof include the bases Inosine (I) and Uracil (U), as well as dUTP and dITP, and these are included within the term "labeled nucleotide analog". It is to be understood that the presence of such alternative nucleosides does not prevent the probe and its target sequence to be essentially
15 complementary to one another as defined above.

Quantitative nucleic acid-based amplification reactions may also be used to detect and quantify specific nucleic acid sequences in complex mixtures as in the present invention. These include the well known Polymerase Chain Reaction (PCR) and Ligase Chain Reaction (LCR) and modifications thereof (see McPherson & Moller, 2006. PCR, second edition.
20 Taylor & Francis Group; Wiedman et al., 1994. PCR Meth Appl; 3:S51-S64). LCR is a method of DNA amplification similar to PCR but differs from PCR because it amplifies the probe molecule rather than producing amplicons through polymerization of nucleotides. Two probes are used per each DNA strand and are ligated together to form a single polynucleotide. LCR uses both a DNA polymerase enzyme and a DNA ligase enzyme to
25 drive the reaction. In a specific application of LCR, the resulting polynucleotide can be amplified by PCR and analyzed separately or, notably when in multiplex samples, hybridized to arrays.

The target for DNA arrays and quantitative nucleic acid-based amplification reactions such as PCR or LCR are nucleic acids, so DNA or RNA. Such nucleic acids include, without
30 limitation, *Srp9* DNA and mRNA and *Srp14* DNA and mRNA.

For the purposes of genotyping the *Srp9* gene with respect to SNP rs12403575 G/A, probes or primers may be employed distinguishing said SNP in a *Srp9* gene. There are many methods for determining polymorphisms in the *Srp9* gene. Suitable methods include, without limitation, primer extension (PinPoint assay, Massexend™, SPC-SBE, or GOOD assay),
35 hybridization (TaqMan assay, bead array, or SNP chip), ligation (combinatorial fluorescence energy transfer (CFET) tags), and enzymatic cleavage (RFLP, Invader® assay), PCR-SSCP (single-strand conformation polymorphism), MRD (mismatch repair detection), BeadArray™,

detection), BeadArrayTM, or SNPlexTM. One of ordinary skill will select the appropriate method and protocol to use.

The invention is also directed to a kit comprising a probe or primer that distinguishes an allele of a SNP in an *Srp9* gene in a sample derived from a subject, wherein the allele is
5 selected from the A or G allele in the SNP rs12403575. Said kit is preferably for determining an increased risk of developing febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's Syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy in a subject.

The probe or primer is not specifically limited in the context of the invention as long as
10 it is capable of distinguishing between the two alleles in the respective SNPs. For easy detection, the probe or primer can be further detectably labelled, such as by fluorescence or detectable materials, such as radioactive materials, chemiluminescence, biotin, or the like.

The kit may further comprise a polymerase, deoxynucleotides, a restriction enzyme, a buffer, or the like for detection. An electronic hardware component, such as arrays (DNA
15 chips), microfluidic systems ("lab-on-a-chip" systems) and so on may also be used for the detection. The kit may further comprise means for determining the amount of a target nucleic acid, and means for comparing the amount with a standard, and may comprise instructions for using the kit to detect the SNP-containing nucleic acid molecule of interest.

The invention further provides for a method for identifying a subject at risk of
20 developing febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the steps of: a) determining in a sample of a subject the sequence of the *Srp9* promoter sequence, wherein the presence of one or more de novo mutations is indicative of a risk of developing febrile seizures, complex febrile seizures,
25 complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

The present inventors found that in a control population of humans de novo mutations in the *Srp9* promoter sequence were very rare and infrequent. In contrast, de novo mutations in the *Srp9* promoter sequence were frequent. This led the present inventors to conclude
30 that the presence of one or more de novo mutation(s) in the *Srp9* promoter sequence of a subject was indicative of a propensity to develop febrile seizures, and would have to lead to a decision to investigate further whether the subject had an increased risk of developing febrile seizures.

In a further aspect, the present invention relates to a method for screening chemical
35 compounds or compositions, said method comprising the step of identifying chemical compounds or compositions targeting the SRP complex, and in particular the SRP9/SRP14

complex. In one embodiment, the present invention deals with a method for screening chemical compounds or compositions interfering with the function of the SRP complex, said method comprising the step of identifying chemical compounds or compositions that suppress expression of SRP9 and/or SRP14 polypeptide, that interact with SRP9 and/or SRP14 polypeptide and thereby prevent formation of the SRP9/SRP14 complex, and/or that interact with the SRP9/SRP14 complex and thereby prevent formation of the SRP complex.

To this end, compounds can be tested on their efficacy to interfere with SRP-mediated translation arrest and/or transport into the endoplasmic reticulum in cell culture. Such studies can be performed in cell systems using known membrane receptor (such as glutamate receptors) proteins as indicators. The method may comprise the steps of: providing a cell or cell line; exposing said cell or cell line to a chemical compound or composition to be tested; determining the level of a selected membrane protein in the endoplasmic reticulum and/or cell membrane of said exposed cell or cell line; comparing the level of said selected membrane protein in the endoplasmic reticulum and/or cell membrane of said exposed cell or cell line with the level of said selected membrane protein in the endoplasmic reticulum and/or cell membrane of the same cell or cell line that has not been contacted with the chemical compound or composition to be tested; in which a decreased level of said selected membrane protein after exposure to said chemical compound or composition is indicative of the chemical compound or composition interfering with the function of the SRP, in particular the SRP9/SRP14 complex. The cell or cell line may be any eukaryotic cell line. The membrane protein may be any protein, and may optionally be labelled, for example, using green fluorescent protein. The chemical compound or composition to be tested may be any chemical compound or composition, such as those contained in a combinatorial library.

The invention also pertains to a compound capable of selectively binding to SRP9 and/or SRP14 polypeptide for use as a medicament. The compound may be an antibody, or a peptide selectively binding to SRP9 and/or SRP14 polypeptide. Preferably, said compound is for use in preventing and/or treating febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. Preferably, said compound blocks the formation of the SRP9/SRP14 complex. The compound, such as antibody, may be administered to a subject in need thereof in an effective amount to achieve the purpose of preventing and/or treating febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

In an embodiment, said method comprises the step of identifying chemical compounds or compositions targeting the SRP9/14 heterodimer (herein also referred to as "SRP9/SRP14 complex"). These could include compounds binding to/scavenging excess SRP9 and/or SRP14, compounds interfering with SRP9/SRP14 interaction, or compounds or compositions
5 that inhibit binding of the SRP/SRP14 complex to SRP complex or to a signal peptide of nascent protein at the ribosome.

In another aspect, the present invention provides for use of therapeutic gene modulation for suppressing the level of SRP9 protein and/or SRP14 protein in a mammal. Gene modulation may be directed at suppressing or down-regulating expression of the *Srp9*
10 gene (modulation at transcriptional level), or at inhibiting or preventing *Srp9* mRNA from being translated into SRP9 protein (modulation at translational level). Said use is preferably for preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

RNA interference is a naturally occurring mechanism for suppressing gene expression and subsequent protein translation. RNA interference suppresses protein translation by either degrading the mRNA before it can be translated or by binding the mRNA and directly preventing its translation. Generally, RNAi has been used to suppress the expression of both gene alleles that lead to the production of a given protein. It is also possible to suppress the
20 expression of only one allele.

Thus, in a further aspect the present invention relates to an *Srp9* and/or *Srp14* nucleic acid modulator selected from the group consisting of antisense DNA, siRNA, miRNA, shRNA, ribozyme specifically binding to and cleaving *Srp9* and/or *Srp14* mRNA and zinc finger nuclease specifically targeting a nucleic acid sequence in the *Srp9* and/or *Srp14* gene
25 resulting in suppression of *Srp9* and/or *Srp14* expression, for use in preventing and/or treating febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

In a further aspect, the invention is concerned with a recombinant nucleic acid
30 construct comprising at least one transcriptional unit comprising:

- a promoter,
- a nucleic acid sequence encoding a gene suppressor selected from:
 - an antisense DNA sequence complementary to the sense strand of the *Srp9* and/or *Srp14* gene;
 - 35 - an interfering RNA molecule which is complementary to *Srp9* mRNA and/or *Srp14* mRNA;

- a nucleic acid sequence encoding a zinc finger nuclease specifically targeting a nucleic acid sequence in the *Srp9* gene and/or *Srp14* gene;
- a nucleic acid sequence encoding a ribozyme specifically binding to and cleaving *Srp9* and/or *Srp14* mRNA; and

5 - a terminator,

for use in preventing and/or treating febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

The interfering RNA molecule may be selected from the group consisting of siRNA,
10 miRNA and shRNA.

The present invention also pertains to a vector comprising a recombinant nucleic acid construct according to the invention for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy. Said vector may comprise
15 more than one transcriptional unit.

Further, the invention is directed to a (host) cell comprising a recombinant nucleic acid construct according to the invention or a vector according to the invention for use in preventing and/or treating febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation,
20 and/or temporal lobe epilepsy.

In an embodiment, the to *Srp9* and/or *Srp14* nucleic acid modulator, recombinant nucleic acid construct, vector, host cell of the present invention are administered to a subject in need thereof in an effective amount to achieve the purpose of preventing and/or treating febrile seizures, complex febrile seizures, complex epilepsy syndromes including FS, such as
25 Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

In yet another aspect, the present invention provides for a method for suppressing the level of SRP9 protein and/or SRP14 protein in a cell, said method comprising the step of including an *Srp9* and/or *Srp14* nucleic acid modulator according to the invention or recombinant nucleic acid construct according to the invention in said cell.

30 In a final aspect, the present invention provides for a kit comprising means for detecting the level of *Srp9* mRNA and/or SRP9 protein and/or *Srp14* mRNA and/or SRP14 protein in a sample derived from a subject. In an embodiment, the kit is for use in identifying a subject at risk of developing febrile seizures (FS), a subject at risk of developing complex FS, a subject at risk of developing a complex epilepsy syndrome including FS, such as
35 Dravet's syndrome and epilepsies with mental retardation, and/or a subject at risk of developing temporal lobe epilepsy.

The kit may comprise a primer pair allowing selective amplification of *Srp9* and/or *Srp14* mRNA, or a fragment thereof. Selective amplification denotes that only (part of) the *Srp9* and/or *Srp14* mRNA is amplified using said primer pair.

Alternatively, the kit may comprise an antibody that specifically binds to SRP9 protein
5 and/or SRP14 protein.

The kit of the present invention is preferably for determining the level of *Srp9* mRNA, SRP9 protein, *Srp14* mRNA and/or SRP14 protein in a sample derived from blood.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

10 It will be clear that the above description and figures is included to illustrate some embodiments of the invention, and not to limit the scope of protection. Starting from this disclosure, many more embodiments will be evident to a skilled person which are within the scope of protection and the essence of this invention and which are obvious combinations of prior art techniques and the disclosure of this patent.

15

Sequence Listing

SEQ ID NO:1 depicts the nucleic acid sequence of the *Srp9* gene in C57BL/6J mice.

SEQ ID NO:2 depicts the nucleic acid sequence of the *Srp9* gene in A/J and CSS1 mice.

SEQ ID NO:3 depicts the nucleic acid sequence of the *Srp9* gene in human.

20 SEQ ID NO:4 depicts the nucleic acid sequence of the *Srp14* gene in human.

SEQ ID NO:5 depicts the amino acid sequence of the SRP9 protein in human.

SEQ ID NO:6 depicts the amino acid sequence of the SRP14 protein in human.

Examples

25

Example 1. Identification of *Srp9* as a susceptibility gene in febrile seizures and TLE.

Methods

30 Animals

C57BL/6J and C57BL/6J-Chr 1A/NaJ (chromosome substitution strain 1, CSS1) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). We generated a CSS1-F2 by intercrossing F1 hybrid animals from a (reciprocal) outcross between CSS1 and C57BL/6J (n=129; 28 males and 31 females; 36 males and 34 females originating from CSS1 and
35 C57BL/6J mothers, respectively), which was pheno- and genotyped. All experimental procedures were approved by the ethical committee of Utrecht University.

FS susceptibility assay

FS susceptibility was determined by inducing hyperthermia in 14-day-old pups by exposure to a warm-air stream and measuring the latency to tonic-clonic seizures (FSL) (Hessel et al., Genes, Brain and Behavior 8, 248-255, 2009). Video/EEG monitoring has proven that the onset of these seizures coincides with FSL to be the most reliable behavioural correlate of the latency to spike-wave discharges in the brain.

Genotyping

- Genomic DNA was isolated from spleen. SNP and microsatellite markers across chromosome 1 were selected based on allelic differences between C57BL/6J and A/J database. SNP genotyping was performed as described (Kas et al, Genes, Brain and Behavior 8, 13-22, 2009). Microsatellite marker genotyping was performed by PCR. SNP genotyping was performed with TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) using automatic genotype detection on an ABI PRISM 9700 Sequence Detection System and SDS 2.2.3 software for allelic discrimination (Applied Biosystems).

Table 1. Known brain-expressed genes in the 1-LOD region of QTL1

	Gene symbol	Region	Gene name
1	<i>1700047M11Rik</i>	184,230,965-184,233,420	RIKEN cDNA 1700047M11 gene
2	<i>Bpnt1</i>	187,156,028-187,181,656	bisphosphate 3'-nucleotidase 1
3	<i>C130074G19Rik</i>	186,695,805-186,707,097	RIKEN cDNA C130074G19 gene
4	<i>Capn2</i>	184,397,393-184,447,614	calpain 2
5	<i>Capn8</i>	184,495,143-184,562,483	calpain 8
6	<i>Degs1</i>	184,205,903-184,212,935	degenerative spermatocyte homolog 1 (Drosophila)
7	<i>Dusp10</i>	185,837,181-185,899,515	dual specificity phosphatase 10
8	<i>Enah</i>	183,835,644-183,949,792	enabled homolog (Drosophila)
9	<i>Eprs</i>	187,186,974-187,252,234	glutamyl-prolyl-tRNA synthetase
10	<i>Fbxo28</i>	184,243,233-184,271,737	F-box protein 28
11	<i>Hlx</i>	186,551,022-186,556,480	H2.0-like homeobox
12	<i>Iars2</i>	187,108,605-187,153,275	isoleucine-tRNA synthetase 2
13	<i>Lbr</i>	183,746,674-183,772,483	lamin B receptor
14	<i>Mark1</i>	186,721,482-186,823,358	MAP/microtubule affinity-regulating kinase 1
15	<i>Mosc1</i>	186,610,655-186,635,192	MOCO sulphurase C-terminal domain containing 1
16	<i>Mosc2</i>	186,636,947-186,670,330	MOCO sulphurase C-terminal domain containing 2
17	<i>Rab3gap2</i>	187,028,121-187,107,986	RAB3 GTPase activating protein subunit 2
18	<i>Slc30a10</i>	187,278,727-187,292,641	solute carrier family 30, member 10
19	<i>Srp9</i>	184,054,868-184,062,538	signal recognition particle 9
20	<i>Susd4</i>	184,693,991-184,826,729	sushi domain containing 4
21	<i>Tlr5</i>	184,884,919-184,906,170	toll-like receptor 5
22	<i>Trp53bp2</i>	184,339,303-184,392,563	transformation related protein 53 binding protein 2

20 Map construction and QTL analysis

Map construction was performed as described earlier (Cox et al., Genetics 182, 1335-1344, 2009). None of the individual markers showed a segregation ratio distortion ($P > 0.05$) in the chi-squared goodness-of-fit-test (AA:AB:BB ratio 1:2:1). Data were tested for normal distribution and transformed where appropriate. QTL location and variance explained by

each locus was determined using MapQTL (version 4.0). LOD scores > 1.65 were considered significant.

In situ hybridization

- 5 Radioactive in situ hybridization was performed as described (van der Hel et al., *Epilepsia* 50, 1717-1728, 2009) and transcript levels were expressed as nCi/g33. CSS1 (n=6) and C57BL/6J (n=6) brains were dissected from naïve P14 mice, snap frozen and stored at – 80°C until coronal (16µm) cryosections were cut. Sense and antisense probes were synthesized using mouse RNA as a template (Primers, Table 2) (Sigma Aldrich Chemie BV, 10 Zwijndrecht, The Netherlands). Hybridization was quantified with ImageJ (version 1.4.0).

Table 2. Primers used for sequencing mouse *Srp9*, mouse in situ hybridization and human quantitative PCR.

Primer	Forward	Reverse	Product size (bp)
Sequence Mouse <i>Srp9</i>			
1	CCATAGCCCCTGCAATATGT	AATTAGATCCGATGCCCTCC	650
2	CTTTCCGCAAACCTCATGACA	GTCACACAGCCACAGAGCAT	641
3	GGCCAGGAGTGAAGACAGAG	ATAGTGCCTGTGCTGCCTTT	847
4	GCCTGAGGGAAAGACTCAAA	GTCTGGAAGTGAAGCATCG	801
5	CTCGTGAGCCTCCGTCTTT	AGGTGCTTCCTAACTGGGAC	673
6	GTGCCCTTTCTTTCTTCTAC	TGGAGAGATGGCTTAGCTGC	592
7	AATTTGCCCTCTGACCACAC	GTTGCCTTAAAAAGGGAGCC	698
8	CCGGCCCTTCTTTTTATTTC	ATTCCCGCACACAAATCCTA	641
9	TCTGGTCCTCGTAGGGCTAA	TTGTGAAAACCCACACTGGA	579
10	GAACCTGGGTCTCTGAACA	AAAGCCAAGACAAGGAGTGG	623
11	CGACTTGGTCCTCATGTCCT	ACCACCCGTACCTGCAATAA	653
12	TGCCTCTGCTGGGATTAAG	CCTGGCTCTGTTTGTCTCA	683
13	GTCTGTTTTCTGGCTGTCC	TTTCTCCCTCTCTCCCCTTC	574
14	GGGAGTTGGGAAGGGAGTAG	AACCTCTGAACCTGGAAGCC	635
15	TTTAATTGGACCTGGCTTCC	CGTTAGAGGGGACATGAGGA	612
16	AGCCTGCTGGAATGATTTTT	TTACTGCCGGATTCTGCTCT	620
17	GGTATGGCACCTGAGAGAGC	TGTGTGTGTTGTAAACGGCA	625
18	TGCCGTTTACAACACACACA	AGGACAACCAGGGCTAGTGA	650
in situ hybridization Mouse			
<i>Srp9</i>	TGAAGGTACGGGTGGTTCTC	CATAACTGCGTCTCGCTCTG	752
<i>Dusp10</i>	TGAAGGTACGGGTGGTTCTC	GGTGGGGACAGACTGAGGTA	466
<i>Bpnt1</i>	GATGACCAAATGCAGCAAGA	GGTGGGGACAGACTGAGGTA	410
<i>Eprs</i>	AGACCTTGTGGTTTGGGTTG	CCCATTCTTACACCCAGGA	500
<i>Enah</i>	GGCAGGACTTGAATCTGGAG	ACACCACCCTCTGAAAGCAC	502
<i>HLX</i>	GCAGAAGGACAAGGACAAGG	CCAGCCAGTGTAGAGGAAGC	468
<i>Mark1</i>	GGATGCACCACACCTGAGTA	AGTCCCAACCACACCTTGTC	558
<i>Rab3gap2</i>	GCAGTTCCCTTGAGCCTTG	GACATCGTCGCTCACTTGAA	796
Quantitative PCR Human			
<i>Srp9</i>	TCCCGGACGTAGGTAGTTTG	CTTCATAGGGTCAGCGAGGT	145
<i>Srp68</i>	GAAAATCCCAGCAAGTTCCA	TGACCAAAGGCAATCAATCA	243

PSMD2	GATTCGTCGTCGCAGAAGCCTAA	GGCACTGATGAGAACCACCT	148
DDX48	AGAACAAGGACCACGGAATG	CACGATTTGAGCCAGCAGCATAA	282

Functional interference studies

Srp9 antisense (T*G*A*G*GCATCGTGGCT*T*C*A*G*G; n=7) (The asterisks (*) represent phosphorothioate bonds in the nucleic acid sequence) or scrambled

5 (T*C*A*G*G*GTACGCGTCGT*A*G*C*G*T*; n=5) (The asterisks (*) represent phosphorothioate bonds in the nucleic acid sequence) oligonucleotides (18 nM in 1.5 µl) (Sigma-Aldrich, Zwijndrecht, the Netherlands) were injected intracerebroventricularly in C57BL/6J mice using a stereotactic device (coordinates: 10 mediolateral +1.0 mm, anteriorposterior -1.0 mm, depth, +2.6 mm, angle 0 degrees) 20h prior to hyperthermia

10 induction (0.3 µl/min, 1.5 µl). Prior to injection mice were anesthetized with Ketamine (75 mg/kg), Medetomidine (1 mg/kg) and Atropine (0.04 mg/kg), antagonized with antisedan 0.5 mg/kg (0.1 ml/10g) and returned to their mother. After hyperthermia animals were sacrificed and brains were dissected and snap frozen for expression studies. Cycloheximide (20 mg/kg; n=9; Sigma, St. Louis, MO, USA), rapamycin (30 mg/kg; n=9; Santa Cruz Biotechnology, Inc.

15 Santa Cruz, CA, USA) or saline (n=9) were injected intraperitoneally (ip) (total volume 0.1 ml/g) in C57BL/6J mice 20 or 30 min before hyperthermia induction, respectively.

Human brain tissue and DNA isolation

20 Hippocampal and neocortical tissue was obtained from pharmacoresistant mTLE patients with complex partial seizures who had surgical resective treatment. Hippocampal tissue from autopsy controls was obtained from the Netherlands Brain Bank. Selection of patients (for clinical data see Table 3), collection and processing of tissue for qPCR analysis was as described (van Gassen et al., *Epilepsia* 49, 1055-1065, 2008). DNA was isolated from

25 neocortex of mTLE patients (n=368, 91 with FS) and from blood of healthy controls (n=730). Informed and written consent was obtained from the patients for all procedures as approved by the Institutional Review Board.

Table 3. Clinical data of patients used for the qPCR analyses.

Patient	Age	Sex	AO/PMD	Epilepsy focus	AEDS	Early life incident	Neuropathological diagnosis
Autopsy							
1	56	F	16	x	x	ND	normal hippocampus
2	32	F	13	x	x	ND	normal hippocampus
3	47	F	4	x	x	ND	normal hippocampus
4	51	M	8	x	x	ND	normal hippocampus
5	52	F	12	x	x	ND	normal hippocampus
Mean	47.6	5F,1M	10				
Non	-						

HS

1	41	M	33	L	LEV, CBZ		vascular malformation
2	31	M	13	L	CBZ, CLB, LTG		tumor
3	47	M	16	R	VPA, LEV, LTG	trauma	posttraumatic epilepsy
4	24	M	21	L	CBZ, LTG, LEV	trauma	posttraumatic epilepsy
5	48	F	26	L	NA		contusio cerebri
6	23	F	12	R	LTG, OXC	trauma	posttraumatic epilepsy
7	37	M	18	R	CBZ, PHT	trauma	posttraumatic epilepsy
8	39	M	4	R	CBZ		tumor
9	51	F	46	R	TMD		tumor
10	36	M	17	R	LEV, VPA		tumor
11	45	M	27	L	LTG, PHT		FCD
12	34	F	4	L	CBZ		cavernoma in cortex
13	28	M	16	R	CBZ, TPR		NA
14	54	M	19	L	LTG, OXC, CLB		ganglioglioma
15	42	F	20	R	CBZ, LTG, LEV		tumor
16	46	F	30	R	CBZ, VPA		FCD
Mean	39.1	6F,10M	20	9R,7L			

HS – FS

1	34	F	6	L	CBZ		MTS W4
2	39	F	28	R	LTG, OXC	meningitis	MTS W4
3	19	M	13	L	OXC		MTS W4
4	26	F	9	R	NA		MTS W4
5	32	F	1	R	LTG	meningitis	MTS W4
6	37	F	11	R	LEV, TPM		MTS W4
7	32	F	14	L	NA		MTS W4
8	19	F	2	L	CBZ, LTG		MTS W4
9	50	M	3	L	CBZ, GBP	meningitis	MTS W4
10	51	M	48	R	NA	meningitis	MTS W4
11	49	F	12	L	OXC, CLB		MTS W4
12	42	M	1	L	LEV, LTG		MTS W4
13	50	M	3	L	CBZ, GBP	meningitis	MTS W4
Mean	36.9	8F,5M	12	5R,8L			

HS + FS

1	37	F	1	R	NA	FS	MTS W4
2	38	M	9	L	CBZ, CLB	FS	MTS W4
3	40	M	1	R	VPA, CLB	FS	MTS W4
4	33	M	11	L	CBZ, AZA	FS	MTS W4
5	24	M	1	L	VPA, CBZ, CLB	FS	MTS W4
6	36	M	5	L	OXC, PGB	FS	MTS W4
7	41	M	6	R	PHT, CLB, CBZ, LTG	FS	MTS W4
Mean	35.6	1F,6M	5	3R,4L			

AO=Age of onset. PMD=post mortem delay in hours. AED=Antipileptic Drugs, PHT=phenytoin, CLB=Clobazam, CBZ=Carbamazepine, LTG=lamotrigine, LEV=Levetiracetam, VPA=Valproic acid, OXC=oxcarbazepine, PGB=Pregabalin, AZA=Acetazolamide, TPM=Topiramate, TMD=Tramadol, NA=information not available, FCD=Focal Cortical Dysplasia

5

Quantitative PCR

qPCR was performed as described earlier (van Gassen et al., Epilepsia 49, 1055-1065, 2008). cDNA was synthesized from hippocampal RNA of human autopsy controls (n=5), and mTLE patients with (HS; n=20) and without sclerosis (non-HS; n=16). In a group of HS-mTLE patients we compared hippocampal RNA of mTLE patients with (+FS; n=7) and without (-FS; n=13) antecedent FS. Primers for SRP9, SRP68 and two reference genes, PSMD2 and DDX48 were from Sigma Genosys (Cambridge, U.K.). Expression data was calculated as normalized ratio's and normalized to two reference genes.

15 Sequencing

Mouse *Srp9* was sequenced as described (Kas et al., Genes, Brain and Behavior 8, 13-22, 2009). Primers 1–18 (Table 2 above) were chosen (Mus musculus *Srp9* gene sequence in Ensembl (ENSMUSG00000026511, 183,755,349 – 187,980,365 bp) to amplify the promoter, 5' UTR, exons, introns and 3'UTR in C57BL/6J, A/J and CSS1 mice (n=3). Human *Srp9* exons, exon-intron boundaries and the promoter region (5 kb upstream) was sequenced with nested PCR (Smits, B.M. et al. Generation of gene knockouts and mutant models in the laboratory rat by ENU-driven target-selected mutagenesis. *Pharmacogenet Genomics* **16**, 159-169 (2006)) as described. Primers (Table 4) were chosen using LIMSTILL (<http://limstill.niob.knaw.nl/>).

10

Table 4. Primers used for nesting PCR to sequence human *Srp9* and the Taqman assay for SNP rs12403575.

Primer	Forward	Reverse	bp
Srp9p-c1-1	AATGAGAGCCCTTGTAAG	CAGCACTGTAGCTGCTTAGAC	553
Srp9p-c1-2	TGTAACACGACGGCCAGT GTCCACCAACATGATTTACG	AGGAAACAGCTATGACCAT CTTGCCAGGAATGTGTGTC	408
Srp9p-c2-1	AATCCATGAAACTTGGAAG	TTATAGGCCAGTCATTGTGC	545
Srp9p-c2-2	TGTAACACGACGGCCAGT CAAGCCAGCAGCACTTC	AGGAAACAGCTATGACCAT AATAGCTGAAGCTGGAGAGC	346
Srp9p-c3-1	GCAGACCTTTGGGAAGATAC	CTCTGTCTGACCTCCTCTG	603
Srp9p-c3-2	TGTAACACGACGGCCAGT CCTCAGGATTAGACTGCTC	AGGAAACAGCTATGACCAT CCAAGACACCAGCCTCTG	471
Srp9p-c4-1	CCTCATAGCATGGAGTTG	GGTGATGAAGAAACCAACC	662
Srp9p-c4-2	TGTAACACGACGGCCAGT GGGTATTGTCCAGGAAGAAC	AGGAAACAGCTATGACCAT GGATTGTCCAGCAAATGG	562
Srp9p-c5-1	GCTTTACCGATCTAGCTTCTG	TGTGGTTATCCTTTCTGCTTC	551
Srp9p-c5-2	TGTAACACGACGGCCAGT CCTGTCACTTCCGGTTATG	AGGAAACAGCTATGACCAT TGAGCTTTCAAACAACTCTG	447
Srp9p-c6-1	CCAAACACACCAAGTGAAC	CACTATCACAAGAAGCAAGG	645
Srp9p-c6-2	TGTAACACGACGGCCAGT GGAATTGTAATAAGGCTCACTG	AGGAAACAGCTATGACCAT CCCAACACTGGGAATTAGAG	462
Srp9p-c7-1	GAGCTAAACAATGGGCACTC	CCCAGTCACACTAGGATGC	654
Srp9p-c7-2	TGTAACACGACGGCCAGT CCCAAATCTCATGTTGAACTC	AGGAAACAGCTATGACCAT TGTGCATGTTTGTACATGG	550
Srp9p-c8-1	ACCTAATGCCACCAAGTATG	TGTGTGTGTGTGTGTGTGTG	573
Srp9p-c8-2	TGTAACACGACGGCCAGT ACAAACATGCACATGTACCC	AGGAAACAGCTATGACCAT TAAAGAGCAATGAGCAGAGG	500
Srp9p-c9-1	TTGGCATTGTCTTAACCTCC	TAGGGTCTGTCTCCAGCAAC	577
Srp9p-c9-2	TGTAACACGACGGCCAGT GTCATTCTGCCCAACTCTG	AGGAAACAGCTATGACCATCATGAGGAAGAAGAAACAAAGG	441
Srp9p-c10-1	TTCTCTTAATCCTGGTTCC	AGACAGGAGACCACCTTCG	752
Srp9p-c10-2	TGTAACACGACGGCCAGT TGACCTTTGTTCTTCTTCC	AGGAAACAGCTATGACCAT ATCCTTGAGGAGAAGGTTTG	682
Srp9p-c11-1	GGCTTCCTTATTCATTTCTCAC	ACTGGTGTTTAGGAGATGAGG	595
Srp9p-c11-2	TGTAACACGACGGCCAGT TTCTCCTCAAGGATCCAAAC	AGGAAACAGCTATGACCAT AAATCTGATCCTCCAAGCAG	501
Srp9p-c12-1	CGATCATGGCTCACTGC	CTACCTACGTCGGGAGTC	689
Srp9p-c12-2	TGTAACACGACGGCCAGT TCCTGCTTGAGGATCAG	AGGAAACAGCTATGACCAT GGATGTTGTCAGGGAGTGG	519
srp9-1-1	CGACTCCCGGACGTAGG	GGTCCATTGTGTTTATTCC	270
srp9-1-2	TGTAACACGACGGCCAGTGAGGCCTTGCTTCTTTAC	AGGAAACAGCTATGACCATGATGGCGGAAGACATC	168
srp9-2-1	ATGGTAGCAAAGGAATGAAC	AAAGTAGCTGCTAGAATGAGG	419

srp9-2-2	TGTAAAACGACGGCCAGTTGTCATTTGTCTTCACTGTTTC	AGGAAACAGCTATGACCATGCATCCTAATGGGTAATCTCC	216
srp9-3-1	ACATGAGTGCTTACCCTTGG	CAGAAAGATGGCTTGAGTCC	315
srp9-3-2	TGTAAAACGACGGCCAGTGTGTTGATTGGTTGGTTG	AGGAAACAGCTATGACCATATTAGCTGAGTGTGGTGGTG	189
srp9-4-1	GAGATAATCTAATACCTAATTCGAAGG	CCTCTAAGCTTCTGAACAGC	584
srp9-4-2	TGTAAAACGACGGCCAGTTCTTCATCAGACCATAGCAG	AGGAAACAGCTATGACCATTTTCTGTCCCAACACTTTCTC	486
rs12403575	GAGGGTGGCCAGTGGCCTCAAATAC	GAGGGTGGCCAGTGGCCTCAAATAC	

Statistical Analysis

Data were analyzed with SPSS (version 15.0) and expressed as means $\pm$ SEM. One Way Analysis of Variance (ANOVAs) was performed with a post-hoc Bonferroni-Dun test.

- 5 Significance levels ($\alpha=0.05$) were corrected with the Dunnett method to account for the multiple (strain, expression, treatment or gene) comparison. Equality of variances was tested with Levene's test. ISH expression, treatment effect of AS oligo's and cycloheximide/ rapamycin treatment were analyzed with an independent t-test ($\alpha=0.05$). P-values (2-tailed) of allele frequencies were calculated by means of chi-square analysis with a 2 x 2
- 10 contingency table. Distribution of genotypes was in Hardy-Weinberg equilibrium.

Results

To identify genetic susceptibility factors for EEG-verified prolonged FS induced by hyperthermia, we performed a phenotypic screen for FS latency (FSL) in a mouse

15 chromosome substitution (CSS) panel C57BL/6J-Chr#A/NaJ and found that chromosome 1 (CSS1) has a strong contribution. Indeed, FSL was higher in CSS1 (915.3 $\pm$ 37.5 s) than in C57BL/6J (631.9 $\pm$ 26.9 s) (Fig. 1A), showing that CSS1 mice were less susceptible to FS than C57BL/6J.

To map genes contributing to reduced FS susceptibility on mouse chromosome 1 we bred a

20 CSS1-F2 generation (n=129). Phenotypic analysis of all individuals showed that the majority of these genetically unique CSS1-F2 mice had a longer FSL than C57BL/6J mice (Fig. 1B). QTL analysis (Table 5) revealed a highly significant peak (LOD=7.6) (Fig. 1C). The 1-LOD support interval⁹ of this QTL1 was located between 183,756,349-187,980,365 bp. This QTL1 interval harboured 22 brain-expressed genes and accounted for 23.9% of the variance

25 in FS susceptibility in the CSS1-F2 population. The heterozygous genotype showed a phenotype intermediate between the two homozygous genotypes, indicating an additive mode of inheritance at this QTL).

Table 5. Co-segregation analysis in the CSS1-F₂ progeny

marker	BB		AA		AB		F		P	
rs32634595	837.6 ±	58.6 (n=31)	851.4 ±	39.1 (n=31)	917.5 ±	37.3 (n=64)	1.681	0.190		
rs4222217	843.9 ±	52.3 (n=37)	893.8 ±	52.9 (n=28)	894.8 ±	35.7 (n=59)	0.396	0.674		
rs32652808	792.6 ±	48.6 (n=33)	903.9 ±	46.5 (n=28)	903.9 ±	36.6 (n=66)	2.091	0.128		
rs30843169	776.6 ±	44.9 (n=36)	913.6 ±	45.4 (n=30)	930.4 ±	39.8 (n=58)	3.608	0.030		
rs13475954	778.7 ±	47.7 (n=34)	923.3 ±	53.4 (n=29)	904.9 ±	37.0 (n=63)	2.739	0.069		
rs31928547	770.4 ±	39.8 (n=36)	938.4 ±	60.6 (n=26)	907.6 ±	36.1 (n=65)	3.594	0.030		
mev22847648	769.3 ±	38.1 (n=37)	948.2 ±	63.7 (n=28)	907.4 ±	38.9 (n=59)	3.947	0.022		
rs30798049	786.6 ±	37.1 (n=36)	942.7 ±	59.5 (n=30)	895.5 ±	36.6 (n=62)	2.810	0.064		
rs31072643	788.1 ±	40.2 (n=33)	1063.0 ±	76.7 (n=26)	846.0 ±	29.1 (n=65)	8.184	0.0001		
rs31884979	761.7 ±	34.5 (n=34)	1076.6 ±	71.4 (n=28)	845.7 ±	28.9 (n=63)	11.453	0.0001		
rs13476246172	722.6 ±	35.1 (n=27)	1109.2 ±	68.7 (n=29)	838.5 ±	26.3 (n=76)	17.721	0.0001		
rs30560577	756.8 ±	47.3 (n=32)	1090.8 ±	69.1 (n=29)	838.4 ±	23.4 (n=66)	13.826	0.0001		
D1Mir165	708.0 ±	30.7 (n=26)	1080.1 ±	64.5 (n=32)	829.3 ±	23.0 (n=76)	18.782	0.0001		
D1Mir315	716.8 ±	30.7 (n=28)	1080.1 ±	64.5 (n=32)	842.9 ±	27.0 (n=69)	16.148	0.0001		
rs31997386	797.4 ±	26.2 (n=37)	1154.8 ±	71.7 (n=28)	830.7 ±	24.1 (n=72)	28.582	0.0001		
D1Mir467	713.3 ±	27.2 (n=26)	1115.1 ±	71.0 (n=30)	835.9 ±	23.9 (n=72)	20.211	0.0001		
D1Mir361	709.4 ±	28.0 (n=25)	1115.1 ±	71.0 (n=30)	835.9 ±	23.9 (n=72)	20.126	0.0001		
D1Mir362	706.3 ±	28.0 (n=25)	1105.8 ±	71.4 (n=31)	836.7 ±	22.7 (n=72)	19.676	0.0001		
D1Mir511	706.7 ±	24.8 (n=29)	1054.9 ±	63.4 (n=35)	852.0 ±	27.3 (n=65)	14.944	0.0001		

To further analyze QTL1, we selected eight brain-expressed candidate genes (Fig. 2A) based on known SNPs differences and differential gene expression (http://www.genenetwork.org/) between the A/J and C57BL/6J (Fig. 2A). Only for *Srp9* we found lower expression in CSS1 compared to C57BL/6J (total brain -23.1%; hippocampus -35.6%) (Fig. 2B). Sequencing of *Srp9* (184,053,433 - 184,063,607 bp) in C57BL/6J, CSS1 and A/J mice identified 1 new SNP in the 3'UTR and 52 polymorphisms in intronic and promoter regions.

Database analysis (WebQTL: http://www.genenetwork.org/) revealed a strong cisQTL for *Srp9* mRNA regulation in the AXB eye tissue (LOD=12.1) and BXD brain (LOD=11.7). Such high cisQTLs (LOD>11) have a mean gene-to-QTL distance of about 450 kb, indicating an upstream region regulating gene expression. Indeed, sequencing 2500 bp upstream revealed a major 210 bp deletion in A/J and CSS1, containing a SINE B2 element (RepeatMasker: http://www.repeatmasker.org/). What sequence differences contribute to differential *Srp9* expression remains to be determined. Interestingly, using the same mapping strategy, we identified *Srp14* as a strong candidate gene for FS susceptibility in QTL2b (LOD=6.2; 1-LOD interval 114,875,854 - 133,817,501) on chromosome 2 (Figure 5). SRP14 forms a functional heterodimer with SRP9 in the SRP complex, which in total comprises 6 proteins. This observation, combined with the differential expression of *Srp9*, prompted us to

prompted us to select *Srp9* for further study.

To investigate a causal relationship between *Srp9* expression levels and FS susceptibility, we microinjected *Srp9* antisense (AS) oligonucleotides intracerebroventricularly (ICV) in C57BL/6J pups 20 hours prior to inducing hyperthermia.

5 This significantly prolonged FSL in C57BL/6J (899.0 ± 77.7 s) compared with scrambled oligonucleotide injected littermates (574.0 ± 23.5 s) (Fig. 3A) and was accompanied by a down-regulation (-16.7%) of *Srp9* transcript levels in the cortex. Thus, *Srp9* down-regulation *in vivo* decreased FS susceptibility and induced the CSS1 FS phenotype in C57BL/6J, providing strong evidence for a functional role of *Srp9* in FS susceptibility in mice.

10 SRP9 is part of the SRP complex, which couples the cytoplasmic protein synthesis machinery to the membrane-bound protein translocation machinery of the endoplasmic reticulum (ER). To independently assess the importance of protein synthesis in FS susceptibility, we injected protein synthesis inhibitors in C57BL/6J pups prior to hyperthermia induction. Cycloheximide significantly prolonged FSL (769.1 ± 41.1 s) compared with saline
15 injected littermates (662.6 ± 28.0 s) (Fig. 3B), as did rapamycin, which inhibits protein synthesis via mTOR (mammalian target of rapamycin) (708.1 ± 35.9 compared to 591.9 ± 11.4 s) (Fig. 3B). Thus, both our genetic and pharmacological studies point to the importance of protein synthesis in conveying FS susceptibility.

Several deletions have been described in the human orthologous region of QTL1
20 (1q41-1q43) presenting in patients with complex seizure phenotypes often including febrile seizures^{13,14}. To investigate a role of *Srp9* in FS susceptibility in humans, we measured *Srp9* transcript levels in hippocampal tissue obtained during surgery performed to treat patients with mTLE. We compared mTLE patients with hippocampal sclerosis (HS), known to have a high incidence of antecedent FS, with mTLE patients without HS and autopsy
25 controls. SRP9 expression (Fig. 4A) was highest in mTLE patients with HS ($Rn=169.3 \pm 17.0$), intermediate in mTLE patients without HS ($Rn=100.0 \pm 14.9$) and lowest in autopsy controls ($Rn=40.4 \pm 6.1$). Expression of SRP68, an SRP protein not directly interacting with SRP9, did not differ between the mTLE patient groups, indicating that not all SRP proteins are differentially expressed. In a separate cohort of HS-mTLE patients we found significantly
30 higher SRP9 levels ($+48.6\%$) in patients with antecedent FS compared with patients without antecedent FS (Fig. 4B). These data strongly suggest that also in humans increased SRP9 expression is linked to FS susceptibility.

In search for a genetic variant associated with FS and mTLE, we sequenced all four exons of the human SRP9 gene (including intron-exon boundaries) and its promoter region
35 (5 kb) in a Dutch mTLE patient cohort ($n=368$) including 91 patients with antecedent FS, and ethnically matched healthy controls ($n=169$) (Fig. 4C, 8). We identified a large number of

new and known variants. We did not find significant differences in frequency of total number of sporadic mutations between groups nor did we find variants in coding sequences that are predicted to affect protein function. However, we found a significant increase in the frequency of sporadic mutations in the promoter region ($p < 0.001$) in patients with TLE (fig. 8). Analysis of the frequency of 5 common SNPs revealed a significant association of a single SNP in the promoter region (rs12403575 G/A) (Fig. 4C, fig. 8) with mTLE. Further analysis of this SNP in the cohort and a larger control sample ($n=730$) resulted in significant association with mTLE ($P=0.01$, ODDS-ratio 1.238) and FS ($P=0.045$, ODDS-ratio=1.321) (Fig 4D).

These data identify SRP9 as risk factor for FS and mTLE, providing compelling evidence for a common genetic predisposition. Correlating SNP genotype and hippocampal SRP9 expression levels in individual mTLE patients, we found a significantly higher SRP9 expression in patients with AA compared to AG genotype (Fig. 4E), indicating that this SNP contributes to regulation of SRP9 expression.

SRP9 is a subunit of SRP, a ubiquitous, highly conserved cytoplasmic ribonucleoprotein complex that plays a key role in targeting nascent membrane and secretory proteins to the rough ER membrane (Walter and Blobel, Federation Proceedings 40, 1557, 1981). SRP consists of six polypeptides and one RNA (7SL) together forming two functional domains, the S-domain primarily involved in recognition of the signal sequence of nascent peptide chains, and the ALU domain (7SL RNA and the SRP9/SRP14 heterodimer) thought to be responsible for elongation arrest facilitating targeting of nascent chains to the ER. SRP lacking SRP9/14 lacks elongation arrest activity, but is still translocation competent, albeit, at lower efficiency. Thus, our data implicate ER-dependent protein synthesis in FS susceptibility.

We confirmed involvement of protein synthesis by showing that reduction of protein synthesis led to a decrease in FS susceptibility using either cycloheximide, which inhibits translation initiation (Baliga et al., Federation Proceedings 27, 766, 1968) or rapamycin, which inhibits activation of eukaryotic translation initiation factor 4B (eIF4B, Burnett et al., PNAS 95, 1432-1437, 1998)) through inhibition of mTOR. mTOR signalling has been implicated in the pathogenesis of tuberous sclerosis complex, a syndrome with a prominent epilepsy phenotype. The rapid effect of protein synthesis inhibitors (20-30 min) indicates involvement of local protein synthesis, possibly in dendrites and/or synapses. Specialized ER has been identified in dendrites and in neurons local protein synthesis plays a key role in regulating

neuronal plasticity.

The precise mechanism by which SRP9 conveys its effect on FS susceptibility needs to be further investigated. Presumably loss of SRP9 from the SRP complex leads to loss of

elongation arrest and decreased translocation efficiency due to the strict time dependence of the functional interaction between polysome and microsomal membrane. Therefore, reduced SRP9 levels could affect the amount and/or composition of proteins targeted to the ER, thus affecting secretion, plasticity and cell growth. Fever and FS, but also other risk factors for mTLE, such as viral infection, stroke or head trauma, are known to disrupt ER function and trigger the ER stress response. ER stress has been implicated in the pathogenesis of various other neurological degeneration syndromes including mTLE, Alzheimer's and Parkinson's disease. Not only ER function, but also post-ER trafficking maybe affected by SRP9 levels. In HELA cells down-regulation of several proteins in the SRP complex resulted in reduced expression of membrane proteins, possibly involving trafficking. Interestingly, temperature-dependent receptor trafficking defects have previously been implicated in FS. Thus, it is tempting to speculate that variations in SRP9 levels observed in the present study, affect the composition of receptors and ion channels at the synaptic membrane in a temperature-dependent manner, resulting in altered susceptibility to FS.

Our data show that susceptibility to FS is, at least in part, determined by susceptibility to increased body temperature (without an infection). We propose that SRP9-mediated changes in FS susceptibility involve temperature-induced changes in ER function and protein trafficking. The identification of SRP9 as FS susceptibility gene implicates (local) ER-dependent protein synthesis as key factor in FS susceptibility and opens new possibilities for early diagnosis and treatment of FS.

Table 6. *Srp9* sequence differences between C57BL/6J and A/J/CSS1 mice. *Srp9* was sequenced (n=3) in the CSS1, A/J and C57BL/6J mice. The *Srp9* sequence of A/J and CSS1 were identical. A/J/CSS1 and C57BL/6J differences are in parentheses (with the nucleotide(s) present in the *Srp9* gene of C57BL/6J being indicated before the slash and the nucleotide(s) present in the *Srp9* gene of A/J/CSS1 being indicated after the slash). The exon region is in bold. Newly identified polymorphisms are in bold and underlined (known polymorphisms are available on <http://www.ensembl.org>). The 210 bp underlined promoter region in bold was not present in the A/J/CSS1:

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ACCAATCTAAAAGAGGTAGGGTTAGAGGGTGTGAACATGATCAAGGCACATTGTGGA
CATGTGTGAAGATGTCATAACGAACTCATTGTTATGTACACTGCTAAAAATGTTTACA
AACTCCTCTTCCAAGAAGGCCCTTTCTGCTGGACTGTCCTTTGTCTCGTCTCGTTTGT
35 CTGCTCTTCAGCGTCCCATAGCCCCTGCAATATGTCCCTTGTTCTAAGGGAATAGAA
GCCTTCTTCTGAAGCTAATCTTATTCACAAGTGACTTTAACCTTC(G/T)TAGAGATGACT
CAGCTAACCAACCAGCCTCCTGGGAAC(T/C)TGAGCTGTAAAGCCCTAATGTTTGAGC
AGACCTTCAAGCATCCCCC(-A)GGGAGGCTGACTTCAGAGTCCCTTTGTT
TCACCCCTTTAAAAAAA(-A)TCAAAATAATT(G/A)CTATCTATTTAGCCCAGGTA(C/
40 T)CATGATGGCCTCTTGCTAGGACTGCCATCATTCTTGTTCCTTCTTCTTTCTTTCTT
TGCTTATTTCTTTTTTAAAAA(-A/G/T)ATTTATTTA(GGGGGGCTGGTGAGATGGC

```

TCAGTGGGTAAGAGCACCCGACTGTTCTTTTGAAGATCCGGAGTTCAAATCCCAGC
 AACCACATGGTGGCTCACAACCATGTGTAAGGAGTGTCTGAAGACAGCTACAGTGT
 ACTTACATATAATAAATAAATAAATCTTAAAAAAAAGGACTGAAGGTTTGTCCCAGA
 GACTGCTCAATGTTTAAAAAA)AAAAAGATTTATTTATTTA(TTT/AAG)ATTTATTTATTTA
 5 TTTATTATGAGTATGCTGTTGCTGTCTTGAGACACACCGGAGGAGGGCATCGGATCTA
 ATTACAGGTGGTTGTAAGGCACCATGTGGTTGCTGGGATTTGAACTCAGGACCTTTGG
 AAGAGTAGTCGGTGTCTTAACTGCTGAGGCATCTCTCCAGCCCTGCTTGTTTATTTT
 TAAGTAGCCCAGTTTGGCCTTGGACTAAATAGCTGGGACTTTCCGCAAACCTCATGACA
 TAAAAAAGAAAAAGAAAGAGCAAAAGAAAAAAGGAAGGAAGGAAGGAAGGAAGAA
 10 AAGAGAAAGAAAATGAATGAAAGGAAAGAAAAGAGAAA(A/C)AATGAAAGAAA(G/T)AA
 AAAGAAAGAGAAAGAAGGAAAAAAGAAAAGAAG(TC/CT)GGGCAGGGGTGGC(G/A)CA(
 C/T)GCCTTTAATCCCAGCACTTGG(G/A)AGGCAGAGGCAGGCGGATTTCTGAGTTCAA
 GGCCAGCCTGGTCTACAGAG(A/T)GAGTTCCAGGACAGCC(A/G)GGGATACACAGA(A/
 G)AAACCCTGTCTTGAAAAACAAACAAACAAACA(---/AAG)AA(C/
 15 G)AAAG(-/A)AAGAAGAAAGAAAAAG(-----/AAAAAGA)AAAAAGAAAAAGATAGGGAA
 GAGGAAGGCAAGATGGAGACCTTGTCTAGAATGCACAATCCTTAGCACTGAAAACCC
 AAAACAATACAAGACAAAACAAAACAAAAAACCCAACAAAACCTGTTTAAAGCTGATCA
 ATGGGGGCTGGTGAGAGGGCTCAGGGTTAAGAGCAC(T/C)GACTGCTCTTCC(A/
 G)GAGGTCATGAATGAGTTCAAATCCCAGCAACCACATGGTGGCTCACAACCATCTGT
 20 AATGAGAAACAAAAAATAAAAAACAACAACAGCAACAACAACAACAAC(C/T)TTTGGGC
 CCGGAT(T/C)GAGGA(G/A)GGGCAGAGCAAGATGGAGAAGGGAAGGGGGAAAAAG(C/
 G)AGCTCAATGGACAGGGGGATGTAATTTTTAGTAAGAGTAAACATTCTGTTGGGCCAG
 GAGTGAAGACAGAGGAAATGAATTACAAGGAGGCATGAAGA(A/G)ATTTCTAGAGGTG
 AAAAATACCCCCATATATTGATTATGACATTAGGCAAGTAGGTATATGCTCTGTGGCTG
 25 TGTGACGTGTGTGTATATGTATTTTTTT(T)CAGAATCCATGATCG(T/C)A
 TATTCTCAAAGCCGATGTTTACTGCACACATAAACTATATACCCCAATAAAATT(T/G)CT
 TTTAAAGCTGTCCTGTGTACACTCTG(C/A)CTGAGG(G/A)AAAGACTCAAATTTTCGTA
 TCCTAGCAGATGCCACAGTGTAGCTTCTTCTCTGTGCTTCTTCTCTTCTCCTCCATTC
 CCTTGAACACTACTGTTT(C/A)TCTTAAGTACACGTACACTGCAGATCTTAAATGT
 30 GCCTAGAGGTCTCGCCCTTCTCTACTCCCTATTCAGTTTGTCTCACCTCTGGGAGCCT
 TGCTCCTTTTTGTTCCACTGACCCGATACAAAAGTCTACCACAGAAAGTTTTTGGTTGC
 GCGTCCGTTATGTCTCCCCAACTCCTTGATTAGGCTTTGAGTGTGACAGCTGCG(C/T)
 CTTGTCATCACCACGACTGTGACTTGGGTACCAGGTACAGAGCTCCTGCTTTGAGT
 GTGACAGCTGCGCCTTGTCTATCACCACGACTGTGACTTGGGTACCAGGTACAGAGC
 35 TCCTTAGTCTTTGAAAGGCAGCACAGGCACTATTAGTCTTTGAAAGGCAGCACAG
 GCACTATCCCCTCCAGCCCTGCAGGCGGCGCTGTGGCAGCCGCCCTCTCCTCTCC(
 C/G)GACCTCTCAGAGCGGAGGCCCCCCCTCCAGCCCTGCAGGCGGCGCTGTGGCA
 GCCGCCCTCTCCTCTCCCGACCTCTCAGAGCGGAGGCCCGCCCCCGGACATCAC
 GCCTCCTGCCATTGCCCCCGACATCACGCCTCCTGC(C/T)CATTGGACAGGCGC
 40 GCGGCCGAGGCTGGCCAATGGCGTGAGCGTCTGTGAGCCTCCGTCTTTGCGCCGCC
 GGAGTGC(A/T)GCGCTGTGGGCGGTTGCGGGAGAGCGGCGGGCCGCCGGGGCGA
 CAGCGCCGTG(A/C)CTCGCTTCGCGGCCTTGTCTCTCGCTGCCTGAAGCCACGATG
 CCTCAGTTCCAGACCTGGGAGGAGTTAGCCGGGCGGCCGAGAAGCTCTACCTGG
 CGGACCCCATGAAGGTGAGTCGCTGCAGGGACCGCCCTGGGGCCCGGGGCTTCTC
 45 TACCCTTGCGCGGCCTGAGCACGCCAGGAAACAGTCGGGCCACCCGGTGCCAC
 TGTCCCTTCGTCTGCTTCCGACTGACCCGGAGACCCAGTGACCCAGCGAGGCGACC
 CCAGCAGCTACAGGCAGGGCGGTTTCCGCAGCGCCGGACCCACAGCGGCC(T/C)G
 CACCCGGGGGAGCTCCTAACTTCCGCTCGGGATCTGGTTGGTTTACGTGTGCTGAG
 TGGGCTCCTCCCC(T/C)GCACCGTCGCTTTCCTGTCCGT(C/G)GCTTAGTTTGTGTC
 50 CCCTTTCCTTTCCTACCC(A/T)GACCCGAGAGTACCAGCTTATAAAATGGTGCTACTGT

ACTGTACGTCCCAGTTAGGAAGCACCTGCTCTACTTTTTTTTTTTTTTTTTT(---
/TTT)GGATAGTGGGGATTTTTAGTCTTTTCTGGACCAGTTGAAACATGGAGACT
GTTTGCACCTCTCACGTTAGACATAAATGTTTGCAAGCGGTTTCATTCCCCAAAGCAAA
CTACCCAGTCCCATCTCAGCAGTTCAGAACA(A/G)GTTCCGGGAGGGGGACACAAAG
5 GTCAGCTTCATCATTCCCTTAAAGGATTGTTGAAGGTATGGTAGACGC(A/G)TGTTTTAT
GAATATACACGTTAAGGAAAAAGAAAAACAAAAAACCCGATGAGCAGCTAACA
ATCTCAAGAACCCGGGCTCAGTGCATAACACGAACTCTGTGTGTGTGTGTGTATCA
TAATGAGACTGCAGAGACACTCAGCATGCAGAACTCACTTAAGGATGGAAAGAGGGA
ATCTACTCCTTTCTGACCACACATCTCTCACACTAACATGTACACACACAG
10 ACACACACA(T/C)GTAAGTAAAAATCAAAAATATATTGGTACTGGCAGCTTGCGGTGG
CAGTACTTACTACAGGATTTTACAGGGAGC(AT)TCTTTTACTTTTGTTGTGAGCATAG
CCTTTAGCAGCTAAGCCATCTCTCCAAGCCCACAGGA(A/G)GCATCTTTTAAGAAGGT
CAGTCCATTAATTCTTACGTATGTCAGTGTGTTGCTTGTGCACATGGGGTGCCTGCCCT
GCTGGGGTCAGAAGAGGGCATTGAATCCCCGGGAAATGGAGTTAGGCATGGGTGTG
15 AGCCACCTTGTGGAGTTAGGCATGGGTGTGAGCCACCTTGTGGGTGCTGGGA(G/A)C
CAAATCTGTGTCTACCTGCATTAGAACCACAGGAAGACCAGAGTAGACAGTAGAACAT
TCGGAACCATCCTTGTGTATTTTAAAGCTCCT(A/G)TCT(T/C)CTCATGCGTCCTAGATG
GGCCTCATTAAATGTCCTGTGGAGATGAGA(G/A)TGATCTT(G/A)AACTCT
GGGTGTTAGCTCCCCCGGCCCTTCTTTTTATTTCTCAGGAACTCAGAGATC(C/T)GCCT
20 GCCTCTGCCTCCC(G/A)AGTGCTGGGATTAAAGGC(G/A)TGTGCCAC(C/T)AC(AC/TG)
CC(G/T)GGCTGCAGGCTCCCTTTTTAAGGCAACATTTAAACTAATGATCTTATTAAGT
GATAATAAATGGCTAGGTAGATTAGGTATTTTGAATTGTGTAGTTTGTGACAGTGGT
GGCTTCTTGTATGTACTTTTCATGTGGTTCATTTCTCAGTTGGGCAGTGTGCTGTAAGG
TGGTATAAGGTTTGTCCAGAAGGGCCTGATACTGACAACAGTATACAGTGTCTGCATT
25 TAGTAAAGAGACTGTAGATACACCAGCACACATTGAGTGACTATTTGAA
GATGTAGTTGCGGGGCCAGTGAAAGTAGAAGTGTGAGGGAGTTGTGATGAGAAGGA
GACACAGAGGAGGAGCTGGCCAGATCTGTCCAGGAGAAAATCCTTGTGGCCTCCAA
ACTTAAGTATAAACCTGGATCTGAACTCCAGTCCATTCTGGTCCTCGTAGGGCTAAC
GAAGACAGTTTGTGCTCTAGGATTTGTGTGCGGGAATTCTGCTTTCTGTACCCAG
30 CACATTCAGTGTGGAACC(-C)AATGTACCTTTTACCCTTTAC(T/C)CTTCCCTT
TATGGCTTGAAGTGAATGCTGTTGCTGGTGAGCTGAAAGAGCCCGTGG(T/C)TGCTC
TGTCTTCCCTTTTAGTCCCTCCCTTACGTTTCTGCTACCGTGGTCTGAGAATACCGACAC
TGCCGTGTCCTCTGCTTAAACCAGTTCCTTCATTGATTACTGAATGAAGTCCT
GA(C/T)TCCAGTCGCACTGTCTTGATTGAGTCATTCTGGCTTCAGACTTGGTGAAGCC
35 ATA(G/T)ATACATCAGCTCTTTGTTGAGAGTGCTTATACTTT(C/T)TTCAGCCTTCTCTAT
ATGAAAACAAAACATGTATTTTCAAAGCCACATCCCAAGAAAAGTGGTTCTTGGCCTC
TTTTTCTTTAGCACTTTTACCAGTATTTTCTTTATAGGACTTTTTTTTTTTT(-T)AAA
(T/-)AACTTAATTCATTTATTTTTCTTGTA(T/A)AAAAACCCTCATATGATA(G/C)CCA
CA(G/A)ATGGAACC(T/G)GGGTCCTCT(G/A)AACAGATACTCCAG(T/G)G(T/G)GGGTTT
40 TCACAAGGTGGCCAGTGAATTCCTGATAAAGAGACTTGATAACAAAGTTTCATACTCC
AGGGGGGGTTTTACAAAGGTGGCCAGTGAATTCCTGATAGGAGACTTGGTGAAACA
GTCTCTTTCCAGAGGTCAGGGGTCAGGTAGCTGTATTTCCGAGGTCGGGGGTCAGGT
AGCTGTAGGTCTTGGAGGTTGCACCACTGGTGGCCTTGGCAGTGTTATCCAGGGTGG
CAGTGCAGCCCCTGGCTGATTAATGGCACTTATCAATATTGGCCATCATTAGTAGTTTT
45 TTGGGCACAGGAGCAGAGACAATGCCAGTGCTTCTGGGGGCAGAAATAAGATGCAC
CAGCACAGAGCCACAGTGGCCTGTCACCTTGCATGGGACATTGTGGGGCTTGCTAGT
CTTGTTCCTCCAGTAGCCTCTCCAAATAGGGACAATGGAGAGCTTGGCCAAGATGGCG
GCCCCTAGATGGCTGTGGGTTTCTCCTTGGAGCATGGAACACCAAGACCAACATGAC
CATTGTAGTTCCCAGTAGTGTCAAAGCCTTGAACC(T/G)GTCCACCGGCCGCCAAGC
50 GTCTGTTTCTGTATGAGCAGGGTTTTAGAACCTCCCTTGAGGGAGGGATGTGCTCAG
GGAAGATGAGTAGTCTGGGAGCTCCACGACTTGGTCCTCATGTCCTTACCTTGGTGA
CGGGGACCCACTCCTTGTCTTGGCTTTGCCTCCAAGAGCTCCCTGGCCTCGTCTGA(T

TCTGA(T/C)CACAGCCCCACGGCTGCCAGATCACTGCCTCCACCTCTTACT(C/T)CT
 GGCCCCAGGTCCCTCCATGCCTCCTGGGGTTCCAGGATCATCTCCATTTCCCGAATAA
 GAGGCTGCCTTTCTAGGACCTTTATTTTAATTCACTCTGTTTTGTAT(T/C)GATTATTTT
 TGGTTGTTGAATTAAGTTCTTAAGTACAGGAACCTTGAC(C/T)
 5)TTTTGTCTCTGTGACTCTATTGAAATGGATGCTTATTTTGAAGTGTAAACAGTATTTTT
 ATCTAACAGTTAAAATCCTTCAGGTTGCCACACTGAACTAACTTTAAGTTTGTGGGGCT
 TTGGGAAGTGTAGGGAGCAGGGAAAGGCTGGTGATTCTTGGTGTTTGTGTTTTGTTTTG
 CTTTGTGTTTTGTTGGGGGGTGTGTTTTGTTTTGTTT(T/G)T(G/C)TTTTCTGAGACAG
 GGTTTCTCTGTCTAGCCCTAGTAGCTATCCTGGAATTCAGTCTAGACCAGGCTGGCCT
 10 CAACTCAGGCCTGCCTCTGCTGGGATTAAAGG(T/C)GTGTGCCACCAGTGCCCTCT
 GTCTGTTGCTGCTTTTAAATAAGTATTTTATTGCAGGTACGGGGTGGTTCTCAAATACA
GGCATGTTGATGGGAATTTGTGTATCAAAGTAACGGATGATCTAGTTGTAAGTATAC
 ACTTTTACTTGTGTTGGGTCGGGGGATTTGAGTTGACTACTTGTGTTGAGGTTGTTTCATGTA
 GAGTCT(G/A)CAGATGACCTACTAGGTGCATGCGTCCCACATGGAAGAACTTGGTTCT
 15 AAAGTTCTTCTTTTAGACTCAACAAATAACGAACCTTGCTCCATGAAGACAGTGTGTCAG
 CTGTGGCTCAGTAGACCTTCTTCAGACCTGGCCAGAGAAACAAGACAACTGAGTAG
 GTTTCCTTCTCATGGAAGAAGCAAATGATTATATAGAGTCTTTGTTTTTGGGACAGGT
 TCTGTCTGTTTTCTGGCTGTCCATGAACTCAGTATGTAG(-----
 /TAG)ACCAGGCCGGCCTCGAACTCAGAGTGCTATTATCAACATATACTACCATGCT
 20 TTGCTCTGTAAATGATGACAAAC(A/C)GAGCCAGGGACTCGTTACTGTCCGTCTGTCT
 GTCTGCCTCCC(/T)CCC(-----/TCCCTTCCCTT)CCTCCCTGTGCAGCCCTCCACT
 GGTA(C/T)TGCTCCTTGTGTGAGCTCCTCCGTGCTGTGATCTCTAGCTCTTTGCTCTTA
 TCACCTCCTGTTCCCTTTTTTGTGGTGTGAAGACC(A/G)TTCTCTGATTTTCTAGCCCTGG
 TA(G/A)CTCTACTTTATGTTGCCTTTGCTTTTCTGTGTTCCCC(G/L)TCTGCTAAT
 25 TATTAGATGTTTCTATTGTGTTTTATAACAAAGGAAAAAGAGCCAAGGATGTGTTTGATA
 A(G/A)AGGCGCGGTATGGTGAGGTGGGAGGGGTGCCTCGGCGGGCCCATGCAGAG
 CCAAGGATGTGTTTGATAA(G/A)AGGCGCGGTATGGTGAGGTGGGAGGGGTGCCTCG
 GCGGGCCCATGCTGAAGTTTCCCTTCCCC(/C)TGAGAGACCAGCTGAAGTTTC
 CCTTCCCCCTGAGAGACCAGCCATAGGACAGGTATAGTGTAGAATAGTGTATTTAGG
 30 GCATGGGAA(A/G)GGGAGTTGGGAAGGGAGTAGAGACAGAAGGGGGTGGGGGAAG
 GAGAGGAGAGGCTGGCCAGGAACATGTGGGGAAGGAGGAGAGGGGAAGGGGAGA
 GAGGGAGAAAGAGTTCAGAGAGAAGAGATGGCCAGGAACATGTGGGGAAGGAGGA
 GAGGGGAAGGGGAGAGAGGGAGAAAGAGTTCAGAGAGAAGAGAGGCAGAGAAAGA
 GAAGGGGCAGAGAGCAAGGGCAGAGAAAGAGAAGGGGCAGAGAGCAAGAGAGAGG
 35 AGGGGGCAAACGGCCCCCTTTATAGTAATCCAGCCATATTAGCGGTTGCC(A/G)TGTA
 ACTGTGGGGTGGAGCCTAGAAGGAATGCTAACAGTTTCCCCATAATACTGCATTTTA
 CCAGCATTCTGGCCTTGGCTATTCTTCTGAGCAGTTCATTTGATTCTGAGTCTGGT
 GCTTCCTATAGCATGTCTGCTTGGGCATTTATGTATGTGGTCCGTCTTTCTTCTGAGGC
 TCTGACTAACTCTGAAGTCAGGTAATTTTCTTCATGCAGCTGTTAGAGTTAGCCTTCC
 40 CAGAGTGGATCTTTTCAGCTCTTCTGCCAGTAGGCATGCTGTC(A/C)ACCACCTGCC
 AGAAACCTGGT(A/G)TCTTAGGGTTTTACTGCTGTGAACAGATACCATGACCAAGTCAA
 GTCTTATAAAGGACAAC(A/G)TTTAATTGG(A/G)CCTGGCTTCCAGGTTTCAGAGGTTCA
 GTCCATTATGAAGGTGGGAGCATGGCAGTGTCCAGGCAGGCATGGTGCAGGAGGAG
 CTGAGAGTTCTACATCTTCACCTGAAGGCTTCCAGGCAGCTAGGATGAGGGTCTTAA
 45 GCCCA(AA/TG)CC(T/C)ACAGTGACATGCCTACTCCAACAAGGCCACATCTCCTAATAG
 TGCCACTCCCTGGGCCAAGCACATACAAGC(AT/CA)TCACAGCTGGCATGGCATTTC
 CCCTTCCCTTCTCCTGTTATGCCTACTGCTGGTTTACTTGAAAAAGTGGCATTTCATC
 CCTTATAATACACA(TGA/----)CA(C/-)ACACACACACACACA
 (CACACACA/-----)CTCTCTCTCTCTCTCTCT(C/T)TCT(C/T)T(CTCTCT/-----)CTCTCT

TCTCCCCC(C/T)CCCCCCCATTCAAGGGTCTTCCACAGCTACACCTGCTGTACTATTG
 AGCATATCTCTAGCCTGCTGGAATGATTTTTAAAAGTGCAAACCTTGATTAATTCCTTTTA
 AAAAATCCTCTTACTTTTGGTATAGCATTGGAAATGTAAATGAGTTAAATACCTAATAAA
 AAATGGAAAAAATCCTCTT(A/-)AACTTCATCCTCATGTCCCCTCTAA(C/T)
 5 GTTTTTTCTACTTTACTTAAGAGACTATTTTTCTCAAGTTGGAAAT(C/A)TATAAATATAT
 AAATTC(C/A)CCTTAATAGTAAA(-/T)AGTCTTCTTTTGTTCCTGATACAGGGTCTC
 ATGA(C/G)CCCACACTAACCTGACTTGCTGTGTATCTGAGAATAATGTTAAACATTTTG
 ACATTTATTTATTTTAGGTGTTTGGGTATTTTGCTGCATGTCTGTGTGCCA(A/G)ATGCA
 TGCCTGTGCCTTAGCAAGCCTGAACAGGGCGCCAGTTCCCTGGAGCTGGA(C/G)CTA
 10 (T/C)AGG(T/C)GGCTGTA(A/G)ACAGTGGGACTTAACTTGCAGTGTTTGA(A/G)AGTTTT
 CTA(-/G)AAAAGTTTCTTTACTTTCTCTATGGCAATGGCATTAAAAATAGA
 AAATTAAGCTAGGATTAAGAAAAGGGTAAGAAAGCAGTTGCTGGCTGCTCGGTATG
 GCACCTGAGAGAGCAGAGCAGAATCCGGCAGTAAAGGCTCCCGCGTGTTCCTAC
 TCACTGGCTTCTGTCACTACTGCTATCTGGATTTGCTCAGTTTGTGTATCTTTCCTTTCT
 15 CTGACAGTGTTTGGTGTACAGAACAGACCAAGCGCAAGACGTAAAGAAGATTGAGA
 AATTCCACAGTCAGTTAATGCGACTTATGGTGGCCAAGGAATCCCGCAATGTCACT
 ATGGAAACAGAATGAATGTTTGCATGAAGACGACTGTT(C/A)CGTT(A/G)TTGGGA
 AGTAATCAGCTTT(T/-)GAAACTGAGAGTGTTGGG(A/G)AGGATATACTTAC(G/A)
 TAA(T/C)GGAGCTGTCAAAGCCGAGAGACCAGCCTGCGTCCTAAAGTTTGCTTTAGA

 GAGTAGGAATGTCGGGGTTTCCAGTTAGAAAACCTTTATTTTTGGAAACGGAATAAA
 AATCTCTTAGAACTTTTGCAGATAATTTGATGTTGGGCAAATATATATAATTATTTTT
 TCTGGTAAATTCATGTCAGTAATTTGTTGAAGAGTTAACAAGAAAAGGTCTTTCTAG
 ATTGTGTCTAAGATGAAATAAAATGTAACCTTTTGTGCTGCAGTGGTATGTTTCTTTTGA
 25 GTACAACATGTATGCCGTTTACAACACACACATGGTAACGGGGATCACATGCTCCA
 TGGTGGCGACGATCCTGTGGTGTGAAGAGCAGGTCTGCTTTACCTTTCTTGCCAGGT
 TCAAACGTGTTTTTAAATGGAAGGGAT(T/C)TGTGGCATCCACTTGCTGAGCGGTGAA
 CAGAGCGAGACGCAGTTATGTGTGCACCGGCAGATACTGACACTGATGGCTGACAGT
 GTGTGCGTCCCTCACGCCGGTGTGCA(A/G)CGTACGCAGTGT(T/G)TACTCAGTGTGG
 30 CTCC(C/T)GCCTCAGACTGAATTAAAGTATTACTATAGCATGCCTCTCAGAACTGCTAG
 CTTCTT(A/C)ATAAACCACAAATCCACCGTTTCTATGTAGCTTGTATTTGTAAAGTCTGA
 GTTTACCGTCTGAATGTAAAGTTCCGTGTTGTCCGCTCACGACGGAAGTGAAGAAGAT
 ACTGCATTCAAGTGTTTTTTGTAAAAGTTTGTGTCATGGTGAATAACTTGAAATTTTATTA
 GTTATAAGAATTTTAGTCTGTTTTAGAGGTCAGTAAATAAAATACAAGCATCCTGG(T/C)
 35 TTTGTTGAAGTTTTGGATTCTTGTATAGGTTTCTTTGTTTCTTCTTTGTTTAGCTA
 GGGTCTCACTAGCCCTGGTTGTCCTGGAACCTCATTGTAGAGGCCAGGCTGGCCTTGA
 ACTTACAGAGA

Example 2. Evidence supporting a role of *Srp14* in the susceptibility for febrile seizures.

Animals

Breeding pairs for C57BL/6/J, C57BL/6J-Chr 2^A/NaJ (CSS2) and C57BL/6J-Chr
 2^{PWD}/Ph/ForeJ (CSS2-PWD) were obtained from The Jackson Laboratory (Bar Harbor, ME,
 45 USA). To produce F₁ hybrids, C57BL/6J and CSS2 males and females were reciprocally
 outcrossed. Thereafter, F₁ hybrids were intercrossed producing 143 F₂ progeny (61

(male:female = 25 : 36) originated from CSS2 mothers and 82 (male:female = 52 : 30) from C57BL/6J mothers.

Animals were kept in a controlled 12 hour light-dark cycle (lights on: 7:00 a.m. to 7:00 p.m) with a room temperature of $22 \pm 1^\circ\text{C}$ and a humidity of 50-70%. Food and water were available *ad libitum* (2111 RMH-TM diet; Hope Farms/Arie Blok BV, Woerden, the Netherlands). All animals were housed in wire topped (Macrolon® Type II) cages (Techniplast, Milan, Italy) with sawdust bedding and a paper tissue for nest building. All experiments were performed according to the institutional guidelines of the University Medical Center Utrecht.

Phenotypic screen for eFS (experimental febrile seizure) susceptibility.

FS susceptibility in inbred mice was measured as described earlier (Hessel et al., Genes Brain and Behavior 8, 248-255, 2009). Prolonged eFS were induced by exposing 14-day-old mice (P14) to a warm-air stream to induced hyperthermia. Susceptibility to eFS was determined by measuring the latency to tonic-clonic seizures (febrile seizure latency = FSL), a phenotypic parameter which has previously been validated by video/EEG monitoring (Hessel et al., Brain and Behavior 8, 248-255, 2009). On P1 litters were culled to 4-6 pups with a balanced male:female ratio, when possible. On P10 a temperature sensitive transponder (IPTT-300, (BioMedic Data Systems) Plexx BV, Elst, The Netherlands) was injected subcutaneously (separation time from the mother < 2 min) in pups weighing > 5.0g. At P14 individual pups (weighing 6.5 - 8.0 gram) were exposed to a pre-heated warm-air stream of $50 \pm 0.5^\circ\text{C}$ until they develop FSL. Core body temperature was measured with a wireless temperature reader (WRS-6007, (BioMedic Data Systems), Plexx BV) throughout the experiment. Mice were immediately sacrificed after hyperthermia and tail, spleen and brain were collected for genotyping. All experiments were performed between 10:00 a.m. – 4:00 p.m. Parents of origin, gender and body weight (BW) were recorded for covariate analysis. FSL of the complete CSS2-F₂ progeny was determined within a period of two months. During that period FSL of C57BL/6J (n=9) and CSS2 (n=9) was also measured to monitor stability of the eFS susceptibility phenotype.

Genotyping and genetic map construction

DNA was isolated from tails as described by Kas et al. (Genes Brain and Behavior 98, 13-22, 2009). DNA concentration was measured on agarose gel and diluted to 10 ng/μl. To generate a genetic map fifteen microsatellite markers distributed over chromosome 2 were chosen from the mouse genome database based on the presence of allelic differences between the A/J and C57BL/6J inbred strains (Mouse Genome Informatics, <http://www.informatics.jax.org>). Microsatellite marker genotyping (Williams et al., 2001) was

performed by PCR. Primers (150 nM forward/reverse) flanking these markers (Sigma-Aldrich, Zwijndrecht, the Netherlands) were used to amplify the DNA (50 µg/reaction) in a Veriti 96 wells thermocycler (PCR system, Applied Bioscience Inc., Foster city, CA, USA) using Super Taq polymerase (SphaeroQ, Gorinchem, The Netherlands: 0.25 units/reaction). The PCR products were separated on 3% agarose gels and visualized by ethidium bromide staining. Two additional TaqMan SNP primers were used for fine-mapping. SNP genotyping was performed as described (*Kas et al.*, Genes Brain and Behavior 98, 13-22, 2009). The two SNP markers were selected using Genenetwork (<http://www.genenetwork.org/>): rs27498297 and rs27434812. Primers (Taqman SNP genotyping Assay, Applied Biosystems, USA) were used to amplify the DNA (10 ng/reaction) using TaqMan Universal PCR Master Mix, Applied Biosystems, USA. SNP analyses were done with a sequence detection system (7900HT, Applied Biosystems, Foster City, CA, USA). Segregation ratio of the genotypes of individual markers was checked by means of the Chi-squared goodness-of-fit-test. None of the markers showed ($p < 0.05$) segregation distortion (AA:AB:BB ratio 1:2:1) for any of the sub-F2 populations. Cox et al. (Genetics 182, 1335-1344, 2009) have constructed a revised genetic map of the mouse genome and demonstrated that utilization of the revised map improves QTL mapping. Therefore, marker positions (in cM) were taken from this map by using the 'mouse map converter' (<http://cqd.jax.org/mousemapconverter/>).

20 QTL mapping

The location of the FS susceptibility QTLs and the variance explained by each locus were determined using the MapQTL® software package, version 4.0 (Van Ooijen et al., 2002). Because the parameters were normally distributed, the interval mapping module was used. Results were expressed as LOD scores. Based on Lander and Botstein (Genetics 121,185-199, 1989), having an average distance between markers of 4.95 cM, and taking into account that a genetic scan was performed across a single, complete chromosome rather than the entire genome, an association was assumed significant when the LOD score was ≥ 1.63 . Output from MapQTL® was converted to figures using the graphics program MapChart. Epistatic interactions for marker pairs were tested with a two-way ANOVA. Homogeneity of variances was tested using the Levene's test. All within-group FSL data were normally distributed and subjected to a one-way ANOVA with genotype-group as main factor.

Statistics

The phenotypic characteristics of C57BL/6J and CSS2 mice were normally distributed and subjected to a two-way analysis of variance (ANOVA) with strain and gender as main factors. A Type IV sum of squares was used for the ANOVA since the data were unbalanced.

unbalanced. Homoscedasticity was tested using the Levene's test, which is a powerful and robust test based on the F statistic. If the ANOVA showed significant effects the group means were further compared with the unpaired Student's t test. The unpaired Student's t tests were performed using pooled (for equal variances) or separate (for unequal variances) variance estimates. The equality of variances was tested with the Levene's test. For the unpaired Student's t test with separate variance estimates, SPSS® uses the Welch-Satterthwaite correction.

Performing many ANOVA's in the F_2 population increases the risk of a Type I error. To avoid this, the level of statistical significance for these ANOVA's was adjusted by using the so-called Dunn—Šidák method ($\alpha = 1 - [1 - 0.05]^{1/\gamma}$; γ = total number of DNA markers [17, $\alpha \approx 0.003$]). In all other cases (i.e. the Kolmogorov-Smirnov one sample test, Levene's test, statistical testing of host and consomic strain, Pearson product moment correlation, Chi-squared goodness-of-fit-test) the probability of a Type I error < 0.05 was taken as the criterion of significance.

Candidate gene selection

The 1-LOD support interval was estimated using linear interpolation calculations. Coordinates were obtained with NCBI Build 37. Genes present in the 1-LOD support interval were selected on <http://www.informatics.jax.org/>. Genes on chromosome 2 previously implicated in human FS or epilepsy were studied using OMIM (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM>) or CarpeDB (<http://www.carpedb.ua.edu/>). Haplotype mapping was done with perlegen (<http://mouse.perlegen.com/mouse/index.html>). SNP differences between A/J and C57BL/6J were studied with genenetwork (www.genenetwork.org) and mouse phenome database (<http://www.informatics.jax.org/>). Pathway analysis on brain expressed genes in all mouse FS susceptibility QTLs we mapped so far, was performed on (<http://bioinfo.vanderbilt.edu/webgestalt>).

In situ hybridization

Radioactive in situ hybridization was performed as described (van der Hel et al., Genes Brain and Behaviour 8, 248-255, 2009). CSS2 (n = 6) and C57BL/6J (n = 6) brains were dissected from naive P14 mice, snap frozen and stored at -80°C . Coronal (16 μm) sections were cut with a cryostat (Leica CM3050, Rijswijk, The Netherlands), collected on glass slides (Superfrost Plus, Thermo Scientific, Braunschweig, Germany) and stored at -80°C . Sense and antisense probes for *Srp14* were synthesized using mouse cDNA as a template. The

primers were designed using the mRNA sequence of *Srp14* provided by the PubMed nucleotide database (<http://www.ncbi.nlm.nih.gov/pubmed>; NM_009273.4) and the online Primer3 program (<http://frodo.wi.mit.edu/primer3/input.htm>): forward primer: 5' GAGAGCGAGCAGTTCCTGAC 3' and reverse primer: 3' GCCACACAGCACAAATCAAA 5'.

- 5 DNA sequences were confirmed by sequencing, and used for the preparation of the probe (BaseClear (Leiden, the Netherlands). Partial cDNA was inserted in a p-GemT easy vector and verified by digestion with restriction enzyme NCO1. Riboprobes were synthesized using T7 and SP6 RNA polymerase and labeled with [α - 33 P] UTP (ICN Biomedicals, Irvine, CA, USA).
- 10 Dehydrated air-dried slides were exposed to X-ray film (Kodak Bio-Max MR, VWR, Amsterdam, The Netherlands) for 3 days with 14 C-microscales (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom). Hybridization of 33 P-labeled probes was quantified with ImageJ 1.43 (Maryland, USA).

15 *Human tissue*

- Hippocampal and neocortical tissue used for qPCR analysis was obtained from patients who had surgical treatment for pharmaco-resistant TLE (van der Hel et al., 2005). Patient details are summarized in supplementary table 3. Informed and written consent was obtained from the patients for all procedures as approved by the Institutional Review Board. For qPCR
- 20 analysis we compared TLE patients with hippocampal sclerosis (HS; n= 6) and without hippocampal sclerosis (non-HS; n= 6) with autopsy control patients (n=7) obtained from the Netherlands Brain Bank (NBB). Hippocampal sclerosis was graded according to Wyler et al. (1992), only patients with severe hippocampal sclerosis (Wyler grade 4) or patients without hippocampal sclerosis (Wyler grade 0) were selected. RNA was isolated as described (van
 - 25 Gassen et al. 2008).

Quantitative RT-PCR

- Srp14* mRNA expression was studied in human brain homogenates. Extracted RNA was DNase treated with RNeasy mini kit (Qiagen, Valencia, CA). cDNA was synthesized with
- 30 superscript 2. The qPCR reaction was performed using the LightCycler (Roche, Almere, NL) and the Fast Start DNA Master PLUS SYBRgreen I kit (Roche). Primer (Sigma Genosys, Cambridge, U.K.) Forward primer: 5' AGGGTACTGTGGAGGGCTTT 3'; Reverse primer: 3' CTGTTGTTGGTGCTGTTGCT 5'.

- Gene expression was calculated as normalized ratio and normalized to two reference genes.
- 35 cDNA from the common reference pool was used for calibration. The reference genes (Proteasome 26S subunit, non-ATPase, 2 (*PSMD2*) and DEAD (Asp-Glu-Ala-Asp) box

polypeptide 48 (*DDX48*) were selected because of their housekeeping function and their microarray expression strength and stability (van Gassen et al., *Epilepsia* 49, 1055-1065, 2008). All samples were analyzed in duplicate and reported as mean $\pm$ standard error of the mean (SEM). Both reference genes gave similar results.

5

Results:

C57BL/6J versus CSS2

First we confirmed that CSS2 (445.1 ± 15.1 sec) mice have a shorter FSL than C57BL/6J
 10 (597.4 ± 36.2 sec) mice (Fig.5, panel A) in a separate group of male and female CSS2 ($n = 9$) and C57BL/6J ($n = 9$) (two-way ANOVA: strain effect, $F_{\text{strain}(1,14)} = 11.867$, $p = 0.0001$; gender effect, $F_{\text{gender}(1,14)} = 0.298$, $p = 0.594$; interaction effect, $F_{\text{interaction}(1,14)} = 0.008$, $p = 0.929$). The time-course of body temperature increase during exposure to hyperthermia did not differ between C57BL/6J and CSS2 mice (Fig. 5, panel B (time $F_{(1,14)} = 1704.63$, $p =$
 15 0.0001 ; time $\times$ group $F_{(1,16)} = 1.363$, $p = 0.264$). There was neither a difference in P14 body weight between the C57BL/6J (7.36 ± 0.24 g) and CSS2 (7.07 ± 0.13 g) (two-way ANOVA: strain effect, $F_{\text{strain}(1,14)} = 3.091$, $p = 0.101$; gender effect, $F_{\text{gender}(1,14)} = 0.439$, $P = 0.518$; interaction effect, $F_{\text{interaction}(1,14)} = 1.137$, $p = 0.304$) nor a difference in the start temperature between the C57BL/6J ($T_{\text{start}} = 36.0 \pm 0.1^\circ\text{C}$) and CSS2 ($T_{\text{start}} = 35.9 \pm 0.2^\circ\text{C}$) (two-way
 20 ANOVA: strain effect, $F_{\text{strain}(1,14)} = 0.202$, $p = 0.660$; gender effect, $F_{\text{gender}(1,14)} = 1.153$, $p = 0.301$; interaction effect, $F_{\text{interaction}(1,14)} = 2.502$, $p = 0.136$)

QTL analysis CSS2

To map the QTLs for eFS susceptibility on chromosome 2, we bred a CSS2- F_2 generation
 25 ($n=143$) by intercrossing F_1 hybrids from a CSS2 $\times$ C57BL/6J outcross. As expected, phenotyping these genetically unique F_2 mice revealed a (left) shift toward a shorter FSL compared to C57BL/6J mice (fig 6a). Two distinct QTLs were mapped on chromosome 2 (fig. 2b). The most proximal QTL2a (LOD-score 4.5) was responsible for 13.5% of the F_2 phenotypic variance and the effect was recessive with respect to the A/J grandparent's
 30 allele. The 1-LOD support interval of QTL2a was 52,581,428 – 66,278,629 bp. QTL2b (LOD score 6.8) was responsible for 19.8% of the F_2 phenotypic variance and the effect is also recessive for the A/J grandparent's allele. The 1 -LOD support interval for QTL2b was 114,875,854 – 133,817,501 bp (fig. 6b). One way ANOVA was performed for each marker and genotype. Taking body weight parent of origin or gender as covariate did not effect the
 35 1-LOD scores significantly (data not shown).

QTL2b

The 1-LOD support interval of QTL2b contains 273 genes (excluding 65 predicted (GM) genes) of which 165 are known to be expressed in brain. To fine-map the QTLs on chromosome 2 we measured FSL in CSS2 from the PWD/Ph donor strain (CSS2-PWD, fig.

5 3) and compared it to FSL of CSS2 and C57BL/6J with a one-way ANOVA ($F_{\text{Strain } 2,25} = 36.080$, $P < 0.0001$). The FSL of CSS2-PWD (1037.1 ± 75.2 sec) is significantly longer compared to the CSS2 (445.1 ± 15.1 sec; $p = 0.0001$) and C57BL/6J (597.4 ± 36.2 sec; $p = 0.001$). Based on haplotype mapping of the three strains (<http://mouse.perlegen.com/mouse/index.html>) the number of potential candidate genes in

10 QTL2b can be reduced to 114, of which 49 are brain expressed. The most interesting of these 49 brain expressed genes are *Il1 β* and *Srp14*.

Il1 β (129.190.306-129.196.875 bp) has previously been associated with FS in mouse (Dube et al., Ann.Neurol 57, 152-155, 2005) and human (Virta et al., Epilepsia 42, 9320-923, 2002; Kira et al., Neurosci.Lett. 384, 239-244, 2005). No known SNP or haplotype differences

15 were found for *Il1 β* between CSS2 and C57BL/6J. Re-sequenced (forward and reverse) of the *Il1 β* sequence in C57BL/6J, A/J and CSS2 (129,189,728-129,197,401 bp) did not identify any new SNPs, and the haplotype did not differ between the strains. To further assess a possible role of IL1 β in FS susceptibility, we injected IL1 β in C57BL/6J mice 45 min before hyperthermia. The FSL of the IL1 β group ($786.5s \pm 308.15$ sec) did not differ from the

20 saline group ($779.2s \pm 144.54$ sec) ($t_{10} = 0.053$, $p = 0.959$). The start temperature of the IL1 β group ($T_{\text{start}} = 35.9 \pm 0.1$ °C) was significantly higher than that of the saline group ($T_{\text{start}} = 34.9 \pm 0.2$ °C) ($t_{10} = -3.972$, $p = 0.003$), confirming the pyrogenic properties of IL1 β . No significant differences were found in the temperature increase during the experiment between the two groups (data not shown). Together these data show that it is highly unlikely

25 that IL1 β contributes to the phenotypic difference between CSS2 and C57BL/6J.

The second interesting candidate gene in QTL2b is *Srp14* (118301579..118305432). SRP14 is part of the ubiquitous signal recognition particle (SRP) and forms a heterodimer with SRP9. The *Srp9* gene is a functional and positional eFS susceptibility gene as identified above after QTL mapping on chromosome 1; *Srp9* shows differential expression between

30 CSS1 and C57BL/6J (Hessel et al., example 1). In situ hybridization revealed a significantly higher *Srp14* expression in the hippocampus of CSS2 (16.7 ± 0.7 KBq/g) compared to C57BL/6J (14.4 ± 0.6 KBq/g) (Figure 7a; $t_{10} = 2.450$, $p = 0.034$).

Interestingly, qPCR analysis of *Srp14* expression in hippocampal homogenates resected from epilepsy patients to treat pharmaco-resistant TLE, showed (fig. 7b) that HS-TLE

35 patients (3.42 ± 1.2) have a higher *Srp14* expression than non-HS TLE patients (1.00 ± 0.08) and autopsy controls (0.96 ± 0.22) ($F_{2,13} = 5.949$, $p = 0.018$; Autopsy vs non-HS, $p =$

0.538; Autopsy vs HS $p = 0.015$; non-HS vs HS $p = 0.012$). No difference between HS and non-HS patients was found in the expression of *SRP68*, another gene within the SRP complex, ($F_{2,18} = 14.346$, $p = 0.001$;; autopsy vs non-HS $p = 0.001$; autopsy vs HS $p = 0.0001$; non-HS vs HS $p = 0.361$) indicating that the increase in *Srp14* is not due to the

5 altered hippocampal morphology related to the sclerosis.

CLAIMS

1. Use of an SRP9 protein and/or an *Srp9* nucleic acid sequence and/or an SRP14 protein and/or an *Srp14* nucleic acid sequence as a diagnostic marker.
2. Use according to claim 1, wherein said diagnostic marker is for febrile seizures,
5 complex febrile seizures, for complex epilepsy syndromes including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or for temporal lobe epilepsy.
3. Use of an SRP9 protein and/or an *Srp9* nucleic acid sequence and/or an SRP14 protein and/or an *Srp14* nucleic acid sequence in identifying a subject at risk of developing
10 febrile seizures (FS), a subject at risk of developing complex FS, a subject at risk of developing a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or a subject at risk of developing temporal lobe epilepsy.
- 15 4. Method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the steps of:
 - a) providing a sample of a subject;
 - 20 b) determining the level of at least one biomarker selected from SRP9 polypeptide, *Srp9* mRNA, SRP14 protein and/or *Srp14* mRNA in said sample;
 - c) comparing the level of said biomarker to a reference level; and
 - d) determining whether the level of said biomarker is indicative of a risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such
25 as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.
5. Method according to claim 4, wherein said subject is an infant or child.
6. Method according to claim 4 or 5, wherein said sample is selected from the group
30 consisting of a whole blood sample, serum, and plasma.
7. Method according to any one of claims 4-6, wherein an increased level of said biomarker is indicative of a risk of developing febrile seizures, complex febrile seizures, a

complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

8. Use of a SNP in the promoter region of the *Srp9* gene, said SNP being selected from the group consisting of rs6688819 (T/C), rs12403575 (G/A), rs16845266 (G/C), rs120398148 (T/C), and rs6659660 (G/A), for determining the risk in a subject of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

9. Use according to claim 8, wherein said SNP is rs12403575 G/A.

10. A method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the steps of:

a) determining in a sample of a subject a SNP in the promoter region of the *Srp9* gene, wherein the SNP is selected from rs12403575 G/A, rs6688819 T/C, rs16845266 G/C, rs120398148 T/C, and rs6659660 G/A, and preferably is rs12403575 G/A.

11. Method according to claim 10, wherein the A allele of rs12403575 G/A is associated with an increased risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

12. Method according to any one of claims 10 or 11, wherein the genotype of said subject for rs12403575 is determined, and wherein an AA genotype is associated with an increased risk of developing febrile seizure, complex febrile seizure and/or temporal lobe epilepsy.

13. Method according to any one of claims 10-12, comprising a further step of providing a sample of a subject.

14. Method according to any one of claims 10-13, wherein said sample comprises or is derived from blood, an amniotic fluid, cerebrospinal fluid, tissue from skin, muscle, buccal or conjunctival mucosa, placenta, gastrointestinal tract or chorionic villi.

15. Method for identifying a subject at risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy, said method comprising the steps of:

- 5 a) determining in a sample of a subject the sequence of the *Srp9* promoter sequence, wherein the presence of one or more de novo mutations is indicative of a risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

10

16. A kit comprising a probe or primer that distinguishes an allele of a SNP in a *Srp9* gene in a sample from a subject, wherein the allele is selected from the A or G allele in the SNP rs12403575.

15

17. Kit according to claim 16 for determining an increased risk of developing febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy in a subject.

20

18. Kit according to claim 16 or 17, wherein the probe or primer is detectably labelled.

19. A method for screening chemical compounds or compositions interfering with the function of the SRP complex, said method comprising the step of identifying chemical
25 compounds or compositions that suppress expression of SRP9 and/or SRP14 polypeptide, that interact with SRP9 and/or SRP14 polypeptide and thereby prevent formation of the SRP9/SRP14 complex, and/or that interact with the SRP9/SRP14 complex and thereby prevent formation of the SRP complex.

30 20. A compound capable of selectively binding to SRP9 and/or SRP14 polypeptide, preferably an antibody capable of selectively binding to SRP9 and/or SRP14 polypeptide, for use as a medicament.

21. A compound capable of selectively binding to SRP9 and/or SRP14, preferably an
35 antibody capable of selectively binding to SRP9 and/or SRP14 polypeptide, for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy

syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

22. Use of therapeutic gene modulation for suppressing the level of SRP9 protein and/or
5 SRP14 protein in a mammal.

23. Use according to claim 22, which is for preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

10

24. *Srp9* and/or *Srp14* nucleic acid modulator selected from the group consisting of antisense DNA, siRNA, miRNA, shRNA, ribozyme specifically binding to and cleaving *Srp9* and/or *Srp14* mRNA and zinc finger nuclease specifically targeting a nucleic acid sequence in the *Srp9* and/or *Srp14* gene resulting in suppression of *Srp9* and/or *Srp14* expression for
15 use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

25. A recombinant nucleic acid construct comprising at least one transcriptional unit
20 comprising:

- a promoter,
- a nucleic acid sequence encoding a gene suppressor selected from:
 - an antisense DNA sequence complementary to the sense strand of the *Srp9* and/or *Srp14* gene;

25 - an interfering RNA molecule which is complementary to *Srp9* mRNA and/or *Srp14* mRNA;

 - a nucleic acid sequence encoding a zinc finger nuclease specifically targeting a nucleic acid sequence in the *Srp9* gene and/or *Srp14* gene;

30 - a nucleic acid sequence encoding a ribozyme specifically binding to and cleaving *Srp9* and/or *Srp14* mRNA; and

- a terminator,

for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

35

26. A vector comprising a recombinant nucleic acid construct according to claim 25 for use in preventing and/or treating febrile seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

5

27. A vector according to claim 26 comprising more than one transcriptional unit.

28. A cell comprising a recombinant nucleic acid construct according to claim 25 or a vector according to any one of claims 26-27 for use in preventing and/or treating febrile
10 seizures, complex febrile seizures, a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or temporal lobe epilepsy.

29. A method for suppressing the level of SRP9 protein and/or SRP14 protein in a cell, said method comprising the step of including an *Srp9* and/or *Srp14* nucleic acid modulator
15 according to claim 24 or recombinant nucleic acid construct according to claim 25 in said cell.

30. A kit comprising means for detecting the level of *Srp9* mRNA and/or SRP9 protein and/or *Srp14* mRNA and/or SRP14 protein in a sample derived from a subject.

20

31. A kit according to claim 30 for use in identifying a subject at risk of developing febrile seizures (FS), a subject at risk of developing complex FS, a subject at risk of developing a complex epilepsy syndrome including FS, such as Dravet's syndrome and epilepsies with mental retardation, and/or a subject at risk of developing temporal lobe epilepsy.

25

32. A kit according to any one of claims 30 or 31, wherein the kit comprises a primer pair allowing selective amplification of *Srp9* and/or *Srp14* mRNA.

33. A kit according to any one of claims 30 or 31, wherein the kit comprises an antibody
30 that specifically binds to SRP9 protein and/or SRP14 protein.

34. A kit according to any one of claims 30-33, wherein said sample derived from a subject is a sample derived from blood.

Fig. 1

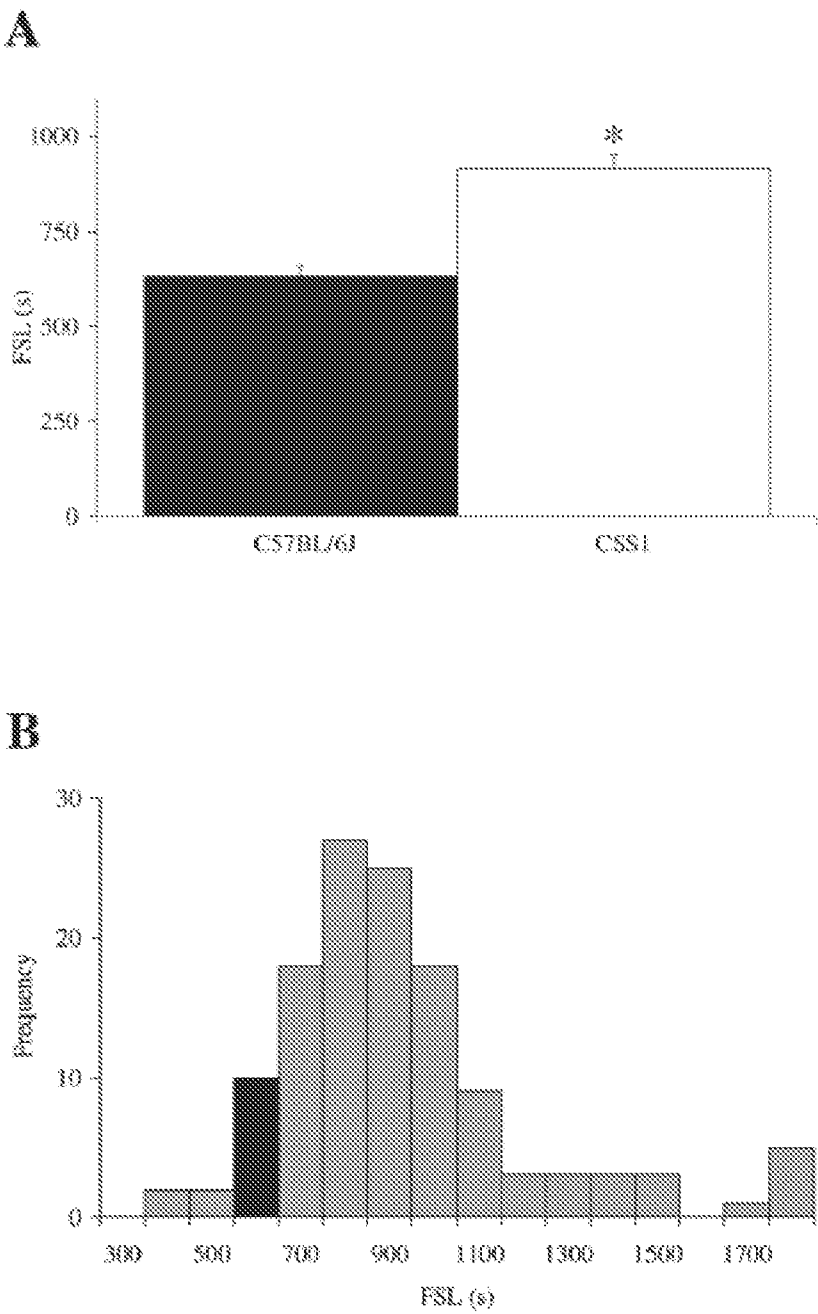


Fig. 1

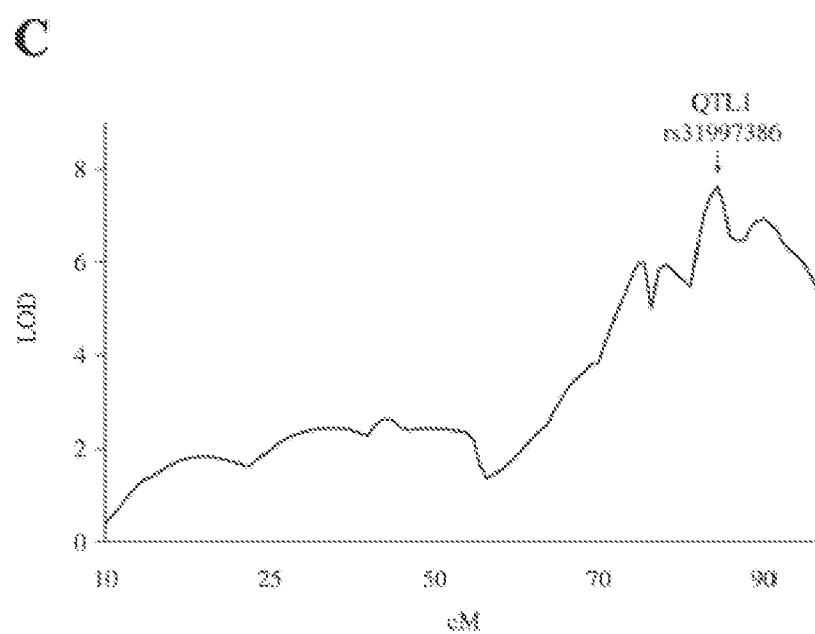


Fig. 2

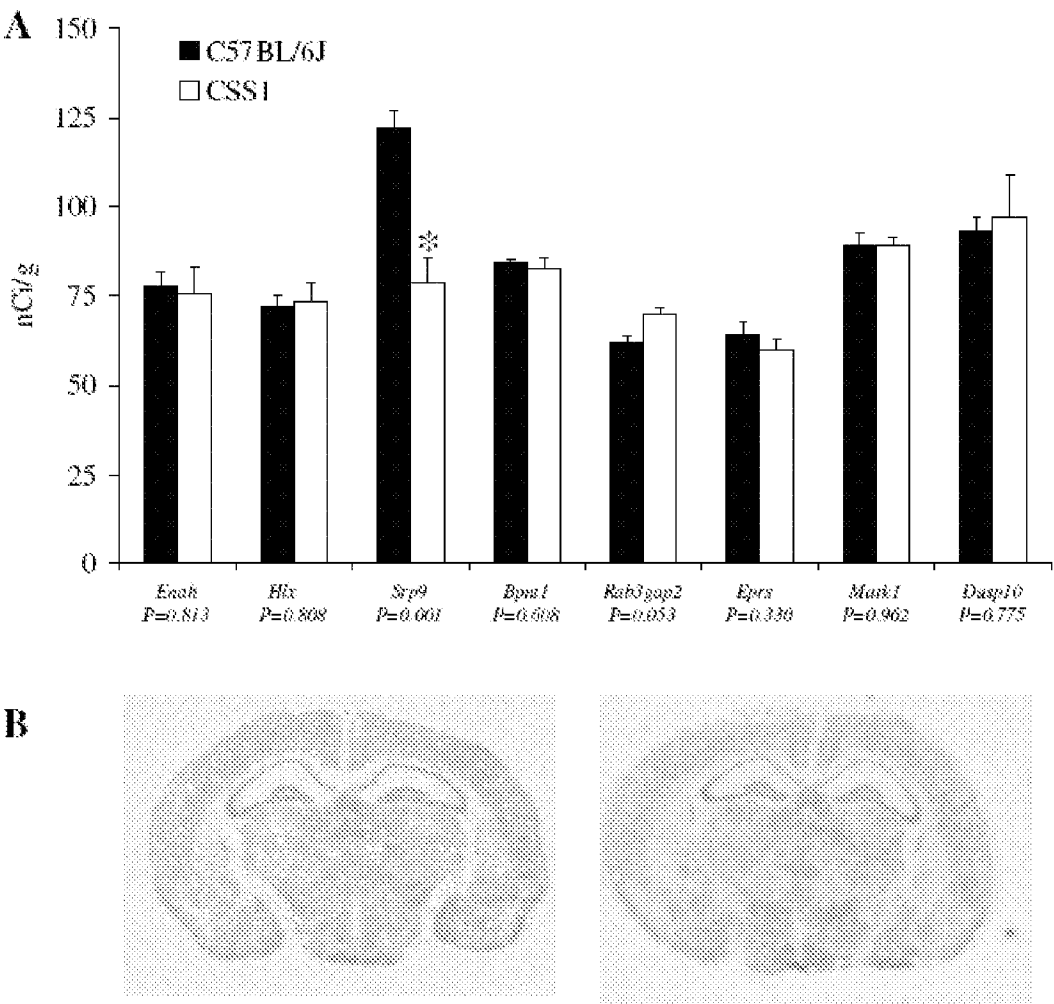


Fig. 3

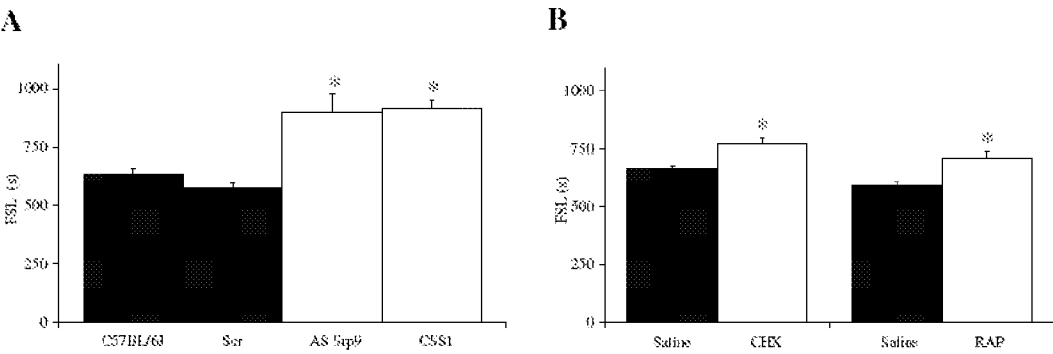


Fig. 4

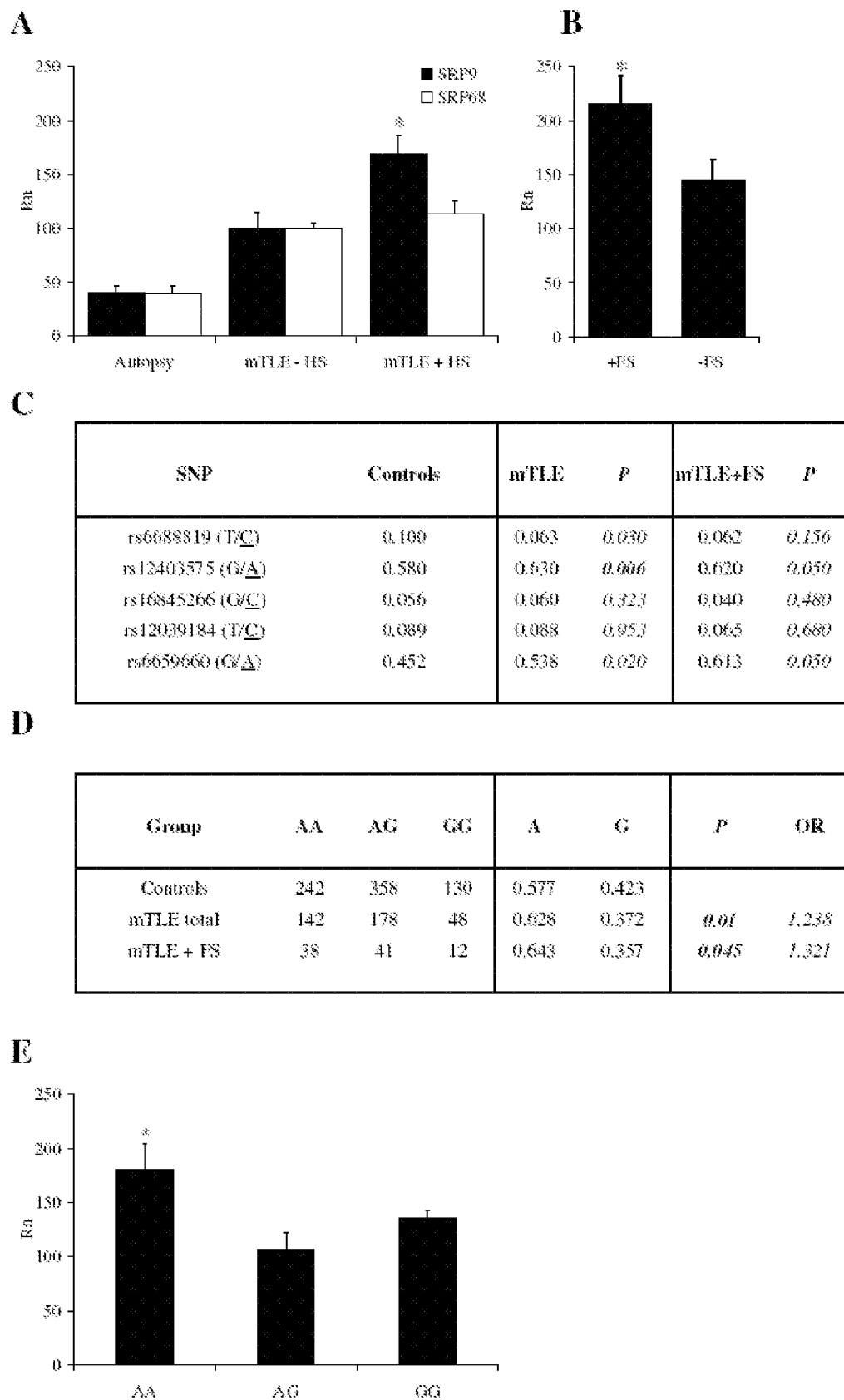


Fig. 5

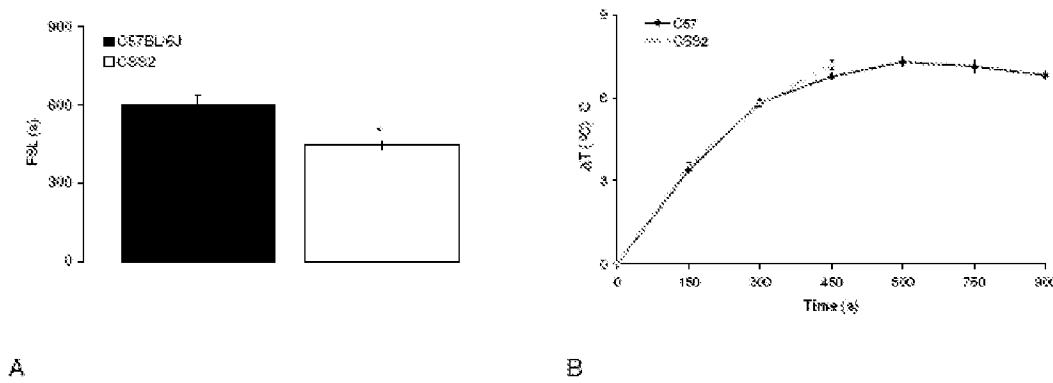


Fig. 6

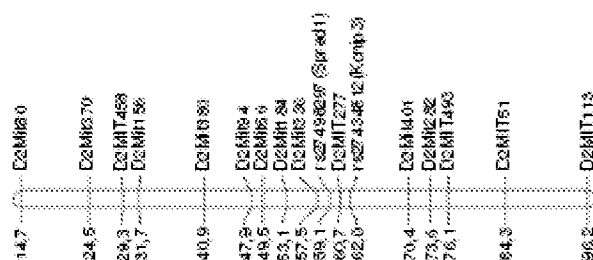
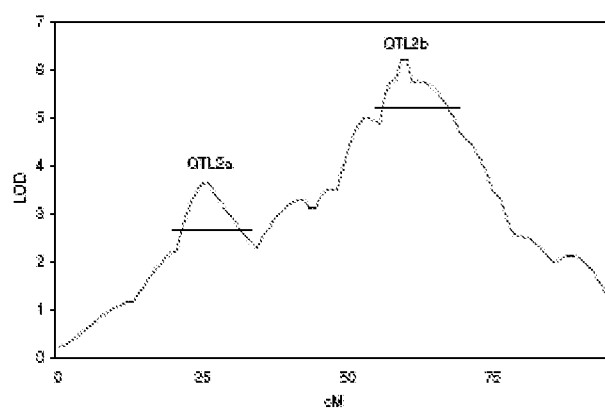
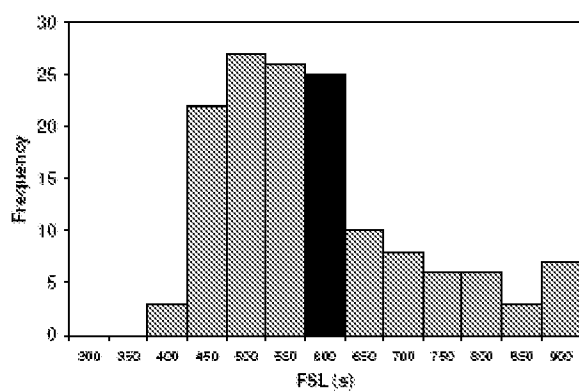


Fig. 7

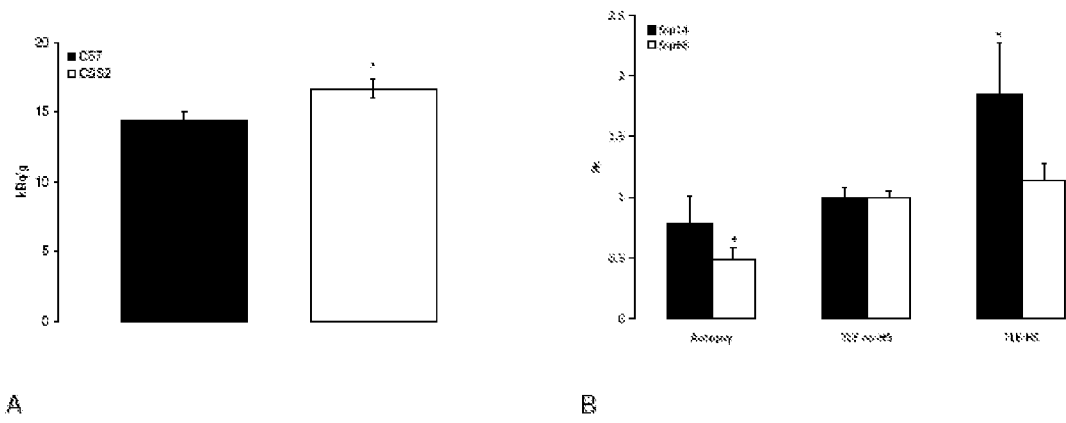
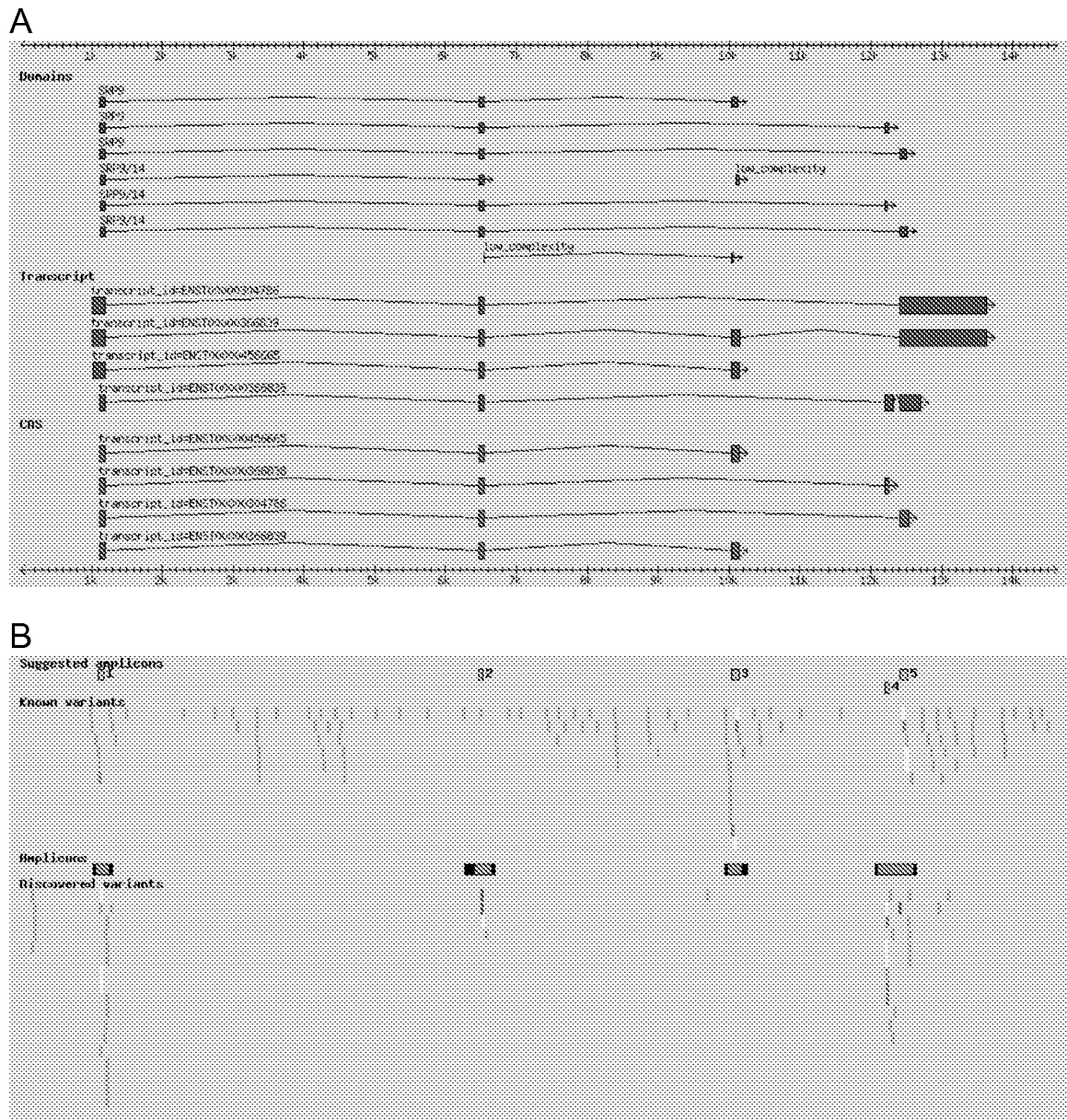


Fig. 8



INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2011/050551

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G01N33/50
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JUNG-HYUN RHO ET AL: "Proteomic Expression Analysis of Surgical Human Colorectal Cancer Tissues: Up-Regulation of PSB7, PRDX1, and SRP9 and Hypoxic Adaptation in Cancer", JOURNAL OF PROTEOME RESEARCH, vol. 7, no. 7, 1 July 2008 (2008-07-01), pages 2959-2972, XP55008502, ISSN: 1535-3893, DOI: 10.1021/pr8000892 the whole document ----- -/--	1



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 September 2011

Date of mailing of the international search report

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Authorized officer:

Dolce, Luca

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2011/050551

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOVIA F ET AL: "The SRP9/14 subunit of the signal recognition particle (SRP) is present in more than 20-fold excess over SRP in primate cells and exists primarily free but also in complex with small cytoplasmic Alu RNAs.", MOLECULAR BIOLOGY OF THE CELL APR 1995 LNKD- PUBMED:7542942, vol. 6, no. 4, April 1995 (1995-04), pages 471-484, XP055008507, ISSN: 1059-1524 the whole document	20-22
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X	----- M VIRTÀ: "Increased frequency of interleukin-1[beta] (-511) allele 2 in febrile seizures", PEDIATRIC NEUROLOGY, vol. 26, no. 3, 1 March 2002 (2002-03-01), pages 192-195, XP55008510, ISSN: 0887-8994, DOI: 10.1016/S0887-8994(01)00380-0 the whole document	1-34
X	----- WO 2010/071881 A1 (UNIV COLUMBIA [US]; PAL DEB KUMAR [GB]; STRUG LISA JOANNA [CA]) 24 June 2010 (2010-06-24) the whole document	1-34
A	----- STEPHEN CUSACK ET AL: NATURE, vol. 408, no. 6809, 9 November 2000 (2000-11-09), pages 167-173, XP55008512, ISSN: 0028-0836, DOI: 10.1038/35041507 the whole document	1-34
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INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2011/050551

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MORI MAYRA ET AL: "Differential early expression of novel genes in the hippocampus after pilocarpine-induced status epilepticus in inbred mouse strains with high and low seizure susceptibility", EPILEPSIA, RAVEN PRESS LTD, NEW YORK, US, vol. 42, no. Supple 7, 1 January 2001 (2001-01-01), page 294, XP008143563, ISSN: 0013-9580 [retrieved on 2003-08-20] the whole document	1-34
X	----- "SNP rs12403575", NCBI dbSNP, 20 April 2004 (2004-04-20), XP55008522, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=12403575 [retrieved on 2011-09-30] the whole document -----	16-18

INTERNATIONAL SEARCH REPORT

International application No

PCT/NL2011/050551

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W0 2010071881 A1	24-06-2010	CA 2747718 A1	24-06-2010
